Inhibition of oxidation of omega-3 polyunsaturated fatty acids and fish oil by quercetin glycosides

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The antioxidant properties of naturally occurring flavonols, quercetin glycosides, were examined and compared with common food antioxidants butylated hydroxytoluene (BHT) and α-tocopherol. Antioxidants were incorporated into selected polyunsaturated fatty acids (PUFA) or fish oil in aqueous emulsions and bulk oil systems. The effectiveness of quercetin was similar to or greater than quercetin glycosides in inhibiting lipid oxidation in the oil-in-water emulsion systems when oxidation was induced by heat, light, peroxyl radical or ferrous ion. In bulk fish oil, C-3 glycosylation enhanced the antioxidant activity of quercetin. The effectiveness of quercetin and its glycosides was greater than that of α-tocopherol in the emulsions. Quercetin and quercetin-3-O-glucoside exhibited a better antioxidant activity than BHT in bulk fish oil; however, the reverse was observed in the emulsions of omega-3 PUFA and fish oil systems in agreement with the polar paradox theory. Quercetin and its glycosides were more effective than α-tocopherol in emulsion systems.

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1. Introduction

Dietary lipids, fatty acid profiles and their balance in food and within the body have received considerable attention in recent years. This is due to a better recognition that low levels of omega-3 fatty acids may be implicated in several chronic diseases (Shahidi & Miraliakbari, 2004, 2005; Simopoulos, 2002). As a result, omega-3 fatty acid-containing functional foods and nutraceuticals have been introduced into the market. However, highly unsaturated fatty acids (HUFA) in such oils are vulnerable to oxidation, thus producing various aldehydes and ketones that render unacceptable colours, odours and flavours in polyunsaturated fatty acid (PUFA) containing foods and nutraceutical products (Nawar, 1996). Moreover, products of lipid oxidation, such as propanal, acrolein and malonaldehyde, among others, possess adverse health effects due to their cytotoxic and genotoxic effects (Esterbauer, Schaur, & Zollner, 1990; Fang, Vaca, Valsta, & Mutanen, 1996). The high rate of oxidation of PUFA can be controlled by the addition of synthetic or natural antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and α-tocopherol. Recently, consumer health consciousness has led to a demand for ‘natural’ alternatives to synthetically produced food antioxidants.

Flavonoids are a sub-group of flavonoids, found ubiquitously in fruits, vegetables and many medicinal and aromatic plants (Rupasinghe, 2008). Quercetin, a common flavonol, has been shown as an effective antioxidant in several in vitro systems such as the oxygen radical absorbance capacity (ORAC) (Ou, Hampsch-Woodill, & Prior, 2001), the ferric reducing antioxidant power (FRAP) (Pulido, Bravo, & Saura-Calixto, 2000) and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays (Kemertelidze, Tsitsishvili, Alaniya, & Sagareishvili, 2000). When compared to other flavonoids, quercetin has been shown to prolong the lag time before the initiation of low density lipoprotein (LDL) oxidation effectively (Safari & Sheikh, 2003). As well, quercetin has also been shown to inhibit lipid oxidation in cereal grains (Viscidi, Dougherty, Briggs, & Camire, 2004) and marine oils rich in PUFA (Wanasundara & Shahidi, 1998; Montero, Giménez, Pérez-Mateos, & Gómez-Guillén, 2005; Nieto et al., 1993). Heat-induced cholesterol oxidation can also be reduced by incorporation of 0.002% (w/w) quercetin (Chien, Hsu, & Chen, 2006).

In plants, quercetin occurs in the glycosylated forms such as glucoside, galactoside, rhamnoside, arabinoside and rutinoside (Herrera & Luque de Castro, 2004). In fruits such as apples, glyco-
sytlation is commonly occur at the C-3 position (Rupasinghe, 2008) but the influence of C-3 glycosylation and the type of sugar moiety on its ability to inhibit omega-3 PUFA oxidation is not clear. Therefore, the objective of this research was to examine the inhibition of oxidation of omega-3 PUFA in emulsion and fish oil by selected naturally occurring quercetin glycosides and to compare their ability with common food antioxidants such as BHT and α-tocopherol, under different oxidation-induced conditions.

2. Materials and methods

2.1. Materials and chemicals

The authentic flavonol compounds were obtained as follows: quercetin-3-O-rhamnoside (quercitrin) and quercetin-3-O-galactoside (hyperin) were from Indofine Chemical Company (Hillsborough, NJ, USA); quercetin-3-O-glucoside (isoorquercitrin) and quercetin aglycone were from Sigma–Aldrich (St. Louis, MO, USA); and quercetin-3-O-rutinoside (rutin) was from Chromadex (Santa Ana, CA, USA). Methyl linolenate (MLN) and docosahexaenoic acid (DHA) were obtained from NuChek (Elysian, MN, USA). The bulk fish oil (Canadian Food Inspection Agency [CFIA] registration number 3529) was a gift from Ocean Nutrition Canada, Dartmouth, NS, Canada. The fish oil was devoid of any antioxidants. The composition of the oil was 17.6% monounsaturates; and 77.6% polyunsaturates [61% eicosapentaenoic acid (EPA), 4.3% DHA] by weight of total fatty acids. Approximately 51% of EPA and DHA were in the triacylglycerol form. BHT, trichloroacetic acid (TCA), 2-thiobarbituric acid (TBA) and α-tocopherol were purchased from Sigma–Aldrich (St. Louis, MO, USA). 2,2’-Azobis(2-amidinopropane)dihydrochloride (AAPH) was acquired from Wako Chemicals (Richmond, VA, USA). Other chemicals were obtained from Fisher Scientific (Ottawa, ON, Canada).

2.2. The aqueous emulsion (oil-in-water) model system

The MLN, DHA and fish oil model systems were adapted from those of Okuda, McClements, and Decker (2005) and Boadi, Iyere, and Adunyah (2003) as follows: MLN or DHA (1.5 mg per mL) was suspended in a buffer solution (0.05 M TRIS–HCl, 0.15 M KCl, and 1% Tween 20, pH 7.0) by homogenisation for 20 s using a Polytron (Santa Ana, CA, USA). Methyl linolenate (MLN) and docosahexaenoic acid (DHA) were obtained from NuChek (Elysian, MN, USA). The bulk fish oil (Canadian Food Inspection Agency [CFIA] registration number 3529) was a gift from Ocean Nutrition Canada, Dartmouth, NS, Canada. The fish oil was devoid of any antioxidants.

2.3. Inductions of oxidation

The oxidation systems were optimised for determination of antioxidant properties of quercetin glycosides, α-tocopherol and BHT under various oxidation–induction conditions. MLN or DHA emulsions (1 mL final volume) were incorporated with antioxidants and then subjected to oxidation under different conditions: heat, light, iron (100 μL of 10 mM FeSO4 for the MLN and 100 μL of 1 mM FeSO4 for the DHA), or peroxyl radical (generated using 100 μL of 100 mM AAPH). Heat-induced oxidation for emulsions was carried out by incubation of test tubes at 50 °C for 10 h using an incubator-shaker oven (model Apollo HP50, CLP Tools, San Diego, CA, USA). Light-induced induction was performed using a 13 W light source (300–640 nm with peaks at approximately 440, 490, 540, 590 and 610 nm; Model Repti Glo 2.0uvB; HAGEN, China) at a distance of 12 cm. The incubation periods for peroxyl radical- and iron-induced oxidation were 20 and 3 h, respectively. These optimum induction periods were based on preliminary experiments that were conducted to study the time-oxidation relationships of the MLN model system (data not presented).

2.4. The bulk fish oil model system

The bulk fish oil model system was prepared by oxidising 90 μL of the fish oil in 13 × 100 mm borosilicate glass tubes with caps. Antioxidants were incorporated by placing 100 μL of the desired concentration of the specific compound in methanol in each test tube, drying the solvent completely under nitrogen, and then mixing with 10 μL of ethanol and 90 μL of fish oil. The final concentrations of quercetin glycosides used were 0.1, 0.5, 1 and 5 mM. Fish oil samples were incubated using the same shaker rotating horizontally (150 rpm) at 70 °C for 3 h.

2.5. Effect of the type of sugar moiety of quercetin glycosides on the inhibition of PUFA oxidation

The antioxidant properties of four naturally occurring quercetin glycosides (quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-rhamnoside, quercetin-3-O-rutinoside), quercetin, BHT and α-tocopherol were determined using 1 mL of emulsion systems of MLN and DHA (mentioned above) with peroxyl radical-induced oxidation (100 μL of 100 mM AAPH). The MLN and DHA emulsions were incorporated with 100 μL of 0.1 and 0.5 mM, respectively, of the antioxidant compounds. The final concentrations of antioxidants, 10 μM for MLN and 50 μM for DHA, were chosen based on the concentration effect as shown in Fig. 1.

2.6. Thiobarbituric acid reactive substances (TBARS) assay

After completion of the duration of the oxidation–induction treatment, TBARS were quantified by a modified method of Boadi et al. (2003) and Okuda et al. (2005), as follows: One-hundred microlitres of 2% BHT in ethanol were added to all test tubes to stop the oxidation process immediately. The TBA reagent (1 mL of 15% (w/v) TCA and 0.375% (w/v) TBA in 0.25 M HCl) was then added and vortexed. The reaction mixture was placed in an 80 °C water bath for 15 min. At the end of this time, the samples were brought to room temperature and centrifuged at 2000g for 10 min (Model 300 Precision, Durafuge, Winchester, VA, USA). The absorbance of the supernatant was then measured at 532 nm using 96-well microplates in the FLUostar OPTIMA plate reader (BMG Labtech, Durham, NC, USA). Percent inhibition of oxidation was calculated based on percentage of the total oxidation experienced by the system without the protection of antioxidants using the following equation:

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\% \text{ Inhibition of oxidation} = \left[ 1 - \left( \frac{\text{sample absorbance}}{\text{control absorbance}} \right) \right] \times 100
\]
2.7. Experimental design and statistical analysis

The experimental design was a completely randomised one with treatments being the type and concentration of antioxidants employed. All experiments were conducted in triplicate, independently twice. A one-way analysis of variance blocked by experimental run and least significant difference (LSD) multiple means analysis was used to determine significant differences among the glycoside treatments, α = 0.05, and assignment of letter groupings with SAS V8 (Cary, NC, USA). Graphical representations were made.
Using SigmaPlot 10.0 (San Jose, CA, USA). Error bars in the graphs represent standard error.

3. Results

3.1. Inhibition of PUFA oxidation by quercetin and quercetin-3-O-glucoside under various inductions

To examine the effect of C-3 glycosylation of quercetin on inhibition of PUFA oxidation, 10, 50 and 100 μM quercetin, quercetin-3-O-glucoside and BHT were incorporated into the aqueous emulsions of MLN and DHA and subjected to the oxidation by heat, light, peroxyl radical and Fe²⁺, as inducers. In general, the higher the concentration of antioxidants, greater was the inhibition of oxidation of MLN and DHA (Fig. 1). In general, BHT was more effective in its inhibition of lipid oxidation than quercetin and its glucoside. However, quercetin and its glucoside at 100 μM were as effective as BHT in inhibiting heat-induced oxidation of MLN. Regardless of the type of antioxidant and induction method, inhibition of oxidation was lower in emulsions of DHA than that of MLN (Fig. 1). Meanwhile, the antioxidant activity of quercetin was similar or better than that of quercetin-3-O-glucoside in the omega-3 PUFA emulsions. The influence of C-3 glycosylation was more distinct when the oxidation was induced by ferrous ion.

3.2. Effect of type of sugar moiety at C-3 on the inhibition of PUFA oxidation

The effect of the type of sugar moiety at C-3 of quercetin was further studied by comparing the antioxidant activity of four quercetin glycosides (quercetin-3-O-glucoside, quercetin-3-O-galactoside, quercetin-3-O-rhamnoside, quercetin-3-O-rutinoside) with their aglycone, BHT and α-tocopherol using the peroxyl radical-induced oxidation of emulsions of MLN and DHA. For the emulsions containing DHA, 50 μM antioxidants were used instead of 10 μM due to the low inhibition of oxidation at the latter level (Fig. 1). Depending on the fatty acid used for the emulsion and the type of sugar moiety, C-3 glycosylation of quercetin had either no influence or a lower effect for inhibiting the peroxyl radical-induced oxidation (Fig. 2). When the sugar moiety was a disaccharide (rutin), its antioxidant activity in DHA emulsion was lower than the corresponding monosaccharide. BHT was significantly more effective in suppressing oxidation than flavonols and α-tocopherol (Fig. 2). α-Tocopherol exhibited a significantly lower inhibition of oxidation of MLN and DHA when compared to other antioxidants tested (Fig. 2).

3.3. Inhibition of heat-induced oxidation of fish oil emulsion and bulk fish oil by quercetin and quercetin-3-O-glucoside

Incorporation of antioxidants in fish oil emulsions exhibited a concentration-dependent antioxidant activity similar to that of MLN and DHA emulsions, but BHT was more effective than flavonols in inhibiting lipid oxidation (Fig. 3A). Quercetin and quercetin-3-O-glucoside at three tested concentrations rendered similar effects (Fig. 3A). In bulk fish oil, quercetin-3-O-glucoside at 100 and 500 μM was more effective than quercetin and BHT (p < 0.001, α = 0.05), but at the 1000 μM concentration both quercetin and quercetin-3-O-glucoside were equally effective (Fig. 3B). The effectiveness of BHT in bulk fish oil was lower than that of quercetin and its glucoside.

4. Discussion

The C-3-glycosylated-quercetins examined in this study are commonly found in fruits and their processing by-products (Rupasinghe, 2008; Rupasinghe & Kean, 2008). An emulsion model system of MLN, DHA and fish oil was used to resemble food products which contain lipids dispersed in an aqueous phase (oil-in-water) such as milk, salad dressings, beverages, soups and sauces (McClements, 2004). Though a concentration-dependent inhibition of oxidation of the omega-3 PUFA and fish oil containing emulsions by quercetin, quercetin-3-O-glucoside and BHT was observed, the antioxidants tested were more effective in MLN emulsion compared to DHA and fish oil emulsions. The observed differences could be, in part, due to the fact that DHA has twice as many double bonds when compared with MLN. The oxidation of PUFA and their esters in aqueous emulsions was found to de-
crease with an increase in the number of bis-allylic positions in the fatty acids involved (Miyashita, Nara, & Ota, 1993). A higher level of UVB-radiation-induced oxidation in arachidonic acid (four double bonds) micelles than MLN micelles was also reported by Carini et al. (1998).

In the emulsion systems, quercetin-3-0-glucoside showed a similar or a lesser effect on the inhibition of oxidation depending on the concentration and oxidation-induction method when compared to quercetin itself. In addition, the type of sugar attached at C-3 of quercetin influenced the effectiveness of inhibition of PUFA oxidation in the emulsion systems. For example, when the oxidation of PUFA was induced by light, inhibition of oxidation by quercetin-3-0-glucoside and its aglycone at all tested concentrations was similar. However, a lesser effect of quercetin-3-0-glucoside compared to its aglycone was observed when the oxidation was induced by Fe2+. It may be speculated that C-3 glycosylation could hinder the ability of quercetin to chelate ferrous ion. In general, similar to the present results of heat- and Fe2+-induced oxidation, quercetin-3-O-rutinoside has been reported to be less effective in inhibiting lipid oxidation than its aglycone in lipid oxidation systems i.e. Fe2+- and peroxyl radical-induced oxidation in a liposomal system (Arora, Nair, & Strasburg, 1998) and Cu2+-induced oxidation of an LDL emulsion system (Brown, Khodr, Hider, & Rice-Evans, 1998), which also agrees with the polar paradox theory (Frankel, 1996; Porter, 1980). The polar paradox theory states that lipophilic antioxidants are more effective at inhibiting oxidation in oil-in-water emulsions, whereas hydrophilic antioxidants are more effective in bulk oil systems. This difference is thought to be due to the interfacial phenomenon, such that the hydrophilic antioxidants are positioned between the oil-air interface and the lipophilic antioxidants are dissolved in the oil droplets and thus at the water-oil interface (Frankel, 1996; Porter, 1980). Glycosylation with the disaccharide moiety, glucorhamnoside, decreased the partition coefficient (1.2–0.37) in an octanol/water mixture, or in other words, increased the hydrophilicity of the molecule (Brown et al., 1998). According to the polar paradox theory, the aglycone should have been more effective than its glycosides in both MLN and DHA emulsion systems employed. However, fatty acid emulsion models produce micellar systems as opposed to droplet systems (Decker, Warner, & Richards, 2005). Quercetin glycosides have also been shown to be less effective than their aglycone in inhibiting oxidation of PUFA in a non-emulsion system and a peroxyl radical-induced oxidation of phospholipid bilayer system (Ioku, Tsushida, Takei, Nakatani, & Terao, 1995).

In DHA emulsion, the effectiveness of flavonols is known to be less polar than flavonols (Frankel, 1996) which according to the polar paradox theory is expected to be a more effective antioxidant in the emulsion system. This discrepancy could be due to the potential micellar form of PUFA emulsions (Decker et al., 2005). Similar to our finding, Becker, Ntouma, and Skibsted (2007) found that quercetin was as effective, if not more so, than α-tocopherol in peroxyl radical-induced oxidation of oil-in-water emulsion, phospholipid liposome and bulk oil. Carini et al. (1998) also showed that a flavonoid mixture containing catechin, catechin oligomers and gallic acid catechin ester from grape seed was as effective as α-tocopherol in a UVB-induced oxidation of PUFA micellar system.

In contrast to the activity of the tested antioxidants in emulsions, the flavonols used were more effective than BHT in bulk fish oil, which is also in agreement with the polar paradox theory in that the more polar antioxidants are more effective in the bulk oil. Similar to the present results, quercetin was shown to be a more effective antioxidant than BHT in heat-induced oxidation of fish oil (Wanasundara & Shahidi, 1998). Naturally occurring flavonols or glycosylation of quercetin seem to increase the antioxidant activity due to the enhanced polarity of the aglycone. The antioxidant nature of phenolic compounds has been shown to provide a balance between the ability of the compound to enter the lipid droplet for preventing oxidation and the amount of phenolic-metal interactions within the aqueous phase (Mei, McClements, & Decker, 1999). Also, the presence of 3,4′-dihydroxy (catechol structure) in B ring, the C2–C3 double bond and the 4-keto group seems to be the most important structural features in scavenging free radicals or chain breaking antioxidant activity (Amic, Davidovic-Amic, Beslo, & Trinajstic, 2003) since the C-3 glycosylation did not diminish the ability of quercetin to inhibit PUFA oxidation. However, further experiments required to understand the effect of the positioning of sugar moiety of flavonols on the ability to inhibit PUFA oxidation in emulsion and bulk oil systems.

5. Conclusions

Although flavonols were found to be less effective in oil-in-water emulsions than, BHT, they were as effective as α-tocopherol. Interestingly, flavonols exhibited a better antioxidant activity in bulk fish oil than BHT. Considering the consumer preference for ‘natural’ products and their existence in a wide array of fruits and vegetables, naturally sourced flavonols such as quercetin glycosides could be used as effective antioxidants for stabilising omega-3 PUFA-containing foods and nutraceuticals while providing potential additional health benefits.
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