

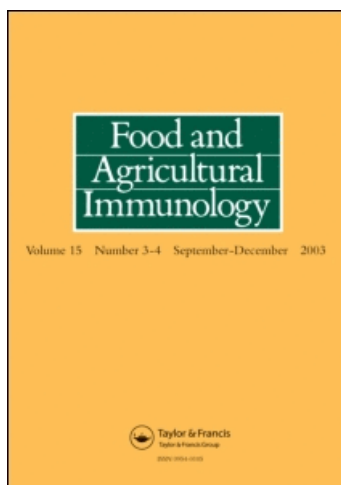
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REVIEW

Immunochemical characteristics of wheat proteins

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The purpose of this paper is to provide a comprehensive overview of wheat protein characteristics and of enzyme-linked immunosorbent assay (ELISA) technique for wheat quality characteristics determination. Literature review is to report the identification of low and high molecular weight subunits of glutenins with ELISA. Immunological assay has been reported to be used as an alternative method to reduce the test time and cost. ELISA is an immunological technique involving an enzyme (a protein that catalyses a biochemical reaction) to detect the presence of an antibody or an antigen in a test sample. Currently available knowledge on immunochemical characteristics of wheat proteins is insufficient. That's why this review is unique in its comprehensive nature for immunochemical characteristics of wheat proteins.

Keywords: immunochemical characteristics; wheat proteins; ELISA

Introduction

Cereals are monocots belonging to the grass family (Gramineae) and 70% of the world's farmland has been devoted to the cultivation of these crops. Wheat, corn, rice and barley are major cereal crops grown in the world. Wheat occupies an important position among cereal grains with respect to production and utilisation. The quality of wheat is generally assessed on the basis of different physical, chemical, biochemical and rheological properties. The products prepared from wheat grains require different quality characteristics. The wheat flour's ability to be processed into different food products is mainly determined by the quantity and quality of gluten proteins (Weegels, van de Pijpekamp, Graveland, Hamer, & Schofield, 1996). The mature wheat grains contain 8–20% protein. The gluten proteins constitute up to 80–85% of total flour proteins and are responsible for imparting elasticity and extensibility character to the dough, essential for the functionality of wheat flours (Shewry et al., 1984). The main constituents of the gluten are gliadins and glutenins contributing a major role in the rheological and bread-making properties of the wheat flour. The unique elastic and cohesive properties of wheat dough are mainly due to glutenin, which determines the end-use quality of the baked products. Enzyme-linked immunosorbent assay (ELISA) may be an effective tool for assessing

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chapatti and bread-making qualities of different wheats. The two most used variants of ELISA are the indirect and the sandwich ELISA. The indirect ELISA utilises two antibodies, one of which is specific to the antigen and the other which is coupled to an enzyme. This second antibody gives the assay its 'enzyme-linked' name, and will cause a chromogenic or fluorogenic substrate to produce a signal. The detection antibody can be coupled to an enzyme or can bind the conjugate (enzyme-linked antibody) that will produce the biochemical reaction (Goldsby, Kindt, Osborne, & Kubly, 2003). Both polyclonal antibodies (PABs) and monoclonal antibodies (MAbs) can be used in ELISA techniques for identification of food components. PABs offer a number of benefits such as recognition of a mixture of different epitopes of the antigens, more tolerance to small changes in the nature of antigen, like polymerisation or slight denaturation, and they are a preferred choice for detection of denaturated proteins. Food authenticity can be carried out through an ELISA test (Liu, Chen, Dorsey, & Hsieh, 2006). The use of ELISA has been reported for the identification of low molecular weight (LMW) and high molecular weight (HMW) subunits of glutenins associated with the bread-making quality of wheat flours (Brett et al., 1993).

Wheat proteins

The wheat proteins are complex in nature but possess unique characteristics which are often attributed to them (Weegels et al., 1996). Most of the technological properties related to dough-making and baking are dependent upon the wheat proteins. The flour-baking quality is also directly related to the type of proteins (Shewry & Halford, 2002).

The wheat contains four types of proteins that include albumins (soluble in water and dilute buffers), globulins (not soluble in water but soluble in saline solutions), prolamins (soluble in 70–90% ethanol) and glutenins (soluble in dilute acid or alkali). Most of the albumin proteins identified from proteomic analyses of mature grain endosperm of wheat (Singh, Blundell, Tanner, & Skerritt, 2001) and barley (*Hordeum vulgare* L.; Finnie, Melchior, Roepstorff, & Svensson, 2002) belong to a family of α -amylase/trypsin inhibitors. The gliadin and glutenin protein fractions consist of storage proteins and are defined as proteins that accumulate during the grain-filling period (Shewry & Halford, 2002).

The gliadins are heterogeneous mixtures of single-chained polypeptides which are, in their native state, soluble in aqueous alcohol. The gliadins can be separated into four groups, denoted as α -, β - and ω -gliadins, when fractionated through polyacrylamide gel electrophoresis at low pH. These are important in intramolecular disulfide bond formation that also renders their stability against aggregate formation. Therefore, they are considered to be less critical for the bread-making potential of wheat flour (Dubcovsky et al., 1997). The gliadin has a good extensibility but is lacking in elasticity which is a fundamental requirement in the dough-making process (Cheftel, Cug, & Lorient, 1985). On the other hand, glutenins are large molecular weight proteins (several millions) which are multi-chained (Graveland et al., 1985). These are elastic in nature, blending of both these proteins in the dough brings a specific elasticity and extensibility which can then be used in the processing of different flour products. Wall (1979) pointed out the relationship between gluten proteins and elasticity of dough also determines the suitability of

dough for bread-making. The ratio of glutenin and gliadin influences the viscoelasticity of the gluten mass (Janssen, Vereijken, & Vliet, 1991) and is further used to correlate the quality of bread or chapatti prepared from different wheat cultivars (Ram & Nigam, 1981; Uthayakumaran, Newberry, Keentok, Stoddard, & Bekes, 1999).

MacRitchie (1980) delineated that the differences in baking quality can be attributed to the whole gluten protein or to a specific protein fraction. Graveland, Bosveld, Lichtendonk, Moonen, and Scheepstra (1982) reported that flour of good bread-making variety Sicco possessed 45% glutenins and 11% glutelins compared with 38% glutenins and 18% glutelins for flour of a poor bread-making variety, such as Tundra. Graybosch and Morris (1990) reported that correlations between end-use quality characteristics (associated with substituted chromosomes) and endosperm storage proteins are reliable only when proteins of both gliadin and glutenin fractions are analysed. Bunce, White, and Shewry (1985) isolated gluten by washing method and reported its composition on dry matter as gliadin 43%, glutenin 39%, other proteins 4.4%, lipid 2.8%, sugars 2.1%, and starch 6.4% with some cellulose and mineral matters.

The wheat quality is assessed by the molecular structure of the wheat storage proteins which, in turn, control the interactions of the proteins during the bread-making process (Bushuk, 1998; Shewry, Tatham, & Halford, 1999). The bread-making quality is significantly altered by variation in composition as well as the protein content of wheat flour (Branlard, Dardevet, Saccomano, Lagoutte, & Gourdon, 2001; Lafiandra, Masci, Blumenthal, & Wrigley, 1999; Weegels et al., 1996).

Wieser and Kieffer (2001) stated that molecular weight distribution of wheat proteins was primarily responsible for the viscoelastic properties of flour dough and furthermore high molecular weight glutenin (HMW; GS) plays the major role. The glutenin proteins (polymeric proteins) are among the largest protein molecules in nature (Wrigley, 1996) with molecular weight of glutenin polymers reaching over 20 million Daltons. These proteins are heterogeneous mixtures of polymers formed by disulphide-bonded linkages of polypeptides (Payne, Corfield, & Blackman, 1979; Payne, Holt, Jarvis, & Jackson, 1985; Thompson, Bishop, Tatham, & Shewry, 1994).

Lawrence and Payne (1983) reported that glutenin fraction of hexaploid (*Triticum aestivum* L.) makes up approximately 40% of the total seed protein and this fraction plays an important role in determining the strength and elasticity of wheat flour dough because it contains protein aggregates of high molecular weight (up to several million) formed by the association of a number of constituent polypeptide chains.

Ohm and Chung (1999) concluded that gluten contents and hydration amounts had significant correlations with water absorption and have a dominant role in bread-making. Complexity in the behaviour of gluten proteins such as adsorption, absorption, displacement and rearrangement during the adsorption process in a gluten-stabilised emulsion. Among the major gluten proteins, gliadin shows higher surface activity than glutenins under acidic conditions. Glutenins are likely to have been adsorbed more tightly than gliadins (Takeda, Matsumura, & Shimizu, 2001).

Many attempts to reveal the structure of the gluten proteins have been carried out, although they have been troubled by the low solubility and lack of crystallinity of the proteins. The solubility properties of gluten proteins are determined by the primary structures of the individual proteins and their interactions by non-covalent

forces (notably hydrogen bonds and hydrophobic interactions; Belton, Gil, Grant, Alberti, & Tatham, 1998) and by covalent disulphide bonds (Shewry & Halford, 2002). The whole protein structure is still far from being clear (Veraverbeke & Delcour, 2002). The mature wheat grains contain 8–20% proteins. The gluten proteins, the gliadins and glutenins, constitute up to 80–85% of total flour protein, and confer properties of elasticity and extensibility that are essential for functionality of wheat flours (Shewry, Tatham, Forde, Kreis, & Mifflin, 1989). The gliadins and glutenins each constitute around 50% of the gluten proteins. Osborne (1907) was the first to classify wheat grain proteins on the basis of their solubility: albumins (soluble in water), globulins (salt), gliadins (aqueous water) and glutenins (dilute acid or alkali). Due to findings of the Osborne fractions being heterogeneous and containing protein types overlapping each other, the methods of protein fractionation have now been improved (MacRitchie et al., 1990b). Now the protein classifying system is based on biological characteristics of the proteins together with their chemical and genetic relationship, leading to different states of aggregation in dissociating solutions (Shewry, Halford, & Tatham, 1992; Shewry & Tatham, 1990). Thus, gliadins are a mixture of monomeric polypeptides and glutenins consist of polypeptides aggregated by disulphide bonds (Shewry & Tatham, 1990; Singh & MacRitchie, 2001). The glutenin fraction is formed of a mixture of polymers, high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). The large glutenin polymers are stabilised by inter-chain disulphide bonds (Field, Shewry, & Mifflin, 1983).

High molecular weight glutenin subunits (HMW-GS)

The HMW-GS account for 5–10% of the total protein (Payne, 1986). HMW-GS are minor components in terms of quality; and are major determinants of gluten elasticity (Tatham, Mifflin, & Shewry, 1985). HMW-GS are closely associated with bread-making quality (Bietz & Wall, 1973).

The HMW-GS have molecular weight ranging from 80 to 160 KDa as evident from SDS-PAGE (Payne, Law, & Mudd, 1980). Their polypeptides with a molecular mass of less than 60 KDa containing glutenins were found to be large polymers linked by disulfide bonds or by non-covalent associations between LMW-GS and HMW-GS. The three to six HMW-GS (Margiotta et al., 1996; Payne & Corfield, 1979) are recognised in hexaploid wheat (Lew, Kuzmicky, & Kasarda, 1992).

Bietz and Wall (1972) conducted single kernel analysis of glutenin through SDS-PAGE and reported the five highest molecular weight subunits (1:133,000 mol wt; 2:104,000 mol wt; 3:93,000 mol wt; 4:86,000 mol wt; and 5:68,000 mol wt) coded by chromosomes 1B (Subunits 2 and 3), 1D (Subunits 1 and 4) and 4D (Subunit 5). They concluded that most of the tetraploid wheat lacks glutenin subunits (GS) 1, 4 and 5 but only Subunit 4 was consistently absent and in many varieties considerable variability also occurred. Hexaploid wheat varieties, however, were more uniform in glutenin-subunit composition than tetraploid wheats. Payne and Lawrence (1983) reported that there was a considerable variation in the pattern of HMW-GS due to the presence of different alleles at each for three gene loci (Glu A1, Glu B1 and Glu D1) which control their synthesis.

HMW-GS make dough elastic and allow them to trap the gas bubbles produced by yeast and to rise which are important in improving quality of final produce

(Cornish, Bekes, Eagles, & Payne, 2006). Cultivars of high bread-making quality have high proportions of these HMG polymers of proteins (Bottomley, Kearns, & Schofield, 1982; Huebbner & Wall, 1976). Application of modern techniques like genetic engineering has been used to change the levels of these proteins and to check their utility; recently field trials were conducted in Idaho and California (Branlard & Dardevet, 1985) and they concluded that bread-making performance has also been related to the glutenin-polymer size distribution.

Yahata et al. (2006) quantified and observed significant correlation of HMW-GS in wheat mill streams with the SDS-sedimentation volume and the mixing properties, which are the respective indices of specific loaf volume and dough strength. Further work in this area has suggested the role of a specific HMW subunit in the contribution of glutenin to bread-making quality. Payne et al. (1979) observed a direct relationship between bread-making and proportion of subunit Glu-1Ax1. Anjum, George, and Charles (2000) studied HMW-GS composition of Pakistani hard white spring wheat grown at three locations for two years and found significant correlations between certain HMW-GS and some quality attributes such as protein, farinograph dough development time, farinograph water absorption, loaf volume and mixograph peak height (Anjum, 1991; Anjum & Walker, 2000). Several studies have shown that the amount and relative proportion of HMW subunit is an important factor in functional performance for bread-making quality (Kolster, Krechting, & Gelder, 1992; Kolster & Vereijken, 1993).

The intrinsic viscosities of glutenins have been shown to be related to bread-making quality (Ewart, 1968). For the past 10–15 years, much of the emphasis has been on defining the molecular basis of bread-making quality in relation to specific polypeptides of the gluten protein complex, especially HMW subunits of glutenin (MacRitchie et al., 1990a,b; Payne, Holt, & Law, 1981). In order to correlate the bread-making potential with HMW subunit composition, two statistical approaches have been used. Based on the SDS-sedimentation volume, Payne (1987) assigned a quality score to each subunit and then individual values were summed to calculate the total quality score for a wheat cultivar, with a maximum score of 10. In another approach, Ng and Bushuk (1988) developed an equation for predicting the unit loaf volume, based on HMW subunit composition of a bread wheat cultivar.

Wieser and Kieffer (2001) demonstrated that correlations of the amount of gluten protein types of 14 cultivars of wheat flours determined on a micro-scale indicated that the maximum resistance of dough and gluten and the gluten index were strongly dependent on the quality of GS in flour. Among HMW-GS, the contribution of X-type GS were more important than those of Y-type GS. The extensibility of dough and gluten was mainly dependent on the ratio of gliadin to total GS, to HMW-GS and LMW-GS. Dough development time showed the highest correlation with total HMW-GS. Bread volume was influenced by the total amount of gluten protein more than by the amount of protein in different groups or of different types, probably because of a rather low range of flour protein contents (8.7–12.0%) within the set studied. Andrews and Skerritt (1996) and Skerritt, Diment, and Wrigely (1985) found that dough strength characteristics (e.g. extensograph, maximum, resistance, farinograph and development time) have been dominantly correlated with HMW-GS content and HMW-GS composition.

Sontag-Stohm and Juuti (1997) studied the relationship between baking quality and HMW and LMW and GS gliadin alleles on Group 1 chromosomes in 75 spring

wheats grown in Finland. They reported 80% of the spring wheats contained HMW-GS allele Glu-(5 + 10), which was associated with higher Pelshenke test values than allele Glu-(2 + 12). Ivanov, Todorov, Stoeva, and Ivanova (1998) reported significant correlations between Glu-1 quality score and some qualitative features. HMW-GS 5 + 10 controlled by Glu-D1 locus demonstrated the highest effect on qualitative characteristics, followed by Subunit 2* controlled by Glu-A1 locus in progenies of wheat crosses. Branlard and Dardevet (1985) determined protein quality in each of the bands of 70 wheat cultivars for HMW-GS composition by SDS-PAGE using densitometry and simply correlated with protein of each of HMW-GS and various flour quality characteristics, alveograph keeping in view strength, tenacity, swelling and extensibility. All of the characteristics were correlated with at least four subunits. Subunits 6 and 8 were the only subunits not correlated significantly with at least one of the characteristics. Subunits 17 and 18 remained favourably correlated significantly with swelling. They suggested that HMW-GS interact in producing the rheological characteristics of the dough and strength of wheat can be improved by using wheat with positive bands for high strength and extensibility. Lorenzo, Kronstad, and Viera (1987) reported that HMW bands 5 and 10 in a homozygous state always were found to be correlated with higher loaf volumes than bands 3 and 12 or the heterozygous state bands 5 and 10 + 3 and 12, contributed by the D genome. They also concluded that the importance of individual subunits in the A and B genome depends on the interaction with the glutenin proteins.

Payne, Lookhart, and Forsyth (1988) evaluated wheat selections, i.e. Shawnee and Ottawa by SDS-PAGE and concluded that the main reason for poorer bread-making quality in Ottawa than Shawnee is that it does not contain a pair of HMW subunits of glutenin coded by genes on chromosome 1D. Later, Ammar, Kronstad, and Morris (2000) evaluated 27 durum wheat genotypes and concluded that genotypes expressing HMW-GS 6 + 8 exhibited better overall bread-making quality compared with those expressing HMW-GS 7 + 8 or 20. Variation in the quality and type of HMW-GS in a variety can strongly influence its bread-making properties (Payne et al., 1988).

Low molecular weight glutenin subunits (LMW-GS)

LMW-GS are among the major components of wheat storage proteins, collectively known as prolamins because of their high content of the amino acids proline and glutamine. The LMW-GS have a molecular weight ranging from 30 to 51,000 using SDS-PAGE (Payne et al., 1980). The LMW-GS most closely resemble γ -gliadins in sequence (Muller, Vensel, Kasarda, Köhler, & Wieser, 1998) and comprise about 20–30% of the total protein (Gupta, Batey, & MacRitchie, 1992) and 15–20 different LMW-GS proteins are recognised in one-dimensional (1D) and two-dimensional (2D) gels of hexaploid wheat (Lew et al., 1992). Despite their abundance, they have received much less research attention than the HMW-GS. This has been mainly due to the difficulty in identifying them in 1D SDS-PAGE gels. The resolution of the problem, which was principally due to overlapping between LMW-GS and gliadins, was largely resolved when Singh and Shepherd (1985) developed a simplified two-step SDS-PAGE method. Earlier, Jackson, Holt, and Payne (1983), using a more complicated 2D electrophoresis procedure, improved resolution and began systematic work on this group of subunits. More recently, Singh, Shepherd, and Cornish

(1991) and Gupta and MacRitchie (1991) reported similar methods to analyse polymeric proteins after prior extraction of monomeric proteins with either 50% propan-1-ol or dimethyl sulfoxide (DMSO), respectively. Advances in the characterisation of LMW-GS have also been enhanced by the production of wheat-rye translocation lines (single, double and triple; Gupta & Shepherd, 1993), permitting the simplification of the electrophoretic pattern for closer study of the alleles of LMW-GS. RP-HPLC has also proved useful for the study of LMW-GS, showing that these proteins have higher hydrophobic surfaces than those from HMW-GS and comparable with the hydrophobic surfaces of gliadins. Recent improvements in capillary electrophoresis, as reported by Bean and Lookhart (2000), allow clear characterisation of all GS. The discovery of a new subunit is still in progress as two new LMW-GS with molecular weights of $\approx 30\text{--}31,000$ Da (Glu-D4 locus) and 32,000 Da (Glu-D5 locus) were reported by Sreeramulu and Singh (1997). These GS could be seen only in alkylated glutenin, and the one encoded at Glu-D5 locus has α -type N-terminal sequence (Gianibelli, 1998). Formerly, Bunce et al. (1985) demonstrated that although SDS-PAGE was a convenient method for determining the relative molecular weight of cereal prolamins, the results, however, should be treated with caution. In another research investigation, MacRitchie, Kasarda, and Kuzmicky (1991) reported that LMW proteins can be separated by gel permeation chromatography into two fractions with molecular weights around 44,000 and 36,000.

Characterisation of LMW-GS that represent about 70% of glutenins and 20–30% of total proteins explicated their ability to form large aggregates that are related to dough strength (Melas, Morel, Autran, & Feillet, 1994). However, the strength and resistance provided by LMW to the dough are half to that of HMW but still correlation coefficients for both of them are in a similar range within the family of glutenin proteins (Wieser & Kieffer, 2001). In contrast, Andrews and Skerritt (1996) found relationships between total GS (LMW-GS), and dough extensibility with several sets of flours and they found poor overall correlations between these proteins and other quality attributes of dough.

The products made from durum wheat, LMW-GS has a certain role to play for the end-use quality particular subunits encoded by loci present on chromosome 1B (Josephides, Joppa, & Youngs, 1987). The best pasta-making characteristics are associated with the presence of a specific allelic form of typical LMW-GS, named LMW-2 (Payne & Lawrence, 1983). This allele also seems to be important for determining bread-making properties (Pena, Zarco-Hernandez, Amaya-cellis, & Kazi, 1994). Most commonly grown durum wheat cultivars have either the LMW-2/g-45 (plus v-gliadin 35) or the LMW-1/g-42 (plus v-gliadins 33, 35 and 38) allelic forms, the latter being associated with poor quality pasta-making properties. LMW-2 also exerts a positive effect on gluten strength when present in hexaploid tritordeum (Alvarez, Campos, Martin, & Martin, 1999). There are indications that the better quality associated with the presence of LMW-2 in durum wheat is mainly due to the fact that the subunits are more abundant than the LMW-1 subunits (Autran & Galterio, 1987; Ovidio, Tanzarella, & Porceddu, 1992). Differences in the total amount of LMW-GS, associated with specific allelic forms, have also been reported to be an important cause of quality differences in bread wheat (Gupta & MacRitchie, 1994). The 42 KDa LMW-GS may also be present in good quality bread wheat (Masci, D'Ovidio, Lafiandra, & Kasarda, 2000), but it is not associated with the LMW-2 group, which does not appear to occur in hexaploid wheat. Similarly, null

alleles of LMW-GS have detrimental effects on these parameters (Benedettelli, Margiotta, Porceddu, Ciaffi, & Lafiandra, 1992). Different allelic forms of LMW-GS seem to play different roles in determining different quality parameters (Luo, Giffin, Branlard, & McNeil, 2001). Thus, the Glu-A3 alleles influence protein content, SDS-sedimentation volume and mixograph midline peak value of New Zealand wheat cultivars, whereas the Glu-D3 alleles do not have any influence on the SDS sedimentation. Lastly, studies have also demonstrated that g-42 and g-45 are only genetic markers for quality (Boggini & Pogna, 1989; Pogna, Lafiandra, Feillet, & Autran, 1988).

Immunochemical characteristics

The quality of wheat proteins can be judged by quantitative assessment of their molecular weights and studying their rheological behaviours along with end-use quality. In recent years, wheat breeders demand simple methods offering thorough assessment of wheat quality using small samples of grain, wholemeal or flour, because initial crosses within breeding programmes may produce many thousands of progeny, each bearing only small amounts (500 mg to 10 g) of grain. Large-scale rheological or other quality testing methods that require many grams of flour and are very labour-intensive cannot be used for wheat quality screening (Andrew, Blundell, & Skerritt, 1993; Skerritt, 1991).

Chromatographic and electrophoretic techniques have proved to be useful in food component identification (Berrini, Berrini, Tepedino, Borromeo, & Secchi, 2006; Mackie et al., 2000; Mayer, 2005). Modern equipment, such as high pressure liquid chromatography (HPLC), mass spectroscopy–gas chromatography (GC–MS) and Fourier transform infrared radioscropy (FT-IR) are in common use in the developed world. The time required to run each sample hinders its application on a large scale (Skerritt, 1991, 1993). The tools of genetics can be used for the assessment of wheat quality and its relationship with end-product quality. The basic antigen–antibody reaction provides a means for very sensitive and analytical procedures. Immunology has been increasingly recognised as a valuable analytical tool. The techniques probing antibody production in response to food intake are in progress (Asensio, Gonzalez, Garcia, & Martin, 2008).

In a number of research investigations, efforts were diverted into the development of immunochemical assays; many techniques have also been developed (Gathumbi, Usleber, Ngatia, Kangethe, & Märklbauer, 2003; Korde et al., 2003; Lipigorngoson, Limtrakul, Suttajit, & Yoshizawa, 2003) as qualitative or semi-quantitative and probably most useful for rapid screening. These types include radio-immunoassay and ELISA. New and innovative approaches for rapid screening methods based on dip-stick-like kits and immunosensors based on transducer techniques such as surface plasmon resonance or the quartz-crystal microbalance, capable of simultaneous detection of different mycotoxins, have appeared in the most recent literature (Van der Gaag et al., 2003). These techniques are important screening tools for the detection of drugs in biological fluids because of its pre-treatment simplicity and high ability to treat many samples in a short time (Bean & Lookhart, 2000).

However, although they are of considerable value in certain instances, these methods are not convenient for routine sample analyses because they are relatively costly, time consuming and complex to perform. Consequently, in the past few years

the identification of food products has been performed primarily by genetic and immunological techniques (Liu et al., 2006; Terzy et al., 2005). The ELISA is the most widely used technique for regulatory purposes in detecting food authenticity because of its specificity, simplicity and sensitivity, among other advantages (Mackie et al., 2000). Indirect and the sandwich ELISA are two broader categories in such analysis. The detection antibody can be coupled to an enzyme or can bind the conjugate (enzyme-linked antibody) that will produce the biochemical reaction (Goldsby et al., 2003). ELISA may be used for qualitative and quantitative estimation of desired groups. Qualitative results provide a simple positive or negative result for a sample, however, the optical density or fluorescent units of the sample are interpolated into a standard curve in quantitative ELISA (Goldsby et al., 2003).

These techniques can be employed in cereals for detection of many physiologically important substances and their correlation. The approach has several advantages; a large number of samples can be analysed in a shorter time with minimal sample volume (Li et al., 2008). In order to carry out specific analyses, prerequisite for an ELISA is the availability of the amount of antibodies, i.e. MAbs and PAbs. Both PAbs and MAbs can be used in ELISA methods for food component identification. PAbs offer a number of benefits such as recognition of a mixture of different epitopes of the antigens and more tolerance to small changes in the nature of antigen (Harlow & Lane, 1999). In contrast, MAbs are a homogeneous population of antibodies produced by hybridoma technology that have defined biological activity, consistent specificity and their production is not limited (Goding, 1996). Both PAbs and MAbs are used in the ELISA variants for food authentication that have been previously described (Harlow & Lane, 1999).

These immunoassays include utilisation of MAbs and PAbs; their responses against specific proteins can be further split against storage (glutenin/gliadin) and non-storage (albumins/globulins) proteins. The reactions which are in direct correlation with product quality and are often attributed to GS like LMG-GS and HMG-GS thus their reaction with antibodies can be further used for quality assessment (Prabhasankar & Manohar, 2002). MAbs are used for specific fraction of these larger protein molecules while PAbs are used for predicting overall behaviour of specific types of proteins. In this context, Ragae, Abdel-Aal, and Noaman (2006) developed MAbs to wheat gluten proteins for use as diagnostics for screening of large numbers of wheat lines for their suitability for processing into bread and other products and as research tools for investigating protein structure and function (Andrews & Skerritt, 1996; Skerritt, 1991). Ragae et al. (2006) further developed an ELISA for the prediction of dough strength using antibodies to HMW-GS extracted from wheat flour samples. Similarly Skerritt (1991) observed a strong relationship between MAbs and HMW-GS containing extracts of flour samples. The relationship between antibody binding and the content of large glutenin polymers confirms to allow the detection of HMW-GS (Gupta et al., 1995). This effect has also been shown earlier in a detailed study using a number of MAbs (Skerritt, 1991). More recently, Leszczyńska, Łacka, Bryszewska, and Brzezińska-Błaszczyk (2008) estimated the usefulness of rabbit polyclonal anti-QQQP peptide antibodies in determination of gluten proteins.

In a sub-continent where the chapatti is used as staple food, an indirect ELISA has been developed for evaluation of the chapatti-making quality of wheat varieties. PAbs against gliadin, low molecular weight glutenin (LMG), and HMG were

developed and utilised in ELISA. Twenty-eight Indian wheat varieties were utilised in the ELISA. Out of these antibodies, an anti-gliadin antibody response was negatively correlated with farinograph water absorption ($(r) -0.89$ at $P < 0.01$), chapatti dough water absorption ($(r) -0.91$ at $P < 0.01$), overall chapatti sensory score ($(r) -0.95$ at $P < 0.01$), chapatti puffed height score ($(r) -0.95$ at $P < 0.01$) and positively correlated with shear value of chapatti ($(r) 0.76$ at $P < 0.01$). Anti-LMG antibody response was not correlated with any of these parameters, whereas anti-HMG response positively correlated with chapatti dough water absorption ($(r) 0.44$ at $P < 0.05$), farinograph water absorption ($(r) 0.45$ at $P < 0.05$), and overall chapatti sensory score ($(r) 0.44$ at $P < 0.05$), and negatively correlated with shear value ($(r) -0.38$ at $P < 0.05$) and chapatti puffed height ($(r) -0.44$ at $P < 0.05$). These results indicate that wheat varieties with good chapatti-making quality have a lower anti-gliadin antibody response (Prabhasankar & Manohar, 2002). The ability of simple antibody-based tests to assess and predict specific aspects of dough can be evaluated using an ELISA that can be further used for the prediction of end-use quality.

Wheat (*Triticum aestivum*) is ranked as a staple food of people all over the world. It occupies an important position among cereal crops with respect to production and utilisation. The varietal trials are common to develop new wheat varieties and maintaining the vigour of old ones. Many tools are used to characterise wheat on the basis of their set criterion. The quality of wheat is generally assessed on the basis of different physical, chemical, biochemical and rheological properties including milling yield, weight, protein, moisture and gluten. The wheat quality depends upon cultivar, climatic conditions and year and is expressed by a variety of physical and chemical tests such as test weight, kernel weight, moisture content, ash content, protein content, SDS, etc.

The wheat flour's ability to be processed into different food products is mainly determined by the contents and quality of gluten proteins. The main constituents of the gluten are gliadins and glutenins. The glutenins exhibit a broad spectrum of molecular weights varying from 40 kDa to several millions. Two major classes of GS have been identified in wheat endosperm by SDS-PAGE, the HMG (80–130 kDa) and the LMG (10–70 kDa). The HMW-GS are a major determinant of the bread-making characteristics of dough, and the LMW-GS also play an important role related to dough resistance and extensibility.

Because the quality of wheat is governed by interaction of many constituents, it is difficult to judge quality by a single test. Immunochemical quality assessment through immunological methods has been increasingly recognised as a valuable analytical tool in measuring functionality and suitability of different wheat varieties for their end use. ELISA may be an effective tool for assessing chapatti and bread-making qualities of different wheats.

Conclusion

The application of immunological methods to study and compare the cereal proteins from different species actually excludes the use of various chemical and rheological tests. Immunochemical quality assessment through immunological methods could provide a vital tool in measuring functionality and suitability of different wheat varieties for chapatti and bread-making quality. Food authenticity can be carried out through an ELISA test which is used for the identification of meat and meat-based

products, fish and seafood, milk and dairy products, and other foods. ELISA may be an effective tool for assessing chapatti and bread-making qualities of different wheats.

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