Journal of Food Composition and Analysis 22 (2009) 563–570

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original Article

Relationship between potato starch isolation methods and kinetic parameters of hydrolysis by free and immobilised α-amylase on alginate (from *Laminaria digitata* algae)

Djaffar Djabali [a,b,*], Naima Belhaneche [b], Boubekeur Nadjemi [a], Virginie Dulong [c], Luc Picton [c]

[a] *Laboratoire sur les produits bioactifs et valorisation de la biomasse, Ecole Normale Supérieure, Département de chimie, BP 92 Kouba, Alger, Algeria*
[b] *Département de génie de l'environnement, Ecole Nationale Polytechnique, 10 Avenue Hacène Badi, El-Harrach, Alger, Algeria*
[c] *Laboratoire Polymères, Biopolymères, Membranes, CNRS UMR 6522, Université de Rouen, F-76821 Mont-Saint Aignan, France*

ARTICLE INFO

Article history:
Received 6 August 2008
Received in revised form 11 October 2008
Accepted 2 November 2008

Keywords:
Potato
Solanum tuberosum
Starch isolation
α-Amylase
Sodium alginate
Covalent immobilization
TOC/TON
Free and immobilised enzyme
Kinetic
Bisulphite and alkaline analysis
Food composition

ABSTRACT

The kinetic study of the hydrolysis of starch, isolated by two methods (bisulphite and alkaline) from potatoes cultivated in the south of Algeria, was carried out by α-amylase (from *Aspergillus oryzae*). The enzyme used is free and immobilised by covalent bonds on alginate in the presence of the 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (EDCI). The kinetics of hydrolysis of the extracted starches obeys the Michaelis–Menten model. The values of k_m in g L^{-1} and V_{max} in g L^{-1} min^{-1} for the starches isolated by the alkaline method are (40.7, 3.8) for free amylase and (70.8, 3.4) for the immobilised enzyme. For the starches extracted by the bisulphite method, the latter kinetic parameters values are (34.2, 2.8) for free amylase and (55.7, 1.7) for the immobilised enzyme. The results show that the kinetic parameters of the hydrolysis are dependent on the conditions of extraction and they are larger for the alkaline method for both the free and immobilised enzyme.

1. Introduction

Starch is a major product of many economically important crops, such as wheat, rice, maize, tapioca, and potato (Agrawal et al., 2005). Starch hydrolysates are the products of either partial or total depolymerization of starch; they are formed as a result of hydrolysis of starch into a low-molecule carbohydrate, taking place under the action of either acids or enzymes (Nebesny et al., 1998). Starch is hydrolysed to glucose, maltose and maltooligosaccharides by α-amylase and β-amylase and other related enzymes (Apar and Özbek, 2005; Fujii and Kawamura, 1985; Goni et al., 1997; Park and Rollings, 1994). Starch susceptibility to enzyme attacks is influenced by several factors, such as amylose and amylopectin content, crystalline structure, particle size and the presence of enzyme inhibitors (Zhang and Oates, 1999) and starch origins.

The results of several authors of the kinetics of hydrolysis of the starches by free amylase are in conformity with the Michaelis–Menten model (Fujii and Kawamura, 1985; Gorinstein, 1993; Karakatsanis and Liakoupoulou-Kiriakides, 1998; Park and Rollings, 1994; Sims and Cheryan, 1992), and the kinetic parameters found vary with the botanical origins of the starches. For the hydrolysis by immobilised amylase, the majority of the authors find an increase in k_m. Some authors find that V_{max} value decreases compared to the free enzyme, and they justify these results by the phenomena of diffusion and the structural changes of the immobilised enzyme (Hasirci et al., 2006; Kumar et al., 2006; Reshmi et al., 2007). Other authors find that the V_{max} values are larger, and that it is, in fact, a more active enzyme (Changa and Juang, 2005; Kara et al., 2005).

The present investigation was undertaken to study the relationship between potato starch susceptibility to free and immobilised α-amylase from *Aspergillus oryzae* on alginate with EDCI, and their two isolation methods; alkaline and bisulphite.

* Corresponding author at: Département de génie de l'environnement, Ecole Nationale Polytechnique, 10 Avenue Hacène Badi, El-Harrach, Alger, Algeria.
Tel.: +213 25420977; fax: +213 21287912.
E-mail address: djabalidjaafar@yahoo.fr (D. Djabali).

0889-1575/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2008.11.001

Table 1
Characteristics of alginates.

	Source	M/G	η[a] 1% (cps)	M_w[b] (g mol^{-1})	M_n[b] (g mol^{-1})
Alginate	*Laminaria digitata*	1.2	379	230,000	160,000

[a] Data given by SKW Biosystems.
[b] Measured by SEC/MALLS.

Potato (Spunta) used as a source of starch was successfully cultivated in Saharan (arid) region of south Algeria (El Oued), with specific soil and climatic conditions.

2. Materials and methods

2.1. Materials

Potatoes (*Solanum tuberosum*) of the Spunta variety grown in south Algeria were obtained. α-Amylase from *A. oryzae*, M = 51,200 g mol^{-1}, 31.8 U mg^{-1}, was purchased from Fluka (Seelze, Germany). Sodium alginate from *Laminaria digitata* algae (M/G = 1.2) was purchased from Degussa (France); the characteristics of alginate are given in Table 1. 1-Ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (EDCI) and glucose 99% were purchased from Sigma (Saint-Quentin Fallavier, France). All other chemicals of high purity were available commercially.

2.2. Starch isolation

2.2.1. Bisulphite method

Starch was isolated using modified procedures of Hung and Morita (2005), Pérez and coworkers (2001), Vasanthan and coworkers (1999), and Yang and Seib (1996). 200 g of potato was cleaned and steeped in bisulphite solution (pH 3) at 58 °C for 2 h, washed with distilled water three times, ground in a blender (IKA Labortechnic A10), filtered with distilled water through a 150 μm sieve, and finally centrifuged at 5500 tr/min for 10 min. After centrifugation, the protein layer was separated. The operations of centrifugation and protein layer separation were repeated until white starch was obtained. The starch was dried at 40 °C for 48 h.

2.2.2. Alkaline method

200 g of potato was cleaned and steeped in sodium hydroxide solution (0.30%, w/v) at 8 °C for 24 h (Beta et al., 2001; Beta and Corke, 2001), washed with distilled water three times, ground in a blender (IKA Labortechnic A10), filtered with distilled water through a 150 μm sieve and centrifuged at 5500 tr/min for 10 min. After centrifugation, the protein layer was separated. The operations of centrifugation and protein layer separation were repeated until white starch was obtained. The starch was dried at 40 °C for 48 h.

2.3. Total starch (TS) in starch extract and yield of extraction (R%)

The proportioning of the starch in extract was carried out by proportioning the glucose produced by enzymatic hydrolysis of the starch dissolved in a solution of KOH, using methods given by Hu et al. (2004), Goni et al. (1997) and Rosin et al. (2002). 50 mg of sample weighed with a precision 0.1 mg was dispersed in 6 mL of KOH (2 M) solution, shaken in a vortex, left for 1–2 h at ambient temperature until total dissolution of the starch, and then neutralised with acetic acid. 3 mL of a buffer solution of sodium acetate 0.4 M (pH 4.75) and 1 mL of the enzyme amyloglucosidase (300 U/mL, Sigma, A-7255) were added, and the mixture was introduced into a thermostated bath (60 °C) for 45 min with occasional shaking. The reactional mixture was diluted in a flask of 50 mL with distilled water. 1 mL of the obtained solution was diluted to 10 mL with distilled water to obtain a glucose concentration lower than 100 μg/mL. The glucose produced was proportioned by the glucose oxidase–peroxidase method, and the concentration of starch was obtained by multiplying the concentration of glucose by 0.9.

The yield of extraction by dry weight of potato is calculated using Eq. (1):

$$R\% = \frac{m(1 - (H\%/100))\mathrm{TS}\%}{50}, \quad (1)$$

where m is the mass of starch extract in g; H% is the starch extract moisture; 50 is a dry mass (in g) of 200 g of potato (moisture of potato is equal to 75%); and TS is the total starch in starch extract.

2.4. Covalent immobilization of enzyme

Na-alginate (M/G = 1.2, 18 g L^{-1}) was dissolved in acetate buffer (pH 4.7). After complete dissolution, EDCI was added under vigorous stirring. The molar ratio of EDCI over Na-alginate (the molar mass of anhydroglucose unit of alginate was used for the calculation of molar ratio) was 0.5. At the end, enzyme was added (the concentration of enzyme in solution is 2 g L^{-1}). The above solution was incubated at 25 °C for 24 h. 0.5 M of NaCl was added at the end of the reaction to eliminate adsorbed enzymes. The reaction products were purified by ultrafiltration through polyether sulfone membrane (cutoff 100,000 g mol^{-1}) under nitrogen at 0.6–0.8 bars for 5 days (ultrafiltration was stopped when the washing Milli-Q water conductivity was low, constant and nearly equal to pure Milli-Q water conductivity) and freeze-dried.

2.5. Characterization of immobilised enzyme

Total organic carbon and total organic nitrogen (TOC/TON) were used to get information about grafted alginate and enzyme covalent immobilization.

2.5.1. Total organic carbon and total organic nitrogen

TOC and TON were measured by Shimadzu TOC-VCSH/CSN analyser. TOC/TON analysis of purified enzyme (dialysed through dialysis membrane D = 25.5 mm, V/L = 5.1 mL/cm, cut off 6000–8000 g mol^{-1} and freeze-dried), of ultrafiltered and freeze-dried Na-alginates before and after immobilization was performed. The differences between the values of total organic carbon and total organic nitrogen of the all analysed products were used to confirm the enzyme immobilization on alginate and calculate the immobilization efficiency.

2.5.2. Immobilization efficiency

The nitrogen concentration in alginate is very small; therefore, we consider the enzyme and EDCI ($C_8H_{17}N_3$) as the only sources of nitrogen in grafted alginate (gr.alg). The calculation of the immobilization efficiency of the enzyme on alginate is based on the calculation of the amounts of nitrogen and carbon in grafted alginate by TOC/TON. As the EDCI can form stable bonds with alginate by rearrangement of activated alginate of the O-acylurea form to the N-acylurea form (Nakajima and Ikada, 1995), a preliminary study of activated alginate with EDCI was carried out.

From Eq. (2), the ratio of carbon on nitrogen in activated alginate is $(T_C/T_N)_{ac.alg}$ ultrafiltered and freeze-dried, allows the