Biophysical Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biophysical Chemistry

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

Effect of high pressure on the saccharification of starch in the tuberous root of sweet potato (*Ipomoea batatas*)

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ARTICLE INFO

Keywords:
High hydrostatic pressure
Gelatinization of starch
Saccharification
Sweet potato
Ipomoea batatas
Food processing

ABSTRACT

We analyzed the effect of high hydrostatic pressure (HHP) treatment on reducing sugar production in the tuberous root of sweet potato (*Ipomoea batatas*), based on pressure-gelatinization of starch and subsequent saccharification by internal amylases. HHP treatment at up to 600 MPa at ambient temperature for 10 min did not apparently affect the reducing sugar concentration in tuberous root. However, HHP treatment at 100 to 500 MPa and 60 °C or 70 °C for 10 min increased reducing sugar concentration as both the pressure and temperature increased. The reducing sugar concentration after HHP treatment at 500 MPa and 70 °C for 10 min was roughly comparable to that of the thermal treatment control (80 °C for 10 min under atmospheric pressure). HHP treatment enabled the gelatinization and enzymatic saccharification of starch in the tuberous root of sweet potato, at a lower temperature than required by thermal treatment at atmospheric pressure.

1. Introduction

The tuberous root of sweet potato (*Ipomoea batatas*) is a popular food that is higher in starch, as well as various vitamins, minerals and protein, than other vegetables [1]. Additionally, sweet potato contains functional components such as anthocyanins, polyphenols, and dietary fiber that have health-protecting properties [2,3]. The tuberous root of sweet potato is commonly processed by thermal treatment, enabling the gelatinization of starch and simultaneous enzymatic saccharification by internal amylases. This processing method not only generates a high concentration of sugar, making the tuberous roots actually sweet, but is also used in the manufacture of a number of other foods, such as baked potato and Japanese tempura.

Consumers worldwide are interested both in non-thermal food processing methods in order to preserve nutritive compounds and in reducing the use of chemical food additives [4]. High hydrostatic pressure (HHP) treatment is one promising alternative non-thermal food processing technology [5]. HHP treatment can inactivate micro-organisms responsible for food deterioration while retaining the original sensory properties and thermolabile nutrients of the raw food materials, as either no heat or mild heat is used during processing [6]. A number of papers have reported microbial inactivation and the improved quality of food by HHP [7–9].

HHP treatment at 100 to 700 MPa can change the structure of starch, like gelatinization, which enables improved enzymatic digestion [10]. And, certain enzymes such as amylases remain active even at 600 MPa [11]. These findings suggest that HHP treatment can non-thermally cause the “pressure gelatinization of starch” and result in the enzymatic saccharification of starch in the tuberous root of sweet potato. However, to date there have been no reports of the application of HHP treatment on food materials for the pressure gelatinization of starch and subsequent saccharification, and our knowledge of the effect of HHP on *in situ* gelatinization and saccharification of starch in food materials remains limited.

In this study, we analyzed the effect of HHP treatment on sugars in the tuberous root of sweet potato. Tuberous roots were subjected to HHP treatment at 100 to 500 MPa while heating at 25 to 80 °C, then the produced sugars were analyzed and the structures of the starch granules were visualized using microscopy.

2. Materials and methods

2.1. Tuberous root of sweet potato

Tuberous root of sweet potato (*Ipomoea batatas* cultivar Beni-azuma) harvested in Ibaraki prefecture, Japan, was purchased from a local

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http://dx.doi.org/10.1016/j.bpc.2017.04.012
Received 1 January 2017; Received in revised form 29 April 2017; Accepted 29 April 2017

market in Niigata City and used for all experiments.

2.2. Thermal treatment under atmospheric pressure

A tuberous root sample was cut into blocks of approximately 10 g each. Each block was vacuum-sealed in a polyethylene-polyamide nylon pouch (Magic-Vac, Flaem Nuova, San Martino della Battaglia, Italy) using a vacuum packing machine (DZ-400T, Hualian Machinery Group, Wenzhou, China). The vacuum-sealed samples were incubated at 60, 70 or 80 °C under atmospheric pressure for 60 min by immersion in a water bath (Thermal Robot TR-2AR, AS One, Osaka, Japan), or incubated at 100 °C for 20 min by immersion in boiling water. The treated sample blocks were stored at − 20 °C until use for the preparation of crude extracts.

2.3. HHP treatment

The vacuum-sealed samples were subjected to HHP treatment (up to 500 MPa) using a high pressure Servo Pressure 500 apparatus (model HPV-50C20-S; Sugino Machine, Uozu, Japan) with a pressure limit of 500 MPa. The inner diameter of the high pressure vessel was 45 mm and the depth was 100 mm. The pressure increase rate was between 1.9 and 2.2 MPa/s. HHP treatment at 600 MPa was achieved using another high pressure apparatus (Kobe Steel, Kobe, Japan) with a pressure limit of 686 MPa. The inner diameter of this high pressure vessel was 65 mm and the depth was 200 mm. For both high pressure apparatuses, water was used as the medium to transmit the pressure to the samples. The sample blocks were treated for 10 min at 100 MPa (at 60, 70 and 80 °C), 200 MPa (25, 60, 70 and 80 °C), 300 MPa (60, 70 and 80 °C), 400 MPa (25, 60, 70 and 80 °C), 500 MPa (60, 70 and 80 °C), or 600 MPa (at 25 °C). The treated sample blocks were stored at − 20 °C until use for the preparation of crude extracts.

2.4. Crude extract preparation

Each thermally or HHP treated sample block was cut into small pieces. A 3 g portion of the sample was added to 30 ml distilled water and homogenized on ice at 12,000 rpm twice for 3 min using an excel-auto homogenizer (model ED-12, Nihon Seiki, Tokyo, Japan). The supernatant was separated from the insoluble material by centrifugation at 890 × *g* (4 °C) for 10 min using a refrigerated centrifuge (model 6200, Kubota, Tokyo, Japan), then the supernatant was further separated from insolubles by centrifugation at 20,400 × *g* (4 °C) for 10 min using a refrigerated micro centrifuge (model MX-301, Tomy Seiko, Tokyo, Japan).

2.5. Analytical methods

The reducing sugar concentration in each crude extract was measured according to the Somogyi-Nelson method [12]. A 150 µl aliquot of the crude extract was mixed with 150 µl of Somogyi's copper reagent (Wako Pure Chemical Industries, Osaka, Japan), heated in boiling water for 10 min, then cooled on ice. To the mixture, 300 µl of Nelson's reagent (Wako Pure Chemical Industries) was added and mixed, then 1800 µl of distilled water was added and the sample was mixed. The color was allowed to develop for 15 min and the absorbance at 500 nm (A_{500}) was measured. Maltose was used as a standard and the reducing sugar concentration in the crude extract was calculated in terms of maltose equivalents.

The sugars in the crude extract were analyzed by high-performance liquid chromatography (HPLC) according to our previous report [13]. An assembled HPLC system used in this study consisted as follows: a degasser (DG 1580-53, Jasco, Tokyo, Japan), a low gradient unit (LG 2080-02, Jasco), an HPLC pump (PU 2080 Plus, Jasco), an auto injector (SIL-20A, Shimadzu, Kyoto, Japan), a system controller (SCL-$10AP_{VP}$, Shimadzu) and a column oven (CTO-$10AC_{VP}$, Shimadzu), equipped with an Inertsil NH2 (5 µm) column (4.6 mm × 250 mm; GL Sciences, Tokyo, Japan) temperature-controlled at 35 °C. Acetonitrile (75% v/v) in distilled water was used as the mobile phase and compounds were eluted isocratically using a flow rate of 1 ml/min. The load volume was 20 µl. A refractive index detector (RID-10A, Shimadzu) was used to detect sugars. Chromatograms were analyzed using Chromato-PRO software (Run Time Corporation, Tokyo, Japan).

2.6. Measurement of amylase activity

Crude extract (3 ml) was sealed in a polyethylene-polyamide nylon pouch (Magic-Vac, Flaem Nuova), then subjected to HHP treatment at 200, 400 and 600 MPa (25 °C) for 10 min. A 1 ml aliquot of each HHP-treated extract or untreated control was mixed with 3 ml of 2% (w/v) soluble starch solution and 2 ml of 0.1 M sodium acetate buffer (pH 5.5), incubated at 50 °C for 10 min, then heated in boiling water for 10 min to inactivate the enzymes. The concentration of reducing sugar in each sample was measured according to the Somogyi-Nelson method and amylase activity was calculated based on the amount of reducing sugar produced.

2.7. Microscopic observation

A section was sliced from each HHP-treated sample block and untreated control, then each section was placed on a slide glass and stained with iodine solution. The stained starch granules were visualized using a Nikon Eclipse 50i microscope (Nikon, Tokyo, Japan) equipped with a Nikon DS-Fi1 camera (Nikon).

3. Results

3.1. Pressure tolerance of the internal amylases in the tuberous root of sweet potato

The pressure tolerance of the internal amylases in tuberous root was evaluated. Crude extract was prepared by water extraction and subjected to HHP treatment at 200 MPa to 600 MPa (25 °C) for 10 min, or left untreated (control), then the amylase activity was assayed using soluble starch as the substrate (Fig. 1). Under atmospheric pressure (approximately 0.1 MPa), the activity of the amylases was approximately 13.5 µmol (tuber with peel) and 13.2 µmol (tuber without peel) of reducing sugar produced in 1 min by 1 g of sample.

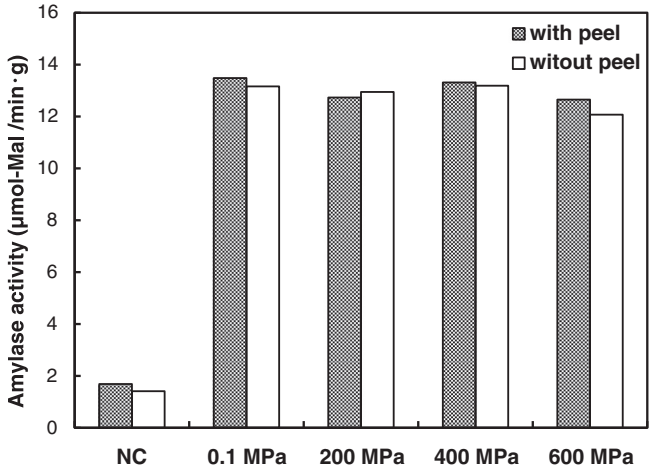

Fig. 1. Amylase activities of crude extracts from the tuberous root of sweet potato subjected to HHP treatment at 200, 400 and 600 MPa (25 °C) for 10 min. The crude extracts were prepared from tuberous root with peel (gray bars) or without peel (open bars). The amylase activities of crude extracts without HHP treatment are designated '0.1 MPa'. Amylase activities of boiled crude extracts are shown as negative control (NC). All values were average from duplicate experiments.