Volume 54(1):19-23, 2010
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Starch to protein ratio and α-amylase activities in grains of different wheat cultivars

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ABSTRACT Starch and total protein contents, α-amylase activity of grains as well as falling number in the flour, an internationally accepted measure of α-amylase activity in the food industry, were compared in wheat (*Triticum aestivum* L.) genotypes which are widely used in the agriculture and breeding in Romania and Hungary. These cultivars were originated from Romania (20 cultivars), Hungary (cv. GK Élet, GK Öthalom and Mv Emese), France (cv. Cappelle Desprez), the USA (cv. Plainsman) and China (cv. Xiang). The starch contents of the grains were high in those cultivars, where the protein contents were relatively low. The highest starch content among the investigated wheat genotypes was found in the Romanian Gruia cultivar, which could be characterized also with low starch degrading α-amylase activity in the grain and with a high falling number. This wheat genotype can be a good candidate for utilization in starch industry. Other genotypes, which had relatively high starch contents and, a low α-amylase activity (or high falling number) were the Romanian Crina and Gloria, the Hungarian GK Öthalom, GK Élet, Mv Emese and the French Cappelle Desprez cultivars.
Acta Biol Szeged 54(1):19-23 (2010)

KEY WORDS

α-amylase activity,
falling number,
total protein content,
starch content,
wheat genotypes

The composition of wheat storage proteins and starch permit the production of wide range of food and industrial products from the processed grain. These storage products, proteins (albumins, globulins and the fractions of glutene, gliadins and glutenins) and starch accumulate in the endosperm and act as a nutrient source for the germinating seedling.

The protein content is an indicator of direct nutritional value and is a key specification for wheat and flour purchasers since the bread-making quality is to large extent determined by the quantity and composition, polymeric amount and size distribution of storage proteins. The genetic improvement of wheat has received considerable attention over the years from plant breeders with the purpose of increasing protein content and yield. In recent years however, there has been an increased interest in starch content and composition of wheat because of the growing industrial demand and the possible production of fuel alcohol from grain.

Starch is the most abundant storage polysaccharide in plants, and occurs as granules in the chloroplast of green leaves and the amyloplast of seeds, pulses and tubers. Starch, is a staple in the diet of much of the world's population, and is also widely used in the Western world in the food and beverage industries as a thickener and a sweetener, as well as having some manufacturing applications in the paper and textile industries. The more prevalent use of starch for industrial purposes will only become economically viable when its use as a raw material rivals those derived from petroleum-based products (Slattery et al. 2000). The use of starch as a renewable and biodegradable polymer is becoming increasingly attractive because of the environmental concerns about the industrial wastes generated from petroleum products and the growing awareness of the potential deleterious consequences of greenhouse gas emissions from these activities. Biofuels can be derived from any substance yielding fermentable sugars. Cereal grains are good feedstock because grains contain a high proportion of starch and can be stored dry for many months, allowing year round processing.

Starch is composed of two different glucan chains, amylose and amylopectin. These polymers have the same basic structure, but differ in their length and degree of branching. Amylose is an essentially linear chain of glucose residues linked via α-1,4 glucosidic linkages, whereas amylopectin is a branched α-1,4: α-1,6 D-glucan polymer, that is made up of a linear glucose backbone with occasional glucose side branches. The ratio of amylose to amylopectin in starch contributes to its physical properties and its functionality varies between species and varieties (Slattery et al. 2000). The percentage of amylose and amylopectin in wheat starches is ca. 25% and 75%, respectively.

Accepted July 6, 2010
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Starch in grains is packed into granules which, based on size, may be classified into large (25-35 μm, A type) and small (2.0-8.0 μm, B type) granule starch. Brosnan et al. (1998) reported, that total starch content is more important for industrial purpose (e.g. alcohol yield) than the relative amounts of large and small granules. In the matter of using starch as feedstock, alcohol processing yield depends on (i) the amount of starch present, (ii) how much of this starch is converted to fermentable sugars, and (iii) the efficiency with which these sugars are fermented into alcohol. Increasing starch quantity directly impact yield in the industrial application.

Starch reserves may be degraded *in vitro* by the action of endogenous amylases. α-amylase is an endoamylolytic enzyme that hydrolyzes the α-1,4-glucosidic linkages of starch; the hydrolytic products have α-configuration. It is found in most reserve tissues during periods of starch mobilization. In germinating cereal seeds, α-amylase secreted from the aleurone cells initiates the degradation of starch granules in the non-living endosperm. It liberates soluble glucans that can be further degraded by other hydrolytic enzymes such as debranching enzymes and β-amylases.

Some α-amylase enzyme is present in the embryo or germ of sound wheat kernels, and α-amylase isoenzymes are exist also in immature wheat. During the development of wheat grains, the α-amylase activity increases and reaches a maximum value about 16 days after heading, then the amylase activity rapidly decreases and reaches a minimum value at the maturation stage. Interestingly, wheat kernel contains a number of albumin components that actively inhibit many α-amylases from sources other than wheat but are inactive with α-amylase of wheat (Pace et al. 1978). When germination begins, the embryo and layers surrounding the starchy endosperm produce α-amylase at an accelerating rate. A severely sprout-damaged kernel contains many thousands of times the amounts of enzyme present in kernels that are in the early stages of germination. Because of this, a wheat grain sample containing very low levels of severely sprouted kernels may exhibit significant amylase activity. α-amylase converts starch into sugars in the sprouting kernel, and similarly breaks down the starch granules in wheat flour when mixed with water to make bread dough.

A genetic defect, named Late Maturity α-amylase (LMA) or Prematurity α-amylase activity (PMAA), present in particular wheat genotypes, was reported by Gale et al. (1983). LMA may result in the accumulation of unacceptable levels of high amylase in grains in the absence of germination or weather damage and it has been inadvertently disseminated to wheat programmes around the world. Grain of these genotypes may develop high pI α-amylase activity either under normal growing conditions or, more commonly, as a result of cool temperature stress (Mrva et al. 2006).

For bakers, the α-amylase activity of the flour is important, because it has negative effects on the dough. Flour damaged by α-amylase holds less water when mixed and the dough absorbs less water during baking. The enzyme also affects gas retention, dough handling and bread texture. Too much α-amylase activity causes wet, sticky dough that is hard to handle in a commercial bakery. The loaf may have large, open holes and the crumb texture is gummy. Gummy bread is difficult to slice and builds up on slicer blades. Loaves are often deformed, hard to package and unattractive to customers. Because of these, the α-amylase activity of flour is a relevant information. In the food industry, falling number is the internationally accepted measure of α-amylase activity. It could be argued that low falling number samples may give more efficient starch conversion because of higher levels of endogenous amylase, but conversely poor quality, e.g. sprouted samples may have already lost starch, and therefore for example alcohol yields might be reduced.

In this work the starch and protein contents as well as α-amylase activities of 26 wheat cultivars mostly of Romanian and Hungarian origin were compared. The investigated genotypes are widely used by the agriculture and breeding in Romania and Hungary. Our aim was to investigate the variations present in these cultivars and find a correlation between the protein and starch content of grains.

Materials and Methods

Plant material

Wheat (*Triticum aestivum* L.) kernels were originated from Romania (20 cultivars), Hungary (cv. GK Élet, GK Öthalom and Mv Emese), France (cv. Cappelle Desprez), the USA (cv. Plainsman) and China (cv. Xiang) from the yield of 2007 year. The grains of Romanian cultivars were kindly provided by the Horticulture Faculty, Banat's University of Agricultural Sciences, Timisoara, Romania; Mv Emese was given by Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary (László Láng); the other plant material were kindly provided by László Cseuz, Cereal Research Non-Profit Company, Szeged, Hungary. Wheat grains were conditioned to equalize the moisture content (15.5%) of each sample.

Determination of starch and protein content

Starch content of the grains was measured by the polarimetric analytical method used in food industry (according to MSZ 6367/13-82, polarimetric Ewers method, in Institute of Food Engineering, Faculty of Engineering, University of Szeged, Hungary), and by the method of Lásztity and Törley (1987). The protein content of the samples was determined also by the method used in food industry (Ma et al. 2007).

Measurements of α-amylase activity

For determination of the α-amylase activities, 0.5 g of the whole grains was homogenized with 50 ml water. For mea-

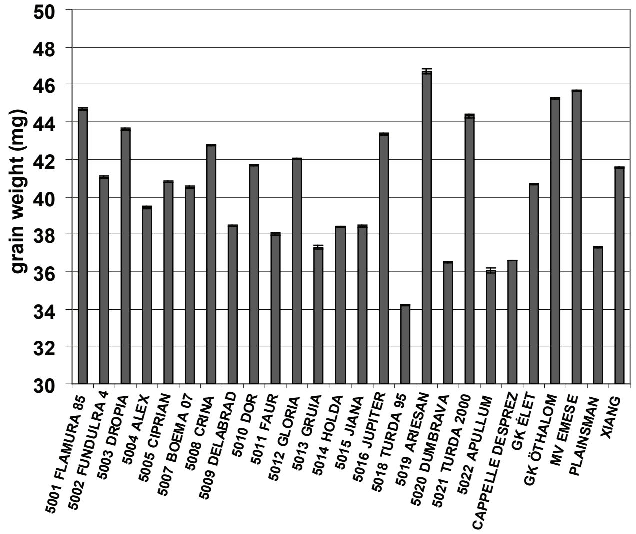

Figure 1. Dry weight of grains of different wheat cultivars.

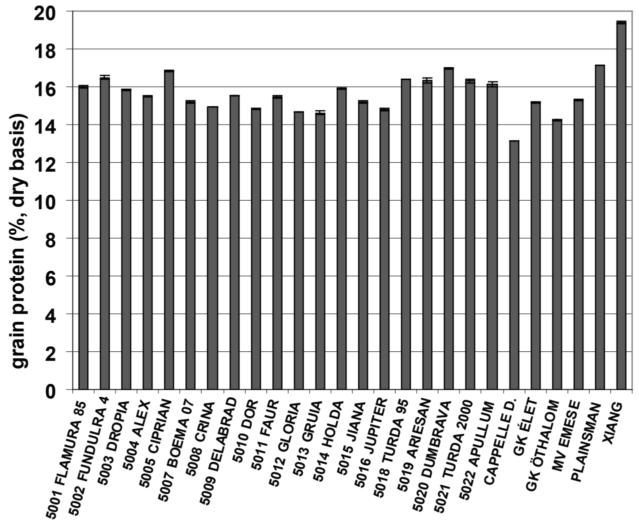

Figure 2. Protein content in % of the grain dry weight of different wheat cultivars.

suring the amount of starch degraded by α-amylase, 15 ml reaction mixture containing 13 mg ml^{-1} of starch and 5 ml of plant extract containing α-amylase activity was used. The reaction was performed at 30°C for 20 minutes. The starch content not degraded by the enzyme, was detected with KI/I_2 solution. For the quantification, calibration curve was made with diluted starch solution in the range of 1.3-13 mg ml^{-1} concentrations.

The α-amylase activity of the wheat flour was also examined by measuring the falling number. This method utilizes the gelatinization of starch polymers in suspension of water and flour. The principle of the falling number method is that the enzyme activity can be indirectly measured by the rheological properties of heated starch suspensions. The procedure involves the agitation by a stirring rod of a meal-water mixture within a precision test tube immersed in a boiling water bath. After 1 min, the stirring rod is released at the top of the tube and falls by its own weight through the viscous suspension. The time, in seconds, needed for the stirrer to travel through a fixed depth of suspension, plus 60 (from the agitation period) is the Hagberg Falling Number (FN). All the measurements were repeated at least three times, and the means ± SD were calculated.

Results and Discussion

The average dry weight of grains displayed a high variability among the investigated wheat cultivars from 34.21±0.019 (cv. Turda) to 46.73±0.136 mg (cv. Ariesan; Fig. 1). However, there were a much less difference in the protein and starch content of the different cultivars. The protein content of the grains on dry weight basis varied between 13.12±0.021 (cv. Cappelle Desprez) and 19.41±0.049% (cv. Xiang; Fig. 2). Determination of the starch content from grain samples by the polarimetric method revealed that the amount of the starch in the chosen varieties was between 60.14 and 66.93% (Fig. 3). According to our results, the Romanian cv. Gruia had the highest starch content, but similarly good level was measured in the Romanian Crina, Boema 07 and Gloria genotypes, or in the Hungarian GK Öthalom and in the French Cappelle Desprez wheat cultivars (their starch content varied between 65.85 and 66.93%). The Xiang genotype contained the less starch, but among the local genotypes, the Romanian Fundulra 4, Ciprian, Turda 95 and Dumbrava had ca. 62 % starch, these can be regarded as wheat cultivars with relative low starch levels (Fig. 3). Using wheat genotypes of different origin made the variability of these parameters greater.

Starch granules are embedded within a protein matrix within the endosperm. Comparing the starch content with the protein content of the grains revealed a strong negative correlation (R^2= 0.82). Due to inverse relation between starch and protein contents, increases in starch amount are correlated with decreases in grain protein content (Fig. 4).

The ratio of starch and various proteins like water soluble albumins and globulins or gluten (gliadins and glutenins) accumulating in wheat grains is determined by the genotype, but environmental factors are also important. Technological quality of wheat is determined by the quantity and quality of reserve proteins and the status of carbohydrate-amylase complex. This means that the degree of the damage to starch granules and the content and activity of α-amylase in the flour determines the flour quality. The extent of starch conversion can be assessed conventionally using the falling number (FN)

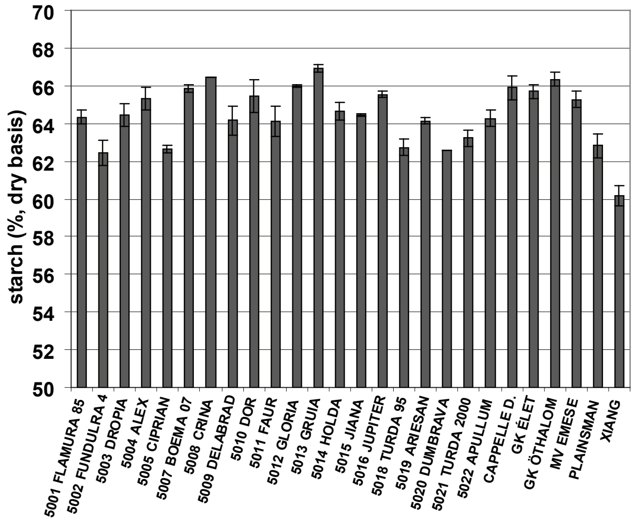

Figure 3. Starch content in % of the grain dry weight in the investigated wheat genotypes measured by polarimetric method.

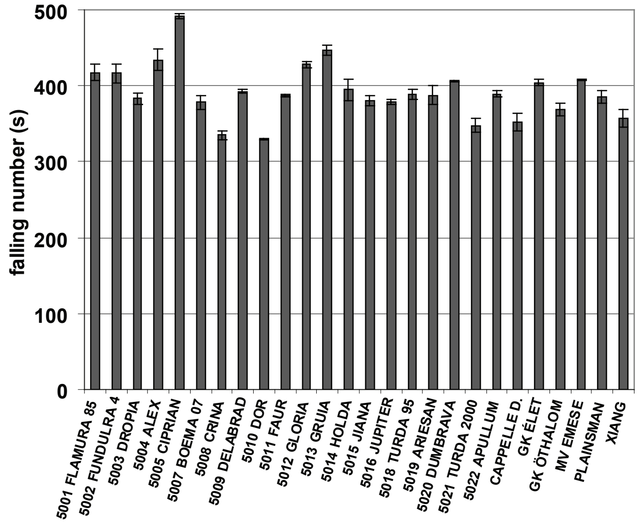

Figure 5. Falling number of the investigated wheat genotypes.

test, which was performed on flour samples. The FN values were between 330 and 492 s (Fig. 5). The Romanian cv. Cyprian had the highest falling number, but the Alex, Gloria and Gruia genotypes had also relatively high FN, which means relatively low amylase activity. Low falling number was measured in the flour of Dor, Crina, Turda 2000 and Cappelle Desprez wheat cultivars.

Many buyers place strict limits on falling number in the wheat they buy. It could be argued that low falling number samples may give more efficient starch conversion because of higher levels of endogenous amylase, but conversely poor quality, e.g. sprouted samples may have already lost starch, and therefore the industrial utilization of the grains might be reduced.

The falling number is a complex parameter, which depends on the α-amylase activity and the properties of starch in the grain. Therefore, we measured the α-amylase activities of the grains also by another method. Measuring the amount of the degraded starch in the samples originated from these wheat grains revealed approximately constant level of α-amylase activities (Fig. 6.). Relatively high activities was detected in the Romanian Flamur 85, Fundulra 4 and Ciprian genotypes,

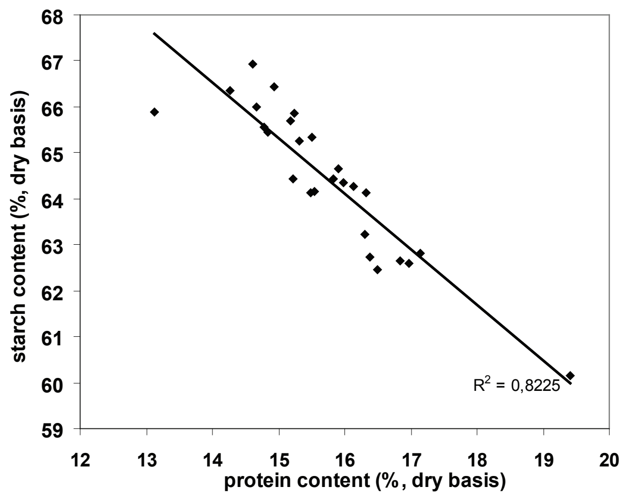

Figure 4. Starch content of grain samples from investigated wheat varieties against total protein content.

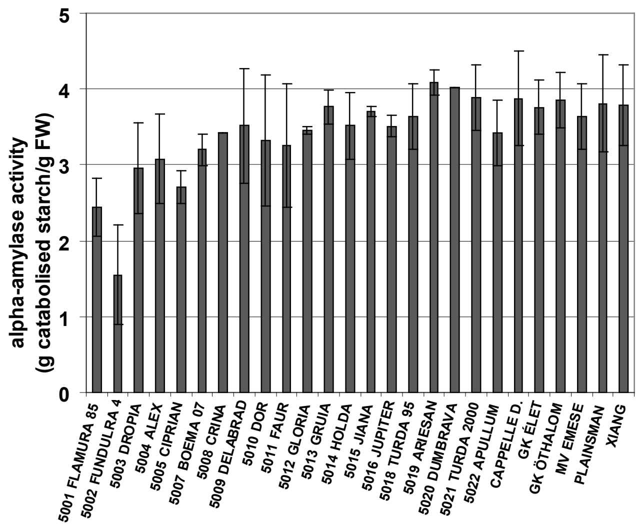

Figure 6. α-amylase activities of the investigated wheat genotypes measured according to the degraded starch by the grain extracts.

and low values were measured e.g. in the Romanian Gruia, Jiana, Ariesan, Dumbrava, Turda 2000 lines, in the Hungarian genotypes (Élet, Öthalom, Emese), in the French Cappelle Desprez and in the American Plainsman wheat.

High endogenous α–amylase activity (and low falling number) can be associated with the pre-harvest sprouting and economic losses of grain dry matter, but may also result in starch conversion to sugars without any visible sprouting damage. The genotypes characterised by high falling number and low starch degrading amylase activity in the grain can be submitted for industrial purpose. For example, cv. Gruia could be characterised by high FN and low α-amylase activities, as expected; so in this case the α -amylase activity correlated with the flour quality.

In summary, using wheat genotypes with different origin displayed high variability in protein and starch content. Generally, the starch content of the wheat grains was high in those varieties, where the protein contents were relatively low. Those genotypes, which had relatively high starch content and, according to our results, a low α-amylase activity (or high falling number) are the Romanian Crina and Gloria, the Hungarian GK Öthalom, GK Élet, Mv Emese and the French Cappelle Desprez cultivars.

The highest starch content among the investigated wheat genotypes was found in the Romanian Gruia genotype, which can be characterized also with a high falling number and low α-amylase activity. This wheat line can be good candidate for utilization in starch industry.

Acknowledgment

This work was supported by the HURO-06-02/006 project and by 2010TÁMOP 4.2.1.B.

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