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Enhancement of Digestion Resistance and Glycemic Control of Corn Starch through Conjugation with Gallic Acid and Quercetin Using the Free Radical Grafting Method

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Abstract: The objective of this study was to synthesize different polyphenol–corn starch complexes including gallic acid–starch and quercetin–starch by conjugating corn starch with gallic acid and quercetin using the free radical grafting method. This process was effective in enhancing conjugations of starch molecules with gallic acid and quercetin (5.20 and 5.83 mg GAE/g, respectively) and imparted promising antioxidant capacity to the phenolic–starch complexes. Significant interactions between these phenolic compounds and corn starch molecules were revealed with an ultraperformance liquid chromatography electrospray ionization Q-time-of-flight mass spectrometry assay. It was revealed that significantly higher levels of resistant starch in the above gallic–starch and quercetin–starch complex samples (11.6 and 15.3 g/100 g, respectively) together with an obvious reduction in glycemic response (7.9% and 11.8%, respectively) observed over the control. Complex samples functionalized with gallic acid and quercetin have exerted modified physicochemical properties, particularly reduction in swelling ability (58.7–60.1%), breakdown viscosity (62.5–67.8%), and setback viscosity (37.7–44.5%). In sum, free radical grafting treatment could be a promising method for imparting corn starch with enhanced resistance to enzyme digestion along with changes in pasting properties for specific food applications.

Keywords: corn starch; gallic acid; quercetin; interaction; starch digestibility; glycemic control

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1. Introduction

Many studies have demonstrated that resistant starch slows digestion rates and reduces glycemic responses when compared with normal starch [1,2]. Hence, increasing resistant starch intake in daily diets could be one of the solutions to decreasing relative health risks. Despite the benefits of consuming resistant starch, its food applications are limited by its possible adverse impacts on texture and other physicochemical characteristics of food products including swelling, viscosity, gelatinization, moisture holding, thickening, and gel forming properties [3,4].

In recent years, considerable effort has been devoted to synthesizing polyphenol-grafted polysaccharides by reacting starch with phenolic acids and flavonoids [5]. In the literature, different phenolic compound–carbohydrate-grafted conjugates such as catechin–alginate, catechin–inulin [6], starch–quercetin [7], gallic acid–chitosan [8], inulin–gallic acid [9], and rice starch–chlorogenic acid [10] have been studied. Through the conjugation of various phenolic compounds to the carbohydrate molecule, the antioxidant activity, digestive enzyme inhibitory activity, and physicochemical and biological properties of

these complexes were greatly enhanced. The grafting of a phenolic compound onto a carbohydrate molecule allows the formation of new functionalized materials and food applications. For instance, grafting gallic acid onto inulin would give this oligosaccharide, which originally does not possess any antioxidant potential, a promising capacity for scavenging free radicals [9]. The approach of conjugating phenolic groups with native carbohydrates imparted peculiar characteristics to these grafted macromolecules, and as a consequence greatly broadened their applications in the food industry [11,12].

Deng et al. have demonstrated that the formation of polyphenol–starch complexes can increase the resistance to the digestion of the starch component by α-amylase and α-glucosidase [13]. The reduction in amylolytic hydrolysis was probably attributed to the enzyme-inhibitory effects of phenolic compounds (like flavonoids and phenolic acids) directly bound to amylose [14]. Therefore, it was speculated that the polyphenol–starch complexes could be regarded as a kind of resistant starch and provide some physiological benefits, such as improvement of glycemic control and lipid profiles [15].

Common graft copolymerization techniques included acid catalyzed condensation, chemical coupling, enzyme catalysis, and free radical grafting treatments [16]. Most of the graft copolymerization techniques involved the use of toxic substrates, which negatively impact the environment and human health. Therefore, exploring an environmentally friendly and non-toxic method for the potential application of graft copolymerization techniques in the food industry would be desirable. Compared with the chemical coupling method, the free radical grafting process was more economic and eco-friendlier, and prevented hydroxyl groups' oxidation [13].

With respect to the free radical grafting process, a mixture of ascorbic acid and hydrogen peroxide redox pair was used to form flavonoid-grafted polysaccharides, such as quercetin-grafted starch [7]. Although hydroxyl radical has long been accepted to be the major free radical to bring about the synthesis of antioxidant-grafted carbohydrate conjugate, it was reported that the reaction was actually mediated by ascorbate radical rather than hydroxyl radical in the redox system [11,17]. The probable interaction mechanism involves the accelerated formation of an ascorbate radical in the presence of hydrogen peroxide, and the generation of carbon-centered radicals along the carbohydrate chain in the redox pair system [18]. In the grafting process, the generated macro-radical on the polymer chain subsequently offers an opportunity for the phenolic compound molecule to link to the macro-radical through a covalent bond.

The aim of this study was to synthesize gallic acid–starch and quercetin–starch complexes by conjugating corn starch with gallic acid and quercetin, respectively, by using the radical grafting method. The amounts of gallic acid and quercetin bound to each gram of complex were quantified using the Folin–Ciocalteu assay. The substantial conjugation of gallic acid and quercetin to corn starch molecules was proofed by using an ultraperformance liquid chromatography electrospray ionization Q-time-of-flight mass spectrometry (UPLC-Q-TOF-MS) analysis. Changes in pasting properties as well as digestibility of phenolic–starch complexes in vitro and in vivo were determined. As relevant research of phenolic–starch complexes using the free radical grafting method remains limited, this work would provide an alternative idea of developing an enzyme-resistant starch material for functional food application and fill a gap in the literature.

2. Materials and Methods

2.1. Phenolic–Starch Complexes Preparation

Phenolic–starch complex samples were synthesized in accordance with the procedures detailed by Cirillo et al. [7] with minor adjustments. Corn starch (S-4126, Sigma-Aldrich, St. Louis, MO, USA), which contained 27% amylose and 73% amylopectin, was used in this experiment. This native starch (10.0 g) was dissolved in 130 mL of a 50% ethanol (v/v) mixture, into which 15 mL of hydrogen peroxide (1.0 M) containing 0.81 g of ascorbic acid were added. After 30 min of stirring, 0.25 g (low dose) and 0.50 g (high dose) of gallic acid

(G) or quercetin (Q) were added, and the mixture solutions were maintained at 25 °C for 24 h.

In this work, phenolic–starch samples prepared with low dose of gallic acid and quercetin were named as FRG-L and FRQ-L, respectively; while those made with high dose of gallic acid and quercetin were named as FRG-H and FRQ-H, respectively. The complex samples collected were washed with distilled water (1:15, w/v) for 1 min before being centrifuged at 2400× g for 10 min. This process was performed twice prior to collecting and lyophilizing the sediments. Blank starch, acting as a control, was prepared with the same conditions but in the absence of phenolic compounds.

According to the literature with some modifications [19], the content of total phenolic compounds bound to the starch backbone was analyzed using the Folin–Ciocalteu reagent procedure. Briefly, 25 mg of complex sample solution was dispersed in 10 mL of distilled water. Into the mixture, one milliliter of Folin–Ciocalteu reagent was added and shaken thoroughly for 3 min. After that, 3 mL of 2% Na_2CO_3 was added, and then the mixture was allowed to stand for 2 h with intermittent shaking in darkness. The absorbance of the reactant was determined with a spectrophotometer at 750 nm. In this experiment, a calibration curve was created using different concentrations of gallic acid solution. The amount of phenolic compounds bound in the complexes was calculated using the standard calibration curve.

2.2. Determination of DPPH Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity of different complex samples was analyzed according to the literature [20] with some modifications. The measurement of DPPH radical scavenging activity was repeated three times for each concentration level. Briefly, 90 mg complex samples were dispersed in 6 mL of ethanol and then 4 mL of ethanol solution of the DPPH (0.6 mM) were added. The mixed solution was incubated in a water bath in the dark at room temperature and, after 30 min, the absorbance of the remaining DPPH was read at 517 nm. In this test, a 6.4 mM Trolox solution was used as positive control. The DPPH radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity} = [(A_0 - A_1)/A_0 \times 100]$$

where A_0 represents the baseline absorbance, and A_1 represents the absorbance of samples.

2.3. Determination of Water Solubility Index and Swelling Power

To estimate the swelling abilities of samples, modifications were made to the procedure outlined by Li et al. [21]. A starch slurry was prepared by mixing 0.5 g of testing sample with 25 mL of distilled water, followed by heating at 90 °C for 30 min. After centrifugation (7000× g) for 20 min, the weight of the precipitate was determined (W_p). The supernatant collected was dried at 105 °C until at a constant weight (W_s). The swelling power and water solubility were calculated using the following equations:

$$\text{Water solubility index } (\%) = \frac{\text{dry weight of supernatant } (W_s)}{\text{weight of sample}} \times 100\%$$

$$\text{Swelling power } \left(\frac{g}{g}\right) = \frac{\text{weight of precipitate } (W_p)}{\text{weight of sample} - \text{dry weight of supernatant } (W_s)}$$

2.4. Determination of Pasting Properties

Using a rapid visco-analyzer (Model RVA-Super 3, Newport Scientific, Warriewood, NSW, Australia), pasting properties of samples mixed 1:9 (w/w) with distilled water were analyzed. The total analysis time was 13 min. The testing sample suspension was held at 50 °C for 1 min, gradually heated from 50 °C to 95 °C at a rate of 6 °C per min, held at 95 °C for 3 min, cooled to 50 °C at the same rate, and then held at 50 °C for 2 min. Pasting

properties, including hot paste viscosity (HP), cold paste viscosity (CP), peak viscosity (P), and pasting temperature (PT) were determined. Afterwards, breakdown viscosity (BD = P − HP) and setback viscosity (SB = CP − P) were calculated.

2.5. Determination of Resistance Starch Content

Relative resistant starch content was analyzed with a kit assay (K-RSTAR, Megazyme Bray, Co. Wicklow, Ireland). The phenolic–starch and control samples (100 ± 0.5 mg) were incubated with 4 mL of mixed solution of pancreatic α-amylase (10 mg/mL) and amyloglucosidase (3 U/mL) for 16 h in a 37 °C water bath with constant shaking. After hydrolysis, the testing samples were washed repeatedly with ethanol (99% and 50% ethanol, v/v) to stop the enzyme reaction, followed by 10 min of centrifugation at 1500× g. The sediment was solubilized in 2 mL of 3.0 M KOH in an ice bath, neutralized with 8 mL sodium acetate (1.2 M) and the resistance starch was hydrolyzed to glucose with amyloglucosidase (0.1 mL, 3300 U/mL). Sample solution was put into a 100 mL quantitative bottle and made up to 100 mL with distilled water. Part of the testing sample solution was taken out and centrifuged at 1500× g for 10 min. A quantity of 100 µL of supernatant and 3 mL of glucose oxidase/peroxidase reagent were mixed well in a glass tube and reacted in a 50 °C water bath for 20 min. In addition, 100 µL of 0.1 M sodium acetate (pH = 4.5) and 100 µL of D-glucose (1.0 mg/mL) were used as blank and standard, respectively. Absorbance was measured with a spectrophotometer at 510 nm after 20 min of incubation at 50 °C. The levels of digested starch and resistant starch were measured as glucose × 0.9. The total starch content was the sum of resistant starch and digested starch. The levels of resistant starch in the complex samples were calculated by the equation below:

$$\text{Resistant starch}\left(\frac{\text{g}}{\text{100 g dry sample}}\right) = \Delta\text{E} \times \frac{\text{F}}{\text{W}} \times 90$$

ΔE = absorbance (reaction) read against the reagent blank.

F = the absorbance obtained for 100 µg of D-glucose in the glucose oxidase/peroxidase reaction was determined.

W = dry weight of sample analyzed.

2.6. Determination of In Vivo Postprandial Glycemic Responses

Forty 12-week-old male Sprague–Dawley (SD) rats weighing 364 ± 38 g obtained from BioLASCO (A Charles River Licensee Corp., Taiwan) were used. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of National Chung Hsing University (IACUC approval number: 110-092). All the SD rats were housed under a light–dark cycle that lasted for 12 h at ambient temperature (22 ± 3 °C), with free access to food and tap water. Rats were randomly split into 5 groups comprising one control group and four sample groups, namely FRG-L, FRQ-L, FRG-H, and FRQ-H groups, which were fed with FRG-L, FRQ-L, FRG-H, and FRQ-H samples, respectively. To prepare the feeding samples, phenolic–starch sample (1 g) was combined with distilled water (5 mL). Animals were given these complex samples by oral gavage after fasting for 12 to 14 h. Blood samples were collected between 0 and 180 min. A glucometer was used to determine blood glucose levels (AB-103G, Apexbio, Taiwan). Utilizing the trapezoidal rule approach established by Matthews et al., the areas under the curve (AUC) of blood glucose throughout the 180 min period were computed [22].

2.7. UPLC-Q-TOF-MS Analysis

Samples were dissolved in 0.02 M phosphate buffer (5 g/100 mL, pH 6.8), heated in a hot water bath at 80 °C for 30 min, and cooled to room temperature. Subsequently, the samples (10 mL) were reacted with α-amylase (0.1 mg/mL, Cat. No. 100447, MP Biomedicals, Ohio, USA) dissolved in 0.02 M phosphate buffer (1 mL, pH 6.8) at 37 °C for 30 min. To terminate the amylolytic hydrolysis reaction, the mixture was boiled for 15 min. Utilizing a dialysis membrane having a cutoff MW of 10,000, the hydrolysate mixture was

dialyzed against distilled water in the refrigerator for 24 h. The dialysate was further ultrafiltered with a Microsep Advance centrifugal devices fitted with Omega™ membrane (PALL Corporation, Port Washington, NY, USA) at a MW cutoff of 1 kDa. The dialysate was ultrafiltered and collected for analysis.

A UHPLC system (Ultimate 3000, Dionex, Germering, Germany) equipped with a BEH Amide column (2.1 × 150 mm, 2.5 mm; Waters, Milford, MA, USA) was coupled with a hybrid Q-TOF mass spectrometer (maXis impact, Bruker Daltonics, Bremen, Germany) with an orthogonal electrospray ionization (ESI) source. The liquid chromatography flow rate was 0.25 mL/min, and 10 mM ammonium bicarbonate and pure acetonitrile were used as solvents A and B, respectively. Sample (5 µL) was injected. The concentration of solvent B was held at 90% for 0.5 min, and gradually lowered to 30% over 6.5 min. After retaining at 30% for 1 min, solvent B was reduced back to 90% and maintained at this percentage for 3 min. The mass spectrometer was operated in negative ion mode using the m/z range 50–1000 at 2 Hz. The capillary voltage of the ion source was set at −3000 V, and the endplate offset was 500 V. The nebulizer gas flow was 1 bar and drying gas flow was 8 L/min. The drying temperature was set at 200 °C. Both funnel 1 radiofrequency and funnel 2 radiofrequency were 400 Vpp. The hexapole radiofrequency was 200 Vpp. The low mass cutoff of quadrupole was 100 m/z. In this experiment, the most six abundant precursors were selected for MS/MS data-dependent acquisition analysis. Activation ions were excluded after 2 spectra and released after 0.5 min.

2.8. Statistical Analysis

The results were expressed as the mean values of three replicates. One-way ANOVA or T-test were used to assess statistical differences among groups. Statistical analysis and chart drawing were completed with SPSS version 20.0 (Armonk, NY, USA), which used a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Phenolic Compound-Binding Capacity in the Complex

The amounts of phenolic compounds (i.e., gallic acid and quercetin) bound within the starch network among different phenolic–starch complex samples were presented in Figure 1. After the Folin–Ciocalteu assay, FRG-L and FRQ-L samples were found to have different levels of total phenolic compound (3.74 and 4.35 mg GAE/g, respectively) bound to the corn starch molecules. It demonstrated that this free radical grafting reaction was a potential approach to synthesizing phenolic compound-grafted corn starch. As the concentrations of phenolic compounds further increased during the free radical grafting treatment, the amounts of gallic acid and quercetin that interacted with corn starch (i.e., FRG-H and FRQ-H, respectively) were found to be increased correspondingly by 39.0% (5.20 mg GAE/g) and 34.0% (5.83 mg GAE/g), respectively. It was inferred that the synthesis of gallic acid- and quercetin-grafted starch conjugates in the ascorbic acid and hydrogen peroxide redox system was probably mediated by ascorbate radical, which generated some carbon-centered radicals on the starch molecules, and thus interacted with the phenolic compound and formed a complex [11]. According to a previous study, the binding efficiency of phenolic compounds would be affected by their concentrations during the conjugation process. For instance, a decreased level of quercetin in the reaction mixture would apparently result in a less effective conjugation formation [7]. Since gallic acid was more vulnerable to oxidative stress than quercetin [23], it might partly explain the relative lesser amount of gallic acid bound in the complex samples including FRG-L and FRG-H.

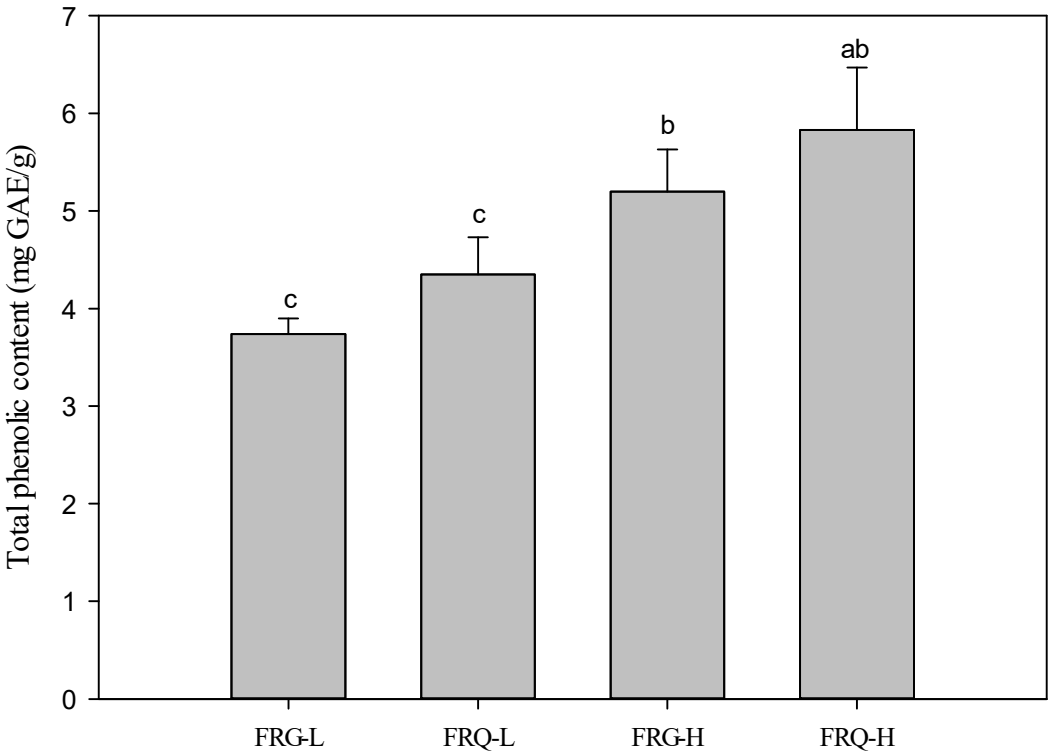

Figure 1. Contents of phenolic compounds bound in different phenolic–starch complexes. a–c: bars with letters are significantly different (Duncan, $p < 0.05$).

3.2. Antioxidant Activity of Phenolic–Starch Complexes

Using the DPPH assay, the free radical scavenging properties of phenolic–starch complexes were compared with the control (Figure 2). The percentage of inhibition of DPPH radical of the FRG-L, FRQ-L, FRG-H, and FRQ-H samples (38.9, 39.6, 49.3, and 55.8%, respectively) were comparable to each other and significantly higher ($p < 0.05$) than the control sample (4.1%). It demonstrated that these potent phenolic antioxidants were capable of imparting a higher antioxidant capacity to the phenolic–starch complexes and agreed with observations obtained from previous research [24,25]. Furthermore, in the findings of Cirillo [7], the DPPH scavenging activity of the quercetin–starch complex, which was synthesized by free radical treatment, was considerably greater than that of the untreated starch sample. It was also reported that the antioxidant activity of maize starch conjugated with condensed tannin could be noticeably enhanced through the conjugation of maize starch with sorghum-condensed tannin [26].

As compared with the FRG-L and FRQ-L samples (Figure 2), a significant increase in the percent inhibition of DPPH radical (by 10.4–16.2%) was observed in both the gallic acid– and quercetin–starch complex samples as the concentrations of interventional phenolic compounds increased. It was worth noting that outcomes of antioxidant activity tests could be influenced by several variables, including assay selection and reactant structure–activity relationships [27]. It is possible that the starch molecules present in phenolic–starch complexes serve as a trapping matrix inside the structures of these complexes and hamper activity assessments. It was speculated that gallic acid and quercetin activities that were restrained by the polysaccharide matrix might be one of the causes of the comparable antioxidant activities across the low dose samples [21].

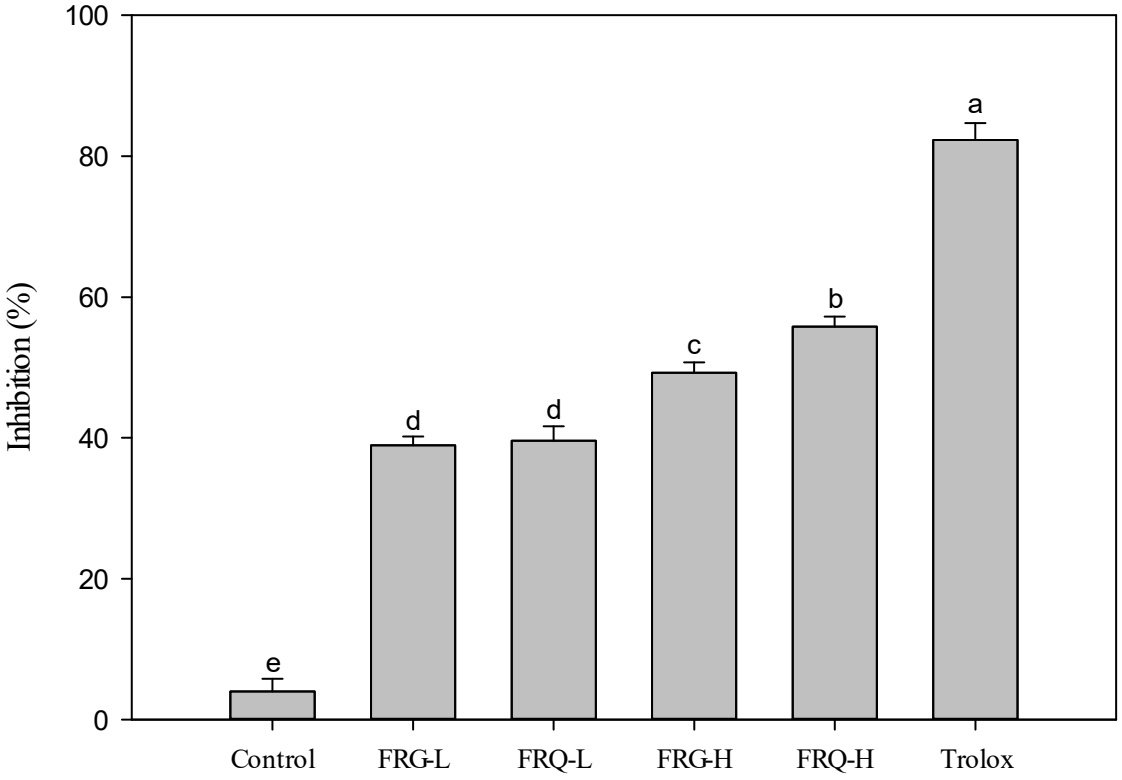

Figure 2. A comparison of the percentage of inhibition of DPPH radicals among different phenolic–starch complexes. a–e: bars with different letters are significantly different (Duncan, $p < 0.05$).

3.3. Characterization of Interactions by UPLC-Q-TOF-MS

LC-MS/MS was used to identify the products formed from the interactions between corn starch molecules and gallic acid as well as quercetin. For the detection of products formed in the gallic–starch complex, the precursor ion (m/z 493.12) was detected with its fragmented ions (m/z 195.04, m/z 237.0533, and 297.0726) at 6.2 min (Figure 3A). A similar precursor ion at m/z 493.12, which was proposed as galloyl diglucoside, was also found in some natural sources such as mango pulp and peel [28]. It was proposed that Hex-Hex-O-galloyl was formed as all of their three observable fragments could match to the in silico fragments derived from Hex-Hex-O-galloyl using the Metfrag Web tool (Figure 4A). For the detection of products formed in the quercetin–starch complex, a precursor ion (m/z 625.14) was observed with its fragmented ions (m/z 297.0726, m/z 327.0825, m/z 357.0917, m/z 429.1109, and m/z 501.1306) at 6.5 min (Figure 3B). In the study of Yeo et al. [29], a deprotonated molecule $[M - H]^-$ at m/z 625 that was suggested as a quercetin diglucoside was identified in lentil hulls. It was proposed that quercetin 3-O-beta-D-glucosyl-(1→2)-beta-D-glucoside (also known as quercetin-3-O-sophoroside) was formed as there were five observable fragments matching to the in silico fragments derived from quercetin 3-O-beta-D-glucosyl-(1→2)-beta-D-glucoside using the Metfrag Web tool (Figure 4B). Possible glucose-derived product was not observed in these spectroscopic experiments. Considering the aforementioned results, the presence of maltose as the glycone unit of gallic acid glycoside as well as quercetin glycoside supported the inference of the apparent interaction and formation of phenolic–starch complexes.

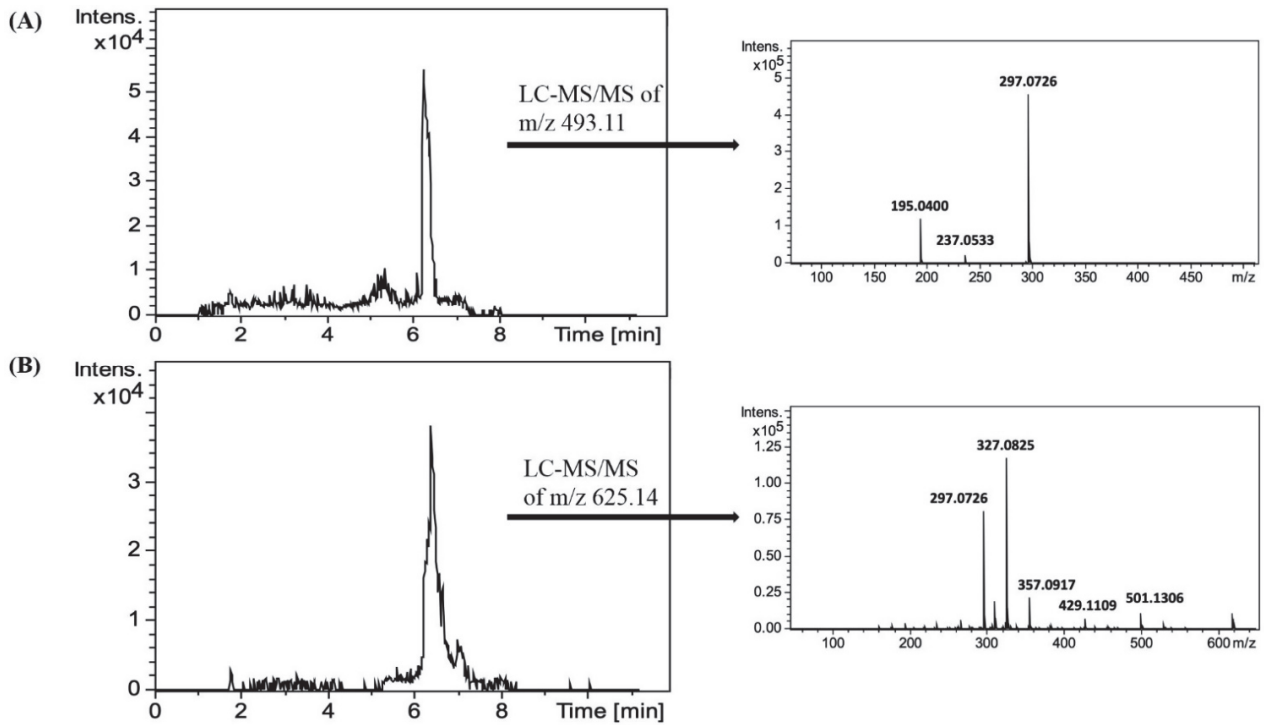

Figure 3. Chromatograms and precursor ion spectra of phenolic–starch complexes by LC-MS/MS. (**A**) m/z 493.11 of the gallic acid–starch complex, (**B**) m/z 625.14 of the quercetin–starch complex.

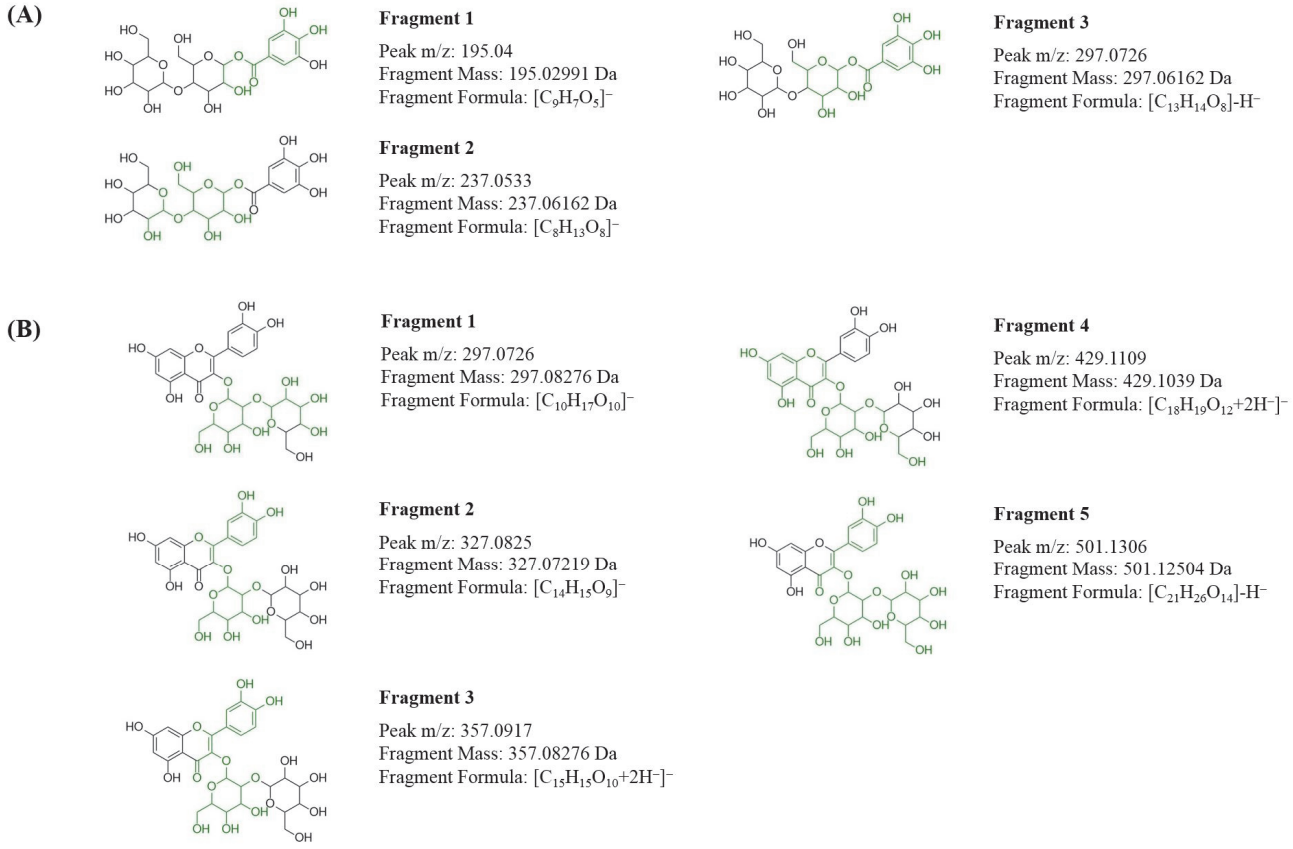

Figure 4. The in silico fragments derived from products using Metfrag Web tool. The fragmented ion structure was highlighted with green color. (**A**) Hex-Hex-O-galloyl, (**B**) quercetin 3-O-beta-D-glucosyl-(1→2)-beta-D-glucoside.

3.4. Resistant to Starch Digestibility

The amylolytic hydrolysis resistances of the FRG-L, FRQ-L, FRG-H, and FRQ-H were compared in Table 1. Our results revealed that the resistant starch content in the corn starch control was 3.0 mg/g. After the graft copolymerization treatment, the resistant starch contents in the FRG-L, FRQ-L, FRG-H, and FRQ-H samples increased ($p < 0.05$) by 2.3-, 2.6-, 3.9-, and 5.1-fold, respectively, over the control. These findings suggested that the direct binding of gallic acid and quercetin within complex samples at the levels of 3.74–5.83 mg/g appeared to be an effective way of decreasing starch digestibility. These outcomes corroborate those of Hernández et al. [30] that the resistance of starch to amylolytic hydrolysis could be enhanced by conjugating the starch ingredient with phenolic compounds. It was believed that the higher resistance of phenolic–starch complex to amylolytic digestion might be attributed to the formation of inner bonding, a higher structure stability, and a higher ability to compete with starch molecules for the active sites of α-amylolytic enzymes [25].

Table 1. Resistant starch contents and in vivo postprandial glycemic responses among different phenolic–starch complexes.

Samples	Resistant Starch (g/100 g)	AUC#
Control	3.0 ± 0.4 d	416 ± 16 b
FRG-L	6.9 ± 1.2 c	405 ± 27 b
FRQ-L	7.7 ± 1.0 c	389 ± 17 ab
FRG-H	11.6 ± 1.3 b	383 ± 33 ab
FRQ-H	15.3 ± 1.0 a	367 ± 13 a

#AUC, represented as arbitrary unit. a–d values (mean ± SD) in the same column with different letters are significantly different (Duncan, $p < 0.05$).

3.5. In Vivo Starch Digestibility

The in vivo starch digestibility result demonstrated that rats' baseline blood glucose levels were steady with an average level of 110–120 mg/dL from 0 min to 180 min. As presented in Table 1, the FRQ-H group showed a marked ($p < 0.05$) drop in the AUC value (−11.8%) compared with the control group (416 arbitrary unit). The AUC values of rats fed with FRG-L, FRG-H, and FRQ-L samples were found to be comparable to each other. The trend of AUC values obtained among the five animal groups was reversely related to the levels of bound phenolic compounds and resistant starch among different complex samples (Figure 1 and Table 1). It was inferred that the apparent reduction in the AUC value as observed in the FRQ-H group might be associated with the relatively pronounced physiological activity of quercetin in relation to gallic acid [31,32]. These results demonstrated that the conjugation of starch with a high dose of quercetin (equivalent to 5.83 mg GAE/g) could effectively render the starch sample's partial inaccessibility to digestive enzymes, resulting in a decreased rate of glucose production. Although some previous studies have demonstrated that both gallic acid and quercetin were capable of lowering plasma glucose and improving insulin sensitivity [33,34], it was inferred that the statistically non-significant changes in the AUC values among the FRG-L, FRQ-L, and FRG-H groups might be influenced by some factors such as type and amount of phenolic compound in the complex samples.

3.6. Swelling Ability and Water Solubility Index

A fundamental understanding of structural relationships between the crystalline and amorphous domains of starch molecules could be provided by their swelling capacity and water solubility index. In Table 2, the swelling ability and water solubility among the corn starch control, FRG-L, FRQ-L, FRG-H, and FRQ-H samples are compared. It was observed that the corn starch control had a swelling ability of 12.0 g/g and a water solubility index of 30.1%. Prior research [35] has reported that the swelling ability of different corn cultivars

ranged from 12.0 g/g to 13.6 g/g, while the water solubility index ranged from 46.2% to 49.4%.

Table 2. Swelling power and water solubility among different phenolic–starch complexes.

Samples	Swelling Power (g/g)	Water Solubility (%)
Control	12.0 ± 0.8 cd	30.1 ± 2.0 c
FRG-L	12.7 ± 0.9 bc	46.0 ± 2.0 a
FRQ-L	11.5 ± 0.2 d	42.6 ± 0.6 b
FRG-H	14.3 ± 0.5 a	48.2 ± 3.4 a
FRQ-H	13.2 ± 0.7 b	47.7 ± 2.2 a

a–d values (mean ± SD) in the same column with different letters are significantly different (Duncan, $p < 0.05$).

As shown in Table 2, the higher-dose samples (FRG-H and FRQ-H) were revealed to exhibit higher ($p < 0.05$) ability to swell than their corresponding lower dose (18.7% for FRG-L and 9.9% for FRQ-L, respectively) after complete gelatinization by heating at 90 °C for 30 min. An elevation in swelling might be partly contributed by the conjunctions between phenolic compounds and starch molecules, resulting in weakened interchain association and bonding forces between starch chains, and thus reinforcing the structure to hold more water inside the phenolic–starch complex [36]. An increase in the swelling ability of phenolic–starch complexes, which was produced by conjugating gallic acid and quercetin onto rice starch using a pre-gelatinization method, was also observed by Han et al. [37]. On the contrary, in the literature of Li et al. [10], an apparently lower swelling ability of phenolic–starch complexes made with the approach of acid-catalyzed condensation than untreated starch was reported. It was hence inferred that the swelling ability of different phenolic–starch complexes were subjected to being different based on the different conjugating methods.

The results in Table 2 also indicated that interventions of phenolic compounds would significantly ($p < 0.05$) increase the solubility of all phenolic–starch samples by 12.5–18.1% relative to the control. The free radical grafting treatment of corn starch could lead to the disintegration of starch particles by hydrogen peroxide and thereby increase the water solubility index [38]. Furthermore, the presence of phenolic compounds in the complex sample would enhance the molecular interactions between water and corn starch, leading to a greater rate of starch disintegration in aqueous environments [39].

3.7. Pasting Properties

Figure 5 shows the visco-amylograms of the FRG-L, FRQ-L, FRG-H, and FRQ-H samples in comparison to the corn starch control. Their pasting behaviors are summarized in Table 3. In comparison to the control, a significant ($p < 0.05$) decrease in peak viscosity (40–60%) and breakdown value (41.9–67.8%) were observed in all four phenolic-starch samples. It was noted that the final viscosity decreased significantly ($p < 0.05$) from 221 cP (control) to 132 cP (FRG-H) or 111 cP (FRQ-H). Final viscosity indicated the ability of starch to form a viscous paste after cooking and cooling. The results revealed that the more phenolic compounds incorporated into the complexes, the lower the final viscosity. In particular, the inclusion of gallic acid and quercetin into the corn starch matrix (at the levels of 5.20 and 5.83 mg GAE/g, respectively) resulted in an apparently less viscous paste after cooking and cooling.

Starch solution's capacity to withstand shear stress during heating is correlated with its breakdown viscosity [40]. A considerable ($p < 0.05$) reduction in the breakdown values among all phenolic–starch complexes demonstrated that these samples exhibited a great resistance to shear thinning throughout the process of heating. This indicated that the conjugation of gallic acid and quercetin to the corn starch network resulted in better ability of these complex samples to tolerate high temperature and shear forces. It was inferred that the reduction in breakdown viscosity induced by free radical grafting would render the phenolic–starch complexes more stable during heating and agitation [41].

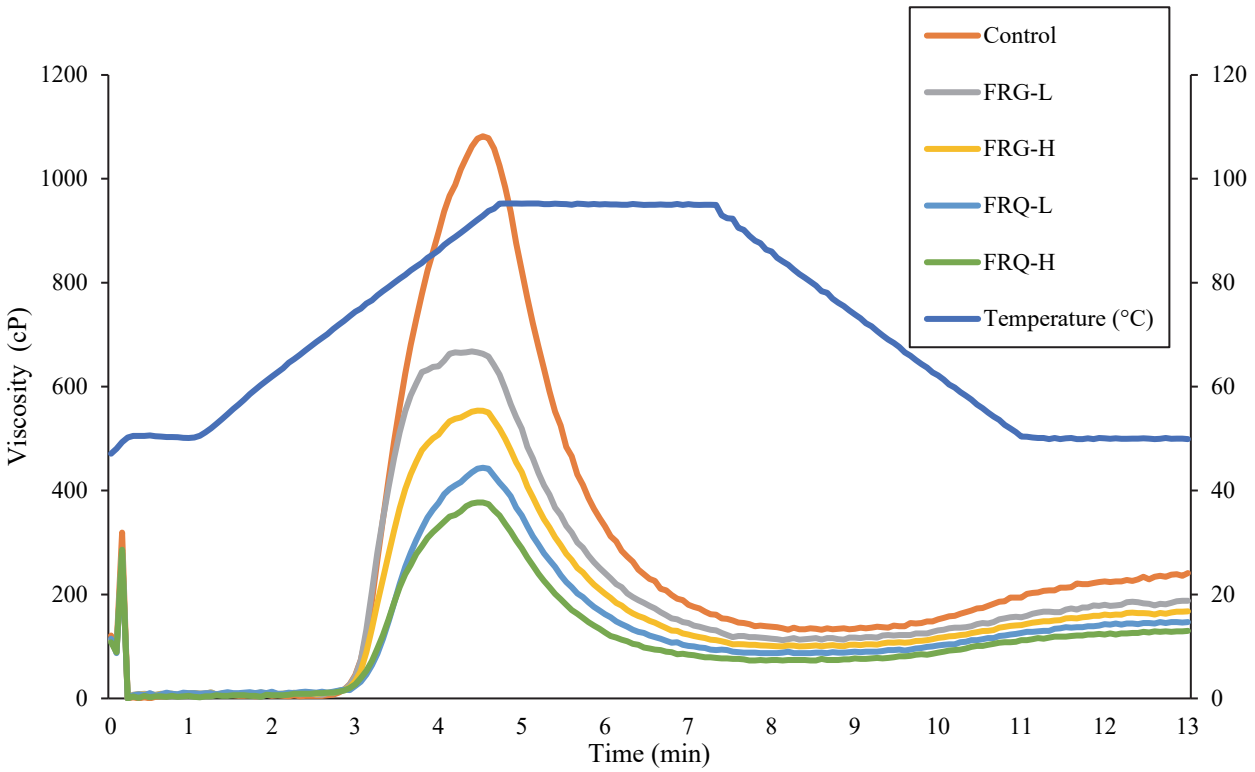

Figure 5. Viscosity profiles among different free radical treated phenolic–starch complexes.

Table 3. A comparison of viscosity behaviors among different phenolic–starch complexes.

Samples	Peak Viscosity (cP)	Breakdown Viscosity (cP)	Final Viscosity (cP)	Setback Viscosity (cP)	Pasting Temperature (°C)
Control	1066 ± 7 e	950 ± 7 e	221 ± 2 e	109 ± 3 d	72.5 ± 0.3 a
FRG-L	640 ± 6 d	552 ± 4 d	163 ± 3 d	75 ± 2 c	72.9 ± 0.4 a
FRQ-L	424 ± 3 c	359 ± 2 bc	126 ± 2 b	62 ± 4 a	75.9 ± 0.4 c
FRG-H	416 ± 3 b	356 ± 7 b	132 ± 2 c	69 ± 3 b	74.5 ± 0.3 b
FRQ-H	352 ± 3 a	306 ± 2 a	111 ± 3 a	60 ± 4 a	76.0 ± 0.1 c

a–e Values (mean ± SD) in the same column with different letters are significantly different (Duncan, $p < 0.05$).

In Table 3, the pasting temperatures of the FRQ-L, FRG-H, and FRQ-H samples were substantially greater ($p < 0.05$) than that of the control. A difference in pasting temperature indicates the varying accessibility of starch granules to hydration during the heating process. The findings from Malhotra et al. showed that starch molecules with higher pasting temperatures might be caused by reinforcing the granular structure of starch through some forms of phenolic–starch conjugations [42]. These additional intermolecular interactions permitted limited accessibility and hence increased the pasting temperature. It could be a hint inferring that the starch molecule in the complex might have a relatively stable structure against a thermal process through conjugation with phenolic compounds.

Setback viscosity occurs due to the recrystallization of amylose molecules and could be applied as a measure of the gelling ability or retrogradation ability of starches [43]. The setback of the control sample was 109 cP at first, which significantly ($p < 0.05$) decreased by 6.4–9.8% to 60–75 cP after the conjugation of gallic acid or quercetin with corn starch. As the contents of gallic acid or quercetin in the complex samples increased, an apparent downward trend was observed in the setback values in comparison to the control. The reduction in setback might be attributable to the interactions between phenolic compounds and leached amylose in the hydrophobic regions [44]. As seen in Figure 5, reductions in retrogradation level along with enhanced paste cooling stability were noted among the phenolic–starch complex samples. In general, retrogradation is less likely to occur in starch

with a smaller setback value. Based on the data, the presence of phenolic compounds might retard the short-term retrogradation of phenolic–starch complexes. It was believed that the thermally stable characteristics of phenolic–starch complexes could be used as ingredients in canned and frozen foods [45].

In contrast to the majority of resistant starches having limited usage in baking due to their poor sensory properties [46], corn starch conjugated with gallic acid or quercetin using the free radical grafting approach was capable of yielding phenolic–starch complexes of improved digestion resistance and glycemic control (Table 1) along with some desirable pasting properties including lower viscosity, thermal stability, and reduced retrogradation.

4. Conclusions

In conclusion, free radical grafting treatments using a hydrogen–ascorbic acid redox pair was applied to accelerate the conjugation of gallic acid or quercetin with corn starch molecules and provide pronounced antioxidant capability to the complex. Analysis using UPLC-Q-TOF-MS confirmed the existence of an apparent intermolecular interaction between phenolic compounds and corn starch molecules. These processes could be an alternative solution to reducing corn starch digestibility by elevating resistance to enzyme digestion and further lowering glycemic response. The inclusion of gallic acid or quercetin (at levels of 5.20 and 5.83 mg GAE/g complex, respectively) within the corn starch network apparently increased the swelling ability, water solubility, and pasting temperature of the complex samples, while a decreased tendency toward retrogradation was observed. Altogether, this process could be considered a promising way to elevate resistance to enzyme digestion, lower postprandial glycemic response, and improve the pasting properties of corn starch, which can employed in a variety of food items with specific functions.

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