Food Hydrocolloids 63 (2017) 265–272

Contents lists available at ScienceDirect

Food Hydrocolloids

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

A response surface analysis of the aqueous leaching of amylose from maize starch

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ARTICLE INFO

Article history:
Received 6 June 2016
Received in revised form
3 September 2016
Accepted 6 September 2016
Available online 9 September 2016

Keywords:
Amylose leaching
Response surface
Leaching temperature
Starch concentration

Chemical compounds:
Amylose (PubChem CID: 53477771)
Amylopectin (PubChem CID: 439207)
Maize starch (PubChem CID: 24836924)
Water (PubChem CID: 962)

ABSTRACT

A response surface analysis with a face centered central composite design was implemented to study the effect of maize (*Zea mays* L.) starch concentration (3.0–7.0% w/v) and leaching temperature (LT, 70–90 °C) on aqueous leaching of amylose (AM) as a way to optimize the conditions for obtaining the highest yield of long chain AM [number average degree of polymerization (DP_n) ranging between 860 and 930] and highest purity. Second order empirical models were fitted via the least squares approach. Negligible terms were removed using backwards model reduction. Negligible lack of fit terms were obtained for the responses total leached carbohydrate and DP_n. The optimization was complemented with a desirability test using the purity of the extracts. As targets for the optimization, maximum leachate yields, $DP_n \approx 900$, and purity >95% were set. The reported contour plots and prediction profilers can be used for tailor made production of leachates. Temperature had the most significant main effect as yields and DP_n increased with temperature at the expense of purity. Purity was highly compromised when treatments were above 85 °C. This was reflected in the high DP_n values (>1500) which suggested the presence of amylopectin material. When using 3.0% (w/v) maize starch suspension at an LT of 81 °C, the largest yield (15.0% of starch) of high DP_n AM chains ($DP_n \sim 900$) and less than 3.3% of non-AM material were obtained.

1. Introduction

Starch is a main source of energy in the human diet (Copeland, Blazek, Salman, & Tang, 2009; Jéquier, 1994). It occurs as granules in cereals, tubers, and legumes where it serves as reserve polysaccharide. It is mainly composed of two glucose polymers: the highly branched amylopectin (AP) and the almost linear amylose (AM) (Delcour & Hoseney, 2010). Starch has a semicrystalline character and thus has both crystalline and amorphous regions. In the crystalline region, AP outer chains reside as clustered double helices. The amorphous regions consist of the branching points of AP and most of the AM chains (Jenkins & Donald, 1995). It has been suggested that some AM chains can be oriented in such way that they disrupt some of the double helices in the crystalline regions (Yuryev et al., 2004).

A common way to fractionate starch into AM and AP is by aqueous leaching. In this technique, a starch suspension is heated in excess water to a temperature above the gelatinization temperature in a way which avoids AP solubilization and does not compromise granular integrity (Doblado-Maldonado, Gomand, Goderis, & Delcour, 2015). At room temperature already, water acts as a plasticizer and makes the granules grow somewhat in size. During subsequent heating, the granules swell further. The mobility of AM in the amorphous regions increases and it starts to leach out of the granules. AP behaves differently. Its crystals melt during the heating process but they remain in the core of the granule remnants. The resulting aqueous and gel phases are further separated with simple procedures such as centrifugation (Banks, Greenwood,

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http://dx.doi.org/10.1016/j.foodhyd.2016.09.006

& Muir, 1991; Shi, Seib, & Lu, 1991).

The conditions (*e.g.* temperature, concentration, starch botanical source, extent of crystal melting) can influence the properties (*e.g.*, chain length, purity, polydispersity) of the leached AM [as previously reviewed by Doblado-Maldonado et al. (2015)]. All these parameters can be handled to tailor the characteristics of the isolated AM. Shi et al. (1991) studied the effect of starch concentration and leaching temperature (LT) on leaching of AM from maize and wheat starches. However, their study was limited to determining yields and provided neither the purity nor the average degree of polymerization (DP) of the leachates. Moreover, the experimental study included the effect of maize and wheat starch concentration at only one LT (i.e., 95 °C). The optimal temperature for leaching was not determined and no evidence was provided on the possible interaction between starch concentration and the LT.

The rationale for optimizing starch fractionation methodologies resides in the high price of AM which has incentivized researchers to explore and/or improve laboratory approaches. The present study was designed to determine the most suitable starch concentration and LT for efficiently isolating AM from maize starch (MS) in terms of yield, DP and purity. These two factors were varied and their combined effect on the properties of AM leachates was assessed. To achieve this, optimization via response surface methodology (RSM) was applied to determine the properties of the leachates based on selected treatment combinations in terms of starch concentration and LT. RSM is a statistical approach used to develop and/or optimize processes or products (Myers, Montgomery, & Anderson-Cook, 2009). Its advantage over that of other factorial methodologies resides in the flexibility in the collection of data. More in particular, based on a certain set of experiments, an empirical model is fitted to predict full data behavior over the experimental range given by the boundaries of the selected factors (Myers et al., 2009). RSM approaches have been extensively applied to starch-related systems since they allow determining the optimal conditions depending on the desired target (i.e., maximal, minimal, equal to …). Processes including enzymatic hydrolysis (Göksungur, Uzunogullari, & Dagbagli, 2011), gelatinization (El-Dash, Gonzales, & Ciol, 1983), and annealing (Ozcan & Jackson, 2003) of different starches have previously been analyzed via RSM. Industrial manufacture of starch-based products, ethanol (Ratnam, Rao, Rao, Rao, & Ayyanna, 2003), glucose (Kunamneni & Singh, 2005) and biomaterials (Galicia-García et al., 2012; Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013) from starch have also been optimized using RSM.

2. Materials and methods

2.1. Samples

Maize (*Zea mays* L.) starch was obtained from Tereos Syral (Aalst, Belgium). MS accounts for 80% of the worldwide starch production (Waterschoot, Gomand, Fierens, & Delcour, 2015c). Moisture content was 11.0% as determined in triplicate using an air-oven method [approved method 44–19.01; (AACCInternational, 2013)]. AM content for this MS sample was 28.0% as determined in triplicate with an iodine binding procedure (Kaufman, Wilson, Bean, Herald, & Shi, 2015). All used reagents, solvents, and chemicals were of at least analytical grade and obtained from Sigma-Aldrich (Bornem, Belgium) unless indicated otherwise.

2.2. Experimental design

A face centered central composite design was implemented as a basis to fit an adequate response surface model. The two factors were starch dry matter (dm) concentration and LT. Three center points, four axial and four factorial points were included. Factor boundaries (starch concentration: 3.0–5.0%, LT: 70–90 °C) were selected based on previous reports on AM aqueous leaching. To the best of our knowledge, only Shi et al. (1991) have studied the effect of starch concentration. They reported that increasing MS concentrations between 0.5 and 4.5% decreased the yield of AM leached at 95 °C. They obtained the highest yield (about 0.25 g leachate in 60 ml dispersion) when using concentrations ranging from 1.5 to 3.0%. However, the authors did not study the effect of concentration at lower LTs (*e.g.*, within the range of AP crystal melting) which may provide extracts of higher purity (Doblado-Maldonado, Gomand, Goderis, & Delcour, 2016). AM leaching takes place when starch granules gelatinize. When exceeding 95% melting, the purity is highly compromised (Doblado-Maldonado et al., 2016). With the above in mind, LTs were here selected within or nearby the range of melting of MS AP crystals to ensure that at least partial gelatinization took place. The selected LTs range from 70 to 90 °C. The lower boundary suffices for partial melting and some AM leaching and at the upper temperature full gelatinization is attained. AM purity was also assessed as a response in the optimization. Main and interaction effects between the two factors were statistically assessed. As responses, the yield, the number average DP (DP_n), and the purity of the AM extract were selected. Each design point was executed in triplicate and the empirical models for the responses were fitted using JMP Pro 11 software (SAS Institute, Cary, NC, USA). Optimization was executed taking into account the obtained models for the responses. The optimized conditions were selected as those that satisfied maximal overall desirability (i.e, highest yield and purity of AM chains with a DP_n of at least 900) [DP_n of AM from MS is 830–960 according to Waterschoot et al. (2015c) and Tester, Karkalas, and Qi (2004)].

2.3. Aqueous leaching of amylose

Aqueous leaching at 70, 80 or 90 °C was carried on for 60 min with mild shaking (50–60 strokes per min) using an aqueous dispersion (30 ml) of 3.0, 4.0 or 7.0% dm native MS in centrifuge tubes (50 ml). In order to prevent oxidation, the headspace of each tube was flushed with nitrogen gas for 10 min before closing. After leaching, the dispersion was cooled by placing the tubes in cold water for 5 min. The gel and aqueous phases were then separated by centrifugation (16,000 *g*, 20 min) and supernatants separated from the gel phase rich in AP and granule remnants.

2.4. Swelling behavior

The close packing concentration (C^*) and the swelling power (SP) of MS were determined as in Eerlingen, Jacobs, Block, and Delcour (1997) with slight modifications. C^* is the concentration at which the swollen granules fill up the space in the suspension (Eerlingen et al., 1997) whereas SP refers to the water holding capacity of starches which itself is corrected for carbohydrate leaching (Leach, Mccowen, & Schoch, 1959). After centrifugation, the gels obtained in the aqueous leaching procedure (*cf.* Section 2.3) were weighed and the supernatants analyzed for total leached carbohydrate (CHL). C^* (%) and SP (g/g) were calculated using Eqs. (1) and (2), respectively. SP takes the presence of interstitial liquid between granules into account (Evans & Haisman, 1980). Swelling data were analyzed using a two factor ANOVA and post hoc tests were applied after a positive omnibus test. Statistical significance was defined at $P < 0.05$.