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Proceeding Paper

Effect of Acid-Extrusion Cooking on Some Properties of Quinoa Starch †

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Abstract: According to the FAO, the economic potential of quinoa relies on the extraction and processing of its by-products. Starch in quinoa represents a major component. Although it has a limited application (due to its low solubility, high reactivity to hydrolysis or reactive hydroxyl groups), certain technological processes can modify or even improve the techno-functional or healthy properties. In this work, the effect of acid extrusion cooking on the molecular, chemical and morphological properties of quinoa starch was evaluated. A quinoa sub product from protein extraction (73% starch) was acid extruded (100 °C and 0, 10 and 40% of citric acid) and milled. Native (NS) and extruded (ES) samples were taken as the control. The resistant starch (RS) and free glucose (FG) content were measured though enzymatic methods. Molecular, structural and morphological characterization was assessed by infrared (IR) spectroscopy, particle size analysis by laser diffraction and optical microphotography. The results showed that an acid esterification at 40% caused a two-fold increase (1.10 g/100 starch dry basis) in the RS content, reduced the FG (mg/100 g db) from 801.36 (NS) to 368.56 and changed the IR spectrum due to the formation of new ester groups at a wavelength of 1712 cm^{-1} (carbonyl groups). Although, no significant differences were observed in the particle size distribution of the samples, microphotographs showed semi-crystalline structures (extruded and citrate starches) formed from native starch (starch aggregates). These data suggest that acid extrusion increased the RS content, formed citrate starch esters and changed the molecular and structural conformation of native quinoa starch. The evaluation of the additional properties would elucidate the effect of these changes on the bio and techno-functional properties.

Keywords: ester; extrusion; microestructure; quinoa; resistant starch; starch; glucose

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1. Introduction

According to the FAO, the potential of quinoa grains relies on procedures of extraction and the processing of quinoa by-products such as protein concentrates and starch, among others. Quinoa seeds have an important content of starch (55 to 60%, dry matter) which makes them appropriate as a carbohydrate source for processing and isolation [1]. Starch has food and non-food applications due to its versatile properties. It is a non-soluble biopolymer at room temperature, but instant starches have been prepared and developed to overcome these characteristics and make it even more useful for different applications [2]. From the nutrition point of view, starch not only provides the carbohydrates and energy necessary for human physiology, but it also contains starch forms that interact differently with the digestive system. Starch may be composed of rapidly digested starch, slowly digested starch and resistant starch (RS). Digestive enzymes do not hydrolyse RS and thus, they can be fermented in the colon and act as a source of dietary fibre. Among the four types of RS, RS4 is produced by chemical modifications and prevents the action of amylolytic

enzymes by blocking their access [3]. In the colon, RS serves as a carbohydrate source for microbial fermentation and the production of short-chain fatty acids, which protect the colon from colorectal cancer. Additionally, a daily intake of food containing RS reduces the caloric value of meals and helps control the release of glucose into the bloodstream, thus reducing the insulin response [4]. Processing technologies for increasing the RS in foods such as cooking, baking, autoclaving and extrusion affect starch gelatinization and the retrogradation phenomena in starch, affecting the crystalline structure [5]. Extrusion is an interesting technique for the production of various food products since it has various advantages, such as being a continuously versatile process, having short processing times, a high yield and it is energetically efficient. Additionally, it can be combined with other treatments, such as different types of hydrolysis with enzymes and acids to give other products [6].

Changes produced during the extrusion process can affect the various properties of starchy materials. Important relations were described between the structural changes and rheological properties such as the texture, water binding capacity and viscoelastic characteristics of some food matrixes [2]. The objective of this study was to evaluate the effect of acid extrusion cooking on the molecular, chemical and structural changes in starch from quinoa seeds.

2. Materials and Methods

2.1. Quinoa Starch

Quinoa starchy material was obtained according to the procedure described by Rueda et al. [7]. The starchy pellet separated after the alkali protein extraction was air-dried (12 h, 40 °C), milled (0.5 mm of mesh) (Kinematica PX-MFC 90D, Bohemia, NY, USA) and stored in sealed-packed bags for a later use.

2.2. Acid Extrusion of Starch

The starch was prepared according to Ye et al. [4]. Aqueous solutions (100 mL) of citric acid at 0, 10 and 40% of the starch basis were mixed with 300 g of quinoa starch. Extrusion was performed in a KE19 Brabender (South Hackensack, NJ, USA) at a 3:1 compression ratio screw and 200 rpm, 26% moisture and 60, 70 and 100 °C for each barrel section.

2.3. Starch, Resistant Starch and Glucose Content Determination

The total and resistant starches were measured using the Megazyme Total Starch Assay Kit and the free glucose was measured using the Megazyme D-Glucose kit.

2.4. FTIR Spectrum of Starch Samples

The Fourier transforms infrared (FTIR) spectra of starch and modified starch samples were obtained using an FTIR spectrophotometer (FT/IR-4100, Jasco International Co. Ltd., Tokyo, Japan). The starch samples were in contact with the universal diamond ATR top-plate and each spectrum, from 4000–400 cm^{-1}, represented an average of the three scans. The absorbance readings were transformed into transmittance and plotted against the wavelength (cm^{-1}) using the software Origin (Origin Lab, Northampton, MA, USA).

2.5. Particle Size Distribution of Milled Starch

The particle size distribution in the samples was analysed by laser diffraction analysis (Malvern Instruments Ltd., Malvern, UK) equipped with an MS 15 Sample Presentation Unit. The particle size distribution was described by the following parameters: the largest particle size (D_{90}), the median diameter (D_{50}), the smallest particle size (D_{10}), Sauter mean diameter (D[3.2]) and mean particle diameter (D[4.3]). Triplicate measurements were performed at room temperature.

2.6. Optical Microphotography Characterization

The morphological characteristics of starch and the particle size of modified starch were evaluated by an Eclipse 90i Nikon wide-field microscope (Nikon Corporation, Tokio, Japan) equipped with a 5-megapixel cooled digital colour camera Nikon Digital Sight DS-5Mc (Nikon Corporation, Tokio, Japan). Reflected light images were achieved with an external USB-LED. The Nikon objective used was the CFI Plan Fluor 4X (MRH00040). The Samples were gently spread over a glass slide with a good particle separation. All microscopy images were acquired by using Nis-Elements Br 3.2 Software (Nikon Corporation, Tokio, Japan).

3. Results and Discussion

3.1. Changes in the Resistant Starch Content and Molecular Characterization

The effect of acid extrusion on the RS content is shown in Table 1. A slight decrease in the RS content was observed when the quinoa starch was extruded. However, the reactive extrusion with citric acid changed drastically the RS content and a reduction was observed at 10%, the extrusion at 40% of citric acid causing nearly a two-fold increase compared to the unextruded sample. A similar RS content was obtained by Neder-Suarez et al. [6] for acid extruded corn starch using a lower citric acid e than that employed in this study, and the moisture content seems to have an important role during the formation of RS. Hasjim and Jay-Lin Jane [8] found that acid-modified corn starch increased the RS amount by 2.5% after extrusion and attributed the increase to the formation of retrograded amylose.

Table 1. Resistant starch and free glucose content of native, extruded and acid extruded samples.

Parameter	QNS	QES0	QAES10	QAES40
Resistant starch (g/100 g starch)	$0.63^{b} \pm 0.08$	$0.56^{b} \pm 0.07$	$0.20^{c} \pm 0.04$	$1.10^{a} \pm 0.09$
Free glucose (mg/100 g sample)	$801.36^{ab} \pm 176.73$	$1064.37^{a} \pm 50.15$	$904.62^{a} \pm 88.31$	$368.56^{c} \pm 6.76$

Data represent mean ± standard deviation ($n = 2$). Values followed by different letters in each row mean statistical difference ($p < 0.05$). QNS: quinoa native starch. QES0: Quinoa extruded starch with no citric acid. QAES10: quinoa acid extruded starch with 10% of citric acid. QAES40: quinoa acid extruded starch with 40% of citric acid.

Free glucose (FG) content also was affected by the reactive extrusion process (Table 1). The extrusion caused a glucose liberation, suggesting a partial hydrolysis. The addition of citric acid diminished significantly the initial FG amount by more than two-fold times. The initial FG was reduced from an 801 to 368 mg/100 g sample after the addition of 40% of acid. These changes indicate not only the partial hydrolysis of starch during the extrusion, but also suggests the liberation of simple sugars and the readily available glucose for a reaction with citric acid.

The esterification reaction between starch and citric acid was assessed by an FTIR spectra of native starch, extruded starch, and acid extruded starch samples and changes in the characteristic functional groups were compared (Figure 1). All spectra had similar patterns and peak absorptions of quinoa starch and flour [1,4,9], however, when comparing the spectra of native and extruded starch against acid extruded starch, a new peak was observed at 1712 cm^{-1} in acid extruded samples and less intense peaks at 3300 and 2900, suggesting the esterification reaction took place in the native quinoa starch. Ye et al. [4] also observed a similar peak at 1730 cm^{-1} in citrate starch samples, after extrusion for carbonyl groups C=O, and they also observed less intense peaks around 3400 and 2930 in citrate starch samples, suggesting the formation of covalent groups between hydroxyl and carboxyl groups and starch molecules.

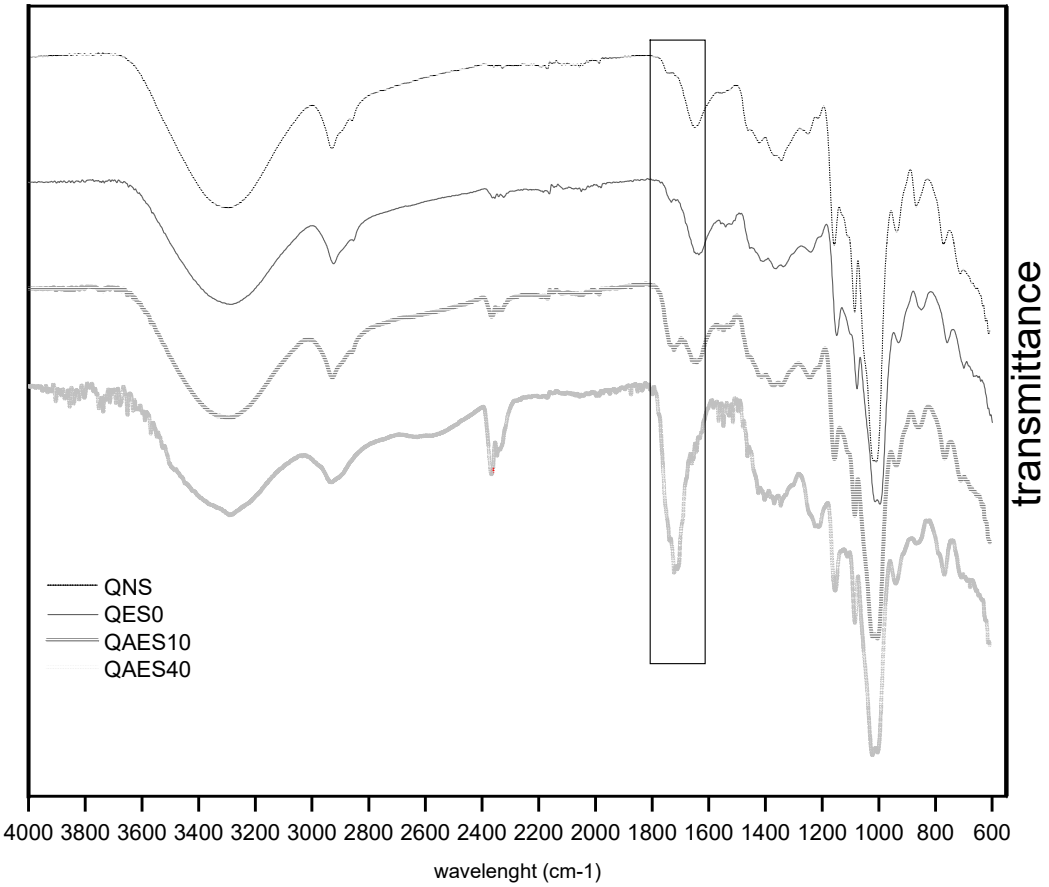

Figure 1. FTIR spectra of quinoa native starch (QNS); quinoa extruded starch (QES0); quinoa acid extruded starch 10% of citric acid (QAES10) and quinoa acid extruded starch at 40% of citric acid (QAES40).

3.2. Effect of Acid Extrusion on Particle Starch Properties

The behaviour and characteristics of food particulate materials, such as flours, depend on the geometry and size distribution of particles. In this study, light scattering and optical microscopy were used to evaluate the properties of native and processed quinoa starch. The particle size distributions of native and modified starch are shown in Table 2. The mean particle size D [4.3] of starch, extruded starch and acid extruded starch ranged from 282 to 321 µm. No significant differences were observed between native starch, extruded and acid extruded starch at 10%. Nevertheless, acid extrusion at 40% diminished significantly the mean diameter to 282.31 ± 4.70. The largest particle size ranged from 517 to 567 µm. Acid extrusion at 40% also diminished significantly ($p < 0.05$) the particle size.

Table 2. Particle size distribution of quinoa starch samples after extrusion and acid extrusion.

Parameter	Unit	Treatments			
		QNS	**QES0**	**QAES10**	**QAES40**
D_{10}	µm	26.81 ^{d} ± 19.12	90.67 ^{bc} ± 2.72	71.09 ^{c} ± 2.73	71.83 ^{c} ± 1.67
D_{50}	µm	319.45 ^{bc} ± 53.48	305.75 ^{bcd} ± 4.87	284.99 ^{bcd} ± 7.94	262.80 ^{d} ± 5.18
D_{90}	µm	560.72 ^{c} ± 4.04	567.63 ^{c} ± 5.47	558.56 ^{c} ± 6.10	517.84 ^{d} ± 6.05
D[3.2]	µm	45.99 ^{d} ± 18.79	145.53 ^{c} ± 10.91	132.78 ^{c} ± 5.17	136.071 ^{c} ± 2.54
D[4.3]	µm	295.8643 ^{c} ± 7.25	321.4907 ^{c} ± 4.09	303.55 ^{c} ± 6.39	282.3167 ^{cd} ± 4.70

Data represent mean ± standard deviation ($n = 3$). Values followed by different letters in each row mean statistical difference ($p < 0.05$). QNS: quinoa native starch; QES0: quinoa extruded starch with no citric acid; QAES10: quinoa acid extruded starch with 10% of citric acid; and QAES40: quinoa acid extruded starch with 40% of citric acid.

Although Table 2 shows similarities between the particle size distribution of quinoa starch flour, optical microphotography of the particulate material showed structural differences among the treatments. The microstructure of the particles is shown in Figure 2. All samples displayed particle diameters below 500 μm, however, starch aggregates can be observed in the native starch sample (Figure 2). Extruded (Figure 2 QES0) and acid extruded samples (Figure 2 QAES) showed a similar geometry but different than the particles observed for the native starch. Additionally, the translucent feature observed for extruded and acid extruded starch shows a clear difference and evidences an effect of citric acid on the starch particles. Figure 2 clearly shows that extrusion and reactive extrusion destroyed the starch aggregates and created amorphous structures. Neder-Suarez et al. [6] also found similar changes in the extruded samples of corn starch and attributed these changes to the gelatinization and dextrinization phenomena produced by a high pressure, temperature and mechanical forces during extrusion. Butt et al. [2] found similar results for rice extruded with citric acid using similar concentrations to the present study, and significant structural changes were reported.

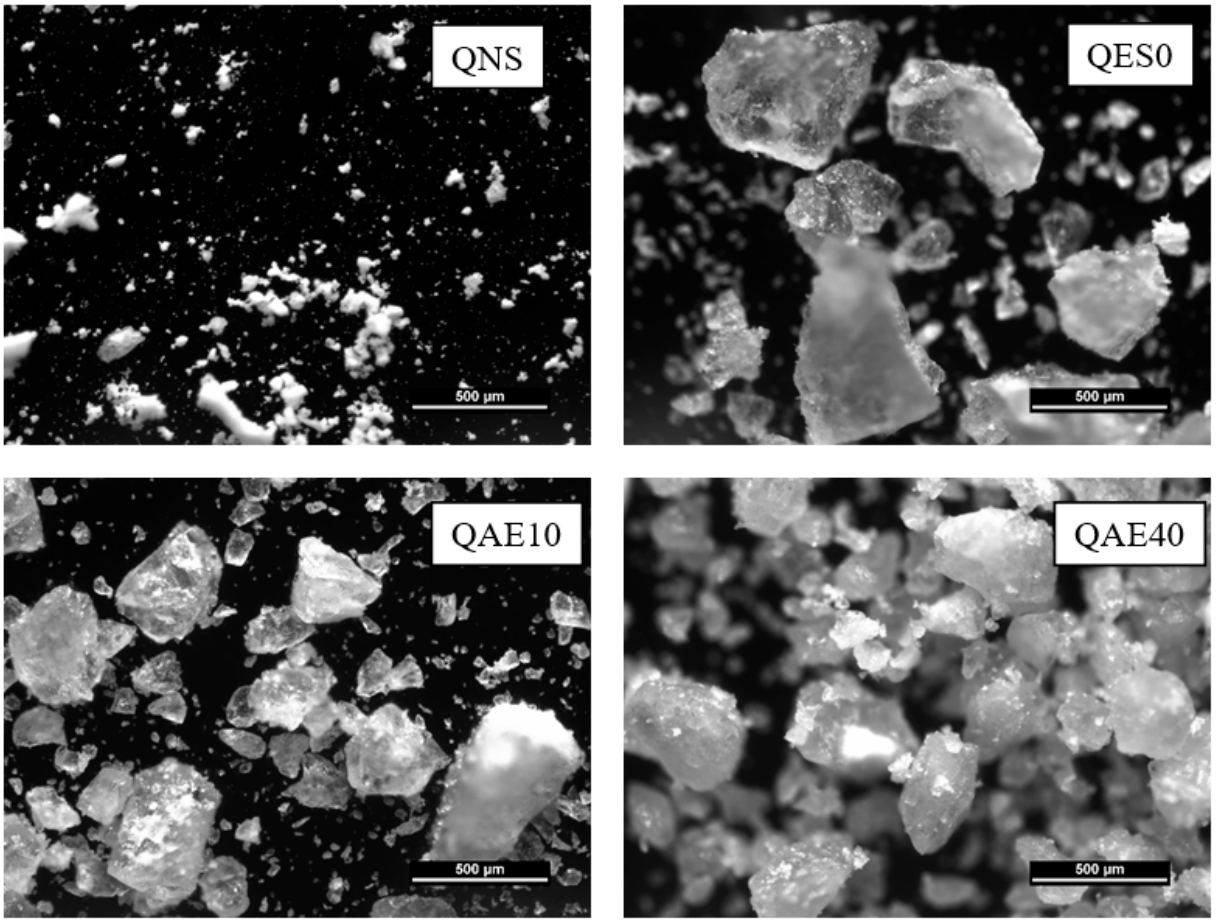

Figure 2. Micrograph images of quinoa native starch (QNS); quinoa extruded starch (QES0); quinoa acid extruded starch 10% of citric acid (QAES10) and quinoa acid extruded starch at 40% of citric acid (QAES40).

4. Conclusions

The results showed that the reactive extrusion of quinoa starch increased the resistant starch content by producing cross-linking reactions between starch and citrate molecules, evidenced by the formations of ester groups, as revealed by the FTIR spectra. The two-fold increase in the resistant starch occurred at a concentration of 40% of citric acid. Acid extrusion cooking also changed the structural morphology of starch particles compared to

native starch and significant three-dimensional conformational changes were shown by the micrographic analysis.

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