JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Extraction and Separation of Water-Soluble Proteins from Different Wheat Species by Acidic Capillary Electrophoresis

ANGELA R. PIERGIOVANNI*

Istituto di Genetica Vegetale-CNR, via Amendola 165/a, 70126 Bari, Italy

Optimization of protein extraction and a capillary zone electrophoresis method for water-soluble protein analysis in wheat is described. The optimal separation was obtained with a 50 μm i.d. $\times$ 27 cm (20 cm to detector) uncoated capillary filled with 0.1 M phosphoric acid/β-alanine, pH 2.5, buffer containing urea (1 M), 0.05% (w/v) hydroxypropylmethylcellulose, and 20% (v/v) acetonitrile. Separation was carried out at 15 kV and 35 °C for 9 min. Extract stability was also investigated within 2 h from the extraction. Good visual peak parameters and a higher sensitivity can be obtained when 30% ethanol is used as an extraction medium. The method was successfully used to analyze extracts obtained from whole and refined meals of six *Triticum* spp. Moreover, the described methodology could be applied to the discrimination of species with different ploidy levels and to the detection of durum wheat adulteration, as well as to screen wheat collections for enzymes involved with the quality of wheat derivatives.

KEYWORDS: Capillary zone electrophoresis; durum wheat adulteration; species differentiation; *Triticum* spp; water-soluble protein extraction and analysis

INTRODUCTION

From a functional point of view, wheat proteins are classified into gluten and non-gluten proteins. The gluten proteins are constituted by gliadins and high and low molecular weight glutenins (HMW-GS and LMW-GS, respectively). The non-gluten proteins, comprised of both albumins and globulins, represent 15−20% of the total proteins and mainly occur in the outer layers of the kernel (*1*). Albumins and globulins are mainly metabolic and structural proteins (*2*); the storage proteins, generically named triticins, are little represented in these fractions (*3*). The high molecular mass albumins include enzymes, such as β-amylase, involved in metabolic activity, while the low molecular mass albumins (LMM albumins) include a variety of trypsin and α-amylase inhibitors that are implicated in plant defense (*4*) and germination (*5*). Some oxidative enzymes such as polyphenoloxidase (PPO) and peroxidase that affect the end-use quality, being responsible for the discoloration of food products, are also components of the water-soluble protein fraction (*6*). Moreover, a number of salt-soluble proteins have been identified as allergens that contribute to baker's asthma, a typical occupational allergic disease that has been in existence since ancient Roman times (*7*, *8*). Albumins and globulins are nutritionally significant, having a higher content of the essential amino acids lysine and methionine as compared to the gluten protein fraction (*9*). As a consequence of their wide range of functions, the non-gluten proteins are encoded by dozens of genes belonging to different families and are located on several chromosomes (*1*). To date, these genes have not been completely characterized with the exception of α-amylase and trypsin inhibitors (*10*).

Despite an increase in the number of DNA markers available for wheat, polymorphic protein markers continue to be valuable as they provide simple and economical assays. Wheat proteins are very difficult to separate and quantify because of their complexity and their interactions. Among the analytical techniques used for the separation of cereal proteins (A-PAGE, SDS-PAGE, 2-DE, and RP-HPLC), capillary electrophoresis (CE) is being used increasingly. CE can be completely automated and does not need toxic reagents or long analysis times. It requires very small sample sizes and small amounts of buffer and allows high-resolution separation. Nowadays, this technique is widely used for gliadin and glutenin analysis (*11*), while very little research has been focused on the development of CE methods for separating the water-soluble proteins. This can be attributed to several reasons. First of all, difficulties are associated with the analysis of water-soluble proteins by means of CE because these proteins have a high tendency to bind to the inner walls of fused silica capillaries as a consequence of their high amounts of basic amino acids (*12*). Moreover, compounds such as free amino acids, DNA, and carbohydrates being partially or completely soluble in water can interfere with CE analysis (*11*). On the other hand, the low polymorphism previously described for wheat albumins greatly discouraged efforts to develop new CE methodologies. However, a recent study demonstrating the utility of isoelectric focusing analysis of albumins to fingerprint wheat cultivars has renewed attention toward this protein fraction (*10*).

* Corresponding author. Tel.: +39 080 5583400; fax: +39 080 5587566; e-mail: angelarosa.piergiovanni@igv.cnr.it.

10.1021/jf063628n CCC: $37.00
Published on Web 04/17/2007

Table 1. Composition of Separation Buffers Tested in this Study[a]

work code	composition of separation buffers	operating conditions
phos	0.050 M sodium dihydrogenphosphate (pH 2.5), 0.05% (w/v) HPMC, 20% (v/v) ACN	10 kV for 10 min, 35 °C, 1 min ramp-up time
phos-gly	0.1 M phosphoric acid/glycine (pH 2.5), 0.05% (w/v) HPMC, 20% (v/v) ACN	12 kV for 10 min, 35 °C, 0.17 min ramp-up time
asp	0.040 M aspartic acid, 0.05% (w/v) HEC, 4 M urea, 20% (v/v) ACN	22 kV for 5 min, 42 °C, 1 min ramp-up time
phos-ala	0.1 M phosphoric acid/β-alanine (pH 2.5), 1 M urea, 0.05% (w/v) HPMC, 20% (v/v) ACN	15 kV for 9 min, 35 °C, 1 min ramp-up time
phos-phy	0.020 M phosphate (pH 6.8), 0.030 M phytic acid sodium salt, 20% (v/v) ACN	12 kV for 18 min, 25 °C, 0.17 min ramp-up time
phos-HSA	0.050 M phosphoric acid (pH 2.5), 0.05% (w/v) HPMC, 0.050 M HSA-Na salt, 20% (v/v) ACN	18 kV for 12 min, 35 °C, 1 min ramp-up time

[a] Only the best operating conditions for each buffer are reported.

Lookhart and Bean (*13*) attributed to water-soluble proteins the early migrating peaks of 70% ethanolic extracts analyzed under separation conditions optimized for gliadin separation. However, under these conditions, the height of peaks related to water-soluble proteins is too low to have an analytical value. Scholz et al. (*14*) studied the accumulation of different wheat protein fractions during the maturation of wheat kernels using a phosphoric/β-alanine buffer (pH 2.5) containing urea, hydroxypropylmethylcellulose (HPMC), and acetonitrile (ACN) as a background electrolyte. Bean and Tilley (*15*) obtained excellent resolutions using a phosphate buffer, pH 2.5, with the addition of hexanesulfonic acid (HSA) and acetonitrile. Finally, Bonetti et al. (*16*) described the simultaneous analysis of albumins and gliadins with a basic buffer consisting of boric acid, sodium tetraborate, phosphoric acid, polyethylene glycol (PEG), and ethanol.

The present research was designed to compare some CE protocols available in the literature for the analysis of water-soluble wheat proteins. Attention was also devoted to the methods used for protein extraction from both whole meal and flour. The development of a reliable CE method for separating these wheat proteins would allow for their regular analysis.

MATERIALS AND METHODS

Wheat Samples. Seeds of six cultivars or accessions belonging to different *Triticum* species were used in this study. The cultivars were 'Norba' (*Triticum durum* Desf.), 'Rio' (*T. aestivum* L.), 'Farvento' (*T. dicoccum* Schrank.), and 'Forenza' (*T. spelta* L.). The tested accessions were one each for einkorn (*T. monococcum* L.) MG 300007 and oriental wheat (*T. turanicum* Jakuz.) PI 192658. Cultivars and the einkorn accessions were obtained from the Istituto di Genetica Vegetale, CNR (Bari, Italy), while the oriental wheat accession was from the USDA. Whole and refined meals were analyzed only for the four cultivars. Seeds were milled in a mill Ciclotec 1093 (Tecator, Sweden) to obtain whole meals, while a Labormill 4RB (Bona, Italy) was used to remove the outer layers of the kernel to obtain the refined meals. As concerns the analysis of artificially adulterated durum wheat semolina, a set of samples was prepared. The adulterant common wheat blend, cv. 'Rio' flour, was added with decreasing percentages (25, 20, 15, 10, and 5%, w/w) in the semolina of the durum wheat cv. 'Norba'. Adulterated samples were extracted in triplicate and processed as described for the uncontaminated ones.

Albumin Extraction. Whole or refined meal (40 mg) was extracted with deionized water (1:5, w/v) for 8 min at room temperature with continuous vortexing. After centrifugation for 10 min at 12 000 rpm, the supernatant was collected and used for CE analysis. The same procedure was used for the extraction with 30% ethanol in water. Gliadin removal before the extraction of water-soluble proteins was carried out as follows. Wheat meal (60 mg) was extracted with cold ethanol (70%) in water (1:5, w/v) for 30 min at room temperature with periodical vortexing. After centrifugation for 15 min at 9000 rpm, the supernatant was discharged, and the pellet was air-dried. The pellet was successively extracted with deionized water as previously described.

CE Analysis. A Beckman P/ACE MDQ (Fullerton, CA) equipped with a UV detector set at 214 nm was used in this study. Separations were carried out using an uncoated fused silica capillary that was 27 cm in length (20 cm to detector) and had a 50 μm i.d. Samples were injected for 5 s at 0.5 psi. The composition of the tested buffers as well as the optimum separation conditions are reported in **Table 1**. All buffers were prepared in 18 MΩ cm distilled and deionized water (Milli-Q water system Millipore, Billerica, MA). All chemicals were analytical reagent grade. The capillary was rinsed at the end of each day by flushing with 0.1 M NaOH for 3 min and with water for 20 min. Beckman Karat 32 software was used for storing, manipulating, and comparing electropherograms as well as for the peak area calculations. For each test, CE analysis was repeated on different extracts on the same days and on different days.

Statistical Analysis. The reproducibility of CE analysis under the different experimental conditions investigated in this study was determined by calculating the coefficient of variation (RSD %) of migration times relative to the prominent peaks detectable in the electropherograms. The calculation was carried out on three runs. As concerns the analysis of artificially adulterated samples, three analyses were realized for each calibration point. The regression equation was calculated using the least-squares method, and confidence intervals ($p < 0.05$) were determined for each adulteration level.

RESULTS AND DISCUSSION

CZE Separation of Water-Soluble Wheat Proteins. The majority of CE methods developed to separate cereal proteins use low pH buffers with one or more additives to improve protein solubility and reduce protein−inner capillary wall interactions that adversely affect the peak resolution. Bean and Lookhart (*17*, *18*) separated wheat albumins with a 50 mM sodium phosphate buffer, pH 2.5, containing HPMC and ACN (**Table 1**). The electropherogram relative to albumins extracted from the whole meal of cv. 'Norba' and analyzed according to the Bean and Lookhart protocol is shown in **Figure 1**, trace **B**. The protein separation was successfully performed over 9.0 min, and although a partial overlap of some peaks occurred, the overall peak resolution was acceptable. The replacement of the high-conductive sodium dihydrogenphosphate with the lower conductivity glycine (**Table 1**, phos-gly buffer) slightly reduced the time of analysis but produced a tangible loss in peak resolution (**Figure 1**, trace **A**). Advantages associated with the use of isoelectric buffers for the analysis of cereal storage proteins are widely described in the literature (*19*, *20*). Isoelectric compounds have pI values that are approximately equal to their pH value in solution, allowing them to provide a good buffering capacity without the need of a co-ion. Because of these unique properties, isoelectric buffers have an extremely low conductivity, allowing very fast protein separations. With a 40 mM aspartic acid buffer (**Table 1**, asp buffer) containing hydroxyethylcellolose (HEC), urea, and ACN (*12*), less than 3 min was sufficient to complete the water extract analysis (**Figure 1**, trace

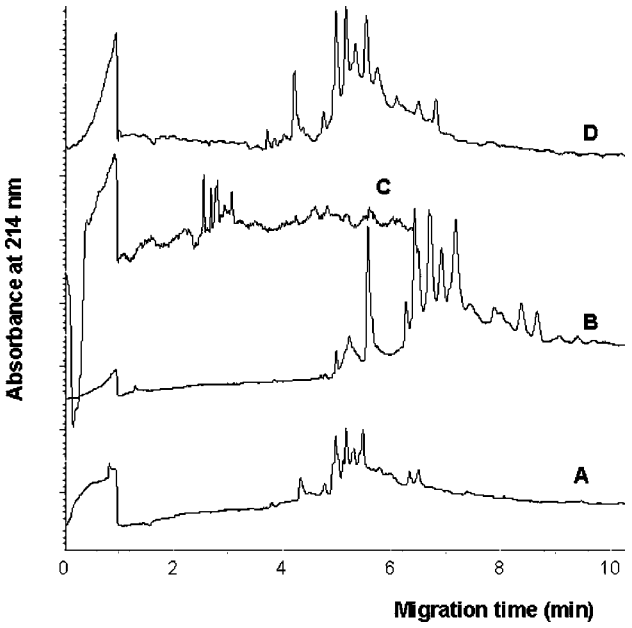

Figure 1. Effect of buffer composition on resolution of water-soluble proteins. The extracts were from whole meal of cv. 'Norba'. Trace **A**: phos-gly buffer; trace **B**: phos buffer; trace **C**: asp buffer; and trace **D**: phos-ala buffer. Buffer composition and separation conditions as in **Table 1**.

C). Moreover, the sharpness as well as the symmetry of peaks were significantly improved as compared to phos-gly, phos, and phos-ala buffers (**Figure 1**, traces **A**, **B**, and **D**, respectively). Unfortunately, the reduced sensitivity of the asp buffer, decreasing the peak height, makes the detection of the less represented protein fractions very difficult. An excellent correspondence of the main peaks is evident between traces **B** and **C** of **Figure 1**, while the late migrating peaks of trace **B** (more than 7 min) cannot be discerned from the noise of trace **C**. This should be taken into consideration because it is a substantial limitation when electrophoretic profiles of different cultivars or wheat species need to be compared. Scholz et al. (*14*) investigated albumin accumulation in wheat kernels by using a phosphate/β-alanine buffer, pH 2.5, with the addition of urea, HPMC, and ACN (**Table 1**, phos-ala buffer). A slight increase of both separation voltage (from 14 to 15 kV) and capillary temperature (from 25 to 35 °C) was found to be beneficial to improve the resolution and separation efficiency reported by Scholz et al. (*14*). The modifications of the operating conditions allowed completion of the water extract analysis in less than 7 min (**Figure 1**, trace **D**). The electrophoretic patterns obtained with phos or phos-ala buffer (traces **B** and **D**, respectively) showed a very high similarity. However, the phos-ala buffer gave slightly superior performances by matching to a shorter time of analysis, good resolution, and acceptable visual peak parameters (i.e., sharpness, height, and presence of tailing). Moreover, the baseline, after passage of the whole train of peaks, returns to the initial value, suggesting that there is no binding of proteins to the capillary wall. Finally, the examination of the current profiles did not reveal the fluctuations in the initial stages of the separation responsible for the drift of migration times from run-to-run observed when the phos buffer was used. An excellent day-to-day reproducibility of the profile was obtained with this buffer (data not shown).

Recently, the alkaline buffer consisting of borate/boric acid/phosphoric acid/PEG/ethyl alcohol has been proposed to analyze albumins and gliadins at the same time (*16*). This buffer gives rise to two distinct migration regions in the electropherogram. Peaks belonging to the fast migration region are mainly albumins, while gliadins are located in the slow migration region. The overall pattern of the albumin region obtained with this buffer was similar to that of the phos or phos-ala buffers, but the separation time was longer, in fact, 15 min was required for completing the run. Moreover, a relatively poor reproducibility, attributed by the authors to the separation conditions (alkaline buffer and 75 μm i.d. uncoated capillary), affected the analysis. Attempts to separate wheat albumins using an uncoated capillary with phosphate buffer (pH 6.8) containing phytic acid sodium salt as a buffer modifier or phosphate buffer (pH 2.5) with the addition of HPMC, 1-HSA sodium salt, and ACN as additives resulted in a poor resolution and in a considerable broadening of protein peaks (data not shown).

Water Extract Stability. It is known that wheat protein fractions may show an instability in the course of their preparation, storage, subsequent redissolution, and electrophoretic analysis (*21*). Albumins contain some enzymes that could become active, when solubilized in water, producing the instability of extracts. For this reason, before an analytical protocol can be successfully used, it is fundamental to check that the protein extracts remain unaltered at least over the time necessary to carry out the analysis. Scarce information is available in the literature about the stability of water-soluble wheat proteins. Bean and Tilley (*15*) reported that modifications of the RP-HPLC chromatogram occur after 10 h of incubation, at room temperature, of water extracts. Scholz et al. (*14*), who analyzed wheat water-soluble proteins by CZE, did not report any information about the extract stability. In this study, albumins extracted from both whole and refined meal of the tested cultivars were repeatedly analyzed over a 2 h period. Between subsequent injections, extracts were held in the sample storage room of P/ACE MDQ, which was kept at 25 °C to avoid their degradation. The overlays of consecutive runs relative to 'Norba' and 'Forenza' cultivars are shown as examples (**Figure 2**). Over the tested period, no appearance or disappearance of peaks was observed in the electrophoretic profile of extracts obtained from whole meals, but changes of patterns are evident. Increasing the incubation time before CE analysis improved the peak visual parameters to a certain extent. The exact reason for this is unknown but is probably related to chemical and/or physical modifications of the co-extracted compounds (e.g., carbohydrates, free amino acid, etc.). Further changes in the electrophoretic pattern involved the height of some peaks in the migrating region between 4.7 and 5.1 min. However, a relationship could exist between the improvement of resolution and the height of these peaks. These data indicate that the impact of extract stability on the electrophoretic profile can be minimized without adopting the time-expensive procedures of stabilization proposed by Bean and Tilley (*15*). Acceptable reproducibility can be obtained by carrying out the CZE analysis within 2 h from the extraction but avoiding beginning the run immediately after extraction to obtain better visual peak parameters. The optimum incubation time was judged to be 1 h. Under these conditions of analysis, the mean variation coefficients of the migration times (RSD %) relative to the traces of **Figure 2** ranged from $\pm$1.7% for the early migrating peak (4.16 and 4.01 min for 'Norba' and 'Forenza', respectively) and $\pm$3.2% for the late prominent migrating peak (5.61 and 5.19 min for 'Norba' and 'Forenza', respectively).

Matrix Effect. Water extracts obtained from whole meals should be regarded as dirty samples because they likely contain, in addition to albumins, some compounds, such as free amino acids, DNA, and carbohydrates that, being water-soluble, are

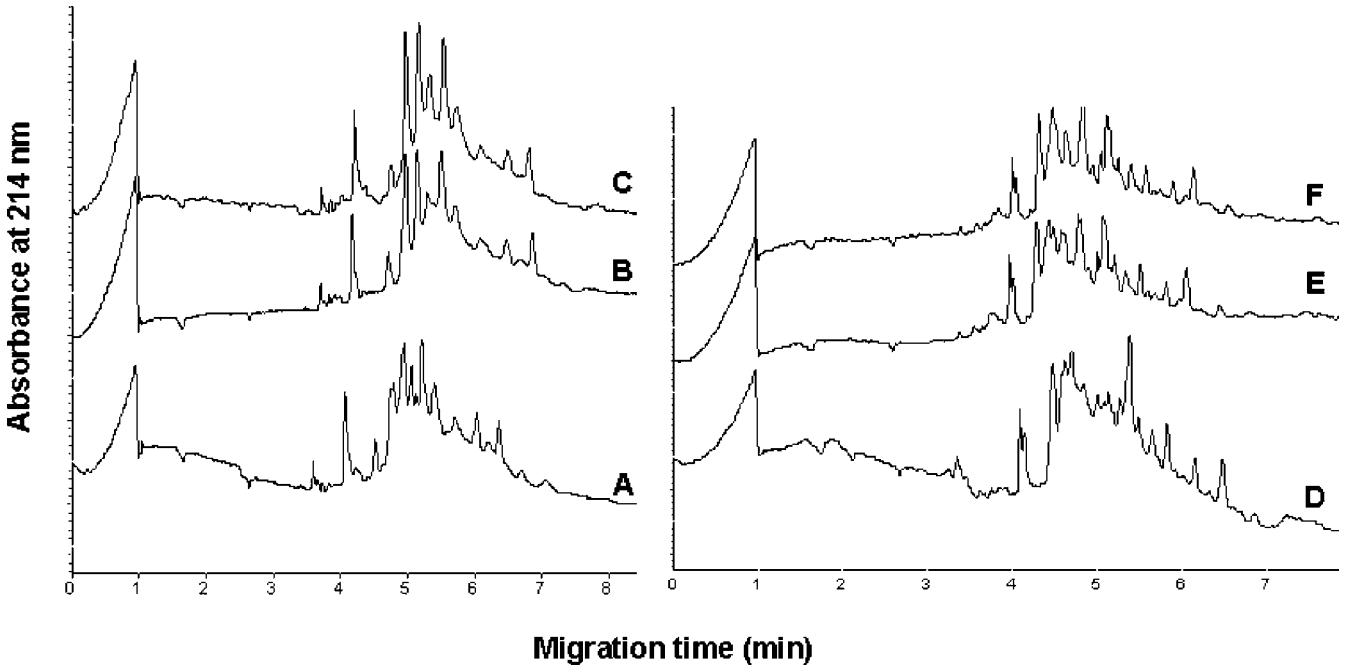

Figure 2. CZE pattern of water-soluble proteins extracted from whole meal repeatedly analyzed over a 2 h period. From the bottom to the top, analyses were carried out at 0, 1, and 2 h after the extraction. Left: cv. 'Norba' and right: cv. 'Forenza'. The phos-ala buffer was used. Separation conditions as in **Table 1**.

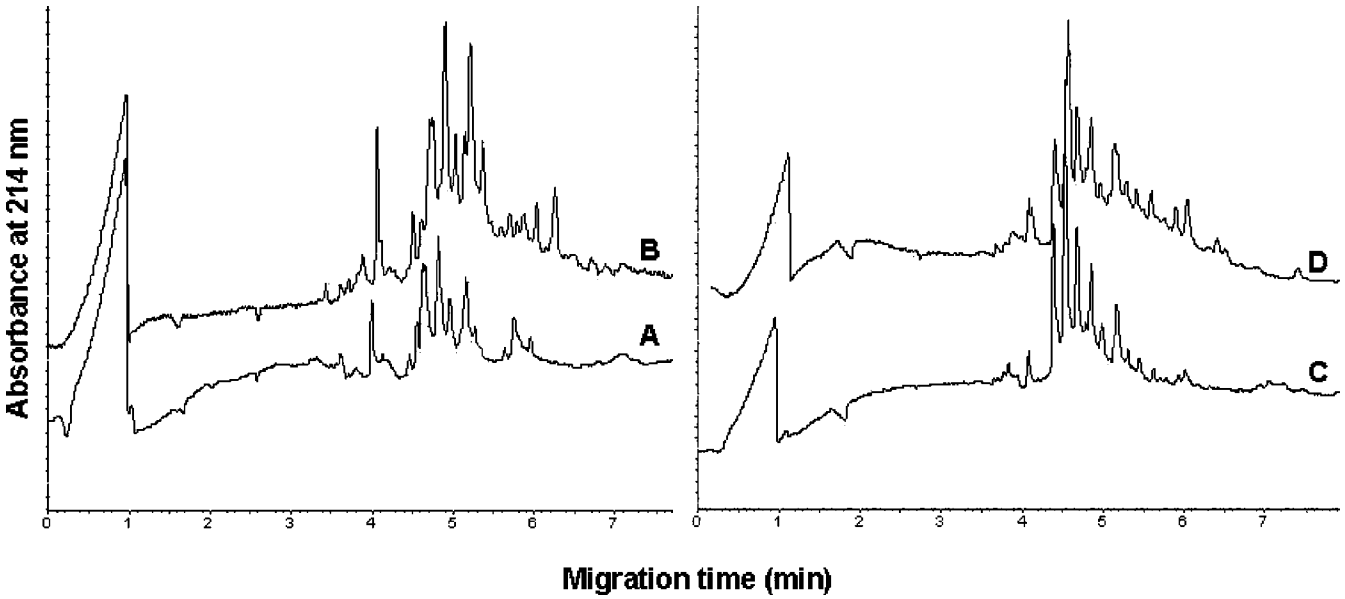

Figure 3. Comparison of water-soluble proteins from whole and refined meal (lower and upper traces, respectively). Left: cv. 'Farvento' and right: cv. 'Rio'. Conditions of analysis as in **Figure 2**.

inevitably co-extracted. Consequently, a remarkable matrix effect on the electrophoretic profile is predictable. How much the profile can be modified by the kind of matrix (i.e., whole or refined meals from which albumins are extracted) is shown in **Figure 3**. For all the tested species, a higher number of peaks was detected in the profiles of extracts from whole meals. In general, several late migrating peaks were missing in the profiles of extracts obtained from refined meals. This suggests that these minor protein fractions are present only in the outer layers of the kernel (the bran), which are removed during milling. At the same time, quantitative differences affecting the early migrating peaks suggested that these fractions are predominant in the endosperm. A remarkable increase of the peak at 4.5 min (**Figure 3**, trace **C**) appeared to be a typical trend in hexaploid wheats, being observed for both 'Rio' and 'Forenza' cultivars. Moreover, a pronounced improvement of the visual peak parameters, such as sharpness and peak resolution, characterized the electrophoretic profiles of the refined meal extracts. It should be noticed that the improved peak resolution would greatly increase the accuracy of the quantitative analyses. In fact, a higher resolution will approximate the peak area calculation to the optimal valley-to-valley integration. Conversely to whole meal extracts, the water-soluble fraction obtained from refined meal is almost completely free of non-protein compounds that adversely affect either the electrophoretic profile or the extract stability over the time. Electropherograms of water-soluble

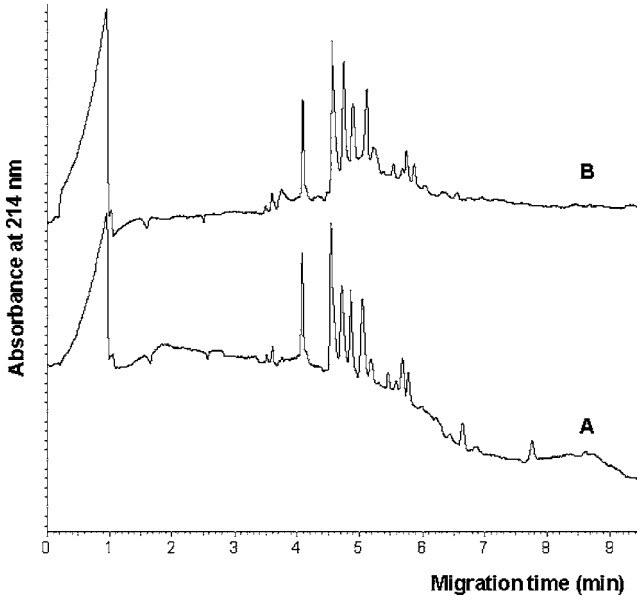

Figure 4. CZE pattern of water-soluble proteins from refined meal of cv. 'Norba' analyzed at 0 and 2 h after the extraction. Conditions of analysis as in **Figure 2**.

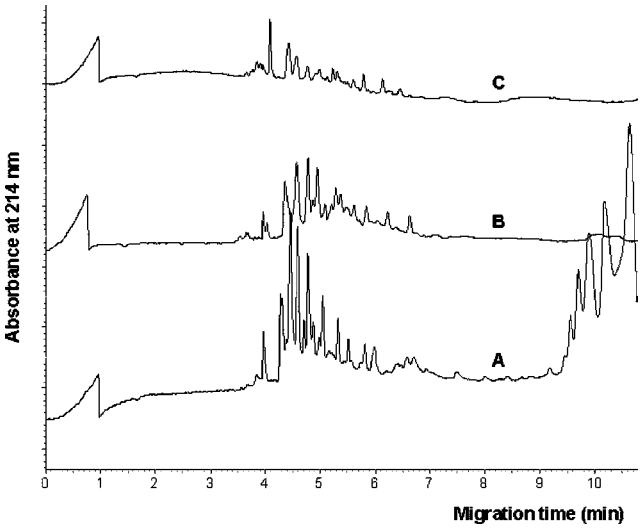

Figure 5. Effect of extraction medium on CZE pattern of water-soluble proteins from cv. 'Rio' whole meal. Trace **A**: 30% ethanol in water; trace **B**: water; trace **C**: sequential gliadin-albumin extraction. Conditions of analysis as in **Figure 2**.

proteins extracted from 'Norba' semolina are shown as examples (**Figure 4**). An excellent separation combined with a high resolution and a good reproducibility of electrophoretic profile was observed over a period of 2 h, if one does not consider the two minor late eluting peaks in the 6.4−7.8 min time window detected only in the first run (immediately after the extraction). The mean variation coefficients of the retention time relative to the early and middle migrating peak (4.07 ± 0.61 and 5.10 ± 1.30%, respectively) were inferior to those reported, in the previous section, for whole 'Norba' meal.

Effect of Albumin Extraction Procedure. The protocol used for the protein extraction can also affect the electrophoretic pattern. In **Figure 5** are compared the profiles of extracts obtained from 'Rio' whole meal according to the following procedures: extraction with 30% ethanol in water (trace **A**);

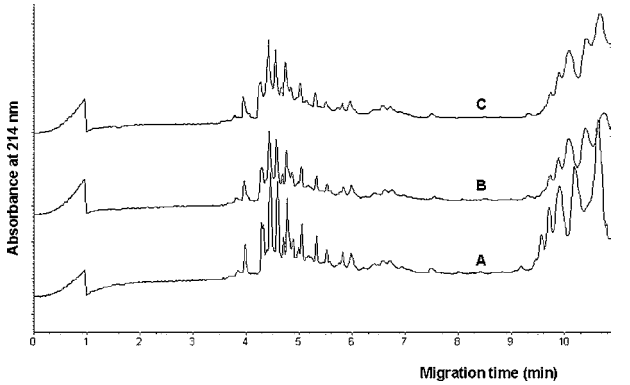

Figure 6. CZE pattern of 30% ethanolic extract from whole meal of cv. 'Rio' analyzed at 0, 1, and 2 h (traces **A**–**C**, respectively) after the extraction. Conditions of analysis as in **Figure 2**.

extraction with water (trace **B**); and gliadin removal prior to albumin extraction with water (trace **C**). As expected, without a preliminary fractionation of proteins, gliadins were partially co-extracted together with albumins when 30% ethanol in water was used as the extraction medium (**Figure 5**, trace **A**). However, this is not a disadvantage because two well-separated regions were detectable in the electropherogram. Peaks belonging to the fast migration region were albumins, while gliadins, migrating slowly, were detected at a longer time of analysis (more than 9 min). Because of the poor resolution associated with the gliadin region, contemporaneous analysis of albumins and gliadins cannot be performed under these separation conditions. In contrast, the albumin region (**Figure 5**, trace **A**, migration time: 4.0−7.0 min) exhibited a very good resolution. The comparison with the profile of aqueous extract (**Figure 5**, trace **B**) clearly evidenced that a better resolution, good visual peak parameters, and higher sensitivity can be obtained when 30% ethanol is the extraction medium. The efficiency of this extraction medium was checked by re-extracting the pellet under the same conditions used for the first extraction. The CE analysis evidenced an incomplete extraction being that the peak intensities of the second extract were about 40% of those recorded for the first extraction step. The removal of gliadins through a pre-extraction resulted in the worst protocol among those compared (**Figure 5**, trace **C**). Peaks were poorly resolved, and their heights were significantly inferior to those of traces **B** and **C** in **Figure 5**. This could be attributed to the partial co-extraction of albumins together with gliadins during the first step of this protocol.

Stability of 30% ethanol extracts was also investigated by analyzing repeatedly the extracts over a 2 h period. As shown in **Figure 6**, the observed reproducibility was excellent over the tested time and certainly superior to that shown, over the same period, for the water extracts (**Figure 2**). The mean variation coefficients of the migration times calculated for the whole train of the peaks never exceeded 0.6%. These results suggest that 30% ethanol should be preferred as an extraction medium of water-soluble proteins especially when whole meals need to be analyzed.

Applications of CZE Analysis of Albumins. Wheat Species Differentiation. To test the ability of the phos-ala buffer to successfully differentiate *Triticum* species, water-soluble proteins extracted from six species were analyzed under the optimized conditions of extraction and analysis. As shown in **Figure 7**, a good separation of protein extracts was obtained for all species. Moreover, the electrophoretic patterns resulted highly differentiated, several differences being detectable in the whole range of

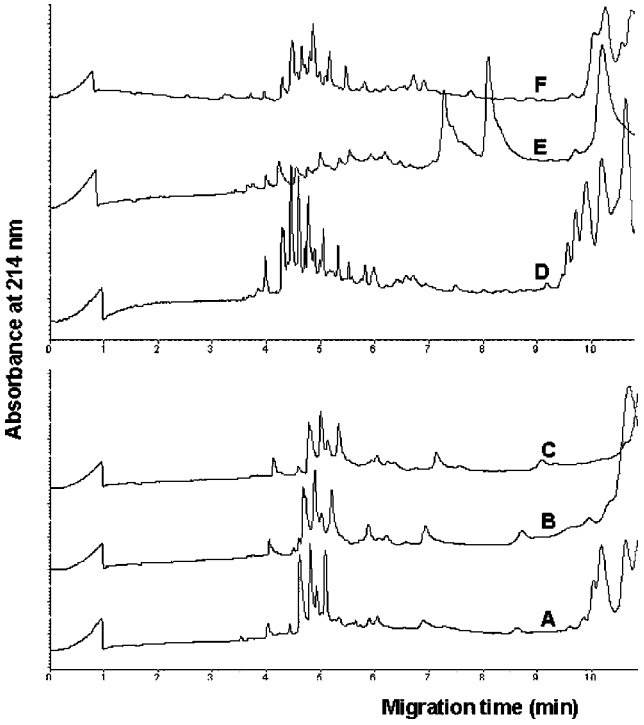

Figure 7. Separation of 30% ethanolic extracts from whole meal of six wheat species. Bottom: trace **A**: durum wheat cv. 'Norba'; trace **B**: emmer cv. 'Farvento'; and trace **C**: oriental wheat accessions PI 192658. Top: trace **D**: common wheat cv. 'Rio'; trace **E**: einkorn accession MG300007; and trace **F**: spelt cv. 'Forenza'. Conditions of analysis as in **Figure 2**.

migration times. The einkorn accession had a unique profile (**Figure 7**, trace **E**) constituted by several minor peaks between 4.0 and 6.6 min. In addition, two poorly resolved clusters were detected at migration times (7.2 and 8.1 min) that were intermediate compared to those of albumins and gliadins. As shown in the figure, no prominent peaks with comparable migration times were observed in the profiles of the other species.

The most notable difference among the electropherograms relative to emmer, spelt, oriental wheat, and common and durum wheat samples was the number of peaks detectable in the 4.0−7.0 min time window. This zone of electropherogram clearly differentiates tetraploid (emmer, oriental, and durum wheat) from hexaploid (spelt and common wheat) cultivars. In fact, more than 15 peaks and shoulders can be detected in the profiles of 'Rio' and 'Forenza' (**Figure 7**, traces **D** and **F**, respectively). Conversely, the main body of albumins relative to the tetraploid 'Norba', 'Farvento', and oriental wheat samples (**Figure 7**, traces **A**−**C**, respectively) was constituted by −five to six pronounced peaks. It should be emphasized that the number of peaks detectable in the profile of wheat species is not affected by the kind of matrix (whole or refined meal) from which extracts were obtained. A further advantage of this methodology is represented by the very short time of analysis. In fact, samples belonging to species with different ploidy levels can be differentiated, on the basis of the main body of water-soluble proteins, with a time of analysis of 7−8 min, allowing higher throughput as compared to the traditional gel electrophoresis. This is very advantageous when a large number of samples (i.e., germplasm collections) need to be analyzed. On the other hand, the screening of a high number of samples for each species will provide useful information on the efficacy of CZE analysis of water-soluble proteins to discriminate species with equal ploidy levels.

Authenticity Test of Durum Wheat Semolina. Pasta made from durum wheat is considered superior in several qualitative aspects to that manufactured from common wheat or a mixture of durum and common wheat. For this reason, Italian law prohibits the manufacturing of pasta for sale in Italy containing more than 3% common wheat. Therefore, it is important to establish quick analytical methods for controlling raw material composition. The official Italian method for the detection and quantification of common wheat adulteration of durum wheat semolina is based on the separation of albumins by native-PAGE or IEF (*22*, *23*). A recent study has shown the usefulness of CZE to check for wheat adulteration (*16*). This method is based on the quantification of two peaks specific for common wheat and not present in the profile of aqueous durum wheat extracts. As previously discussed, the basic buffer used by Bonetti et al. (*16*) and the phos-ala buffer used in this study give electrophoretic patterns with high similarities. This suggested that CZE analysis of water-soluble proteins carried out with acidic buffers could be suitable to detect semolina adulteration. Potential diagnostic peaks are the early migrating ones (4.3−4.5 min) markedly pronounced in the profiles of hexaploid species (**Figure 3**, trace **C**) but are very little represented (**Figure 3**, trace **A**) or absent (**Figure 4**) in the tetraploid ones. The analysis of a set of artificially adulterated semolina samples has shown that the area as well as the height of both diagnostic peaks increased with the adulteration level. These direct relationships enabled straight lines to be constructed between level of adulteration and area of diagnostic peaks. Correlation between adulteration level and area of diagnostic peaks resulted in being better than that between adulteration level and height of peaks ($r > 0.95$ vs $r < 0.85$). Moreover, a lower adulteration threshold (5% w/w) was found to be associated with the late migrating diagnostic peak (4.5 min). For this reason, this peak seemed to be more appropriate to accurately quantify the adulteration level in the studied range according to the following equation:

$$\text{diagnostic peak area} = 198.00 + 142.00\% \text{ adulteration } (R^2 = 0.973)$$

When raw material needs to be analyzed, an assay based on CZE is certainly faster and cheaper with respect to the methods based on DNA analysis that were recently proposed (*24*, *25*), the use of which could be restricted to the analysis of processed food, such as pasta. Prospects for new assays for the detection of durum wheat adulteration depend on their validation through ring tests among different laboratories. In fact, any technique intended to be used routinely requires that the measured component be independent of the wheat cultivar and be determined in an accurate and reproducible manner. The presented CZE method could be appropriate for raw material analysis, while DNA based assays are useful to check pasta.

Conclusion. This study showed that acidic buffers permit the separation of hydrophilic wheat albumins with a good selectivity and efficiency. With respect to the analysis on PAGE and RP-HPLC, CZE is less time-consuming, requires a very low sample volume, and does not produce large organic solvent waste. The protein separation was complete in less than 8 min with the phosphate/β-alanine buffer system. This buffer provided good resolution and repeatability. Moreover, the presented protocol would allow fast differentiation of *Triticum* spp. with different ploidy levels and can be used for detecting and estimating the adulteration of durum semolina with flour from common wheat varieties. More work is needed to develop further applications of this methodology. For example, it should be useful in screening wheat enzymes, once identified in the

electropherograms, for a relationship to end-use quality such as food coloration and baking quality.

LITERATURE CITED

(1) Singh, J.; Skerritt, J. H. Chromosomal control of albumins and globulins in wheat grain assessed using different fractionation procedures. *J. Cereal Sci.* **2001**, *33*, 163−181.

(2) Wrigley, C. W.; Bietz, J. A. Proteins and amino acids. In *Wheat Chemistry and Technology*; Pomeranz Y., Ed.; American Association of Cereal Chemistry: St. Paul, MN, 1987; pp 159−252.

(3) Singh, N. K.; Shephered, K. W.; Langridge, P.; Gruen, L. C. Characterization of triticin, a legumin-like protein in wheat endosperm. *J. Cereal Sci.* **1991**, *13*, 207−219.

(4) Garcia-Maroto, F.; Marana, C.; Mena, M.; Garcia-Olmedo, F.; Carbonero, P. Cloning of cDNA and chromosomal location of genes encoding the three types of subunits of the wheat tetrameric inhibitor of insect α-amylase. *Plant Mol. Biol.* **1990**, *14*, 845−853.

(5) Shewry, P. R.; Lafiandra, D.; Salcedo, G.; Aragoncillo, C.; Garcia-Olmedo, F.; Lew, E. J. L.; Dietler, M. D.; Kasarda, D. D. N-terminal amino acid sequences of chloroform-methanol-soluble proteins and albumins from endosperms of wheat, barley, and related species. *Fed. Eur. Biol. Soc. Lett.* **1984**, *175*, 359−363.

(6) Goesaert, H.; Brijs, K.; Veraverbeke, W. S.; Courtin, C. M.; Gebruers, K.; Delcour, J. A. Wheat flour constituents: how they impact bread quality and how to impact their functionality. *Trends Food Sci. Technol.* **2005**, *16*, 12−30.

(7) Gomez, L.; Martin, E.; Hernandez, D.; Sanchez-Monge, R.; Barber, D.; Pozo, V.; Andres, B.; Armentia, A.; Lahoz, C.; Salcedo, G.; Palomino, P. Members of the α-amylase inhibitor family from wheat endosperm are major allergens associated with baker's asthma. *FEBS Lett.* **1990**, *261*, 85−88.

(8) Fränken, J.; König, W. Analysis of allergic rye and wheat flour components in baker's asthma disease. *J. Allergy Clin. Immunol.* **1991**, *87*, 201−205.

(9) Framon, P.; Salcedo, P.; Aragoncillo, C.; Garcia-Olmedo, F. Chromosomal assignment of genes controlling salt-soluble proteins (albumins and globulins) in wheat and related species. *Theor. Appl. Genet.* **1984**, *69*, 167−172.

(10) Singh, J.; Appels, R.; Sharp, P. J.; Skerritt, J. H. Albumin polymorphism and mapping of a dimeric α-amylase inhibitor in wheat. *Aust. J. Agric. Res.* **2001**, *52*, 1173−1179.

(11) Bean, S. R.; Lookhart, G. L. Recent advances in the HPCE of cereal proteins. *Electrophoresis* **2001**, *22*, 1503−1509.

(12) Capelli, L.; Forlani, F.; Perini, F.; Guerrieri, N.; Cerletti, P.; Righetti, P. G. Wheat cultivar discrimination by capillary electrophoresis of gliadins in isoelectric buffers. *Electrophoresis* **1998**, *19*, 311−318.

(13) Lookhart, G. L.; Bean, S. R. Separation and characterization of wheat protein fractions by high-performance capillary electrophoresis. *Cereal Chem.* **1995**, *72*, 527−532.

(14) Scholz, E.; Ganzler, K.; Gergely, S.; Salgo, A. Use of capillary electrophoresis to monitor wheat maturation. *Chromatographia* **2002**, *56*, 127−130.

(15) Bean, S. R.; Tilley, M. Separation of water-soluble proteins from cereals by high-performance capillary electrophoresis (HPCE). *Cereal Chem.* **2003**, *80*, 505−510.

(16) Bonetti, A.; Marotti, I.; Catizone, P.; Dinelli, G.; Maietti, A.; Tedeschi, P.; Brandolini, V. Compared use of HPLC and FZCE for cluster analysis of *Triticum* spp. and for identification of *Triticum durum* adulteration. *J. Agric. Food Chem.* **2004**, *52*, 4080−4089.

(17) Bean, S. R.; Lookhart, G. L. Faster capillary electrophoretic separations of wheat proteins through modification to buffer composition and sample handling. *Electrophoresis* **1998**, *19*, 3190−3198.

(18) Bean, S. R.; Lookhart, G. L. Ultrafast capillary electrophoretic analysis of cereal storage proteins and its applications to protein characterization and cultivar differentiation. *J. Agric. Food Chem.* **2000**, *48*, 344−353.

(19) Righetti, P. G.; Bossi, A.; Olivieri, E.; Gelfi, C. Capillary electrophoresis of peptides and proteins in acidic, isoelectric buffers: recent developments. *J. Biochem. Biophys. Methods* **1999**, *40*, 1−15.

(20) Righetti, P. G.; Gelfi, C.; Bossi, A.; Olivieri, E.; Castelletti, L.; Verzola, B.; Stoyanov, A. V. Capillary electrophoresis of peptides and proteins in isoelectric buffers: an update. *Electrophoresis* **2000**, *21*, 4046−4053.

(21) Islas-Rubio, A. R.; Singh, H.; Chittrakorn, S.; MacRitchie, F. Stability of wheat proteins in solution. *J. Cereal Sci.* **2006**, *43*, 169−174.

(22) Resmini, P. A new method for the identification and dosage of common wheat flour in durum wheat flour and pasta. *Tec. Molitoria* **1967**, *19*, 145−150.

(23) Resmini, P.; De Bernardi, G. A fast electrophoresis method for the identification of common wheat adulteration in durum wheat semolina and pasta. *Tec. Molitoria* **1976**, *27*, 97−109.

(24) Alary, R; Serin, A; Duviau, M. P.; Joudrier, P; Gautier, M. F. Quantification of common wheat adulteration of durum wheat pasta using real-time quantitative chain reaction (PCR). *Cereal Chem.* **2002**, *79*, 553−558.

(25) Terzi, V.; Malnati, M.; Barbanera, M.; Stanca, A. M.; Faccioli, P. Development of analytical systems based on real-time PCR for *Triticum* species-specific detection and quantitation of bread wheat contamination in semolina and pasta. *J. Cereal Sci.* **2003**, *38*, 87−94.

Received for review December 14, 2006. Revised manuscript received March 13, 2007. Accepted March 16, 2007. Contribution n.86 from Institute of Plant Genetics-CNR, Bari, Italy.

JF063628N