ELSEVIER

Journal of Chromatography A, 881 (2000) 23–36

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Electrophoresis of cereal storage proteins☆

S.R. Bean[a], G.L. Lookhart[a,b,*]

[a]*Department of Grain Science and Industry, Kansas State University, Manhattan, KS 66506, USA*
[b]*US Department of Agriculture, Agricultural Research Service, Grain Marketing and Production Research Center, Manhattan, KS 66502, USA*

Abstract

Cereal proteins have been studied by a number of analytical techniques over the years. One of the major methodologies utilized by cereal chemists has been electrophoresis. Starting with moving boundary electrophoresis and progressing to slab gels and high-performance capillary electrophoresis, innovative methods have been developed to provide high resolution separations of difficult to separate proteins. Sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE), acid–PAGE, isoelectric focusing, free zone CE, and even high-resolution two-dimensional HPLC–HPCE methods have been developed to separate cereal proteins. This review focuses on electrophoretic methods for separating and characterizing cereal storage proteins.

Keywords: Cereals; Reviews; Proteins

Contents

☆Cooperative investigations, US Department of Agriculture, Agricultural Research Service, and the Department of Grain Science and Industry, Kansas State University. Contribution 00-184-J, Department of Grain Science and Industry, Kansas State Agricultural Experiment Station, Manhattan, KS 66506, USA. The US Department of Agriculture, Agricultural Research Service, Northern Plains Area, is an equal opportunity/affirmative action employer and all agency services arer available without discrimination. Mention of firm names or trade products does not constitute endorsement by the US Department of Agriculture over others not mentioned.

*Corresponding author.

0021-9673/00/$ – see front matter
PII: S0021-9673(99)01270-4

1. Introduction

1.1. Importance of separating cereal proteins

Agriculture is one of modern humanity's most ancient practices and ''can perhaps justifiably claim to be among the most important of human activities [1].'' In fact, cereal grains produce the natural resources for ''two of man's oldest technologies – the baking of bread and the fermentation of alcoholic beverages [2].'' Bread, produced from the cereal grain wheat, may be considered one of the first processed foods [3].

Cereal grains are important foods in every country, either directly as human food or indirectly as animal feeds [4]. While cereal grains are important sources of energy, cereals are also a primary provider of protein, often out-producing all other major crops in annual protein yield (on a dry mass basis) [5]. Cereal proteins also contribute important functional properties to many foods. The best example of this is wheat, whose gluten proteins have unique viscoelastic properties that enable wheat flour doughs to be made into bread and many other unique products [6].

Because cereal proteins have important nutritional and functional roles, cereal proteins have been intensively studied for many years. For over 250 years chemists have attempted to relate the amount, composition and structure of cereal proteins to product end-use properties [7,8].

While in some instances knowing the total protein level of a given cereal may provide useful information, much more can be learned by fractionating, or separating, the proteins in some manner. This can be done by selective extraction of cereal proteins with various solvents. For cereal proteins, virtually all extraction schemes are based on the pioneering work of Osborne [8]. While selective solvent extraction may provide useful answers, it often does not produce ''pure'' classes of proteins and provides only limited quantitative information.

To gather more information, higher resolution techniques were employed, typically either chromatography or electrophoresis. High-performance liquid chromatography (HPLC) has developed into a widely used and useful tool for the separation of cereal proteins. For a complete review of this subject, the reader is directed to Kruger and Bietz [9]. This current review will focus on the electrophoretic methods for separating cereal proteins, with an emphasis on recent development and improvements in methodology.

1.2. Cereal protein classification, extraction and handling

Cereal proteins have typically been separated into classes based primarily on the work of Osborne [8], who divided wheat proteins into four classes. While originally used for wheat, Osborne's classification has since been applied to virtually all of the cereals, albeit often modified for different cereals. The ''Osborne fractionation'' scheme divides cereal proteins into water soluble proteins (albumins), salt soluble proteins (globulins), alcohol soluble proteins (prolamins), and acid or base soluble proteins (glutelins). These extractions are typically performed sequentially, that is the water soluble proteins are removed, followed by the salt soluble proteins and so on.

Because of its widespread use and acceptance, this classification scheme, or a variation thereof, has been widely used to extract the various protein classes for subsequent electrophoretic analysis. Extraction and handling of cereal proteins has been covered in a number of reviews [10–12]. An excellent review on protein solubilization in general for electrophoresis is also available [13].

While widely used, and for the most part, a useful classification scheme, it is now generally recognized that considerable overlap between the Osborne protein classes occurs. Because of this, alternative classification schemes have been developed for many cereals. Shewry et al. [14] proposed an alternative nomenclature for wheat proteins. Maize and sorghum proteins have also had substantial work done on classification systems [15] and protein classes are now defined based on solubility, structure, amino acid composition, and size [15,16]. This work has also led to improved extraction procedures for maize and sorghum storage proteins [17].

Whatever extraction scheme is used, researchers should be aware of potential overlap between fractions and clearly state the conditions used for protein extraction and subsequent electrophoretic analysis.

Cross contamination should also be considered when quantitating the various protein classes of cereals.

Aside from classification and nomenclature difficulties, several other considerations should be taken into account. Phytate in cereals can potentially interfere with protein extraction and analysis, this has been demonstrated in rice [18]. Likewise tannins in sorghum may interfere with protein extraction [19]. In most cereals, a large part of the storage proteins are disulfide bonded into large polymeric networks, so a reducing agent is necessary to extract these proteins for electrophoretic analysis. To prevent disulfide bonds from reforming, free sulfhydryls are often alkylated to block them. The use of alkylating reagents may impart a charged group to the proteins, which could impact the proteins electrophoretic mobility. It should be remembered however, that excess alkylating reagent will increase the ionic strength of the sample plug and thus could interfere with sample stacking during electrophoresis. This may be one reason why alkylated glutenins have been reported to have lower resolution than unalkylated samples when separated by free zone capillary electrophoresis (FZCE) [20].

Sample stacking is very important in high-performance capillary electrophoresis (HPCE), and care should be taken to minimize the salt concentrations of samples where possible. This may be one reason why globulin proteins show poor separation when separated by FZCE [21]. Buffer salts may also contribute to baseline disturbances and lowered resolution in sodium dodecyl sulfate capillary electrophoresis (SDS-CE) separations [22], leading to the suggestion that samples be extracted only with unbuffered SDS–β-mercaptoethanol (ME) solutions when preparing glutenins for SDS analysis [22]. The organic solvents typically used in cereal storage protein extraction also provide a stacking benefit in HPCE. Because of the lower conductivity of the alcoholic sample plugs, lower conductivity exists in the sample plug relative to the surrounding buffer, which provides excellent stacking. This effect has been taken advantage of when using low conductive isoelectric buffers [23]. Organic solvents such as acetonitrile (ACN) have also been found to provide excellent stacking even in the presence of high salt levels [24,25]. Several pH mediated stacking methods have been developed for HPCE [26], including one method for separations at low-pH buffers [27]. This last method and ACN/salt stacking methods [24,25] have been used successfully with albumin and globulin proteins of wheat that were extracted in the presence of high salt levels (Bean and Lookhart, unpublished results). Due to the impact of the sample matrix on electrophoresis, especially HPCE, the solvents and buffers used for sample preparation should be carefully considered.

Finally, sample stability should be considered when preparing samples for analysis. Since aqueous alcohols are often used for extracting cereal proteins, care should be taken that the solvents cannot evaporate during storage or analysis. Stability of extracts should be examined and reported as well as the time between extraction and analysis.

2. Polyacrylamide gel electrophoresis

Early electrophoretic methods for separating cereal proteins included moving boundary electrophoresis [28] and starch gel analysis [29]. Two later forms of electrophoresis have developed into widely used methods, these are SDS–polyacrylamide gel electrophoresis (PAGE) and acid (A)–PAGE. SDS–PAGE is routinely used for separating proteins from all classes of cereals, whereas A–PAGE has been used mainly for cultivar ‘‘fingerprinting.’’

2.1. Sodium dodecyl sulfate–polyacrylamide gel electophoresis

SDS–PAGE was first introduced for the separation of wheat proteins by Bietz and Wall [30]. Since that time, SDS–PAGE has been widely used for separation proteins from all cereals. For wheat, SDS–PAGE is particularly valuable for the separation of high-molecular-mass glutenin subunits (HMM-GS), a class of proteins linked to bread quality [31]. SDS–PAGE separations have been used to help classify proteins from other cereals such as barley [10,32].

SDS–PAGE provides a relatively low cost, high throughput method for analyzing cereal proteins. It does have the disadvantages of needing post-separation staining to visualize the bands and quantitation can be difficult. Quantitation issues for wheat pro-

teins have been addressed in a number of papers [33,34]. SDS–PAGE also suffers from the need to use acrylamide, a potent neurotoxin.

SDS–PAGE formulations vary widely depending on the type of proteins being separated. For wheat proteins, proteins are often separated in gels varying from 6% to 17%, depending on the protein fractions being separated [10,32]. Gradient gels have also been used [35–37]. Precast gradient gels have also been used to separate wheat proteins [37,38], which provide convenience and can provide very rapid separations [38]. For specific classes of wheat proteins, slightly different gel formulations have been used. Glutenins are often separated on gels similar to those of total proteins. In these types of gels, HMM-GS are usually well separated from the other proteins, even in total protein extracts (see for example [39]). Low-molecular-mass glutenin subunits (LMM-GS) have been difficult to easily separate, primarily because of their similar sizes to gliadins. Several methods have been published that have dealt specifically with the separation of LMM-GS (e.g., Refs. [40,41]). Likewise, specific methods have been developed for improved separations of the HMM-GS [39,41–43].

SDS–PAGE methods for barley proteins include both straight percentage gels and gradient gels (reviewed in [10,44]). Oat and rice prolamins have also been successfully separated in SDS–PAGE gels (reviewed in [10,45,46]).

For maize and sorghum, different concentrations of SDS–PAGE gels are used. Typically gradient gels of high %T, such as 15 to 20% are used[1] (e.g., Ref. [17]). Because of the higher hydrophobicity of these proteins, urea is often added to the gels, presumably to help maintain protein solubility [15,17,19].

A relatively new form of SDS–PAGE was recently introduced for the analysis of large glutenin polymers. This type of SDS–PAGE was termed ''multi-stacking'' electrophoresis and used several layers of increasing concentrations of stacking gels placed on top of the separating gel [47]. When unreduced glutenin samples were analyzed by this separation technique, protein polymers too large to enter the different stacking gel interfaces would be trapped. This has allowed better separations of these large glutenin aggregates [47].

[1]T=(g acrylamide+g *N,N′*-methyllenebisacrylamide)/100 ml solution.

2.2. Acid–polyacrylamide gel electrophoresis

A–PAGE separates proteins at acidic pH without the use of the charged detergent SDS, therefore separation should generally be based on differences in protein charge density, not mass as in SDS–PAGE. Some of the first electrophoretic separations of cereal proteins were done in starch gels at acidic pH [29]. Since then A–PAGE has been widely used to separate a variety of cereal proteins.

For wheat, A–PAGE is most often used to separate gliadins, typically for ''fingerprinting'' cultivars [10,32]. As with SDS–PAGE, both straight concentration as well as gradient gels have been used and even precast gels [10,32,48]. Several papers have investigated the relationships between gel properties and protein separations [49–51].

In addition to gliadins, glutenins have also been successfully separated by A–PAGE. Morel [52] described an A–PAGE method with urea (to aid in solubility) that provided good resolution of glutenins. Preparative A–PAGE methods have also been described for other wheat gliadin and glutenin proteins [53,54].

Barley hordeins have been successfully separated in A–PAGE gels, allowing various barley cultivars to be successfully differentiated [44]. Likewise, oat and rice prolamins have been successfully separated by A–PAGE, allowing cultivars to be differentiated [45,55]. Maize and sorghum proteins have also been successfully separated with A–PAGE, although these proteins require the addition of urea to the gels to help maintain protein solubility [19]. Resolution for maize and sorghum proteins in A–PAGE is generally low, however [15].

2.3. Isoelectric focusing

In addition to SDS–PAGE and A–PAGE, isoelectric focusing (IEF) has been used to separate storage proteins from several different cereals. Due to the nature of IEF separations, protein solubility during the separation is often difficult to maintain. For this reason strong protein solublizers are typically added to the gels. For cereal proteins, as with most

proteins, 8 *M* urea seems to be one of the most widely used reagents [10,56].

Because urea has the potential to modify proteins during IEF [57], it may not be the best choice of reagents. Righetti [58] recommended aqueous alcohols as a possible additive for IEF of cereal proteins, since the ampholytes used to generate the pH gradients necessary for IEF are soluble in aqueous alcohols; this would avoid the problems associated with using urea. Several alcohols and organic solvents have been used in IEF separations of cereal proteins including 50% 1-propanol [59], 60% ethanol [60], 45% ethanol [61] and 30% ethylene glycol [62].

2.4. Two-dimensional gels

Two-dimensional (2D) electrophoresis has provided extremely high resolution separations of cereal storage proteins. Many reports of 2D systems based on the original method of O'Farrel have been reported (for selected examples see Refs. [63–65]). In addition to these traditional methods, novel combinations of gel types have been used for 2D separations. 2D A–PAGE–SDS–PAGE separations have been reported [52] as have 2D native PAGE separations at acidic and basic pH [66]. Note in this case, an organic solvent, dimethyl sulfoxide (DMSO), had to be used when performing the separations at basic pH to maintain protein solubility.

3. High-performance capillary electrophoresis

In addition to slab gels, HPCE is starting to be used for cereal storage protein separations. HPCE utilizes small inner diameter (typically 25 to 100 μm) capillaries for separation. These capillaries, due to their high surface area to volume ratios allow extremely high voltage separations, up to 30 kV in most commercial instruments. These high voltages allow for rapid, high efficiency separations, often reaching over $1 \cdot 10^6$ theoretical plates/m. HPCE also generates computerized data that can be easily quantified, stored and analyzed, and most commercial instruments are completely automated, allowing unattended operation. HPCE also generates little hazardous waste. Thus, HPCE appeared to have considerable potential for cereal protein analysis. HPCE combines the high resolution of electrophoresis with the automation and ease of use of HPLC, and can give high resolution, rapid separations of proteins.

3.1. Free zone capillary electrophoresis

To date, most of the HPCE separations of cereal storage proteins has been done with FZCE. FZCE separates proteins mainly on differences in their charge densities, producing analagous separations as A–PAGE. In fact several studies have shown that FZCE separations at acidic pH separate cereal storage proteins in a similar manner as A–PAGE, with various protein subclasses showing the same migration order [67–71].

At least four different methodologies have been utilized for FZCE separations of cereal proteins. Werner et al. [67] employed a commercial cationic reagent that dynamically coated the inside of the capillaries to produce positively charged capillary surfaces. An aluminum lactate buffer, pH 2.3, was then used as a separation buffer. Resolution was sufficient enough to discriminate wheat cultivars in 20 min.

Early work was also done using a borate buffer, pH 9 containing 1% SDS and 20% acetonitrile for wheat protein separations [72–74]. While this buffer produced good resolution, Bietz and Schmalzried [74] reported poor reproducibility with this buffer. Other authors have also employed this buffer for characterizing gliadins from wheat [75–77].

To overcome the repeatability problems of the alkaline borate buffer, Bietz and Schmalzried [74] tested a 100 m*M* sodium phosphate buffer. This buffer improved resolution and repeatability. Lookhart and Bean [78] later used small inner diameter capillaries (20 μm) in combination with the sodium phosphate based buffer. These smaller capillaries allowed the use of higher separation voltages (22 kV) and produced faster separations without significant loss of resolution (Fig. 1). Later developments with the sodium phosphate buffer included the use of organic solvents as buffer additives, where 20% ACN was found to produce the highest resolution for separation of gliadins [79]. Several zwitterionic and non-ionic detergents were also

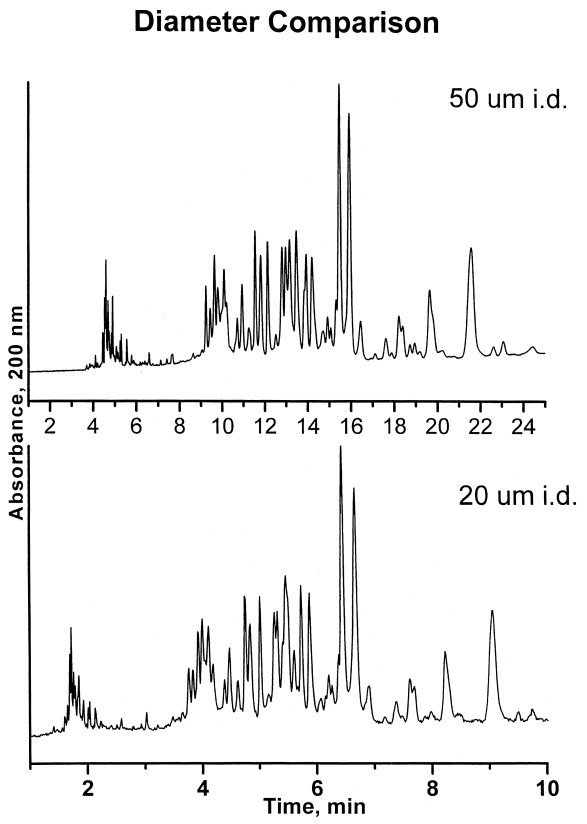

Fig. 1. Comparison of capillary diameters. From Ref. [78], with permission.

tested as buffer additives [79]. The addition of 26 m*M* laurylsulfobetaine (SB 3-12), a zwitterionic detergent, greatly improved the resolution of wheat glutenins as well as oat prolamin and rice prolamin separations [79]. The sodium phosphate buffers and separation conditions were modified slightly to study gliadin subclasses of Argentinian wheats [70] and spelt wheat gliadins [80]. This buffer system was also adapted for the separation of wheat proteins related to kernel hardness [81].

While sodium phosphate-based buffers produced high resolution separations, this buffer generated substantial current due to the high conductivity of the sodium ions [82]. This in turn limits the voltage that can be applied, unless small diameter capillaries are used, which was the approach taken by Lookhart and Bean [78]. Using small inner-diameter capillaries, however, reduces the sensitivity and increases the opportunity for the capillaries to become plugged.

To overcome these limitations two approaches have been reported. Lookhart and Bean [83] reported the use of glycine or β-alanine to replace the sodium phosphate in the separation buffers. Due to the lower conductivity of these buffers the currents generated were ~50% lower than the sodium-based buffers [83]. Since mobility is inversely related to conductivity [84], these buffers resulted in more rapid separations than the sodium-based buffers (Fig. 2). The phosphate glycine buffer (with 20% ACN added) was capable of discriminating cereal cultivars and produced good separations of all classes of wheat proteins [84] as well as oat and rice prolamins [84]. This same buffer (phosphate–glycine–ACN) was later used to separate barley hordeins as well [69]. The phosphate–glycine buffer, modified by the addition of 60% ACN was used to successfully separate maize and sorghum proteins (Fig. 3) [71]. The higher levels of ACN were necessary to maintain protein solubility of the hydrophobic maize and

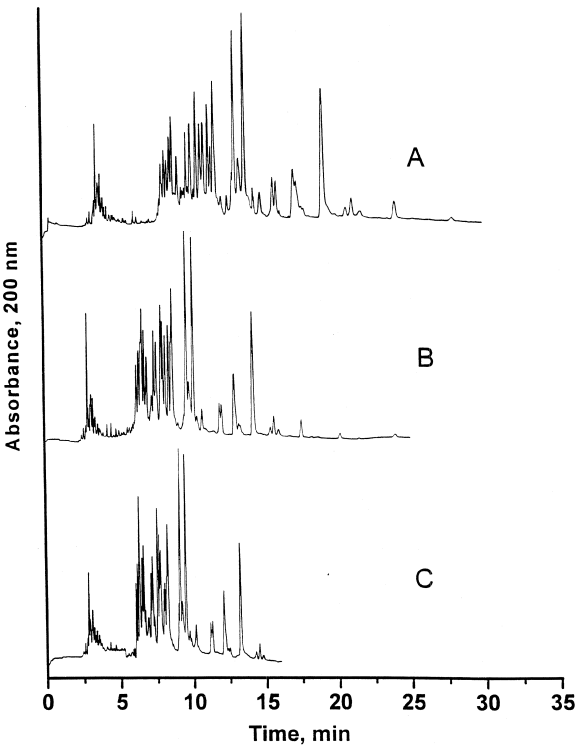

Fig. 2. Effect of replacing sodium phosphate (A) with β-alanine (B) or glycine (C) in acidic phosphate buffers. From Ref. [83], with permission.

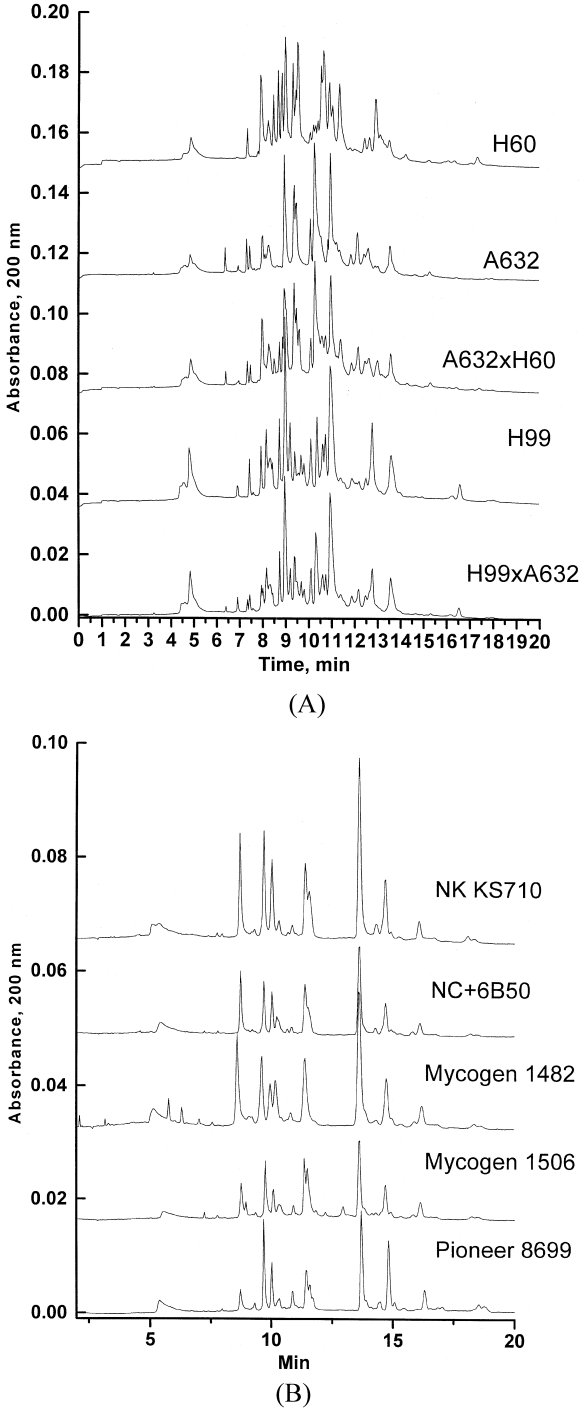

Fig. 3. Maize genotype (A) and sorghum genotype (B) separations in 100 m*M* phosphate–glycine buffers containing 60% ACN as an additive to maintain protein solubility. From Ref. [71], with permission.

sorghum proteins, and eliminated the need to use high levels of urea in the buffer, which can be problematic. Due to the low current generated with this buffer, separations were successfully made in 50 μm I.D. capillaries, thus not limiting the sensitivity [71]. The phosphate–β-alanine buffer was also used to study gliadin proteins during kernel development in wheat [85].

A second approach to overcome the limitations of sodium-based buffers has been the use of isoelectric buffering compounds. These unique compounds have an isoelectric point roughly equal to their pH in solution, which means they can buffer without the need for a co-ion [86]. Furthermore, the amphoteric nature of these compounds means that they produce very little current, even at extremely high voltages [86]). For a complete discussion on the use of these buffers the reader is directed to Righetti et al. [86].

Capelli et al. [87] first applied isoelectric buffers to the separation of wheat proteins. These researchers utilized 40 m*M* aspartic acid as the isoelectric buffering compound and 8 *M* urea as a buffer additive. Due to the relatively low ionic strength of aspartic acid extremely high voltages were used, 30 kV (1 kV/cm), in larger diameter capillaries (50 μm), overcoming the sensitivity problems with the smaller diameter capillaries (20 or 25 μm). Strangely, acetonitrile was not found to be an effective buffer additive when used with the aspartic acid buffer, unless used in combination with 4 *M* urea (Fig. 4). This buffer system was capable of differentiating

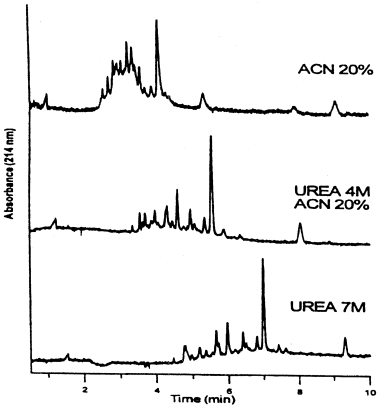

Fig. 4. Wheat gliadin separations in 40 m*M* aspartic acid buffers. From Ref. [87], with permission.

wheat cultivars in roughly 10 min with good repeatability (less than 4% variation in migration times) [87].

The aspartic acid–8 *M* urea buffer system was later applied to the separation of maize storage proteins with high resolution [88] (Fig. 5). This buffer was later used in combination with statistical methods to differentiate maize genotypes [89].

In addition to aspartic acid, iminodiacetic acid (IDA) has also been used as an isoelectric buffer for cereal storage proteins. IDA was discovered by Bossi and Righetti [90] and used to separate peptides and was recommended as an alternative isoelectric buffer for proteins [87]. IDA has a lower pH in solution than aspartic acid, so may provide less problems with wall interactions than aspartic acid [87].

The use of IDA buffers was optimized by Bean and Lookhart [91] for the separation of prolamins and glutelins (storage proteins) from wheat, oats, rice, rye and barley. Due to the high voltages that could be used with this buffer, 30 kV, extremely rapid separations were possible, even in 50 μm capillaries. Storage proteins from most cereals tested could be separated in ~3 min with high resolution. An example of glutenins from wheat are shown in Fig. 6. Excellent repeatability was reported with this buffer, for run-to-run as well as day-to-day and capillary-to-capillary [91]. Albumin and globulin proteins had to be removed by pre-extraction to obtain good repeatability and stable separations with this buffer, however [91].

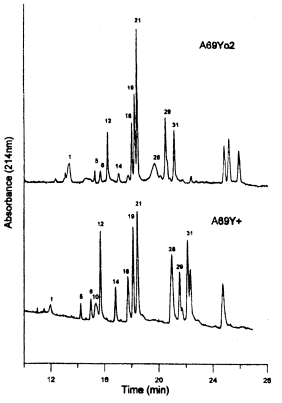

Fig. 5. Separation of maize zeins in 40 m*M* aspartic acid–8 *M* urea. From Ref. [89], with permission.

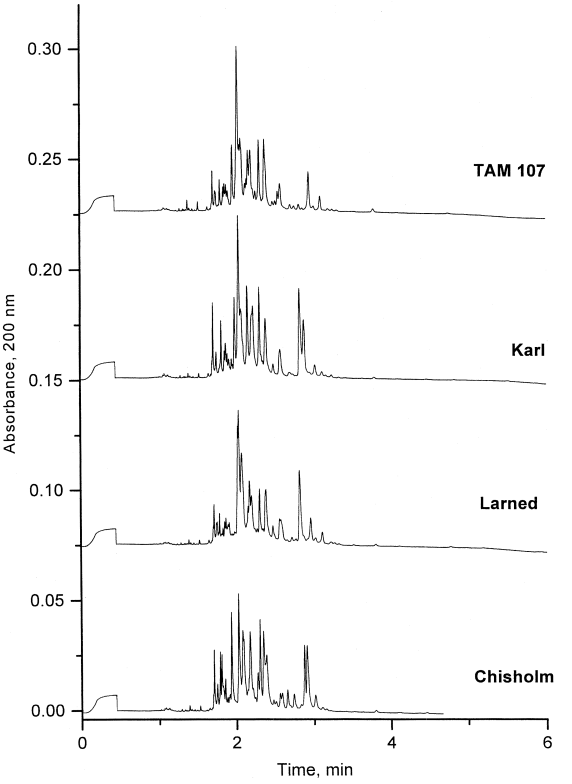

Fig. 6. Separation of wheat glutenins in isoelectric IDA based buffers. From Ref. [91], with permission.

3.2. Sodium dodecyl sulfate capillary electrophoresis

One of the most widely used separation techniques for cereal storage proteins is SDS–PAGE. Likewise, one of the first reports of using HPCE to separate proteins was in the SDS-CE mode, where protein–SDS complexes were separated on the basis of their molecular mass. The principles of these types of separations and available general methodologies have been reviewed in a number of papers [92–95].

For cereal proteins, relatively little work has been done with this mode of separation. Most work has utilized a commercial sieving reagent, ProSort. This reagent consists of a linear poly(acrylamide) in a neutral buffer [96]. Optimization studies with wheat proteins found that it was necessary to add an organic modifier(s) to the buffer; either 5% methanol and 5% glycerol [67] or just 5% methanol [22]. While this reagent has provided good separations of

wheat proteins, to the best of our knowledge it is no longer commercially available.

Several other polymer systems have been utilized to separate wheat proteins including dextran [97,98], a commercial reagent from Beckman [99], a commercial reagent from Bio-Rad [98] and linear polyacrylamide [67,98]. An example of wheat glutenins separated by SDS-CE is shown in Fig. 7. It is interesting to note that the migration order of the HMM-GS is not the same in SDS-CE as that in SDS–PAGE [22,67,98]. The exact reason for this is unknown.

Bean and Lookhart [98] compared four different polymers for SDS-CE separations. All polymers produced acceptable separations, though lower resolution was found when using polyethylene oxide (PEO) as the sieving polymer. This polymer produced excellent separations of standard M_r markers proteins, however [98]. The estimated M_r of wheat proteins separated in the PEO buffer system showed extremely high masses and this combined with the poor resolution, (despite acceptable separations of M_r marker proteins) suggested that PEO–wheat protein interactions may have occurred. This same phenomenon, and almost identical separations, were also obtained when using the Beckman SDS-CE kit (Bean and Lookhart, unpublished results). Researchers using PEO systems or commercial systems that employ PEO should use caution when estimating M_r values from SDS-CE separations.

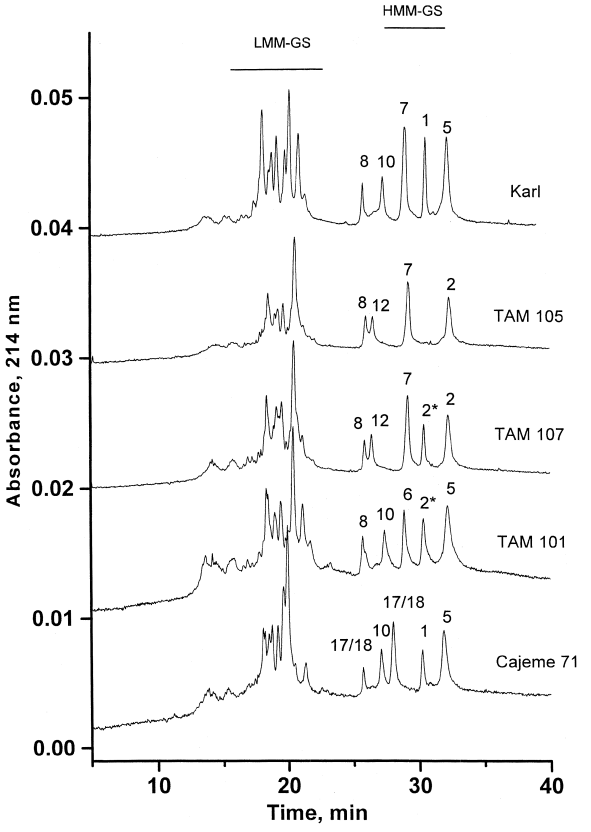

Fig. 7. SDS-CE separation of glutenins in Bio-Rad SDS-CE reagent modified by the addition of 15% ethylene glycol. From Ref. [98], with permission.

Bean and Lookhart [98] demonstrated that the addition of ethylene glycol to the separation buffer improved the resolution of the HMM-GS in the Bio-Rad commercial polymer as did methanol. However, the ethylene glycol did not have the problems of evaporation that the methanol had and did not cause precipitation of the polymer when added to the buffer, unlike methanol. As of yet, no experimental data has been reported explaining why wheat proteins show improved separations in SDS-CE when organic modifiers are added to the separation buffers, though several hypotheses have been put forward [98].

In addition to wheat, at least one other SDS-CE separation of cereal proteins has been reported. Parris et al. [100] reported the use of the Bio-Rad commercial reagent for the separation and quantitation of maize zeins. This method allowed the zein content of maize to be reliably quantitated.

3.3. Two dimensional high-performance liquid chromatography–high-performance capillary electrophoresis

In addition to the one-dimensional (1D) methods discussed above, methods for high-resolution 2D HPLC–HPCE methods have been reported [20]. This method has been successfully used to separate gliadins and glutenins from wheat [20] as well as hordeins from barley [69]. This method requires no special interfaces between the HPLC and HPCE instruments, instead an automated fraction collector is used to collect fractions throughout the HPLC separation. These fractions are then loaded directly into the HPCE instrument and separated. Computer

software is used to combine the data into a comprehensive 2D map, similar to those of traditional 2D gel electrophoresis. Data can also be combined into 3D surface plots to provide quantitative information. An example of wheat gliadins separated by this 2D procedure is shown in Fig. 8. This procedure can be almost entirely automated and requires no specialized equipment. With the use of HPCE buffers that allow extremely rapid separations [91], the 2D HPLC–HPCE separations would require less total time to complete. All possible combinations of modes of HPLC–HPCE should also be possible, providing a great deal of flexibility in separating cereal storage proteins.

3.4. Repeatability

One concern when using HPCE to separate pro-

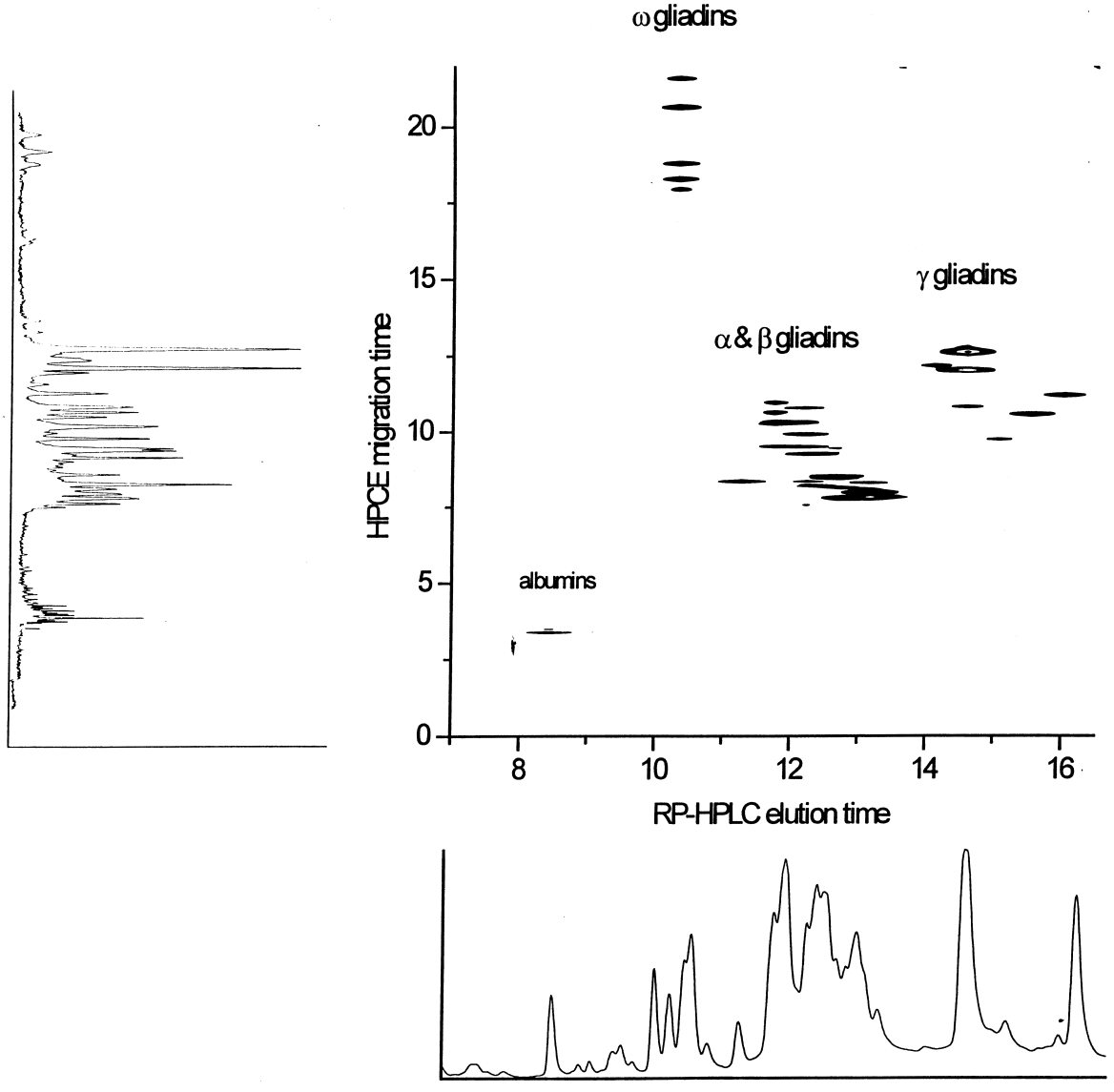

Fig. 8. Two-dimensional RP-HPLC–FZCE separations of gliadins. From Ref. [20], with permission.

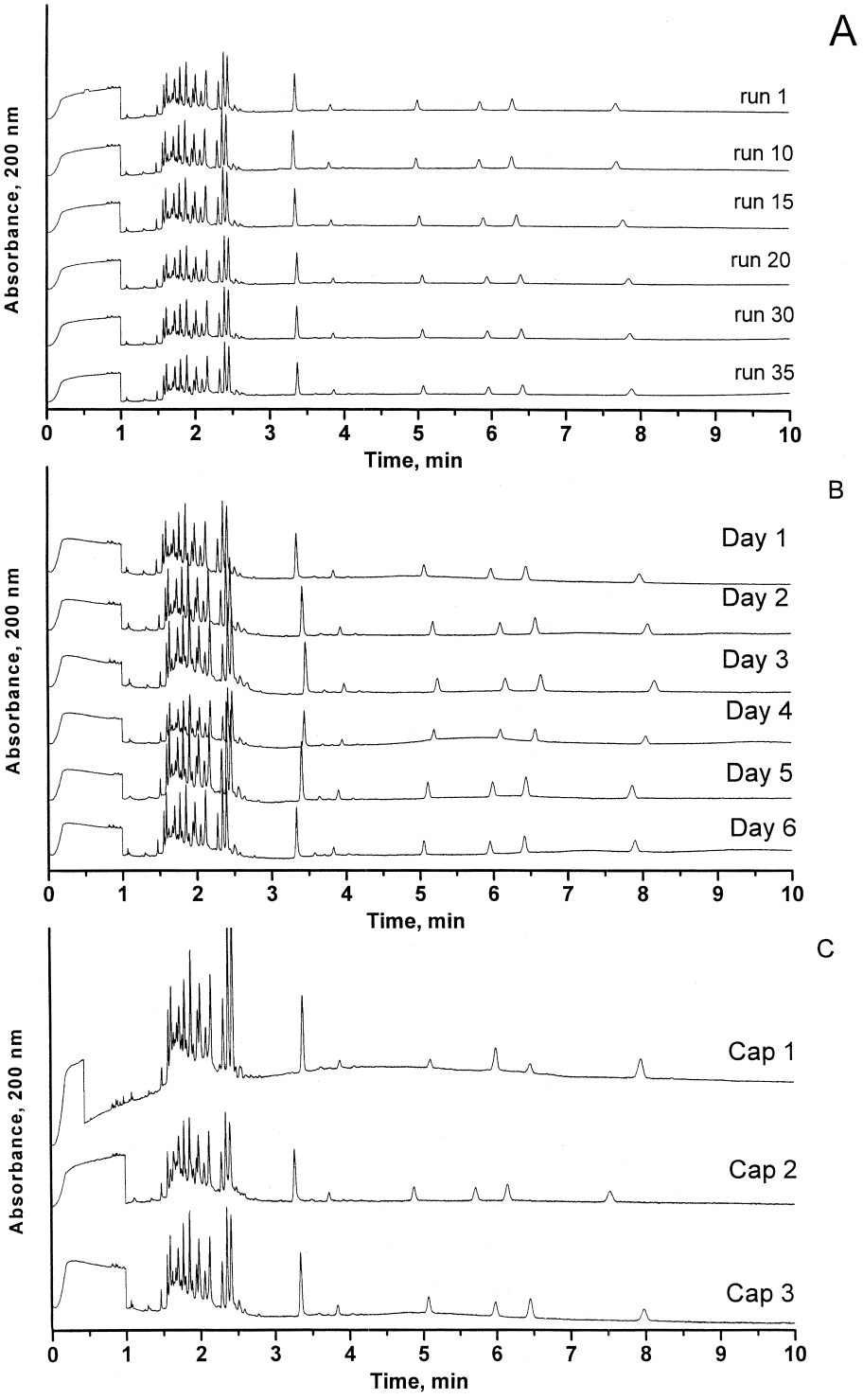

Fig. 9. Run-to-run (A), day-to-day (B), and capillary-to-capillary (C) repeatability of FZCE separations of wheat proteins. From Ref. [91], with permission.

teins is repeatability. HPCE is capable of producing extremely high resolution separations, however, if these separations are not reproducible, the technique is not useful. Thus a number of papers have addressed the repeatability of cereal protein separations [69,71,74,76–79,81,83,87,89]. In all cases repeatability has been found to be excellent, often with migration time RSDs less than 1% (e.g., Refs. [79,91]). Even day-to-day and capillary-to-capillary repeatability is good, with RSDs less than 5% for a number of cereals [69,71,91,98]. An example of the repeatability possible with HPCE is shown in Fig. 9.

A wide variety of capillary rinsing protocols have been followed [69,71,74,76–79,81,83,87,89]. In HPCE, it is generally a good idea to rinse the capillary with solutions of similar pH as the separation buffer and avoid using solutions of higher or lower pH. For wheat proteins, most often separated at acidic pH, rinsing with an acid [79,83] or separation buffer provides good repeatability [83].

Bean and Lookhart [83] reported that albumin and globulin proteins, or perhaps some other water or salt soluble compound in flour, binds to the capillary walls and required strong post-separation rinses to obtain good repeatability. Therefore, removal of these substances by pre-extraction allowed capillaries to only be rinsed with separation buffer between runs, thereby reducing total separation time [83]. Capillary rinsing protocols were specifically studied in a number of reports [76,79,83]. Bean and Lookhart [83] reported the use of a simple equilibration protocol that used only separation buffer and was very rapid (~30 min with 25 μm I.D. capillaries). This rinsing protocol can be shortened to 10 min for 50 μm I.D. capillaries (Bean and Lookhart, unpublished results) and has not failed to successfully equilibrate a capillary in over 150+ different capillaries (Bean and Lookhart, unpublished results). Thus, HPCE is a rugged, reliable method for separating cereal storage proteins [101].

4. Conclusions

Cereal storage proteins have been intensively studied for a number of years. One of the major methods for separating these proteins has been electrophoresis. Through the years a number of different electrophoretic methods and techniques have been adopted for the characterization and study of this interesting group of proteins. Slab gels have played a major role in helping to understand the relationships between these proteins and their many nutritional and functional roles in human and animal feeds.

Capillary electrophoresis is now starting to be used and should provide new information and opportunities for the separation and study of cereal storage proteins. Starting with two papers published in 1992, now over 35 papers have been published using HPCE to study cereal proteins, with roughly half of those appearing in 1998 and 1999. The number of research groups now employing HPCE to study cereal proteins has increased steadily, showing the growth of HPCE.

Improvements in methodology over the years has led to faster separations and higher resolution of cereal storage protein separations for both PAGE and HPCE. Continued development and improvement will hopefully continue to lead to a better understanding of these proteins.

References

[1] R.J. Cooke, in: C.W. Wrigley (Ed.), Identification of Food Grain Varieties, AACC, St. Paul, MN, 1995, p. 1.
[2] B.J. Miflin, J.M. Field, P.R. Shewry, in: J. Daussant, J. Mosse, J. Vaughan (Eds.), Seed Proteins, Academic Press, London, 1983, p. 255.
[3] S.P. Cauvain, in: S.P. Cauvain, L.S. Young (Eds.), Technology of Bread Making, Blackie, London, 1998, p. 1.
[4] J.A. Bietz, J.E. Kruger, in: J.E. Kruger, J.A. Bietz (Eds.), High Performance Liquid Chromatography of Cereal and Legume Proteins, American Association of Cereal Chemists, St. Paul, MN, 1994, p. 1.
[5] P.I. Payne, in: J. Daussant, J. Mosse, J. Vaughan (Eds.), Seed Proteins, Academic Press, London, 1983, p. 223.
[6] F. MacRitchie, in: J.E. Kinsella (Ed.), Advances in Food and Nutrition Research, Academic Press, San Diego, CA, 1992, p. 1.
[7] C.H. Bailey, Cereal Chem. 18 (1941) 555.
[8] T.B. Osborne, The Proteins of the Wheat Kernel, Carnegie Institute, Washington, DC, 1907.
[9] J.E. Kruger, J.A. Bietz (Eds.), High-Performance Liquid Chromatography of Cereal and Legume Proteins, AACC, St. Paul, MN, 1994.
[10] G.L. Lookhart, in: K.J. Lorenz, K.K. Kulp (Eds.), Handbook of Cereal Science and Technology, Marcel Dekker, New York, 1991, p. 441.

[11] G.L. Lookhart, J.A. Bietz, in: J.E. Kruger, J.A. Bietz (Eds.), High-Performance Liquid Chromatography of Cereal and Legume Proteins, AACC, St. Paul, MN, 1994, p. 51.
[12] C.W. Wrigley (Ed.), Identification of Food Grain Varieties, AACC, St. Paul, MN, 1995.
[13] T. Rabilloud, Electrophoresis 17 (1996) 813.
[14] P.R. Shewry, A.S. Tathum, J. Forde, M. Kreis, B.J. Miflin, J. Cereal Sci. 4 (1986) 97.
[15] A. Esen, J. Cereal Sci. 5 (1987) 117.
[16] J.M. Shull, J.J. Watterson, A.W. Kirleis, J. Agric. Food Chem. 39 (1991) 83.
[17] B.R. Hamaker, A.A. Mohamed, J.E. Habben, C.P. Huang, B.A. Larkins, Cereal Chem. 72 (1995) 583.
[18] B.O. Juliano, A. Hussain, A.P. Resurreccion, W. Bushuk, Cereal Chem. 68 (1991) 317.
[19] J.R.N. Taylor, L. Schussler, J. Sci. Food Agric. 35 (1984) 1.
[20] S.R. Bean, G.L. Lookhart, Cereal Chem. 74 (1997) 758.
[21] G.L. Lookhart, S.R. Bean, Cereal Chem. 72 (1995) 527.
[22] K.H. Sutton, J.A. Bietz, J. Cereal Sci. 25 (1997) 9.
[23] L. Capelli, F. Forlani, F. Perini, N. Guerrieri, P. Cerletti, P.G. Righetti, Electrophoresis 19 (1998) 311.
[24] Z.K. Shihabi, Electrophoresis 19 (1998) 3008.
[25] Z.K. Shihabi, J. Chromatogr. A 853 (1999) 3.
[26] B.J. Wanders, F.M. Everaerts, in: J.P. Landers (Ed.), Handbook of Capillary Electrophoresis, CRC Press, Boca Raton, FL, 1994, p. 111.
[27] C. Schwer, F. Lottspeich, J. Chromatogr. 623 (1992) 345.
[28] R.W. Jones, N.W. Taylor, F.R. Senti, Arch. Biochem. Biophys. 84 (1959) 363.
[29] J.W. Woychik, J.A. Boundy, R.J. Dimler, Arch. Biochem. Biophys. 94 (1961) 477.
[30] J.A. Bietz, J.S. Wall, Cereal Chem. 49 (1972) 416.
[31] P.I. Payne, K.G. Corfield, J.A. Blackman, Theor. Appl. Genet. 55 (1979) 153.
[32] G.L. Lookhart, C.W. Wrigley, in: C.W. Wrigley (Ed.), Identification of Food Grain Varieties, AACC, St. Paul, MN, 1995, p. 55.
[33] P. Kolster, C.F. Krechting, W.M.J. van Gelder, J. Cereal Sci. 15 (1992) 49.
[34] G. Hou, P.K.W. Ng, Cereal Chem. 72 (1995) 545.
[35] B.A. Marchylo, Can. J. Plant Sci. 67 (1987) 945.
[36] B.A. Marchylo, K.A. Handel, V.J. Mellish, Cereal Chem. 66 (1989) 186.
[37] D.D. Kasarda, K.M. Woodard, A.E. Adalsteins, Cereal Chem. 75 (1998) 70.
[38] C.W. Wrigley, P.J. Gore, H.P. Mansau, Electrophoresis 12 (1991) 384.
[39] Z. Zhen, D. Mares, J. Cereal Sci. 15 (1992) 63.
[40] D. Khelifi, G. Branlard, J. Cereal Sci. 13 (1991) 41.
[41] R.B. Gupta, K.W. Shepherd, Theor. Appl. Genet. 80 (1990) 65.
[42] R.A. Graybosch, R. Morris, J. Cereal Sci. 11 (1990) 201.
[43] D.Y. Huang, K.J. Khan, Cereal Sci. 27 (1998) 237.
[44] R.J. Cooke, in: C.W. Wrigley (Ed.), Identification of Food Grain Varieties, AACC, St. Paul, MN, 1995, p. 221.
[45] B.O. Juliano, in: C.W. Wrigley (Ed.), Identification of Food Grain Varieties, AACC, St. Paul, MN, 1995, p. 265.
[46] D.M. Peterson, W.K. Heneen, B. Mattson, in: C.W. Wrigley (Ed.), Identification of Food Grain Varieties, AACC, St. Paul, MN, 1995, p. 239.
[47] K. Khan, L. Huckle, Cereal Chem. 69 (1992) 686.
[48] C.W. Wrigley, P.J. Gore, H.P. Mansau, Appl. Theor. Electrophoresis 3 (1992) 69.
[49] K. Khan, C.E. MacDonald, O.J. Banasik, Cereal Chem. 60 (1983) 178.
[50] G.L. Lookhart, D.B. Cooper, B.L. Jones, Cereal Chem. 62 (1985) 19.
[51] K. Khan, A.S. Hamada, J. Patek, Cereal Chem. 62 (1985) 310.
[52] M.H. Morel, Cereal Chem. 71 (1994) 238.
[53] A. Curioni, M.H. Morel, L. Furegon, R. Redaelli, D.B. Peruffo, Electrophoresis 16 (1995) 1005.
[54] M. Rumbo, F.G. Chirdo, S.A. Giorgieri, C.A. Fossati, M.C. Anon, J. Agric. Food Chem. 47 (1999) 3243.
[55] G.L. Lookhart, Cereal Chem. 62 (1985) 345.
[56] P.G. Righetti, A.B. Bosisio, Electrophoresis 2 (1981) 65.
[57] P.H. O'Farrel, J. Biol. Chem. 250 (1975) 4007.
[58] P.G. Righetti, Isoelectric Focusing – Theory, Methodology and Applications, Elsevier, Amsterdam, 1983.
[59] A. Curioni, A.D.B. Peruffo, N.E. Pogna, Electrophoresis 11 (1990) 462.
[60] A.V. Lebedev, V.P. Neudachin, V.G. Zima, V.G. Ryadchikov, Sci. Tools 23 (1976) 22.
[61] U. Stenram, F. Acevedo, M. Larsson-Raznikiewicz, Food Chem. 36 (1990) 305.
[62] I. Sondergaard, K. Jensen, B.N. Krath, Electrophoresis 15 (1994) 584.
[63] D.A. Dougherty, R.L. Wehling, M.G. Zeece, J.E. Partridge, Cereal Chem. 67 (1990) 564.
[64] M.G. Lei, G.R. Reeck, Cereal Chem. 63 (1986) 111.
[65] N.G. Anderson, S.L. Tollaksen, F.H. Pascoe, L. Anderson, Crop Sci. 25 (1985) 667.
[66] D. Lafiandra, D.D. Kasarda, Cereal Chem. 62 (1985) 314.
[67] W.E. Werner, J.E. Wiktorowicz, D.D. Kasarda, Cereal Chem. 71 (1994) 397.
[68] G.L. Lookhart, S.R. Bean, Cereal Chem. 72 (1995) 527.
[69] G.L. Lookhart, S.R. Bean, B.L. Jones, Electrophoresis 20 (1999) 1605.
[70] M. Rumbo, S.A. Giorgieri, J. Cap. Electrophoresis 5 (1998) 39.
[71] S.R. Bean, G.L. Lookhart, J.A. Bietz, J. Agric. Food Chem. (2000) in press.
[72] H.E. Schwartz, R.H. Palmeri, J.A. Nolan, R. Brown, Separation of Proteins and Peptides by Capillary Electrophoresis: An Introduction, Beckman Instruments, Fullerton, CA, 1992.
[73] J.A. Bietz, E. Schmalzried, Cereal Foods World 37 (1992) 555.
[74] J.A. Bietz, E. Schmalzried, Lebensm. Wissensch. Technol. 28 (1995) 174.
[75] Y. Yan, G. Liu, Acta Agronom. Sinica 25 (1999) 237.
[76] Y. Yan, G. Surlan-Momirovic, S. Prodanovic, D. Zoric, G. Liu, Euphytica 105 (1999) 197.
[77] Y. Yan, G. Liu, in: Seed Industry and Agric. Develop., Int. Annual Meeting, China, 1997, p. 608.

[78] G.L. Lookhart, S.R. Bean, Cereal Chem. 72 (1995) 42.
[79] G.L. Lookhart, S.R. Bean, Cereal Chem. 73 (1996) 81.
[80] T. Schober, S. Graber, M. Kuhn, Deut. Lebensm. Rundsch. 94 (1998) 297.
[81] L. Day, P. Greenwell, S. Lock, H. Brown, J. Chromatogr. A 836 (1999) 147.
[82] J.C. Reijenga, T.P.E.M. Verheggen, J.H.P.A. Martens, F.M.J. Everaerts, J. Chromatogr. A 744 (1996) 147.
[83] S.R. Bean, G.L. Lookhart, Electrophoresis 19 (1998) 3190.
[84] S. Hjerten, L. Valtcheva, K. Elenbring, J.L. Liao, Electrophoresis 16 (1995) 584.
[85] J. Zhu, K. Khan, Cereal Chem. 76 (1999) 261.
[86] P.G. Righetti, C. Gelfi, M. Perego, A.V. Stoyanov, A. Bossi, Electrophoresis 18 (1997) 2145.
[87] L. Capelli, F. Forlani, F. Perini, N. Guerrieri, P. Cerletti, P.G. Righetti, Electrophoresis 19 (1998) 311.
[88] P.G. Righetti, E. Oliveri, A. Viotti, Electrophoresis 19 (1998) 1738.
[89] E. Olivieri, A. Viotti, M. Lauria, E. Simo-Alfonso, P.G. Righetti, Electrophoresis 20 (1999) 1595.
[90] A. Bossi, P.G. Righetti, Electrophoresis 18 (1997) 2012.
[91] S.R. Bean, G.L. Lookhart, J. Agric. Food Chem. (2000) in press.
[92] A. Guttman, Electrophoresis 17 (1996) 1333.
[93] C. Heller, J. Chromatogr. A 698 (1995) 19.
[94] E. Kenndler, H. Poppe, J. Cap. Electrophoresis 1 (1994) 144.
[95] T. Takagi, Electrophoresis 18 (1997) 2239.
[96] W.E. Werner, D.M. Demorest, J. Stevens, J.E. Wiktorowicz, Anal. Biochem. 212 (1993) 253.
[97] E. Scholz, G. Pogany, K. Ganzler, G.W. Laurie, J. Cap. Electrophoresis 4 (1997) 287.
[98] S.R. Bean, G.L. Lookhart, J. Agric. Food Chem. 47 (1999) 4246.
[99] N.J. Pollard, C.W. Wrigley, F. Bekes, MacRitchie, Plant Varieties Seeds 11 (1998) 187.
[100] N. Parris, L. Dickey, J. Craig, Cereal Chem. 74 (1997) 766.
[101] S.R. Bean, J.A. Bietz, G.L. Lookhart, J. Chromatogr. A 814 (1998) 25.