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Možnost uporabe nizkoamiloznega škroba ajde kot nadomestek za maščobe

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Izvleček
V tem članku so predstavljene morfološke razlike med škrobnimi zrni navadne ajde kv. 'Siva' in škrobnimi zrni ajde rastlin, ki kažejo nizkoamilozne lastnosti, s pomočjo uporabe vrstične elektronske mikroskopije (SEM). Navadna ajda v endospermu vsebuje majhna poligonalna škroba zrnca, katerih velikost se giblje v velikosti 4-8 mikrometrov. Škrobna zrna v endospermu ajde, ki kaže nizkoamilozne lastnosti, vsebuje večinoma okrogla škrobna zrna v velikosti 3-6 mikrometrov. Piknje katere smo opazili na površini škrobnih zrn ajde z nizkoamiloznimi lastnostmi, so najverjetneje posledica visoke aktivnosti amilaz. Te morfološke razlike so najverjetneje posledica nizke koncentracije amiloze v mutiranih škrobnih zrnih. SEM analiza slike je uporabno orodje za razvrščanje posameznih zrn ajde v mešanicah z nizko in normalno vsebnostjo amiloze in bi se lahko uporabila za ločevanje normalnega od nizkoamiloznega škroba endosperma. Sferični naravni škrob, s premerom zrn, podobnim lipidnemu micelu imajo potencial za uporabo kot nadomestki maščob z ali brez dodatnega brušenja. Povpraševanje po ogljikovih hidratih, maščobah, ki temeljijo na nadomestkih je v zadnjem času v porastu in škrob endosperma ajde, ki kaže nizkoamilozne lastnosti lahko najde komercialno aplikacijo.

Ključne besede: škrob ajde, *Fagopyrum esculentum*, nizko-amilozni mutant, vrstična elektronska mikroskopija, morfologija škrobnih zrn, nadomestila za maščobe

Buckwheat mutant starch granules for potential application as fat replacer

Abstract
In this article, morphological differences between normal buckwheat cultivar 'Siva' endosperm starch granules and low amylose mutant endosperm starch granules using scanning electron microscopy (SEM) are represented. A normal buckwheat endosperm contains small polygonal starch granules ranging in size from 4 to 8 μm. Low amylose mutant endosperm contained mostly spherical starch granules ranging in size from 3 to 6 μm. Pinpricks observed on the surface of mutant starch granules may be a result of high amylase activity. These morphological differences

may have resulted from low concentration of amylose in the mutant starch granules. SEM image analysis is a useful tool for classification of individual buckwheat grains in the mixtures of low and normal amylose material. and could be used in grain processing to separate normal from low-amylose starch endosperm. Spherical native starches with granule diameter similar to those of lipid micelles have a potential for use as fat replacers with or without further grinding. With the growing demand for carbohydrate-based fat replacers, starch from buckwheat low amylose mutant may find commercial applications.

Key words: Buckwheat starch, *Fagopyrum esculentum*, Low amylose mutant, Scanning electron microscopy, Starch granule morphology, fat-replacer

1 Introduction

Common buckwheat (*Fagopyrum esculentum*) could not be classified as true cereals. The seeds of common buckwheat contain a cereal-like starchy endosperm. Buckwheat starch granules are polygonal, containing 25% of amylase as determined by iodine colorimetry, having a gelatinization temperature of 61-65 °C, and a high cooling paste viscosity (Kim et al., 1977). Buckwheat groat carbohydrate percentage ranges from 67.8 to 70.1% (Steadman et al., 2001; Li & Zhang, 2001), about 55% of carbohydrate is starch (Steadman et al., 2001). The apparent amylose content (15.6-17.9%) and the true amylose content (25.5-26.5%) differ considerably (Skrabanja & Kreft, 1998). This difference may indicate a presence of a large amount of longer chain amylopectins that have a tendency to complex with iodine (Yoshimoto et al., 2004). Buckwheat amylopectins contain a higher proportion of long chains in comparison to cereal amylopectins (Noda et al., 1998).

The relation of amylose to amylopectin is especially important in buckwheat as it is connected with the technological and nutritional value of buckwheat products (Skrabanja et al., 2001; Kreft & Skrabanja, 2002). Buckwheat may be responsible for the formation of retrograded starch during the traditional hydro-thermal procedure used for production of buckwheat groats (Skrabanja & Kreft, 1998; Skrabanja et al., 1998). Buckwheat seeds are used to prepare many food products and are particularly popular in Japan, Korea, China, Russia, and Central and Eastern Europe. Buckwheat seeds are a rich source of antioxidants and trace elements, including selenium (Kreft et al., 2002; Fabjan et al., 2003; Stibilj et al., 2004; Smrkolj et al., 2006; Kreft et al., 2006; Ikeda et al., 2006). Buckwheat seed milling fractions contain various proportions of endosperm, embryo, and maternal tissues, each of which has a different composition (Steadman et al., 2001; Bonafaccia et al., 2003a; Bonafaccia et al., 2003b; Skrabanja et al., 2004).

Several investigations of physicochemical buckwheat starch properties have been completed in the past (Kim et al., 1977; Alekseeva et al., 1979; Soral-Smietana et al., 1984; Li et al., 1997; Qian et al., 1998; Zheng et al., 1998; Skrabanja et al., 2001). The detailed structures of pericarp, spermoderm, aleurone layer, and starchy endosperm of buckwheat achene were studied by SEM (Pomeranz & Sachs, 1972). In a cross-section through the kernel of tetraploid buckwheat cultivar (cv.) 'Bednja 4n', the prismatic structure of the cells packed with round or polygonal starch granules can be observed in the endosperm. In the peripheral part of the endosperm, starch granules are strongly imbedded in the matrix. Cells containing polygonal structures are quite coherent; when the kernel is broken, the breakage plane passes mainly along the cell walls (Javornik & Kreft, 1980). SEM studies have shown granules of buckwheat starch to be round or polygonal and smaller than wheat starch granules. X-ray diffraction of buckwheat starch showed a pattern typical for that of cereal starches (Lorenz & Dilsaver, 1982). The starch granules isolated from five buckwheat cultivars from Russia, Poland, and Germany, were spherical, oval and polygonal in shape (Quian & Kuhn, 1999; Wijngaard et al., 2007). In buckwheat endosperm, starch granules are located in large, rigid cells, surrounded by relatively thin cell walls. Similar observations are reported by Pomeranz and Sachs (1972). Buckwheat contains only small polygonal granules ranging in size from approximately 4 to 8 μm. The starch packing depends on the location in the buckwheat endosperm. Starch in the endosperm cells located directly next to the cotyledons is packed more loosely than the more compact structure observed in other areas of the kernel.

To the best of author's knowledge, the differences in buckwheat endosperm starch granules between normal and and low amylose mutants has not been described so far. It shows a need for a method which would allow the screening of low amylose single seed endosperms in mixtures of seeds, for selection of different grains in buckwheat breeding, and to evaluate mixtures of varieties in grain processing.

Aqueous dispersions of small starch particles are known to produce a creamy, smooth texture which mimics fat properties (Jane et al., 1994; Biliaderis et al., 1993; Malinski et al., 2003). Fat-mimic properties of starch granules result from the association of water-carbohydrate molecules and association of the released linear amylose molecules, which can give rise to viscosity increases and gelation (Sanderson, 1981; Yackel & Cox, 1992).

The purpose of this investigation was to characterize low amylose buckwheat grains in regard to the size of starch granules and their ability to be deformed during milling.

2 Materials and methods

2.1 Plant materials

In the experiments the endosperm starch of common buckwheat (*Fagopyrum esculentum* Moench) cv. 'Siva' and endosperm starch of a low amylose mutant, selected from a population of cv. 'Siva', were examined. Buckwheat cv. 'Siva' and the low amylose mutant were grown on the experimental plot of the Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia.

2.2 Starch isolation

Prior to dissection, the unhusked buckwheat seeds were soaked for 1 hour in distilled water. Using a magnifying glass, the husk was removed from each seed and the seeds were cut in half.

2.3 Determination of total amylose

Total amylose in buckwheat endosperm was determined by a spectrophotometric procedure after Morrison and Laignelet (1983). Total amylose content was determined after the removal of lipids through defatting of starch with 98% ethanol. Prior to spectrophotometric determination, the samples were dissolved in urea-dimethylsulphoxide (UDMSO). Aliquots of lipid-free samples in UDMSO solution were diluted and mixed with I_2-KI solution. After 15 minutes, 635 nm absorbances were measured.

2.4 Scanning electron microscopy (SEM)

Buckwheat seed halves were glued on specimen stubs using conducting silver tape, and coated with a thin gold layer. Samples were examined by a Jeol JSM 840A scanning electron microscope at an accelerating potential of 7 kV.

3 Results and Discussion

3.1 Total amylose concentration

Low amylose mutant contained 6-12% of true amylose in the endosperm starch; in comparison, normal common buckwheat cv. ‘Siva’ had 22-25% of true amylose in the endosperm starch.

3.2 Buckwheat seed cross-section

As observed by SEM, the embryo is located in the centre of the seed and has two folded cotyledons (Fig. 1). This is in agreement with Kreft and Kreft (2000), who performed a computer-aided three-dimensional reconstruction of the buckwheat seed. The cotyledons are doubly curved. In the proximal part they approach the testa only with their margins, whereas in the distal part the two cotyledons extend tightly parallel to the testa.

Fig. 1. Cross section of a buckwheat seed as observed by scanning electron microscopy.
(Source: author)

3.3 Endosperm cells in normal buckwheat (cv. ‘Siva’)

As observed by SEM, the endosperm starch granules of cv. ‘Siva’ are polygonal (Fig. 2). The granule size distribution ranges from 4 to 8 μm. Starch granules are placed tightly together in prismatic cells (Fig. 2). Cells containing polygonal structures are quite coherent; when the kernel is broken, the breakage plane passes mainly along the cell walls. This is in agreement with the report of Javornik and Kreft (1980). In the center of the seed the starch granules are not as tightly packed in the cells as they are in the peripheral cells close to the aleurone layer.

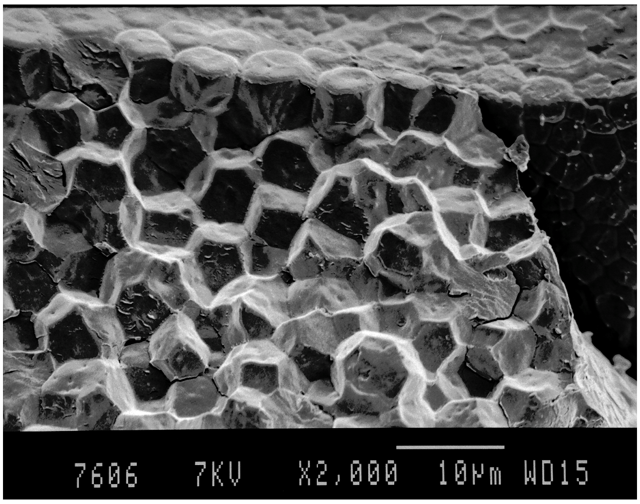

Fig. 2. Prismatic endosperm cells and polygonal starch granules of buckwheat cv. 'Siva' endosperm, observed by scanning electron microscopy.
(Source: author)

3.4 Endosperm cells in the low amylose mutant

SEM observations show spherical starch granules in the endosperm of the low amylose mutant (Fig. 3A). The granule size distribution ranges from 3 to 6 μm. Spherical starch granules are seen in the middle of the endosperm and also in the peripheral cells, close to the aleurone layer (Fig. 3B). Starch granules are packed in prismatic cells (Fig. 3B). Pinpricks are visible on the surface of the mutant starch granules , possibly caused by the high amylase activity (Fig. 3C). In comparison, starch granules from soft maize endosperm have a rough appearance randomly distributed pores on the surface (Dombrink-Kurtzman & Knutson, 1997). Mutant starch granules are more loosely packed in comparison to the normal cv. 'Siva' endosperm starch granules. Flattened starch granules and broken starch granule were observed in several buckwheat mutant endosperms, possibly deformed by mechanical pressure (Figs. 3D). This can be ascribed to empty spaces between amylopectin molecules within the starch granules caused by low amylose amounts and missing amylose molecules between the amylopectin layers. Waxy starch granules in wheat are also less resistant to mechanical damage than normal starch granules (Bettge et al., 2000). Dombrink-Kurtzman and Knutson (1997) suggested that there are differences in susceptibility to physical damage caused by the beam of an electron microscope; the resistance of granules to wrinkling and fracturing under exposure to the electron beam appears to decrease with decreasing amylose content in starch granules. For example, the granules of soft maize endosperm have low amylose content, exhibit more surface pores, and are more susceptible to wrinkling in an electron beam compared to the granules of the hard endosperm (Dombrink-Kurtzman & Knutson, 1997).

The partially hydrolyzed granular starch exhibits fat-mimic characteristics for use in reduced calorie processed foods (Lindeboom et al., 2004). Fine-granule starch with diameter of 2 μm or in size similar to that of liquid micelle could be used as fat substitutes, without further processing (Daniel and Whistler, 1990; Glicksman, 1991; Lucca and Tepper, 1994; Setzer and Racette, 1992). Fig. 3B shows that the interior of the buckwheat starch granule of low amylose mutant is not completely filled with starch - in the middle there is an empty space. The properties of *waxy* starches demonstrate that amylose synthesis is not required for the formation of semi-crystalline granules, and its synthesis takes place within a pre-existing amylopectin matrix as the granule is formed (Denyer et al., 1999; Dauvillèe et al., 1999; Tatge et al., 1999; Denyer et al., 2001).

In this study the morphological differences between normal buckwheat cv. 'Siva' endosperm starch granules and buckwheat low amylose mutant endosperm starch granules were observed.

Morphological differences occurred because of the decreased concentration of amylose in the starch granules. SEM analyses revealed the difference between the starch granules of the cv. 'Siva' and low amylose mutant selected from genetic material of cv. 'Siva'. Author suggests that SEM image analysis could be one of the tools for distinguishing between normal and low amylose starches, and in searching for new low amylose mutants.

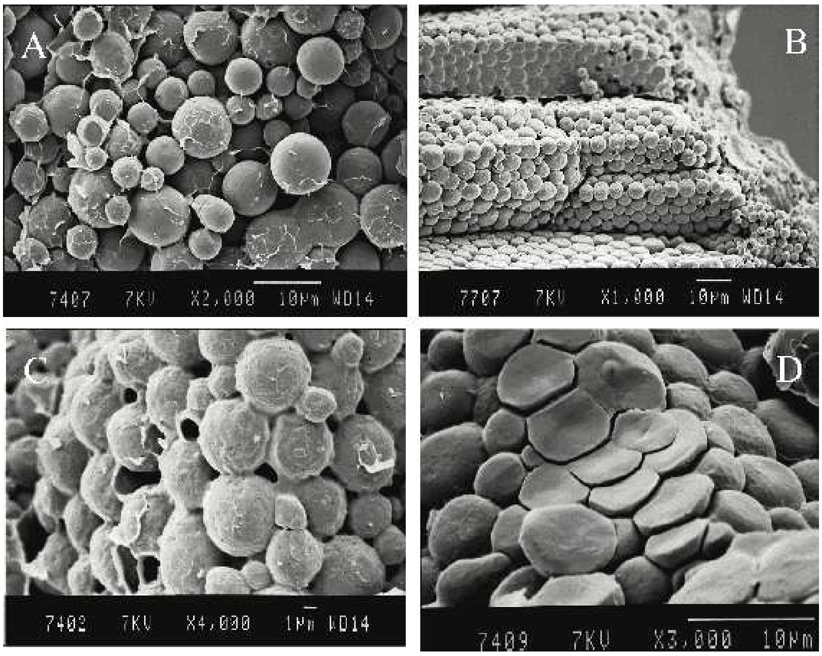

Fig. 3. Endosperm starch granules of buckwheat low amylose mutant; A - Round shaped endosperm starch granules of buckwheat low amylose mutant; B - Round shaped endosperm starch granules of buckwheat low amylose mutant in cells close to the aleurone layer; C - Pinpricks on the surface of endosperm starch granules of buckwheat low amylose mutant; D - Flattened endosperm starch granules of buckwheat low amylose mutant.
(Source: author)

4 Conclusion

The relation of amylose to amylopectin is very important in buckwheat as it determines the technological and nutritional value of buckwheat products. Using SEM we were able to observe the difference between the starch granules of the low amylose mutant in comparison to the original genotype cv. 'Siva'. Morphological differences of starch granules between the low amylose mutant and normal buckwheat cv. 'Siva' occur due to the low amylose concentration in the mutant starch granules. The starch granules in the low amylose mutant endosperm are mostly spherical with size distribution ranges from 3 to 6 μm. Pinpricks observed on the surface of mutant starch granules may be caused by the high amylase activity (Fig. 3C). Starch granules in the mutant are loosely packed in comparison to the normal cv. 'Siva' endosperm. Our results suggest that starch granules from buckwheat low amylose mutant were less resistant to wrinkling and fracturing than starch granules from normal buckwheat. This caused by empty space between amylopectin molecules within the starch granules resulting from the diminished amylose molecules allocation between amylopectin layers.

The properties of low amylose mutant starch should be taken into the account in research and technological procedures using either type of buckwheat starch material. Spherical native starches with granule diameter similar to those of lipid micelles can have potential use as fat replacers with or without further processing. With the growing demand for carbohydrate-based fat replacers, starch from buckwheat low amylose mutant may find commercial applications.

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