THE WETTABILITY AND SURFACE FREE ENERGY OF ACORN STARCH GELS ISOLATED BY ALKALINE AND ENZYMATIC METHODS

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Abstract

Wettability may be a convenient parameter providing information on surface properties of starch gels surface. In food technology, a better understanding of the surface properties of gels can be useful in developing new food products and food components. The gelatinized starch has the property of forming gels, and when it is dehydrated, it forms a hard, transparent, bright, and resistant film. Starch biofilms are produced from gelatinization and after retrogradation of starch. This work aimed to study acorn starch gels wettability and their surface free energy, as well as the colour.

Quercus suber and *Quercus rotundifolia* fruits were dry and flour were produced. Starch was isolated from acorn flours by an alkaline and enzymatic laboratory scale methods. Starch gels films were prepared with starch suspensions of 1.2 and 3% from the isolated acorn starches. Contact angles were measured on dry starch gel films. Colour of films was measured using a colorimeter and the classification by CIELAB system.

The isolation method influenced the wettability of the acorn starch films form heated 3% starch suspensions. The acorn films presented high contact angles, meaning that films are hydrophobic, with less water affinity, for both starch isolation method, between 69.9–74.3° for *Q. rotundifólia* and 68.6–70.2° for *Q. suber*. The surface energy was different for acorn species and isolation methods, and generally it is low, which means that acorn starch presented high intramolecular interactions forces. Films also presented similar transparency and were yellow-brown in colour. Acorn starch films isolated through enzymatic method were browner.

Keywords acorn fruits, starch, films, isolation methods, contact angle.

Introduction

Acorn fruits from *Quercus suber* (QS) and *Quercus rotundifolia* (QR) are important forestry resources in the Central and Southern regions of Portugal, but they can also be important in Greece, Italy and France (Grove, Rackham, 2001). Most of the fruit production goes to animal feeding, mainly of Iberic pigs. These fruits are also consumed in other European countries (Rakic et al., 2006), and there are many different kinds of commercially available processed acorn products, including breads, cakes, soups, snacks, noodles and jelly, which are comprised principally of acorn flours (Kim, Yoo, 2009).

Starch as a natural component, contributes to the characteristic properties of food products such as texture, viscosity, gel formation, adhesion, binding, moisture retention, film formation and product homogeneity in different products like sauces, puddings, confectionary, and a variety of low-fat products. Starch is the main component of acorn flour, 31.4% (Correia, Beirão-da-Costa, 2012), suggesting that these fruits can be a good alternative to conventional starch resources, such as cereals and tubers. However, the properties of starches are not only dependent on the starch source but also highly dependent on the history of the starch itself (Wichmann et al., 2007) as for instance the extraction procedures. It is known that extraction procedures affect both the chemical composition and physical properties of starch, which justify the interest of studying the most suitable one for each individual raw material.

Many uses of starch involve heating it in water, which leads to granule hydration, swelling, and at a high enough temperature, solubilisation of starch molecules. The most important starch properties, with respect to its utilisation, are often related to the properties of gelatinized pastes. The gelatinized starch has the property of forming gels, and when it is dehydrated, it forms a hard, transparent, bright, and resistant film. Starch biofilms are produced from gelatinisation and after retrogradation of starch.

Wettability may be a convenient parameter providing information on surface properties of starch gels`surface. The wettability of a solid surface can be determined by a relatively simple method, measuring the so-called contact angle (Adamson, 1990). The study of starch gels`wettability and their surface free energy may be useful for food science and pharmacology fields. In food technology, a better understanding of the surface properties of gels can be useful in developing new food products and food components. In pharmacology, wettability can help to explain the drug delivery systems and mucosa adhesion (Bialopiotrowicz, 2003). Furthermore, an intense search for new renewable sources to produce edible and biodegradable materials is observed. Edible and biodegradable natural polymer films offer alternative packaging with lower environmental costs. The main renewable and natural biopolymer films are obtained from polysaccharides, lipids and proteins. Furthermore, starch is considered one of the most promising raw materials for developing biodegradable plastic to reduce the environmental impact of plastic wastes, especially from packaging (SinhaRay, Bousmina, 2005).

The objective of this work was to study acorn starch films colour characteristics, their wettability and their surface free energy, important parameters for food and non-food industries.

Materials and Methods

Sampling

Quercus suber Lam. (QS) and *Quercus rotundifolia* Lam. (QR) acorns were collected in "montados", located in Idanha-a-Nova (Centre East of Portugal). The acorns were harvested at full maturity. Three sets of 2 kg were randomly collected for each species. Preparation of acorn fruits was performed as described by Correia and Beirão-da-Costa (2012).

Starch isolation methods

Starch was isolated similarly as from sorghum flours following two methods (Correia, Beirão-da-Costa 2012):

- *Alkaline pH using successively three sieves (A)*

The flours (120 g) were soaked in 2 volumes of 0.25% NaOH at 5ºC for 24 hours. Suspensions were homogenised and screened through a 180 µm sieve. The procedure was then repeated twice. The precipitate was screened successively in 75 and 53 µm sieves. The mixture was centrifuged in a Universal 16 centrifuge (Hettich Zentrifugen Company, Germany) at 800 g 15 minutes, the mucilaginous layer was scraped away and the precipitate was then suspended in water. This last step was repeated twice. Isolated starches were dried for two days at 40 °C in a FD 115 Binder ventilated drying chamber (with an air flow of 300 m^3 per hour).

- *Enzymatic method (E)*

Protease from *Aspergillus oryzae* was purchased from Sigma Chemical Co. One unit of protease was defined as the amount of enzyme that liberated 1.0 µmol of tyrosine per minute from casein at pH 7.5 at 37 °C. The protease was added (900 units) to 120 g individual flours. Water was added (360 mL) and the slurry was adjusted to pH 7.5 (with 0.1 M NaOH or 0.1 M HCl). The slurry was incubated at 37 °C for a period of 2 hours. The slurry was then centrifuged in the same conditions as previously mentioned. The starch fraction was suspended, washed with water (200 mL) and filtered through a 53 µm sieve. The filtrate was centrifuged. The supernatant and tailings were discarded and the starch dried as reported above.

The yields and starch purities are shown in Table 1.

Table 1

Yield and purity of starches isolated from acorn flours through alkaline and enzymatic methods

Sample	Isolation method	Yield, %	Purity, %
QR	Alkaline	88.5 a	98.1 a
	Enzymatic	86.9 b	97.6 a
QS	Alkaline	87.6 a	98.0 a
	Enzymatic	85.4 b	97.8 a

Starch gels and preparation of their films

Starch gels and preparation of their films of 1, 2 and 3% were obtained from the isolated acorn starches, according to Silva et al. (2007). Two hundred milliliters of water suspension of a proper amount (e.g. 1, 2 and 3%) of dry starch powder were poured in a small (250 mL) stainless steel reactor vessel. The vessel was immersed in a thermostated bath at 85 °C and its content was continuously stirred mechanically. After reaching 85 °C, the heating continued during 40 minutes at continuous stirring. The obtained gel was cold, and 15 mL of gelatinised starch was placed in a plastic Petri dish, with 110 mm of diameter and 15 mm of thick. The Petri dish was placed in a dry oven with controlled temperature, at 25 °C for 18 hours (the time necessary to remove the excess of water).

Contact angle measurements

Contact angles were measured on dry starch gel films. The contact angle for MilliQ water (Millipore, Mosh-laim, France), formamide pure Fluka AG (Switzerland), ethylene glycol p.a. POCh Gliwice (Poland), and glycerol p.a. POCh Gliwice (Poland) was determined on acorn gel films. Contact angle measurements were carried out by using a contact angle instrument, Contact Angle System OCA20 (Dataphysics, Germany) with multiple portions, and the Sessile and Captive drop method was applied. A 0.1 mL droplet of water (or the other used liquids) was placed on the sheet surface and the image of the drop was captured by a CCD digital video camera. The contact angles were measured on both sides of the drop and averaged. Samples of 30 mm of length and 5 mm of width were applied on a glass surface using double-sided adhesive tape. At least 30 measurements were done for each experiment. The measurements were made at room temperature in a closed box.

Colour parameters of acorn starch films

The colour of all gel films was measured using a handheld tristimulus colorimeter (Chroma Meter-CR-400). The parameters measured were the brightness L*, which varies between 0 and 100 (from black to white, respectively), and the coordinates of opposed colour: a* and b*, which vary from –60 to +60, where the a* assumes negative values for green and positive values for red, while b* assumes negative values for blue and positive for yellow. From L* a* b*, the chroma (c*) and hue angle (h°) were determined. Chroma or saturation, c* (0–60), measures how dull / vivid the object colour is; hue angle, h° (0–360°), expresses the characteristic/dominant colour (0 – red / purple; 90 – yellow; 180 – bluish / green). Films colour measurements were taken by putting films on white paper. The total colour change (ΔE), was the parameter considered for the overall colour difference evaluation, between a sample and the reference fruit (without storage, designated with an index 0):

$$\Delta E = \sqrt{(L_0 * - L*)^2 + (a_0 * - a*)^2 + (b_0 * - b*)^2} \quad (1)$$

At least 30 measurements were done for each experiment.

Statistical analysis

The obtained results were subject to a one-way analysis of variance (ANOVA) test using the Statistic® vs 8 software. The separation of means or significant difference comparison of all parameters was tested by the Tukey's HSD test. The level of significance used for all the statistical tests was 95%.

Results and Discussion

The isolation methods lead to acorn starches with high purity and yields (Table 1), and the alkaline method was considered to be the better one, mainly because of the higher yields. Considering the experimental conditions for film formation, the 3% gel concentration was the only sample with good results, producing films without breakage. The films showed different colour characteristics depending on the starch source and the isolation method (Figure 1 and Table 1).

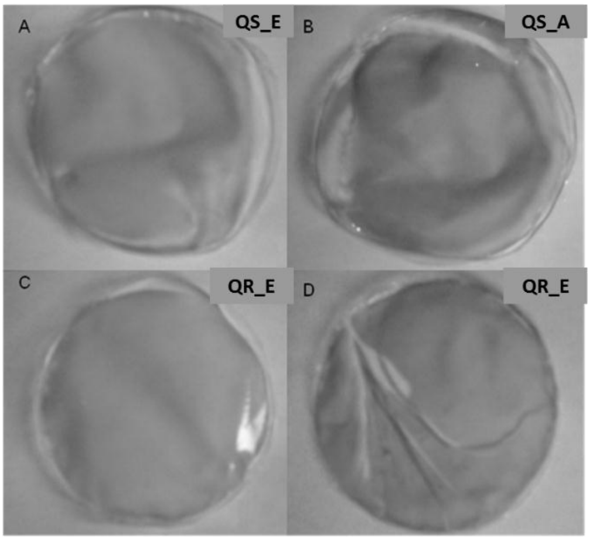

Figure 1. Appearance of acorn starch films(S)
A – Starch alkaline isolation method, E – Starch enzymatic isolation method

The QS_A film seen to be the most distinguished one (Figure 1). The colorimetric results showed that the starch isolation method influences the films colour characteristics, but in different ways. The acorn films presented high transparency and predominant yellow colour (Table 2). QS films presented high absolute values of a* parameter, meaning that they assumed green colour. The QR films formed from starch isolated through enzymatic method were more vivid than the others. The encountered colour difference might be classified according to Drlange (1994) as great (ΔE between 6.0 and 12.0). The results showed that either for the variety QS_A, which corroborate the image showed in Figure 1.

Table 2

Colour parameters of sorghum and corn films

Sample	L*	a*	b*	c*	h°	ΔE
QR_A	89.06 c	-0.35 b	8.25 c	8.25 c	92.42 c	11.51 c
QR_E	90.82 a	0.03 a	3.68 b	3.68 b	89.39 b	6.63 b
QS_A	87.07 b	-0.56 d	12.83 a	12.83 a	92.51 c	16.50 a
QS_E	89.00 c	-0.48 c	8.74 c	8.74 c	93.13 a	12.02 c

Means sharing the same letters in column are not significantly different from each other (Tukey's HSD test, p<0.05).

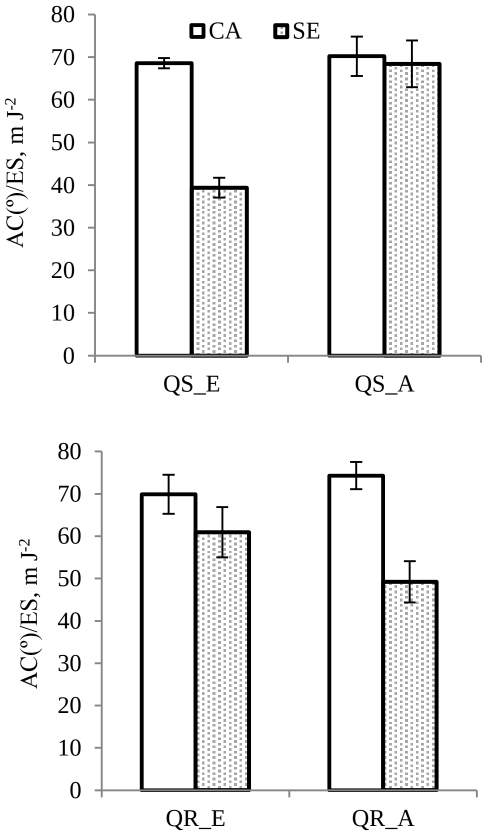

Figure 2. Contact angle (CA) and surface energy (SE) of acorn starch films
E–Enzymatic method, A–Alkaline method

The contact angle of the biofilms formed from sorghum starch presented higher values than commercial corn starch (46°) (Gonçalves et al., 2012), meaning that the acorn films surface is more hydrophobic, with less water affinity (Fig. 2). It could be noticed that the starch isolation method did not affect significantly this characteristic.

The surface energy was greatly influenced by the isolation method but differently and oppositely for QS and QR. Generally all the films presented low (or intermediate values of surface energy, when compared to other tested material, such as sorghum (38.0 and 40.7, respectively for alkaline and enzymatic isolation method) and commercial corn (90.0) starches (Gonçalves et al., 2012) which mean that acorn starches presented lower or intermediate intramolecular interactions forces. This characteristic is lower for QS_E and QR_A.

The results also showed that the interaction forces dispersed and polar part are quite different between species and between isolation methods. For all samples the polar part was high, with the exception of QS films form from starch isolated through enzymatic method (Fig. 3) and this could be related with the lower values for surface free energy (combination of dispersed and polar interactions forces). It was also noticed that for QS films produced from starch isolated from enzymatic method the dispersed component was higher.

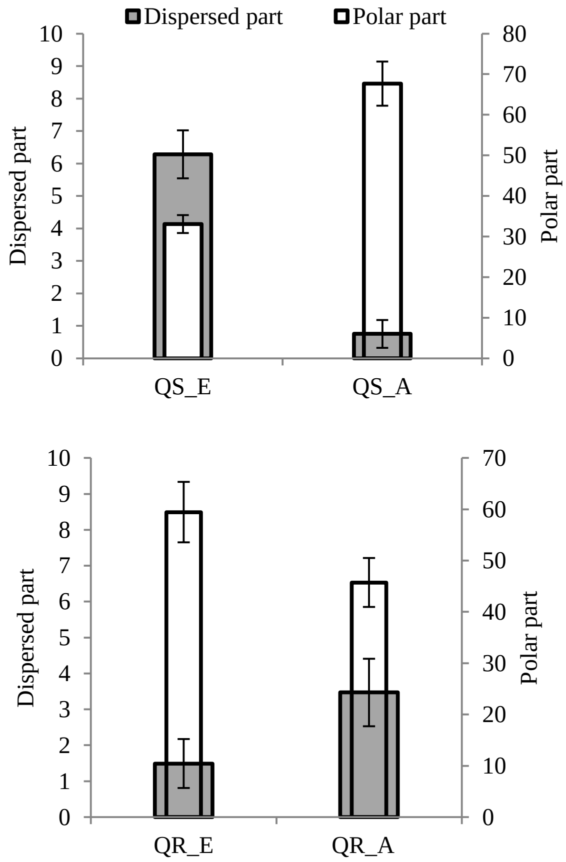

Figure 3. Surface energy of polar and dispersed parts of sorghum and corn starch films

E – Enzymatic method, A –Alkaline method

Conclusions

The results showed that acorn starch could form biodegradable films and appears as a low-cost renewable polymer.

Generally, the isolation method influenced the wettability and colour of the acorn starch films form heated 3% starch suspensions in different ways, and the studied characteristics of acorn films depend also on acorn species.

The acorn films presented high transparency, which is a good characteristic, with a predominant yellow colour, mainly in *Quercus suber* films formed with starch isolated by alkaline method. This yellow colour could influence the surface colour of the product.

Acorn films presented a hydrophobic surface with low water affinity. Moreover, the surface energy was low / intermediate which means that acorn starch presented weak intramolecular interaction forces.

For all samples the interaction force polar part was high, with the exception of QS films from starch isolated through enzymatic method, which could be related with the lower values for surface free energy.

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