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Extraction of wheat endosperm proteins for proteome analysis☆,☆☆

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Abstract

Total protein extracts of wheat endosperm are widely used for the analysis of the highly abundant gliadins and glutenins. In this review, the most popular total endosperm extraction methods are compared for their effectiveness in proteome coverage. A drawback of total endosperm extracts is that the enormous dynamic range of protein abundance limits the detection, quantification, and identification of low abundance proteins. Protein fractionation is invaluable for improving proteome coverage, because it reduces sample complexity while enriching for specific classes of less abundant proteins. A wide array of techniques is available for isolating protein subpopulations. Sequential extraction is a method particularly suited for subfractionation of wheat endosperm proteins, because it takes advantage of the specific solubility properties of the different classes of endosperm proteins. This method effectively separates the highly abundant gliadins and glutenins from the much less abundant albumins and globulins. Subcellular fractionation of tissue homogenates is a classical technique for isolating membranes and organelles for functional analysis. This approach is suitable for defining the biochemical processes associated with amyloplasts, specialized organelles in the endosperm that function in the synthesis and storage of starch. Subproteome fractionation, when combined with 2-DE and protein identification, provides a powerful approach for defining endosperm protein composition and providing new insights into cellular functions.

Keywords: 2-DE; Albumins; Amyloplasts; Endosperm; Gliadins; Globulins; Glutenins; Proteome; Wheat

1. Introduction

Wheat flour is the main ingredient in most types of breads, pastries, and pastas worldwide, because of its unique protein composition (reviewed: [1]). All-purpose flour is the finely ground endosperm of the wheat grain that is separated from the bran (aleurone, seed coats, pericarp) and germ (embryo) during the milling process. Flour contains predominantly starch (approximately 70–80% dry weight) and protein (approximately 10–15% dry weight). Approximately 80% of the endosperm protein is comprised of the gluten proteins, which have unique elasticity and extensibility properties that determine flour functionality. The gluten proteins consist of the monomeric gliadins and polymeric glutenins that, in turn, are comprised of high molecular weight and low molecular weight-glutenin subunits. Wheat grain research has focused on detailed analysis of the gluten proteins to better understand those aspects of protein composition that account for the unique properties of flour [2]. Although the non-gluten protein classes, the albumins and globulins, are a smaller percentage of endosperm protein, they have important roles in cellular metabolism, development, and responses to environment. The unparalleled resolving power of 2-DE has made it the method of choice for analysis of the complex protein populations of the endosperm [2]. Proteomic approaches utilizing 2-DE have provided new insights into protein composition of the endosperm [3–6], processes involved in grain development [7,8], effects of environment on grain fill [9–13], chromosomal locations of genes [14–16], and potential markers for genotype identification and stress tolerance [17–19]. The majority of these studies utilized total protein extracts, which are appropriate for the analysis of the abundant gliadins and glutenins that dominate the endosperm proteome. In this review, the most popular total endosperm extraction methods

Abbreviations: 2-DE, two-dimensional polyacrylamide gel electrophoresis; IEF, isoelectric focusing; IPG, immobilized pH gradient; PAGE, polyacrylamide gel electrophoresis

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are compared to illustrate their effectiveness in the analysis of the endosperm proteome. Since the gliadins and glutenins mask all but a few of the more abundant non-gluten proteins in total protein extracts, two approaches, depletion of high abundance proteins and cell fractionation, are described that extend endosperm proteome coverage respectively to the albumins and globulins and amyloplast proteins.

2. Total protein extraction methods

Extraction of proteins from plant samples is challenging. Plant cells often contain proteases that, if active in the extraction buffer, reduce and alter protein populations. Plant cells also contain various non-protein components that interfere with protein separation during electrophoresis, causing streaking and smearing of the 2-DE patterns. Among these components are cell wall and storage polysaccharides, lipids, phenolics, salts, nucleic acids, and a broad array of secondary metabolites [20]. The optimal extraction procedure must minimize protein degradation and eliminate non-protein components that interfere with protein separation during electrophoresis. The most common methods used to prepare total protein extracts from plant tissues are urea, SDS, TCA, and phenol. In this section, proteins were extracted from flour by each of these methods and the 2-DE patterns compared (Fig. 1). Proteins were precipitated from each sample and quantified by the procedure of Lowry et al. [21] as described in Hurkman and Tanaka ([22,23]; see also Section 3.1]. Protein extracts were solubilized in urea buffer (9 M urea, 4% NP-40, 1% DTT, and 2% ampholytes) and centrifuged at 16,000 × *g* for 10 min (Eppendorf Centrifuge 5415C, Brinkman Instruments, Inc., Westbury, NY) to remove insoluble material. Equal amounts of protein (18 μg) were loaded onto the IEF gels and 2-DE carried out according to Hurkman and Tanaka [22,23].

Although urea buffers are most often used to solubilize proteins for IEF, they can also be used to extract proteins directly from wheat flour [25–27]. For Fig. 1A, flour was extracted essentially by the method of Payne et al. [24] as summarized in Fig. 2. Fifty milligram of flour was suspended in 200 μl of urea buffer (2 M urea, 10% glycerol, 65 mM DTT, and 20 mM Tris, pH 8.0), the suspension incubated at room temperature for 1 min, and insoluble material removed by centrifugation at 16,000 × *g* for 10 min (Fig. 2). The resulting 2-DE pattern (Fig. 1A), like that of Payne et al. [24], contains four protein regions. The proteins in region 1 are the high molecular weight-glutenin subunits, in region 2 the ω-gliadins [28], in region 3 the α- and γ-gliadins and low molecular weight-glutenin subunits, and in region 4 the low molecular weight-albumins and globulins. The albumins and globulins consist of many more proteins (compare Figs. 1A

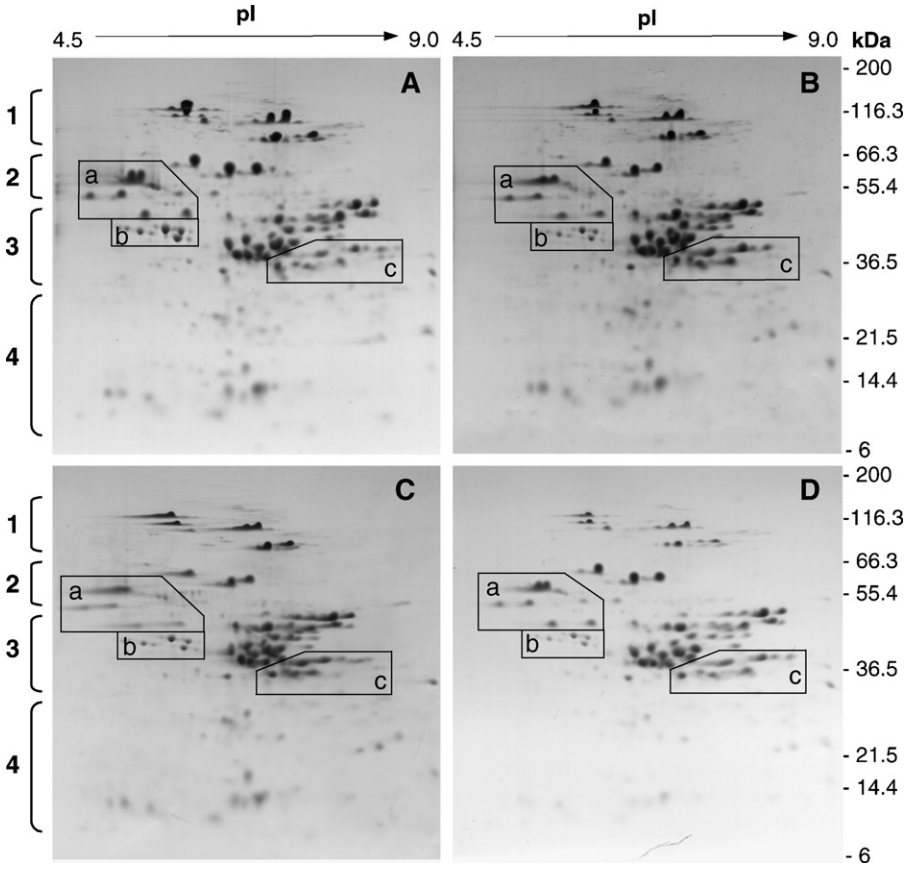

Fig. 1. 2-DE comparison of wheat flour proteins extracted with urea, SDS, TCA, or phenol. (A) Urea-soluble proteins. (B) SDS-soluble proteins. (C) TCA/acetone-insoluble proteins. (D) Phenol-soluble proteins. Numbered brackets indicate region 1, high molecular weight-glutenin subunits; region 2, ω-gliadins; region 3, the α- and γ-gliadins and low molecular weight-glutenin subunits; and region 4, low molecular weight-albumins and globulins. Boxes a–c highlight differences and similarities in the gel patterns.