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Starch isolation from chestnut and acorn flours through alkaline and enzymatic methods

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ABSTRACT

Two different procedures were used to isolate chestnut (*Castanea sativa* Mill.) and acorn (*Quercus suber*) starch. Starch was extracted from the flour of these dried fruits by: (i) low shear at alkaline pH and successively using three sieves (A3S) at different centrifugation conditions (velocity and time) and (ii) enzymatic treatment at low shear (ENZ) at a different amount/digestion time of protease. In both cases a Central Composite Rotatable Design (CCRD) was used as an experimental design. Results were treated through the Response Surface Methodology (RSM). Based on maximum yield values and the degree of purity, the best modified conditions encountered were applied in a new trial and mass balance was established for both isolation procedures. The best centrifugation conditions encountered for the A3S isolation method were $800 \times g$ during 15 min, and for the ENZ method 900 units of protease despite the incubation time, for the same centrifugation terms. The yields reached with these experimental conditions for the A3S and ENZ methods were 83.9% and 79.9% for chestnut and 88.5% and 86.9% for acorn, respectively. Isolated starches shown to be higher in purity, presented values of 98.3% and 96.3% for chestnut and 98.1% and 97.6% for acorn, respectively for the A3S and ENZ methods.

Keywords: Chestnut; Acorn; Starch; Isolation method; Yield; Composition

1. Introduction

Starch is a relatively cheap raw material with physical and chemical properties that have multiple uses in many food and non-food applications. *Castanea sativa* Mill. var Longal and *Quercus suber* are rich starch materials, with a starch content of $32.3 \pm 0.9\%$ and $31.4 \pm 0.5\%$, respectively when fruits, without shell, were dried at 60 °C, according to Correia et al. (2009a,b).

Starch can be extracted using different processes, depending on the plant source and end use of the starch. Besides, the yield in isolated starch in order to be economically viable must be accomplished without significant modification to the starch granules. Extraction procedures affect both the chemical composition and physical properties of starch. These changes in starch properties, and even in starch granule structure, resulting from the extraction procedure, are a reflection of the nonrigid organisation of starch granules (Sing et al., 1997). In a previous study, four different methods for chestnut and acorn starch isolation (Correia and Beirão-da-Costa, 2010) were tested, based on the simplicity, efficiency, time consumption and use of low hazardous chemicals. Those methods are based on low/high shear at alkaline pH and enzymatic treatment at low shear, followed by centrifugation and sieving. Three of them could be classified as physicochemical methods. In these methods a first step included a treatment with water or alkaline solution at a low or high shear. The alkaline treatment at a low shear showed the best results. Another tested method with good physicochemical and functional properties, but not as good as the alkaline method, was an enzymatic method at a low shear. So, these two methods (later designated as original methods) were found to be the most promising, producing high yields, pure starch and high consistency of starch pastes, along with a less damaged structure of the starch granules. The centrifugation conditions used in both methods were $1400 \times g$ during 20 min. Centrifugation is an important factor since it is used, even in industrial scale starch extraction, for separation of starch from other components, mainly fat and protein (Liu, 2005; Andersson et al.,

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2001). In fact, this parameter has a high range of applied values, since $1000 \times g$ until $10{,}000 \times g$ of speed and different centrifugation times, 10–30 min (Conforti and Lupano, 2007; Demiate et al., 2001; Lan et al., 2008; Lim et al., 1992; Perez et al., 1993; Zhao and Whistler, 1994), which can influence starch characteristics (Lim et al., 1992). Some times, in early stages of separation/purification starch processes, chemical and enzymatic treatments may be applied to make the starch isolation easier from other compounds and these could influence starch properties (Seetharaman and White, 2004). In the above mentioned isolation methods, a treatment with 0.25 M NaOH at 5 °C during 24 h was applied for A3S method, and for the ENZ method was used 600 unit of protease with a digestion time of 6 h (Correia and Beirão-da-Costa, 2010). There are other methods that applied different alkali concentrations and soaked times, and for the enzymatic treatment the type of enzymes, quantity and incubation time follow the same tendency (Borght et al., 2005; Lee et al., 2007; Lim et al., 1992; Radosavljevic et al., 1998). Consider that these steps could influence starch yield and purity and consequently starch characteristics, some deeper study must be done in order to find the better conditions for starch extraction from raw materials.

In order to optimise these isolation conditions, a Central Composite Rotatable Design (CCRD) of the experiment was used. For example, this optimisation step was carried out using the Response Surface Methodology (RSM). This technique is a collection of mathematical and statistical techniques, that are useful for modelling and analysis in applications where a response of interest is influenced by several variables, and the objective is to optimise this response (Montgomery, 1996). This is a powerful technique for testing multiple process variables because fewer experimental trials are used, when compared with the study of one variable at a time. Interactions between variables can also be identified and quantified (Kristo et al., 2003). This is probably the most widely used and successful optimisation technique based on designed experiments (Bas and Boyachy, 2007).

The present study reports the optimization of the two mentioned isolation methods, alkaline and enzymatic methods, in order to enhance a higher yield and purity of chestnut and acorn starches, resulting in economic profits. Consequently by improving conditions we expect to reduce the extraction time and damaged starch for a potential use in food and non-food industry.

2. Materials and methods

2.1. Materials

Chestnut (*C. sativa*) var. Longal fruits were collected from Soutos da Lapa a Protected Designation of Origin region, Portugal. Acorns from *Q. suber* were collected in Idanha-a-Nova region, Portugal. Three sets of 1 kg each of chestnuts and acorns fruits were randomly harvested at the maturity stage. Samples were stored in dark conditions at 4 °C until experiments began. Before milling, fruits were prepared as follow: (1) pre-dehydration at 40 °C/24 h in a FD 115 Binder ventilated drying chamber (with an air flow of 300 m^3/h), (2) peeling and chopping into little pieces, (3) drying in the same equipment at 60 °C (before starch granules achieved the gelatinisation temperature, and the best encountered drying temperature (Correia et al., 2009a,b), for a final a_w value of 0.2 ± 0.05, with a moisture content of 8 ± 1%. Dried fruit fragments were then milled in a SK 100 Cross Beater Retsch hammer mill to pass a 1 mm sieve.

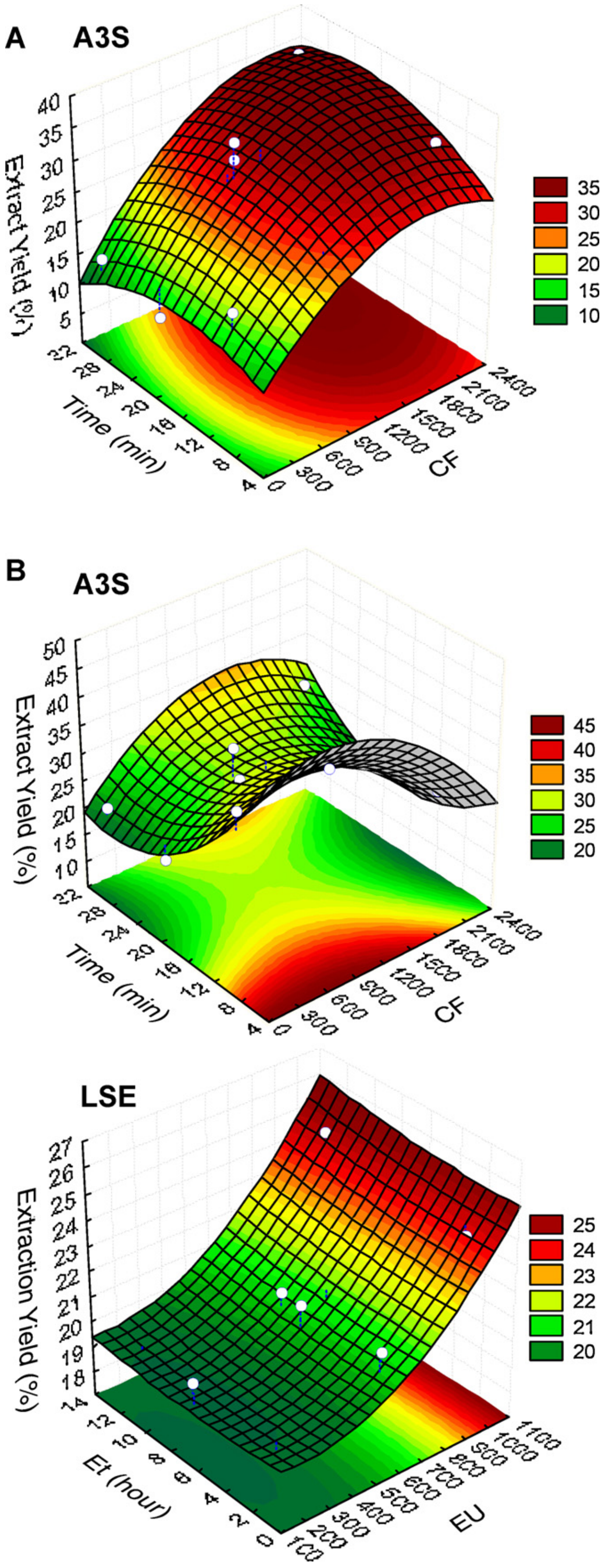

Fig. 1 – Extraction yield response surfaces for A3S and ENZ isolation methods for chestnut (A) and acorn (B).