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Physicochemical interactions between rice starch and caffeic acid during boiling

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ABSTRACT

Defining the physicochemical interactions that may occur during fortification of starchy foods may be of great importance in food science. In this study, DSC and ^{1}H NMR techniques were acquired in order to examine the potential interactions between rice starch and caffeic acid, after following a hydrothermal treatment that may be used for rice fortification applications. According to DSC studies, significant changes were observed in starch thermal characteristics depending on the amount of caffeic acid added in starch-water mixture prior to heating. These changes could be attributed to the phenolic acid being probably embedded into starch-water matrix during heating and this may have altered its thermal properties and stability. Moreover, NMR studies of hydrothermally treated samples containing rice starch or caffeic acid and their mixtures showed a possible interaction of rice starch polysaccharides through H-bond formation with the phenolic acid.

1. Introduction

The interactions between phenolic compounds and carbohydrates or other macromolecules, for example proteins, have been studied extensively in the past. A big part of the relevant literature is focused on the binding of phenols inside the plant tissues they come from, especially their interactions with cell wall carbohydrates such as cellulose and pectins (Le Bourvellec, Bouchet, & Renard, 2005; Padayachee et al., 2012; Bautista-Ortin, Cano-Lechuga, Ruiz-Garcia, & Gomez-Plaza, 2014). In addition, polyphenolic molecules of high molecular weight such as tannins, anthocyanins, flavonoids and their interactions with food macromolecules seemed to have attracted the attention of researchers, probably due to their large size and complex structure which offer a number of potential binding sites (Jakobek, 2015; Le Bourvellec & Renard, 2012). On the contrary, phenolic acids, which possess one aromatic ring and a simpler structure, and their potential interactions have not been studied at the same extent.

Starch is a macromolecule that consists of amylose (linear) and amylopectin (branched), each of them possessing different chemical properties. In particular, only amylose has been found to present the ability to interact with other compounds of specific size and structure by obtaining a helical conformation and forming inclusion complexes, known as v-amylose complexes (Bulpin, Welsh, & Morris, 1982; Conde-Petit, Escher, & Nuessli, 2006). In this case, ligands such as lipids and aromatic compounds can be entrapped either inside a hydrophobic cavity in a single left-handed amylose helix which may contain 6–8 glucose units per turn (V_{6I}, V_7 or V_8 conformations) or both inside V_6 helix and in the area between the helices (conformations V_{6II} and V_{6III}). The ligands are mainly bound to amylose cavity via hydrophobic interactions, while extra H-bonds and *van der Waals* forces may stabilize the complex structure (Obiro, Ray, & Emmambux, 2012).

Amylose-ligand complex formation can be confirmed by using several different techniques among which are the measurement of iodine binding capacity, as well as Differential Scanning Calorimetry (DSC) and X-ray Diffraction (XRD) methods. Most types of amylose complexes present a distinct melting point at temperatures higher than 90 °C, which is due to their partial crystalline structure, and is measured using DSC techniques. These techniques can also offer indirect proof of starch-ligand interactions by determining changes in thermal behavior of starch during gelatinization and retrogradation processes at the presence of other compounds (Wu, Chen, Li, & Li, 2009; Zhu & Wang, 2012). Till today references about possible amylose-phenol inclusion complex formation remain scarce and questionable (Karunaratne & Zhu, 2016; Lorentz et al., 2012).

On the other hand, there is plenty of literature insisting that there could be interactions among starch and phenolic compounds in the form of their binding at the outer part of amylose and amylopectin molecules, mainly through H-bonds between the OH groups of amylose,

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amylopectin and the corresponding phenolic OH groups, without formation of helices. During food fortification processes the presence of phenolic molecules could induce the formation of new H-bonds not only with starch components but also with proteins, water and sugars which also possess OH groups inside the food matrix (Sivam, Sun-Waterhouse, Waterhouse, Quek, & Perera, 2011; Sivam, Waterhouse, Zujovic, Perera, & Sun-Waterhouse, 2013; Zhu, Cai, Sun, & Corke, 2009). The confirmation of such interactions, which do not include helix formation between starch and phenols, has been done in the past using DSC, XRD, NMR, FT-IR techniques, as well as by measuring the alteration of specific rheological properties of starch or its in vitro digestibility, after mixing with phenolic compounds (Sivam, Sun-Waterhouse, Perera, & Waterhouse, 2013; Wu, Lin, Chen, & Xiao, 2011; Zhu, 2015). Among these techniques, only NMR and FT-IR present the ability to offer specific data on the structure of the involved compounds and their potential complexes at a molecular level. In addition, NMR is considered to be a non-destructive technique which can be applied for the study of molecular interactions in a direct way. All other mentioned techniques provide rather complementary results. In general, the understanding of fortified food structure could be a very crucial factor for the development of modern food products, as it may help food scientists take a better control of their sensory characteristics.

In a recent study (Igoumenidis & Karathanos, 2016) white milled rice was enriched with a number of phenolic compounds which presented good thermal stability, as fortified rice grains maintained at least the 24% of all phenolics they obtained via fortification process, even after being rehydrated using excess water at boiling temperatures. That result implied that rice components may have the examined phenolic molecules bound inside the rice matrix. Moreover, caffeic acid was shown to be the predominant phenolic acid among others being entrapped inside rice grains. Given that rice starch constitutes the greatest part of white rice grain, the aim of this study was to evaluate the potential interactions that may occur between rice starch and caffeic acid molecules during rice fortification, by following a boiling process in an aqueous solution of this phenolic acid. DSC and ^{1}H NMR spectroscopy were implemented in order to determine the potential modifications in rice starch-caffeic acid-water matrix structure.

2. Materials and methods

2.1. Materials

Caffeic acid and rice starch of non-waxy type that contained 7.5–13% (w/w) moisture, < 1% protein and < 0.6% ash, according to product specifications, were obtained from Sigma Aldrich (St. Lewis, MO, USA). Deuterated dimethylsulfoxide (DMSO-*d6*) solvent was also purchased from Aldrich (St. Lewis, MO, USA).

2.2. Differential Scanning Calorimetry (DSC)

Thermal properties of rice starch, caffeic acid, as well as their mixtures at specific mass ratios, were evaluated using a Differential Scanning Calorimeter (DSC-4000, Perkin-Elmer, Inc., Waltham, Massachusetts, USA). Mass ratios of starch:caffeic acid were selected to be 0/1, 5/0, 5/0.3, 5/0.5, 5/0.8, 5/1 and 5/2, which correspond to 100, 0, 6, 10, 16, 20 and 40% (w/w) respectively of caffeic acid related to the mass of rice starch in each sample. All mixtures of starch and caffeic acid in the above ratios were weighed inside DSC stainless steel pans and an excess of double-deionized water was injected in each sample so that solid to liquid mass ratio was 1:4. According to literature, melting of starch crystallites becomes highly cooperative at excess water conditions (≥80% w/w) inside DSC samples (Biliaderis, Page, Slade, & Sirett, 1985). All samples were thoroughly mixed, using a stainless steel needle, and then they were hermetically sealed and kept at room temperature for 24 h prior to DSC analyses. An empty pan was used as reference in each scan and calibration of the instrument was done by using an indium standard.

All samples were initially heated in a temperature range of 10–130 °C at a rate of 10 °C/min and the onset (T_0), peak (T_p) and conclusion (T_c) temperatures, as well as the enthalpy (ΔH_g) of gelatinization were measured during this scan. At the end of the first heating process, sample temperature was kept at 130 °C for 1 min and afterwards each sample was cooled down to 10 °C at a constant temperature rate of 10 °C/min. Cooling of samples was followed by their immediate reheating, using same temperature range and heating rate as in the first scan, so as to investigate the potential appearance of reversible thermal phenomena which could be induced by the presence of the phenolic acid inside rice starch-water matrix. At the end of reheating process, samples were stored at 5 °C for 20 days and then rescanned, under the same range of temperature (10–130 °C) using a lower heating rate (5 °C/min), so that retrogradation of starch could be studied at the presence or the absence of caffeic acid. All types of analyses were carried out in triplicate.

2.3. Sample preparation for ^{1}H NMR studies

Rice starch-caffeic acid standard mixtures, at several mass ratios (1:1, 2:1, 4:1, 8:1, 40:3 and 20:1), were boiled inside a metal pot in the presence of excess water for 20 min, so as to simulate the boiling process of white milled rice inside an aqueous herbal extract which was applied in a recent study (Igoumenidis & Karathanos, 2016). In particular, 200 mg of rice starch was weighed and boiled using 100 mL of double deionized water and at the end of boiling process the remaining solution was cooled down to room temperature (gradually) and stored (−80 °C) for freeze drying (Scientz-N Series freeze-dryer, Ningbo, China). The same boiling and freeze drying processes were followed for caffeic acid (200 mg) solution in water (100 mL), as well as for starch-caffeic acid mixtures diluted in the same portion of water (100 mL) at the above mass ratios.

2.4. ^{1}H NMR spectroscopy

A small portion of all above freeze dried samples (2.5 ± 0.1 mg) was re-diluted in 0.7 mL of DMSO-*d6* to achieve better solubility and transferred into 5 mm NORELL 509-UP-7 NMR tubes in order to undergo ^{1}H NMR analysis. DMSO-*d6* was chosen in the present study as it is probably the most common organic solvent used for the dissolution of starch, as well as for the study of hydrogen bonding in carbohydrates (Bekiroglu, Kenne, & Sandstrom, 2003; Christofides & Davies, 1985; Ko, Shim, & Kim, 2005). Spectra were recorded at 25 °C on a 600 MHz Varian spectrometer using a ^{1}H–^{19}F/^{15}N-^{31}P 5 mm PFG AutoX Dual Broadband, VT, 600NB probe and following standard Varian pulse sequences. For ^{1}H experiments, 32 scans were recorded (ns), spectral width (sw) was set to 9615 Hz with a relaxation delay of 1 s and receiver gain (RG) equal to 22. The 90° pulse was calculated at 7.9 μs. 2D HSQC experiments were run with 30,166 Hz spectral width for ^{13}C, 8 scans and 400 t1 increments. NMR spectra were processed using Mnova software (MestReNova v: 9.0-12821, 2013-Mestrelab Research).

2.5. Statistical analysis

Results of DSC analyses concerning characteristic temperatures and enthalpies of specific thermal events were expressed as the averages of three obtained measurements per sample ± standard deviation (SD). In addition, statistical analysis on the measured gelatinization temperatures and enthalpies was performed using the Statistical Package IBM SPSS statistics 22 software and was based on one-way ANOVA, while statistical significance was calculated at a confidence level of 95%. Post hoc analysis using Tukey's Test at a 5% error probability was also applied in all samples.