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Application News

MALDI-TOF mass spectrometry

Analysis of Branching Degree of Starch with MALDI-TOF MS

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User Benefits

- Simple and quick sample preparation analysis
- The MALDI-MS enables uncomplicated interpretation of the branching degree and chain length of starch
- The method enables high throughput

■ Introduction

Starch is widely used in food industry as thickener, emulsion stabilizer or to increase storability, just to name the most common applications (1-2). But its use is not limited to food production but also in textile, paper, pharmaceutical, and packing industry (2). Its use as plasma volume expander turns starch even to a relevant analyte in doping control (3). Starch contains 20–30% amylose and 70–80% amylopectin (4-5). Amylose and amylopectin are polysaccharides composed of α-D-glucopyranosyl units. Amylose has a linear structure that is linked via α-1,4 glycosidic compounds. Amylopectin has a branched structure with α-1,4 glycosidic compounds. The branch points are linked with α-1,6-glycosidic linkages. To adapt the properties of the starch, a wide variety of modifications are carried out, often using derivatization (5). Enzymatic digestion is carried out for the analysis of the products. The enzyme isoamylase cleaves the α-1,6-glycosidic bonds. This leads to the debranching of starch (6).

The chain length distribution of the debranched starch is usually determined using gel permeation chromatography (GPC) (7). The Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI TOF MS) is an alternative measurement method. The MALDI-8020 analysis system offers simple and quick analysis with structural information to analyze the chain length distribution of starch.

■ Sample preparation

The starch sample undergoes enzymatic cleavage by isoamylase. The resulting sample is then diluted with water (1:10) and heated to 90 ºC. 2 mg/mL sodium trifluoroacetate (NaTFA) in water is used as a cation donator. The matrix solution is formulated at a concentration of 5 mg/ml of 2,5-dihydroxybenzoic acid (DHB) in a solvent mixture of acetonitrile / water / TFA (50:50:0,1). 0,5 µL of each of the three solutions were spotted on the target. The target is stored until the solvent is evaporated.

■ Measurement Conditioins

The preparate sample is analyzed with the MALDI-8020 –System shown in Fig. 1. Table 1 shows the measurement conditions.

Fig. 1 MALDI-8020 benchtop mass spectrometer

Table 1 Analysis conditions for MALDI-8020

Mass range	: 500 – 20000
Laser repetition rate	: 200 Hz
Laser shots per spectrum	: 500
Pulsed extraction	: 1000
Blanking mass	: Off

■ Results

The spectra of two distinct starch samples, as illustrated in Fig. 2, exhibit a spectrum of repeating units with a mass difference of 162, which was absent in the spectra of the blank sample. The mass difference of 162 equals one α-1,4 glycosidic unit. There is a difference in the chain lengths between starch samples #1 and #2. Spectrum 1 exhibits signals in the range from m/z of approximately 1340 to 3930, corresponding to 8 to 24 repeating units. The base peak is around m/z 1990 and aligns with 12 repeating units. Contrastingly, Spectrum 2 shows signals from m/z around 690 up to m/z 3120, corresponding to 4 to 19 repeating units. The signals representing more repeating units showed increasing and decreasing intensity. The base peak was

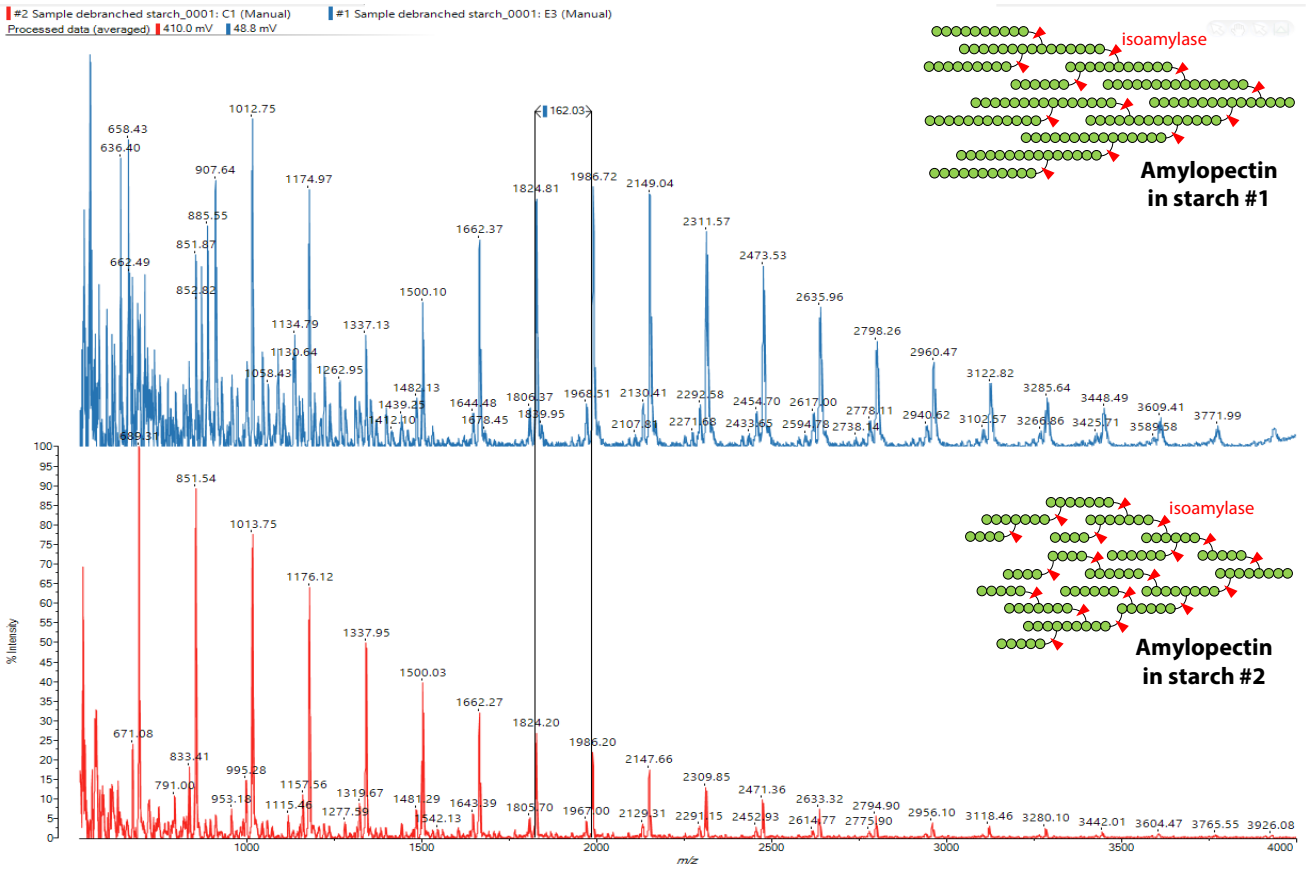

Fig. 2 Sample spectra of debranched starch. #1 in blue and #2 in red.

the first peak, which represented 4 repetition units. The blank sample of the enzyme after incubation with water showed a few signals with a different mass difference, possibly showing clusters of the matrix DHB and Na. Similar spectra were recorded before and after heating the sample, but the signal intensity was a bit lower and more reproducible after heating, which could be explained by the better homogeneity of the sample solution after heating.

■ Conclusion

A MALDI-TOF method for measuring the starch sample was developed. By cleaving the branches of the starch, the chain length distribution can be seen in the MS spectrum, which allows conclusions to be drawn about the chain length and branching degree of the sample. The method developed with MALDI is significantly faster than the conventional method of GPC.

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05-SCA-295-007-EN First Edition: Sep. 2024

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