Food Research International 42 (2009) 1426–1433

Contents lists available at ScienceDirect

Food Research International

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

Properties of enzyme modified corn, rice and tapioca starches

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ARTICLE INFO

Article history:
Received 24 April 2009
Accepted 26 July 2009

Keywords:
Hydrolyzed starches
α-amylase
Enzyme modified starches
Corn starch
Rice starch
Tapioca starch
Dextrose equivalent
Spray drying

ABSTRACT

Corn, rice and tapioca starches were partially hydrolyzed by treating the starch dispersions with heat stable α-amylase. Dextrose equivalent (DE) of 8–12 was achieved by hydrolyzing the starch samples (10–20% w/v) for 30 min at 90 ± 2 °C. Scanning electron micrographs showed that starch granules had broken down to smaller particles. High performance liquid chromatography with refractive index detection indicated that oligosaccharides with broad molecular weight distributions are present in the reaction products. Hydrolyzed starch dispersions were analyzed for their rheological properties. The storage modulus values (G′) for 20% solid containing slurries were 7373 and 1470 Pa for untreated and enzyme treated samples, respectively, indicating a marked decrease in solid properties due to enzyme action. The complex viscosities (η^*) for native corn starch and hydrolyzed corn starch were 8243 and 1637 Pas, respectively, which indicate that the enzyme treatment decreases the overall resistance of the sample to flow such that the product can spread easily. Further 13C CP/MAS NMR and FTIR studies revealed the loss of ordered structures in the enzyme modified starches. Free flowing fat substitute in the form of fine powder was prepared by spray drying the hydrolyzed starch slurry.

1. Introduction

Starch degrading enzymes have been used to modify the physico-chemical properties of polysaccharides to achieve the desired functional properties. The most important tool in providing a saccharide with a specific composition is the use of starch hydrolyzing enzymes. Starches from various botanical origins differ slightly in amylose content, chain length distribution, molecular weight and the number of chain per cluster. It has been reported that wheat and corn maltodextrins with dextrose equivalent 2–3 could be prepared by heterogeneous bacterial α-amylase digestion (Mc Pherson & Seib, 1997). A fat mimetic maltodextrin was also produced by heterogeneous hydrolysis of potato starch by α-amylase (Richter, Schierbaum, Augustat, & Knoch, 1976). The three most widely used α-amylases are all isolated from Bacillus i.e. *B. amyloliquefaciens*, *B licheniformis*, and *B. stearothermophilus,* and differ with respect to the specificity by which they hydrolyze the $\alpha(1 \rightarrow 4)$ linkages in starch. Their temperature optimum is in the range of 60–90 °C and the pH optimum 6–7. The starch hydrolysis products are industrially produced by enzyme reactions from a dissolved solution of starch (upto 40% w/w). However, the concentration at which the hydrolysis reaction takes place influence the saccharide composition (Marchal, Beeftink, & Tramper, 1999). Industrially produced maltodextrins normally consist of a broad distribution of both linear and branched molecules. The objective of the present work is to study the hydrolysis of starch with enzymes at different conditions and characterize the products in terms of structural, functional and rheological aspects.

2. Materials and methods

2.1. Materials

Corn, tapioca and rice starches were procured from the local market of Mysore, India. Thermostable α-amylase from *Bacillus licheniformis* was procured from Hi Media Laboratories Pvt. Ltd., Mumbai, India. All other chemicals were procured locally and they were of analytical grade.

2.2. α-amylase assay

Alpha amylase activity was measured according to the method of Bernfeld (1955). One unit of activity is defined as the amount of enzyme that catalyzed the liberation of reducing sugar equivalent to one micro mole of maltose or glucose per minutes under assay conditions.

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doi:10.1016/j.foodres.2009.07.025

2.3. Enzyme hydrolysis

Corn, tapioca and rice starch dispersions of 10%, 15% and 20% solids (w/v; dry solid basis) were gelatinized in a steam jacketed kettle separately at 95 ± 2 °C for 15 min with moderate and continuous stirring. Gelatinized starch dispersions were partially hydrolyzed with heat stable α-amylase (1.33 IU/g starch) dissolved in 200 ppm calcium chloride solution, at 90 ± 2 °C for 30 min. The reaction was terminated by adjusting the pH to 3.0 with 1 N HCl. The hydrolyzed starch samples were neutralized to pH 6.5–7 with 1 N NaOH at 60 °C according to the method of Mc Pherson & Seib, 1997.

2.4. Dextrose equivalent (DE)

The degree of hydrolysis was measured as an increase in the content of reducing sugars. The results were compared to a calibration curve based on standard glucose (Miller, 1959). DE represents the percentage of hydrolysis of the glycosidic linkages present in starch. It was calculated using the following equation

$$\mathrm{DE} = 100 \times \left(\frac{\text{Reducing sugar, expressed as glucose}}{\text{Total carbohydrate}}\right)$$

2.5. Spray drying of partially hydrolyzed starches

Modified starch dispersions were dried in a spray drier (Model No. BE 1216, Bowen Engineering, NJ, UAS). The dispersion was suitably diluted to ensure proper atomization. Depending on the starch concentration, two to three fold dilution of dispersion was necessary for easy flow and uniform spray through the atomizer. Inlet and outlet temperatures were set at 165–170 °C and 100–105 °C, respectively. The resulting spray dried powder was white in colour and had a moisture content of about 1.5%. The powder was free flowing and hygroscopic.

2.6. Estimation of sugars

Total carbohydrate content of the starches and reducing sugar content of partially hydrolyzed starch samples, drawn at 5 min time intervals during enzymatic treatment, were assayed by phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and di-nitro salicylic acid method (Miller, 1959), respectively. The glucose released from partially hydrolyzed starches was quantified by comparing with calibration curve of standard glucose. Moisture and lipid contents of starch samples were determined by the AOAC methods (1975).

2.7. HPLC analysis

The free sugars from partially hydrolyzed starches were extracted with 70% aqueous ethanol, concentrated by rotary evaporation and quantified by HPLC (Model LC-10ATVP, Shimadzu, Japan) according to the method of Mc Ginnis & Fang, 1980. The column used was aminopropyl (Phenomenex, CA, USA). Isocratic elution was performed using acetonitrile-water (70:30, v/v) solvent system at a flow rate of 1 ml/min. The solvent was delivered using LC-10ATVP pump (Shimadzu, Japan). Refractive index detector (RID-10A, Shimadzu, Japan) was used to detect the sugars. Data signals were acquired and processed on a PC running the Class VP software (Shimadzu, Japan). Sugars were extrapolated from pure glucose, maltose and fructose standard curves. Twenty microliter injections were made in each run and peak areas were used for all calculations. Under the experimental conditions, glucose and fructose were eluted together due to their close retention times.

2.8. Determination of rheological parameters

A controlled stress rheometer (Model # RT 10, Haake, Karesruhe, Germany) was used to determine the dynamic oscillation properties and flow behavior of samples (starch slurries/dispersions). The parallel plate attachment was employed to determine the rheological behavior of these starch slurries. Their concentrations are 10%, 15% or 20% (dry solid basis). They were then mixed manually in a gentle manner by a glass rod for 5 min to have uniform sample. Latter, they were carefully loaded between 30 mm parallel plates with a gap of 1 mm, and excess sample was trimmed off. The starch samples used for rheological measurements are unmodified and enzyme modified corn starch slurries. A thin layer of paraffin oil was gently applied to the edge of the exposed sample to prevent loss of moisture. For dynamic oscillatory tests, the storage modulus (G′), and complex viscosity (η^*) were determined during a frequency sweep varying from 0.01 to 45 Hz at a constant stress of 25 Pa after determining the linear viscoelastic range employing stress sweep tests; all experiments were performed in the linear viscoelastic zone. The G′, and η^* values at an angular velocity of 6.28 radian (equivalent to a frequency of 1 Hz) were chosen for comparison of results. All tests, in triplicate, were conducted at a temperature of 25.0 ± 0.1 °C by employing a circulatory water bath, supplied by the rheometer manufacturer.

To determine the flow properties, all measurements were conducted at 25 ± 0.1 °C on duplicate samples. The flow curves were generated using a stress sweep from 50 to 500 Pa to generate 40 shear-rate/shear-stress data points. The yield stress was noted from the flow curve as the stress to initiate flow. Apparent viscosity was reported corresponding to a shear rate of 100 s^{-1}.

2.9. Structural studies by scanning electron microscope (SEM), solid-state NMR and FTIR

Spray dried partially hydrolyzed starch samples and their corresponding unhydrolysed samples were scanned at 500×–2000× magnification to observe the effect of cleavage of glycosidic bonds on the ultrastructure of starch particles due to enzyme treatment according to the method of French, 1984. Starch particles were coated with gold in a sputter coater and scanned in a scanning electron microscope (Model#435 VP, Leo Electron Microscopy, Cambridge, UK). Representative photomicrographs are presented for comparison among samples. Bruker made, 1997 DSX-300 MHz equipment was used for solid-state NMR (13 C Cross polarization Magic Angle spinning experiment) according to the method of Gidley & Bociek, 1985. FTIR spectra of unmodified and enzyme modified starches were obtained on FTIR spectrophotometer (Model # Nicolet 5700, Thermo Electron Corporation, USA). The dry starch powders were used to record the spectra in the transmission mode from 4000 to 400 cm^{-1} using deutrated triglycerine sulphate detector according to the method of Dupuy, Wojciechowski, Ta, Huvenne, & Legrand (1997). For each sample three spectra were recorded and computed single-beam spectra at 4 cm^{-1} resolution before Fourier transform.

3. Results and discussion

3.1. Enzymatic hydrolysis of starches

Moisture content of starches used for enzymatic hydrolysis ranged from 9.9% to 11.6% (d.b.), total carbohydrate from 77.6% to 83.2% and lipid content varied from 0.1% to 0.5%. A progres-