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Molecular and Physico-chemical Basis of Breadmaking - Properties of Wheat Gluten Proteins : A Critical Appraisal

BHUPENDAR SINGH KHATKAR*[1] AND J. DAVID SCHOFIELD

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Wheat gluten consists mainly of the storage protein of wheat endosperm, i.e., gliadin and glutenin. Upon hydration and during processing, gliadin and glutenin interact to form a unique viscoelastic gluten network, which is envisaged as being necessary for holding the gases and for producing a light porous crumb textured bread. Recent work has confirmed that the elastic properties of gluten are due to the glutenin fraction, whilst the viscous properties come from the gliadin fraction. An appropriate balance in the amount of these two major protein components of wheat gluten is required for achieving the desired bread quality. Variations in the composition and physical properties of the glutenin polypeptides appear to be largely responsible for the differences in the gluten viscoelasticity and breadmaking potential among wheat cultivars. Recently, exploratory results have indicated an association of gliadin polypeptides with breadmaking quality. Using improved protein separation and purification techniques, physical methods and genetic engineering, a beginning has been made to understand the structure-functional relationship of wheat gluten proteins, but much remains to be explored in the years to come.

Keywords : Wheat gluten, Gliadin, Glutenin, Viscoelasticity, Breadmaking quality.

Wheat is the world's most important cereal crop in terms of production (550-600 million tonnes per annum) and nearly two-third of it is used for human consumption; the rest one-third is utilised for seed, feed and non-food applications (Shewry and Tatham 1994). The quantity and diversity of enjoyable and satisfying products made from wheat are remarkable. These include various types of breads, biscuits, cakes, doughnuts, pasta products, breakfast cereals and different varieties of *chapati roti*. Most wheat is consumed in the form of various types of baked goods in most countries of the world (D'Appolonia 1993; Shewry et al. 1994). Furthermore, it is generally agreed that the uniqueness of wheat is due to its breadmaking quality, bread (leavened or unleavened) having been an important staple food for thousands of years. Among plant crops, only wheat flour and to a limited extent, rye flour has the ability to form a dough that retains gases and produces a baked product, particularly leavened bread, with the desired eating qualities (Lasztity 1980; Shewry et al. 1994).

Wheat flour is known to be a complex mixture of starch (70-80%), proteins (8-18%), lipids (~2%), pentosans (~2%), enzymes and enzyme inhibitors and other minor components (MacRitchie 1984; Pomeranz 1988). A good bread quality flour encompasses an optimum blend of all these constituents. However, the technological importance of wheat flour is attributed mainly to its gluten proteins (water-insoluble complex proteins). This stems from the fact that upon fractionating wheat flour into gluten, starch, lipid and water-soluble; gluten alone possesses viscoelastic properties, i.e., it exhibits rubber-like characteristics, Also, if the gluten proteins are removed from the flour, then the property of forming a viscoelastic dough is lost (Ewart 1972; Wall 1979; Bushuk and MacRitchie 1989). It is generally agreed, therefore, that the unique viscoelastic properties of gluten proteins are responsible for uniqueness of wheat flour (Tatham et al. 1984; 1985; Khatkar et al. 1995). Inter-cultivar differences in breadmaking potential may also be linked to the differences in gluten viscoelasticity (Kasarda 1989; Schofield 1994; Khatkar et al. 1995). Accordingly, a thorough and reliable characterisation of wheat gluten is fundamental to a consideration of the development and/or suitability of a wheat cultivar for specific areas of end-use.

Cereal scientists have been trying hard for many decades to understand the structure of the gluten proteins and the mechanism of interaction, within themselves and with other flour constituents, to convert a wheat flour dough into a viscoelastic system. Also, recently, efforts have been made to understand how a viscoelastic gluten network retains gas and how the expanded dough is transformed into bread. In this review, these aspects of wheat gluten proteins are critically

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discussed in different sections.

CLASSIFICATION OF WHEAT GLUTEN PROTEINS

An optimum combination of flour constituents (proteins, carbohydrates, lipids, enzymes, etc.), ingredients and processing conditions enables wheat flour dough to be baked into a bread with a light porous crumb texture. Nevertheless, there are considerable differences in breadmaking quality among wheat cultivars and research has shown that these are due primarily to the differences in their protein fractions (MacRitchie 1992; Schofield 1994).

Proteins of wheat endosperm can be separated into non-gluten forming and gluten forming groups, when a wheat flour is wetted and mixed with water. The term gluten refers to a viscoelastic protein mass, which is recovered after aqueous washing-out of starch and water-soluble components from wheat flour. When dried, it yields a cream-coloured free-flowing powder of high protein content (75-80%) and bland taste. When rehydrated, it regains its original viscoelastic properties. The non-gluten forming proteins are albumins and globulins, which are washed out to a large extent along with the starch during the gluten-starch separation step accomplished normally with 0.5M NaCl (MacRitchie 1984). A dialysis step is often used to separate the albumins and globulins. Dialysis against water causes the globulins to precipitate, while the albumins remain in solution. The non-gluten protein class accounts approximately 10-20% of the total flour proteins and their amounts are relatively constant from one flour to another (Eliasson and Larsson 1993).

Traditionally, gluten forming proteins, which represent 80-90% of the total proteins of wheat flour, have been classified into two major groups, viz., gliadin and glutenin, based on their extractability and unextractability, respectively, in aqueous alcohol (Osborne 1907). The terms, gliadin and glutenin, are the specific names for the wheat proteins, corresponding to the generic terms prolamin and glutelin of all cereal proteins. It is believed that the names, gliadin and zimone, were first coined as early as in 1819 by Taddei for aqueous alcohol extractable and unextractable fractions of gluten, respectively. The name glutenin was proposed later by Osborne, in preference to Taddei's zimone, because the latter term was based on the Greek word for ferment, implying an enzyme activity (Shewry et al. 1986). The term gliadin was retained, however.

Neither of these groups are considered to consist of pure proteins and a considerable amount of overlap between these groups may occur, depending on the exact extraction conditions, such as time, temperature and type, as well as concentration of alcohol used (Miflin and Shewry 1979). There is no fundamental basis for classifying the gluten proteins on the basis of aqueous alcohol extractability, although such a classification does have technological significance. Gliadin and glutenin are known to impart entirely different physical properties to the gluten network in a wheat flour dough. Gliadin behaves mainly as a viscous liquid, when hydrated, and confers extensibility, allowing the dough to rise during fermentation, whereas glutenin provides elasticity and strength, preventing the dough from being over-extended and collapsing either during fermentation or in baking (MacRitchie et al. 1990; MacRitchie 1992; Khatkar et al. 1995).

An alternative classification has been proposed by Shewry et al (1986) that reflects biological, chemical and genetic relationships among component polypeptides of the gluten complex. They divided gluten proteins into three main categories, namely, sulphur-poor prolamins, sulphur-rich prolamins and high molecular weight (high M_r) prolamins (Fig. 1). All these gluten protein groups are considered typical prolamins, because they are rich in proline and glutamine amino acid residues and are extractable, at least partially, in aqueous alcohol, particularly after addition of reducing agent.

Gliadins : Gliadins are usually classified into four main sub-categories, α-, β-, γ- and ω-gliadins, in decreasing order of electrophoretic mobility under acidic conditions and increasing order of relative molecular mass (M_r) (Jones et al. 1959; Woychik et al. 1961).

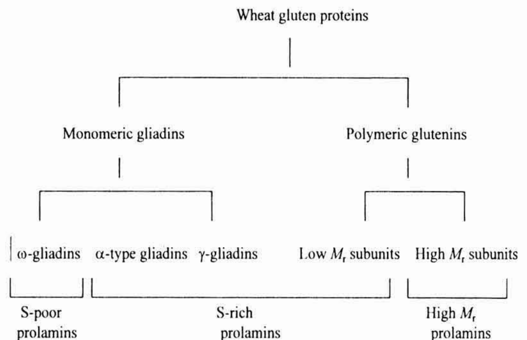


Fig. 1. Classification and nomenclature of wheat gluten proteins as proposed by Shewry et al (1986)

Introduction of new protein separation techniques, such as two-dimensional electrophoresis and reversed-phase high performance liquid chromatography (RP-HPLC), have made it possible now to separate gliadins into many individual polypeptides. In two separate studies, both involving about 70 wheat cultivars, 49 gliadin bands (Branlard and Dardevet 1985a) and 60 gliadin bands (Campbell et al. 1987) were identified. RP-HPLC could resolve more than 30 separate gliadin polypeptides (Bietz 1985).

Amino acid sequencing has revealed that the α- and β-gliadins are structurally closely related polypeptides and, therefore, both of these gliadin polypeptides have been classified into one-group, α-type gliadins. The amino acid compositions and M_rs of α-type gliadins are quite similar to γ-gliadins. However, these groups of gliadin polypeptides show some striking differences from ω-gliadins (Shewry et al. 1986).

The ω-gliadins are rich in glutamine, proline and phenylalanine, these three residues representing about 80% of the total residues, but contain few or no methionine and cysteine (sulphur containing amino acid residues). On the other hand, the α-type and γ-gliandins are relatively rich in sulphur containing amino acids, but have relatively fewer proline, glutamine and phenylalanine residues. The M_rs of α-type and γ-gliadins are between 30,000-45,000, whereas, ω–gliadins generally have M_rs of 44,000-80,000. The γ-gliadins, in general, have somewhat higher M_rs than α-type gliadins (Tatham et al. 1990; Schofield 1994).

Glutenins : The polypeptide components of glutenin are fractionated into low and high M_r subunits by sodium dodecyl polyacrylamide gel electrophoresis (SDS-PAGE) under reduced conditions. The high M_r subunits (also known as A subunits) of glutenin are the largest polypeptides and contain the highest level of glycine residues among gluten proteins. Their M_rs measured by SDS-PAGE, range from 80,000-160,000. However, SDS-PAGE over-estimates the M_rs and the true M_rs obtained from the amino acid sequences, give much lower range of about 63,000-88,000 (Anderson et al. 1988). The high M_r subunits are further divided into x-type and y-type. The x-type subunits are somewhat larger polypeptides (true M_rs 83,000-88,000), while the y-type subunits are smaller with M_rs of 67,000-74,000 (Shewry and Tatham 1990; Tatham et al. 1990).

The low M_r subunits of glutenin, after reduction of the disulphide bonds, can be divided into two main groups; a major group of basic proteins (B subunits) with M_rs of 42,000-51,000 and a minor group (C subunits) with M_rs of 30,000-40,000. Because of their similar molecular sizes, the low M_r glutenin subunits are difficult to separate from α-type gliadins and γ-gliadins by SDS-PAGE. This has hampered studies aimed at determining the functional importance of low M_r glutenin subunits in contrast to those of high M_r subunits. However, electrophoretic and solvent fractionation techniques have been developed recently to separate the low M_r glutenin polymers from monomeric gliadins and high M_r glutenin polymers (MacRitchie et al. 1990).

Among electrophoresis techniques, Singh et al (1991) and Gupta and MacRitchie (1991) have developed simplified procedures for separating low M_r subunits of glutenin. These methods provide excellent separation of low M_r subunits in a background free from monomeric proteins, but they are not suited for preparing large amounts of purified protein groups for functional studies. For this purpose, solvent fractionation schemes, recently developed by Marion et al (1994) and Melas et al (1994) are used.

Marion et al (1994) developed a new and improved method, using the detergent Triton X-114, which, according to them, provides an excellent separation between gliadin and glutenin. Melas et al (1994) have introduced a protein fractionation scheme, based on selective precipitation with acetone, which yields large amounts of purified low and high M_r glutenin polymers. It has long been thought that the gliadin/glutenin ratio, low/high M_r glutenin subunit ratio and the properties of these individual group of polypeptides are primarily responsible for the inter-cultivar variation in breadmaking quality (Kasarda 1989; MacRitchie et al. 1990; Schofield 1994; Khatkar et al. 1995).

STRUCTURAL PROPERTIES

The structural features of proteins depend on composition and sequences of their amino acids, which determine their ability to participate in chemical reactions through covalent and non-covalent interactions. Disulphide bonds are the principal covalent bonds within and between gluten polypeptides and they are of considerable importance technologically. Non-covalent interactions include ionic, hydrogen, as well as van der Waals interactions and are generally much weaker than covalent bonds. Non-covalent interactions, especially hydrogen bonds, are also of considerable technological importance for gluten proteins. In

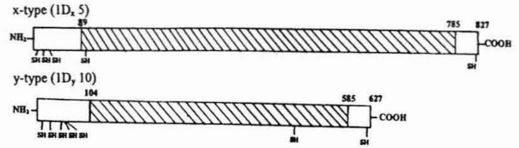
addition, hydrophobic interactions play an important role and occur between apolar groups of amino acid chains. The most significant covalent bonds, evident in the structure of gluten proteins, are disulphide linkages. Gliadins have either intramolecular (as in α-type and γ-gliadins) or no (ω-gliadins) disulphide linkages, whereas these are both inter- and intramolecular in glutenin (Wall 1979; Shewry et al. 1986; Hoseney 1994).

Hydrogen bonds are formed as a result of the affinity of hydrogen atoms in hydroxyl, amide or carboxyl groups for oxygen in carboxyl or carbonyl groups. Gluten proteins are rich in highly polar amino acids, in particular glutamine, which constitutes over 33% of the amino acids present in gluten. The amide group in glutamine actively participates in hydrogen bonding and in fact, forms two hydrogen bonds per glutamine residue (Schofield and Booth 1983). Gluten proteins also contain large proportions (~30% of total amino acid residues) of apolar amino acids, such as phenylalanine and proline, which are considered potential sources of hydrophobic interactions. The apolar residues in amino acids are associated with one another in aqueous medium. The high average molecular weight of glutenin polymers and their strong aggregation tendency are due to (i) inter-polypeptide chain disulphide bonds, (ii) the hydrogen bonding potential of the unusually large numbers of glutamine side chains, (iii) the potential for apolar bonding of the many apolar side chains and (iv) the low ionic character of gluten proteins (Wrigley and Bietz 1988).

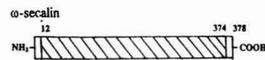
Another important feature of gluten proteins is that they have a very low charge density. This is due to their low level of basic amino acids, such as lysine, histidine, arginine as well as tryptophan and also due to the fact that glutamic as well as aspartic acids occur mainly as amides (Kasarda et al. 1976). As consequence of this low charge density, the wheat gluten proteins are not repelled by mutual charge repulsion and associate strongly by non-covalent interactions. Such behaviour is important to baking technology in that it results in the ability of gluten proteins to form viscoelastic gluten and a gluten film network that is essential for gas retention.

In recent years, a great deal of knowledge about the structure of gluten proteins has been acquired through the application of gene sequencing techniques. The major information has come from cloning of genes for gliadin and glutenin polypeptides and deduction of complete amino acid sequences

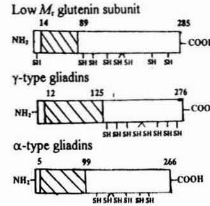
HIGH M_r PROLAMINS



S - POOR PROLAMIN



S - RICH PROLAMINS



— Repeats SH - Cysteine residue

Fig. 2. Schematic comparison of the amino acid sequences of typical gluten proteins of wheat and ω-secalin of rye (a homologue of ω-gliadins). Representations are very approximately to scale. From Shewry et al (1992, 1994) and Shewry and Tatham (1990)

from the DNA sequences of the respective genes. Comparison of these sequences (Fig. 2) reveals that all types of gluten protein polypeptides, in general, have at least two or three distinct structural domains, that is, a central repetitive domain, flanked by non-repetitive C- terminal and N-terminal domains (Shewry et al. 1994).

The S-rich prolamins are characterised by a shorter repetitive domain and a longer C-terminal domain compared with high M_r prolamins and S-poor prolamins. S-rich prolamins have their cysteine residues in the C-terminal domain, the only exception being the low M_r subunits, which also contain one cysteine residue in the N-terminal domain. S-poor prolamins have no cysteine residues, as indicated from ω-secalin of rye, a homologue of wheat ω-gliadins.

In contrast, high M_r prolamins have the majority of their cysteine residues (3 and 5 in x-type and y-type subunits, respectively) in the N-terminal domains and only one in the C-terminal domain. Several y-type subunits also have one additional residue in the central repetitive domain towards the C-terminal end and x-type subunit 5 has one residue close to the N-terminal end of its central domain (Shewry and Tatham 1990; Tatham et al. 1990).

Total numbers and relative positions of cysteine residues are important for polymer size and the different polymerisation behaviour of gliadins and glutenin subunits. MacRitchie (1992) has suggested that the presence of cysteine residues in the form of intra-molecular disulphide bonds in gliadins and inter- and intra-molecular disulphide bonds in glutenin subunits is due to the fact that gliadins have even numbers of cysteine residues, whereas glutenin subunits have odd numbers of residues (analyzed so far) within a single structural domain (Fig. 2). He has also mentioned that the relative position of cysteine residues and the manner of chain folding are also important for this difference. Among the S-rich prolamins, the polymerisation behaviour of low M_r subunits of glutenin is attributed to their ability to form inter-molecular S-S bonds, which distinguish them from gliadin polypeptides.

Inter-molecular disulphide linkages are considered responsible for giant size of glutenin polymer. However, it has been proposed that inter-molecular S-S bonds are limited, i.e., only two per polypeptide chain connecting each chain in a head-to-tail fashion to the next chain, thus forming a linear macropolymer of glutenin known as 'concatenations' (Fig. 3A), as suggested by Ewart (1968, 1977, 1978, 1979). Based on Ewart's findings and recent understanding of the structure of glutenin subunits, in particular high M_r subunits, several hypothetical models for glutenin polymer have been proposed (Khan and Bushuk 1978; Graveland et al. 1985; Kasarda 1989; Gao et al. 1992).

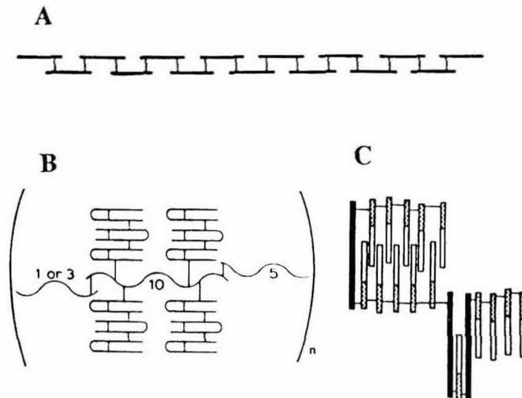


Fig. 3 Schematic illustration of the models of gluten polymer according to: A, Ewart (1968); B, Graveland et al (1985); C, Kasarda (1989). In Fig 3A, horizontal lines represent glutenin polypeptides and vertical lines represent S-S bonds. In Fig 3C, long solid lines indicate high M_r glutenin subunits and in low M_r glutenin subunits, unrepetitve domain is shown by the hatched areas, whereas the unhatched region represents the repetitive domain.

According to the model of Graveland et al (1985), repeat units of a y-type subunit 10 assume central position to which two different x-type subunits, 1 or 3 on one end and 5 at the other end, are linked via S-S bonds, forming a linear backbone of high M_r glutenin A subunits (Fig. 3B). This model is, in partial agreement with the linear glutenin hypothesis, suggested by Ewart (1979). However, it differs from Ewart's model in having four clusters, each comprising two low M_r glutenin B subunits and one low M_r glutenin C subunits attached laterally to the y-type subunit via S-S bonds.

Kasarda (1989) has proposed that glutenin subunits polymerise in a random manner. In his model, the high M_r subunits are linked indirectly via low M_r subunits (Fig. 3C). This implies that the glutenin polymer chain propagates through low M_r subunits, linked by S-S bonds in the unrepetitive C-terminal domains in tail-to-tail manner than head-to-tail. The model does not account for the occurrence of oligomers of high M_r subunits identified by several researchers (Graveland et al. 1985; Gao et al. 1992; Werner et al. 1992).

The model of Khan and Bushuk (1978) depicts S-S bonds (both inter-and intra-molecular) and secondary forces (hydrogen bonds and hydrophobic interactions) involved in the formation of the glutenin polymer. The more recent model of Gao et al (1992) suggests the presence of rheologically effective and ineffective S-S linkages in the glutenin polymers. This was proposed on the basis of their Farinograph and SDS-PAGE results.

Unfortunately, none of these models describes the underlying mechanism, involving interaction of glutenin with gliadin and/or non-gluten components, such as lipids and carbohydrates, which might be essential to explain the complete model of the gluten network. Hence, the models proposed so far are highly simplified and obviously suggest that much remains to be investigated to arrive at a comprehensive model for gluten network.

IMPORTANCE OF WHEAT GLUTEN IN BREADMAKING

Protein quantity and quality: It has long been established that the rheological properties and breadmaking performance of wheat flours are related to the quantity and quality of their proteins. The relationship between protein content and

breadmaking performance (measured as loaf volume) is essentially linear within a cultivar over the normal range of protein contents encountered in commercial wheat flour (Finney and Barmore 1948). The differences in the slopes of the regression lines among wheat cultivars reflect differences in their protein quality. Cultivars that have high breadmaking performance are characterized by higher slopes.

When the total protein content of a wheat grain increases, the total amount of gluten proteins also increases, but the amount of the non-gluten forming proteins (i.e., albumins and globulins) changes very little. For this reason, there is a close positive relationship between the total protein content and gluten content of wheat flours. Thus, wheats of high-protein contents usually have a higher proportion of gluten proteins compared with those having lower protein contents.

Fractionation and reconstitution studies (i.e., experiments in which the different flour constituents are first separated and then systematically exchanged between reconstituted flours of good and poor quality) have confirmed that the gluten proteins are primarily responsible for quality differences among wheat cultivars (Finney 1943; MacRitchie 1978; Booth and Melvin 1979). Similar studies have generally shown that the non-gluten proteins do not play a significant role in the variation in breadmaking quality that occurs amongst different cultivars of wheat. It has been suggested, in fact, that these proteins may be omitted from reconstituted flours without detrimental effects on their baking qualities, provided steps are taken to ensure that gas production is not limited (Kasarda et al. 1976; MacRitchie 1984).

Gluten fractions: A number of research workers have made considerable efforts over many years to elucidate as to which specific gluten protein fraction is responsible for flour quality. The relative importance of gluten fractions in breadmaking is assessed usually either by a correlation approach, which involves surveying a range of genotypes, or by the fractionation and reconstitution technique. The correlation studies give clues about the association of protein composition with breadmaking quality. However, the fractionation and reconstitution technique provides direct evaluation of gluten protein functionality. Reconstitution studies, on the other hand, are not considered suitable for evaluating the relative contributions of individual polypeptides. For this purpose, genetic lines are used in which a specific polypeptide is either not expressed or in which expression is enhanced (McRitchie 1984; MacRitchie et al. 1990; MacRitchie 1992).

In reconstitution experiments, gluten proteins are first separated into sub-fractions and then either their amounts are varied in a given flour or equivalent amounts of these fractions are interchanged between flours of diverse breadmaking quality. The solvents most commonly used for this purpose are aqueous ethanol (70%, v/v) and dilute acids, such as acetic acid, lactic acid and hydrochloric acid. Using these solvents, most gliadins are recovered in the extracted phase on centrifugation and glutenin remains in the unextractable phase. In reconstitution experiments, it is important to take precautions in order to preserve the functionality of the flour components. Guidelines for achieving this have been described by MacRitchie (1985). This is generally checked by reconstituting all the flour constituents in the same proportions as in the original flour and then testing their dough mixing properties and carrying out a baking test to ensure that their quality parameters are unchanged (MacRitchie 1984). Earlier reconstitution studies (Hoseney et al. 1969 a,b; Finney 1979) indicated that glutenin proteins are responsible for mixing requirements and gliadin proteins for breadmaking performance (as measured by loaf volume) of wheat flours.

Orth and Bushuk (1972) used a modified Osborne scheme to fractionate the flour proteins of 26 cultivars, each grown at four different sites. They observed that loaf volume was inversely related to the amount of dilute acid-extractable glutenin and directly related to the proportion of acid-unextractable glutenin. In addition, they showed that loaf volume had no obvious relationship with the amount of gliadin protein. They concluded that the breadmaking potential of a flour is related to glutenin proteins.

Subsequent studies (MacRitchie 1980 a,b; Hamada et al. 1982; Chakraborty and Khan 1988 a,b; MacRitchie 1987 a,b; Gupta et al. 1992) confirmed that differences in the glutenin fraction primarily account for the quality differences among wheat cultivars. As a result, research on the glutenin proteins has been intense. Before discussing the role of glutenin polypeptides in relation to breadmaking, the importance of the gliadin/glutenin ratio will be considered.

Gliadin/glutenin ratio: Differences in the gliadin to glutenin ratio among wheat cultivars have long

been considered an important source of inter-cultivar variation in physical properties and breadmaking quality (Kasarda 1989; Schofield 1994). The technological significance of gliadin and glutenin in breadmaking has been attributed to their contribution to dough extensibility and elasticity, respectively. Doughs that are too elastic and inextensible or *vice versa* give poorer breadmaking performance than do doughs that have an appropriate balance of extensibility and elasticity.

On this basis, it has been considered that an appropriate balance of these fractions must be responsible for the breadmaking potential of wheat flours. However, evidence on this has been inconclusive owing to the lack of suitable techniques for transforming the aqueous ethanol (70%, v/v) separated gliadin and glutenin fractions back into their original form in the gluten network.

Molecular size distribution: Molecular size distribution has been considered another important factor in relation to functional properties of a flour. Evidence for this was presented by MacRitchie (1987 a,b). He fractionated glutens from a number of flours into ten fractions by successive extraction with HCl (0.625-1.5 mM). The results of SDS-PAGE indicated that the proportion of high molecular weight proteins (i.e., glutenin) increased in successive fractions. Similar results were obtained by SE-HPLC (Cornec et al.1994).

These fractions have been added (1%, w/w) to a base flour in order to evaluate their influence on mixing time and breadmaking performance in an optimised baking test. The results indicated that the earlier fractions, rich in low molecular weight proteins (mainly gliadin), decreased mixing time and loaf volume. In contrast, the later fractions with high proportions of high molecular weight proteins (mainly glutenin) increased mixing time and loaf volume. The trend was reversed for the last fractions. This was attributed mainly to the fact that the proportion of total high molecular weight proteins decreased slightly in the last fractions. These results further indicate the importance of the polymeric glutenin fraction for breadmaking quality.

Gel filtration chromatography studies have indicated that the glutenin fractions of flour with longer mixing times have a higher average molecular weight than the glutenin fractions of short mixing flours (Huebner and Wall 1976). Similar results were obtained by Tanaka and Bushuk (1973) and by Bottomley et al (1983). Flours with short mixing times give doughs that break down rapidly during mixing and that are easily extensible. Such flours are considered unsuitable for breadmaking. Conversely, flours with longer mixing times are noted for doughs of high mixing stability and greater resistance to extension and perform well in breadmaking. However, flours that require very long mixing times may prove undesirable under practical bakery conditions.

Protein extractability results often support gel filtration chromatography results in the sense that gluten proteins from poor quality flours have higher extractability in dissociating reagents than those from good quality flours, indicating that gluten proteins from poor quality flours might have lower average weights or lesser tendency to interact with themselves (MacRitchie 1992).

Glutenin polypeptides: In order to understand the molecular basis of breadmaking quality, numerous studies have been conducted during the last decade on the structural aspects and the role of glutenin subunits, specifically the high M_r subunits, in breadmaking. These studies used genetic lines in which specific polypeptides are either not expressed or enhanced. This interest in high M_r subunits of glutenin stems largely from the pioneering work of Payne and colleagues, who demonstrated correlations between the presence or absence of particular high M_r subunits and breadmaking performance in a number of studies involving wheats from different countries and progenies of crosses between cultivars of diverse quality (Payne et al. 1979; 1981; 1987a,b; 1988).

Upto 20 different high M_r subunits have been identified in different bread wheats. Each bread wheat cultivar contains 3-5 high M_r subunits, which together account for about 1% of the dry weight of the mature endosperm of a wheat grain (Payne et al. 1987b). This indicates that, although high M_r subunits are quantitatively minor, they are, nevertheless, functionally important polypeptides of gluten proteins (Shewry et al. 1992).

The high M_r subunits of glutenin are encoded at the *Glu*-1 loci on the long arms of the chromosomes 1A, 1B and 1D. These loci are designated as *Glu*-A1, *Glu*-B1 and *Glu*-D1, respectively. *Glu*-1 quality scores are assigned to the individual subunit or subunit pair based on their values determined by the SDS-sedimentation test, an indirect measure of breadmaking quality (Table 1).

The genes for x-type and y-type subunits are closely linked in the case of the 1D alleles and some

TABLE 1. Glu-1 QUALITY SCORES ASSIGNED TO INDIVIDUAL HIGH M_r SUBUNITS OR SUBUNIT PAIRS OF GLUTENIN

Locus			
Glu-A1	Glu-B1	Glu-D1	Glu-1 score
-	-	5+10	4
1	17+18	-	3
2*	7+8	-	3
-	13+16	-	3
-	7+9	2+12	2
-	-	3+12	2
Null	7	4+12	1
-	6+8	-	1
-	20	-	1

Scores are ranked from 1 to 4, indicating poor and good bread making quality, respectively (Payne 1987).

1B alleles, and therefore, *Glu*-1 scores are assigned to subunit pairs rather than individual subunits for such alleles. The *Glu*-1 score of a cultivar is calculated by summing the scores of the subunits, it contains (Payne et al. 1987b). The *Glu*-1 scores of bread wheats usually range from 3 (for poor breadmaking quality) to 10 (for good breadmaking quality).

The *Glu*-1 score is positively correlated with breadmaking performance and 47-60% of the variation in breadmaking quality could be accounted for by the variation in high M_r subunits of glutenin (Payne et al. 1987b; Lukow et al. 1989; Khatkar et al. 1996). Correlation studies (Payne et al. 1987a; Lawrence et al. 1988) with near-isogenic lines [i.e., lines that differ in polypeptide(s) expressed at one locus] have shown that the composition and quantity of high M_r subunits of glutenin largely control the mixing time and breadmaking performance of a flour. It is also noted that the good bread wheats contain superior high M_r subunits, such as subunits 5+10, 1, 2*, 17+18 and 7+8 and the poor breadmaking wheat cultivars generally have inferior high M_r subunits, such as 2+12, 6+8, 20 or null (i.e., silent) allele (Campbell et al. 1987; Cressey et al. 1987; Ng and Bushuk 1988; Lukow et al. 1989; Singh et al. 1990; Dong et al. 1992; Gupta and MacRitchie 1994; Preston et al. 1992).

Although it is now evident that the high M_r subunits of glutenin play a significant role in breadmaking performance, the basis of the differential effect of various glutenin subunits on breadmaking quality of a flour remains to be defined. According to Gupta and MacRitchie (1994), the alleles at the *Glu*-B1 and *Glu*-D1 loci produce high M_r subunits (17+18 and 20 or 5+10 and 2+12) in similar amounts. Their results indicate that the qualitative differences in high M_r glutenin subunits may be responsible for the differences in the breadmaking quality among wheat cultivars. This is consistent with the previous observations that the superior subunit 5 has one additional cysteine residue (Greene et al. 1988) and has a greater M_r than the inferior subunit 2 (Shewry et al. 1992). The superiority of the subunits 17+18 over subunit 20 in the case of genome B has been attributed to the number and/or position of the cysteine residues (Tatham et al. 1990; Margiotta et al. 1993). Furthermore, on the basis of amino acid sequence differences in the region towards the C-terminal ends of the repetitive domains of high M_r glutenin subunits 10 and 12, it has been postulated that subunit 10 may form a more regular β-spiral structure than subunit 12 and this, in turn, may lead to improved elastic properties (Flavell et al. 1989).

Other researchers have suggested that there may be quantitative as well as qualitative effects that contribute to the differences in quality exerted by different high M_r subunit alleles. For example, in the case of subunits encoded at *Glu*-A1, Halford et al (1992) showed that the presence of either $1A_x1$ or $1A_x2^*$, when compared with a null allele, resulted in an increase in the proportion of high M_r glutenin subunit protein from about 8 to 10% of the total, which is associated with improved quality.

In other reports, it has been argued that it is the quantity of glutenin subunits and not the composition that is important (Kasarda 1989). Studies have indicated that the high M_r subunit pairs 5+10 and 2+12 failed to show the expected differences in dough strength and breadmaking performance in many genotypes (Singh et al. 1990; Gupta et al. 1991; 1994). Bread wheats, such as 'Egret' and 'Halberd', contain superior subunit pair 5+10, yet produce weak dough (Singh et al. 1990), whereas inferior subunits 2+12 are found in French good breadmaking wheats, such as 'Courtot', 'Hardi' and 'Capitole' (Khatkar et al. 1996) and also in many Australian good breadmaking quality wheats, for example, 'Cook', 'Gabo', 'Oxley' and 'Timgalen' (Singh et al. 1990).

Association of low M_r subunits of glutenin and gliadin polypeptides with breadmaking quality have also been reported (Branlard and Dardevet 1985a, b; Campbell et al. 1987; Cressey et al. 1987; Gupta et al. 1994). These results indicate that high M_r subunits are not the only determinants of breadmaking quality. Therefore, there is a need to

obtain more information on the role of low M_r glutenin subunits in breadmaking. Such information may help to resolve the apparent anomalies experienced in the study of high M_r polypeptides and breadmaking performance of wheat cultivars.

High/low M_r glutenin polypeptides ratio: Low M_r subunits account for a large proportion (~75%) of glutenin polymers, while the high M_r subunits represent only about 25% of glutenin (Gupta et al. 1994; Wrigley 1994). This and the fact that the high M_r subunits alone failed to predict the functional properties for many wheat cultivars mentioned earlier, suggests that the relative proportion of high and low M_r subunits of glutenin must be considered to explain the inter-cultivar variation in the baking performance better.

Gupta et al (1991) assessed the relative contributions of low and high M_r subunits to dough strength (measured as Brabendar Extensigraph maximum dough resistance, R_{max}) in a study that involved genetic lines that were deficient in varying numbers of low (*Glu*-3) and high (*Glu*-1) M_r subunits of glutenin. They suggested that both types of glutenin subunits contribute to the dough strength. However, the slope of the regression line was much steeper for variation in the amount of high M_r subunits than that for variation in low M_r subunits, suggesting that high M_r subunits contribute more to dough strength than low M_r subunits, when compared on an equal weight basis.

A bread wheat with strong dough properties (cv. 'Halberd') has been reported to contain a higher ratio of high/low M_r subunits than a wheat flour with weak dough (cv. 'Israel M68') (Gupta et al. 1992). Both these cultivars have very similar proportions of glutenin in their proteins. This indicates that the variation in the ratio of high/low M_r subunits may lead to wide variation in the functional properties of wheat cultivars. However, more experimental evidence is required to confirm this hypothesis.

Dynamic rheology of wheat gluten

Basic concepts: Dynamic rheological tests provide information on the basic rheological properties of the material, such as viscosity (the ease of deformation) and elasticity (the ease of recovery). Wheat gluten or flour dough exhibits a combination of these properties and therefore, is classified as a viscoelastic material (Bushuk 1985). In all dynamic rheological measurements, material of interest is deformed and the force exerted and the deformation achieved are measured. The magnitude of the force that has to be applied to the material to deform it depends on the area over which it is applied. Instead of the force, it is more relevant to use the force per unit area, which is called stress and calculated as:

$$\tau = F/A \quad \text{- - - - - - - - - - -} \quad (1)$$

Where τ = stress, F = force acting upon the sample and A = area over which the force acts. Stress is measured in Pascals or N/m^2. The unit 'Pascal' has replaced the former unit, $dyne/cm^2$, which had previously been used for stress. 1Pa = 10 $dyne/cm^2$.

There are two types of stresses, those that act in a direction parallel to the material surfaces they deform, called shear stresses and those that act in a direction perpendicular to the surfaces of material they deform, called normal stresses or tensile stresses (Ferry 1980). The tensile stress is normally denoted by σ and the shear stress by τ. A stressed material often undergoes deformation and the rheological term associated with deformation is strain, a measure of relative displacement between the particles of a material (Bushuk 1994). Strain is the ratio of two quantities with units of length. Therefore, it is dimensionless (Menjivar 1990).

In the simplest case, the rheological property of interest in a solid is its elasticity and in liquids, it is viscosity. Elasticity is the property of a material by virtue of which, after deformation and upon removal of stress, tends to recover part or all of its original dimensions. The elasticity of a material is described by shear modulus, which is denoted by G. Mathematically this can be stated as:

$$G = \tau/\gamma \quad \text{- - - - - - - - - - -} \quad (2)$$

This is equivalent to Hooke's law and a solid, which has a constant modulus of elasticity is called Hookean or an ideal solid. The energy of deformation, in an ideal solid, is fully recovered, when the stresses are withdrawn. Ideal fluids such as liquids and gases deform irreversibly. In other words, they flow. The energy of deformation is dissipated in fluids in the form of heat and it cannot be recovered just by releasing stresses. An ideal fluids obeys Newton's law, where stress is directly proportional to the rate of the resultant strain:

$$\tau = \eta \, d\gamma/dt \quad \text{- - - - - - - - -} \quad (3)$$

where η is the coefficient of viscosity of a fluid and $d\gamma/dt$ is the rate of strain. Deformation in a liquid is called flow and since flow is a rate process, it is best expressed as a rate of strain. An ideal

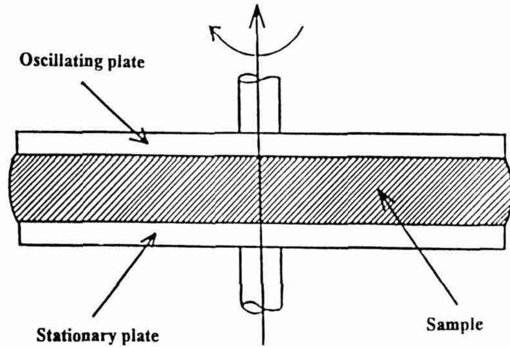


Fig. 4. Shear deformation between parallel plates (Redrawn from Menjivar 1990)

fluid with constant viscosity is also called Newtonian.

Viscoelasticity: Most foods, in general, cannot be categorised as ideal solids or ideal liquids. They are neither perfectly viscous nor perfectly elastic, but are viscoelastic. Hydrated wheat gluten and wheat flour are described as viscoelastic materials, i.e., materials that exhibit both liquid-like and solid-like characteristics.

To study a viscoelastic material, dynamic rheometry often uses a parallel plate geometry, the material being placed between the plates (Fig. 4). The upper plate is subjected to forced oscillation of known amplitude and frequency and the lower plate is attached to a torque transducer. When such material is subjected to sinusoidally oscillating

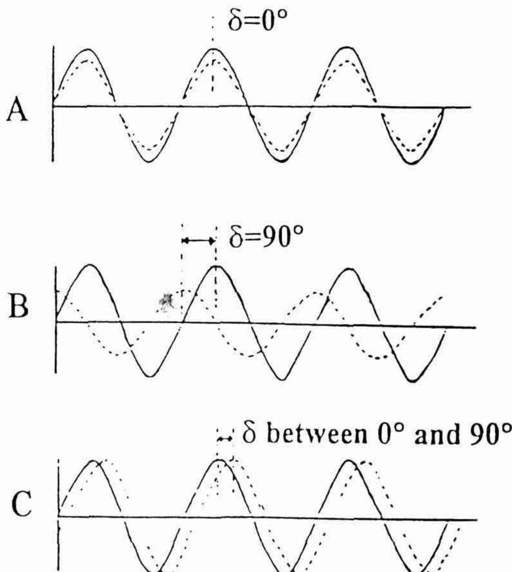


Fig. 5. The response of an elastic solid (A), a viscous liquid (B) and a viscoelastic material (C) in a dynamic experiment. Solid lines represent stress and broken lines represent strain signal. (Redrawn from Mitchell 1980)

stress, as in a controlled stress rheometer, the measured strain signal is neither exactly in phase with the stress (as it would be for a perfectly elastic solid) nor 90° out of phase (as it would be for a perfectly viscous liquid), but is somewhere between 0° and 90° as illustrated in Fig. 5 (Mitchell 1980). In such material, some of the energy input is stored and recovered in each cycle and some is dissipated as heat. Material, possessing such characteristics, is called viscoelastic.

The dynamic rheological parameters G', G" and the ratio of G" to G', which is known as the loss tangent or tan δ are used to characterise the rheological behaviour of a material. G' and G" are called the storage modulus (elastic character) and the loss modulus (viscous character), respectively and in general, are functions of the frequency (ω). G' is taken as a measure of the energy stored and recovered in cyclic deformation, whereas G" is related to the energy dissipated or lost as heat in a cycle of deformation (Ferry 1980). Tan δ indicates the relative contributions of the viscous (G") and elastic (G') characteristics of the material. When the material behaves more like a solid, i.e., when the deformation is essentially elastic and reoverable, the elastic character, G' exceeds the viscous character, G" and tan $\delta < 1$. Conversely, when the material behaves more like a liquid or viscous system, then the viscous character, G", dominates and tan $\delta > 1$.

The study of the frequency dependence of G' and G", known as the mechanical spectrum, yields useful information for discriminating glutens from different wheats. Fig. 6 shows the mechanical spectra of glutens from 'extra strong' (cvs 'Glenlea', 'Aubaine' and 'Laura'), strong (cvs 'Courtot', 'Katepwa' and 'Hardi') and weak (cvs 'Corin' and 'Riband') wheat cultivars. There are consistent significant differences in the magnitude of the elastic moduli (G') for the three groups of glutens. G' and G" values are in the order 'extra strong' glutens > strong glutens > weak glutens. Further evidence for differences in gluten dynamic rheological behaviour being due to wheat cultivar also comes from the reports of Attenburrow et al (1990) and He and Hoseney (1991b).

The curves of G' and G" versus frequency (Fig. 6) for these glutens could be interpreted as being indicative of the mechanical spectral characteristics of a composite network structure, in which different junction zones, resulting from gliadin-glutenin, glutenin-glutenin and gliadin-gliadin interactions, co-exist and contribute to the viscoelastic properties

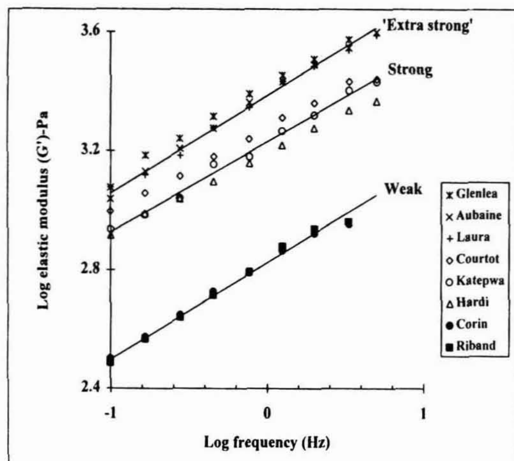


Fig. 6. Mechanical spectra of glutens, obtained using controlled stress rheometry, from different wheat cultivars. (Khatkar 1996)

of glutens at different frequency (or time) scales. Qualitatively, glutens showed very similar behaviour, i.e., both moduli increased moderately with increasing frequency. Quantitative differences in the magnitudes of the dynamic moduli among different groups of glutens may be attributable to differences in the nature and density of covalent and non-covalent cross-links, variation in the rheology of gluten sub-fractions, gliadin/glutenin ratio and differences in the relative quantity and quality of glutenin high M_r subunits (Khatkar et al. 1995).

Differences in the dynamic rheological properties of glutens may also arise due to differences in molecular weight distribution. To study the effect of molecular weight distribution on gluten rheology, Cornec et al (1994) prepared a series of gluten sub-fractions by sequential extraction with increasing concentrations of dilute HCl (0.3-5 mM), as described by MacRitchie (1987 a,b). The results obtained by dynamic measurements in shear suggested that the viscoelasticity of sub-fractions correlate strongly with the proportion of the high M_r proteins (i.e., glutenin polymers), as determined by size exclusion-high performance liquid chromatography(SE-HPLC).

Similar results were shown in another study by Popineau et al (1994), using near-isogenic lines of the wheat 'Sicco', which differed only in high M_r glutenin subunit composition. According to Popineau et al (1994), the presence of subunits 2+12 instead of subunit pair 5+10 in the near-isogenic wheat lines from 'Sicco' caused a marked overall drop in both gluten G' and G" values and deletion of the subunits encoded at the *Glu-Al* and *Glu-Dl* loci further resulted in a large decrease in gluten viscoelasticity. Likewise, Gupta et al (1995) found a significantly greater influence of subunits 5+10 on the elasticity of gluten than those of subunits 17+18. It was also noted that *Glu-1* triple deletion led to a substantial decline in gluten viscoelasticity. In these investigations, the differential effects of high M_r glutenin subunits were attributed to the fact that the loss of subunits 5+10 in genetic lines caused a significantly greater reduction in the large polymers than those of subunits 2+12 or 17+18 as determined by SE-HPLC. This observation is consistent with the suggestion that subunit 5 of cv. 'Cheyenne' contains an additional cysteine residue compared with subunit 2 of cv. 'Chinese spring' (Shewry et al. 1992), which could lead to the formation of a large, highly cross-linked polymer system conferring greater elastic character on the gluten network. However, the relative contribution of subunits 5 and 10 to quality associated with subunit pair 5+10 is still a matter of speculation.

In a recent study by Khatkar (1996), the elastic modulus, G', classified the wheat cultivars into three groups on the basis of the subunits encoded at the *Glu-Al* and *Glu-Dl* loci. Null alleles at the *Glu-Al* and subunits 2+12 at the *Glu-Dl* locus were found to be related to poor elastic character. The presence of subunit 2* in cultivars containing subunits 2+12 or 3+12 increased elasticity substantially. Cultivars having subunits 2* or 1 at the *Glu-Al* and subunit pair 5+10 at the *Glu-Dl* demonstrated the highest modulus of elasticity. These results are in accordance with the findings of Popineau et al (1994) and Gupta et al (1995).

The quantity of high M_r glutenin subunits is another important determinant of gluten viscoelasticity. Although it is well established that the amount of total high M_r glutenin subunits play an important part in determining breadmaking quality, the quantitative relationship of these subunits with gluten viscoelasticity has not been extensively explored. Recent experimentations have shown that the relative quantities of total high M_r glutenin subunits showed a second degree polynomial relationship with the G' values of glutens, which explained 74% of the variation in the elastic character of the glutens. Similarly, the high to low M_r glutenin subunit ratio showed a significant ($R^2 = 0.60$; $P < 0.009$) direct relationship with gluten elastic character, again emphasising the relative importance of high M_r glutenin subunits for gluten viscoelasticity (Khatkar 1996). It is

speculated that the enhanced elasticity of gluten caused by increasing the proportion of the high M_r subunits of glutenin might be linked to the fact that they have been proposed as forming the backbone of the glutenin network (Graveland et al. 1985; Shewry et al. 1992; Schofield 1994). Therefore, increasing the concentration of these subunits might provide conditions conducive to extensive cross-linking and branchng. The type of subunits, indeed, may also enhance cross-linking and branching, thus, in effect, raising the average molecular size of the polymer and hence the elasticity.

Seemingly some progress has been made in fundamental studies of gluten rheology, but, much remains to be explored. For example, the precise molecular basis of the fundamental mechanical properties of gluten proteins is still largely unknown, although a number of hypothesis have been put forward, as discussed in the next section.

THE BASIS OF GLUTEN VISCOELASTICITY

A wheat flour dough is largely composed of starch, proteins, lipids, water and air cells (Bohlin and Carlson 1980). If the gluten proteins are removed from the flour, then the property of forming a viscoelastic dough is lost (Bushuk and MacRitchie 1989). It is generally agreed, therefore, that the gluten proteins form the framework of the dough structure. When flour is wetted, the formation of gluten is mainly caused by a complex interaction between the endosperm storage proteins, viz., gliadin and glutenin.

Usually, the elastic properties of gluten are ascribed to the glutenin fraction, whereas the viscous properties come from the gliadin fraction. It is understood that the gluten of wheat owes its unique viscoelastic behaviour to an appropriate balance in the amounts of gliadin and glutenin proteins, but variations in the composition and elastic properties of the glutenin proteins appear to be largely responsible for the differences in the gluten viscoelasticity among wheat cultivars. For this reason, it is widely believed that the viscoelasticity of gluten is controlled by the glutenin proteins (Kasarda 1989; Shewry et al. 1994; Schofield 1994).

The 'linear glutenin hypothesis' proposed by Ewart has received wide acceptance in explaining the viscoelasticity of gluten and flour doughs. This hypothesis has passed through different stages of sophistication (Ewart 1968, 1972, 1978, 1979). According to Ewart, the glutenin polypeptide chains form long linear 'concatenations' with two S-S bonds connecting each chain in a head-to-tail fashion to the next chain. The 'concatenations' of glutenin molecules are assumed to participate in the formation of a three-dimensional entangled network structure.

The entanglement may be purely physical and/ or at the point of entanglement, known as nodes, non-covalent interactions might occur to form cross-links (branching). The branching of glutenin polymers by S-S bonds is also not ruled out (Kasarda et al. 1985; Tatham et al. 1985). The effectiveness of entanglement and cross-linking will depend on the length of the glutenin 'concatenations', which, in turn, depends on the number and distribution of covalent bonds and non-covalent interactions.

Under stress conditions, the entangled and non-covalently cross-linked structure in glutenin offers resistance to deformation, which is manifested in increased elasticity (elastic modulus, G') during controlled stress rheometry. It also enables glutenin polymers to recoil after stress conditions are withdrawn. Viscous flow depends predominantly on molecular slippage at nodes and the labile nature of weak secondary forces acting between glutenin polymers. Sulphydryl-disulphide bonds interchange and mechanical scission of S-S bonds may also contribute to viscous flow. Mechanical scission of S-S bonds probably occurs, when the rate of deformation exceeds the rate at which molecular slippage or SH/SS interchange can occur, resulting in glutenin polymers being subjected to stress values more than their elastic limit. The viscous nature of gliadins may be attributable to the absence of effective entanglements owing to their low M_rs. According to Ewart (1989), gliadin polypeptides help molecular slip, acting analogously to ball bearings.

Tatham et al (1984; 1985) put forward a spiral hypothesis to explain the elasticity of glutenin. This hypothesis is based on the structural analogy of high M_r subunits of glutenin to the mammalian connective tissue protein, elastin in which the elasticity is attributed to the presence of ß-spiral structure. Circular dichroism spectroscopy and structural prediction studies conducted by Tatham et al (1984; 1985; 1990), Shewry and Tatham (1990), and Shewry et al (1992) have indicated that the central repetitive domain of high M_r glutenin subunits are rich in ß-turns. ß-turns are

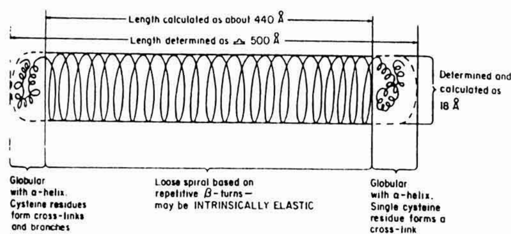


Fig. 7. A hypothetical structural model for the high M_r subunits of glutenin. Source: Shewry et al (1992)

hypothesised to occur so frequently and regularly that they have been suggested to form a β-spiral structure, as in elastin (Fig. 7). β-spiral structure is assumed to be intrinsically elastic.

It has been suggested that under stress conditions, β-spirals undergo deformation and on release of stress, β-spiral structures resume their energetically favourable conformation (Fig. 8). Experimental evidence for β-spiral conformation of the high M_r glutenin subunits has been obtained using scanning tunnelling microscopy of a purified high M_r glutenin subunit (1Bx 20) from durum wheat (Miles et al. 1991). There is no direct evidence as yet for the elastic nature of β-spiral, however.

Recent experiments have shown that the high M_r subunits of glutenin are not elastin-like in their interaction with water (Belton et al. 1994). Belton (1994) has suggested that elasticity of glutenin is primarily due to the occurrence of extensive network of intermolecular hydrogen bonding along the central repetitive domain. According to Belton (1994), shear forces acting on the aggregated high

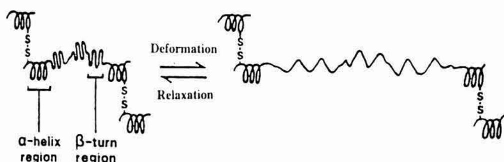


Fig. 8. Schematic illustration of how linear glutenin molecules confer elasticity to gluten and flour doughs. The glutenin polypeptides are joined head-to-tail via S-S bonds in a linear chain. The glutenin polypeptides polymerise into a linear chain by intermolecular S-S bonds between the cysteine residues located in the α-helical regions near N- and C- terminal ends of high M_r glutenin subunits. The central domain is thought to be rich in repetitive β-turns which form stable β-spiral structure. Under stress conditions, the β-spiral structures undergo deformation and on release of stress, the β-spirals resume the energetically more favourable original conformation. The presence of cysteine residues at either end of glutenin molecules allows deformation/reformation to occur in the central spiral region (Source: Schofield 1986; Shewry et al. 1992)

M_r glutenin subunits disrupt the hydrogen bonds, but they reform after stress conditions are withdrawn, and thus restore the original energetically favourable conformation structure of glutenin. This is similar to the basis of gluten elasticity hypothesised by Ewart some years ago (Ewart 1968, 1972, 1978, 1979). It has also been argued that the spiral structure of high M_r of glutenin is based on γ-turns rather than β-turns (Kasarda et al. 1994). Thus, there is still a need for a more complete structural model to explain the viscoelastic behaviour of gluten and flour doughs.

IMPORTANCE OF GLUTEN VISCOELASTICITY IN GAS RETENTION AND BREADMAKING

The technological significance of wheat flour is related to the viscoelastic nature of its flour-water dough, which retains gas produced during fermentation and in the early stages of baking. The ability of a viscoelastic dough to retain gas results in a light porous crumb-textured bread. Wheat gluten proteins are believed to be primarily responsible for the viscoelastic behaviour of dough and hence for the uniqueness of wheat flour. Proteins of other cereals such as corn, rice, rye, etc. do not have the ability to produce such viscoelastic dough. Flours from other cereals, therefore, fail to retain gas and give very dense and small loaves (He and Hoseney 1991a).

The breadmaking process is accomplished through three basic operations i.e., mixing, fermentation and baking (Bloksma 1990). Mixing transforms the flour and water into a cohesive viscoelastic dough. Another key function of the mixing operation is the incorporation of air. Air is essential, as it introduces the gas cells into which the carbon dioxide produced by the yeast fermentation diffuses. The importance of these gas cells emerges from the fact that yeast cannot produce new gas cells. Therefore, if no air cells were present, the crumb grain of the final bread would be very coarse with only a few large cells.

Dough is developed as a result of mixing to high work input levels and at high speed in mechanical dough development systems, such as the Chorleywood Process, or by mixing to low work inputs at low speeds plus long fermentation in bulk in 'traditional' breadmaking systems (Axford et al. 1963). Such developed dough is capable of retaining the carbon dioxide produced by the yeast during fermentation and in the early stages of baking. Baking rheologically transforms an expanded viscoelastic dough foam into an elastic bread

sponge. Flour-water dough retains gas owing to its discontinuous discrete cellular foam structure, whereas baked bread is a continuous open-celled sponge that is permeable to gases (Baker 1939; Hoseney 1994).

Understanding the structure of the gluten proteins and how they interact with themselves and other flour constituents to convert a wheat flour into a viscoelastic system, still largely remains a challenging problem. Also, much remains to be learned about how a viscoelastic dough retains gas and how the expanded dough is transformed into bread. However, some progress has been made in this area of research and in the discussion that follows, the role of gluten proteins in gas retention is explained. The discussion below, although based on available evidence, may be somewhat speculative.

From a colloidal point of view, in a mixed flour-water dough, the hydrated gluten proteins form the continuous phase with starch and air cells as the discontinuous phase embedded in it and the yeast cells are dispersed throughout the aqueous dough phase. The 'free' water in the dough represents the aqueous dough phase, which dissolves the water-solubles and acts as the medium for chemical reactions and for dissolving carbon dioxide up to its saturation point (Hoseney 1994).

Yeast ferments sugars and continuously produces carbon dioxide in the aqueous dough phase. When the aqueous dough phase is saturated with carbon dioxide, most of the carbon dioxide diffuses into the air cells that are formed in the dough during mixing. This is attributed to the fact that the rate of diffusion of carbon dioxide in the dough and hence its ultimate evaporation into the surrounding atmosphere is slow. This is due to the presence of continuous gluten proteins film, pentosans and lipids (Hoseney 1984).

The diffusion of carbon dioxide into gas cells increases the pressure within gas cells that provides the driving force for dough expansion. The viscous flow properties of dough owing to its monomeric proteins (mainly gliadins) allow the gas cells to expand. The expansion of gas cells releases the pressure within gas cells, though, a little pressure does exist in the dough system during fermentation. The pressure in gas cells has been reported to be slightly greater than the atmospheric pressure i.e., about 1.01 atmosphere (Bailey 1955; Bloksma 1981). This small over-pressure results from the surface tension at the gas dough interface and the resistance of dough to deformation, i.e., expansion (Bloksma and Bushuk 1988).

During baking, the temperature increases, water evaporates from the liquid dough phase into the gas cells, carbon dioxide and ethanol produced by the yeast and dissolved in the liquid dough phase also diffuse into the gas cells, which together result in an overall increase in the pressure within the gas cells. The viscous components, i.e., monomeric proteins (mainly gliadins) allow gas cells to expand to equalise the internal gas cell pressure, whereas the elastic components, i.e., polymeric proteins (mainly glutenins) provide strength to prevent the gas cells from over-expanding, and thus preventing the rupture of the gluten film network, enveloping the gas cells.

In a flour-water dough containing insufficient amounts of good quality polymeric proteins, the gluten network would be too extensible, allowing uncontrolled expansion of gas cells, which would ultimately rupture the continuous gluten film network and thus continuity between gas cells would be established. The continuity between gas cells or the discontinuity in the gluten film network is seen as pin holes at the dough surface. This results in the rapid loss of carbon dioxide even at a very low temperature during baking and very early end of the oven spring. Consequently, bread of low loaf volume and dense open crumb is produced. On the other hand, good quality flour-water dough with enough strength retains carbon dioxide, until more advanced stages of baking, i.e., ~70°C. This produces a good bread loaf volume and even crumb texture on completion of baking.

According to He and Hoseney (1991b), differences in the losses of carbon dioxide between poor ('KS 501097') and good ('CI 12995') quality flour doughs are not related to changes in the starch or to changes in the gluten proteins but appear to reside in the inherent differences in the gluten proteins. The ratio of monomeric to polymeric proteins and composition as well as properties of glutenin subunits in flour appear crucial to the gas retention and dough expansion. Dynamic rheological studies reported in this review showed that gluten-water doughs from poor quality flours had lower elasticity and greater viscosity than those from good quality flours (Khatkar et al. 1995; Khatkar 1996). Similar results were obtained by He and Hoseney 1991b).

CONCLUSIONS AND FUTURE RESEARCH NEEDS

As has been discussed in this review, the technological significance of wheat flour is related

to the physico-chemical and structural properties of wheat gluten proteins. In this context, particular attention has been focussed upon the glutenin fraction of wheat gluten, since it is qualitative and quantitative variation in this fraction that accounts for a substantial proportion of the variation in gluten viscoelasticity and breadmaking quality that occurs amongst wheat cultivars. In the past, genetic lines have proved to be powerful tools in investigating the effect of specific high M_r subunits on wheat dough and gluten functionality. Although it is now evident that the high M_r subunits of glutenin play a significant role in gluten viscoelasticity and breadmaking performance, the basis of the differential effect of various glutenin polypeptides on functionality of a wheat flour remains to be defined. Also, little is known about the role of low M_r glutenin subunits and gliadin subgroups in gluten viscoelasticity and breadmaking potential. Therefore, further research is needed in this area as well.

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A Comparative Study on the Hypolipidemic Activity of Eleven Different Pectins

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Inclusion of pectin-rich fruits and vegetables can be regarded as one of the primary prevention strategies of hyperlipidemia. But, it has been found that all pectins do not show effective hypolipidemic activity. Eleven pectins were screened among which pectins from *Carica papaya*, *Coccinia indica*, *Lycopersicum esculentum* and *Cucumis sativus* showed highly significant hypolipidemic activity in albino rats, while others were less significant or insignificant in their action. Detailed studies on elucidating the mechanism of action of these pectins revealed that hypolipidemic activity was mainly due to the lower rate of absorption and higher rate of degradation and elimination of lipids. The increased activity of plasma Lecithin Cholesterol Acyl Transferase activity (LCAT) in rats fed pectin can account for the significant decrease in the concentration of cholesterol. The higher activity of lipoprotein lipase in the adipose tissue and heart may be responsible for the decreased concentration of triglycerides in serum of rats fed pectin.

Keywords: Pectin, Hypolipidemia, Hypercholesterolemia, Lipoprotein lipase, HMG CoA reductase.

Clinical trials have clearly shown that the aggressive treatment of hypercholesterolemia decreases the incidence of cardiovascular events, including fatal myocardial infarction and improves the overall survival of patients, who have coronary artery disease (La Rosa et al. 1990). In mild to moderate cases of hyperlipidemia, the treatment invariably begins with a low fat, low cholesterol diet before initiation of drug therapy. Metabolic studies have shown that dietary factors like fibre contribute to the hypolipidemic effect and the soluble fibres like pectin and guar gum are receiving considerable attention for their hypolipidemic activity (Stasse-Wolthuis et al. 1980; Behall et al. 1984; Judd and Truswell 1985). Though many reports are available on the hypolipidemic effects of pectins from apple (Richter et al. 1981), grape fruit (Cerda et al. 1988), pear fruit (Fernandez et al. 1990) and citrus (Vargo et al. 1985), information on the hypolipidemic activities of pectins from many fruits and vegetables consumed by majority of the population in India is scanty. There is a need for increasing public awareness of the rich sources of pectins, which can be taken as a preventive measure in the treatment of dyslipidemia. The present study was focussed on pectins from 11 different sources to evaluate their hypolipidemic activity. Further investigations on the mechanism of action were carried out with the most beneficial pectin.

Materials and Methods

Male albino rats (Sprague-Dawley strain, weighing 80-100g) were separated into 12 groups. Group I was treated as control and groups II to XII as experimental, supplied with 5% pectin from *Citrus limon* (lemon), *Coccinia indica* (little gourd), *Carica papaya* (papaya), *Cucumis sativus* (cucumber), *Abelmoschus esculentus* (ladies finger), *Annacardium occidentale* (cashew apple), *Psidium guajava* (guava), *Annona squamosa* (custard apple), *Cucurbita maxima* (pumpkin), *Trichossanthes anguina* (snake gourd), *Lycopersicum esculentum* (tomato), respectively. Further studies were also carried out to investigate the mechanism of action of pectin from *Coccinia indica* fed at 5% levels. Pectins were isolated from the above sources as described by Presanna Kumar et al (1993).

The rats were housed in polypropylene cages and given standard diet (Gold-Mohur rat feed) or pectin containing diets for 45 days. At the end of this period, they were deprived of food overnight, stunned by a blow at the back of the neck and killed by decapitation. Blood and tissues were removed and taken in ice-cold containers for the various estimations. Standard procedures were adopted for analysing different parameters as described previously (Gomathy et al. 1989).

Results and Discussion

Data indicated that there was no significant difference in diet consumption (10 g/day/rat) or weight gain (25 g/rat/week) in the rats received pectin compared to the control animals. The concentration of cholesterol decreased significantly in the serum samples of all experimental groups,

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TABLE 1. CONCENTRATIONS OF CHOLESTEROL, PHOSPHOLIPIDS AND FREE FATTY ACIDS IN SERUM AND TISSUES OF RATS FED VARIOUS PECTINS (VALUES EXPRESSED IN mg/100g TISSUE EXCEPT IN THE CASE OF SERUM WHERE IT IS mg/100ml)

Attribute	Groups					
	I	II	III	IV	V	VI
			Cholesterol			
Serum	65 ± 2.6	41 ± 1.0^{a}	35 ± 0.7^{a}	51 ± 1.5^{a}	51.2 ± 1.5^{a}	62 ± 2.3
Liver	345 ± 13.8	306 ± 8.6^{b}	287 ± 6.6^{a}	285 ± 5.7^{a}	315 ± 9.8	313 ± 9.4
Heart	240 ± 10.0	233 ± 8.0	228 ± 7.8	237 ± 8.1	234 ± 8.2	235 ± 8.2
Aorta	140 ± 5.4	132 ± 5.1	124 ± 3.9^{b}	110 ± 3.1^{b}	138 ± 5.3	136 ± 5.1
Kidney	360 ± 13.7	357 ± 13.6	348 ± 12.9	352 ± 13.4	358 ± 13.6	361 ± 13.7
	VII	VIII	IX	X	XI	XII
Serum	57 ± 2.0^{b}	50 ± 1.5^{a}	52 ± 1.7^{a}	49 ± 1.4^{a}	55 ± 1.9^{b}	46 ± 1.3^{a}
Liver	315 ± 9.8	340 ± 13.2	287 ± 6.6^{a}	329 ± 11.2	320 ± 10.2	292 ± 6.1^{a}
Heart	239 ± 9.1	207 ± 5.2^{b}	232 ± 8.8	219 ± 7.0	233 ± 8.9	196 ± 3.9^{a}
Aorta	135 ± 5.1	119 ± 3.9^{a}	137 ± 5.2	138 ± 5.2	131 ± 4.4	116 ± 3.3^{a}
Kidney	363 ± 13.9	287 ± 5.7^{a}	350 ± 12.9	355 ± 13.6	326 ± 10.3	337 ± 1.8
			Phospholipids			
	I	II	III	IV	V	VI
Liver	2210 ± 88.4	1833 ± 55.0^{a}	1833 ± 55.0^{a}	1846 ± 55.4^{b}	2140 ± 79.2	1907 ± 68.7^{b}
Aorta	1139 ± 44.1	1036 ± 36.3	496 ± 10.9^{a}	855 ± 23.9^{a}	1133 ± 45.3	867 ± 25.2^{a}
Heart	2020 ± 80.8	2014 ± 78.6	1905 ± 70.5	2060 ± 82.4	2023 ± 80.9	1895 ± 60.6
Kidney	2225 ± 89.0	1295 ± 34.9^{a}	1314 ± 39.4^{a}	2227 ± 89.1	2255 ± 90.2	2223 ± 88.9
	VII	VIII	IX	X	XI	XII
Liver	2196 ± 87.8	2191 ± 86.3	1926 ± 71.1^{b}	1991 ± 78.9	1935 ± 72.3^{b}	1764 ± 35.2^{a}
Aorta	952 ± 29.5^{b}	1008 ± 33.2	966 ± 30.2^{a}	1054 ± 39.0	1022 ± 35.1	800 ± 22.5^{a}
Heart	1996 ± 75.9	1992 ± 74.8	1878 ± 58.1	1963 ± 70.2	1859 ± 54.5	1712 ± 40.1^{a}
Kidney	1892 ± 66.2^{b}	1680 ± 50.2^{a}	2165 ± 85.8	2213 ± 88.4	1965 ± 80.5	1574 ± 48.9^{a}
			Free Fatty Acids			
	I	II	III	IV	V	VI
Liver	272 ± 10.9	252 ± 8.9	170 ± 3.2^{a}	247 ± 8.7	223 ± 6.9^{a}	172 ± 3.5^{a}
Heart	191 ± 7.6	171 ± 6.2	165 ± 5.1^{b}	171 ± 6.2	120 ± 2.4^{a}	167 ± 5.4^{b}
Aorta	40 ± 1.5	41 ± 1.6	40 ± 1.5	39 ± 1.2	40 ± 1.5	41 ± 1.6
Serum	81 ± 3.2	72 ± 2.2	65 ± 1.1	73 ± 2.4	66 ± 1.3^{a}	68 ± 1.8^{b}
	VII	VIII	IX	X	XI	XII
Liver	247 ± 8.7	249 ± 8.7	218 ± 6.5^{a}	246 ± 8.6	248 ± 8.8	161 ± 2.8^{a}
Heart	180 ± 6.8	184 ± 7.0	191 ± 7.5	181 ± 6.9	187 ± 7.1	171 ± 6.2
Aorta	39 ± 1.2	37 ± 0.9	39 ± 1.1	38 ± 1.0	39 ± 1.1	34 ± 0.6^{a}
Serum	75 ± 2.6	63 ± 1.0^{a}	43 ± 0.6^{a}	43 ± 0.6^{a}	61 ± 0.9^{a}	40 ± 0.4^{a}

Average of the values from 10 rats ± SEM ; Groups II to XII have been compared with group I ; a = $p < 0.01$, b = $0.01 < p < 0.05$

as compared to the control group (Table I). In the liver, the reduction of cholesterol was highly significant in groups III, IV, IX and XII (17%, 17%, 16% and 15%, respectively), as compared to the animals from group I (control). In the aorta, cholesterol was found to be significantly decreased in groups III, IV, VIII, and XII (12%, 22%, 15% and 17%). In the heart, most of the experimental groups showed no significant reduction in cholesterol. The concentration of triglycerides showed significant reduction in the livers of all experimental groups except IX and X (Fig.1). Phospholipid levels were significantly lowered (Table 1), as compared to control animals in the livers of groups II, III, IV, VI, IX, XI and XII (17%, 17%, 16% ,14%, 13%, 13% and 12% respectively). In the aorta, significant reduction was observed in experimental groups III,

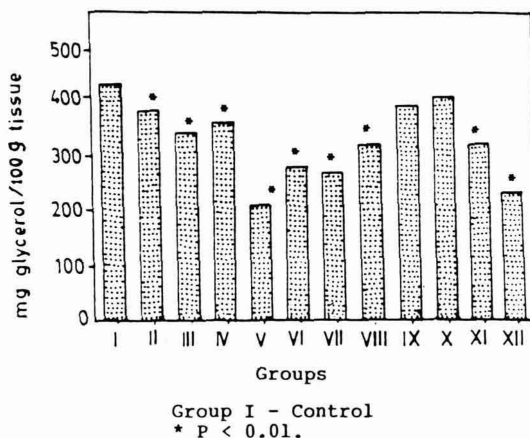

Fig. 1. Triglyceride levels in liver of rats fed various pectins

TABLE 2. ACTIVITY OF HMG CoA REDUCTASE IN LIVER AND INTESTINE OF RATS FED PECTIN FROM *COCCINIA INDICA*

	Groups	
	I (Control)	II (+ pectin)
Activity of HMG CoA reductase in liver (HMG CoA)/(Mevalonate)	3.14 ± 0.07	2.27 ± 0.05ᵃ
Activity of HMG CoA reductase in intestine (HMG CoA)/(Mevalonate)	1.98 ± 0.04	2.13 ± 0.05

Average of the values of 6 rats in each group ± SEM.
Group II is compared with Group I; a = p > 0.01

IV, VI, VII, IX and XII (56%, 25%, 24%, 16%, 15% and 30%). Only group XII showed significant decrease (15%) in the heart. In the kidney, significant reduction was noticed in groups II, III, VII, VIII and XII (42%, 41%, 15%, 24% and 29%, respectively). Concentration of free fatty acids was reduced significantly (Table 1) in the serum samples of groups V, VI, VIII, IX, X, XI and XII (19%, 16%, 22%, 47%, 47%, 25%, and 50%, respectively), as compared to control animals. In the liver, significant reduction was observed in groups III, V, VI, IX and XII (37%, 18%, 37%, 20% and 40%), as compared to animals from group I. In the heart, groups V and VI showed significant reduction. Fatty acid levels showed no significant alteration in the aorta except in group XII, where significantly decreased value of free fatty acid (16% reduction) was seen as compared to the control group. The enzyme, HMG CoA reductase showed significant increase in activity in the liver of rats administered pectin (Table 2).

The activities of the enzyme, lipoprotein lipase

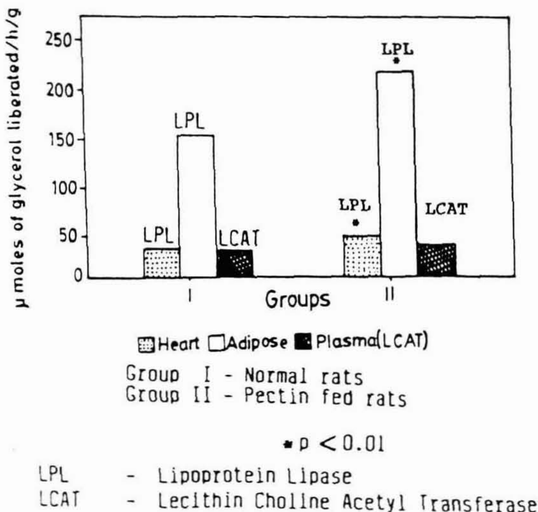

Fig. 2. Activities of lipoprotein lipase of heart and adipose and those of plasma LCAT

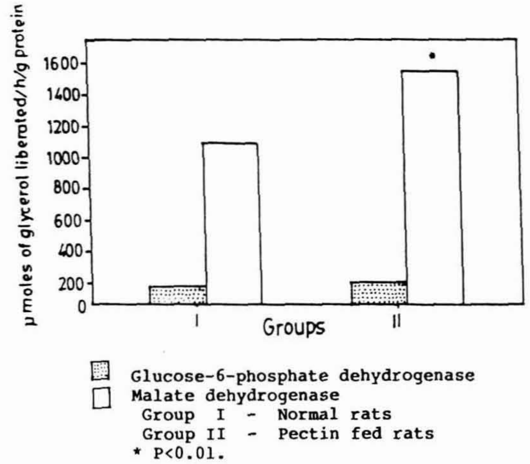

Fig. 3. Activities of lipogenic enzymes of liver

of the heart and the adipose tissue and that of plasma LCAT were increased in rats administered pectin, when compared to control rats (Fig. 2). The activities of lipogenic enzymes, glucose-6-phosphate dehydrogenase and malate dehydrogenase were increased in the livers of rats administered pectin compared to control rats (Fig. 3). The incorporation of labelled (^{14}C) acetate into free cholesterol fraction was significantly higher in the liver, while it was found to be lower in the serum of rats administered pectin (Table 3). The ester cholesterol, triglycerides and phospholipids showed a significantly lower incorporation of (^{14}C) acetate in both serum and liver. The concentration of hepatic and faecal bile acids and faecal neutral sterols were increased significantly in experimental animals given pectin (Fig. 4).

Pectin ingestion did not adversely affect the growth or body weight of the animals. Pectin feeding

TABLE 3. *IN VIVO* INCORPORATION OF (^{14}C) ACETATE IN RATS FED PECTIN FROM *COCCINIA INDICA* (VALUES EXPRESSED AS COUNTS/MT/G TISSUE)

	Groups	
Serum	I (Control)	II (+ pectin)
Free cholesterol	817 ±21.5	670 ±17.7ᵃ
Ester cholesterol	565 ± 5.1	326 ± 8.8ᵃ
Triglycerides	952 ±25.2	763 ±20.6ᵃ
Phospholipids	408 ±10.8	333 ± 8.9ᵃ
Liver		
Free cholesterol	1321 ±35.0	1675 ±44.8ᵃ
Ester cholesterol	453 ±12.2	264 ± 7.0ᵃ
Triglycerides	832 ±22.2	484 ±13.7ᵃ
Phospholipids	4710 ±126.0	2728 ±73.9ᵃ

Average of the values of 6 rats in each group ± SEM.
Group II is compared with group I; a = p > 0.01

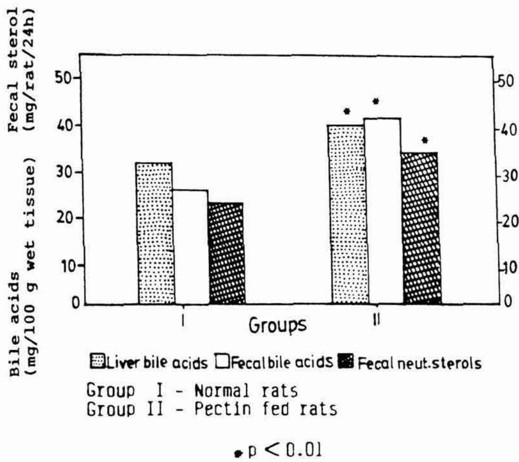

Fig. 4. Concentrations of bile acids and faecal neutral sterols

also resulted in improving dyslipidemia and better utilisation of lipids. Tinker et al (1994) reported that pectin extracted from prunes lowered plasma and liver cholesterol levels in hyperlipidemic rats.

Two hypotheses have been proposed for the mechanism of action of pectin on serum lipid levels. The first hypothesis was by Kay and Truswell (1977), who suggested that pectin caused a negative sterol balance by its viscosity and by interfering with the reabsorption of bile acids in the ileum. The second hypothesis by Anderson and Chen (1979) has implied that fermentation of pectin in the large intestine produces volatile fatty acids, which are absorbed into the portal vein and possibly suppressed hepatic cholesterol synthesis. Further studies on prickly pear pectin indicated that the decreased plasma and hepatic cholesterol concentration of animals are not explained by differences in cholesterol absorption, but rather are due to mechanisms that alter hepatic cholesterol homeostasis, resulting in lower plasma LDL concentrations (Fernandez et al. 1990). The higher activity of hepatic HMG CoA reductase and higher incorporation of labelled acetate into hepatic free cholesterol in rats administered pectin indicate that pectin lowers lipid levels by some other mechanism. Increased rate of cholesterogenesis may not raise the body cholesterol levels, as endogeneously synthesized cholesterol is the preferred substrate for bile acid synthesis (Balasubramanian et al. 1973; Staple and Gurin 1954). Reports on the effect of pectin on hepatic HMG CoA reductase are contradictory. Schartz et al (1983) reported that intestinal cholesterol and phospholipid synthesis was suppressed by long-term pectin administration. On the contrary, Arjmandi et al (1992) suggested an increase in the rate of cholesterogenesis on pectin feeding. Another group of investigators (Hexeberg et al. 1994) also reported similar results on their studies on lipid metabolism in heart and liver of rats fed pectin, which corroborate with data obtained from the present study. Lutton (1976) explained the stimulatory effect of HMG CoA on the basis of his experiments, where a decrease in bile acid reabsorption stimulated cholesterol synthesis in the liver.

The increased activity of plasma LCAT in pectin-fed rats can be accounted for the significant decrease in the concentration of cholesterol in the tissues. The higher activity of lipoprotein lipase in the adipose tissue and heart may be responsible for the decreased concentration of triglycerides in sera of rats fed pectin.

The ability of dietary pectin to bind bile acids in the gastrointestinal tract is believed to be a major involvement in lipid metabolism. Sadam and Danielsson (1985) have suggested that increase in faecal elimination of neutral sterols by ingestion of pectin is balanced by an increase in hepatic sterol synthesis. The enhanced faecal neutral sterol excretion may cause liver depletion of cholesterol, which results in the higher rate of liver cholesterol synthesis. Concerning the mechanism of action, it can be suggested from the above observations that the hypolipidemic activity of pectins may be mainly due to the decreased absorption of cholesterol from the intestine and increased rate of degradation and elimination of lipids.

The above experiments indicate that pectins from papaya, little gourd, tomato and cucumber are the most beneficial pectins in reducing lipid levels. A recent study on the effect of dietary fibre mixture on glucose and lipid metabolism and on mineral absorption in rats indicates that in fibre mixtures, the source, rather than the amount of fibre, generally affects absorptive and metabolic parameters (Galibois et al. 1994). Pectins vary greatly in their hypolipidemic action. The variation in hypolipidemic effect of pectins from different sources may be attributed to the differences in structure, for example the degree of methylation, acetylation etc. Investigations on structure activity relationships are being carried out in this laboratory to understand the underlying mechanism for variation in the effectiveness of pectins on lipid metabolism.

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Preparation of Sweet Potato Flour and Its Fermentation to Ethanol

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A sweet potato variety ('Red') contained 14-15% starch, 3.0 to 3.5% free sugars and 1.0 to 1.2% pectin on wet weight basis. Hand peeling of steamed sweet potato gave on processing better yield of dry flour (5.1 kg) per 20 kg of fresh sweet potato than lye peeling (4.1 kg) and abrasive peeling (4.8 kg) methods. Addition of pectinase (60 U/ml) followed by gelatinisation before mashing of sweet potato or its flour facilitated better conversion of starch to fermentable sugars by amylases. Fermentation of the mash with *Saccharomyces cerevisiae* CFTRI 101 by modified Toyama's method yielded more ethanol (83.0 g/kg, 0.49g/g of sugar, % theoretical yield 95.3) than that of CFTRI method and Toyama's method. The use of *Endomycopsis fibuligera* IFO 0104 culture filtrate as source of amylases, yielded almost same amount of ethanol (81.0g/kg, 0.49g/g, % theoretical yield 95.6) as that of modified Toyama's method. A wine-like product with ethanol upto 8.6% (w/v), desirable aroma and colour was also prepared.

Keywords: Sweet potato flour, Peeling methods, Starch, Pectin, Sugars, Ethanol fermentation, Amylases, Pectinase, *Endomycopsis fibuligera*, Wine.

India produces about 1.15 million tonnes of sweet potato (*Ipomea batatas* L), as compared to the world's production of 124.3 million tonnes per year (Anon 1994), which is mostly produced in developing countries. It is used as a staple food after cooking or baking, or as *shochu* a fermented food (Tamaoka et al. 1971) in certain developing countries. Surplus quantities of this starch-rich material is also utilised for the production of fuel and potable alcohol (Zhang 1983; Wu and Bagby 1987; Kurup 1992). Attempts to ferment raw sweet potato by the use of yeast (Saha and Ueda 1983) and *Aspergillus niger* enzymes (Zhang 1983) were made to economise the process of ethanol production. Fermentation of mash of dried sweet potato to ethanol was also carried out successfully in packed-bed reactors containing immobilized yeast (Bingjun et al. 1995).

In order to preserve surplus quantities of a locally available sweet potato variety ('Red'), studies on economic preparation of flour and its efficient conversion to ethanol as compared to that of fresh sweet potato were carried out and the results are presented in this paper.

Materials and Methods

Sweet potato ('Red' variety) was purchased from the local Mysore market. *Saccharomyces cerevisiae* CFTRI 101 was obtained from the Department of Food Microbiology, CFTRI, Mysore. The amylolytic yeasts, *Endomycopsis fibuligera* (IFO 0104, IFO 0103) were received from National Centre of Industrial Microorganisms (NCIM), Pune, India. The other amylolytic yeast *Schwanniomyces castellii* ATCC 26077 was received from Labatt Brewing Company Ltd., Ontario, Canada, and *Schw. alluvius* UCD 54-83 was procured from Prairie Regional Laboratory, Saskatoon, Canada. The enzymes α-amylase, glucoamylase and pectinase were purchased from Anil Starch, Ahmedabad, India.

Estimation of starch, sugars and pectin : Enzymatic hydrolysis of starch (Srikanta et al. 1987a) and estimations of reducing sugars (Shaffer and Somogyi 1933) and pectin (AOAC 1975) were carried out by standard methods. Estimations of α-amylase (Manning and Campbell 1967), glucoamylase (Pazur and Ando 1959) and pectinase (Ashwell 1957) were also made, wherever necessary. Paper chromatography of starch hydrolyzed products was done by using the procedure of Trevelyan et al (1950).

Preparation of sweet potato flours : A flour was prepared from 'Red' variety of sweet unpeeled potato, by slicing, drying and milling to a particle size of 40 BS (British Standard) mesh in an Apex mill. Flours were also prepared after removing the peel by abrasive peeling (Mechanical Potato Peeler, Mather and Platt, UK), hand peeling (after steaming for 10 min) and lye-peeling (after cooking in IN NaOH for 10 min) methods. After peeling in case of abrasive method, the sweet potato was cooked by steaming for 10 min. All the above samples with 23-25% solids were mashed and dried by using drum drier (Dunford and Elliott, UK) at 121°C. The dried flakes were pulverized as above. All the flours were stored in sealed polythene bags at ambient

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conditions until use.

Hydrolysis of whole sweet potato and its flour: One kg of whole sweet potato (or 250g flour in 750 ml water), was steamed for 10 min, cooled, mashed and treated with α-amylase and glucoamylase according to the method of Srikanta et al (1987b). The treatment period was increased from 4 h to 44 h in order to completely hydrolyse starch.

In the case of Toyama et al (1984) method, the tubers were soaked for 16 h 40 min with 0.2% H_2SO_4 and the tubers alone were mashed and macerated with 0.02% (60 U/ml) pectinase (polygalacturonase) at pH 4.5. The mash was treated with 0.1% α-amylase at 80°C for 20 min, followed by glucoamylase 0.5% at 40°C for 4 h. After supplementation with nutrients and potassium metabisulphite (0.1%), it was fermented by yeast.

In the new method, the sweet potato mash was treated with 0.02% pectinase for 5 h 45 min and gelatinized at 80°C for 15 min. It was then treated with 15% culture filtrate (30 U/ml α-amylase and 80 U/ml glucoamylase) of *Endomycopsis fibuligera* IFO 0104 or IFO 0103 at pH 5.5 for 4 h at room temperature.

Preparation of culture filtrate : E. fibuligera strain(s) was inoculated into sterile 3% wheat bran medium (pH 6.0) and grown for 4 days at 30°C on a rotary shaker. The filter-sterilized culture filtrate was used as source of amylases.

Fermentation of sweet potato mash : One hundred ml of the hydrolyzed or unhydrolyzed mash (pH 4.5) with nutrients (Toyama et al. 1984) was taken in 250 ml conical flask and sterilized at 121°C for 15 min. It was inoculated with 10 ml of yeast culture(s), prepared by growing the yeasts in the hydrolyzed and diluted sterile mash (1 in 10) with nutrients. The inoculated sample was fermented at 30°C for 3 days at stationary conditions.

Ethanol estimation : Ethanol in the centrifuged supernatant was estimated by gas chromatograph (Shimadzu 16A, Japan), using flame ionisation detector (FID) and Poropak Q column (100 to 120 mesh) (Anthony 1984). The percentage v/v of ethanol was converted to percentage w/v by multiplying with a factor 0.79.

Colour measurement : Colour intensity of the wine-like product was measured by spectrophotometry at 450-500 nm.

Results and Discussion

Proximate composition of sweet potato : The 'Red' variety of sweet potato contained 14.0 to 15.0% starch and 3.0 to 3.5% free sugars amounting to 17.0 to 18.5% fermentable carbohydrates on wet weight basis. It also contained 1.0 to 1.2% pectin on wet weight basis. However, certain Japanese varieties contain higher starch contents upto 34% on wet weight basis (Saha and Ueda 1983).

TABLE 1. PREPARATION OF SWEET POTATO FLOURS BY USING DIFFERENT METHODS

Method	Starting material, kg	After Peeling, kg	Final dry product (flour)			Water consumption for peeling, 1/20 kg
			Weight, kg	Colour	Moisture, %	
Hand peeling	20	18.0	5.1	Light yellow	11.0	0.0
Lye peeling	20	15.7	4.1	Deep yellow	9.3	80.0
Mechanical abrasive peeling	20	17.9	4.8	Light brown	12.6	60.0
Control 1 (no peeling)	20	20.0	5.4	Yellowish	12.0	0.0
Control 2 (no peeling and cooking)	20	20.0	5.5	Light yellow	11.0	0.0

Preparation of sweet potato flours by using different methods : Since fresh sweet potato cannot be stored for a long time, attempts have been made in the present study to prepare dry flour for better storage and preservation. Of the three methods of peeling, hand-peeling was found to be the best, as it yielded about 5.1 kg dry flour from 20 kg of fresh sweet potato processed, as compared to the methods of mechanical abrasive-peeling (4.8 kg) and lye-peeling (4.1 kg) (Table 1). Both lye-peeling and mechanical abrasive-peeling methods were not only energy intensive, but also required 60-80 litres of process water per 20 kg of sweet potato processing (Table 1). There was loss of about 7-8% solids in the case of hand peeled-flour, which was mainly due to the peel. The losses in the other two methods were more due to uncontrolled peeling, which removed some fleshy material as well. However, hand-peeling method is advisable, only if human labour is available at a cheaper cost.

Maltose production during sweet potato processing : The water extracts of the above flours were subjected to descending paper chromatography for the qualitative determination of free sugars, especially maltose. The samples prepared by hand-peeling, lye-peeling and mechanical abrasive-peeling methods were subjected to heat processing during steam cooking and drum drying. Due to this heat treatment, there was high concentration of maltose produced in the samples. The control 1 samples

TABLE 2. ETHANOL PRODUCTION FROM WHOLE SWEET POTATO USING VARIOUS METHODS

Method	Time taken for hydrolysis, h	Glucose, g/kg	Ethanol, g/kg	Residual sugars, g/kg	Theoretical yield, %[b]	yield y=p/s, g/g[a]
CFTRI* method (control)	48.5	176.0	73.0	12.5	87.5	0.446
Modified Toyama's						
a. Without pectinase	21.0	110.0	50.4	8.3	97.2	0.496
b. With pectinase	21.0	180.8	83.0	10.1	95.3	0.486
Toyama's method	21.0	107.0	52.6	1.2	97.5	0.497
New** method	20.0	170.0	81.0	3.8	95.6	0.487

* No pre-treatment with pectinase

** *E. fibuligera* IFO 0104 culture filtrate was used in place of commercial α - amylase and glucoamylase

[a] Yield is defined as g of ethanol produced per g of glucose utilized

[b] It was calculated by the formula $\frac{\text{Yield}}{\text{Theoretical yield}} \times 100$

with peel contained trace amounts of maltose, although the samples were subjected to heating as above. This indicates that peel might interfere in the activity of thermostable β-amylase in sweet potato (McArdle and Bowkamp 1986). However, control 2 samples with peel did not contain any maltose, as they were not subjected to high heat treatment, but only dried at 40°C in cross-flow drier. The maltose so generated will add on to the flavour of the flour, which can be used as a breakfast carbohydrate with milk.

Ethanol production from whole sweet potato using various methods : In a preliminary experiment, fresh sweet potato was hydrolyzed by using commercial enzymes and fermented by *S. cerevisiae* CFTRI 101 as per the conventional method, developed by CFTRI, Mysore (Srikanta et al. 1987a) for tapioca fermentation. As the process took more than two days for hydrolysis itself, giving 73g alcohol per kg of fresh sweet potato on fermentation by yeast (Table 2), an improved Toyama's method (Toyama et al. 1984) was followed in the fermentation of fresh sweet potato, which yielded even less ethanol (52.6 g/kg of sweet potato), wherein the starch was hydrolyzed over a period of 21 h. This method involved pectinase treatment before hydrolysis with amylases. Of various levels of pectinase tested, 0.02% (60 U/ml) was found to be optimum in facilitating better hydrolysis of starch. Toyama's method was further modified by introducing gelatinisation (80°C for 10 min) as a pre-treatment, after pectinase treatment. With this pre-treatment the alcohol yield increased to 83 g/kg sweet potato. However, without pectinase treatment, this method yielded very low (50.4 g/kg) alcohol. This indicates that pectinase treatment of sweet potato mash and gelatinisation followed by hydrolysis with amylolytic enzymes are necessary for optimum yield of fermentable sugars. In the new method, the commercial enzymes were replaced with culture filtrate of *E. fibuligera* IFO 0104 and with pectinase and gelatinistion treatments given as above, it was possible to obtain 81 g ethanol per kg of sweet potato, - an yield almost equivalent

TABLE 3. HYDROLYSIS OF SWEET POTATO AND ITS FLOUR BY CONVENTIONAL ENZYMATIC METHOD AND FERMENTATION TO ETHANOL (WINE)

Method	Volume of fermented broth, ml	Residual sugars, g %	Residue wet weight, g %	Moisture content of residue, %	Colour		Alcohol, % w/v
					Type	Intensity	
Sweet potato							
Autoclaved							
Hand-peeled (100g)	90.0	1.8	21.5	82.4	Brown	0.14	8.6
Lye-peeled (100g)	80.0	0.6	15.0	81.4	Deep brown	0.80	6.8
Unautoclaved							
Hand-peeled (100g)	90.0	1.0	32.0	80.0	Yellow	0.13	8.4
Lye-peeled (100g)	80.0	1.0	16.0	76.0	Yellow	0.13	6.6
Sweet potato flour (25%)							
KMS* (500 ppm)	100.0	4.4	17.0	75.0	Yellow	0.14	5.2
Not autoclaved	106.0	3.4	16.0	80.0	Yellow	0.14	6.7
Autoclaved	86.0	1.0	19.0	77.0	Brown	0.30	7.5

Sweet potato hydrolyzed with commercially available amylases and fermented by *S. cerevisiae* CFTRI 101

* Potassium metabisulphite

TABLE 4. HYDROLYSIS OF SWEET POTATO FLOUR (25%) BY CULTURE FILTRATE AND FERMENTATION TO ETHANOL BY CERTAIN YEASTS

Method	Residual sugars, g %	Residue wet weight, g %	Ethanol, % w/v
Mixed culture of *E. fibuligera* IFO 0104 and *S. cerevisiae* CFTRI 101	0.41	32.0	2.1
Culture filtrate of *E. fibuligera* IFO 0104 saccharification (2 h) followed by fermentation by *S. cerevisiae* CFTRI 101	1.18	30.0	8.4
Culture filtrate of *E. fibuligera* IFO 0104 simultaneous saccharification and fermentation with *S. cerevisiae* CFTRI 101	0.31	38.0	3.4
Mixed culture of *E. fibuligera* IFO 0103 and *S. cerevisiae* CFTRI 101	2.03	34.0	2.8
Culture filtrate of *E. fibuligera* IFO 0103 saccharification (2 h) followed by fermentation by *S. cerevisiae* CFTRI 101	0.70	34.0	4.3
Culture filtrate of *E. fibuligera* IFO 0103 simultaneous saccharification and fermentation with *S. cerevisiae* CFTRI 101	0.43	47.0	3.8
Mixed culture of *Schw. castellii* ATCC 26077 and *S. cerevisiae* CFTRI 101	2.05	35.0	3.2
Mixed culture of *Schw. alluvius* UCD 54-83 and *S. cerevisiae* CFTRI 101	0.85	32.0	0.0

to modified Toyama's method (Table 2). In this regard, gelatinization might have two functions to perform, namely softening of starch facilitating the activities of α-amylase and glucoamylase and activating thermostable β-amylase for converting part of the starch to maltose. However, none of the above amylases had the capability to hydrolyse raw sweet potatoes.

Fermentation of whole sweet-potato or its flour to a wine-like product : As sweet potato contained 18–19% fermentable carbohydrates, it was possible to ferment the whole sweet potato mash after hydrolysis with commercial enzymes to a wine-like product. The results presented in Table 3 clearly show that both autoclaved and unautoclaved samples of sweet potato could be fermented to a wine-like product, containing 6.6 to 8.6% (w/v) alcohol. Hand-peeled samples gave higher amount of alcohol than the lye-peeled samples, or the latter lost weight during lye-peeling treatment. Unautoclaved samples gave wine samples having preferable bright yellow colour, whereas autoclaved samples were brown in colour.

Hand-peeled sweet potato flour was also used in the fermentation and similar results were obtained. Addition of potassium metabisulphite (550 ppm) reduced the ethanol production to some extent. There was no problem in the extraction of juice from the fermented samples, as all of them were pre-treated with commercial amylases (Table 3).

In order to reduce the additional cost due to the use of commercial enzymes for conversion of starch to fermentable sugars, amylolytic yeasts and their culture filtrates were used in the fermentation along with *S. cerevisiae* strains. Two strains of *E. fibuligera* IFO 0103 and IFO 0104 and their culture filtrates as well as both the methods, namely saccharification followed by fermentation and simultaneous saccharification and fermentation were employed in the experiments. The results in Table 4 show that the method of saccharification with the culture filtrate of *E. fibuligera* IFO 0104 followed by fermentation with *S. cerevisiae* CFTRI 101 gave the highest yield (8.4% w/v) of ethanol from 25% sweet potato flour, as compared to the other treatments, such as the use of mixed cultures and simultaneous saccharification with *E. fibuligera* IFO 1013 fermentation with *S. cerevisiae* CFTRI 101. Similar experiments with other amylolytic yeasts were not very encouraging.

The culture filtrate of *E. fibuligera* IFO 0104 is cheaper, as it is produced by using a simple wheat bran medium. The alcohol production from sweet potato using this culture filtrate containing both α-amylase and glucoamylase and acting optimally at room temperature, is expected to be more economical than using the commercial amylases. However, scale up studies and cost calculations are needed to prove this point.

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Soymilk Substitution on Quality Attributes of Mozzarella Cheese Made from Buffalo Milk

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Effect of adding soy solids to buffalo milk on the sensory, textural and biochemical characteristics of Mozzarella cheese was studied. Cheese samples made from 90:10, 85:15 and 80:20 blends of buffalo and soymilks were rated acceptable upto 21 days at 7 ± 1°C. Moisture contents of cheese samples increased, as the proportion of soy solids increased, whereas protein, fat and ash contents followed the reverse trend. Hardness, springiness, gumminess and chewiness values were significantly ($p<0.01$) lower for the 80:20 blend and decreased during storage. Cheese samples made from 90:10 blend had the same stretchability as the control, but the 85:15 and 80:20 blends exhibited slightly lower values. Meltability values decreased slowly during storage. Glycolysis, proteolysis and lipolysis increased at a much faster rate in soy Mozzarella cheese samples compared to control.

Keywords: Mozzarella cheese, Soybean, Marzyme, Meltability, Textural changes, Biochemical changes.

Utilization of soybeans is generally considered to be one of the more promising means of alleviating the shortage of good quality protein in developing countries (Aworh et al. 1987). Attempts have been made to replace milk solids by soy solids for the preparation of dairy products (Trantik and Jadsic 1982; Aworh et al. 1987; Yadav et al. 1994). Yand and Taranto (1982) prepared Mozzarella cheese analogues from soybeans and studied their textural characteristics. The present investigation was undertaken to prepare Mozzarella cheese by supplementing buffalo milk with soy solids and assess its effect on flavour, biochemical and textural characteristics.

Materials and Methods

Buffalo milk, obtained from the Livestock Research Centre of this University, standardized to 4% fat had 3.68% protein. Soymilk prepared by the method of Nelson et al (1976) contained 1.3% fat, 3.26% protein and 7.64% total solids.

Marzyme, produced from *Rhizomucor miechei*, obtained from Miles Laboratories, Inc. Madison, WI, USA was used as rennet.

Mother cultures of *Streptococcus thermophilus* (YH-S) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (YH-L), obtained from the National Dairy Research Institute, Karnal, India, were used in equal proportions (1:1) for the preparation of bulk culture in sterilized skim milk.

Buffalo and soymilks at ratios of 100:0, 90:10, 85:15 and 80:20 were used to prepare Mozzarella cheese as depicted in Fig. 1. The cheeses were evaluated for sensory, biochemical and textural

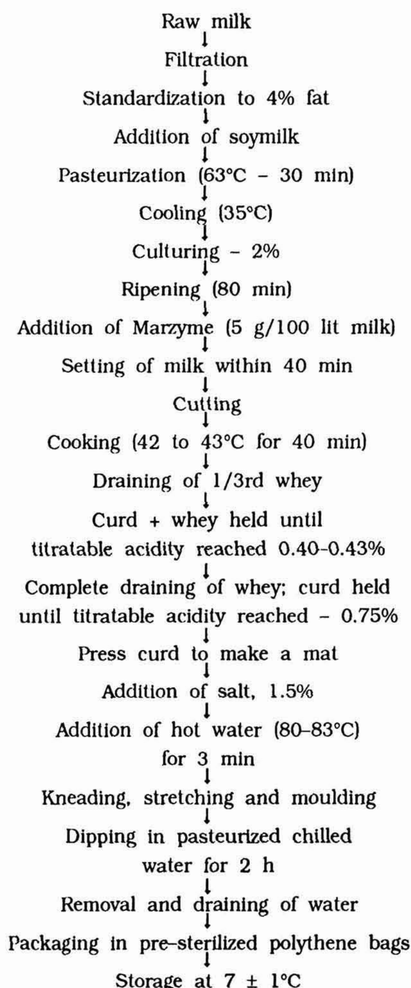

Fig 1. Flow diagram for Mozzarella cheese preparation

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characteristics during storage at 7 ± 1°C at 7 days interval.

Sensory evaluation : Cheese samples made from different blends of buffalo and soymilks were evaluated by 8 trained panelists for colour (3), body and texture (5) and flavour (10), using a 18-point score card (Duthie et al. 1980). The samples stored at 20 ± 1°C were served to panelists randomly for three replications of the study. Shelf life was defined as the number of days for which the cheese samples could be stored before the mean flavour score fell below 6 and samples scoring less than 6 were considered to have pronounced taste defects and were rated sub-normal.

Chemical analysis : Cheese samples were analyzed for proximate composition using standard procedures (AOAC 1965, 1975; AACC 1976). Titratable acidity, pH (AOAC 1975), tyrosine value (Hull 1947) and free fatty acids (Deeth and Fitz-Gerald, 1976) were estimated in cheese samples stored at 7 ± 1°C.

Textural characteristics of cheese : An Instron Universal Testing machine (Model 6021, Instron Inc. Canto, USA) was used to study the textural characteristics of the cheese samples (Bourne 1978). The Instron cross-head fitted with a vertical reciprocating movement at a constant speed of 1 mm/sec with a stroke length of 5.00 mm. The maximum clearance between the moving horizontal plates of the machine was 10 mm and the minimum clearance was 5.0 mm, giving a 50% uniaxial parallel plate. A cubical cheese sample of 10 mm was prepared and subjected to two cycle compression (Max 0.50 strain) to give first and second bite at 9 ± 1°C.

Stretchability was assessed by the 'Stretch Test' described by Kosikowski (1982). The stretchability was graded on a 5-point arbitrary scale, where 5 represented the best stretchable characteristics.

The meltability of Mozzarella cheese was determined according to the procedure of Arnott et al (1957). The specimens were stored at 4°C until testing. The specimen, 17 x 17 mm was placed on a glass pertri dish and then placed in laboratory oven maintained at 100°C for 0 to 25 min. The initial height of specimen was 17 mm. The centre height of each specimen was measured immediately after removal from the oven with the help of needle and scale.

Statistical analysis : A two factor completely randomized design was used for statistical analysis of data obtained for sensory and textural profile analysis of cheese samples stored at 7 ± 1°C (Snedecor and Cochran 1967).

TABLE 1. PHYSICO-CHEMICAL ATTRIBUTES OF MOZZARELLA CHEESE PREPARED FROM DIFFERENT BLENDS OF BUFFALO AND SOYMILK AND LOSSES OF SOLIDS IN WHEY

Attributes, %	Blends of buffalo and soymilks 100:00	90:10	85:15	80:20
Moisture	50.05	51.34	53.10	53.52
Protein	24.98	23.60	23.17	22.80
Fat	22.43	21.40	20.48	20.33
Ash	2.18	2.12	2.04	2.00
Salt	1.30	1.45	1.48	1.50
Yield, % dry weight basis	28.48	29.68	30.95	31.51
Stretchability	4	4	3	3
Losses of solids in whey %				
Total solids	5.23	5.43	5.57	5.69
Fat	0.30	0.35	0.35	0.45

Average of 3 trials

Results and Discussion

The proximate composition of Mozzarella cheese made from buffalo and soymilk blends is shown in Table 1. Moisture content, which was higher than the control, increased from 51.3 to 53.5%, as the proportion of soymilk in blends was increased. This has been attributed to hydrophilic nature of soyprotein, contributing to higher moisture in the cheese (Noyes 1969). Levels of protein, fat and ash in the cheeses decreased slightly, as the level of soymilk in the blends increased. The yield (%, dry weight basis) was lowest (28.5) and highest (31.5) in control and sample made from the 80 : 20 blend. Kosikowski (1982) obtained 11.5% yield of Mozzarella cheese having 53.6% moisture.

Losses of total solids and fat in whey during the manufacture of cheese from the different blends of buffalo and soymilks ranged from 5.23 to 5.69 and 0.30 to 0.45%, respectively (Table 1). Losses of total solids in whey reported by Breene et al (1964) and Shukla and Ladkani (1989) ranged from 6.72 to 8.88%. Fat losses in whey did not increase appreciably, as the proportion of soymilk in the blends increased.

Stretchability rating of the control and the cheese made from the 90:10 blend was same i.e., 4, but decreased to 3 for cheeses made from 85:15 and 80:20 blends. The decrease in stretchability has been attributed to less percentage of casein available for the conversion to mono-calcium-para

TABLE 2. SENSORY AND BIOCHEMICAL CHARACTERISTICS OF MOZZARELLA CHEESE MADE FROM DIFFERENT BLENDS OF BUFFALO AND SOYMILK DURING STORAGE AT 7 ± 1°C

Characteristics	Blends of buffalo and soymilks																			
	100:00 Days					90:10 Days					85:15 Days					80:20 Days				
	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28	0	7	14	21	28
Sensory																				
Flavour, 10 points	9.3	9.2	7.8	6.8	3.3 M	8.7	8.3	7.7	6.7	2.2 B, M	8.3	8.2	7.3	6.3 A	2.8 B, M	7.5	7.2	6.8	5.5	2.3
Body and texture, 5 points	5.0	4.8	4.7	4.5	3.8	4.8	4.5	4.3	4.0	3.3	4.0	4.2	4.1	3.3	3.8	3.7	3.7	3.5	3.3	3.0
Colour, 3 points	3.0	2.9	2.9	2.9	2.8	3.0	2.8	2.8	2.8	2.7	3.1	3.0	2.8	2.8	2.7	3.0	2.8	2.8	2.7	2.6
Biochemical																				
pH	5.4	5.4	5.3	5.3	5.3	5.4	5.4	5.3	5.3	5.2	5.4	5.4	5.3	5.2	5.2	5.4	5.4	5.3	5.2	5.1
Titratable acidity, % lactic acid	0.6	0.6	0.6	0.7	0.8	0.6	0.6	0.7	0.8	1.0	0.7	0.7	0.7	0.8	1.0	0.7	0.7	0.8	0.8	1.0
Tyrosine value, mg/100 g, cheese	7.3	9.9	11.7	12.5	19.3	7.5	10.8	12.5	12.5	20.8	9.0	11.0	13.0	14.2	24.3	10.0	11.5	14.2	15.8	26.0
Free fatty acids, milli equiv/ 100 g, fat	0.9	1.1	1.3	1.9	2.0	0.9	1.3	1.4	2.3	2.8	0.9	1.2	1.8	2.5	3.6	0.9	1.2	2.2	2.8	3.9

A - Acidic flavour, M - Mouldy flavour ; B - Bitter flavour

Average of 3 trials

caseinate by the addition of soy solids (Kosikowski 1982).

Sensory characteristics : Mozzarella cheese samples made from buffalo and soymilk blends (90:10, 85:15, 80:20) rated normal and secured 8.7, 8.3, 7.5 flavour points, respectively against the highest score (9.3) for the control (Table 2). Flavour rating decreased during storage, irrespective of treatments. Cheese samples made from the 80 : 20 blend rated sub-normal on the 21st day and criticised for mouldy and bitter taste, whereas the 90 : 10 and 85 : 15 samples were rated acceptable upto 21 days and then found sub-normal thereafter. Significant differences ($p<0.01$) were observed between control and experimental cheeses made from 80 : 20 blend with respect to flavour and body and texture during the entire storage period. All experimental cheeses, irrespective of treatment, received significantly ($p<0.01$) lower colour rating, as compared to control for sensory characteristics after 21 days of storage at 7 ± 1°C. The body and texture of Mozzarella cheese samples changed from rough and fibrous to smooth during storage (Kindstedt et al. 1988).

Texture profile analysis of Mozzarella cheese made from blends of buffalo and soymilk during storage at 7 ± 1°C are shown in Fig. 2. Initial values of hardness ranged from 9.40 to 10.18 N, being lowest for the 80:20 and highest for the 90:10 blends, whereas control sample showed maximum hardness (20.87 N). Hardness values decreased significantly ($p<0.01$) during storage, irrespective of treatment. After 28 days, the hardness values of the cheeses ranged from 4.570 to 10.183 N, being highest (10.183 N) for the control. Lower values for hardness of the soy cheese sample can be attributed to higher and lower amounts of moisture and casein, respectively. Creamer and Olson (1982) observed weakening of the matrix of cheese by dissolution of protein due to a high moisture content and a significantly negative correlation coefficient between hardness and moisture content of the cheese (Chen et al. 1979). Hardness values for the control cheese differed significantly ($p<0.01$) from soy-fortified cheeses, which decreased during storage, irrspective of treatment. This may be attributed to microbial growth and enzymatic activity, which in turn, resulted into degradation of proteins and salt diffusion (Cervantes et al. 1983). Hardness values of Mozzarella cheese from buffalo milk decreased significantly ($p<0.01$) from 42.9 to 20.1 N during storage of 30 days (Ghosh and Singh 1991a).

Mozzarella cheese samples made from the different blends of buffalo and soymilks showed cohesiveness in the range of 0.577 to 0.582 in comparison to a value of 0.592 for the control. Cohesiveness did not differ significantly ($p>0.05$) among the treatments and with respect to control. This could be attributed to more or less the same level of denatured whey proteins in cheese samples (Thakar et al. 1991). Cohesiveness decreased ($p<0.05$) in all cheese samples during storage (Ghosh and Singh 1991a).

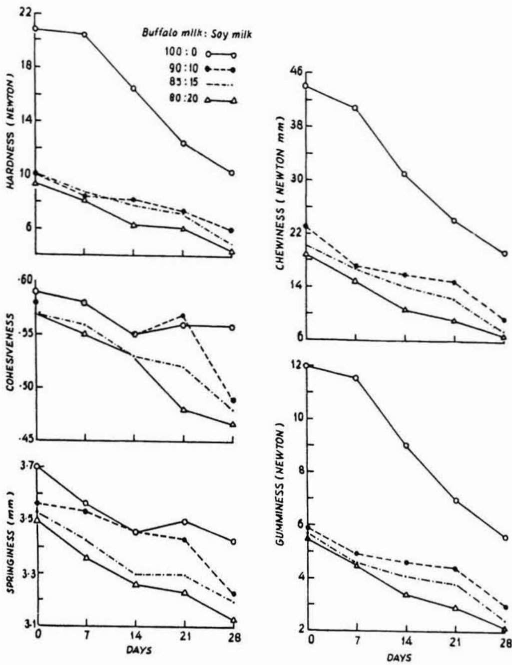

Fig. 2. Textural characteristic of Mozzarella cheese made from different blends of buffalo and soymilks during storage at 7 ± 1°C

Initial values of springiness ranged from 3.50 to 3.56 mm, being lowest for the 80:20 and highest for the 90:10 blends of buffalo and soymilks, respectively, whereas the control sample had a value of 3.70 mm. The springiness in the cheese samples changed significantly ($p<0.01$) during storage and ranged from 3.133 to 3.433 mm, being highest in control. Higher springiness values of control are due to lower moisture in samples (Tunick et al. 1991). The decrease in the springiness of cheddar cheese due to proteolysis has been reported by Fedrick and Dulley (1984). The dependence of springiness on the stereo-structure and intermolecular reaction of the protein network has been shown by Lee and Rha (1978).

Mozzarella cheese made from different blends of buffalo and soymilks had gumminess values in the range of 5.53 to 5.97 N. These values were significantly lower ($p<0.01$) than that of the control. Gumminess decreased significantly ($p<0.01$) during storage and ranged from 2.16 to 3.02 N at the end of 28 days.

Chewiness of the control cheese was significantly higher ($p<0.01$) than that of the experimental cheeses. ANOVA showed that the chewiness of soy cheese samples did not differ significantly ($p>0.01$) among the levels of soymilk supplementation. Chewiness decreased significantly ($p<0.01$) in all the samples during storage and ranged from 6.8 to 9.8 N mm, whereas control sample had 19.7 N mm.

The meltability characteristics of Mozzarella cheese are shown in Fig. 3. The meltability values on day 1 after 25 min of incubation at 100°C were highest for the control, followed by samples made from 90:10, 85:15 and 80:20 blends of buffalo and soymilks. The samples exhibited more or less the same trend in meltability during storage. However, control decreased at slow rate. These observations contradict the findings of Ghosh and Singh (1990), who claimed that meltability increased during storage, whereas Kindstedt et al (1988) observed that meltability remained same and showed no consistent pattern of change during storage.

Biochemical changes in Mozzarella cheese

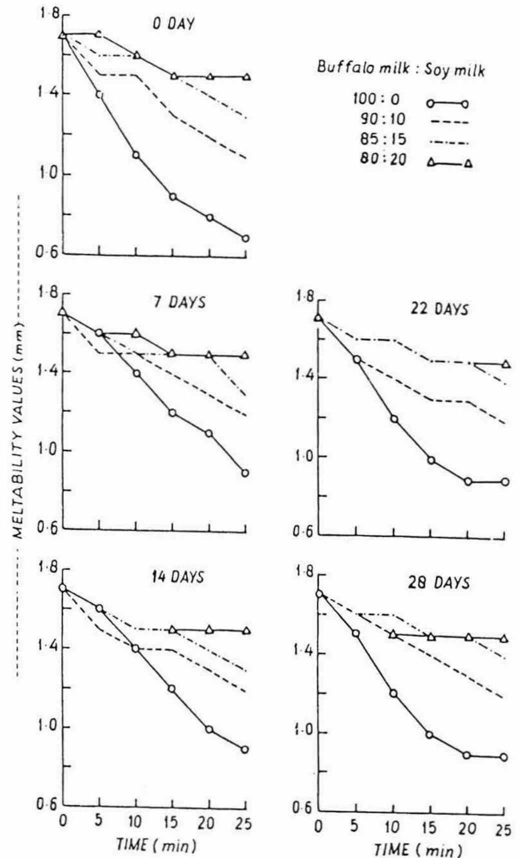

Fig. 3. Meltability in Mozzarella cheese made from different blends of buffalo and soymilks during storage at 7 ± 1°C

samples are presented in Table 2. The initial pH values ranged from 5.38 to 5.42, being lowest for the 80 : 20 blend and highest for the control. pH decreased by 0.02 unit upto 7 days and decreased thereafter at a much faster rate. The present observations are in accordance with the findings of Matteo et al (1982) and Ghosh and Singh (1991b), who reported a decrease in pH during storage. Titratable acidity at 0 day ranged from 0.56 to 0.67%. Ghosh and Singh (1991b) reported a titratable acidity value of 1.06% after 28 days. The present results demonstrated a gradual increase in acidity during storage and was more pronounced in the experimental samples containing soy solids. The increase in acidity of soy cheese samples is probably due to activation of lactic acid bacteria by soy solids (Metwalli et al. 1982). Proteolysis expressed as tyrosine value, ranged from 7.33 to 9.99 mg/100 g cheese on 0 day, being lowest and highest in control and sample made from 80:20 blend, respectively. Tyrosine values increased at a very slow rate upto 21 days and thereafter at a faster rate, reaching reaching 25.99 from 15.83 mg/ 100 g at the end of 28 days. The present results confirm the findings of Gangopadhyay and Thakar (1991). The concentration of free fatty acids increased during storage, as the proportion of soy milk in the blends increased. Wood (1987) observed low concentration of FFA in Mozzarella cheese, which did not change appreciably during storage.

It is concluded that Mozzarella cheese made from 90:10 and 85:15 blends of buffalo and soymilks had normal sensory and textural characteristics, whereas 80:20 blend gave a subnormal quality of cheese with significantly lower sensory and textural characteristics. The shelf life of control and samples made from 90:10 and 85:15 blends rated acceptable upto 21 days.

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Studies on Physico-chemical Composition, Packaging and Storage of Blackgram and Greengram *Wari* Prepared in Uttar Pradesh

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Wari is a partially fermented indigenous traditional savoury food and used as an adjunct in curries. It is being prepared mostly on cottage or home scale. A survey of the units manufacturing blackgram and greengram *wari* was conducted in three important towns of Uttar Pradesh. Data revealed wide variations in the physico-chemical attributes of blackgram and greengram *wari* procured from the manufacturing units. Physico-chemical parameters of different batches of blackgram and greengram *wari* were determined and compared with the standard values. Sorption-isotherm studies revealed that ERH of blackgram and greengram *waris* were 61.4 and 56.8% at 32±2°C with initial moisture contents of 9.6 and 10.2%, respectively. Packaging and storage studies showed changes in moisture content and appearance of moulds or insects during storage at room temperature. Products had a shelf life of 6 months at room temperature in 120 gauge polypropylene bags.

Keywords: *Wari*, Savoury product, Physico-chemical composition, Blackgram, Greengram, Sorption-isotherm.

The preparations of Indian sweetmeat, savoury snack products and other delicacies have been associated and deeply entwined with social customs, rituals and have been developed into culinary art. Among these, *wari*, a partially fermented legume-based product, is manufactured on cottage scale. Generally, *wari* is prepared from split blackgram or greengram along with ingredients like dried fenugreek leaves, coriander powder, cumin seeds, red chilli powder and black pepper. There is no regular statistics available on production of these products used as adjuncts in curry preparation. Literature survey has revealed that work has been done on manufacture, packaging and storage of *wadian* prepared in Punjab state (Pruthi et al. 1983) and quality standards have been drafted (Pruthi et al. 1981).However, published information on the method of preparation, proximate composition, packaging requirement and storage behaviour of *wari* manufactured in Uttar Pradesh and its comparison with proposed standards is rather scanty. Therefore, there is a need to evaluate the quality of such products being manufactured in the State of Uttar Pradesh on cottage scale. The present paper reports results on these aspects.

Materials and Methods

Method of manufacture of blackgram and greengram wari : The traditional method employed for manufacture of *wari* in Uttar Pradesh is as follows :

Split pulses are soaked in ample amount of water for about 20-30 min and scrubbed by hand in order to remove husk and foreign materials, washed thoroughly and soaked again in water overnight. The drained pulses are then ground, in a wet grinder with small addition of water, till it becomes a paste. At times, ash gourd shreddings are also added during wet grinding. The paste is whisked well, until it becomes light and fluffy due to incorporation of air. The resultant paste is mixed with required quantity of spices. A general recipe for the preparation of *wari* includes blackgram split pulse (450 g), water (550 g), ash gourd shreds (100 g), black pepper whole (20 g), cumin seeds (20 g), red chilli powder (10 g), dried fenugreek leaves (100 g), dried coriander seeds (100 g) and a little quantity of asafoetida, nutmeg and cinnamon. The resultant fluffy batter is taken in hand and divided into small lumps weighing 50-60 g and kept over a wooden frame made of round sticks smeared with oil, maintaining a distance of 1-2 inches between lumps and sun-dried for 4-6 days depending upon condition of weather. They are turned over by hand after 2-3 days.

More or less the same procedure is adopted for greengram *wari* (with or without addition of common salt and spices) except that the ashgourd shreddings are not added to the paste and the size of the lump is smaller than that of blackgram *wari*. The technological data obtained during preparation of blackgram and greengram *wari* from manufacturing unit are presented in Table 1.

Physico-chemical composition of wari : Samples of blackgram and greengram *wari* were directly procured from different production centres in three

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TABLE 1. TECHNOLOGICAL DATA FOR PREPARATION OF BLACKGRAM AND GREENGRAM *WARI*

Particulars	Blackgram *wari*	Greengram *wari*
Moisture in pulse, %	10.0 - 11.0	10.0 - 11.0
Moisture in paste, %	65.0	60.0
Whisking time, min	15	15
Drying ratio	2.5:10	2.3:10
Time taken for sun-drying, days	4 - 6	3 - 4
Unit weight. of *wari*, g	Max 31.0	2.0
	Min 17.0	0.9
	Average 23.0	1.2
Moisture in dried product, %	8.0 - 9.0	8.0 - 9.0
Colour of the finished product	Light grey to Light yellow	Dull yellow to Light yellow

important towns of Uttar Pradesh namely Agra, Allahabad and Lucknow. All samples were powdered separately by hand-operated grinder and analyzed for their physico-chemical attributes as per AOAC methods (AOAC 1984). The results of these data were statistically analyzed for variance to find out the level of significance as per the method given by Steel and Torrie (1980).

Frequency distribution for various chemical parameters of blackgram and greengram *wari* have been computed, based on results of analysis of 24 samples (Table 2). The results were assessed for compliance with proposed standards.

Sorption-isotherm studies : In order to ascertain the influence of moisture on keeping quality of *wari* prepared from blackgram or greengram, equilibrium relative humidity (ERH) studies were carried out at room temperature (32 ± 2°C), using appropriate saturated salt solutions (Rockland 1960). Equilibrium moisture content (EMC) of these samples were determined, when there was no further loss or gain in weight., ERH of the sample was determined by plotting the graph between EMC and RH (Fig 1).

Storage studies : Storage studies of blackgram and greengram *wari* were conducted at room temperature (28 to 40°C and 35 to 87% RH). About 200 g of *wari* were packed in 100, 250 gauge low density polyethylene (LDPE), 120 gauge polypropylene (PP) bags of 25 x 20 cm and plastic jars (PET jars of 500 g capacity) and stored for 8 months. The changes in moisture contents were recorded at regular intervals during storage.

Results and Discussion

A survey in three towns of Uttar Pradesh revealed that a number of unregistered units are engaged in the manufacture of *wari* on cottage scale. The technological data collected from manufacturing units during preparation and drying of blackgram and greengram *wari* are presented in Table 1. The data revealed that the initial moisture contents of split blackgram and greengram ranged from 10-11% and the initial moisture contents of the resultant paste after wet-grinding were 65.0 and 60.0%, respectively. The paste, when subjected to whisking, incorporated air into batter, which not only provided porous texture but also gave a shine to the dried *wari*. Whisking of the batter also resulted in the formation of fine air cells, which got fixed upon drying. The total sun-drying time

TABLE 2. VARIATIONS IN PROXIMATE COMPOSITION OF BLACKGRAM AND GREENGRAM *WARI* MANUFACTURED IN UTTAR PRADESH

Characteristics	Blackgram *wari*			Greengram *wari*		
	Range of variation	Mean ± SE	Proposed standards	Range of variation	Mean ± SE	Proposed standards
Moisture, %	5.0 - 9.6	6.3 ± 1.6	Max. 11.0 (24)	3.7 - 11.9	7.8 ± 2.2	Max. 12.0 (24)
Ether extractives, %	0.2 - 0.4	0.3 ± 0.2	Nil	0.2 - 0.5	0.4 ± 0.2	Nil
Protein, %	13.1 - 21.3	17.3 ± 2.5	Min. 16.0 (16)	14.7 - 21.0	17.4 ± 2.1	Min. 16.0 (16)
Total ash, %	3.4 - 5.5	4.4 ± 0.6	Max. 4.5 (13)	3.0 - 6.8	4.2 ± 0.6	Max. 8.0 (24)
Salt as NaCl, %	Nil	Nil	Nil	0.4 - 2.2	0.7 ± 0.5	Max. 6.0 (24)
Acidity as lactic acid, %	0.6 - 1.9	1.3 ± 0.7	Max. 1.7 (20)	0.4 - 1.2	0.7 ± 0.1	Max. 1.0 (20)
pH	4.4 - 5.0	4.6 ± 2.0	Max. 5. (24)	4.8 - 5.9	4.9 ± 2.3	Nil
Acid insoluable ash, %	0.2 - 0.6	0.4 ± 0.1	Max. 0.4 (16)	0.3 - 1.0	0.6 ± 0.2	Max. 1.0 (24)
Carbohydrates by difference, %	77.5 - 61.3	70.4	-	78.0 - 58.7	69.5	-

Each value is average of two determinations and each parameter had 24 replicates

Figures mentioned in parantheses are number of samples conformed to standard values

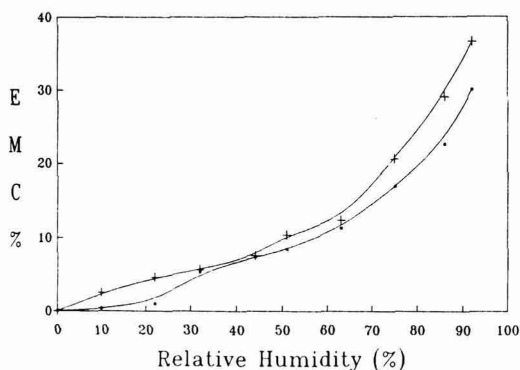

Fig. 1. Sorption curve for *wari*
• Blackgram *wari* ; + Greengram *wari*

required was about 3 to 6 days to attain the moisture level of 8.0 to 9.0% in the dried product. The dehydration ratio was found to be 2.5:1.0 and 2.3:1.0 for blackgram and greengram *wari*, respectively. The average unit weight of the dried blackgram *wari* ranged from 17 to 31 g, when 50–60 g (approx.) of paste was used, while in case of *wari* prepared from greengram, the average unit weight varied between 0.9 and 2.0 g for 5–10 g paste. While the cost of production of blackgram and greengram *wari* was about Rs. 38 and Rs. 32 per kg, the selling rates were Rs. 49 and Rs. 39 per kg, respectively.

Physico-chemical composition of wari : The range and mean values of each chemical parameter of *wari* prepared commercially from blackgram and greengram are presented in Table 2.

Blackgram wari : In freshly prepared commercial samples of blackgram *wari*, the moisture content ranged from 5.0 to 9.6%, ether extractives from 0.2 to 0.4%, protein from 13.1 to 21.3%, total ash from 3.4 to 5.5%, acid insoluble ash from 0.23 to 0.55%, acidity (as lactic acid) from 0.6 to 1.9%, pH of aqueous extracts from 4.4 to 5.0 and carbohydrates by difference from 61.3 to 77.5%.

Greengram wari : In freshly prepared commercial samples of greengram *wari*, the moisture content ranged from 3.7 to 11.9%, ether extractives from 0.2 to 0.5%, protein from 14.7 to 21.0%, total ash from 3.0 to 6.8%, common salt from 0.4 to 2.2%, acid insoluble ash from 0.30 to 0.97%, acidity (as lactic acid) from 0.40 to 1.2%, pH from 4.8 to 5.9 and carbohydrates by difference from 58.7 to 78.0%.

Results have revealed that variation in chemical composition of blackgram and greengram *wari* is significant. Data also indicate that both types of *wari* are good sources of protein, carbohydrates and minerals.

Frequency distribution for various chemical parameters (Table 2) analyzed for 24 samples of *wari* prepared from blackgram pulse revealed that moisture content of all samples ranged between 5.0 and 9.6% and conformed well to the proposed standards (max 11.0%). The total ash contents of only 13 samples out of 24 were within the range of 3.4 and 4.5% with an average of 4.4% and conformed to the proposed standards for *wari*, while the remaining 11 samples were having more than the prescribed limits (max. 4.5%). The variations (0.23 to 0.55%) in acid insoluble ash contents of 16 samples were within the proposed limits (max 0.4%), while the remaining 8 samples showed higher values. The protein contents of 16 samples conformed to the proposed specifications (min. 16.0%), while 8 samples showed lower values. Acidity contents of 20 samples of blackgram *wari* were in the range of 0.6 to 1.7% and conformed well to the standards (max 1.7%), while the remaining 4 samples had slightly higher values. pH of aqueous extract of all the all 24 samples ranged between 4.4 and 5.0 and conformed to the proposed limits (max 5.7).

The analysis of different commercial samples of *wari* made from blackgram in Uttar Pradesh has shown that most of the chemical attributes conform to the proposed standards except for the total ash and acid insoluble ash contents.

The values for various chemical parameters like moisture, total ash and acid insoluble ash contents of all the 24 commerical samples of greengram *wari* prepared in Uttar Pradesh were within the proposed limits. Out of 24 samples analyzed, only 10 samples contained common salt. But, these samples conformed to the proposed limits (max 6.0%), while majority of the commercial samples of *wari* (14 samples) did not contain common salt. Majority of the freshly prepared greengram *wari* samples (16 samples) had protein contents in the range of 16.4 to 21.0% and conformed well to the proposed limits (min 16.0%), while the remaining 8 samples showed slightly lower values. Acidity range of 20 samples of greengram *wari* fell between 0.4 and 0.9% and conformed to the proposed limits, while only 4 samples were having higher values (max 1.0%). It is thus clear that most of the samples conformed to the proposed standards.

It is suggested that manufacturers should thoroughly clean split *dhals* with sufficient quantity

TABLE 3. CHANGES IN MOISTURE CONTENT OF FRESHLY PREPARED BLACKGRAM AND GREENGRAM *WARI* DURING STORAGE AT ROOM TEMPERATURE (28-40°C, RH, 35-87%)

Package gauge	Storage period, months				
	0	2	4	6	8
	Moisture, %				
Blackgram *wari*					
100 LDPE	9.5	8.6	11.3	13.8	ND
250 LDPE	9.5	9.4	9.5	9.8	10.1
120 PP	9.5	9.2	9.6	10.2	10.9
Plastic jars (PET)	9.5	9.4	9.6	9.6	9.9
Greengram *wari*					
100 LDPE	9.6	8.7	11.3	13.4	ND
250 LDPE	9.6	9.2	9.4	10.0	10.8
120 PP	9.6	9.2	9.4	10.6	11.4
Plastic jars (PET)	9.6	9.5	9.7	9.9	10.3

LDPE : Low density polyethylene, PP : Polypropylene,
ND : Not determined. The values are mean of 2 determinations

of water in order to remove the adhering sand and other foreign matters, which may contribute to increase in acid insoluble ash content. It is further suggested that the product may be dried in mechanical cross flow dehydrator, having perforated trays smeared with little quantity of edible oil in order to avoid dust, sand and other foreign matters during sun-drying. Product may be dried in dehydrator, maintaining an initial temperature of 70°C for about 2 h for the quick removal of moisture from *wari* paste. After 2 h, *wari* samples may be turned over in order to accelerate drying. The total drying time could be 8-10 h in order to attain 8-9% moisture content in the final product, with no sand or dust particles (Pruthi et al. 1983).

Sorption behaviour of wari : The sorption behaviour of *wari* prepared from blackgram and greengram is presented in Fig 1. The ERH of blackgram and greengram *wari* were 61.4% and 56.8% at initial moistures of 9.6% and 10.2%, respectively. Both the products remained good with respect to colour, texture (crisp to break by hand) and overall acceptability upto 63% RH, when compared with freshly prepared *wari* sample, but became brittle below 51% RH. When exposed to 75% RH, blackgram and greengram *wari* attained EMC values of 16.9 and 20.6%, respectively and became very soft and unacceptable. Blackgram and greengram *wari* developed mould at moisture levels between 22.6 and 36.6% (RH 86-92%). The samples of *wari* made from blackgram and greengram were acceptable upto EMC of 11.3 and 12.4% respectively.

Storage studies : Data presented in Table 3 revealed that the moisture contents of blackgram and greengram *wari* packed in 100, 250 gauge LDPE and 120 gauge PP bags and plastic jars, decreased upto two months of storage and then increased gradually. This may be due to variation in atmospheric relative humidity (RH), which ranged from 35-87% during the storage period. Greater fluctuations in moisture loss or gain were noticed in 100 gauge LDPE bags as compared to other packaging materials used. The 250 gauge LDPE, 120 gauge PP bags and plastic jars were considered superior to 100 gauge LDPE bags with respect to the extent of loss of moisture from blackgram and greengram *wari* during storage. All packages were free from insect infestation upto 6 months except 100 gauge LDPE bags.

Wari prepared from blackgram and greengram retained the light yellow colour, crisp texture throughout the storage period of 6 months at room temperature, when packed in 250 gauge LDPE bags, 120 gauge PP bags and plastic jars (PET jars). Among the packaging materials, 250 gauge LDPE and 120 gauge PP bags showed no significant changes in the keeping quality of the product. However, 120 gauge PP packs were preferred due to their economy and better transparency.

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Influence of Water Activity Adjustment on Sorption Characteristics, Acceptability and Microbial Stability of *Khoa*

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The influence of water activity adjustment in *khoa* was evaluated in relation to sorption characteristics, product acceptability and microbial stability. Isotherm parameters were determined using G.A.B. equation. The monolayer moisture content increased from 3.292 g water/100 g solids for unblended *khoa* of 0.96 a_w to 9.1561 g water/100 g solids for *khoa*-blend adjusted to 0.866 a_w using 15% sucrose, 4% glycerol and 2% starch. The parameters of texture profile analysis, viz., cohesiveness, springiness, gumminess and chewiness of the above *khoa*-blend decreased during storage as compared to other *khoa*-blends and was found organoleptically acceptable even on the 8th day of un-refrigerated storage. The microbial growth rate constants for total bacterial counts, yeast and mould counts and spore counts decreased with decrease in water activity.

Keywords: *Khoa*, Water activity adjustment, Sorption isotherm, Microbial stability.

The need for reliable sorption data has always arisen in the formulation of shelf-stable food products. Humectants, which reduce the water activity of food without adversely affecting their taste and rheology, greatly improve the marketability of the foods by extending their shelf life. In addition to their ability to bind water, some humectants also exhibit other desirable effects in a food system, as a result of their antimicrobial properties, sweetening capacity and texturizing characteristics (Labuza et al. 1974).

Khoa is a heat-coagulated milk product prepared by partial dehydration of milk. It forms the basis of several other products of significant economic value in the Indian sub-continent. About 2.8 million tonnes of whole milk are converted into *khoa* in India alone (Chatterjee and Acharya 1987). However, due to poor refrigeration, inadequate packaging and post-handling contamination, *khoa* becomes a highly perishable product. The water activity of freshly made *khoa* is 0.96 (Sawhney and Cheryan 1988), which is optimal for growth of most bacteria. Several humectants such as sugars, salts, polyhydric alcohols have been used to reduce the water activity of foods (Karel 1976, Sinskey 1976). It has been established that the water acitivity of *khoa* could be modified by the addition of propylene glycol (Sawhney et al. 1991), sucrose (Sawhney et al. 1992) and glycerol (Sawhney et al. 1994). This study was undertaken to evaluate the influence of water activity adjustment on sorption characteristics, product acceptability and stability of *khoa*.

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Materials and Methods

Preparation of khoa : Buffalo milk obtained from Institute's herd was standardized to 6% fat before preparation of *khoa* in an open steam jacketted pan. The average composition of *khoa* was in % : total solids, 61.7; fat, 23.1; lactose, 20.1; protein, 15.8 and ash, 2.7.

Moisture content in *khoa* was determined by the procedure of De and Ray (1952). Fat content was determined by the procedure based on Mojjonier method of MIF (1959). Protein content was determined by semi-micro Kjeldahl method (Meneffee and Overman 1940).

Preparation of test samples : Four types of humectants most commonly used in intermediate moisture food (IMF) products, viz., surcrose, glycerol, starch and propylene glycol were chosen to adjust water activity in *khoa*. Three combinations of the above humectants (AR grade) were developed to lower the water activity of *khoa* to various values between 0.96 and 0.80. Humectants were added to a pre-determined amount of *khoa* at 80°C and thoroughly blended with a small measured amount of water to make a homogeneous mixture. Different *khoa*-humectant combinations and their initial water activities are shown in Table 1. Pre-sterilized pouches of metallized polyster, each containing 50 g of test sample, were prepared for each of the *khoa*-humectant combination. Pouches were sealed aseptically and stored at 25°C and 70% RH for rheological, microbiological and organoleptic evaluations at different storage intervals. Three replicates of each test were conducted.

TABLE 1. *KHOA*-HUMECTANT COMBINATIONS, INITIAL WATER ACTIVITY AND SET OF PARAMETERS CALCULATED USING G.A.B. EQUATION

Sample	Composition	(% w/w)	Initial water activity	G.A.B. parameters			RMS[b], %
				w^a_m	k	c	
1	*Khoa*	100	0.960	3.2920	0.8784	23.5631	11.85
2	*Khoa*	82.5					
	Sucrose	15.0	0.906	4.0675	1.0029	12.1433	6.45
	Glycerol	2.0					
	Propylene glycol	0.5					
3	*Khoa*	79.0					
	Sucrose	15.0	0.866	9.1561	0.7876	4.4525	5.81
	Glycerol	4.0					
	Starch	2.0					
4	*Khoa*	68.0					
	Sucrose	30.0	0.825	4.5188	1.0368	3.2366	12.55
	Glycerol	2.0					

[a] g water/100 g solids, dry basis
[b] as per equation 2

Measurement of sorption equilibrium : The sorption isotherm of each of the *khoa*-humectant combination listed in Table 1 was measured by static moisture gain/loss from the test samples. The sorption device and procedure as recommended by Wolf et al (1985) and described by Sawhney et al (1992) were used for sorption equilibrium. As most of the samples in each isotherm were expected to desorb moisture, the effect of hysteresis was neglected. The water activity of each test sample was measured by a water activity tester (Rotronic Hygroskope, Zurich, Switzerland) and checked by graphic interpolation method.

Sensory and rheological evaluations : Sensory evaluation was carried out by a panel of 6 trained judges using a 9-point Hedonic scale.

Rheological measurements : These were made using an Instron Universal Testing Machine, fitted with 100 N load cell. Cylindrical samples of *khoa* (dia 1.9 cm, height 2 cm, cross-sectional area 284 mm^2) were compressed at 26°C. The results are expressed per unit cross-sectional area, i.e., per mm^2.

Microbiological analysis : The *khoa* samples were subjected to microbiological analysis for enumeration of total plate counts, spore counts and yeast and mould counts by the methods prescribed in Indian Standards (1968).

Results and Discussion

Sorption isotherms : The water sorption isotherms for different *khoa*-humectant blends showed the typical type-II sigmoidal shape common to most food products (Fig. 1). The equilibrium moisture content increased gradually at lower water activities (upto 0.45) followed by a steep increase above a_w 0.85. The isotherms for the samples adjusted to lower water activities shifted to the left. The effect was very marked at water activities

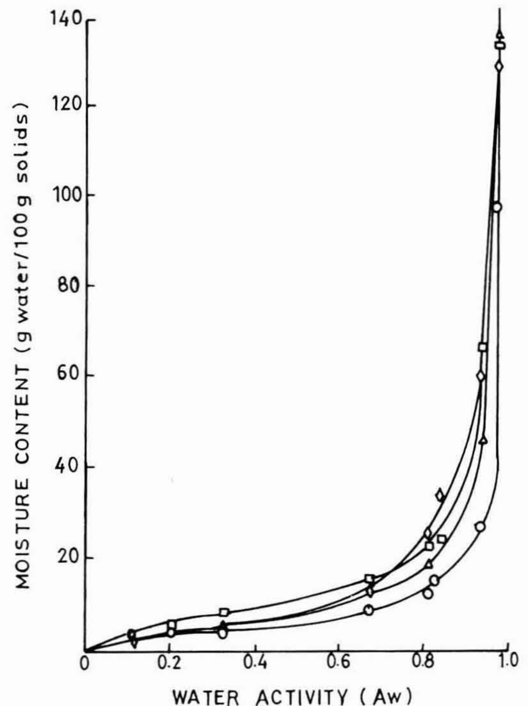

Fig. 1. Moisture sorption isotherms of *khoa* and *khoa*-humectant blends at 25°C : O, Δ, □, ◊ denote samples I II III and IV, respectively

between 0.6 and 0.85. This shift played an important role in the stability of the stored product at a given relative humidity. *Khoa* samples containing 30% sucrose and 2% glycerol showed a different trend below a_w 0.75.

Isotherm parameters : Various equations for fitting water sorption isotherms of foods have been reviewed by Chirife and Iglesias (1978). Sawhney and Cheryan (1988) reported that the Guggenheim-Anderson-deBoer (GAB) equation was the theoretical model giving the best fit for *khoa* in the water activity range of 0.11 to 0.9. Hence, sorption data for *khoa* and *khoa*-humectant blends were expressed mathematically, using GAB equation as described by Bizot (1983).

$$\frac{a_w}{W}=\frac{k(1-C)}{W_mC}a_w^2+\frac{C-2}{W_mC}a_w+\frac{1}{W_mCk} \quad \text{----- (1)}$$

Where, W is the equilibrium moisture content, W_m is the monolayer moisture content, C and GAB are sorption constants and k is the GAB sorption constant related to multilayer properties.

The accuracy of fit was checked by calculating the root mean square percent error (RMS%).

$$RMS\% = \sqrt{1/n\,\Sigma_1^n (w_{exp}-w_{cal})^2} \times 100 \quad \text{------ (2)}$$

Where, W_{exp} are experimental values for moisture contents and W_{cal} are moisture contents calculated using equation (1).

The values of monolayer moisture contents and the GAB constants C and k calculated for different *khoa*-humectant blends are presented in Table 1. Low RMS% values indicate a fairly good fit of data to GAB equation. The monolayer value of moisture content was highest (9.1561 g water/100 g solids) in the samples adjusted to a_w 0.866 by blending the *khoa* with 15% sucrose, 4% glycerol and 2% starch. The monolayer moisture content for this combination was almost three times higher than that of unblended *khoa*. The other *khoa*-humectant combinations showed no remarkable increase in the monolayer moisture contnet compared to unblended *khoa*.

Rheological characteristics : The rheological characteristics of *khoa*-humectant blends were measured in terms of texture profile analysis parameters. It involved two different force measurement (hardness and gumminess), two different energy measurements (adhesiveness and chewiness), one distance measurement (springiness) and one dimensionless ratio (cohesiveness). Table 2 shows the texture profile analysis parameters for different *khoa*-humectant blends. Samples containing 30% sucrose were too pasty for textural studies. Hardness of all humectant-blended *khoa* samples increased during storage, whereas, a decreasing trend was found for adhesiveness. The values of cohesiveness, springiness, gumminess and the chewiness increased during storage in the case of unblended *khoa* and the *khoa* containing 15% sucrose, 2% glycerol and 0.5% propylene glycol. These properties, however, showed a decreasing trend for *khoa* samples blended with 15% sucrose, 4% glycerol and 2% starch (a_w 0.866).

Sensory attributes : Average sensory scores of different *khoa*-humectant blends at different storage periods are presented in Table 3. Addition of the different humectants to *khoa* had almost no effect on the body and texture, flavour, colour and appearance of *khoa* during the initial stages of storage. The average score decreased on or after the 4th day of storage. The flavour scores for unblended *khoa* (a_w 0.96) and *khoa* containing 15% sucrose, 2% glycerol and 0.5% propylene glycol (a_w 0.906) decreased and the samples were unacceptable after 2 days. The flavour score of *khoa* blended with 30% sucrose and 2% glyerol (a_w 0.825) remained acceptable upto 6th day of storage. All the sensory attributes of *khoa* blended with 15% sucrose, 4% glyerol and 2% starch (a_w 0.866) remained unchanged upto the 4th day of storage. Though

TABLE 2. TEXTURE PROFILE ANALYSIS PARAMETERS OF *KHOA* ADJUSTED TO DIFFERENT WATER ACTIVITIES

Testure profile parameters	Storage period, days										
	0.960 A_w			0.906 A_w			0.866 A_w				
	1	2	4	1	2	4	1	2	4	6	8
Hardness, mN	18.93	20.81	20.85	17.49	17.63	19.73	19.47	20.77	20.91	20.94	21.08
Cohesiveness	0.14	0.16	0.16	0.11	0.11	0.13	0.12	0.12	0.11	0.10	0.10
Adhesiveness, mN	1.40	1.20	1.10	2.00	2.01	2.01	3.10	3.10	2.60	2.70	2.70
Springiness, mN	1.60	2.10	2.70	1.20	1.20	1.40	1.10	1.05	1.00	1.00	1.00
Gumminess, mN	2.61	3.32	3.35	1.88	1.92	2.46	2.25	2.23	2.28	2.09	2.10
Chewiness, mN mm	4.17	6.97	9.04	2.25	2.20	3.44	2.47	2.34	2.28	2.09	2.10

TABLE 3. SENSORY SCORES OF *KHOA* ADJUSTED TO DIFFERENT WATER ACTIVITIES

Storage period,	0.960 A_w			0.960 A_w			0.866 A_w			0.825 A_w		
days	a	b	c	a	b	c	a	b	c	a	b	c
1	7.5	8.0	8.3	7.5	8.0	8.0	8.0	8.0	8.0	7.5	7.0	7.3
2	6.0	8.0	8.0	6.0	8.0	8.0	8.0	8.0	8.0	8.0	7.0	7.5
4	2.5	8.0	8.0	4.3	8.0	8.0	8.0	8.0	8.0	8.3	7.3	7.5
6	-	-	-	-	-	-	7.3	7.5	8.0	7.0	7.0	7.3
8	-	-	-	-	-	-	6.5	7.5	8.3	5.5	7.0	8.0

a - Flavour, b - Body and texture, c - Colour and appearance

the average scores of this sample decreased thereafter, the sample still remained acceptable with flavour score at 6.5, body and texture at 7.5 and colour and appearance at 8.3 on the 8th day of storage, which was comparable to that of the unblended *khoa* on the 2nd day of storage.

Microbial growth rate constants : Data on the increase in microbial populations during storage were calculated as per the 1st order reaction kinetics :

$$\ln \frac{N}{N_0} = kt \qquad (3)$$

Where, N is the microbial count at time 't', N_o is the initial microbial count and K is the microbial growth rate constant.

The microbial growth rate constants for total bacterial counts, spore counts and yeast and mould counts for *khoa* adjusted to different water activities are presented in Table 4. Growth rate constants for three types of microbes decreased with decrease in water activity. This decrease was noticeable for yeast and mould counts and total bacterial counts for which the constants decreased from 0.63 at 0.96 a_w to 0.06 at 0.825 a_w and from 1.04 at 0.96 a_w to 0.67 at 0.825 a_w, respectively. The effect of reducing water activity on growth of spores was not very marked.

In order to prevent microbial spoilage, the water activity of food product should be kept below 0.9 and even lower than this, if yeast and mould spoilage is a problem (Erickson 1982). According to Fig. 1, 0.9 a_w of unblended *khoa* corresponds to a moisture content of 20% dry basis, that is, a solid content of more than 82% wet basis. To achieve this in *khoa*, additional heat desiccation may be impractical due to fouling of the heat exchanger. The process would become more energy intensive and would result in deterioration of the sensory qualities of the product. In addition, texture is an important property of *khoa* products. A fairly high moisture content is required to obtain the desired soft and moist texture. *Khoa*-blend adjusted to 0.866 a_w by adding 15% sucrose, 4% glycerol and 2% starch had a monolayer moisture content of 9.16 g/100 g solids, which is significantly higher than that of unblended *khoa*. This indicates that the above humectant combination would help in retaining the desired moisture content in *khoa* to maintain its soft and moist texture during storage.

Textual qualities play an important role in the consumer's response to a product. It is interesting to note that textural properties like cohesiveness, springiness, gumminess and chewiness decreased during storage in *khoa* blended with 15% sucrose, 4% glycerol and 2% starch. Reduced cohesive forces make the product less gummy. Decreased values for gumminess and chewiness indicate that less energy is required to disintegrate and to masticate a food product to a state ready for swallowing. Addition of glycerol also improves the flavour attributes in *khoa* (Sawhney et al. 1994). This is perhaps the reason as to why the above sample remained acceptable in sensory evaluation even after 8 days of storage. Reducing the water activity of *khoa* with the addition of propylene glycol adversely affects the flavour of the product beyond the 0.5% concentration level (Sawhney et al. 1991). The rheological properties of the product are adversely affected, if the *khoa* is adjusted to lower water activity by increasing the sucrose content, as observed for *khoa* blended with 30% sucrose and 2% glycerol (a_w 0.825). Microbiologically, *khoa* is more sensitive to the growth of yeast and moulds.

TABLE 4. MICROBIAL GROWTH RATE CONSTANTS FOR *KHOA* ADJUSTED TO DIFFERENT WATER ACTIVITIES

Type of micro-organism	A_w 0.960	0.906	0.866	0.825
Total bacterial counts	1.04	1.02	0.83	0.67
Spore counts	0.58	0.44	0.42	0.40
Yeast and mould counts	0.63	0.60	0.14	0.06

Khoa adjusted to 0.866 a_w and 0.825 a_w have shown remarkably low growth rate constants for yeast and mould counts, and imparted microbial stability to the product. Based on the above results, it can be concluded that *khoa* samples adjusted to 0.866 a_w, with a humectant combination of sucrose, glycerol and starch is microbiologically stable, rheologically comparable to unblended *khoa* and has an improved flavour to enhance the product acceptability. The stability could further be increased, if efforts are made to use high quality milk with low initial microbial load. Other additives could possibly be used in conjuction with the above humectants, provided the regulatory and safety aspects are satisfied.

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Effect of Spices and Maillard Reaction Products on Rancidity Development in Precooked Refrigerated Meat

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Lipid oxidation in mutton, pork and chicken cooked in the presence of various spices, salt and Maillard reaction products (MRPs), followed by refrigerated storage was studied. Mutton was found to be least susceptible to rancidity development (Warmed over-flavour) compared to chicken and pork. Clove and MRPs exhibited very good antioxidative effects in all the three species, compared to salt and other spices. Studies in a model system, comprising methyl linoleate indicated that clove and MRPs were very effective in arresting the build up of secondary oxidation products, mainly formed during refrigerator storage of cooked meat. They were also found to affect the extent of release of non-haem iron during cooking of meat, which is believed to be the primary catalyst accelerating lipid oxidation.

Keywords: Ranicidity, Cooked and refrigerated meat, Retardation by spices, Maillard reaction products, Non-haem iron.

Lipid oxidation is a major problem in processed meat and meat products. Pre-cooked refrigerated meats are known to develop rancidity, commonly known as Warmed over-flavour (WOF), which becomes perceptible within 24-48 h of storage at 5°C (Igene and Pearson 1979; Gray and Pearson 1987). WOF has been reported to be caused by a catalytic mechanism involving some form of iron (Eriksson 1982). Cured meats are found to be less susceptible to this defect due to the strong antioxidative effect of nitrite (Morrissey and Tichivangana 1985).

In India, mutton and chicken are the more preferred meats than pork and beef. Generally, uncured meats are used for domestic and institutional catering. High temperature cooking such as frying, grilling etc., is reported to be beneficial to minimise WOF (Huang and Greene 1978; Vasundhara and Honikel 1992). This study was undertaken(i) to evaluate the antioxidative role of commonly used spices such as clove, cinnamon, cardamom and salt and also of Maillard reaction products (MRPs) formed from sugar-amino acid interactions, both in cooked and refrigerator stored (5°C) mutton, chicken and pork, (ii) to study the degradation of methyl linoleate, a primary substrate for lipid oxidation and (iii) to examine the changes in total iron and non-haem iron during cooking and refrigerator storage of mutton in the presence of additives.

Materials and Methods

Chemicals and equipments: Dextrose, solvents and chemicals were of AR grade procured from local suppliers. Methyl linoleate, tetra ethoxy propane (TEP) and glycine were from Sigma Chemical Co., USA. All the optical density measurements were done, using Bausch and Lomb spectronic 20 (USA).

Methyl linoleate degradation was determined in GC (Chromatography Instruments Co., Baroda) fitted with a 10 x 1/16" SS column packed with 'Famex' operated at 200°C with a N_2 flow of 45 ml/min and H_2 flow of 30 ml/min, using flame ionization detector (FID). The injector and detector temperatures were maintained at 240°C.

Sample preparation : Fresh mutton, chicken and pork (leg portions) were procured from a local butcher shop (4-5 h post-mortem) deboned and cut into 1 cm cubes. Meat samples (50g) were separately transferred to polypropylene (PP) pouches (10" x 6") containing 10 ml water. Freshly powdered spices such as clove (250 mg %-T_1), cinnamon (520 mg %-T_2), cardamom (650 mg %-T_3) and salt (2 g %-T_5) were added individually to different pouches and the levels of additives were as per those prescribed for meat curry (ASC specifications 115B, 1972).

In addition, one set of sample contained only meat pieces and no added water, to which preformed MRPs present in 10 ml water were added (T_4). MRPs were prepared by refluxing 40 mg glucose and 25 mg glycine in 10 ml water for 2 h on a sand bath (100-110°C). Losses in water were periodically restored and the final volume was maintained at 10 ml. Changes in pH and non-enzymatic browning were monitored before and after heating in order to maintain the same reaction conditions for MRP formation. In this manner, 2 sets with 5 different treatments (all in multiples of 4), in 3 different species of meat were cooked in boiling water bath for 35 min and cooled to room temperature. Of these samples, one set (duplicate) for each type of treatment was immediately taken up for analysis (set-A), while the other set containing the remaining

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two samples for each treatment was stored at 5°C for 48 h prior to analysis (set-B). Three such batches were prepared and analysed.

Thiobarbituric acid reactive substances (TBARS) in cooked and stored meat: TBARS were estimated using 25 g ground meat sample from set A and set B (Tarladgis et al. 1960). Samples were separately taken in round bottom flasks and pH was adjusted to 1.5 with 4 N HCl and made up to 100 ml with distilled water. Samples were steam-distilled and 50 ml distillate was collected. Distillate (25 ml) was used for colour development along with different aliquots of tetra ethoxy propane standards. Optical density was measured at 538 nm and TBARS values were calculated and expressed as mg/kg sample.

Effect of additives on methyl linoleate degradation (model system): Chloroform solution (10 ml) containing a weighed amount (around 10 mg) methyl linoleate was mixed with 5 g microcrystalline cellulose in a 100 ml capacity RB flask. Chloroform was removed by gentle evaporation under vacuum. Water (10 ml) was added to each flask except for the one containing MRP, since MRPs were already present in 10 ml water. The contents were thoroughly mixed, followed by the addition of 50 mg clove, 100 mg cinnamon, 125 mg cardamom and 500 mg salt separately for each. The flasks were heated on a boiling water bath for 35 min and cooled. For each additive treatment, 4 samples were prepared, out of which two were immediately used for analysis (after heating and cooling) and other two were stored at 5°C for 48 h and analyzed.

Oxidative degradation in methyl linoleate in the above model system was monitored by measuring TBARS. Samples containing methyl linoleate and other additives were subjected to steam distillation and TBARS were measured using 25 ml distillate and expressed as µg/g sample.

Measurement of undegraded methyl linoleate was monitored by GLC. The samples immediately after heating and also after storage were extracted with a total volume of 25 ml chloroform repeatedly and chloroform layer, containing methyl linoleate along with its degradation products were pooled, dried and concentrated to remove the solvent. The residue was then redissolved in 1 ml chloroform and 0.5 µl was used for GLC analysis in which the intact methyl linoleate peak alone (identified from its Rt) was quantified and percentage loss was calculated.

Changes in total iron and non-haem iron in pre-cooked mutton immediately after cooking and after storage: In one set of experiments, mutton was cooked with spices, MRP and salt as described earlier. The samples immediately after heating and after storage (5°C/48 h) were blended. Sample (2x1g in duplicate) was taken for iron estimation. For total iron, the sample was converted into ash in a muffle furnace (450-500°C) and the residue was carefully dissolved in 1 ml concentrated HCl and treated with hydroxylamine hydrochloride and 1,10 - phenanthroline reagent (Edward 1979) to develop pink colour due to metal complexation and OD was read at 510 nm. A standard graph was prepared using known quantity of ferrous sulphate solution in the same way. Total iron and non-haem iron were calculated and expressed as mg per 100g sample.

Results and Discussion

Data on the changes in TBARS (measure of lipid oxidation) in mutton, chicken and pork, cooked in the presence of various spices, MRP and salt, both immediately after cooking and also after 2 days storage at 5°C are shown in Table 1. Among the 3 species, mutton exhibited the lowest susceptibility to oxidation, as indicated by the TBARS value immediately after cooking. Addition of spices and MRP reduced the oxidation during cooking to nearly 60-70% in the case of pork and chicken compared to control, while in the case of mutton, there was no measurable TBARS immediately after cooking, thereby indicating the

TABLE 1. CHANGES IN TBARS (mg/kg SAMPLE) IN DIFFERENT MEAT SPECIES UPON COOKING IN THE PRESENCE OF VARIOUS ADDITIVES ON STORAGE AT 5°C FOR 48 HOURS

Sample	Mutton		Chicken		Pork	
	Cooked	Cooked and stored	Cooked	Cooked and stored	Cooked	Cooked and stored
Control	198.0 ± 50.9	1461.0 ± 163.20	703.4 ± 98.2	3226 ± 706.0	704.32 ± 32.1	3016 ± 50.0
With clove	nil	198.0 ± 70.16	181.7 ± 35.0	499.8 ± 72.0	295.3 ± 32.1	295 ± 30.1
With cinnamon	nil	284.0 ± 66.40	340.8 ± 32.1	1363.2 ± 128.5	386.2 ± 32.0	727 ± 25.7
With cardamom	nil	284 ± 60.42	363.5 ± 10.0	1408 ± 64.6	385 ± 3.0	795 ± 25.7
With MRP	nil	75.8 ± 32.60	295.26 ± 32.1	568 ± 160.6	340.8 ± 160.0	454 ± 192.0
With salt	198.8 ± 15.1	686.5 ± 183.00	545.28 ± 50.0	3044 ± 110.0	408 ± 40.1	4276 ± 115.0

retardatory effect of spices and MRP. Salt was not effective in all the 3 species, although the TBARS values in these samples were less than those in control. Upon storage of cooked meat at 5°C, in all the species of meat, there was nearly 6-7 fold increase in TBARS value for control, followed by salt-treated samples. Among the different spices, clove was the most effective in reducing oxidation during storage in all the 3 species. In clove-treated mutton and chicken, there was nearly 2 - fold increase compared to initial values, while in pork, there was no detectable change. Al-Talay et al (1987) have also reported that clove has the highest antioxidative potential among the spices. Effect of MRP on lipid oxidation was nearly comparable to that shown by clove. But, it had an additional advantage in that it did not impart any characteristic smell to meat as done by spices including clove. Wide variations were seen in TBARS values from batch to batch, as indicated by high standard deviations from sample to sample. Also, the differences in rancid odours with respect to immediately after cooking and after storage and between different additive treatments were clearly indicated. Sensory analysis on a 5-point Hedonic scale (1- most rancid, 2- moderately rancid, 3- little rancid, 4- no rancidity, acceptable, 5- nil, no rancidity, most acceptable) also indicated that there was very little rancid smell in MRP-treated sample (sensory score 4) compared to control (score 2). In spice-treated samples, dominant flavour note characteristic of each spice was easily detectable. Still rancid note could not be totally masked in some cases e.g., in cinnamon and cardamom-treated samples, which scored sensory scores between 2-3. This was more pronounced in chicken. The MRPs used in the present study were early reaction products, as indicated by their browning index and pH and were found to exhibit desirable antioxidative effects, comparable to some of the potential spices such as clove. Though TBA measurement is indicative of rancidity development, it cannot always be related with WOF intensities, since WOF is dependent on various factors such as heating temperature, duration and presence of additives.

Fig. 1 shows the antioxidative potentials of spices, MRP and salt on methyl linoleate degradation in model system. Methyl linoleate concentration selected in this study was based on the normal level of occurrence in mutton. The level of addition of spices and salt was selected after several trials. Immediately upon heating, slight differences were observed in different treatments with respect to loss of methyl linoleate compared to control and it varied from 11–24%. But on storage, the loss of methyl linoleate in control increased to 4.5 times and followed by salt and cardamom-treated samples. Clove, cinnamon and MRP-treated samples exhibited smaller losses compared to control. In GLC analysis,

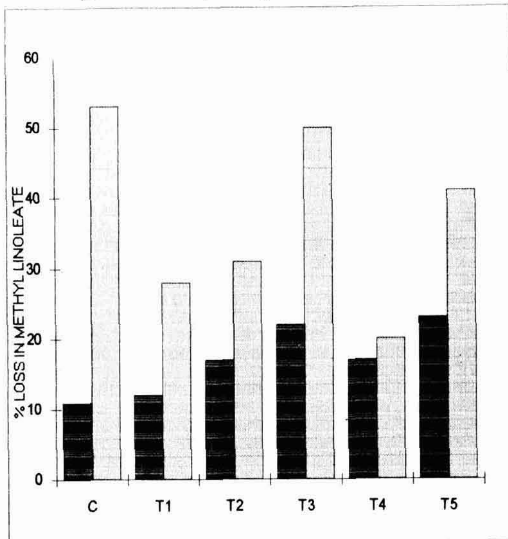

Fig. 1. Loss of methyl linoleate (by GLC) when heated in the presence of additives.
C - Control; T_1 - with clove;
T_2 - with cinnamon; T_3 - with cardamon;
T_4 - with MRP; T_5 - with salt
Immediately after heating,
Heated and stored at 5°C for 48 h

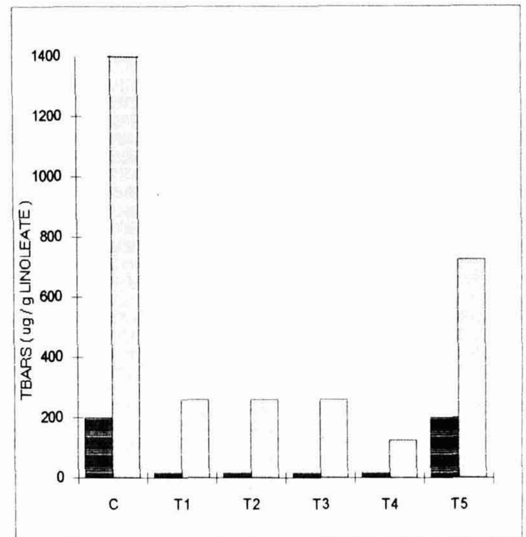

Fig. 2. Oxidative changes in methyl linoleate (as TBARS values) when heated in the presence of additives
Legends for Figures are as in Fig 1

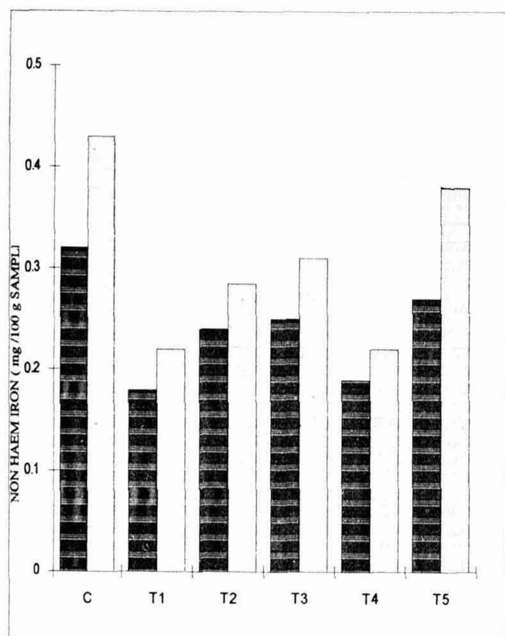

Fig. 3. Changes in non-haem iron in cooked and stored mutton Legends for Figures are as in Fig 1

in addition to the undegraded methyl linoleate peak, there were a few additional peaks probably corresponding to oxidation products and other chloroform-soluble volatile compounds from spices. The latter were not quantified. The retardatory effect was more pronounced with respect to oxidative breakdown of methyl linoleate in heated and stored samples (Fig 2). Immediately on heating, except in control and salt-treated sample, there was no measurable oxidative degradation (TBARS value) in spices and MRP-treated samples. On storage, the increase in TBARS was found to be nearly 6 times in control and 3.5 times in salt-treated sample, while in spice and MRP-treated samples, TBARS value was comparatively very small. Smallest increase was noticed in MRP-treated sample. These results clearly indicate that spices and MRP do retard the formation of secondary oxidation products formed mainly during storage at low temperature. Methyl linoleate is reported to be the primary substrate for oxidation in meat and hence its degradation was followed to monitor the onset of rancidity in pre-cooked meat.

Non-haem iron is reported to be the primary catalyst causing oxidation. Changes in non-heam iron in mutton, cooked in the presence of spices, salt and MRP and stored at 5°C is shown in Fig 3. Immediately after cooking, the total iron in control and salt-treated sample was comparatively higher than that in spice and MRP-treated samples. During storage, there was a further increase in iron in all the samples with treated ones showing lower degree of increase than control. Similarly, non-haem iron content, which is believed to be the primary catalyst, showed differences with regard to treated samples and control. In the presence of spices and MRP, the extent of release of non-haem iron was reduced by 30-40% immediately after cooking and also after storage. MRP and clove-treated samples showed the lowest levels of non-haem iron. These results indicate that both clove and MRP are effective in reducing TBARS in all the 3 species of meat and also in methyl linoleate model system and influence the release of non-haem iron.

Interaction studies on iron with spices and MRP are needed to determine their role and mechanism by which they affect lipid oxidation. It can be concluded that spices such as clove and MRP prepared from glucose-glycine interaction may be beneficially used to minimise the rapid onset of rancidity (WOF) in pre-cooked refrigerated meat by retarding the release of non-haem iron.

Acknowledgement

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Nutritional and Toxicological Evaluation of Wild Apricot Pomace

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Potential toxicity and nutritive value of wild apricot pomace (WAP) was evaluated in a 4 week feeding study in rats. WAP is a rich source of protein and contains major essential and non-essential amino acids. It also contains low levels of the cyanogenic glycoside-amygdalin. Rats fed 30% WAP as dry feed with 4 or 15% casein showed growth inhibition, as evidenced by poor weight gain and food efficiency ratio, whereas wetting of the meal with water ameliorated these effects. The results of this study revealed that WAP is a rich source of good quality protein and any toxin present in it, e.g., amygdalin, could be detoxified by wetting with water. There were also no histological manifestations in the organs examined, attributable solely to the feeding of WAP in rats.

Keywords: Wild apricot pomace, Cyanogenic glycoside, Amygdalin, Detoxification, *Prunus armeniaca*, Proximate composition.

The shortage of edible proteins and oils in India has stimulated studies on tree-borne oilseeds. *Prunus armeniaca*, Family: Rosaceae, includes many varieties of cultivated and wild apricots. Wild apricot locally called *chullu*, is found in the hilly regions of States of Himachal Pradesh, Kashmir and Uttar Pradesh in India. It is a tree of moderate size, about 10 meters tall (Wealth of India 1969). The fruit of wild apricot has a round shape and is about 5 cm in diameter. It is unpalatable and generally not consumed, because it contains high amounts of acids and low amounts of sugars. Wild apricot is utilized by the tribal people for the preparation of a distilled alcoholic liquor and the oil is used for cooking and burning. The seeds yield about 27% of kernels. Potential availability of the kernels in India is about 40,000 tonnes and the kernel yields about 47% of oil. The kernels are bitter due to the presence of a cyanogenic glycoside amygdalin (Montgomery 1969). The nutritional quality of the protein of the wild apricot pomace (WAP) and toxicity were evaluated in a 4 week feeding study in rats after determining its chemical composition. The prospect of detoxifying the pomace, by addition of water, was also looked into, as the cyanogenic glycosides are hydrolyzed in the presence of endogenous enzymes. WAP was incorporated in the diets of rats at 30% level, containing either 4 or 15% casein, and fed either as a dry or wet food.

WAP analysis : Wild apricot seeds, obtained from National Oilseeds and Vegetable Oils Development Board, Ministry of Agriculture, Government of India, Gurgaon, were decorticated. The kernels were powdered and extracted in Soxhlet apparaus for 30 h with hexane. The defatted powder was dried and the cyanogenic glycoside was estimated by titrimetric method as described in AOAC (1984). The amino acid composition of the defatted pomace was determined on the LKB 4151-Alpha plus amino acid analyzer (LKB Biochrom, Cambridge, UK), after acid hydrolysis of the sample (6N HCl, 20 h at 110°C), as per the basic procedure of Moore and Stein (1954).

Feeding study in rats : Thirty six rats of Haffkine Wistar strain were distributed in 6 groups (1-6), each consisting of 6 animals (3 males and 3 females). Group 1, serving as control, was fed a purified diet containing 15% casein, 4% salt mixture, 6% groundnut oil, 6% cellulose and the rest comprising starch and vitamins. Of the remaining 5 groups, experimental groups 2 and 3 were given diets containing 15% casein with 30% WAP, as dry powder or mixed with water (80% by weight of the diet), respectively.

To assess the potential of WAP as a protein source, rats of two groups were fed diets containing 4% casein and 30% WAP as the dry powder (group 5) or mixed with water (80% by weight of the diet) (group 6). As a control for protein deficiency, rats of group 4 were fed a purified diet containing only 4% casein. The incorporation of WAP at 30% level in the purified diets was achieved by replacement of cellulose and part replacement of starch. All the diets contained 4% salt mixture and 6% groundnut oil. The levels of protein in the diets were 12, 24, 24,3.2,15.2 and 15.2% for groups 1,2,3,4,5 and 6, respectively. The animals were housed individually under controlled environment (temperature 23±3°C)

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humidity 40-70% and light/dark cycle 12 h) and received food and water *ad libitum*. Food consumption and body weights of the animals were recorded daily.

Urine was collected from all the rats for qualitative analysis of albumin and reducing sugars. At the end of 4 weeks of study, blood was collected from all the rats under ether anaesthesia. Blood haemoglobin and cell counts were carried out by standard methods (Hunter and Bomford 1956). Serum albumin, total proteins, cholesterol, transaminases, glucose, urea and triglycerides were estimated by RA 50 autoanalyser (Miles India) using reagents (Miles India). The quantitative data were analyzed using Student's *t*-test. Selected organs (liver, kidney, heart, spleen and testes) were weighed and these organs along with stomach, jejunum, thyroid, adrenals, urinary bladder and lungs were taken for histological examination after a detailed necropsy. Tissues were processed using conventional histological methods.

Proximate composition and amino acid analysis: WAP consisted of (%): moisture 12.1, oil 0.50, proteins 40.6, ash 4.36, crude fibre 12.46, HCN 0.03 and carbohydrates by difference 29.95. The presence of amygdalin, the cyanogenic glycoside, in the pomace makes it bitter, toxic and unpalatable. The amino acid composition of the pomace (Mmoles/100g) is as follows:- aspartic acid 23.7, theronine 8.9, serine 19.1, glutamic acid 73.4, glycine 21.6, alanine 15.6, valine 11.3, arginine 35.8, cystine 0.8, methionine 2, isoleucine 7.4, leucine 20.9, tyrosine 7.3, phenylalanine 6.1, lysine 6.8 and histidine 5.4. Methionine and lysine contents are more in WAP compared to those in the cultivable variety of apricot meal (Kamel and Kakuda 1992).

Body weight and food intake : All the animals survived the experimental period. The control

TABLE 1. MEAN WEIGHT GAINS, BLOOD ANALYSES AND ORGAN WEIGHTS OF RATS (±SEM)

Groups	1	2	3	4	5	6
Body wt., wt. gain, Food consumed and FER[a]						
Initial weight, g	42.2 ± 4.5	42.3 ± 5.0	42.8 ± 5.7	42.3 ± 6.0	42.0 ± 5.8	42.3 ± 5.7
Final weight, g	131.3 ± 4.5	79.7 ± 3.5[b]	148.2 ± 10.0	45.8 ± 6.9[b]	71.7 ± 4.4[b]	131.1 ± 11.0
Weight gain, g	89.1 ± 5.5	37.4 ± 2.2[b]	105.4 ± 6.8	3.5 ± 1.8[b]	29.7 ± 2.8[b]	88.8 ± 5.8
Food consumed, g	276 ± 6.0	184 ± 6.5b	268 ± 8.6	140 ± 6.9[b]	161 ± 4.1b	268 ± 10.0
FER[a]	0.323 ± 0.2	0.203 ± 0.01[b]	0.393 ± 0.02[d]	0.025 ± 0.01[b]	0.184 ± 0.01[b]	0.330 ± 0.11
Haematology and Biochemistry						
Haemoglobin, g/dl	14.5 ± 0.20	14.4 ± 0.30	14.9 ± 0.10	13.0 ± 0.50[d]	13.9 ± 0.20	14.4 ± 0.20
PCV	47.6 ± 0.20	46.0 ± 1.20	48.0 ± 1.40	42.7 ± 2.90[d]	44.0 ± 1.40	48.5 ± 1.40
RBC, millions/mm^3	5.84 ± 0.10	5.71 ± 0.12	6.14 ± 0.10	5.12 ± 0.66	5.95 ± 0.24	5.93 ± 0.31
WBC, mm^3	4730 ± 246	5250 ± 304	4916 ± 192	4566 ± 766	5025 ± 161	5262 ± 130
Albumin, g/dl	3.9 ± 0.12	3.73 ± 0.12[c]	3.73 ± 0.03	2.67 ± 0.12[b]	3.62 ± 0.17	3.55 ± 0.08
Protein, g/dl	7.0 ± 0.10	6.5 ± 0.10[c]	7.2 ± 0.30	5.0 ± 0.10[b]	5.9 ± 0.60	6.9 ± 0.20
Glucose, mg/dl	74 ± 10.0	65 ± 10.0	73 ± 10.0	58 ± 13.0	74 ± 26.0	73 ± 7.00
Urea, mg/dl	16.3 ± 1.30	26.0 ± 0.90[b]	27.0 ± 0.80[b]	16.7 ± 4.20	22.0 ± 3.30[d]	15.9 ± 1.40
Cholesterol, mg/dl	80.2 ± 4.50	56.9 ± 1.60[b]	67.3 ± 1.30	59.1 ± 3.10[b]	79.2 ± 4.40	76.7 ± 5.90
SGOT, units/l	170 ± 21.0	160 ± 10.0	169 ± 26.0	253 ± 19.0[d]	218 ± 8.00	198 ± 18.0
SGPT, units/l	35 ± 4.00	36 ± 4.00	34 ± 3.00	38 ± 2.00	41 ± 2.00	39 ± 2.00
Alkaline phosphate, units/l	575 ± 45.0	640 ± 17.0	444 ± 65.0	493 ± 40.0	514 ± 101.0	538 ± 68.0
Organ weights g/100g body weight						
Liver	4.33 ± 0.06	4.74 ± 0.19	4.66 ± 0.16	4.45 ± 0.24	4.88 ± 0.17[c]	4.17 ± 0.04
Kidney	0.84 ± 0.03	0.94 ± 0.06	0.83 ± 0.04	0.79 ± 0.08[c]	0.97 ± 0.03[c]	0.77 ± 0.03
Heart	0.41 ± 0.02	0.43 ± 0.03	0.38 ± 0.02	0.56 ± 0.04[c]	0.46 ± 0.03	0.38 ± 0.02
Spleen	0.29 ± 0.02	0.30 ± 0.02	0.27 ± 0.03	0.23 ± 0.03	0.34 ± 0.02[d]	0.24 ± 0.02
Testes	1.17 ± 0.07	1.03 ± 0.13	0.90 ± 0.09	0.67 ± 0.13[d]	1.03 ± 0.10	1.01 ± 0.04

[a] Weight increase per g of food consumed
[b] Significantly different from group 1 $p < 0.001$
[c] Significantly different from group 1 $p < 0.02$
[d] Significantly different from group 1 $p < 0.05$

Group 1 = Control – 15% casein dry food
Group 2 = WAP 30% + 15% casein dry food
Group 3 = WAP 30% + 15% casein wet food
Group 4 = Control - 4 % casein dry food
Group 5= WAP 30% + 4% casein dry food
Group 6 = WAP 30% + 4% casein wet food

animals fed 4% casein diet (group 4), appeared weak and showed poor hair growth. The animals fed 30% WAP either with 4% (group 5) or 15% casein (group 2) as dry food and animals fed control diet with 4% casein (group 4) showed significantly lower body weight, weight gain, food consumption and food efficiency ratio (FER) compared to those fed control diet with 15% casein (group 1) (Table 1). The reduced food intake in the above experimental groups may be due to the unpalatable nature of the pomace in dry form. The animals fed WAP as wet feed with 15% casein (group 3) showed significantly higher FER compared to that of the control group 1. Thus, WAP fed as dry food in diets, was growth inhibitory even in the presence of 15% casein and offered poor FER, whereas the rats fed WAP as wet food in diet containing 4% casein (group 6) showed normal growth compared to that of control (group 1). These findings indicate that the protein in WAP is of good quality, comparable to casein and can sustain growth. The kernel powder also seems to be adequately detoxified by wetting with water, as the animals fed WAP as a wet diet containing 15% casein (group 3) showed significantly higher FER compared to that of control (group 1). The rats fed 4% casein (group 4) showed poor weight gain and FER, as expected, and the incorporation of WAP in the diet and fed in the wet form (group 6), ameliorated these deficiencies.

Haematology and clinical pathology : The haemoglobin content and packed cell volume (PCV) of blood samples of the animals (Table 1) fed control diet with 4% casein (group 4) were significantly less as compared to those of control (group 1). There were no other significant differences in the haemoglobin, RBC, WBC and PCV between the groups fed WAP diets and control diet. There were no significant differences in the mean values of blood constituents between the control (group 1) and the two groups fed WAP in diets as wet food (groups 3 and 6), except for increases (within normal limits) in blood urea levels of animals fed WAP in diet containing 15% casein (group 3) (Table 1). The animals fed WAP as dry food with 15% casein (group 2) showed significantly lower serum protein, cholesterol and higher (within normal limits) urea levels. The animals fed control diet with 4% casein (group 4), showed significantly lower serum albumin, protein, cholesterol and significantly higher SGOT levels, which could be due to the low dietary protein content. The animals fed WAP pomace in diet with 4% casein as dry feed (group 5), showed higher (within normal limits) serum urea contents as compared to those fed control diet with 15% casein (group 1). Albumin and reducing sugars were not present in urine samples.

Organ weights : The relative weights of heart of rats in the group fed control diet with 4% casein (group 4) and liver, kidney and spleen weights in the group fed 30% WAP with 4% casein diet as dry food (group 5) were significantly higher compared to those fed control diet (group 1) (Table 1). Weights of kidney and testes of the animals fed diet with 4% casein (group 4) were significantly lower as compared to control (group 1). Weights of liver, kidney, spleen, testes and heart of rats fed WAP in the wet form with 4 or 15% casein (groups 3 and 6) showed no significant differences as compared to control (group 1).

Histopathology : The control group-1 fed 15% casein showed slight disorganization of the cord, occasional intracellular vacuolation in the liver, slight shrinkage of glomerular tuft of the kidney and focal consolidation of the alveolar tissue in one or two animals. The control group 4, fed 4% casein, showed the above features in the livers of all the animals, with higher intensity along with obliteration of the sinusoides. Atrophy of seminiferous tubules with loss of spermatozoa in 2 males, and erosion of the tips of intestinal villi in 1 female, were also observed. These findings are similar to those reported previously (Gandhi et al.1994).

The experimental group 2, (30% WAP with 15% casein in the dry form), besides showing most of the manifestations observed in the control group 1, also showed general nuclear pyknosis, moderate obliteration of sinusoides, increase in liver kupffer cells, presence of megalocytes and loosening of white pulp in the spleen and focal atrophy of seminiferous tubules with lack of spermatozoa in few animals. In group 3, fed 30% WAP with 15% casein in the wet form, the histological manifestations were similar to those of the control group1. However, deposition of haemosiderin pigments was noticed in the spleen of 1 female rat.

Feeding of 30% WAP with 4% casein in the dry form (group 5), produced similar histological changes as seen in the 4% casein control (group 4) with no increase in either intensity or incidence. In rats fed 4% casein and 30% WAP as wet feed (group 6), the histological manifestations were milder than those observed in group 4 and group 5. Thus, feeding of WAP neither brought about any characteristic histopathological lesion nor

aggaravated the adverse effect due to consumption of low protein diet. In fact, it ameliorated the effects of protein deficiency to some degree, which was further corroborated by the weight gain and high FER observed in the groups 3 and 6. Amygdalin present in the pomace is hydrolyzed after the addition of water into gentiobiose, HCN and benzaldehyde in the presence of enzymes β-glucosidase and oxynitrilase (Concon 1988). HCN, escapes in the atmosphere leaving the material palatable and non-toxic. After an initial 2 h of soaking with water, the material does not show any HCN in a qualitative colour reaction (AOAC 1984). The benzaldehyde has a tendency to oxidise to benzoic acid, which is considered to be safe. In the present study, HCN released endogenously in the body, subsequent to ingestion of WAP in the dry form (groups 2 and 5), was not found to be lethal to rats. The reported lethal doses of HCN in humans range from 0.5 to 3.5 mg/kg body weight (Concon 1988). Thus, from the present study, it may be inferred that WAP is a rich source of protein with all the essential amino acids and can sustain growth. The cyanogenic glycoside can be detoxified by wetting the meal and such a meal has immense potential as a source of protein, both in human food and animal feed. However, plants storing cyanogenic glycosides (e.g. *Prunus serotina*), have been reported to induce deformities and abortions in domestic animals (Keeler 1983). Therefore, a long term feeding study with WAP is warranted to rule out doubts in terms of any reproductive toxicity, teratogenicity or carcinogenicity potential, before it can recommended for use as a food/feed ingredient.

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Effect of Rigor State, Curing and Tumbling on Physico-chemical and Organoleptic Properties of Goat Ham

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Twelve live African Dwarf goats with average weight of 20 kg were slaughtered, dressed, and the bone-in and boneless hams from these carcasses were obtained to study the effects of rigor state, curing (dry-cure and immersion-cure) and tumbling methods (intermittent and continuous) on the composition and organoleptic properties. Regardless of processing methods, pre-rigor meat had less ($P<0.05$) protein and ash and more moisture contents than post-rigor counterpart. An inverse trend was observed with the cooking losses. The dry-cured hams exhibited lower pH and WHC, as compared to the immersed and tumbled ones. The products processed from tumbled meat were juicier, more tender, pink and produced better products than their equivalent non-tumbled counterparts. The methods of tumbling had no significant ($P>0.05$) effect on the sensory parameters of the products.

Keywords : Rigor, Curing, Tumbling, Goat, Ham, Organoleptic properties.

Heat processing has numerous advantages in relation to the quality of the final products (Taylor et al. 1982). The addition of salt to meat facilitates the extraction of myofibrillar protein and thus increases the binding properties of meat (Paterson et al. 1988). The ability to retain more moisture is superior for hot-boned meat in the presence of salt (Lamkey et al. 1986).

A recent development in meat curing is mechanical tumbling (Cassidy et al. 1978). It results in the extraction of protein exudates, which act as binder during thermal processing. By destroying the muscle cells, tumbling facilitates the diffusion and the dispersion of curing ingredients into the meat and consequently results in a more uniform colour formation (Percel et al. 1982). Maesso et al (1970) studied the effect of some physical treatments on quality of poultry loaves and observed that mechanical beating of chicken meat for 3 min caused significant increase in its binding properties.

Most of the work on heat processing, curing and tumbling has been carried out with pork shoulders and hams (Chow et al. 1986; Van Laack et al. 1989). Although, numerically goats are important in the semi-arid zone of Africa (Wilson 1984), little information is available on the use of goat meat in processed meat products.

The purpose of this study was to evaluate the effects of curing and tumbling on quality of goat hams as influenced by the state of rigor.

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Meat obtained from 12 African Dwarf goats, average weight 20 kg and raised in the same environment was used in the present study. Four animals were slaughtered at a time. The meat from each of the eight legs from these animals were allocated at random to each of the sub-treatments. Tumbled legs were hand-deboned before curing by continuous and intermittent method, while dry-cured and immersion-cured legs were not deboned. Heat processed meat was cured within 2 h following the exsanguination, while the meat to be processed cold was held for 24 h in a cooler at 2°C before any further operation.

Dry-cure : A dry curing mixture containing nitrite salt (95%), dextrose (2.5%) and phosphate (2.5%) was rubbed uniformly over the surface of the meat at the rate of 80 g per kg. The ham was then kept in a cooler at 25°C for 2 days in a perforated plastic bucket. After curing, the meat was rinsed of the excess salt with tap water and held for an additional 2 days in the same cooler for salt equalization.

Immersion-cure : The hams were injected with curing brine containing 10 g nitrite salt (9.94 g NaCl and 0.06 g $NaNO_2$), 2.5 g dextrose and 2.5 g phosphate (commercial polyphosphate, 565 g P_2O_5/kg) in 100 g solution. The meat was injected to 115% of their green weight, using a two-needle injector pump (Gudin type 2 model, France). After injection, they were held in a cover pickle (same concentration as the injected brine) for two days in the same cooler for salt equalization.

Tumbling : The boneless and trimmed goat hams were injection-pumped to 115% by weight

with a solution (same composition as the brine used for the immersion of cured hams). The injected meal was held in a cover pickle (same concentration as the injected brine) for 2 days, then taken out and held for another two days in a coller at 2°C. The meat was tumbled either continuously for 4 h or intermittently for 6 h (40 min on, 20 min off). Tumbling was accomplished with a tumbler (CMIA-Bodson model, France) operated at 28 rpm.

Following the curing treatments, the dry-cured and immersion-cured hams were cooked directly, while the tumbled legs were put into plastic lined metal moulds before cooking. Cooking was performed in a water bath at 85°C to an internal temperature of 72°C using an electric cooker (Corpo-160 model, France). After cooking, the hams were showered with tap water for 10 min. The cooled and tumbled hams were then removed from the moulds. All the hams were individually wrapped in cellophane and stored in a 2°C cooler until subsequent analyses.

Chemical and physical analysis : Sections of *Quadriceps femoris* muscle were removed from each non-tumbled sample just prior to and after cooking and cooling. For the tumbled hams and due to the disruption effect of tumbling, sections of meat were taken from each ham of each treatment irrespective of muscle position. The meat was then chopped in a kitchen grinder (Moulinex, France) and used for analyses. Moisture, fat, protein, ash, salt and residual nitrite contents were determined (AOAC 1980), pH was determined using pH meter (Jenway 3220 model, Bioblock France). Water holding capacity (WHC) was determined by the method of Lin et al (1974). After cooking and cooling, each ham was allowed to drain for 10 min, prior to weighing. Percentage cooking loss was determined by taking the weights of the hams before and after cooking and cooling and expressing the difference in weights as percent of the original weight.

Shear force determination : *Semitendinosus* muscle was excised for non-tumbled hams, while for the tumbled samples, section of meat was taken irrespective of the muscle position. Shear force values were measured using the Warner-Bratzler apparatus.

Sensory evaluation : Samples for sensory evaluation were taken from *Semimembranosus* muscle excised from the bone-in hams, while for the tumbled ones, samples were taken irrespective of muscle position. The samples were presented to 15 trained panel members for evaluating flavour, juiciness, tenderness, colour, saltiness and overall acceptability on a 9-point Hedonic scale.

Statistical analysis : The data were statistically analysed using analysis of variance for a split-plot design (Steel and Torrie 1980) and in case of significant effects, the means were compared using Duncan's test.

The results presented in Table 1 demonstrate that the state of rigor had a significant ($P<0.05$) effect on the moisture content of the non-tumbled goat hams, but had no significant ($P>0.05$) effect on the proximate composition of the tumbled products. Moore et al (1992) found no significant difference in proximate analysis of country ham due to rigor state. Moisture and ash contents of the tumbled hams were significantly higher ($P<0.05$) and their protein contents were significantly lower than those of the non-tumbled ones. Percel et al (1982) reported that tumbling significantly increased % moisture of liver loaves, when compared to immersion. Theno et al (1978) reported that tumbling disrupted muscle cells, thus facilitating the diffusion of curing ingredients into the meat. This could explain the higher ash content of the tumbled hams over the non-tumbled ones. In contrast, Babji et al (1982) found no significant difference in chemical composition of the tumbled salted and non-tumbled salted turkey breast muscle. The greater ash content of the dry-cured hams compared to the immersion ones may be the result of the greater diffusion of curing ingredients associated with the dry-curing.

The effects of rigor state, curing and tumbling on physico-chemical properties of hams are also presented in Table 1. In all the treatment groups, the pre-rigor hams had higher pH and WHC values compared to the post-rigor ones. In contrast to the results obtained in this study, Babji et al (1982) found no difference in the pH and WHC of tumbled and non-tumbled turkey breast muscle. The shorter period of tumbling (30 min), they utilized, was certainly not enough for cellular disruption associated with tumbling and this could be the reason for the low lesser pH and improvement in water retaining ability of the products. The differences observed in the cooking losses of the various products could be attributed to variations in their WHC. The lower shear force values for the tumbled, compared to the non-tumbled samples may be attributed to the well known muscle fibre disruption effect of tumbling (Theno et al. 1978). The

TABLE 1. EFFECT OF RIGOR STATE, CURING AND TUMBLING ON COMPOSITION* AND PHYSICO-CHEMICAL PROPERTIES OF RAW AND COOKED GOAT HAMS

Attributes, %	Rigor state	Curring methods		Tumbling methods 4h	
		Dry-cure	Immersion-cure	Continuously	Intermittently
Raw					
Moisture	Pre	71.10 ± 1.10^{cd}	74.75 ± 1.15^{bd}	78.15 ± 1.20^{a}	78.85 ± 1.15^{a}
	Post	69.85 ± 0.95^{ce}	71.97 ± 0.90^{be}	77.85 ± 0.95^{a}	77.90 ± 1.40^{a}
Fat	Pre	2.42 ± 0.25^{a}	2.10 ± 0.20^{b}	1.60 ± 0.20^{b}	78.85 ± 1.15^{a}
	Post	2.51 ± 0.35^{a}	2.20 ± 0.25^{a}	1.74 ± 0.30^{b}	77.90 ± 1.40^{a}
Protein	Pre	22.65 ± 0.90^{ad}	20.25 ± 0.75^{bd}	15.91 ± 0.81^{c}	16.40 ± 0.95^{c}
	Post	24.20 ± 0.85^{ac}	21.95 ± 0.95^{be}	16.45 ± 1.09^{c}	17.20 ± 1.10^{c}
Ash	Pre	3.60 ± 0.25^{b}	2.87 ± 0.40^{c}	4.70 ± 0.25^{a}	4.75 ± 0.35^{a}
	Post	3.75 ± 0.40^{b}	2.95 ± 0.35^{c}	4.85 ± 0.30^{a}	4.92 ± 0.40^{a}
Cooked					
Moisture	Pre	66.20 ± 0.70^{cd}	70.17 ± 0.75^{bd}	72.03 ± 0.80^{a}	72.70 ± 0.95^{a}
	Post	65.08 ± 0.87^{ce}	68.95 ± 0.95^{be}	71.70 ± 0.70^{a}	71.80 ± 1.05^{a}
Fat	Pre	2.80 ± 0.30^{a}	2.70 ± 0.40^{a}	1.90 ± 0.40^{b}	1.85 ± 0.25^{b}
	Post	2.90 ± 0.35^{a}	2.55 ± 0.35^{a}	1.95 ± 0.35^{b}	1.80 ± 0.30^{b}
Protein	Pre	27.10 ± 0.95^{ad}	25.30 ± 0.80^{bd}	21.05 ± 0.90^{c}	21.05 ± 0.85^{c}
	Post	28.50 ± 0.80^{ac}	26.97 ± 0.75^{be}	22.07 ± 1.90^{c}	21.95 ± 0.95^{c}
Ash	Pre	3.80 ± 0.35^{b}	2.95 ± 0.25^{c}	4.85 ± 0.30^{a}	4.88 ± 0.35^{a}
	Post	3.90 ± 0.40^{b}	3.15 ± 0.35^{c}	4.90 ± 0.40^{a}	4.98 ± 0.20^{a}
Physico-chemical properties					
pH pre-cooked	Pre	5.85 ± 0.08^{cd}	6.47 ± 0.07^{ad}	6.05 ± 0.06^{bd}	6.03 ± 0.09^{bd}
	Post	5.65 ± 0.06^{ce}	6.10 ± 0.05^{ac}	7.85 ± 0.95^{a}	5.86 ± 0.05^{be}
pH post-cooked	Pre	6.05 ± 0.07^{cd}	6.60 ± 0.09^{ad}	6.22 ± 0.10^{b}	6.24 ± 0.06^{b}
	Post	5.88 ± 0.08^{ce}	6.40 ± 0.07^{ac}	6.15 ± 0.09^{b}	6.18 ± 0.12^{b}
WHC,% pre-cooked	Pre	50.12 ± 2.20^{cd}	58.18 ± 2.10^{bd}	84.50 ± 3.10^{ad}	85.30 ± 2.90^{ac}
	Post	47.80 ± 2.70^{ce}	52.20 ± 2.80^{be}	80.45 ± 2.30^{ac}	82.15 ± 2.50^{ac}
Cooking losses,%	Pre	25.82 ± 1.20^{ad}	20.25 ± 1.70^{bd}	16.85 ± 1.30^{cd}	16.02 ± 1.40^{cd}
	Post	27.20 ± 1.60^{ac}	23.35 ± 2.10^{be}	19.10 ± 1.95^{ce}	18.95 ± 1.75^{ce}
Shear force, kg	Pre	6.10 ± 0.70^{a}	5.80 ± 0.60^{a}	3.31 ± 0.55^{b}	3.24 ± 0.60^{b}
	Post	6.30 ± 0.95^{a}	5.95 ± 0.80^{a}	3.45 ± 0.75^{b}	3.30 ± 0.90^{b}
NaCl, %	Pre	2.62 ± 0.25^{b}	2.10 ± 0.35^{c}	3.10 ± 0.25^{a}	3.12 ± 0.40^{a}
	Post	2.75 ± 0.30^{b}	2.15 ± 0.20^{c}	3.15 ± 0.35^{a}	3.18 ± 0.25^{a}
$NaNO_2$, ppm	Pre	98.80 ± 2.15^{bd}	94.60 ± 2.50^{cd}	103.70 ± 2.15^{a}	102.65 ± 2.15^{a}
	Post	95.55 ± 2.60^{be}	90.75 ± 0.85^{ce}	102.85 ± 2.95^{a}	101.72 ± 2.50^{a}

* Means ± Standard error

^{abc} Means in the same row with different superscripts are significantly different (P<0,05)

^{de} Means in the same column, within rigor state and composition with different superscripts are significantly different (P<0,05)

increases in salt and residual nitrite levels of the tumbled products are in agreement with the reports of Percel et al (1982), who found that by increasing the migration of salt into the meat, tumbling increased the residual nitrite levels of the corresponding product. As judged by the sensory panel members, the sensory characteristics of the goat hams were not affected by the state of rigor or the tumbling methods. However, the tumbled products were juicier, more tender, pink and produced slightly better products than cured counterparts.

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Tryptophan Contents of Total (True) Proteins and Protein Fractions, Albumins and Globulins of Tribal/Little Known/Under-exploited Indian Legumes

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Tryptophan contents of total (true) proteins, protein fractions, albumins and globulins, were determined. Tryptophan contents of total proteins ranged from 5.8 g kg^{-1} (*Mucuna atropurpurea*) to 20.3 g kg^{-1} (*Prosopis chilensis*). Tryptophan contents of *Abrus precatorius* 'white variety', *Acacia nilotica*, *Prosopis chilensis*, *Vigna aconitifolia* and *V. sinensis* were found to be greater than those present in whole hen's egg protein (16.2g kg^{-1} protein). In most of the presently investigated legumes, the albumin fractions seemed to be a rich source of tryptophan as compared to globulins.

Keywords : Tryptophan, Tribal legumes, True proteins, Albumins, Globulins.

Tryptophan is the second limiting amino acid in cereals (Oser 1951). The content of tryptophan in seed proteins that might complement cereals should, therefore, be considered, whenever seed protein sources are evaluated (Sastry and Muray 1986). The data on various other essential amino acids of the little known/tribal/wild legume seed proteins are available (Janardhanan and Lakshmanan 1985; Marry Josephine and Janardhanan 1992; Rajaram 1990; Ravindran and Ravindran 1988; Siddhuraju et al. 1992; Vijayakumari et al. 1993), whereas no information is available regarding the levels of tryptophan in the above mentioned protein sources.

In view of this, tryptophan contents of the total seed proteins and protein fractions, albumins and globulins, of various legumes consumed by different Indian tribal sects and their wild progenitors belonging to the genera, *Abrus*, *Acacia*, *Bauhinia*, *Canavalia*, *Cassia*, *Entada*, *Indigofera*, *Mucuna*, *Parkia*, *Parkinosnia*, *Phaseolus*, *Pongamia*, *Prosopis*, *Sesbania*, *Tamarindus*, *Vigna* and *Xylia*, have been determined in the present study.

Mature seed materials were collected from different tribal pockets of India including Andaman and Nicobar Islands (Rajaram 1990; Siddhuraju 1994; Vijayakumari 1994). *Vigna vexillata* and *V. sinensis* were procured from Pulses Breeding Station, School of Genetics, Tamil Nadu Agricultural University, Coimbatore. The seeds were powdered separately in a Willey Mill to 60 mesh size. The seed powders were extracted with hexane to remove lipids and dried before use.

The total (true) proteins were extracted by the method of Rajaram and Janardhanan (1990). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA). The albumin and globulin fractions of seed proteins were extracted following the method of Murray (1979). The concentrations of above said total protein and protein fractions were estimated (Lowry et al. 1951). For determining the tryptophan content of seed proteins, duplicate aliquots containing known amounts of protein were dispersed into glass ampoules together with 1.0 ml of 5M NaOH. The ampoules were sealed and incubated at 110°C for 18 h. After incubation, the tryptophan contents of the alkaline hydrolysates were determined colorimetrically (Spies and Chambers 1949).

The values obtained for tryptophan contents of total proteins and albumin and globulin fractions are given in Table 1. Tryptophan concentrations of total proteins ranged from 5.8 g kg^{1} protein for *Mucuna atropurpurea* to 20.3 g kg^{-1} protein for *Prosopis chilensis*. In *Abrus precatorius* 'white variety', *Acacia nilotica*, *Prosopis chilensis*, *Vigna aconitifolia* and *V. sinensis*, the tryptophan contents were found to be greater than whole hen's egg proteins (16.2 g kg^{-1} protein). When compared with soybean, the seeds of *Abrus precatorius* 'black variety', *Acacia leucophloea*, *Bauhinia malabarica*, *Canavalia gladiata* 'white variety', *Indigofera linifolia*, *Mucuna pruriens*, *M.utilis*, *Phaseolus lunatus* 'brown variety' and *Xylia xylocarpa* contained more or less equal or relatively, higher values of tryptophan.

Information regarding the tryptophan contents of albumin and globulin protein fractions of commonly cultivated legumes are available (Gupta and Wagle 1978; Bhatty 1982; Sastry and Murray 1986). The ranges of values obtained for albumins and globulins are 3.3-21.1 and 3.6-14.2 g kg^{1}

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TABLE 1. TRYPTOPHAN CONTENT OF THE TOTAL (TRUE) PROTEINS, AND PROTEIN FRACTIONS (ALBUMINS AND GLOBULINS) OF THE VARIOUS LITTLE KNOWN/ UNDEREXPLOITED LEGUME SEEDS, g kg⁻¹ PROTEIN*

Name of the legume	Total (true protein)	Albumins	Globulins
Abrus precatorius L			
'Black variety'	12.2	5.2	7.1
'Red and black-dotted variety'	18.0	10.7	12.0
'White variety'	16.6	9.6	6.2
Acacia leucophloea (Roxb) Willd	12.1	7.4	13.5
A. nilotica (L) Del	18.7	20.9	8.2
Alysicarpus rugosus (Willd) DC	9.2	12.5	9.0
Atylosia goensis (Dalz)	8.8	19.3	6.3
Bauhinia malabarica (Roxb)	13.0	15.2	trace
B. purpurea L	7.8	20.1	trace
B. racemosa Lamk	9.7	8.4	7.9
B. tomentosa L	6.0	7.8	3.4
B. vahlii W & A	8.4	trace	10.9
Canavalia ensiformis DC	9.6	19.4	10.3
C. gladiata (Jacqi) DC.'White variety'	12.6	8.7	8.6
C. gladiata (Jacqi) DC.'Brown variety'	10.3	8.4	7.6
Cassia laevigata Wild	10.2	7.8	6.4
C. obtusifolia L	8.2	15.6	12.1
Entada scandens Benth	10.9	12.6	08.2
Indigofera linifolia (L.F.) Retz	12.8	9.1	10.4
Mucuna atropurpurea DC	5.8	14.6	04.2
M. gigantea (Willd) DC	6.9	08.7	04.1
M. pruriens (L) DC	13.5	12.5	10.3
M. utilis Wall ex Wight	13.1	9.7	12.6
Parkia biglandulosa W & A	trace	8.2	7.1
P. roxburghii G Don	11.9	5.6	10.2
Phaseolus lunatus L			
Brown variety'	12.6	19.2	10.3
'Black variety'	10.8	14.3	10.5
'Red variety'	8.0	10.2	11.7
Prosopis chilensis (Molina) Stunz	20.3	trace	13.6
Parkinsonia aculeata L	10.4	7.2	trace
Pongamia pinnata (L) Pierre	15.3	10.3	9.0
Sesbania bispinosa (Jacq) W.F. Wight	9.1	11.1	14.2
Tamarindus indica L	9.6	7.4	3.7
Vigna aconitifolia (Jacq) Marechal	16.7	16.2	4.3
V. capensis Walp	12.6	7.1	10.2
V. glabrescens Marechal	7.5	9.8	5.2
V. sesquipedalis (L) Verdc	11.7	8.3	4.9
V. sinensis (L) Savi. ex Hassk	18.2	19.7	7.3
V. sublobata (Roxb) Babu et. Sharma	10.5	8.1	7.6
V. trilobata (L) Verdc	9.4	trace	11.5
V. umbellata (Thumb) Ohwi and Ohashi (Var. K_1)	10.6	21.1	6.1
V. vexillata (L) A. Rich	11.3	7.0	12.1
Xylia xylocarpa (Roxb) Taub	13.6	12.2	9.2
FAO/WHO (1990) Suggested pattern	10.1	–	–
Whole hen's egg[a]	16.2	–	–
Soybean[a]	13.7	–	–

[a] Reported by Oyenuga and Fetuga (1975)

* Average value of duplicate determinations.

proteins, respectively. The albumin fraction of *Acacia nilotica, Atylosia goensis, Bauhinia purpurea, Canavalia ensiformis, P. lunatus* 'brown variety', *Vigna sinensis* and *V. umbellata* is known to contain higher amounts of tryptophan than the cultivated pulses like *Cicer arietinum* (Sastry and Murray 1986) and lentil, *mung*bean and faba beans (Bhatty 1982). In the tribal pulses like *Abrus precatorius* 'red and black-dotted variety', *Acacia leucophloea, Mucuna utilis, Phaseolus lunatus* 'red variety', *Prosopis chilensis, Sesbania bispinosa, Vigna trilobata* and *V. vexillata,* the globulin fraction contains, relatively, higher amount of tryptophan, when compared with the respective albumin fractions and FAO/WHO (1990) recommended pattern. In general, albumin fractions of most of the presently investigated legumes seemed to be a rich source of tryptophan compared to globulin fractions. This is in corroboration with the previous reports in some of the cultivated legumes (Sastry and Murray 1986). Thus, tryptophan-rich little known legumes may be incorporated into a diet to complement this amino acid deficiency of the major cereals.

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Production and Quality Characteristics of Nigerian *Agidi* Supplemented with Soy Flour

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Agidi, normally made from fermented maize, was prepared from fermented maize flour, supplemented with up to 20% dry milled, full-fat soy flour. Soy-supplementation of the maize significantly increased the protein content of the *agidi* products from 9.06 in the unsupplemented products to 19.08% in the 20% supplemented product. There were also marked increases in the fat, ash and energy values of the products. The pH increased from 3.81 in the unsupplemented product to 51.7 in the 20% soy-supplemented product, while the titrable acidity decreased from 0.132% to 0.094%. The viscosity of the flour blends decreased significantly with increase in soy flour. The flours reconstituted very well in boiling water. Soy-supplementation significantly affected the colour, consistency and overall acceptability of the products. The study revealed that the optimum level of soy-supplementation of fermented maize for *agidi* production was 15%.

Keywords : Nigerian-*Agidi*, Maize, Soybean, Supplementation, Sensory quality.

Ogi, one of the popular maize products consumed widely in Nigeria, is a starchy mash obtained by soaking and wet extraction of maize (Umoh and Fields 1981; Akingbala et al. 1981). If *ogi* is cooked with water to produce a semi-solid product, it is called *agidi* (Umoh and Fields 1981). *Agidi* is eaten by both infants and adults. It could be eaten alone or with vegetable soup or stew as well as with *moi-moi* or *akara* (steamed or fried bean cake). *Agidi* has added advantage over *ogi*, as it could be eaten cold, allowing its preparation long before use, unlike *ogi*, which should be eaten warm, thereby requiring fresh preparation. Earlier attempts with a view to improving the nutritional qualities of these maize-based products were concentrated on *ogi* (Adeniji and Potter 1978; Eka 1978; Banigo and Akpapunam 1987; Akobundu and Hoskins 1987; Akinyele and Fasaye 1988).

Soybean, grown extensively in Nigeria, represents an inexpensive and abundant source of good quality protein (Wolf and Cowan 1971). It has a high potential for supplementing the protein quality of *agidi*. Hence, the present study was aimed at producing a maize-soy flour blend for *agidi* production and to determine the physico-chemical and sensory properties of the product.

Preparation of maize flour : Cleaned white maize grains purchased from a local market in Makurdi, Nigeria were steeped for two days in tap water (1:2 w/v) at room temperature (30 ± 2 °C), wet milled in a commercial maize mill and filtered through cheese cloth with excess water (steep water was used to minimize nutrient loss). The by-product (residue) on the cheese cloth was discarded, while the bucket was covered with cheese cloth and the contents were allowed to settle for 10h at room temperature (30° ±2°C). Water was decanted from the starch slurry, which was, then, sun-dried on an aluminium tray and milled into flour in a corn mill (Model Corona-2N). The milled sample was sieved into fine flour with cheese cloth.

Preparation of soy flour : Soybeans used for the study were purchased from a local market in Makurdi, Nigeria. The beans were sorted, cleaned and blanched in water at 90°C for 7 min. The blanched beans were soaked in 0.5% $NaHCO_3$ solution (Johnson and Snyder 1978) for 6 h. The soaked beans were dehulled, sun-dried, and milled into flour in a corn mill. The flour was sieved through cheese cloth to obtain fine and uniform particle-size flour.

Blend formulation : Five flour blends were prepared by mixing maize and soy flours in the proportions of 100:0, 95:5, 90.10, 85:15 and 80:20, respectively. All the samples and blends were thoroughly mixed in a Kenwood food processor (Model KM 201, England) and were stored at 4°C, until used.

Preparation of agidi : The slurry, containing 30 g of flour blends and 150 ml water was cooked in an aluminium pot for 5 min with constant stirring at about 85°C on an electric stove. The highly viscous paste formed was poured into a 250 ml glass beaker and allowed to cool for about 1 h during which it solidified into a gel (*Agidi*).

Chemical and physical analyses : Crude proteins (N x 6.25), fat, ash and moisture contents

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of the blends were determined by the AOAC (1984) methods. Carbhydrate content was estimated by difference and the energy value was calculated, using Atwater factor (4 x protein, 9 x fat, and 4 x carbohydrate).

The pH values of the samples were determined by mixing 10g samples with 10 ml distilled water. The mixture was left at room temperature (30°C ± 2°C) for 30 min and the pH values of the supernatants were measured. Titratable acidity as lactic acid was determined by titrating 10 ml aliquots of the supernatant from the pH determination with 0.1N NaOH to phenolphthalein end-point.

Reconstitution index was determined, using the method described by Akpapunam and Markakis (1981). The viscosity of the product was determined by the slightly modified method of Mosha and Svanberg (1983). A gruel was prepared by heating each sample of the flour (7% DM) in water to a cooking temperature of 83°C within 6 min with continuous stirring. The gruel was kept at this temperature for 10 min with constant stirring. It was then, transferred to a water bath at 40°C and the viscosity was measured using Brookfield Viscometer (Model LV8, Viscometer UK Ltd.).

Sensory evaluation: Colour, consistency, flavour and general acceptability of the *agidi* samples were evaluated by a 22-member panel, using a 5-point Hedonic scale with 1 representing the least score and 5 the highest. Analysis of variance (ANOVA) was performed on the data to determine differences, while the least significant test was used to detect significant differences among the means (Steel and Torrie 1960).

There was a significant increase in the proteins of the *agidi* samples with increase in proportion of soy flour (Table 1), indicating that addition of soy flour to maize flour would greatly improve the protein nutritional quality of *agidi*. Soy-supplementation of the maize flour increased the crude fat, ash and energy values of the *agidi* products, while the carbohydrate contents decreased with increase in the amount of soy flour in the blends. The results showed that addition of soybean improved the overall nutritional quality of *agidi*. This is desirable for the majority of the population, who cannot afford other highly nutritious and expensive foods.

Supplementation of soy flour into maize flour significantly increased the pH of the flour blend as well as the respective *agidi* samples (Table 1).

TABLE 1. PHYSICO-CHEMICAL PROPERTIES (% ON DRY BASIS) AND SENSORY CHARACTERISTICS OF MAIZE/SOYBEAN *AGIDI*

Attributes	Maize: soybean blends				
	100:0	95:5	90:10	85:15	80:20
Crude proteins	9.06^{a}	11.29^{b}	13.10^{c}	15.82^{d}	19.08^{c}
Crude fat	4.30^{b}	4.95^{b}	5.29^{ab}	6.15^{a}	6.89^{a}
Ash	0.90^{b}	0.95^{b}	1.05^{ab}	1.11^{a}	1.18^{a}
Carbohydrates (by difference)	85.74	82.81	80.56	76.92	72.85
Energy, kcal/100g	418	421	422	426	430
pH	3.81	4.10	4.60	5.10	5.17
Titratable acidity*	0.132	0.120	0.112	0.105	0.09
Reconstitution index, ml	1.06	1.10	1.10	1.16	1.26
Viscosity, cps	11,100	10,600	9,600	9,000	8,200
Sensory characterstics					
Colour	4.64^{a}	4.14^{ab}	3.95^{b}	3.45^{bc}	3.05^{c}
Consistency	4.73^{a}	4.05^{a}	4.25^{a}	4.23^{a}	3.50^{b}
Flavour	4.32	4.18	4.06	4.18	3.91
Overall acceptability	4.64^{a}	4.05^{a}	3.85^{b}	3.68^{bc}	3.41^{c}

Means in column with same letters are not significantly different ($p<0.05$)

* As lactic acid

This has some significant implications for *agidi* in terms of shelf life and flavour. Since lower pH in foods suggests longer shelf-life and more sour taste, it would appear from this study that increased soy supplementation would result in a product with shorter shelf-life and undesirable taste. The titratable acidity (TA) of *agidi* flour and *agidi* product showed similar trends as pH. TA values of the *agidi*, which varied from 0.132% in the unsupplemented flour to 0.094% in the blends containing 20% soy flour are in agreement with the those reported by Adeyemi and Backley (1986) and Umoh and Fields (1981). Changes in acidity would have similar effects as the pH on perishability and flavour *agidi*.

Reconstitution index (RI) and viscosity profiles of *agidi* flours from the maize/soy flour blends are shown in Table 1. Soy supplementation increased the RI of the flours. It would appear that soy flour was more resistant to swelling than maize flour. This may result in different gelatinization rates of the flours with the partially gelatinized flours settling out of the mixture. However, with the exception of the blend containing 20% soy flour, there were no significant differences among the samples with respect to RI. Thus, the sedimentation effect was not pronounced, implying that *agidi* flour with good reconstitution could be produced from

maize/soy flour blends. The viscosity of the blends decreased steadily with increase in soy-supplementation. The blends containing 15 and 20% soy flours differed significantly from those containing 0, 5 and 10% soy flours, respectively. The decrease in viscosity of the samples implied reduced gel strength, which may be due to changes in starch, lipid, ash and protein contents of the blends with soy-supplementation (Singh and Singh 1991; Narayana and Narasinga Rao 1982; Zobel 1984). The significance of the reduction in viscosity of the *agidi* flours is that more flour concentrations would be required to form the same gel as the unsupplemented *agidi* and this would mean more nutrient in the *agidi*.

The mean scores for the sensory evaluation of the *agidi* samples are shown in Table 1. The unsupplemented *agidi* was consistently rated higher than the soy-supplemented *agidi* samples with respect to colour, consistency, flavour and overall acceptability. As the level of soy flour in the blends increased from 10 to 20%, the *agidi* products became progressively darker in colour. The samples were rated significantly lower than the unsupplemented *agidi*. The colour change was due probably to the yellowish-brown colour of the soy flour. The supplemented *agidi* products were grainy in texture, compared to the smooth texture of the unsupple-mented product. This is an indication of incomplete gelatinization, which was demonstrated by the slightly higher reconstruction index values of the soy-supplemented flours. It is important to note that there were no significant differences among the products with respect to flavour. In general, all the products were acceptable, as evidenced by the high scores of 3 and above recorded for the samples.

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Occurrence of Pesticide Residues in Market and Farm Gate Samples of Vegetables in and around Bangalore City

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Periodic monitoring of market and farm gate samples of different vegetables was carried out in and around Bangalore City during 1993 and 1995 for the extent of pesticidal contamination. The market samples of vegetables, monitored for DDT and HCH residues, have shown widespread contamination, mainly with isomers of HCH and traces of the metabolites of DDT. Residues of HCH were, however, within safe limits. The monitoring of farm gate samples showed positive contamination with endosulfan and monocrotophos residues in 40% of samples. While endosulfan residues were within safe limits, monocrotophos residues were detected at bioconcentrations. Synthetic pyrethroids proved to be having a favourable residue profile.

Keywords : Farm gate, Market vegetables, Pesticide residues, Organochlorine, Insecticides, Monocrotophos.

Repeated application of pesticides on vegetables often result in the build up of their residues and seldom at bioconcentrations (Handa 1992). Regular consumption of such contaminated vegetables can cause toxic health hazards to the consumers (Bindra 1971). It is, therefore, desirable to monitor the extent of contamination of market basket samples of vegetables as well as farm gate samples of vegetables through periodical survey by analysis of pesticide residues to guage the extent of persistence. Present studies were the continuation of the exercise from the point of view of proper management of such toxic residues.

Sampling of market basket samples of vegetables : Vegetable samples of 6 extensively consumed vegetables namely, okra, brinjal, tomato, French bean, cabbage and cauliflower were collected from 3 main vegetable markets of Bangalore City periodically during January–May 1993. The gross samples (approximately 2 kg) of each of the vegetables were drawn afresh and immediately transported to the laboratory for processing to analyse the residues of organochlorine hydrocarbon insecticides.

Sampling of farm gate samples of vegetables : Farm gate samples of 8 vegetables namely, okra, brinjal, tomato, French bean, cabbage, cauliflower, cucumber and fieldbean were collected periodically during August 1994–February 1995 at harvest from 4 locations around Bangalore City. In all, a total of 50 samples of the above vegetables were collected for monitoring of residues of various insecticides being used during fruit bearing stage of the vegetables. The background information on pesticidal schedules being followed to control insect pests at the farm for each of the vegetable was also collected. The samples were then brought to the laboratory for processing for residue analysis.

Extraction, clean up and estimation of residues: Market basket samples of all the vegetables were processed for residue analysis of two main organochlorine insecticides, dichlorodiphenyltrichloroethane and hexachlorocyclohexane, along with their metabolities/isomers by extraction in acetone through blending in high speed, explosion proof Waring blender. The cleanup of residues from other plant co-extractives was carried out by solvent partitioning from a hexane layer, followed by column adsorption chromatography adopted from the standardized technique (Luke et al. 1975). The quantitative analysis of residues was, thereafter, carried out by gas liquid chromatography using Varian GC-3600 model, equipped with electron capture detector over 2 m stainless steel column packed with 1.5% OV 17+1.95% OV 210 liquid phase. The other standardized parameters were: nitrogen as carrier gas at a flow rate of 40 ml/min.; oven temperature of 190° C for column, 220° C for injector inlet and 240° C for detector, for the simultaneous multi-residue detection of para-para-dichlorodiphenyldichloroethylene, para-para-tetrachlorodiphenylethane, para-para-dichlorodiphenyltrichloroethane; α, β and γ isomers of hexachlorocyclohexane.

Farm gate samples of different vegetables were processed for residue analysis of endosulfan, monocrotophos, fenvalerate, cypermethrin and deltamethrin insecticides, based on the background information of insecticidal schedules followed at

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different farms. The suitable extraction solvents/ solvent mixtures viz., n-hexane + isopropyl alcohol for endosulfan, acetone for monocrotophos and acetone + hexane for fenvalerate, cypermethrin and deltamethrin were used during the extraction of their residues through blending. The residues from the insecticides were, then, cleaned up by solvent partitioning and column chromatography for endosulfan and monocrotophos, following the procedures of Jones and Riddick (1952) and synthetic pyrethroids as per Awasthi (1985). The quantitative determinations of the residues from all above insecticides were carried out by gas liquid chromatography at the standardized parameters on Varian GC-3600 model as per BIS specifications for endosulfan (BIS 1989) and monocrotophos (BIS 1986) and for pyrethroids at the reported conditions (Awasthi 1985). The efficiency of extraction, clean up and determination procedures of residue analysis on spiked control samples of different vegetables ranged between 87 and 92% recovery for endusulfan, monocrotophos, dichlorodiphenyltrichloroethane and hexachlorocyclohexane, while that of synthetic pyrethroids between 92 and 94%.

The monitoring of market basket samples of different vegetables revealed widespread contamination with residues of the two chlorinated hydrocarbon insecticides-dichlorodiphenyltrichloroethane and hexachlorocyclohexane (Table 1). Data indicate positive contamination to the extent of 77.7% of the samples in the quantitative range of residues from traces of total dichlorodiphenyltrichloroethane, identified as para-para- dichlorodiphenyltrichloroethylene, para-para- tetrachlorodiphenylethane and para-para-dichlorodiphenyltrichloroethane. Hexachlorocyclohexane residues were identified as α, β and γ isomers. Total hexachlorocyclohexane residues ranged between 0.0014 and 0.1867 ppm against the prescribed maximum residue limit (MRL) of 3.0 ppm for vegetables (Parmar and Dureja 1990). Present studies confirm the occurrence of dichlorodiphenyltrichloroethane and hexachloro-cyclohexane residues in market samples of various vegetables, reported from time to time across the country at varying concentrations (Agnihotri et al. 1974; Lal et al. 1980; Dahia and Chauhan 1982; Chauhan et al. 1983; Dube and Nath 1991). However, in the present studies, the extent of the persistence of residues was within safe levels from all the contaminated samples. But the detection of γ-hexachlorocyclohexane in some of the vegetables is a cause of concern, since its use has been restricted on vegetables (Anon 1996). Among the vegetables monitored, hexachlorocyclohexane residues were detected in most of the samples of okra, brinjal, cauliflower, French bean and cabbage. Out of the major isomers of hexachlorocyclohexane, α and γ isomers were predominantly detected, while β isomer was detected in fewer samples only. The persistence of α and γ isomers reflect fresh

TABLE 1. STATUS OF PESTICIDE RESIDUE CONTAMINATION IN MARKET SAMPLS OF DIFFERENT VEGETABLES

Vegetables	Markets	Average residues of HCH and DDT, mg kg^{-1}							
		α-HCH	β-HCH	γ-HCH	Total HCH	pp-DDE	pp-TDE	pp-DDT	Total DDT
Okra	1	0.0032	ND	0.0034	0.0066	ND	ND	ND	ND
	2	0.0038	ND	0.0019	0.0057	ND	ND	ND	ND
	3	0.0020	ND	0.0075	0.0095	ND	ND	ND	ND
Tomato	1	ND	ND	ND	ND	ND	ND	ND	ND
	2	ND	ND	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND	ND	ND
Brinjal	1	0.0037	ND	0.0051	0.0088	Traces	ND	Traces	Traces
	2	0.0006	ND	0.0020	0.0026	Traces	ND	Traces	Traces
	3	ND	ND	ND	ND	Traces	Traces	Traces	Traces
Cabbage	1	ND	ND	ND	ND	ND	ND	ND	ND
	2	0.0116	ND	ND	0.0116	ND	ND	ND	ND
	3	0.0074	ND	ND	0.0074	ND	ND	ND	ND
Cauliflower	1	0.0864	0.0543	0.0460	0.1867	Traces	Traces	Traces	Traces
	2	0.0696	0.0406	0.0190	0.1292	Traces	Traces	Traces	Traces
	3	0.0855	0.0321	0.0542	0.1718	Traces	Traces	Traces	Traces
French bean	1	0.0027	ND	0.0013	0.0040	Traces	ND	Traces	Traces
	2	0.0031	ND	0.0013	0.0044	Traces	ND	Traces	Traces
	3	0.0006	ND	0.0008	0.0014	Traces	ND	Traces	Traces

ND - Not detectable

TABLE 2. STATUS OF PESTICIDE RESIDUE CONTAMINATION AT HARVEST IN FARM GATE SAMPLES OF DIFFERENT VEGETABLES

Monitoring period	Locations of vegetable farms	Vegetables	Pesticides detected	Range of residues, mg kg^{-1}	Samples contaminated
August 1994	1 Hesaraghatta Village	Tomato	Deltamethrin	ND	0/5
	2 Shivakote Village	Tomato	Endosulfan	0.17 - 0.33	3/5
September 1994	1 Hesaraghatta Village	Tomato	Deltamethrin	ND	0/5
	2 Shivakote Village	Tomato	Endosulfan	0.27 - 0.41	4/5
October 1994	1 Thamarasanahalli Village	Brinjal	Endosulfan	0.55 - 0.84	5/5
December 1994	1 Hesaraghatta Village	French bean	Monocrotophos	0.16 - 0.42	3/5
		Cauliflower	Fenvalerate	ND	0/5
January 1995	1 Hesaraghatta Village	Field bean	Deltamethrin	ND	0/5
February 1995	1 Shivakote Village	Cucumber	Monocrotophos	0.27 - 0.75	5/5
	2 Hesaraghatta Village	Okra	Cypermethrin	ND	0/5

ND- Not detectable

contamination most probably during transportation or storage stage in the vegetable yards, as these isomers make bulk of hexachlorocyclohexane dust and dissipate faster as compared to isomer of hexachlorocyclohexane.

The status of pesticide residues in farm gate samples of different vegetables at harvest (Table 2) showed that approximately 40% of samples were contaminated mainly with residues of endosulfan and monocrotophos, which were used close to harvest. While monocrotophos residues were detected on French bean and cucumber, endosulfan residues persisted on tomato and brinjal only. Residues from deltamethrin applied on tomato and dolicos, fenvalerate on cauliflower and cypermethrin on okra were, however, below the detectable limit of 0.1 ng. Quantitatively, endosulfan residues were well within the safe limit of 2.0 ppm (Parmar and Dureja 1990), but monocrotophos residues were found to persist in excess of the prescribed 0.2 ppm MRL (Parmar and Dureja 1990) on French bean and cucumber. Field contamination at harvest has also been reported in many vegetables (Dethe et al. 1995).

Such vegetables loaded with pesticide residues at biconcentrations on consumption over a period of time may cause health hazards and therefore, it is recommended that suitable waiting periods (Awasthi 1993) be followed for the residue decay to critical maximum residue limits. Further, it is also suggested to follow effective decontamination practices (Awasthi 1986) at domestic levels before cooking that may help in eliminating toxic residues significantly. Synthetic pyrethroids appear to possess a more favourable residue pattern.

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Suitability of New Soybean Cultivars in the Production of Soy Milk

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Six new varieties of soybeans were evaluated for their suitability for the preparation of soy milk. The ranges of different constituents of soy milk samples prepared from various new varieties of soybeans were: yield of milk 725.0-802.5 ml/100g, total solids 5.78-6.48%, proteins 2.81-3.45%, fat 1.42-1.65%, calcium 20.34-27.12 mg/100g, phosphorus 30.81-40.19 mg/100g, iron 0.67-0.90 mg/100g, phenols 25.0-33.7 mg/100g, titratable acidity 0.121-0.157%, pH 6.29-6.77, viscosity 3.8-5.2 cps and specific gravity 1.024-1.030. Organoleptic evaluation of products revealed that soy milk prepared from variety 'PK-472' and 'PK-416' had greater overall acceptability than other preparations.

Keywords : Soybeans, Soymilk, Physical characteristics, Chemical composition, Minerals, Phenols, Quality.

Varietal variations in soybeans are known to influence the quality characteristics of products prepared from them (Snyder and Kwon 1987). The suitability of soybean varieties for preparation of different soy products has been investigated in detail in countries like USA, Japan and China (Smith et al. 1960; Wang et al. 1983). Though soybean cultivation was introduced in India during mid sixties, information on quality characteristics of Indian varieties is limited. A number of soybean varieties developed by plant breeders in the country have been evaluated (Kapoor et al. 1975; Jain 1985; Gupta et al. 1976; Reddy and Mital 1992). In an earlier communication, physical and chemical characteristics of 6 new varieties of soybeans have been reported (Saxena et al. 1994). The present communication reports quality characteristics of soy milk prepared from these varieties. Such information would be useful in selecting suitable varieties for soy milk manufacture.

Dry, mature seeds of soybean varieties 'PK-262', 'PK-416', 'PK-471' 'PK-472' 'PK-515' and 'PK-564' grown during kharif season 1990-91 were procured from Crop Research Centre of the University, cleaned and stored in air tight containers at ambient temperature (20-25°C), until use. Soy milk was prepared according to the procedure described by Grover et al (1983) with the modification that whole soybeans were soaked in water (bean to water ratio 1:8) containing 0.15% glucono-δ-lactone.

Moisture, proteins (Nx6.25) and fat contents in soy milk were determined according to AOAC (1984) procedures, whereas specific gravity, phosphorus, iron and phenols were estimated as per methods described by Ranganna (1986). Calcium content and acidity (expressed as lactic acid) were determined as per AACC (1976) methods. Viscosity of soy milk was measured in centipoise at 25 ± 2°C with Brookfield Synchrolectric viscometer using spindle No. 1 and speed of 60 rpm. The pH of the samples was determined using a digital pH meter.

Soy milk samples were evaluated organoleptically by a taste panel of 24 members drawn from this department. The panelists were presented sweetened soy milk (7% sugar) chilled to 4°C and were asked to evaluate the samples for overall acceptability on a 9-point Hedonic scale, where a score of 9 represents 'extreme liking' and 1 denotes 'extreme disliking'. The data were analyzed statistically using analysis of variance technique (Larmond 1977).

Under identical conditions of extraction, yield of soy milk from various varieties was different (Table 1). However, their total solids and protein contents were similar. Varieities 'PK-416' and 'PK-564' gave maximum and minimum yields of soy milk, respectively. The fat contents of various soy milk samples were statistically different ($P<0.05$). Soy milk prepared from varieties 'PK-416' and 'PK-472' exhibited maximum fat content. Comparison of protein content of soy milk obtained in this study with protein content of whole soybeans (Saxena et al. 1994) showed a high positive correlation (0.8868) between the two. Since soy milk is not a standard product, its composition varies widely, depending upon the raw material and processing conditions used (Bourne et al. 1976; Wang et al. 1983; Lim et al. 1990). The differences observed in the present study are attributed to varietal differences.

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TABLE 1. YIELD, COMPOSITION, PHENOL CONTENT, PHYSICO-CHEMICAL PROPERTIES AND OVERALL ACCEPTABILITY OF SOY MILK PREPARED FROM DIFFERENT VARIETIES

Attribute	Variety						
	'PK-262'	'PK-416'	'PK-471'	'PK-472'	'PK-515'	'PK-564'	CD at 5% local
Yield of milk, ml/100g	735.00	802.50	740.50	785.00	772.50	725.00	15.80
Total solids, %	6.45	5.78	6.47	6.19	6.42	6.48	NS
Proteins, %	3.15	2.81	3.17	2.82	3.18	3.45	NS
Fat, %	1.48	1.65	1.54	1.65	1.42	1.55	0.08
Calcium, mg/100g	25.74	20.34	24.32	20.80	22.47	27.12	NS
Phosphorus, mg/100g	35.47	30.81	37.47	31.29	40.19	37.86	1.73
Iron, mg/100g	0.91	0.68	0.67	0.67	0.89	0.95	0.05
Total phenols, mg/100g							
Untreated	39.50[a]	41.70[a]	41.90[a]	42.10[a]	40.50[a]	41.20[a]	-
Treated with glucono-δ-lactone	29.80[b]	30.50[b]	28.00[b]	33.40[b]	25.00[b]	33.70[b]	-
% recovery untreated	50.49	48.71	47.33	45.90	49.19	49.61	-
Treated with glucono-δ-lactone	38.09	35.62	31.63	36.41	30.36	40.58	-
Titratable acidity, %	0.16	0.15	0.14	0.14	0.12	0.16	0.02
pH	6.31	6.58	6.29	6.77	6.45	6.32	0.12
Viscosity, centipoise	5.20	3.80	4.50	4.00	4.50	5.00	0.41
Specific gravity	1.02	1.03	1.02	1.03	1.4	1.03	0.01
Overall acceptability	6.34[bc]	7.58[a]	6.75[b]	7.92[a]	5.79[c]	5.88[c]	0.66

Means followed by different letters differ significantly at 5% level. Hedonic scale rating 1 dislike extremely; 2 dislike very much; 3 dislike moderately; 4 dislike slightly; 5 neither like nor dislike; 6 like slightly; 7 like moderately; 8 like very much; 9 like extremely
Results of composition expressed on fresh weight basis (w/w)

The extraction of fat was greater than that of protein in all the soy milk preparations. Maximum extraction of both protein and fat was observed in variety 'PK-416', whereas minimum recovery was recorded for variety 'PK-262'. Interestingly, recovery data of total solids of various varieties were in a narrower range (53-55%) than those of protein (57-66%) and fat (62-71%). Extraction of protein in soy milk in the present study was lower than the values reported by Wang et al (1983), Jain (1985) and Vijaya Nand (1987) who found recovery of 63.93–71.99%. The differences are attributed to variations in extraction conditions used by them.

The mineral content in soy milk prepared from different varieties varied considerably (Table 1). Soy milk prepared from variety 'PK-564' contained the highest quantity of iron, whereas phosphorus was highest in soy milk, prepared from variety 'PK-515'. Soy milk from varieties 'PK-416' and 'PK-472' had relatively low amounts of the three minerals. Among the three minerals, extractability of calcium from whole soybeans was better (62.9–69.4%) than for phosphorus (32.2–59.2%) and iron (48.6–56.1%). In general, the recovery of the three minerals was maximum in soy milk prepared from variety 'PK-515'.

Soy milk prepared without using glucono -δ-lactone during soaking step contained significantly higher amounts (39.5–42.1 mg/100g) of phenols ($P<0.05$) than those prepared without the use of glucono-δ- lactone (Table 1). Apparently, glucono - δ- lactone was effective in reducing extraction of phenols in soy milk. The reduction was maximum in variety 'PK-515'. It was also seen that about 45-50% phenolic compounds of the whole soybeans were recovered in plain soy milk. However, when glucono-δ-lactone was used during soaking at the rate of 0.15%, only 30-40% of total phenols were extracted into soy milk. Glucono-δ-lactone, therefore, inhibited the action β-galactosidase enzyme responsible for production of daidzein and genestein (Matsuura et al. 1989).

Soy milk samples were found to differ in various physico-chemical characteristics (Table 1). Soy milk prepared from varieties 'PK-262' and 'PK-515' had maximum titratable acidity, whereas soy milk of variety 'PK-472' exhibited maximum pH. Viscosity values were maximum and minimum for soy milk preparations from varieties 'PK-262' and 'PK-416', respectively. Soy milk of variety 'PK-515' exhibited maximum specific gravity. All these values are similar to those reported by Reddy and Mital (1992) for various Indian varieties.

The mean acceptability scores for various soy milk preparations varied from 5.79 to 7.92. Soy milk prepared from variety 'PK-472', was found

most acceptable, followed by variety 'PK-416'. The penelists recorded that soy milk prepared from variety 'PK-472' was almost devoid of beany flavour and exhibited appealing white colour.

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A Mathematical Model For Vibrofluidized Beds for Drying Casein

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Different factors that affect moisture loss process in vibrofluidized bed were correlated into a mathematical model. Trials were conducted with casein. The data were analyzed and presented graphically. The graphs of drying rate versus under root of temperature and two third power of diameter of the particles showed linearity as predicted by the model. The drying time predicted by the model varied the actual values by 2 to 25%. The model will be useful in designing industrial fluidized bed systems for drying casein.

Keywords : Mathematical model, Vibrofluidized beds, Casein, Moisture ratio, Drying factor.

Fluidization technology offers a series of advantages (Hovmand 1987). Its application ranges from processing of various dairy and food products (Espie et al. 1984; Hovmand 1987; Towler 1987) to steam generation (Holstrom 1983) and even in atomic energy sector, this technique is applied (Priestley 1962). Lately, the fluidization technology has been modifed into a number of systems such as mechanically agitated, centrifugal, vibrofluidized bed etc., and large number of products are processed using this technology, combining product improvement and energy savings. So far, the research on fluidized bed drying is based on trial and error method. Very few mathematical models are available for fluidized bed drying systems. In the present study, different parameters that affect the moisture removal process in vibrofluidized bed were correlated into a mathematical model.

A schematic diagram of the equipment is shown in Fig. 1. The tray has a perforated bottom plate of 0.0552 m^2 (0.24m x 0.23m) area and is vibrated by the vibration generator (8) with the help of a lever arm. It is made of wood and has a screen of 80 gauge (equivalent to 180 μ aperture), as its bottom plate. Heating elements are arranged into three different sets, containing 1, 2 and 4 elements to get different temperatures of air. They are of Nickel-chromium wire, coiled over a porcelain rod. Both the amplitude and frequency can be controlled by the frequency generator (9).

Materials : Casein samples were collected from Students Training Dairy of Sheth M.C. College of Dairy Science, Anand. The samples were ground in a domestic mixer and screen analyzed with standard test sieves (ISS-460-1962). Required size sample was selected, moistured and used for drying trials.

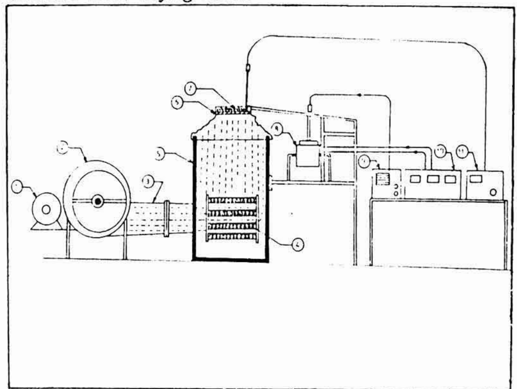

Fig.1. Schemtic diagram of vibrofluidizer

1. Motor
2. Air blower
3. Air duct
4. Heating elements
5. Drum
6. Tray
7. Product
8. Vibration
9. Frequency generator
10. Digital temp. indicator

Parameters : In the present study, effect of vibrations, temperature of air, velocity of air, mass of the product and diam of the particle was studied. Vibration frequency was adjusted to 20.5 Hz to get resonance at fundamental mode. When the resonance is at fundamental mode, optimum amplitude is obtained with minimum input. Vibrations were provided with the help of generator and an oscillator/power amplifier (Model-3516. Industrial Electronics Pvt. Ltd., Bangalore). Amplitude was kept constant by controlling the input voltage. This was observed on Cathode Ray Oscilloscope (Philips-PM 3200). In all the runs, vibratory acceleration (Av) of 16.5908 m/s^2 was maintained. Velocity of air was varied by controlling the blower suction opening. Some more details on parameters and notations are given in Table 1.

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TABLE 1. DETAILS OF PARAMETERS, NOTATIONS AND UNITS

Notation	Name	Unit	Value/range of selected parameters
Ab	Area of the bed	m^2	0.0552
Av	Vibratory acceleration	m/s^2	16.5908
Dp	Diameter of the particle	m	1.015, 1.85, 3.075, 4.375
Et	Total energy of air	J	--
L	Latent heat of vaporization	J/kg	--
Mm	Average moisture content	% dry basis	--
Mo	Initial moisture content	% dry basis	-- --
mo	Initial moisture content	kg	--
mp	Mass of the product	kg	0.5, 0.7, 0.8, 1.0
mpd	Mass of bone dry product	kg	--
mpm	Mean mass of the product	kg	--
T	Time	s	--
u	Velocity of air	m/s	0.2, 0.47, 0.7, 0.93
Vb	Volume of the bed	m	--
-	Temperature of air	°C	31.6, 49.4, 65.4, 83.4

Samples were collected at 5 min. interval. In order to get representative samples, the bed was mixed for a period of 10s before taking the sample. The collected samples were analyzed for moisture using ISI method (IS:1167-1965). The moisture ratio (Mm/Mo) is expressed as:

$$\frac{Mm}{Mo} = f\,(Et/Ab,\ Vb,\ Av,\ Dp,\ mpm,\ mpd,\ mo.L,\ T)$$

By carrying out dimensional analysis, we get

$$\frac{Mm}{Mo} = K \left[\frac{(Et/Ab)^3 VB^{10/3}\ mpm^4\ Av^6}{Dp^4\ mpd\ (moL)^6}\right]^{1/6} T$$

Moisture ratio = drying constant X drying factor

The absence of constant drying rate period was noticed in the present study. In general, the hindrances for faster heat and mass transfer processes are stagnant boundary conditions, diffusional mechanism, which is relatively slow process, stagnant bed of material itself etc. The advantage of vibrations is derived in the form of avoiding stagnancy in the boundary conditions, vigorous mixing of the bed etc. This is confirmed by obtaining initial drying rates in the presence and absence of vibrations as 41.5 and 34.8 kg/kg. h. m^2,respectively at 0.7 m/s velocity.

Fifteen trials were conducted with each having two replications. The average of two replications was considered for analysis. All the 131 data points were plotted on a graph as shown in Fig. 2. The

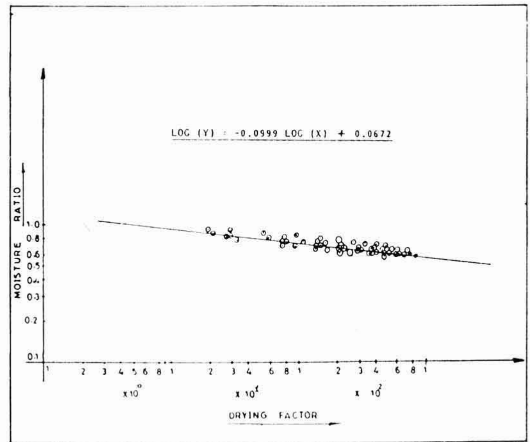

Fig. 2. Graphical presentation of the data analysed with the model

characteristic equation was derived by least squares method. The regression equation is:

$$\text{Log}\ (Y) = -0.0999\ \text{Log}\ (X) + 0.0672.$$

The correlation coefficient is -0.92. The validity of the relationship was verified by conducting experiments at different operating variables. For general range of parameters studied, the values of drying time predicted by the model varied the actual values by ± 2 to 25%. However, in two trials of high temperature and low particle diameter, the predicted values varied by 70% and 45%, respectively. As per the model, the drying rate is directly proportional to under root of temperature and inversely proportional to 2/3rd power of diameter of the particle. The collected data on analyzing and plotting on the graph showed the same trend confirming the model. The model will be useful in designing industrial fluidized bed system for drying casein.

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Controlled Fermentation of Vegetables Using Mixed Inoculum of Lactic Cultures

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Six isolates of Lactic Acid Bacteria (LAB) were selected on the basis of their homo- and hetero-fermentative character, salt tolerance and rate of acid production. The controlled fermentation of brined beet, carrot, cabbage, cucumber, cauliflower, ginger, green chilli, onion, radish and turnip was carried out using the combination of the 6 isolates. The fermented brined vegetables attained the desired acidity within 4 days at 28-30 °C and could be preserved at 28-30°C by adding 0.1% sorbic acid to the brine.

Keywords: Acid production, Controlled fermentation, Lactic acid bacteria, Vegetables.

Indigenous fermented foods are the state-of-the-art in many developing countries. Traditional pickles made from locally available seasonal vegetables are in increasing demand, as they are nourishing, have a longer shelf-life and are available throughout the year. However, the natural fermentation of vegetables, depending on the indigenous flora of raw vegetables, is a time consuming process and may result in spoilage, if the desired lactic acid bacteria are not present in sufficient numbers. The development of specific starter cultures with desirable properties is of prime importance to achieve consistent quality of the fermented brined vegetable. Commercially, starter cultures have been employed only to a limited extent for the preservation of vegetables.

Controlled fermentation of brined cucumber using pure cultures of Lactic Acid Bacteria (LAB) has been achieved in the West and is known to prevent economic loss due to pickle spoilage such as bloater, softness and off colours (Etchells et al. 1964). Several reports on controlled fermentation of vegetables have indicated the use of either one or two LAB cultures or in combination with yeasts (Azizi and Ranganna 1993; Fleming et al. 1978; Vaughn 1982; Daeschel et al. 1988; Yamani 1993). Pederson and Albury (1961) demonstrated that bloating, weight recovery and visual appearance of the cucumber pickles correlated with the relative influence of the homo-and hetero-fermentative species. There is limited information in the literature on the use of mixed inoculum of LAB as starter cultures for fermentation of vegetables (Daeschel and Fleming 1984).

The present study was undertaken with the objective of developing a mixed starter culture comprising homo-and hetero-fermentative LAB to bring about rapid fermentation of various vegetables in brine and preserve the product in an acceptable condition.

Isolation of LAB : The lactic cultures were isolated from naturally prepared sauerkraut (2.25% salt) and cucumber (8% brine) pickle at various stages of fermentation on Rogosa agar plates. Six isolates were identified and maintained according to the methods described by Sneath et al (1986) and Garvie (1984).

Selection of LAB: The homo-and hetero-fermentative ability of the isolates was determined using the differential medium, suggested by McDonald et al (1987) and Gibson's semi-solid agar butts (Harrigan and McCance 1976). Rate of acid production by each LAB isolate was studied by adding the washed cell suspension to 10 ml brine containing 4% NaCl, 0.1% $CaCl_2$ and 1% sucrose to attain the cell density of 9×10^8 cells/ml. Titratable acidity was determined according to the procedure described by Fleming et al (1984) and expressed in terms of % acidity. Rogosa agar plates containing 0%, 2%, 4%, 6% and 8% NaCl were streaked with each of the 6 isolates to study their salt tolerance. The LAB cultures were also screened for bacteriocin production using the agar spot test, as described by Schillinger and Lucke (1989).

Preparation of cell suspensions : Each of the 6 cultures was grown on Rogosa agar slant for 18h at 28–30°C under 5% CO_2. Saline suspension of each was prepared and their optical density was adjusted to 0.25 OD. units at 530 nm using an ERMA AE-11N photoelectric colorimeter and mixed in equal proportion to obtain the mixed inoculum. Two ml of this mixture, when inoculated in 10 ml sterile brine, gave a final cell density of 9×10^8 cells/ ml. The sterile brine used throughout the study contained 4% NaCl (salt), 1% sucrose (sugar)

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and 0.1% $CaCl_2$ to impart colour, texture and taste to the fermented product (Buescher and Burgin 1988).

Controlled fermentation of vegetables: Vegetables selected for fermentation were washed thoroughly in clean water, peeled and cut into uniform pieces. These pieces were then blanched in boiling water for 2 min and transferred into sterile conical flasks. The inoculated brine was poured over the pieces (45g/60ml; 75% w/v). Uninoculated brine controls for each type of vegetables were prepared similarly.

The vegetables were allowed to ferment for 4 days at 28-30°C during which aliquots of the brine were withdrawn at 24h intervals and analyzed for pH and acidity as described by Fleming et al (1984).

Preservation of the fermented vegetables : After 4 days of fermentation, the brine from all the pickles was drained off. The fermented vegetable pieces were rinsed in sterile saline and resuspended in sterile 4% brine, 0.1% $CaCl_2$ and 0.1% sorbic acid.

Sensory evaluation : All the fermented vegetables were analyzed for organoleptic acceptability using the triangle test (Lees 1975). Fifteen panel members consisting of both males and females in the age group of 23-48 years were selected for sensory evaluation of the samples. They were asked to rate different quality attributes by assigning scores ranging from 1-5 namely 1=unacceptable; 2=poor; 3= acceptable; 4= good and 5=best.

The 6 cultures *Lactococcus lactis* BM-12, *Pediococcus pentosaceus* BM-13, *Lactobacillus brevis* BM-14, *Lactobacillus plantarum* BM-15, *Leuconostoc mesenteroides* BM-16 and *Leuconostoc mesenteroides* BM-17 were selected on the basis of their biochemical characteristics, salt tolerance and rate of acid production (Table 1 and Fig.1) Three of the cultures were homofermentors and the other three were heterofermentors. All the 6 showed good growth in 4% salt, but *L. lactis* BM-12 and *L. plantarum* BM-15 were sensitive to 6% salt (Table 1). Each of the 6 cultures showed acid production in brine reaching maximum acidity in 4 to 5 h (Fig.1).

TABLE 1. BIOCHEMICAL CHARACTERISTICS AND SALT TOLERANCE OF THE LAB ISOLATES

Biochemical characteristics	BM-12	BM-13	BM-14	BM-15	BM-16	BM-17
Gas from glucose	-	-	+	-	+	+
HHD@ medium	H	H	HT	H	HT	HT
Arginine hydrolysis	+	+	+	-	-	-
Growth at 45°C	-	+	-	+	ND	ND
Dextran production	-	-	-	-	+	+
Acid from						
Glucose	A	A	A	A	A	A
Sucrose	A	-	A	A	d	A
Fructose	A	A	A	A	A	A
Maltose	A	A	A	A	A	A
Mannose	A	A	A	A	A	A
Xylose	d	A	d	A	-	A
Galactose	d	A	A	A	-	-
Lactose	d	A	A	A	-	-
Growth in salt						
0%	+	+	+	+	+	+
2%	+	+	+	+	+	+
4%	+	+	+	+	+	+
6%	d	+	+	d	+	+
8%	-	-	-	-	-	-
Bacteriocin production	-	-	-	-	-	-

@ Differential medium for homo-(H) and hetero-fermentation (HT)
A Acid production
d Delayed acid production/growth
ND Not determined

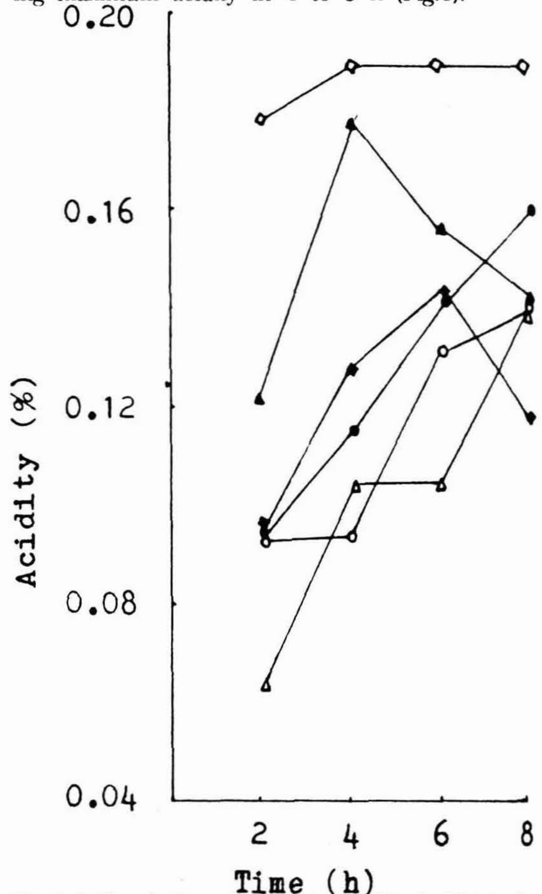

Fig. 1. Acid production in brine containing 4% salt, 0.1% $CaCl_2$ and 1% sugar by LAB isolates BM-12 (o), BM-13 (●), BM-14 (Δ), BM-15 (▲), BM-16 (◇) and BM-17 (◆)

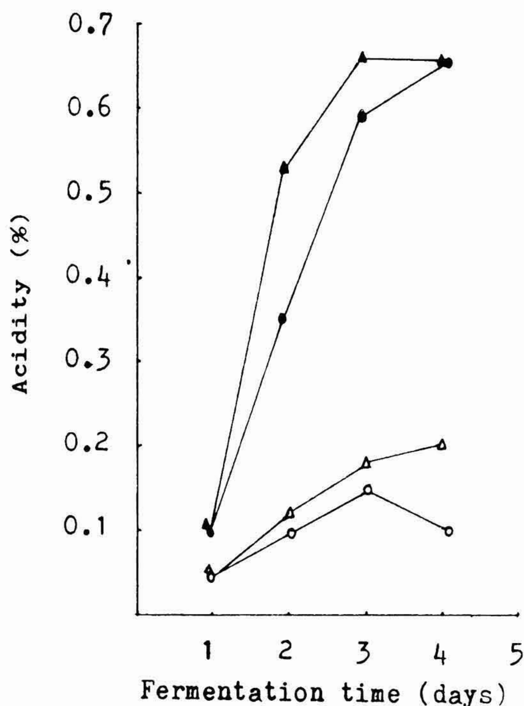

Fig. 2. Acid production in the brine of control carrots (o) and cucumbers (Δ),and of carrots (•), and cucumbers (▲), inoculated with the 6 LAB.

When the mixed inoculum of the 6 LAB cultures (9 x 10^8 cells/ml) was added to the brine of carrots and cucumbers, a rapid increase in the acidity was observed, attaining a maximum of 0.6 to 0.7% in 4 days (Fig.2). At the same time, the pH of the inoculated brine of the carrots and the cucumbers decreased from 6.0 to 3.0. The controls, however, showed only 0.2% acidity and pH 5.0 in the same period. An undesirable film of yeast was also observed on the 4th day in the controls, thus indicating that inoculation of LAB could prevent the development of undesirable organisms presumably due to acid production. Yamani (1993) also observed a gradual increase in yeast numbers in the brine to which no LAB were added.

Koli and Kulkarni (1973) had observed 0.8% acidity after 9 days of fermentation in cucumbers inoculated with only *Leuconostoc mesenteroides*. In another study on turnips, 0.5% acidity was attained after 16 days of fermentation, when inoculated with either *L.plantarum* or *L. mesenteroides* (Yamani 1993). In the present study, desired acidity in carrots and cucumbers was obtained within 4 days, when inoculated with the mixture of 6 LAB in 4% brine, 0.1% $CaCl_2$ and 1% sugar. Based on these results, 8 different vegetables viz., beet, cauliflower, ginger, green chilli, onion, radish, cabbage and turnip were individually subjected to controlled fermentation using the mixed LAB. The cut pieces of the vegetables permit rapid diffusion of salt and release of nutrients, which induce acid production by the LAB (Fleming et al. 1978). The final acidity after 4 days of fermentation was measured in the inoculated and the control vegetables. There was nearly 50% increase in acidity in the inoculated brined vegetables, as compared to their controls (Table 2).

The LAB fermented vegetables were preserved in 4% salt, 0.1% $CaCl_2$ and 0.1% sorbic acid, which prevented yeast growth. After 2 months of storage at 28-30°C, the fermented vegetables did not show any visible spoilage. All the vegetables retained their natural colour and were found to be acceptable with respect to appearance, flavour, crispness, sourness and taste, when presented to the taste panel for organoleptic evaluation (Table 2).

TABLE 2. PERCENT ACIDITY AND SENSORY CHARACTERISICS OF FERMENTED VEGETABLES

Vegetable	Control*	Test **	Appearance	Flavour	Crispness	Sourness	Taste
Beet	ND	ND	4.3 ± 0.7	3.6 ± 0.6	3.9 ± 0.2	3.6 ± 0.6	3.4 ± 0.2
Carrot	0.3	0.7	4.0 ± 0.6	3.4 ± 0.6	3.7 ± 0.9	3.4 ± 0.5	3.4 ± 0.5
Cucumber	0.2	0.7	3.6 ± 0.8	3.6 ± 0.8	3.8 ± 1.1	3.0 ± 0.4	3.2 ± 1.0
Cauliflower	0.3	0.7	4.1 ± 0.7	3.6 ± 0.8	3.5 ± 0.9	3.6 ± 1.1	3.6 ± 0.9
Ginger	0.3	0.6	3.5 ± 0.5	2.8 ± 0.6	3.2 ± 0.7	2.2 ± 0.7	3.1 ± 1.0
Green chilli	0.3	0.6	3.7 ± 0.7	3.6 ± 0.5	3.6 ± 1.1	3.0 ± 1.1	3.7 ± 0.8
Onion	0.2	0.6	4.0 ± 0.6	3.6 ± 0.8	3.3 ± 0.3	3.2 ± 0.7	3.4 ± 0.5
Radish	0.2	0.5	4.1 ± 0.3	3.6 ± 0.7	3.5 ± 0.5	2.7 ± 0.7	3.3 ± 0.7
Sauerkraut	0.2	0.7	3.5 ± 0.2	3.4 ± 1.0	3.5 ± 0.5	3.0 ± 0.8	2.9 ± 0.8
Turnip	0.5	0.9	3.8 ± 0.2	3.6 ± 0.7	4.1 ± 0.2	3.8 ± 0.3	3.6 ± 0.4

* Blanched vegetables uninoculated

** Blanched vegetables, inoculated with consortium of 6 LAB

ND = Not determined due to inherent pink colour of the beet brine

± = SD

The fermented brined vegetables can be used for preparing a variety of pickles by addition of spices and condiments to suit the tastes of the consumer. Thus, using controlled fermentation, losses in seasonal vegetables due to lack of cold storage facilities can be prevented and at the same time the vegetables be made more palatable.

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Surface Heat Transfer Coefficient In Steam Cooking of Vegetables

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Surface heat transfer coefficients in steam cooking of vegetables under transient conditions were evaluated. The temperature ratio (θ) was determined using the experimental temperature at the vegetable core. Fourier number (N_{fo}) was determined with thermal diffusivity (α), cooking time (t) and radius of the cylinder (r). Having obtained the value of Fourier number, the Biot number (Bi) was evaluated at each value of (θ) by using the Hessler's Chart. From these values, the corresponding surface heat transfer coefficient (h) was calculated. The average values of h for carrot, knolkhol and radish were 306, 307 and 298 W/m² C, respectively.

Keywords: Heat transfer coefficient, Vegetable cooking, Biot number, Fourier number.

It is important to know the change in the temperature of the thermal centre of the product and temperature distribution during the process as a function of time and external conditions, to define the process and design of the processing equipment. Accurate prediction of unsteady or transient state temperature distributions in foods during heating is important in both distribution and optimal use of heating process. In order to predict thermal changes, diffusional losses, energy consumption and the optimum process and equipment designs, data for the heat transfer properties of vegetables are required. Currently, there is a lack of such data, particularly in the relevant blanch (cooking) temperature ranges and heating media (Selman 1987).

Most of the studies carried out previously dealt with thermal processing of foods both in-container and aseptic processing, (Chang et al. 1990; Sastry 1984) and assumed negligible restriction to heat transfer. However, the temperature profile in food material is dependent on convective heat transfer coefficient at the fluid particle interface (Ramaswamy 1982). Alhamadan et al (1990) have investigated natural convection heat transfer between water and a mushroom-shaped particle using a simulated aluminium particle. However, the equations used are applicable to Bi <0.1. When Biot number is <0.1, there is negligible internal resistance to heat transfer, in which case the temperature is uniform throughout the interior of the object. (Singh and Heldman 1993). But, this is not true in the case of the vegetables as indicated in the present study (Fig.1). Vinod and Bera (1995) have estimated the surface heat transfer coefficient of faba bean puffed with sand, assuming radial and axial heat transfer for short cylindrical geometery.

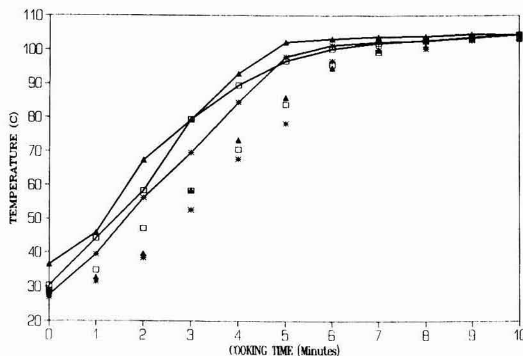

Fig. 1. Temperatures distribution of vegetables
Δ carrot; • knolkol; □ radish.

Nevertheless, the validity of these equations as they apply to cooking of vegetables has not been much attempted. Hence, the objective of this study was to determine surface heat transfer coefficients and define their importance in the cooking of vegetables.

Fresh carrot (*Daucus carota*) 'Desi' variety, knolkol (*Brassica oleracea*) 'Caolorapa' variety and radish (*Raphanus sativus*) 'White and stout' variety were obtained commercially on the day of the experiment and washed. Vegetables were washed and cut into cylindrical pieces of 20 mm dia and 40 mm length, using a cork borer and a sharp knife. Two special needle (1mm dia) type Copper-Constantan thermocouples were inserted, one at the core and the other at the surface. Three replicates were taken. All the thermocouples (3 x 2) along with the retort thermocouple was

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connected to a data logger (Model CDL 102 of Century Instruments).

The basic procedure was to heat the water in the retort until steam generation, keeping the cylindrical vegetables suspended by a specially constructed fixture to ensure proper heat convection around the vegetable, after steam generation. The retort's lid was closed securely and the outlet valve opened. The entire experiments were carried out at atmospheric pressure to simulate the actual continuous cooker.

The major mode of heat transfer was natural convection. The specific heat (Cp) of individual vegetables were determined by its individual composition, as described by Singh and Heldman (1993). The values of thermal conductivity (k), density (ρ) and thermal diffusivity (α) were calculated, as described by Toledo (1991). These values were estimated as the mean of initial product temperature (25°C) and the final temperature of 100°C. The food composition values were obtained from Gopalan et al (1991).

Temperature ratio (θ) was calculated from the temperature data T, Ta and Ti with Ti=25°C. T and Ta were determined with a range as indicated in Table 1. Fourier number (N_{fo}) was determined with thermal diffusivity, cooking time and radius of the cylinder. Having obtained the value of Fourier number, the line of 1/Bi, where Bi is the Biot number could be located at each value of (θ) in the Hessler's Chart. From the corresponding value of 1/Bi, the value of surface heat transfer coefficient h was calculated and the values for 3 vegetables is shown in Table 1.

Since the L/D ratio is >1, most of the heat transfers radially and only a small amount of heat transfers axially (Singh and Heldman 1993; Geankoplis 1978). Hence for an infinite slab, (Ta-Ta-Ti)=0.99 i.e., nearly equal to 1. Hence, one dimension heat conduction is considered in the radial direction only, as given by Geankoplis (1978).

TABLE 1. SURFACE HEAT TRANSFER COEFFICIENT (H)

Vegetable	Ta, C	T, C	t, min	θ	NFo	1/Bi	h, W/m²C
Carrot	103.0	86.0	5	0.220	0.576	0.20	306.75
	104.1	94.6	6	0.120	0.691	0.20	306.75
	105.0	100.9	8	0.051	0.922	0.20	306.75
Knolkhol	103.0	90.2	6	0.167	0.617	0.20	306.75
	104.0	98.3	8	0.072	0.823	0.21	292.14
	105.0	103.2	10	0.022	1.028	0.19	322.90
Radish	102.1	95.6	6	0.083	0.711	0.19	335.32
	104.5	99.2	7	0.067	0.829	0.25	254.84
	105.0	103.2	10	0.023	1.186	0.21	303.38

The equation for finite cylinder is given below,

$$\frac{(Ta-T)}{(Ta-Ti)_{finite\ cylinder}} = \frac{(Ta-T)}{(Ta-Ti)_{infinite\ cylinder}} * \frac{(Ta-T)}{(Ta-Ti)_{for\ slab}}$$

becomes

$$\frac{(Ta-T)}{(Ta-Ti)_{finite\ cylinder}} = \frac{(Ta-T)}{(Ta-Ti)_{infinite\ cylinder}}$$

Where,

T = Core temperature of the material at any time 't' (C)

Ta = Temperature of the heating medium (C)

Ti = Initial temperature of the material (C) and ambient temperature

As suggested by Vinod and Bera (1995) for low thermal conductivity (k<1) and when exposed to high heat transfer conditions during steaming, there is a temperature gradient within the vegetable. This is also indicated in Fig 1. The analytical solutions of unsteady state heat transfer

$$\frac{\partial Ta}{\partial t} = \frac{\partial^2 T}{\partial x^2}$$

is complicated. Hence, Hessler's Chart, which gives various values of temperature ratio (θ) at various Fourier numbers (Nfo) and Biot numbers (Bi) can be a simple and convenient procedure for determining the value of heat transfer coefficients. Fig 1 indicates the existence of temperature gradient for 3 vegetables with respect to cooking time. Similar curves have been obtained by Cacace et al (1994) in the thermal processing of particulate foods. It can be observed that there is some initial lag in the temperature curve upto 2 min for carrot and Knol-khol, indicating the fibrous nature of the outer core, which offers more resistance for heat penetration. This effect however, is absent in case of radish. It can also be observed that the surface temperature trend in all the three vegetables is similar. This indicates that all three vegetables are exposed to similar heating conditions.

Table 1 indicates the values of heat transfer coefficients and the values are in the range of 255 W/m²C to 323 W/m²C and the average heat transfer coefficients were 306, 307 and 298 W/m² C for carrot, knol-khol and radish, respectively. It

can be observed that the average Biot number is >0.1, indicating the existence of temperature gradient within the vegetable. Since the Biot number (Bi) lies between 0.1 and 40, there is a finite internal and external resistance to heat transfer.

In the large scale blanching or cooking of vegetables, one of the important factors required for the design of equipment is the heat transfer coefficient (h). Heat transfer coefficient indicates the efficiency of steam utilization. It also influences the process parameters. In the present study, surface heat transfer coefficients in steam cooking of vegetables under transient conditions were evaluated. The results of this work with respect to heat transfer coefficient data in the steam cooking of vegetable are important to understand the heat transfer mechanism during cooking.

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Characteristics of Cultured Milks, Yoghurt and Probiotic Yoghurts Prepared from Pre-refrigerated Milks

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In raw milk samples stored at refrigeration temperature (7±1 °C), the physico-chemical changes increased, as the storage period increased leading to clot-on-boiling test positive by 6th day of storage. The counts of all types of psychrotrophic bacteria greatly increased, while the counts of other types of bacteria appreciably increased during refrigerated storage. Acidophilus-cultured milk prepared from refrigerated milk had higher values in respect of all characteristics, when compared to that prepared from fresh milk. Lactis-cultured milk prepared from refrigerated milk recorded higher values in respect of some attributes. However, diacetylactis-cultured milk prepared from refrigerated milks generally had lower values in respect of all attributes. Short set yoghurt, long set yoghurt and probiotic yoghurts registered higher values, when refrigerated milks were used for their preparation.

Keywords : Cultured milks, Yoghurt and probiotic yoghurts, Refrigerated milks, Psychrotrophic counts.

Refrigerated storage of raw or heat processed milk prior to its conversion into products not only extends its shelf life but also provides greater convenience and flexibility in the manufacturing schedules (Reinheimer et al. 1989), allowing sufficient time to the manufacturer to test its suitability for end use, for example, presence of antibiotic residues and culture activity (Tamime 1981). Refrigerated storage allows growth of the psychrotrophic bacteria and when they multiply in large numbers, they cause structural changes in the constituents of milk by elaborating enzymes (Cousin and Marth 1977a; Rowe et al. 1990). These changes include breakdown of proteins and lipids, decreased pH etc., all of which may influence the performance of starter cultures in milk and affect the final quality of fermented milks. This communication describes the characteristics of various fermented milks such as cultured milks, yoghurt, and probiotic yoghurts prepared from milk, which had been previously held as raw milk at refrigeration temperature.

Pooled raw cow milk samples obtained from the University's cow-herd (Holstein-Friesien crosses) were brought to the laboratory and stored at 7 ± 1°C for upto 6 days in an incubator. During the storage period, the raw milk samples were drawn and tested for physico-chemical properties such as clot-on-boiling (COB), methylene blue reduction test (MBRT), pH, titratable acidity (TA) as % lactic acid, solids-not-fat (SNF) and fat (ISI 1981). Free fatty acid (FFA) content was estimated by the method of Fraskel and Tarassuk (1955), while tyrosine value (TV) was determined as per the method described by Hull (1947). Milk samples were also tested for bacteriological counts such as total, proteolytic and lipolytic psychrotrophic counts (Prabha and Shankar 1994), penicillin-resistant agar counts and violet red bile agar counts (Harrigan and McCance 1976).

Lactic cultures used in this study included *Lactobacillus acidophilus* 111, *Bifidobacterium bifidum* ATCC 11863, *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* spp. *diacetylactis* DRC, *Streptococcus salivarius* ssp. *thermophilus* STW and *Lactobacillus delbruckeii* spp.*bulgaricus* LBR. These cultures were obtained from the culture collection maintained in the department. All these cultures were grown in yeast glucose litmus chalk milk for upto 30 h at either 30°C, or 37°C depending on their optimum temperature and stored at -20°C as stock cultures. Working cultures prepared as above in autoclaved plain skim milk were stored at 7°C.

Fresh and refrigerated milks were used for the preparation of cultured milks, yoghurt and probiotic yoghurts. Milk samples standardized to 3.5% fat and 8.5% SNF were heat-treated at 90°C for 10 min. and cooled to room temperature. Appropriate starter cultures were inoculated at 1% (v/v) level either singly or in combination and incubated at suitable temperature. Long set method (20 h at 30°C) was followed for the preparation of various probiotic yoghurts in order to provide sufficient time for the cultures to grow and multiply in high numbers. Preliminary studies indicated that *L. acidophilus* and *B. bifidum* would not grow to sufficient numbers, when short set method (42°C/4h) was followed. All the fermented milks were tested for pH, TA, FFA and TV as described earlier.

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Starter counts (SC) of all the samples were determined using yeast glucose agar (Harrigan and McCance 1976), while sensory scores for flavour, firmness, body and texture were determined by a panel of tasters for a total of 10.

Chemical and bacteriolgical changes occurred in refrigerator milk during storage are shown in Table 1. Raw milk samples stored at 7°C became positive for COB test on the 6th day. The 6th day sample showed increased values in respect of TA, FFA and TV, to indicate acid-production, lipolytic and proteolytic changes taking place during the storage. It may also be seen that total psychrotrophic counts increased from 6.31 to 9.13 log_{10} cfu/ml at the end of the 6th day. Similarly, proteolytic and lipolytic psychrotrophic counts increased to 8.99 and 8.78 log_{10} cfu/ml, respectively. Gram-negative rod counts (expressed as penicillin resistant agar counts) and VRBA counts were also increased during the storage period.

Growth of all these types of bacteria during low temperature storage may have been responsible for the changes in the chemical composition of milk. Findings of the present study are in accordance with the observations of Hankin et al (1977); Muir et al (1978); Griffiths et al (1988) and Rowe et al (1990).

TABLE 1. CHEMICAL AND BACTERIOLOGICAL CHANGES OCCURRING IN POOLED COW RAW MILK DURING STORAGE AT REFRIGERATION TEMPERATURE (7±1°C).

Attribute	Storage periods, days			
	0	2	4	6
Physico-chemical				
Clot-on-boiling	-ve	-ve	-ve	+ve
Methylene blue reduction test, h	5	4	2	0.50
pH	6.68	6.60	6.52	6.15
Titratable acidity, as % lactic acid	0.15	0.17	0.18	0.25
Free fatty acids (ml. of 0.025N NaOH for 10 ml. of sample)	1.05	1.95	2.10	2.92
Tyrosine value, mg/5g sample	0.17	0.16	0.20	0.32
Bacteriological log_{10}cfu/ml				
Total psychrotrophic counts	6.31	7.36	7.78	9.13
Proteolytic psychrotrophic counts	6.27	7.34	7.57	8.99
Lipolytic psychrotrophic counts	6.00	7.08	7.14	8.78
Penicillin resistant agar counts	6.36	6.95	7.11	7.19
Violet red bile agar counts	5.69	6.51	7.19	7.56

Raw milk samples, which had been previously refrigerated, were subsequently used as the base for the preparation of various fermented milks. Three different individual cultures were used in the preparation of cultured milks and their characteristics are presented in Table 2. The acidophiluscultured milk prepared from

TABLE 2. CHARACTERISTICS OF CULTURED MILKS YOGHURTS AND PROBIOTIC YOGHURTS PREPARED FROM PRE-REFRIGERATED COW MILKS

Pre-refrigeration period, days	pH	Titratable acidity, % LA	Free fatty acids, ml	Tyrosine value, mg	Starter counts, log_{10} cfu/ml	Sensory scores, 10
Acidophilus-cultured milk (*L. acidophius* 111)						
0	4.70	0.70	1.50	0.24	7.69	5.4
2	4.60	0.75	2.10	0.30	8.55	5.9
4	4.60	0.75	2.10	0.33	8.61	6.6
Lactis-cultured milk (*L. lactis*)						
0	4.97	0.60	3.60	0.79	8.92	8.0
2	4.80	0.65	3.00	0.76	9.34	8.0
4	4.70	0.70	3.00	0.73	9.50	8.1
Diacetylactis-cultured milk (*L.diacetylactis* DRC)						
0	4.10	1.0	3.15	0.87	9.20	8.2
2	4.14	0.95	3.00	0.49	9.04	7.9
4	4.25	0.90	3.00	0.78	8.95	7.9
Short-set Yoghurt (42°C/4h) (*S. thermophilus* STW + *L.bulgaricus* LBR)						
0	4.70	0.69	2.25	0.21	7.74	8.4
2	4.60	0.75	2.25	0.375	7.90	8.7
4	4.50	0.79	2.25	0.695	8.47	8.9
Long-set Yoghurt (30°C/20h) (*S. thermophilus* STW + *L.bulgaricus* LBR)						
0	4.30	0.90	3.30	0.21	8.49	8.1
2	3.85	1.20	4.95	0.48	8.67	8.6
4	3.75	1.30	4.65	0.98	8.94	8.7
Acidophilus-Yoghurt (30°C/20h) (*S. thermophilus* STW + *L.bulgaricus* LBR) + L. acidophilus 111)						
0	3.95	1.10	4.10	0.29	8.41	7.4
2	3.90	1.15	4.25	0.29	8.64	8.3
4	3.90	1.15	4.10	0.45	8.62	8.3
Bifidus-Yoghurt (30°C/20h) (*S. thermophilus* STW + *L.bulgaricus* LBR) + *L. acidophilus* ATCC 11863)						
0	3.95	1.10	3.70	0.43	8.60	5.4
2	3.70	1.35	5.25	0.72	8.79	5.9
4	3.65	1.40	6.60	1.30	8.83	7.6
Acidophilus-Bifidus Yoghurt (30°C/20h) (*S. thermophilus* STW + *L.bulgaricus* LBR) + *L.acidophilus* 111 + *B.bifidum* ATCC 11863)						
0	3.85	1.20	3.30	0.49	8.73	6.0
2	3.80	1.20	3.00	0.49	8.79	6.5
4	3.65	1.40	7.80	1.00	8.92	7.6

unrefrigerated milk had the lowest TA, FFA, TV, SC and SS. As the pre-refrigeration period of raw milk increased, the achidophilus-cultured milk prepared from it showed higher values with respect to all the attributes. In respect of lactis-cultured milk, FFA and TV were higher, when unrefrigerated milk was used, but TA, SC and SS values were higher, when refrigerated milks were used. It is interesting to note that *L. diacetylactis* has performed better in unrefrigerated milk as compared to refrigerated milk, indicating that on refrigeration, certain changes especially lipolytic changes occurring in raw milk might have retarded the growth of *L. diacetylactis* . Kalogridou-Vassiliadou (1989) has reported that fatty acids especially caprylic and lauric acids have the ability to retard the growth of lactococci.

The characteristics of five different types of yoghurts and probiotic yoghurt prepared from refrigerated milks are also shown in Table 2. Both short set yoghurt prepared from refrigerated milks had higher values in respect of all attributes, when compared to yoghurts prepared from unrefrigerated milks. Acidophilus-yoghurt prepared from refrigerated milk generally had higher characteristics than that prepared from unrefrigerated milks. As regards bifidus-yoghurt, it can be seen that 4-day old refrigerated milk was found to be most suitable followed by 2-day old refrigerated milk and unrefrigerated milk. Both 2-day old refrigerated milk and unrefrigerated milk exhibited nearly similar characteristics, when used for the preparation by acidophilus-bifidus yoghurt. The increased activity of lactic cultures in refrigereated milks has been attributed to heat stable enzymes and protein degradation products elaborated by psychrorophic bacteria during refrigerated storage (Cousin and Marth 1977b; Kielwein (1982). Similar enhanced growth and lactic acid production by yoghurt cultures in refrigerated milk have been observed by Tayfour et al (1981).

The results of the present study indicate that instead of fresh milk, refrigerated milk stored upto 4 days could be most suitable for the production of quality fermented milks.

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Physical, Physiological and Cooking Qualities of Pearl Millet and Their Correlations

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Physical, physiological and cooking qualities of pearl millet seeds were studied. The data showed positive and significant correlations between cooking time and weight/size and volume of 500 seeds. Path-coefficient analysis revealed that 500 seed weight, seed volume, hydration capacity and hydration index were the most important parameters for cooking time. These relationships enable to screen and determine large number of pearl millet genotypes as longer, normal and shorter cooking time types.

Keywords: Pearl millet, Physical quality, Physiological and cooking characters, Correlation.

Pearl millet (*Pennisetum glaucum* (L.) R.Br.) is the fourth most important cereal of India after rice, wheat and sorghum. It provides cheap staple food with comparatively more nutrients (proteins, fat, carbohydrates, minerals etc.) to millions of poor people, cattle and poultry. Also, it is considered as the 'food of the people' in Sudan. The nutritional value, flavour, odour and texture of the cooked product are important to determine its acceptance and suitability as human food. Cooking time is also an important aspect of cooking quality. There is no published information on the cooking quality or cooking time of pearl millet. In the present communication, results on the relationship between physical, physiological, cooking qualities and cooking time of pearl millet are presented.

Thirty six genotypes (12 hybrids, 12 inbreds and 12 open pollinated varieties) were used as the experimental materials. Seed size/500 seed weight (g), seed volume (ml/seed), density (g/ml), hydration capacity (g/seed), hydration index (g/seed/g), swelling capacity (ml), swelling index (ml/seed/ml) and cooking time (min.) on each genotype were recorded in duplicate in 3 replications. Data were subjected to analysis of variance, correlation coefficient (Johnson et al. 1955) and path analysis (Dewey and Lu 1959).

Mean squares due to genotypes were significant for all characters in hybrids (H), inbreds (I), open pollinated varieties (V) and genotypes (G), indicating that sufficient variation existed among the materials for the parameters and the data can be processed further.

The phenotype correlation coefficients between physical and physiological characters are presented in Table 1. The results clearly indicated that 500 seed weight was significantly positively correlated with seed volume (H, I, V and G), hydration capacity (I and G) and cooking time (G). Seed volume was significantly positively correlated with hydration capacity (I and G) and cooking time (G). Seed density, showed significantly positive association with swelling index (V and G) only. Hydration capacity showed significantly positive association with hydration index (H, I and G). Swelling capacity also showed significantly positive association with swelling index (H and G).

It is apparent that many of the characters/measurements show significant positive or negative association because of their nature of distribution. When more variables are considered, their significant associations are complex to understand the importance of each character. So, the path-coefficient analysis would provide an efficient means of separating both direct and indirect causes of association and permit critical examination of the specific forces, acting to produce a given correlation and measure the relative importance of each causal factor.

The component characters taken into consideration in path analysis showed significant associations with cooking time and the results are presented in Table 2. Of the 8 characters, 500-grain weight (I), seed volume (V) and hydration capacity (H and G) showed maximum positive direct effect on cooking time, whereas, hydration index (H and G), seed volume (I) and swelling capacity (V) showed maximum negative direct effect on cooking time. The positive association between 500-seed weight and cooking time (G) was largely contributed by the direct effect of 500-seed weight itself. Indirect effects of 500-seed weight via seed-volume (H and V) and hydration capacity (H and G) were also

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TABLE 2. PHENOTYPIC CORRELATION COEFFICIENTS BETWEEN PHYSICAL AND PHYSIOLOGICAL CHARACTERS OF PEARL MILLET

Character		500-seed weight	Seed volume	Density	Hydration capacity	Hydration index	Swelling capacity	Swelling index	Cooking time
500-seed weight, g	H	1.00	0.86**	-0.11	0.45	0.10	0.47	-0.11	0.51
	I	1.00	0.87**	-0.02	0.64*	0.08	0.25	-0.60*	0.15
	V	1.00	0.77**	0.04	0.49	-0.28	0.24	-0.38	0.50
	G	1.00	0.81**	-0.01	0.50**	-0.07	0.29	-0.40**	0.37*
Seed volume, ml/seed	H		1.00	-0.42	0.57	0.23	0.36	-0.22	0.51
	I		1.00	-0.47	0.74**	0.30	-0.00	-0.66*	-0.01
	V		1.00	-0.52	0.45	-0.15	0.09	-0.63*	0.47
	G		1.00	-0.51**	0.55**	0.09	0.14	-0.57**	0.35*
Density, g/ml	H			1.00	-0.44	-0.30	0.24	0.50	-0.17
	I			1.00	-0.36	-0.45	0.49	0.29	0.17
	V			1.00	-0.17	-0.30	0.34	0.68*	0.04
	G			1.00	-0.27	-0.35	0.32	0.54**	-0.04
Hydration capacity,g/seed/g	H				1.00	0.83**	0.34	-0.01	0.28
	I				1.00	0.78**	0.26	-0.37	-0.13
	V				1.00	0.64*	0.33	-0.15	0.08
	G				1.00	0.75**	0.25	-0.24	0.15
Hydration index, g/seed/g	H					1.00	0.11	-0.03	-0.03
	I					1.00	0.18	0.01	-0.20
	V					1.00	-0.03	-0.08	-0.35
	G					1.00	0.03	-0.06	-0.12
Swelling capacity, ml	H						1.00	0.79**	0.37
	I						1.00	-0.03	0.22
	V						1.00	0.66*	0.03
	G						1.00	0.49	0.22
Swelling index, ml/seed/ml	H							1.00	0.06
	I							1.00	0.07
	V							1.00	-0.23
	G							1.00	-0.05
Cooking time, min	H								1.00
	I								1.00
	V								1.00
	G								1.00

*, ** significant at 5% and 1% levels, respectively

TABLE 2. CORRELATION COEFFICIENTS DIRECT (DIAGONAL) AND INDIRECT (OFF DIAGNAL) EFFECTS OF VARIOUS CHARACTERS ON COOKING TIME

Character		500-seed weight	Seed volume	Density	Hydration capacity	Hydration index	Swelling capacity	Swelling index	r with cooking time
500-seed weight, g	H	0.22	0.10	0.01	0.19	-0.05	0.04	-0.00	0.51
	I	1.71	-0.96	0.01	-0.57	0.03	0.08	-0.16	0.15
	V	0.03	0.81	0.01	0.04	0.05	-0.12	-0.29	0.50
	G	0.17	0.10	0.00	0.11	0.02	0.02	-0.06	0.32*
Seed volume, ml/seed	H	0.19	0.12	0.05	0.25	-0.11	0.03	-0.02	0.51
	I	1.49	-1.10	0.35	-0.66	0.09	-0.00	-0.17	-0.01
	V	0.03	1.06	-0.15	0.04	0.03	-0.06	-0.47	0.47
	G	0.14	0.12	0.07	0.13	-0.03	0.01	-0.09	0.35*
Density, g/ml	H	-0.02	-0.05	-0.11	-0.19	0.14	0.02	0.04	-0.17
	I	-0.03	0.52	-0.74	0.32	-0.14	0.12	0.08	0.17
	V	0.03	-0.55	0.29	-0.02	0.06	-0.24	0.51	0.04
	G	-0.00	-0.06	-0.14	-0.06	0.12	0.03	0.08	-0.04

(Contd...)

TABLE 2. CONTD..

Character		500-seed weight	Seed volume	Density	Hydration capacity	Hydration index	Swelling capacity	Swelling index	r with cooking time
Hydration capacity/g/seed	H	0.98	0.02	0.05	0.44	-0.40	0.03	-0.00	0.29
	I	1.09	-0.82	0.22	-0.90	0.24	0.09	-0.10	-0.13
	V	0.02	0.48	-0.05	0.09	-0.12	-0.23	-0.11	0.08
	G	0.09	0.07	0.39	0.23	-0.25	0.02	-0.04	0.15
Hydration index,g/seed/g	H	0.22	0.03	0.03	0.36	-0.48	0.00	-0.00	-0.03
	I	0.14	-0.33	0.34	-0.71	0.31	0.06	0.00	-0.20
	V	-0.01	-0.15	-0.09	0.06	-0.18	0.00	-0.00	-0.35
	G	-0.01	0.01	0.05	0.17	-0.33	0.00	-0.01	-0.12
Swelling capacity, ml	H	o.10	0.04	-0.31	0.15	-0.05	0.09	0.07	0.37
	I	0.42	0.00	-0.37	-0.23	0.05	0.34	-0.01	0.22
	V	0.01	0.10	0.10	0.03	0.01	-0.71	0.49	0.03
	G	0.05	0.02	-0.05	0.06	-0.01	0.08	0.07	0.22
Swelling index, ml/seed/ml	H	-0.02	-0.03	-0.06	-0.00	0.02	0.02	0.38	0.06
	I	-1.03	0.73	-0.22	0.33	0.00	-0.01	0.26	0.07
	V	-0.01	-0.67	0.20	-0.01	0.00	-0.47	0.74	-0.23
	G	-0.07	-0.07	-0.08	-0.05	0.02	0.04	0.15	-0.05

* significant at 5% level

positive. The significant and positive association between seed volume (G) and cooking time (G) was because of the direct effect of seed volume itself. The indirect effects of seed volume through 500-seed weight and hydration capacity (G) were also high and positive.

The path coefficient analysis showed better understanding of the contribution of different characters e.g., the hydration capacity and hydration index had non-significant correlation with cooking time, whereas, path analysis showed that these were important characters. It may be concluded from the combined results of correlations and path analysis that 500-seed weight, seed volume, hydration capacity and hydration index were the most important parameters for cooking time for pearl millet.

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Preparation and Evaluation of *Acidophilus* Yoghurt

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A yoghurt-like product was prepared using *Lactobacillus acidophilus* strain 301, having antagonistic activity towards enteric pathogens and strain 1899, exhibiting hypocholesterolemic effect. It was compared with a regular yoghurt for its quality characteristics. Both the products were found to be almost identical in compositional, textural and sensory attributes. However, acetaldehyde content in acidophilus yoghurt was much less than in the regular one.

Keywords: *Lactobacillus acidophilus, Streptococcus salivarius* sub sp. *thermophilus, Lactobacillus delbrueckii* sub sp. *bulgaricus*, Textural characteristics, *Acidophilus* yoghurt, Regular yoghurt.

Lactobacillus acidophilus fermented food products have been recommended as a dietary adjunct because of their beneficial effects on health. These include stabilization of gut microflora, antagonistic activity towards intestinal and foodborne pathogens, control of serum cholesterol level, prevention of colon cancer and stimulation of host immune mechanism (Perdigon et al. 1986; Gilliland 1989; Mital and Garg 1992). Milk, an essential component of our diet, serves as an excellent medium for the preparation of fermented products using *L. acidophilus*. However, *L. acidophilus* strains considerably vary in their activity and growth characteristics in milk. Johnson et al (1987) suggested that strain that could produce sufficient acid in milk with desirable traits should be used for the manufacture of *acidophilus* products. In an earlier study, Gupta et al (1996) found that *L. acidophilus* strain 301 produced sufficient amount of acid and also showed inhibitory activity towards enteric pathogens, whereas strain 1899 exhibited hypocholesterolemic effect. The present communication reports the preparation of a yoghurt-like product, using combination of these strains. The product thus prepared was also compared with a regular yoghurt for its acceptability.

Lactobacillus acidophilus strains 301 and 1899, *Streptococcus salivarius* sub sp. *thermophilus*-H and *Lactobacillus delbrueckii* sub sp. *bulgaricus*-W were procured from Culture Collection Centre, National Dairy Research Institute, Karnal, India. The cultures were maintained by fortnightly transfers in sterilized litmus milk and held at 4 ± 1°C between transfers. The cultures were sub-cultured in sterile litmus milk 2-3 times prior to use. The cultures were examined regularly for purity and activity.

Fresh cow milk obtained from Livestock Research Centre of the University was used. For testing *L. acidophilus* strains for acid production individually and in combination, 400 ml. of sterilized milk (110°C for 10 min.) was tempered to 37°C and was inoculated with 16-18 h culture at the rate of 1% in case of individual strains and 0.5% of each, when acid production by the strains in combination was studied. Samples were withdrawn aseptically at pre-determined intervals and analyzed for acid production.

Milk was preheated to 60°C. Thereafter, 6% sugar and 0.1% carboxymethylcellulose were added and the contents were again heated to 90°C for 15 min. The milk was then cooled to 37°C, inoculated with active cultures of *L. acidophilus* 301 and 1899 at the rate of 1.5% each for *acidophilus* yoghurt preparation and incubated at 37°C for 8 h. Regular yoghurt was prepared in a similar manner using *S. salivarius* sub sp. *thermophilus* - H and *L. delbrueckii* sub sp. *bulgaricus*-W as the fermenting organisms at an incubation temperature of 42°C for the same period.

Acid production in the samples was determined by the method of Atherton and Newlander (1977) by titrating 18 g of the sample in duplicate with 0.1 N NaOH, using phenolphthalein as the indicator. The changes in pH were directly measured using a digital pH meter. Fat, ash, and moisture contents were determined by AOAC (1975) procedures. Nitrogen was determined by Kjeldahl method and a conversion factor of 6.38 was used to convert nitrogen to protein. (AOAC 1975). Sharp extraction method for soluble nitrogen (Kosikowski 1966) and modified Lane and Eynon method for total and reducing sugars (Ranganna 1986) were used. Acetaldehyde content (ppm) in the samples

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was estimated according to the method of Robinson et al (1977).

Total viable and coliform counts were determined according to APHA (1978) procedures using lactic agar and violet red bile agar, respectively. Pour plates were prepared in duplicate and incubated at 37°C for total viable and at 32°C for coliform counts.

The samples were analyzed for textural characteristics using Instron universal testing machine (Instron Inc., Canton, MA, USA), according to the procedure described by Bourne (1968). Instron crosshead fitted with 100 N load cell and 25 mm dia plunger was cycled with a vertical reciprocating movement at a constant speed of 50 mm/min. and a stroke length of 10 mm. The X and Y scales of the plotter were set at 10 min. and 2 N, respectively.

A uniform and representative 50 g portion of the product was subjected to two cycles of compression to give a first and second bite. The texture profile curves were plotted with time (min) on the X-axis and force (N) on the Y-axis and used to determine hardness, cohesiveness, springiness, gumminess and chewiness.

Freshly prepared samples of *acidophilus* and regular yoghurts were evaluated for appearance, colour, texture, body, flavour and overall acceptability, using a 9 point Hedonic scale, where 1 represented dislike extremely and 9 represented like extremely (Larmond 1977). The taste panel consisted of 12 semi-trained members selected from the staff and students of the department. The samples were cooled to 4 ± 1°C and served at random to the same panelists for two replications of the study. Significance of the Hedonic test data (means of two replicates) was evaluated using 'T' test (Larmond 1977).

Fig. 1 shows acid production by *L. acidophilus* strains 301 and 1899 individually and in combination. The former produced greater amount of acid than the latter. In contrast, acid production by combination of the two (1:1) was greater than attained by either of the strain individually.

Table 1 shows product characteristics of regular and *acidophilus* yoghurts. Both the products had more or less the similar quantities of major and minor constituents. However, acetaldehyde content was about one and a half times more in regular than in *acidophilus* yoghurt. Marshall and Cole (1983) reported that *L. acidophilus* elaborated the enzyme alcohol dehydrogenase, which converted most of the acetaldehyde to alcohol. As a

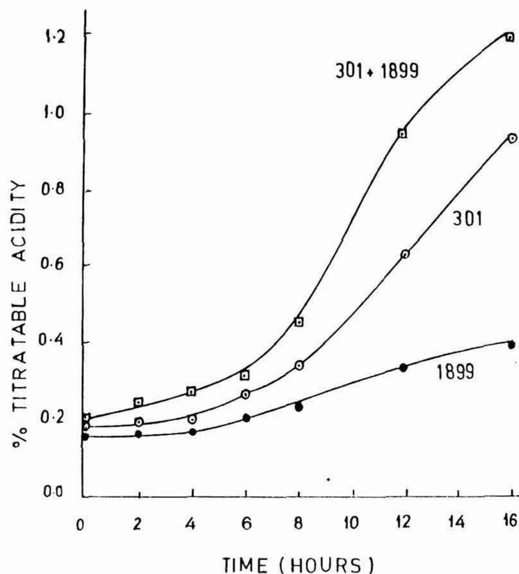

Fig. 1. Acid production by *L. acidophilus* strains individually and in combination

TABLE 1. PRODUCT CHARACTERISTICS OF REGULAR AND *ACIDOPHILUS* YOGHURT

Chemical	Regular yoghurt	*Acidophilus* yoghurt
Moisture, %	82.00 ± 1.50	81.00 ± 1.10
Total sugars, %	9.10 ± 0.21	9.60 ± 0.26
Reducing sugars, %	4.00 ± 0.15	4.20 ± 0.05
Fat, %	4.00 ± 0.20	4.00 ± 0.10
Protein, %	3.85 ± 0.04	3.70 ± 0.16
Ash, %	0.70 ± 0.04	0.70 ± 0.02
Soluble nitrogen, %	0.16 ± 0.05	0.11 ± 0.04
Acetaldehyde, ppm	29.40 ± 7.53	16.60 ± 3.80
Titratable acidity, %	0.95 ± 0.05	0.80 ± 0.07
pH	4.10 ± 0.08	4.40 ± 0.07
Textural		
Hardness[a], force in Newton	0.56 ± 0.05	0.51 ± 0.06
Cohesiveness[b], A_2/A_1	0.22 ± 0.03	0.28 ± 0.04
Springiness[c], cm	0.40 ± 0.00	0.47 ± 0.30
Gumminess[d], force in Newton	0.13 ± 0.26	0.15 ± 0.03
Chewiness[e], force in Newton	0.05 ± 0.01	0.07 ± 0.02
Microbiological		
Total viable counts, cfu/ml	2.00×10^9	1.60×10^9
Coliforms, cfu/ml	Nil	Nil

Values are mean ± SD of 3 determinations. [a]Peak force of first compression cycle. [b]Ratio of area under the second peak A_2 and the area under the first peak A_1. [c]Width of the second down stroke curve. [d]Hardness x cohesiveness. [e]Gumminess x springiness.

result, lesser quantities of acetaldehyde were present in *acidophilus* products. Hickey et al (1983) observed that the enzyme threonine aldolase would convert excess threonine to acetaldehyde via glycine without concomitant increase in alcohol dehydrogenase activity. Therefore, acetaldehyde content of *acidophilus* yoghurt could be enhanced by fortifying the processed milk with threonine (Marshall 1987). The total viable counts in *acidophilus* yoghurt was 1.60 x 10^9/ml. This number of viable *L. acidophilus* in the product was adequate to achieve the desired results (Gilliland et al. 1978). Coliforms were not detected in either of the product.

No significant differences in the textural charcteristics of both the products were observed (Table 1). Organoleptic evaluation revealed that both the products were almost identical with respect to colour, flavour, appearance, texture, and body characteristics and overall acceptability with a score ranging from 7.4 to 7.8 on the 9 point-Hedonic scale (data not presented).

The results show that *acidophilus* yoghurt, comparable in acceptability to regular yoghurt, could be prepared by using combination of appropriate *L. acidophilus* strains. Selection of strains having beneficial traits would also impart therapeutic value to the product.

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Physico-chemical Characteristics and Processing Quality of Newly Introduced Seven Tomato Cultivars into Jordan in Comparison with Local Variety

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The physico-chemical characteristics and suitability for processing into *puree* of tomato fruits from 7 cultivars ('Riogrande', 'Piccaduli', 'Turkish round', 'Lima', 'Savio', 'Marreha' and '378-87'), newly introduced into commercial cultivation in Jordan were compared with the local variety ('Rohaba'). Results showed that 'Rohaba' variety had better fruit weight with lowest yield, followed by 'Turkish round', which had intermediate yield. The maximum yield was shown by 'Lima' cultivar, but its fruit was small. The juice yield was maximum in 'Riogrande', while others were comparable. The total soluble solids were higher in 'Savio' than other cultivars. The acidity was maximum in 'Turkish round' followed by 'Rohaba' variety. The *puree* prepared from 'Turkish round' cultivar performed better than 'Rohaba' or the other cultivars in sensory evaluation due to higher consistency and colour. 'Piccaduli' was the next best variety.

Keywords: Tomato, Physico-chemical Characteristics, Composition, Cultivars, *Puree*, Processing.

Tomato (*Lycopersicon esculentum*, Mill, is a leading crop in Jordan, the annual production of which has increased from 275.6 in 1991 to 231.3 thousand metric tonnes in 1993 (Anon 1993) to meet the increasing demand by the 4 tomato processing plants. Tomatoes are consumed directly as salads, in meals cooked into soups and cooking recipes, or processed into juice, ketchup, whole peeled tomato and tomato paste. In the present investigation, *puree* was selected as a test product, because it is an intermediate product for juice, ketchup and paste manufacturing (Sethi and Anand 1986; Gowda et al. 1994). The purpose of this study was to compare the newly introduced tomato cultivars with a local variety by determining their physical properties, chemical composition and to test their suitability for processing and stability during storage.

Materials: Seeds of 7 tomato cultivars ('Riogrande', 'Piccaduli', 'Turkish round', 'Lima', 'Savio', 'Marreha' and '378-87') and local cultivar ('Rohaba') were sown and nursery was raised at Jordan University of Science and Technology experimental station. When the seedlings were 35 days old, they were transplanted, spaced at a distance of 0.5 m within rows. Each cultivar was replicated 3 times (25 plants per replicate) in a randomized complete block design (RCBD). The harvesting of fruits was started from 2 months. At the peak of harvest season (mid harvest period), a representative composite sample of red-ripe fruits were collected for each replicate and transferred to the laboratory for analysis.

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Physical properties: Twelve fruits of each replicate were randomly selected and the average fruit weight, fruit volume (by water displacement), specific gravity (weight in air/weight in water) were recorded. Fruits were blanched for 4 min in boiling water, peeled and the percentage of peel and pulp (including seeds) were determined. Another lot of fruits was used to count number of locules and afterwards, the green portions were trimmed, cut into small pieces and the juice, then, was extracted. The % weight of fruits and the percentage yield of juice were computed. The total soluble solids (TSS) in the juice were determined by a hand refractometer (0-32°B) at room temperature and the necessary corrections for temperature were applied. The pH, titratable acidity (TA), total solids of juice, reducing sugars and ash content were determined by AOAC (1984) methods, while the refractive index (RI) was determined by using a bench top refractometer. The % insoluble solids (ISS) were calculated from the weight of insoluble solids left on a pre-weighed filter paper after filtering the soluble solids.

Preparation of puree: Six kilograms of tomato fruits from each replicate, were washed, cored and cut into pieces. These were heated to boil in their own juice for 15 min and then passed through a 30 mesh stainless steel sieve to collect the juice. The juice was concentrated in an open pan by heating on a hot plate to obtain a TSS of 14°B, then filled hot into clean sterilized 200 ml bottles, screw capped and processed for 15 min in boiling water. The *puree* was analyzed for various chemical constituents. The sensory quality of the *puree* was

assessed by a panel of 15 judges, using a Hedonic scale, with scores 30, 30 and 40 for colour, consistency and flavour, respectively. The colour of the fresh and *puree* stored for 6 months was evaluated by measuring L, a, b, and a/b colour values by Colorgrad system instrument (model 05, Parma, Italy).

Statistical analysis: All the data collected were statistically analyzed using the randomized complete block design with three replicates and the means were compared at a probability of 5% level (Steel and Torrie 1982).

Physical properties: The physical properties of tomato fruits are shown in Table 1. Fruit weight varied significantly in the investigated cultivars. 'Rohaba' cultivar had a significantly higher weight than others, followed by 'Turkish round'. 'Piccaduli' had the lowest fruit weight. Fruit volume followed

TABLE 1. PHYSICO-CHEMICAL CHARACTERISTICS OF TOMATO CULTIVARS AND THEIR CORRESPONDING *PUREE* AT 0 AND 6 MONTHS STORAGE

	Tomato cultivars								
	'Rohaba'	'Riogrande'	'Piccaduli'	'Turkish round'	'Lima'	'Savio'	'Marreha'	'378-87'	LSD
Yield, T/ha	20.900	38.100	25.500	36.700	45.100	29.700	41.800	39.90	13.740
Fruit weight, g	256.400	76.100	60.600	173.900	65.300	75.700	68.400	93.50	35.100
Volume, ml	264.700	78.300	63.300	177.000	68.700	80.000	75.700	95.00	36.450
Specific gravity, g/ml	0.967	0.970	0.950	0.977	0.950	0.940	0.903	0.77	NS
Peel, %	1.800	2.700	1.700	1.300	1.900	3.300	3.000	2.90	1.135
Pulp, %	98.200	97.300	98.300	98.700	98.100	96.700	97.000	97.10	1.135
Juice, %	91.600	97.200	90.500	94.800	88.400	90.700	87.100	84.20	8.391
Locule No	14.700	2.000	3.000	7.300	3.000	2.700	2.000	4.30	1.682
Total soluble solids, %	5.500	5.100	4.300	4.300	4.000	5.900	4.700	4.60	1.256
pH	4.600	4.400	4.300	4.400	4.300	4.500	4.300	4.30	0.157
Titratable acidity, %	0.300	0.300	0.200	0.500	0.100	0.200	0.300	0.30	0.248
°Brix	4.200	4.300	4.100	4.200	4.300	4.200	4.200	4.20	0.303
Vitamin C, mg/100g	17.900	27.700	24.700	19.800	8.600	11.900	8.400	8.90	8.523
Reducing sugars, %	4.000	1.100	2.600	2.700	4.100	4.000	2.900	2.80	1.465
Ash, %	0.700	0.600	0.500	0.600	0.500	0.600	0.500	0.60	0.199
Puree yield, %	27.200	28.600	25.000	23.900	24.900	27.900	25.800	24.10	6.858
			***Purees* fresh, 0 month storage**						
°Brix	16.300	15.100	14.900	16.100	14.800	15.400	14.200	14.200	2.288
pH	3.900	4.000	4.100	4.000	4.000	3.900	3.900	3.900	0.078
Titratable acidity, %	1.200	1.600	1.600	1.600	1.700	1.500	1.400	1.600	0.266
Refractive index	1.357	1.356	1.355	1.358	1.354	1.354	1.354	1.355	NS
Specific gravity, g/ml	1.027	1.023	1.025	1.026	1.025	1.027	1.023	1.026	NS
Total soluble solids, %	15.500	14.300	14.500	15.400	14.200	14.300	14.000	14.300	1.793
Reducing sugars, %	4.600	3.400	5.100	2.400	3.800	4.100	1.400	3.700	0.769
Ash, %	2.000	5.600	3.900	6.100	4.600	6.200	5.100	5.600	1.531
Insoluble solids, %	3.500	2.800	2.900	4.000	3.400	2.600	2.600	2.900	1.466
			6 months storage						
°Brix	16.300	16.800	15.600	16.500	16.400	15.500	15.800	14.000	2.843
pH	4.000	3.900	3.900	3.900	3.800	4.200	4.100	4.400	0.166
Titratable acidity, %	1.800	1.800	1.900	2.000	1.600	1.400	1.700	1.800	0.551
Refractive Index	1.358	1.357	1.357	1.358	1.358	1.356	1.357	1.358	NS
Specific gravity, g/ml	1.037	1.037	1.035	1.037	1.035	1.033	1.041	1.033	NS
Total soluble solids, %	16.800	17.300	15.800	17.500	17.600	16.500	17.100	15.300	3.005
Reducing sugars, %	7.400	6.500	10.400	8.100	8.700	5.900	9.500	5.600	1.424
Ash, %	1.700	1.600	1.500	1.900	1.900	1.600	1.600	1.700	0.629
Insoluble solids, %	3.500	3.900	3.300	3.900	5.300	5.200	5.300	5.100	1.441

NS = Not significant

• All values are based on 90 % moisture content

a similar trend as fruit weight. Specific gravity for these cultivars showed no significant differences at P < 0.05. The peel and peel contents varied significantly between cultivars, the number of locules was highest in 'Rohaba' (14.7) and lowest in 'Riogrande' and 'Marreha' (2.0), which was significantly different (P< 0.5). The % juice content varied significantly and ranged from 84.2 (in cv '378-87') to 97.2 % ('Riogrande'). The highest yield of fruits per hectare was produced by 'Lima' (45.1). It was almost twice that of 'Rohaba', which gave the lowest (20.9 Ton/ha) among these cultivars, but the yields varied significantly at P<0.05.

Chemical composition of fruits: The total solids (TSS), pH, titratable acidity (TA), Brix of juice (°B). vitamin C, reducing sugars (RS) and ash content of tomato fruits are shown in Table 1. The TSS content was maximum in 'Savio', while it was the lowest in 'Lima'. The acidity varied from 0.1 to 0.5%, but the differences were statistically significant. The Brix values for the tomato fruits were not significantly different, but large variations were observed with respect to vitamin C content, the lowest value being in 'Marreha' and the highest in 'Riogrande'. 'Rohaba' variety possessed significantly higher vitamin C content than 'Lima', 'Savio', 'Marreha' and '378-87'. The reducing sugar content was the highest in 'Lima' and 'Riogrande' had the lowest. The differences between °B and TSS values are dependent on insoluble solids of the tomato fruits, which are usually very low in fully ripe fruits (Gowda et al. 1994).

Chemical composition of puree: Table 1 shows data on the chemical composition of *puree* prepared from the different tomato cultivars. No significant differences were observed in *puree* yield, TSS, refractive index specific gravity, TSS and insoluble solids in the *puree* prepared from the different tomato cultivars, but pH, TA, reducing sugars and ash content showed significant differences in freshly prepared *puree*. The chemical analysis of *puree*, which was carried out after 6 months of storage, showed very slight variation in the results obtained for freshly prepared *purees*, but maintained the same trend of differences between cultivars.

Sensory evaluation: Sensory evaluation of *puree* was carried out without altering the chemical composition of the different samples, which differed in 1°Brix in TSS. The data are shown in Table 2. The 'Turkish round' cultivar was best suited for processing, since it scored maximum for colour, consistency, flavour and overall acceptability. This was followed closely by 'Piccaduli' cultivar. The fresh *puree* prepared from the local cultivar also was close to 'Turkish round' and the variation between them was not significant at P < 0.05. Total scores of 'Rohaba' *puree* were low due to low color and consistency scores, apart from flavour.

Evaluation of puree *colour:* Data on puree colour measurements for L, a, b, a/b values are

TABLE 2. SENSORY AND COLOUR EVALUATION OF TOMATO *PUREE* PREPARED FROM TOMATO CULTIVARS AT 0 AND 6 MONTHS STORAGE

Tomato cultivar	Sensory scores			
	Colour (30)	Consistency (30)	Flavour (40)	Total (100)
	Fresh, 0 month storage			
'Rohaba'	24.20	24.70	33.10	82.00
'Riogrande'	25.40	24.40	25.50	75.30
'Piccaduli'	25.60	22.20	32.70	80.30
'Turkish round'	25.90	27.10	32.70	85.70
'Lima'	24.90	23.70	31.10	79.70
'Savio'	23.80	22.80	31.40	78.70
'Marreha'	25.70	`8.30	31.70	75.70
'873-87'	19.90	20.60	30.50	71.00
LSD (P< 0.05)	3.84	5.46	5.81	9.11
	6 months storage			
'Rohaba'	21.40	20.60	31.40	76.10
'Riogrande'	23.40	23.90	26.00	73.30
'Piccaduli'	23.60	23.20	33.20	80.00
'Turkish round'	25.50	25.70	33.80	85.00
'Lima'	24.10	21.60	28.80	75.50
'Savio'	23.90	22.20	31.70	72.80
'Marreha'	24.50	223.00	32.40	79.80
'378-87'	23.60	24.80	31.40	79.80
LSD (P< 0.05)	4.57	6.42	5.77	11.20
	Fresh, 0 month storage (Colour values)			
		a	**b**	**a/b**
'Rohaba'	30.80	26.10	18.70	1.39
'Riogrande'	30.30	28.50	18.40	1.55
'Piccaduli'	27.10	27.20	16.70	1.63
'Turkish round'	31.20	28.50	19.00	1.49
'Lima'	26.90	24.90	16.40	1.51
'Savio'	27.30	23.10	16.70	1.39
'Marreha'	27.60	25.70	17.00	1.54
'378-87'	30.80	20.90	19.30	1.08
LSD (P< 0.05)	3.61	3.64	2.35	0.22
	6 months storage			
'Rohaba'	23.8	21.9	14.2	1.54
'Riogrande'	24.8	19.6	14.7	1.33
'Piccaduli'	22.4	19.7	13.5	1.46
'Turkish round'	23.5	19.9	14.2	1.40
'Lima'	21.6	20.6	12.9	1.60
'Savio'	23.1	22.8	13.9	1.64
'Marreha'	22.8	19.0	13.7	1.39
'378-87'	25.1	21.6	15.0	1.44
LSD (P< 0.05)	3.03	5.88	1.67	0.35

shown in Table 2. The fresh *puree* of 'Piccaduli' cultivar possesed the maximum a/b value, followed by 'Riogrande'. After 6 months storage, there were no significant differences in a/b value, for all cultivars. 'Savio' cultivar had the highest a/b value probably due to high red colour value. Except for L values, a, b and a/b values were slightly lower than colour values reported by Porretta (1993).

In conclusion, the physico-chemical and processing characteristics of the investigated tomatoes varied significantly. These cultivars can be divided into 3 groups according to the purpose as follows:- (i) 'Turkish round' and 'Piccaduli' for processing (ii) 'Savio, 'Marreha' and '878-87' for fresh fruit trade and (iii) 'Rohaba', 'Riogrande' and 'Lima' for both purposes.

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CARBOHYDRATES AS ORGANIC RAW MATERIALS III: Edited by H. Van Bekkum, H. Roper, and A.G.J. Voragen. Published by VCH Verllagsgesellschft mbH, Weinheim, Germany and VCH publishers Inc., New York, USA. 1996, pp 315, Price-not mentioned.

The book with 16 chapters mainly deals with the utilization of carbohydrates as organic raw materials and gives a clear perspective of the current research status going on in this important area. Various chapters in this book are the extended versions of the lectures given by eminent scientists working on carbohydrates, during the Third International Workshop on Carbohydrates as Organic Raw Materials at The Netherlands in November 1994. As many of the authors, who have contributed in this book are from the industry, this book is mostly practically oriented and will be very valuable for scientists working in the field of upgrading renewable resources.

Utilization of large amounts of by-products produced by the beet sugar and cereal processing industries with special reference to structural characteristics, physical properties and enzymatic modification of the chemical structure are well presented in the first chapter. The second chapter gives an overview on starch properties, its food and non-food applications and on starch-based raw material requirements in industry. The state-of-art on the metal-catalyzed oxidation and hydrogenation of carbohydrates and some industrial applications of the final product have been discussed in depth in the third chapter. There are two chapters in this book, one on inulin and the other on lactose, highlighting different aspects concerning production, analytical aspects, physico-chemical, nutritional and functional properties, major food applications as well as some future developments of these two unique carbohydrates. Evaluation of how carbohydrates and other agricultural raw materials can effectively be converted into existing or new chemical compounds by fermentation are discussed in detail in chapter 6. Chapter 7 gives the updated information of the production of lactic acid by fermentation and downstream processing aspects involved therein, as well as its application in food, pharmaceuticals and cosmetic industries. Chapters 8 and 9 deal on the synthesis of new saccharide polymers and resistant starch and provide an in-depth coverage on the importance of unsaturated monosaccharides for use in radical polymerization reactions and the physiological implications of the consumption of resistant starch by humans, which is comparable to dietary fibres. The possible industrial utilization of amylose for the encapsulation of guest molecules by introducing different substituents into the amylose chain with a variety of guest molecules are presented in great detail with schematic representation in chapter 10. The book has two chapters devoted to carbohydrate-based surfactants, in which several important parameters of non-ionic carbohydrate-derived surfactants and a comparison of the surfactant properties with conventional surfactants are discussed in great depth. The last chapter of the book describes some basic aspects of the chemistry of oxygen-based bleaches and the use of bleach activators with special focus on carbohydrate-based bleach activators.

The book is very informative and covers all major aspects on the utilization of carbohydrates as raw material for product development and is very well presented. The extensive references given at the end of each chapter are comprehensive. In short, it is a book written by internationally renowned scientists from industry and academic sectors and is a valuable source of information of immense practical interest. It will remain as a very useful reference book for many years.

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FRUIT PROCESSING: Edited by D. Arthey and P.R. Ashurst, Published by Blackie Academic and Professional, Wester Cleddeus Road, Bishopbriggs, Glasgow, G64 2HZ, UK, 1995, pp 248, Price £ 65/-

The book contains the following chapters written by professionals involved in fruit growing, post-harvest handling and processing. It is a useful introductory book on fruit science and technology.

Introduction to fruit processing: (R.B. Taylor, Borthwicks Flavours, UK): A global survey of fruit processing is given, including the origin of the fruit and its processing from cottage scale to modern automatic plant. Technical and commercial factors affecting fruit processing are discussed. Different fruit types and the effects of their structure and physico-chemical characteristics like texture, distribution of edible and non-edible portions, composition on processing methods and the products made therefrom are presented. Ripening changes vis-a-vis ethylene production, respiration, flavour genesis and composition are presented. Influence

of variety, environmental factors and processing changes on quality, including flavour are discussed. Finally, the threats and opportunities in the global market are highlighted especially since citrus, pomace and berry fruits are now grown in many parts and shortages and gluts in one part affecting the market globally. There are also dangers of existing sophisticated adulteration, which requires newer tools of detection such as SNIF NMR.

Fruit and human nutrition: (P.C. Fourie, Stellenbosch Institute of Fruit Technology, S. Africa): This chapter discusses the chemical composition of various fruits with respect to their nutritional value. A discussion on the fruits as source of essential nutrients especially the vitamins, minerals and dietary fibre is given. Finally, the losses of nutrients in processing are discussed and the importance of proper storage and processing techniques on minimising the losses of nutrients is stressed.

Storage, ripening and handling of fruit: (Beattie B. and Wade N., NSW Agric., Hort. Res. and Advisory Station, Australia): Definition of maturity and ripening is given with respect to chemical changes and the eating quality of fruits. Respiratory climacteric is explained with examples. Ethylene causes changes in fruits including artificial ripening and degreening. Temperature affects physiology of fruits and so, could be used beneficially to control post-harvest changes. Chilling temperature is quite beneficial, but some tropical fruits respond unfavourably. Various methods of pre-cooling and advantages of faster cooling methods such as pressure, hydro- and vacuum-cooling are described. Useful hints in the management of cold room for fruit storage, including maintenance of high humidity to prevent moisture loss from fruits, dry coil evaporators to minimise water condensation, good insulation to prevent leakage of heat, which lowers humidity etc. are given. Effects of lower O_2 and increased CO_2 on prolonged storage of fresh fruits are discussed for various fruits. Controlled atmosphere (CA) technology is described, giving methods of creating CA. Modified atmosphere is created by using plastic laminates and is useful during transport. Some discussion on maintenance of quality during storage/handling is given vis-a-vis microbial spoilage and its control, disorders due to improper temperature and nutrient deficiency and the mechanical damage due to handling and insects.

Production of non-fermented fruit products: (P. Butledge, CSIRO Food Res. Lab, Australia): Fruit juice is prepared from high quality fruits, not suitable for fresh market. Maturity and variety affect the juice quality. Various methods for preparing juices from temperate fruits and tropical fruits are described. Extraction and pressing machinery have been described. Clarification by centrifuge or finishers, followed by the use of fining agents, enzymes and ultrafiltration is presented with examples. Comparing several methods of preservation such as thermal, aseptic, chemicals, freezing and membrane filtration, merits of these are highlighted and machinery described. Critical factors in the use of plastic bottles are given. Concentration incurs losses of volatile flavours. So, essence is recovered before concentration and added back to concentrate. Various evaporators including multi-stage, multi-effect, thermal-accelerated-short-time (TAST) and centrifugal have been elaborated. Listing different products from fruit juice, a discussion on means of detecting the adulteration is presented. Use of NIR has been suggested for the same.

Cider, perry, fruit wines and other alcoholic fruit beverages: (B. Jarvis, H.P. Bulmer Ltd., UK): Tracing the history of wine production and of various alcoholic products from fruit are listed. Discussion is mainly on cider (apple wine). After characterising the cider apples, the management of orchards, practices and care in harvesting and pressing of apple to obtain juice are described. Fermentors, wooden and modern ceramic or resin-lined MS, glass reinforced plastic and SS of even 200,000 gal. are used. Tall vats cause excess hydrostatic pressure, slowing down *S. cerevisiae* activity. Apple juice alone or with sugars or pear juice can be fermented to yield alcohol content of 7 to 12%. SO_2 is added to control contaminants. Secondary malo-lactic fermentation gives better flavour. Final processing includes blending, clarification. Standards of National Association of cider makers are given. Specialty ciders are described. Detailed microbiology related to cider is given in which mechanism of yeasts fermentation, microbes in secondary fermentation, conrol of contamination by SO_2 and microbial spoilage of cider are discussed. Sparkling ciders contain added CO_2. Chemistry of fermentation process and changes in chemical composition, affecting cider flavour and quality are discussed. Description of perry making from pear juice and wines from various fruits including juice preparation, fermentation, matura-

tion, blending, clarification etc. is given. Variations like fortified and sparkling wines and brandy and liquors are detailed.

Production of thermally processed and frozen fruit: (G. Burrows, Hillsdown Hold. plc, UK): Care in selecting raw material, causes of deterioration and control are given. Importance of hygiene in cannery is emphasised. Raw material testing, preparation of fruit like peeling and blanching by different methods and machinery, can filling, exhausting and its effect on vacuum, seaming and thermal processing are described. Different types of retorts, water/steam, batch/continuous, still/agitated etc. are compared. Canning processes for fruits and their products are given. Bottles and jars are used to a limited extent. Freezing of fruits discusses the mechanism of action of low temperature on microbes and the importance of rapid freezing. Various freezing processes such as room, blast, spiral belt, fluidized bed and liquid nitrogen freezing are compared. Care in storing frozen fruit products at recommended temperature and in proper packaging is emphasised. Discussion on aseptic packaging, its advantages, the process essentials and the different packaging types and forms used is also given.

The manufacture of preserves, flavourings and dried fruits: (R.W. Broomfield, UK): After discussing legal standards of jams and other products, importance of fruits quality is emphasised. Technono-legal aspects of fruits used fresh, frozen or chemically preserved are discussed. Jams are prepared from various fruits with sugar, acid and pectin. Standards, recipes and preparation of jams, jellies, marmalades etc. are described. Fruits are also preserved by coating with sugar to prepare candies. Making of glace cherry and candied citrus peel is described. Drying is an important method of preserving fruit. Processes of drying grapes and some tree fruits are detailed. Finally, preparation of tomato *puree* is given, including care to minimise the losses of colour and vitamins.

The by-products of fruit processing: (R. Cohn and A.L. Cohn, Ruth Cohn Rulek Ltd., Israel): By-products are prepared to minimise cost and the waste disposal problems. About 40-65% of citrus fruit is peel and albedo from which by-products such as essential oil, carotenoids, cellulose, pectins and flavonoids as well as pulp wash concentrate can be recovered. Processes of preparing pulp wash concentrate and various by-products from citrus fruits are described. Removal of limonin and naringin from by-products is necessary for debittering. Processes of debittering and commercial preparation of hesperidin and naringin are given. Citrus oils are important flavour materials and different methods of preparation, distillation and concentration are described. Chemistry of different pectins is discussed with commercial production from fruit waste.

Water supplies, effluent disposal and other environmental considerations: (M.J.V. Wyman, First Effluent Ltd., UK): Water is an important ingredient in fruit processing industry. Its quality largely determines the quality of products. There is also waste water, generated needing disposal. After listing sources of water used in fruit industry, different treatment methods are described to produce water, which meets microbial and other chemical and physical standards. Different uses of water in fruit industry needs different qualities. Their standards are given. The treatment is not only important from environmental point but also there are legal restrictions on quality of water that can be released for disposal. Physical, chemical and microbial treatments are described.

The book tries to describe many aspects in the limited space, which makes it a very good introductory book at undergraduate level. Thee are some excellent discussions given, especially on the microbiological and chemical aspects of fruit wine preparation with special emphasis on cider and the role of SO_2. Even the chapter on fruit juice with machinery and operations description of apple and orange juice preparation is noteworthy. Some modern developments are described such as essence recovery processes, membrane clarification and pasteurisation, aseptic and other methods of thermal processing. It also gives some useful discussions on tropical fruits such as papaya, mango and guava. Other chapters containing worthwhile details are one on storage, ripening and handling. The first gives very useful suggestion for recovery from waste and the second gives excellent discussion on post-harvest care of fruits.

I would also recommend this book for research scientists for these extremely useful aspects covered in the book.

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QUALITY CONTROL FOR FOODS AND AGRICULTURAL PRODUCTS - Second Edition, Edited by J.L. Multon. Translated by Denis Dochain, VCH Publications, D 69469, Weinheim Bundesrepublik, Deustschland Federal Republic of Germany, 1996, pp 286 Price DM. 198/-

The book consists of 14 chapters on general principles of practical metrology, sampling and statistical control, comparison of methods, reliability and maintenance of measurement devices, On-line control and problems with sessions, deontology in the choice and use of methods for efficient control, data acquisition and computer use, automation and standardisation of methods of analysis as well of control systems, circuits of the analysis and their statistical interpretation, reference materials, quality control and management and control approach and philosophy in the plants.

Effective introduction to the concept of metrology in terms of why measure and what to measure is dealt with in chapter 1. Further details regarding the accuracy and precision of measurements, the standardisation of measuring systems for various parameters' hierarchy in measuring methods, absolute methods, calibration methods, empirical methods, errors that occur in measurements, repeatability and sampling fluctuations have been effectively dealt with.

Chapter 2 provides useful information on statistical sampling, estimation, quality control of the final product and control during production. Control by variables as well as atrributes, operating characteristic curves with examples, final product inspection through single, double, progressive sampling procedures and setting up of control charts, both static and dynamic, have been explained with examples.

Comparison of methods of analysis, accuracy, precision, repeatability, reproducibility and calibration have been discussed in chapter 3. Uses of linear regression for calibration or comparison of two given methods are discussed at length.

Chapter 4 highlights quality analysis using instruments to tackle the well known variation problems in agricultural products. The various analytical instruments, requirements in terms of their sensitivity and reliability, maintenance and repair by the customer service has been brought home very well.

On-line control and problems with sessions and use of biosensors and the needs for 'real time' and 'non destructive' measurements in various unit operations of primary and secondary processing of food have been effectively discussed in chapter 5.

Chapter 6 deals with deontology in the choice and use of methods with the objective of obtaining efficient control. The easiest and the most cost-effective methods for adaptation have been discussed from the point of view of on-line quality control.

Data acquisition methods, computer-aided methods and the common statistical methods applicable for data analysis have been dealt with in chapter 7.

The methods and tools, which presently are being used for the automation for specific tasks, the importance of functional analysis for the implementation of the automatic control system have been discussed in chapter 8. Also, it has been attempted to highlight the future requirements for automation in the laboratory. Use of robotics and setting up an automated sensory analysis facility have been emphatically stressed.

Chapter 9, the Standardisation Organisations, French, European and International, their activities and the process of standardisation of methods for analysis have been discussed in detail. Main criteria used for the characterisation of any method have been described as accuracy, sensitivity, reliability and precision to which, ease of implementation, performance rapidity, equipment cost and overall cost need to be added. The types of control methods required in the food industry have been identified as physical, chemical, physico-chemical, microscopic , microbiological and sensorial. The procedures, the establishment rules and the tools are also recommended to be used on co-ordination basis.

Circuits of analysis and the Bureau Inter professional d' etudes analytiques (BIPEA) example deals with comparison of tests among several laboratories for the same samples and the dispersion of the results that occur because of sampling, the methods and the laboratory. Certification of the laboratories has also been discussed in chapter 10.

The next three chapters (11-13) highlight the essential statistical methods for interpretation of the circuits of the analysis, reference materials, quality control and management.

The last chapter on control approach and philosophy in the plants is a wrap-up, stating that in the present situation of the rapidly changing world, the notion "control" is out of date and has

to be replaced by "quality and controlling process of their quality".

On the whole, the book is a good compilation of several important aspects of quality control for foods and agricultural products, which will be useful for achieving accreditation of laboratories. The steps to provide baseline structures of analytical laboratories are clearly brought home. Important references have been provided. The appendices on legal measuring units and their conversion factors with a global reference will be most useful.

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NUTRITIONAL BIOCHEMISTRY- By S. Ramakrishnan and S. Venkat Rao, Published by T.R. Publications (P) Ltd., 57, South Usman Road, PMG Complex (II Floor), T.Nagar, Madras-600 017, 1995 pp. 246. Price Rs. 95.00.

Food is a basic need for all human beings. Proper nutrition helps in eliminating many deficiency diseases in developed countries, but unfortunately, the developing and underdeveloped nations are still facing the problems of malnutrition and undernutrition. Women and children are particularly vulnerable sections of society, who are placed at a disadvantageous position with respect to nutrition. It is time for us to introspect about facts and myths of good nutrition. The 'Nutritional Biochemistry' by Prof. S. Ramakrishnan and Dr. S. Venkat Rao provides an opportunity for recalling the importance of balanced diets to fulfil the societal goals of the government in terms of 'Food with good nutrition for all at affordable cost'.

The contents of the book are divided into 2 sections. Section A deals mostly with the concept of balanced diet, nutritional situation, nutritional disorders and nutritional value of Indian foods. These chapters deal with the basic components of food, measurement of nutritional properties, energy values, biological values of proteins, proteins and calorie malnutrition, essential fatty acids, vitamins and minerals. A chapter is also devoted to safety aspects of naturally occurring toxicants and antinutritional factors in foods. Some information is also given on the ways for inactivating these food toxicants. Nutritional disorders found in India are also highlighted in a separate chapter. Extensive data are also given on the nutritional value of Indian foods. Section A concludes by analysing nutrition situation in India.

Section B contains 15 chapters on topics of general interest on food science and nutrition. Various interesting possibilities such as role of sugars in atherogenesis, protein concentrates from Lichens to combat malnutrition, obesity as cause for various diseases, importance of essential fatty acids in human health, the versatile role of vitamin C in health and prevention of diseases, vital role of iron and damage it can cause if abused, the facts and myths about excess intake of hormones, vitamins, minerals, fasting and feasting, exercise, influence of coffee on heart diseases and deleterious effects of alcoholism are all dealt with in a concise manner.

The various topics dealt in the book are of considerable importance to graduate and postgraduate students of Food Science, Nutritional Sciences, Home Science, Biochemistry and other related areas. The subject matter is written in a concise and easily understandable way. Even though the chapters in section B raises some interesting points on nutrition and health, the references given in support of discussions are fairly old and in some chapters no references are given at all for further reading. The recent and important references on these various topics would have given an opportunity for a curious student to further dig into recent developments in the area. These shortcomings, however, do not come in the usefulness of the book for majority of the students. The book is an useful addition to libraries and for personnel collection of students dealing with various aspects of Nutritional Biochemistry.

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