



Analytical Methods

New determination method of amylose content in potato starch

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ABSTRACT

Thermal stability of potato starch depends on amylose content. Temperature at 50% mass loss from thermogravimetric analysis was used to determine the starch components. The same analysis was carried out to analyse the samples from potato, rice, wheat and high amylose. The results (26.9% of amylose content for potato, 29.1% for rice and 20.9% for wheat) were compared with results obtained from the blue value method and enzymatic method. Satisfactory compatibility for potato and rice was obtained. For such kind of samples thermogravimetric analysis can be used for amylose determination.

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1. Introduction

Starch is a mixture of amylose and amylopectin. The amylose fraction is essentially linear, whereas amylopectin is highly branched. Two starch components have different properties and are not suitable for the same applications (Zobel, 1988).

The relative proportion of amylose and amylopectin varies considerably within plant species, plant organs, and depends on organ development and growth conditions. The amount of both starch components has influence on many various properties of these materials such as: swelling capacity (Hermansson & Svegmarm, 1996; Sandhu, Singh, & Malhi, 2005), water solubility (Sandhu et al., 2005), water-binding capacity (Sandhu et al., 2005), barrier and mechanical properties of starch films (Rindlav-Westling, Stading, Hermansson, & Gatenholm, 1998) and microscopic properties (Hermansson & Svegmarm, 1996). In humans and other monogastric animals amylose is slower digested than amylopectin, so blood glucose and insulin level are lower after a meal high in amylose, satiety is maintained longer and the next meal is likely to be smaller (Heijnen, van Amelsvoort, & Westrate, 1995; Holt & Brand Miller, 1995).

There are different methods of starch components determination.

The enzymatic method (McCleary, Solah, & Gibson, 1994) is highly specific, but may underestimate starch in materials containing starch resistant to enzymatic hydrolysis or gelatinisation. A procedure is both expensive and time consuming and thus not suitable for routine analysis.

In the method proposed by Scott, Jane, and Soundararajan (1999) differences in carbon isotope ratios in amylose and amylopectin were used. Generally the natural materials ^{13}C contents are low and differences between amylose and amylopectin in these contents rather small, the stable carbon isotope ratios are expressed in relative terms using international PDB (Pee Dee Belemnite) standard.

Near-infrared (NIR) spectroscopy can lead to estimate the proportion of both components (Fertig, Podczek, Jee, & Smith, 2004). Single wavelength absorbencies versus concentration plot calibrations are rarely possible with NIR spectra because of overlapping absorbencies between various components. The simplest calibration models are typically based on multiple linear regression, using the absorbance at two or more wavelengths (Fertig et al., 2004). Near infrared spectra often exhibit a baseline shift caused by variations in sample compaction and scatter from the particle surface. To minimize the influence of these parameters the raw spectra are usually subjected to mathematical pre-treatments before developing calibration models.

The technique of high-performance size-exclusion chromatography is now used widely to estimate the relative amounts, and the apparent molecular weights of amylose and amylopectin in native starch (Bradbury & Bello, 1993; Jackson, Choto-Owen, Waniska, & Rooney, 1998). This method is useful for the precise identification of molecular mass fraction starch components between amylose and amylopectin. Next debranching enzyme was used (Bradbury & Bello, 1993). This technique is time consuming and not suitable for routine analysis.

In the literature, dependences between amylose content and melting temperatures of samples (Sievert & Wuesch, 1993), degree

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of crystallinity (Matveev et al., 2001) and enthalpy from differential scanning calorimetry (Matveev et al., 2001) are known. However, these dependences are not well correlated (R^2 factor by enthalpy is 0.5, where R is the linear correlation coefficient).

The most important method to calculate the proportion of both components is the blue value method (McCready & Hassid, 1943; Mohammadkhani, Stoddard, & Marshall, 1998). This method takes advantage of differences in iodine complexes creation. The complex amylose-iodine is blue, and amylopectin-iodine is red. Spectrophotometric or colorimetric measurements of blue value intensity can lead to calculation of amylose content. The iodine reaction has been proposed to quantify the amylose content in starches using measurements made at one wavelength (600 nm) (McCready & Hassid, 1943). The simultaneous determination of both components was described for potato starch using two-wavelength analysis (Hovenkamp-Hermelink et al., 1988). Later, the six-wavelength technique was used too (Jarvis & Walker, 1993; Sène, Thévanot, & Prioul, 1997). This old method is today still most commonly used. It is not easy and requires many labour and time consuming processes.

Thermal properties of different starches were often characterized (Aggarwal & Dollimore, 1996; Rath & Singh, 1998; Seetharaman et al., 2001; Singh, Johnson, White, Jane, & Pollak, 2001; Srichuwong, Sunarti, Mishima, Isono, & Hisamatsu, 2005; Teramoto, Motoyama, Yosomiya, & Shibata, 2003; White, Abbas, Pollak, & Johnson, 1990). The differences between thermal resistance of amylose and amylopectin in corn starch were described (Aggarwal & Dollimore, 1997; Kapuściak, Ciesielski, Koziół, & Tomasik, 2001; Teramoto et al., 2003) but they were not used to determine the proportions of both components.

The aim of presented work was to present a new, simple, rapid and accurate method suitable for simultaneous determination of amylose and amylopectin. This method makes use of the thermal resistance differences of the two starch components that can be detected in a standard thermogravimetric measurement. These differences are described elsewhere (Teramoto et al., 2003; Kapuściak et al., 2001; Aggarwal & Dollimore, 1997) and are caused by the structure dissimilarities of the amylose and amylopectin.

2. Experimental

2.1. Materials

Starches (from potato, wheat and rice), amylose and amylopectin from potato, were purchased from Fluka, Germany.

High amylose (70%) starch were obtained from Megazyme Ltd., Ireland.

Citric acid, natrium hydroxide, pure iodine and kalium iodine were from POCH, Poland.

Acetic acid, sodium hydroxide, sodium azide, sodium acetate, sodium chloride, $\text{CaCl}_2 \times 6\text{H}_2\text{O}$, $\text{MgCl}_2 \times 6\text{H}_2\text{O}$, $\text{MnCl}_2 \times 4\text{H}_2\text{O}$ were obtained from Chempur, Poland.

All samples before use were dried overnight at 60 °C.

Amylose-amylopectin K-AMYL Kit was obtained from Megazyme Ltd., Ireland.

The blends of amylose and amylopectin were prepared in Narva Vibrator, Germany.

2.2. Methods

2.2.1. Thermogravimetric (TG) method

The thermal analysis of all samples were carried out with Perkin-Elmer TGA 7 thermal analyser in a platinum measuring cell, with the use of Pyris programme for data handling. Measurements were performed in a nitrogen atmosphere (gas flow $20 \text{ cm}^3 \text{ min}^{-1}$) with the heating rate 15 °C min^{-1} . The samples (about 5 mg) were heated up to 650 °C, starting from room temperature. All measurements were repeated at least three times. For each course $T_{50\%}$ was determined and after that the average value was calculated. TG method offers very repeatable results and the calculation error is about 1%.

2.2.2. Blue value (BV) method

The analysis was performed according to the procedure described elsewhere (Mohammadkhani et al., 1998). BV method was used as a reference method to verify the results obtained from TGM. Both calibrations were made for the same points for amylose-amylopectin blends.

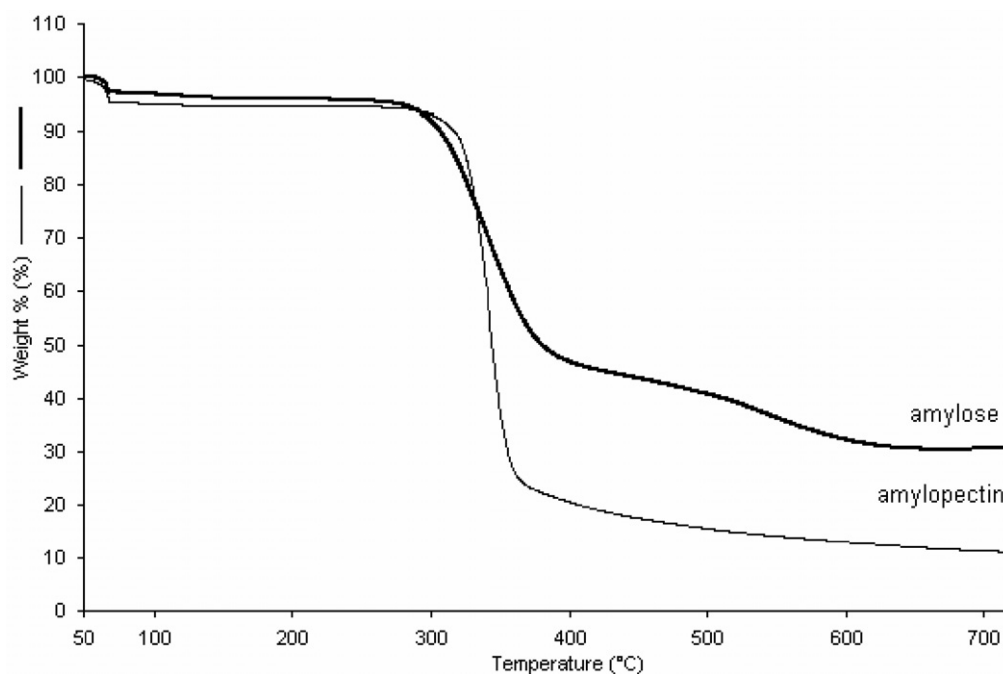


Fig. 1. Thermograms of amylose and amylopectin from potato.

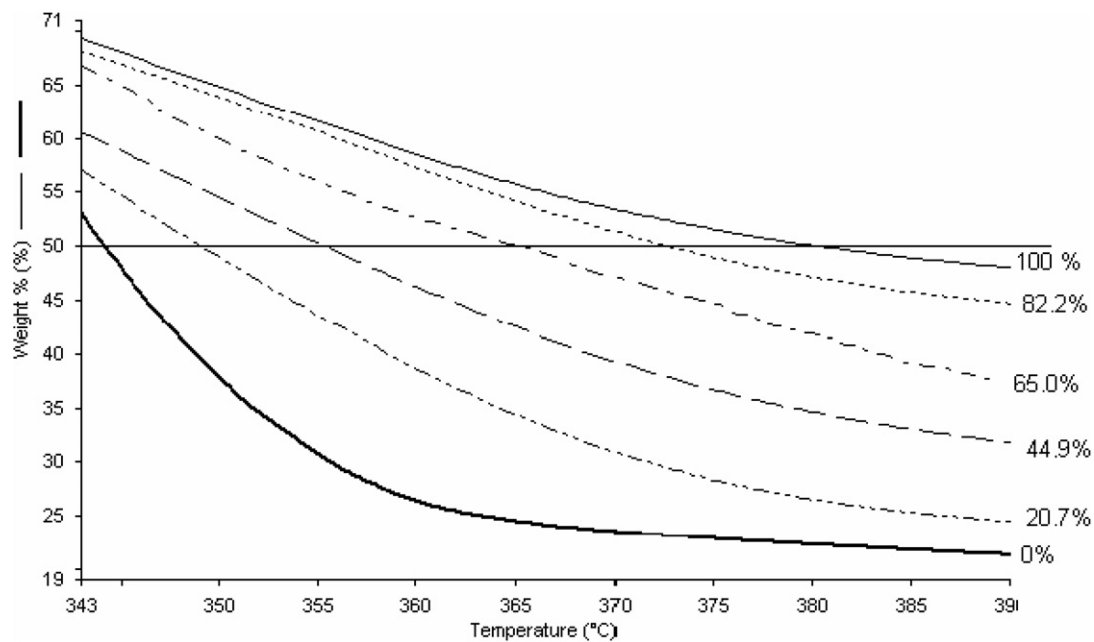


Fig. 2. Representative thermogravimetric curves of amylose-amylopectin blends, in analysed range, with varying weight percent of amylose.

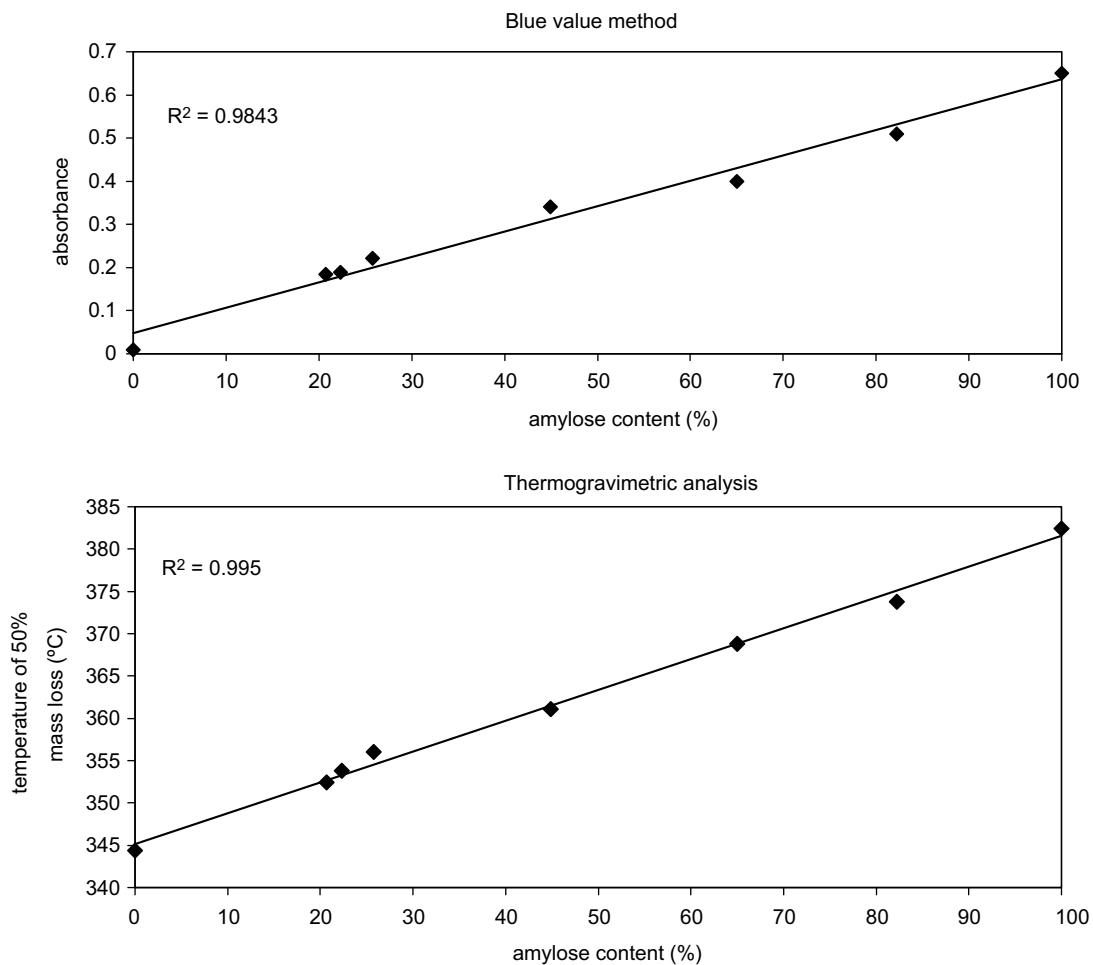


Fig. 3. Calibration curves of amylose determination obtained using thermogravimetric (50% of mass loss temperature) and the blue value method.

2.2.3. Enzymatic method (EN)

Enzymatic test was carried out using amylose–amylopectin K-AMYL Kit obtained from Megazyme, Ireland according to the assay procedure.

2.2.4. UV-Vis measurement

The UV spectra were recorded in solution using spectrometer UV-Vis Lambda 2 (Perkin–Elmer).

3. Results and discussion

The results of thermogravimetric analysis of amylose and amylopectin are presented in Fig. 1. Fig. 2 shows TG plots of starches with various known amylose content.

From Figs. 1 and 2 one can conclude that the thermal stability of starches clearly depends on the proportion of both components. The obtained results were used to prepare the calibration curves. The calibration process using temperatures of 50% mass loss has been compared with the calibration from the blue value method, the one of the most popular methods of amylose content determination.

The both calibration curves are presented in Fig. 3.

As one can see from the results presented in Fig. 3, the calibration curve obtained from TG method has good linearity, and better R^2 factor, as from the blue value method.

The temperature of 50% mass loss was used to determine the amylose content in commercial potato starch. The results were compared with results obtained using the standard blue value and enzymatic method (Table 1).

As seen in data presented in Table 1, all methods show similar values of amylose content for potato starch. Taking into account that the necessary procedure in BV and especially in EN method are very difficult, laborious, time consuming and demand a lot of reagents – the TG method is particularly noteworthy.

Additionally samples from wheat and rice were compared and it was found that the compatibility in these cases is good too. The same results have been obtained for the rice sample and for wheat the result of TG method is situated in the middle (between the result from BV method and enzymatic method). Generally, it is known that there are differences in thermogravimetric results between starches from different sources (Aggarwal & Dollimore, 1996). It is possible that making calibration curve for TG method using amylose and amylopectin from wheat we could obtain much better compatibility for wheat starch results too.

Similar investigation was made for commercial starch (not from mixtures) with a known, high content of amylose. The results (69.9% for BV method, 73.0% for EN method and 70.8% for TG method) show that for commercial, high content amylose starch sample the obtained values are very near to the amylose content given by the supplier (70%). As we can see, the synthetic mixtures can be used as calibration substances for natural products.

Differences between thermal properties of various linear and branched polymers are described in the literature (Feng, Nintz, Augsten, & Jones, 1998; Li & Kissel, 1998; Ouchi, Ichimura, & Ohya, 2006). In some courses the differences were also found for maize

(Rath & Singh, 1998) and corn (Aggarwal & Dollimore, 1997) amylose and amylopectin.

Further investigation should check TG method at other heating rates, at different stages of decomposition (to reduce the duration of the procedure), also in other inert gases and by changing gas flows.

4. Conclusions

This study has clearly demonstrated that the thermal stability of potato starch samples really depends on amylose content. Using temperature of 50% mass loss from TG thermograms it is possible to determine the proportion of both starch components.

TG method gives satisfied results for rice and high amylose content commercial samples too. Doubtful can be the results obtained for wheat starch, and the method for this type of sample needs to be elaborated in the future.

The values of R^2 factor in calibration curves of the TG method and the blue value method are almost the same. Suchlike, as the calculated content of amylose in analysed starch sample using the two methods.

Using the temperature of 50% mass loss for calculations all the thermogram is not required what can diminish the measurement time even 50%. Big measurement accuracy in novel thermogravimetric apparatus practically exclude instrumental and measuring errors and enables to obtain accurate as well as repeatable results.

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Table 1

Results of measurements of percent amylose contents using the blue value, enzymatic and thermogravimetric method

Type of starch	Method		
	Blue value ^a	Enzymatic ^a	Thermogravimetric ^a
Potato	26.0	26.3	26.9
Rice	29.1	29.1	29.1
Wheat	18.1	23.4	20.9

^a Results based on at least three independent analysis.

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