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Analysis of correlations between contents of protein fractions in wheat endosperm models and their mechanical resistance

J.A. Budny [a, *], J. Fornal [b], W. Obuchowski [c]

[a] Department of Physics and Biophysics, University of Warmia and Mazury in Olsztyn, ul. Oczapowskiego 4, 10-719 Olsztyn, Poland
[b] Department of Chemical and Physical Properties of Food, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland
[c] Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Poland

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ABSTRACT

The mechanical strength of wheat endosperm and its laboratory models is determined by the types and contents of endosperm components. Studies on endosperm models enable controlling contents of endosperm components and then determining correlations between the model's components and their mechanical resistance. In this article we present a research on correlations between contents of protein fractions and mechanical resistance of wheat endosperm models. In contrast to previous reports, we showed that the starch fraction was the main factor influencing the mechanical resistance of the analyzed models. Simultaneously, we demonstrated no correlations between the contents of protein fractions in starch and a wheat hardness index. In addition, we showed some correlations between contents of protein fractions in the models and their mechanical resistance. However, these correlations were so weak that they should be treated as secondary.

1. Introduction

The hardness index is one of the most important parameters of the technological suitability of wheat grain (Salmanowicz et al., 2012). Essential in the studies of the technological usefulness of wheat grain is to determine the effect of individual components of wheat endosperm on the hardness index. The available reports on correlations of individual components of wheat grain hardness provide a picture of multi-factorial interactions between the components, giving a resultant mechanical resistance of endosperm. The object of research has become virtually all groups of compounds found in wheat kernel. However, the protein, as one of the main macro-components of the grain has become the focus of interest of researchers due to observable differences in the structure at the interface of endosperm starch granule – protein matrix. The stronger binding of the starch granules with the protein matrix was observed for the wheat varieties with a high hardness index than for these with of a low hardness index. Studies on correlations between protein content and the mechanical properties were, however, inexplicit (Amoroso et al., 2004; Bloch et al., 2001; Chang et al., 2006; Chen et al., 2005; Corona et al., 2001; Csóti et al., 2005; Giroux et al., 2000; Greenblatt et al., 1995; Hogg et al., 2004; Lillemo and Ringlund, 2002).

It was not until the discovery of a protein fraction with the molecular weight of 15 kDa in the starch (the so-called "Prime starch"), originating from wheat varieties with low hardness index (Greenwell and Schofield, 1986; Morrison et al., 1992), that a partial explanation has been reached regarding the source of variation in hardness. Research efforts were first directed to investigate the effect of this fraction on the strength of the endosperm (Malouf and Hoseney, 1992; Malouf et al., 1992), which was followed by the characterization of polypeptides being components of this fraction. Initially, the fraction of these proteins was called friabilin, but successive studies (Morris et al., 1994; Oda and Schofield, 1997) demonstrated that the components of this fraction include, among others, puroindolines A and B (Blochet et al., 1991). Results of a research conducted by Malouf (Malouf et al., 1992) suggested that the presence of proteins (friabilin) on the surface of starch resulted in a reduced strength of the models. However, a later work (Budny et al., 2005) demonstrated that the protein on the surface of starch is an artifact created during hydroseparation of flour ingredients.

On the other hand, in research on biochemical data contributing to variation in the hardness of wheat grain, one of the problems is

* Corresponding author.
E-mail address: jaroslaw.budny@uwm.edu.pl (J.A. Budny).

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An alphabetical list of abbreviations

ANOVA	Analysis of Variance
CM-like	protein fraction which is soluble in KCl-buffer and ammonium acetate solution in methanol
Fr07To15	the fraction with particle size of 15–7 μm;
Fr15To40	the fraction with particle size of 40–15 μm
Gluten	cross-linked, insoluble in water, wheat protein complex
Glutenic	protein fraction which is insoluble in KCl-buffer and soluble in SDS-buffer
Hi40	the fraction with particle size above 40 μm
Lo07	the fraction with particle size below 7 μm
LSD	Least Significant Difference
Metabolic	protein fraction which is soluble in KCl-buffer and insoluble in ammonium acetate solution in methanol
NSHi	insoluble in water fraction of non-starch and non-gluten components, having particle sizes in the hydrated state above 70 μm
NSLo	insoluble in water fraction of non-starch and non-gluten components, having particle sizes in the hydrated state below 70 μm
PCA	Principal Component Analysis
Remaing	protein fraction which is insoluble in KCl-buffer and SDS-buffer and soluble in 0.1 M NaOH solution
SKCS	Single Kernel Characterization System
Starch	wheat starch fraction
WSC	Water-Soluble Compounds and non-sedimented under conditions of the separation

the influence of environmental and variety-species factors (Sadowska et al., 2001). These include: variable contents of chemical components, various grain maturity, as well as the shape and size of grain. To eliminate the effect of these factors on grain strength test results and mechanical resistance to the action of external forces, studies are conducted on endosperm models formed under laboratory conditions. To minimize the impact of the shape, different three-dimensional solids were used, which were made by wheat grain processing. For example, Glenn and Johnston 1992 as well as Dobraszczyk et al., 2002 used cylindrical shapes, whereas Haddad et al., 1998 used rectangular shapes. Other researchers (Sadowska et al., 1999; Konopka et al., 2004, 2005a,2015b) applied microscale techniques to measure the representative areas of breakthroughs of wheat grain. A drawback of the above methods is, however, the impossibility of controlling the chemical composition of grain during the growing season, as the composition of the model depends on grain samples collection.

One possible solution to this problem is to create a model of wheat endosperm from the components which were obtained by water-separation of wheat endosperm (Malouf and Hoseney, 1992; Malouf et al., 1992). In the first part of the experiment, Malouf showed that models made with reconstructed flour, retained the inter-cultivar differences in the mechanical strength. In the second part of the study, he studied the mechanical strength of the models made with reconstructed flour based on different variants of the proportions of the ingredients. In this way, he showed that the ingredient responsible for the mechanical strength of the model was the fraction of starch. Then, bearing in mind the reports about different contents of friabilin in starch from hard and soft wheat cultivars, he made models using starch deprived of protein. After the removal of proteins, the models showed a higher mechanical resistance than the reference models (without any changes). Results obtained by Malouf suggested the decrease in the mechanical resistance of models was due to the presence of proteins (including friabilin) on the surface of starch granules.

It seems, however, that Malouf and coworkers used an overly simplified method of separation of wheat endosperm components. As a result of the separation, they extracted only three fractions: compounds soluble in water, gluten and starch, whereas Wolf (1964) managed to separate the components of wheat endosperm into five fractions: water-soluble compounds, gluten, a gelatinous matrix of pentoglycans, prime starch and a tailing residue (a fraction of slow sedimenting compounds). The starch obtained via the Wolf's method contains almost entirely starch granules of “A” type (their size is greater than 10 μm). While the tailing residue consists of B type starch granules (their size is below 10 μm) and non-starch components, including friabilin (Budny et al., 2005). Therefore, Budny et al. (2005) proposed a modification of Wolf method. The starch obtained with this modified method contains both types of starch granules, with unchanged ratio of their content in relation to their ratio before separation, whereas the fraction of non-starch components contains an insignificant of starch granuloma. These authors showed that the protein on starch surface is an artifact created during hydroseparation of flour ingredients.

In this work, except for hydroseparation, the crushed wheat endosperm was fractionated by air (pneumoseparation) into four fractions, which differed in the composition and size of particles. In this paper, we present results of the analysis of correlations between the mechanical resistance of models of wheat endosperm and protein content in the fractions of endosperm as well as correlations between the above-mentioned mechanical resistance and levels of these protein fractions in the examined models.

2. Materials and methods

2.1. Species and varieties of wheat

In our study, we used three batches of wheat grain, including two hexaploid wheat varieties - Triticum vulgare and one variety of tetraploid wheat – the species Triticum durum. Lots of wheat were chosen that clearly differed in grain hardness. The first sample of wheat grain (T. vulgare), called Banti, was acquired from the collection of the Agricultural Plant Breeding company “Seeds of Kobierzyce” LCC, Poland. The second sample, called Koksa, was acquired from the collection of the Plant Breeding Strzelce LLC company, Poland. Grains of wheat came from a lot of Canadian wheat trade, denoted by the supplier as Amber Durum – further referred in this article as Durum. All samples of grain were derived from the grain harvest in 2003. Selected physico-mechanical properties of the grain are shown in Table 1.

2.2. Preliminary analyses and milling

Prior to milling, grain was determined for moisture content (Polish Approved Standards PN-ISO 712:2002. Polish Committee for Standardization, Warsaw, Poland). Then the grain conditioned to a moisture content of 14.5% was characterized using an SKCS analyzer (Perten 4100, AACC International. 2000. Approved Methods of the American Association of Cereal Chemists, 10th Ed. Method 55-31. The Association: St. Paul, MN.), and further was determined for the content of vitreous grains (Polish Approved Standards PN-70R-74008:1970. Polish Committee for Standardization, Warsaw, Poland). The grinding was performed using a laboratory mill (Quadrumat Junior, Brabender), equipped with a sieve for bran sifting, with mesh sizes of 236 μm (marked 70 GG). The