Food Hydrocolloids 32 (2013) 52–63

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Physicochemical and morphological characterization of different starches with variable amylose/amylopectin ratio

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ARTICLE INFO

Article history:
Received 24 July 2012
Accepted 29 November 2012

Keywords:
Confocal-laser-scanning-microscopy (CLSM)
Starch gelatinization
Image processing
Fluorescent staining
Rapid-visco-analysis (RVA)

ABSTRACT

Starch-rich raw materials are widely used in the food industry. Their functionality and end-use applications are markedly influenced by starch characteristics. Starches with varying amylose (AM) and amylopectin (AP) content are of particular interest due to their ability to influence and modify the texture, quality and stability of starch-based food products. The present study shows the influence of the AM/AP content on physicochemical and morphological properties of a range of starches (Maize = 3%, 23%, 71%; Potato = 2%, 21%; Wheat = 28; Barley = 3%, 25% AM content w/w of starch).

Starches have been analyzed in terms of their chemical composition, water retention capacity, morphological characteristics, and pasting/thermal properties. The changes in starch granule morphology during gelatinization were monitored by confocal laser scanning microscopy (CLSM). The different analysis revealed that waxy-starches (AP>90%) had a high water retention capacity (1.2–1.5 times higher) and developed higher paste viscosities (up to 40% for maize; 43% for barley). The swollen granules were highly susceptible to mechanical breakdown and solubilized faster. Higher AM contents showed inhibition of an extensive granule swelling and lowered the paste viscosity. The exceptional integrity of the high-AM starch even prevented its gelatinization at atmospheric pressure. Significant differences in physicochemical and morphological properties between the starches from regular, high-AM and waxy strains have become evident, no direct relationship between the AM/AP contents and the internal growth ring structures of the starch granules could be identified by CLSM. The waxy starches had a higher gelatinization temperature (up to 2 °C) and enthalpy (up to 20%), which indicates a higher crystalline and molecular order.

1. Introduction

Starch is the major energy storage reserve carbohydrate synthesized in many parts of plants and represents the second most abundant biopolymer on earth, next to the organic compound cellulose (Eliasson, 2004). It plays an important role as functional material in the food and non-food industries and serves as an essential source of energy for human and animal nutrition. Starch granules contain numerous components, which can be divided into two groups: the first comprises of the major components amylose (AM) and amylopectin (AP) and the second is made up of the minor components of starch (proteins, lipids and minerals) (Copeland, Blazek, & Salman, 2009; Tester, Karkalas, & Qi, 2004). The ratio of the two α-glucans in starch granules as well as their molecular structure influence e.g. the solubility, gelatinization temperature, viscosity, gelation and retrogradation properties of starch and therefore represent major parameters for the quality, texture and stability of starch- or flour-based products (Blazek & Copeland, 2008).

In general, the ratio of AM to AP and their structural variability strongly depend on the botanical origin. Regular starches contain approximately 70–80% AP and 20–30% AM, waxy starches less than 10% AM and high-AM starches more than 40% AM (Tester et al., 2004). In order to obtain starches with specific pasting properties and other technologically relevant characteristics, several approaches have been made which aimed to increase the granule AM or AP content. Such breeding programs are based either on non-genetic modification in terms of traditional breeding and selection of agronomically well-adapted varieties or on genetic modification or rather manipulation of the expression of genes involved in starch-biosynthetic pathway (Blazek & Copeland, 2008; Morell & Myers 2005).

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http://dx.doi.org/10.1016/j.foodhyd.2012.11.032

Various analytical methods have been used to estimate the AM and AP contents of starch and to determine the molecular structures of these two starch polysaccharides (Herrero-Martínez, Schoenmakers, & Kok, 2004; Kugimiya & Donovan, 1981; Mestres, Matencio, Pons, Yajid, & Fliedel, 1996; Moorthy, Andersson, Eliasson, Santacruz, & Ruales, 2006; Wang, Li, et al., 2010; Wang, Yu, et al., 2011), (Grant, Ostenson, et al., 2002; Kobayashi, Schwartz, & Lineback, 1985; Scott, Jane, & Soundararajan, 1999; Stawski, 2008). In comparison, different imaging techniques which have proven to be suitable for analyzing starch granule surface or internal structure are light microscopy including polarizing, bright-field (Atkin, Cheng, Abeysekera, & Robards, 1999), and fluorescence microscopy (Goossens, Derez, & Bhar, 1988), atomic force microscopy (Baldwin, Adler, Davies, & Melia, 1998) and electron microscopy including scanning (Jane, Kasemsuwan, Leas, Zobel, & Robyt, 1994) and transmission electron microscopy (Yamaguchi, Kainuma, & French, 1979). But in all cases there were not any morphological characterization as well as quantification of different AM/AP content by microscopic analysis as well as correlations between analytical and microscopic analysis. As a basis for the determination of the AM/AP content of total starch by confocal laser scanning microscopy (CLSM), an enzymatic method based on the specific formation and precipitation of AP-Concanavalin A (Con A) complexes, after a pre-treatment of the sample to solubilize resistant starch and to remove lipids and free D-glucose was used. In this study, CLSM, which uses laser technology in combination with fluorescence imaging, was used to represent first the visualization of starch granule morphology with the objective for the determination of the AM/AP content.

Different starches are used in many applications in the food industry and therefore in almost all applications, starches were heated before use. When starch granules are heated in the presence of water, several changes at molecular and structural level occur. These alterations of starch structure are related to the granule-specific gelatinization behavior, which is strongly affected by a number of inherent characteristics: granule composition (in particular the ratio of AM to AP), granule size, granule molecular architecture (especially the AP branch chain lengths and distribution) and molecular weight of AM and AP molecules (Jane, Chen, et al., 1999; Lindeboom, Chang, & Tyler, 2004). The progress and extent of the thermal gelatinization of starch are affected by a wide range of external factors, including applied heating temperature, moisture content, heating time, heating rate, presence of further ingredients in food or non-food products, processing conditions, starch preparation and storage conditions (Beck, Jekle, & Becker, 2011; Spigno, & De Faveri, 2004). AP is suggested to be responsible for granule swelling while the fraction of AM–lipid complexes may retard the swelling and induce an increase in gelatinization temperature (Jane et al., 1999). Former publications are dealing with the visualization of gelatinized starches as well as analysis of pasting properties and thermal changes, but without combined consideration.

In this study, morphological, rheological and thermal characteristics of different starches were studied. Thereby, CLSM has been used to investigate the granule characteristics as well as the gelatinization behavior of starch granules visualized and analyzed by a micrograph processing tool. These values were combined with pasting properties analyzed by rapid visco analyzer (RVA) and thermal changes measured by differential scanning calorimetry (DSC). The main objectives of this study are to characterize and compare the morphological and physicochemical properties of selected starches of cereal and tuber origin. The samples have either been currently used in the food industry or were less established therein and significantly ($p \leq 0.05$) differ in AM/AP content. On the basis of the morphological characteristics and physicochemical properties, an effect of the AM/AP content on the sample properties is going to be evaluated.

2. Materials and methods

2.1. Starch samples

The starch samples used and their respective sources were maize starch (National Starch & Chemical GmbH, Hamburg, Germany), high-AM maize starch (HYLON® VII, National Starch), waxy maize starch (AMIOCA™ National Starch), potato starch (AVEBE FOOD, Veendam, The Netherlands), waxy potato starch (ELIANE™ 100, AVEBE FOOD), wheat starch (Merck KGaA, Darmstadt, Germany), barley starch (Grain Processing Corporation, Lowa, USA) and native waxy barley starch (Lyckeby PU 91 000, Kampffmeyer Nachf. GmbH Ratzeburg, Germany) which are a selection of important starches for the food industry. The selected starches evinced following AM contents (w/w of starch) Maize = 3%, 23%, 71%; Potato = 2%, 21%; Barley = 3%, 25%.

2.2. Analysis of chemical and physicochemical properties of samples

The sample moisture content (%) was determined thermogravimetrically using the moisture analyzer MLB-50-3 (Kern & Sohn GmbH, Balingen-Frommern, Germany) by weight loss from the initial weight. The crude protein content of the starches and flours was determined on the basis of the Kjeldahl method using a Kjeltec™ 2400 Auto Analyzer Unit. For the conversion of the percentage nitrogen to crude protein content, the factor 6.25 was used. The crude lipid content (i.e. free lipids) was quantified using the AACC Method No. 30-25.01 (2010). The ash content was determined according to the ICC Standard Method No. 104/1 (1990) and related to the dry matter of the sample substance. The water retention capacity (WRC) was determined by measuring the water uptake of the samples (at approx. 20 °C) according to the standard AACC Method No. 56-11.02. It is expressed as percent weight of solvent retained by the sample in a gel pellet after centrifugation and decantation related to the sample weight on a 14% moisture basis. For the determination of the AM/AP content of total starch, the AM/AP assay procedure, utilizing the commercially available kit (Megazyme International Ireland Ltd.), was followed according to the recommendation of the manufacturer. This enzymatic method is based on the specific formation and precipitation of AP–Concanavalin A (Con A) complexes, after a pre-treatment of the sample to solubilize resistant starch and to remove lipids and free D-glucose. The test kit includes relative standard deviations of <5% for pure starches. The total starch content of the samples was measured enzymatically using an assay kit according to the standard AACC Method No. 76.13. The starch content measurement includes the major components amylose and amylopectin, the minor components of starch (protein, minerals, and lipids) are not detected. The damaged starch content of the samples was determined using an assay kit in accordance with the AACC Method No. 76-31.01 (2010). The method is based on the enzymatic susceptibility of damaged starch granules. Each measurement was performed in duplicate.

2.3. Visual characterization by confocal laser scanning microscopy (CLSM) and micrograph analysis

Aqueous sample suspensions (10 g kg^{-1}) were prepared by dispersing 50 mg of the sample in an appropriate volume of distilled water while gently stirring for 2 min. Aliquots (500 μL) of the suspension were transferred into 1.5 mL microtubes and 40 μL of aqueous Nile Blue solution (0.1 g 100 mL^{-1}) were added. After mixing thoroughly by repeated pipetting up and down, the stained solutions were incubated at 20 °C for 3 h. The swollen and gelatinized starch samples were prepared by heating the starch–water