Development of New Starch Formulations for Inclusion in the Dietotherapeutic Treatment of Glycogen Storage Disease [†]

Raquel Selma-Gracia [1,2], José Moisés Laparra Llopis [2] and Claudia Monika Haros [1,*]

[1] Instituto de Agroquímica y Tecnología de Alimentos (IATA), Consejo Superior de Investigaciones Científicas (CSIC), Av. Agustín Escardino 7, Parque Científico, 46980 Paterna, Valencia, Spain; raquelselgra@iata.csic.es

[2] Molecular Immunonutrition Group, Madrid Institute for Advanced Studies in Food (IMDEA-Food), Ctra. de Canto Blanco n° 8, 28049 Madrid, Spain; moises.laparra@imdea.org

[*] Correspondence: cmharos@iata.csic.es

[†] Presented at the 2nd International Conference of Ia ValSe-Food Network, Lisbon, Portugal, 21–22 October 2019.

Abstract: In this study, the thermal properties of quinoa and maize starch were evaluated and related to their digestibility. Lower gelatinisation and retrogradation parameters were obtained in quinoa starch, suggesting a better susceptibility to the disruption of the crystalline structure. These results were accompanied with a higher percentage of hydrolysis in raw quinoa, reaching more twofold higher than in raw maize starch. Besides, the slopes calculated by a Lineweaver-Bürke transformation showed similar values in raw quinoa and maize starches. Taken together, these characteristics of quinoa starch could provide more digestible benefits than the current treatment, raw maize starch, in glycogen storage disease patients.

Keywords: maize starch; quinoa starch; thermal properties; starch hydrolysis; glycogen storage disease

1. Introduction

Previous research has shown variability in the susceptibility to digestion depending on structural differences in starches from different sources [1]. A crystalline structure is an important factor to take into account in digestibility, and can be modified by a gelatinisation process [2]. These altered structural changes rely on starch type and, as a result, each starch shows a different digestibility [3]. Besides, the extent of digestibility has been known to be related to the degree of polymerisation (DP) of amylopectin, affecting functional properties of the starches, such as thermal parameters, as well as their digestibility [4]. The identification of new sources of starch to obtain an improved digestibility than the current treatment, raw maize starch, could help in minimising digestive and metabolic disturbances for patients with glycogen storage diseases (GSDs).

2. Materials and Methods

2.1. Starches

Commercial maize starch was provided by ACH Food Companies (Argo, IL, USA). Red quinoa starch was obtained in the laboratory by wet milling [5]. Enzymes were purchased from Sigma-Aldrich: α-amylase (EC 3.2.1.1, A3176-1MU, St Louis, MO, USA, 16 U/mg), amyloglucosidase from

Aspergillus niger (EC 3.2.1.3, 10115, Buchs, Switzerland, 60.1 U/mg) and pepsin (EC 3.4.23.1, P7000, Gillingham, UK, 480 U/mg).

2.2. Thermal Properties

Gelatinisation and retrogradation properties were determined using differential scanning calorimetry (DSC) (Perking-Elmer DSC-7, Norwalk, CT, USA). The procedure was done according to the method described by Haros et al. [6] with slight modifications. Water:starch ratio was 3:1 and calorimeter scan conditions were kept at 25 °C for 1 min and then heated from 25 °C to 120 °C at 10 °C/min. To analyse retrograded starch, the samples were stored in a refrigerator for a week and the same process was repeated.

2.3. Preparation of Samples for Digestion

Aliquots (100 mg) of starch samples were weighed and 1 mL of water was added. Raw starches stayed in unheated water and gelatinised starches were kept at 100 °C for 5 min, as a positive control.

2.4. In Vitro Starch Digestion and Glycaemic Index (GI) Estimation

The rate of starch hydrolysis was evaluated according to the method described by Goñi et al. [7] with modifications. Briefly, 10 mL HCl-KCl buffer (pH 1.5) and 400 µL of a solution of pepsin (0.1 g/mL) were added and starches were kept in a shaking water bath at 37 °C for 1 h. Afterwards, 19.6 mL Tris-Maleate buffer (pH 6.9) and 1 mL of a solution containing α-amylase (0.01 g/mL) were added. Aliquots were taken from 0 to 120 min and the enzyme was inactivated. Finally, glucose, area under the curve (AUC), hydrolysis index ((HI) and GI were determined by spectrophotometry according to a commercially available enzymatic kit (D-Glucose Assay Procedure, K-GLUC 07/11, Megazyme) [8]. All the reagents used were analytical grade or better.

2.5. Statistical Analysis

Multiple ANOVA and Fisher's least significant difference (LSD) were carried out for the thermal properties and Tukey's test for the digestion values. The statistical analyses were realised with the software Statgraphics Centurion XVI, and the significance level was established at $p < 0.05$.

3. Results and Discussion

Gelatinisation parameters were determined in maize and quinoa starch, and quinoa displayed lower values than those of maize (Table 1). Thermal parameters have shown a positive correlation with the DP of amylopectin, where maize starch presented a higher DP (12–18) than the quinoa DP (8–10) [4,9]. When retrogradation parameters were measured (4 °C) after 7 days, quinoa starch exhibited the highest resistance to retrogradation, as the short chains of amylopectin in quinoa could have contributed to a lower rearrangement of the starch structure. The proportion of these shorter amylopectin chains in quinoa could have affected the crystalline structure, resulting in a higher susceptibility to enzymatic action [9].

Table 1. Preliminary gelatinisation parameters by differential scanning calorimetry (DSC) *.

Starch	Gelatinisation				Retrogradation			
	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)	To (°C)	Tp (°C)	Tc (°C)	ΔH (J/g)
Maize	65 ± 1 [b]	70 ± 1 [b]	76 ± 1 [b]	13 ± 1 [b]	44 ± 1 [b]	54 ± 1 [b]	63 ± 1 [b]	3.5 ± 1 [a]
Quinoa	51 ± 2 [a]	59 ± 1[a]	69 ± 1 [a]	10 ± 1 [a]	37 ± 1[a]	47 ± 1 [a]	56 ± 1 [a]	1.6 ± 1 [a]

[a] Mean ± standard deviation, n = 3. Values in the same column followed by the same letter are not [significantly different ($p < 0.05$); [b] To: Onset temperature, Tp: Peak temperature, Tc: Conclusion temperature, ΔH: Enthalpy change; Mean ± standard deviation, n = 2. * Selma-Gracia et al. [10].

The digestibility of starch represents an important parameter to consider in the severity and clinical manifestations of GSD. Only 30% of raw maize starch was hydrolysed, unlike raw quinoa,

which obtained high proportions of hydrolysis from the beginning, reaching up to around 70% (Figure 1). From the accumulated curves of hydrolysis, the Lineweaver-Bürke transformation [8] was calculated to estimate the kinetics of hydrolysis. For raw starches, close values for the slope of the plotted lines for maize (slope = 2.6 SH/min) and quinoa (slope = 2.2 SH/min) (Table 2) were calculated. Thus, similar slope values, but a higher hydrolysis of raw quinoa, could imply the maintenance of optimal glucose levels for a longer time with quinoa starch compared to maize starch. Besides, raw quinoa starch could help in improving the digestive inconveniences derived from the need to consume high amounts of raw maize starch in patients with GSD. When starches were heat treated, gelatinised samples displayed significant differences between the kinetics of hydrolysis: maize, slope = 11.7 SH/min; quinoa, slope = 3.9 SH/min. These differences were reflected mainly in the higher proportions of hydrolysis calculated for gelatinised maize starch within the first 20 min (Figure 1).

Table 2. Preliminary hydrolysis of starches by α-amylase.

Starch	Treatment	TSH$_{120}$ (%)	AUC	HI (%)	GI	Slope (SH/min)
Maize	Raw	30 ± 3 [a]	2975 ± 323 [a]	39 ± 4 [a]	61 ± 2 [a]	2.6 ± 0.2 [a]
	Gelatinised	73 ± 4 [bc]	7408 ± 572 [c]	97 ± 7 [c]	93 ± 4 [c]	11.7 ± 2.1 [b]
Quinoa	Raw	67 ± 4 [b]	5741 ± 606 [b]	75 ± 8 [b]	81 ± 4 [b]	2.2 ± 0.6 [a]
	Gelatinised	82 ± 2 [c]	7153 ± 167 [c]	94 ± 2 [c]	91 ± 1 [c]	3.9 ± 0.5 [a]

[a] Values in the same column followed by the same letter are not significantly different ($p < 0.05$); [b] TSH$_{120}$: Total starch hydrolysed at 120 min, AUC: Area under the curve of starch digestion from 0 to 120 min, HI: Hydrolysis index, GI: Glycaemic index; [c] Slope was calculated using the Lineweaver-Bürke transformation.
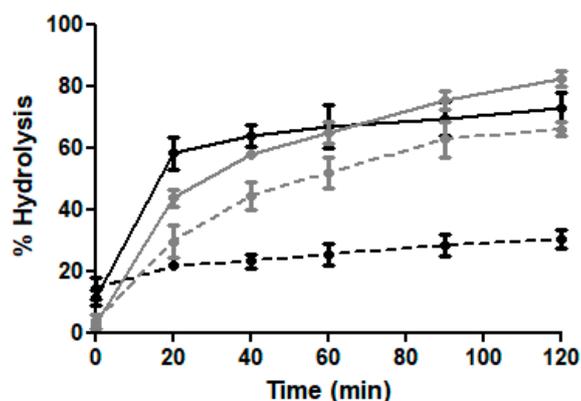
Figure 1. Kinetics of hydrolysis in raw starches (A) and gelatinised starches (B). Symbols: ■ Maize 100 °C starch; ● Raw maize starch; ■ Quinoa 100 °C starch; ● Raw quinoa starch. Selma-Gracia et al. [10].

However, although quinoa starch presented a greater susceptibility to digestion, gelatinised quinoa obtained about 25% less hydrolysis than gelatinised maize after 20 min, reaching higher total hydrolysis and, as a result, a slower hydrolysis rate.

4. Conclusions

The abovementioned results indicated that thermal parameters showed a reverse trend with hydrolysis, where quinoa displayed higher susceptibility to digestion than maize. The high hydrolysis and low slope kinetics from raw quinoa could suggest a potential starch for extending normoglycaemia in GSD patients. However, it would be necessary to evaluate the physiological consequences of this starch in an in vivo test.

References

1. Rosin, P.M.; Lajolo, F.M.; Menezes, E.W. Measurement and characterization of dietary starches. *J. Food Compos. Anal.* **2002**, *15*, 367–377, doi:10.1006/jfca.2002.1084.

2. Ahmadi-Abhari, S.; Woortman, A.J.J.; Oudhuis, A.A.C.M.; Hamer, R.J.; Loos, K. The influence of amylose-LPC complex formation on the susceptibility of wheat starch to amylase. *Carbohydr. Polym.* **2013**, *97*, 436–440, doi:10.1016/j.carbpol.2013.04.095.

3. Ratnayake, W.S.; Jackson, D.S. A new insight into the gelatinization process of native starches. *Carbohydr. Polym.* **2007**, *67*, 511–529, doi:10.1016/j.carbpol.2006.06.025.

4. Srichuwong, S.; Sunarti, T.C.; Mishima, T.; Isono, N.; Hisamatsu, M. Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility. *Carbohydr. Polym.* **2005**, *60*, 529–538, doi:10.1 016/j.carbpol.2005.03.004.

5. Ballester-Sánchez, J.; Gil, J.V.; Fernández-Espinar, M.T.; Haros, C.M. Quinoa wet-milling: Effect of steeping conditions on starch recovery and quality. *Food Hydrocoll.* **2019**, *89*, 837–843, doi:10.1016/j.foodhyd.2018.11.053.

6. Haros, M.; Blaszczak, W.; Perez, O.E.; Sadowska, J.; Rosell, C.M. Effect of ground corn steeping on starch properties. *Eur. Food Res. Technol.* **2006**, *222*, 194–200, doi:10.1007/s00217-005-0102-2.

7. Goñi, I.; Garcia-Alonso, A.; Saura-Calixto, F. A starch hydrolysis procedure to estimate glycemic index. *Nutr. Res.* **1997**, *17*, 427–437, doi:10.1016/s0271-5317(97)00010-9.

8. Sanz-Penella, J.M.; Laparra, J.M.; Haros, M. Impact of α-amylase during breadmaking on in vitro kinetics of starch hydrolysis and glycaemic index of enriched bread with bran. *Plant Foods Hum. Nutr.* **2014**, *69*, 216–221, doi:10.1007/s11130-014-0436-7.

9. Srichuwong, S.; Curti, D.; Austin, S.; King, R.; Lamothe, L.; Gloria-Hernandez, H. Physicochemical properties and starch digestibility of whole grain sorghums, millet, quinoa and amaranth flours, as affected by starch and non-starch constituents. *Food Chem.* **2017**, *233*, 1–10, doi:10.1016/j.foodchem.2017.04.019.

10. Selma-Gracia, R.; Laparra, J.M.; Haros, C.M. Potential beneficial effect of the hydrothermal treatment of starches from different sources on the in vitro digestion. *Food Hydrocol.* **2020**, *103*, 105687, doi:10.1016/j.foodhyd.2020.105687.