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Mechanisms by which flavonol aglycones inhibit lipid oxidation better than glycosylated flavonols in comminuted muscle tissue

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ABSTRACT

The effect of glycosylation on the ability of flavonoids to inhibit lipid oxidation in comminuted muscle tissue has not been previously examined. This work examined the ability of quercetin and quercetin-β-D-glucoside to inhibit lipid oxidation in mechanically separated turkey (MST). Quercetin inhibited formation of lipid peroxides and thiobarbituric acid reactive substances (TBARS) more effectively compared to quercetin-β-D-glucoside during frozen storage ($p < 0.05$). The possible mechanisms that cause glycosylation to decrease inhibition of lipid oxidation were also examined. Hydroxyl radical scavenging activity was similar when comparing quercetin and quercetin-β-D-glucoside, which indicated that the free hydroxyl group in 3 position of C ring in quercetin did not enhance its hydroxyl radical scavenging ability. Since muscle membrane lipids are susceptible to lipid oxidation, the ability of quercetin and quercetin-β-D-glucoside to incorporate into cellular membranes was studied. After adding quercetin and quercetin-β-D-glucoside to minced chicken muscle, flavonol content in the membrane fraction was determined. Around 32% of added quercetin partitioned into the membranes whereas quercetin-β-D-glucoside was not detected in the membranes. Similar trends were observed when each flavonol was added to isolated membranes. These studies suggest that glycosylation of flavonols weakens their ability to inhibit lipid oxidation in muscle tissue partly by decreasing the amount of flavonols in the membrane phase. In order to understand whether metal chelation by flavonols is a likely mechanism involved in the inhibition of lipid oxidation in MST, the role of endogenous metals in promoting lipid oxidation was examined. Addition of the metal chelators ethylenediamine tetraacetic acid (EDTA) and tetrakis(2-pyridylmethyl) ethylenediamine (TPEN) to MST did not inhibit lipid oxidation, which suggests that endogenous metals present in MST were not promoters of lipid oxidation. Hence it seems unlikely that the mechanism of inhibition by flavonols involved metal chelation in the comminuted muscle.

1. Introduction

Muscle foods are susceptible to lipid oxidation which negatively affects the quality and economic value of the muscle products. Incorporation of antioxidants into foods can effectively retard lipid oxidation (Labuza, 1971). In our previous work, we found that a fraction isolated from an amphiphilic extract prepared from cranberry juice powder was an effective inhibitor of lipid oxidation in mechanically separated turkey (MST). The compound responsible for the inhibitory activity was then identified as quercetin. It was evident from these studies that glycosylated quercetin in the extract did not contribute to the efficacy of the isolated fraction in MST (Kathirvel, Gong, & Richards, 2009).

For a better understanding of how glycosylation of flavonols affects the antioxidant activity in comminuted muscle tissue systems, a comparative study between quercetin and quercetin-β-D-glucoside was conducted. Quercetin and quercetin-β-D-glucoside belong to the class of flavonoids known as flavonols. Quercetin and quercetin-β-D-glucoside are structurally similar flavonols except for the presence of a glucose moiety at 3-hydroxy position in quercetin-β-D-glucoside (Fig. 1). Flavonols are widely distributed in plant foods (Bahorun, Luximon-Ramma, Crozier, & Aruoma, 2004; Justesen, Knuthsen, & Leth, 1998; Vvedenskaya & Vorsa, 2004) and have received considerable attention for their antioxidant properties (Crozier et al., 2000; Mira et al., 2002; Plumb, Price, & Williamson, 1999). The structure–activity relationship of flavonols depends on the test system used to determine lipid oxidation. The antioxidant activity of flavonols has been extensively studied in methyl lineolate, liposome systems and fish lipids (Hopia & Heinonen, 1998; Liao & Yin, 2000; Pazos, Gallardo, Torres, & Medina, 2005). Free radical scavenging and metal chelation have been described to be the key factors responsible for the antioxidative

Abbreviations: MST, mechanically separated turkey; TBARS, thiobarbituric acid reactive substances; HPLC, high performance liquid chromatograph; EDTA, ethylenediamine tetraacetic acid; TPEN, tetrakis(2-pyridylmethyl) ethylenediamine.

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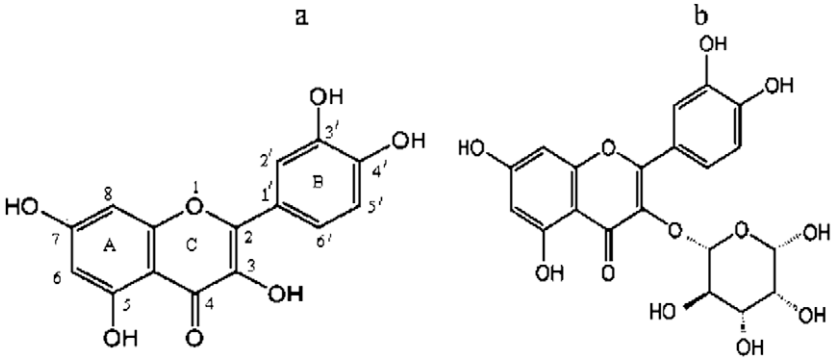

Fig. 1. Structure of (a) quercetin and (b) quercetin-β-D-glucoside.

activities of flavonols (Bors, Heller, Michel, & Saran, 1990; Mira et al., 2002).

Partitioning of flavonols in octanol and water phases has been reported (Rothwell, Day, & Morgan, 2005) and has been described as a useful way to determine the behaviour of flavonols towards the membrane phase. In muscle foods, membrane phospholipids are believed to be the most susceptible lipid fraction to lipid oxidation compared to neutral lipids (e.g. triacylglycerols), mainly due to the higher degree of fatty acid unsaturation and increased surface area of the membranes (Pikul, Leszczynski, & Kummerow, 1984). Partitioning of antioxidants to sites where the lipids are most susceptible to oxidation might be important in reducing the extent of lipid oxidation in muscle foods. Studies that probe the importance of flavonoid partitioning in muscle membranes are still lacking. The purpose of the work here was to compare the ability of two commonly occurring flavonols in plant foods, quercetin and quercetin-β-D-glucoside to inhibit lipid oxidation in mechanically separated turkey (MST) as well as to assess the possible mechanisms by which these flavonols exhibit their antioxidative properties in muscle tissue. MST was used as a substrate for oxidation study mainly due to its high susceptibility to lipid oxidation and use as a raw material in formulation of processed meats such as bologna and wieners. This study helps in the better understanding of how structural changes influence the antioxidant activity of flavonols in a muscle tissue matrix.

2. Materials and methods

2.1. Materials

Mechanically separated turkey (MST) was obtained from Kraft-Oscar Mayer (Newberry, SC) and was vacuum packaged and stored at −80 °C until use. Broiler chicks (Cornish Rock) obtained from Sunnyside Farms, WI were raised in the Poultry Research Lab (PRL), UW-Madison. Chicken breast muscle used for partitioning studies was harvested from broiler chickens raised in the PRL. Sealable, polyethylene bags (10 cm × 15 cm) and vacuum pouches (3 ml standard barrier) were purchased from Koch Supplies (Chicago, IL). Ethanol (absolute, 200 proof) was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, Kentucky). 2-Thiobarbituric acid, sodium chloride, potassium hydroxide, sodium hydroxide, disodium ethylenediamine-tetraacetic acid, propyl gallate, potassium dihydrogen phosphate, deoxyribose, ferric chloride, ferrous sulphate, hydrogen peroxide, sodium ascorbate, Proteinase, HEPES buffer (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid buffer hemisodium salt), ethylene diamine tetraacetic acid (EDTA), tetrakis(2-pyridylmethyl) ethylenediamine (TPEN), quercetin dihydrate and quercetin-β-D-glucoside were procured from Sigma A/S (St. Louis, MO). Ammonium thiocyanate, barium chloride, hydrochloric acid, sulphuric acid, trichloroacetic acid, sodium carbonate, methanol, 1-butanol and chloroform were purchased from Fisher Scientific (Chicago, IL). The solvents used were of HPLC grade.

2.2. High Performance Liquid Chromatograph (HPLC) apparatus and chromatographic conditions

HPLC analysis of flavonols were performed on a Agilent 1100 HPLC system equipped with an Agilent 1100 series binary pump and photodiode array detector (Agilent, Wilmington, DE). A C18 reversed phase column (Discovery 5 μm, 25 cm × 0.46 cm i.d.) (Supelco, Bellefonte, PA) was used. The binary solvent system used was: Solvent A, 0.1% trifluoroacetic acid in water and solvent B, 0.1% trifluoroacetic acid in methanol. To determine the flavonols that partitioned into the membranes, membrane lipids were extracted using chloroform:methanol (1:1 v/v) and NaCl solution and both the chloroform and methanol/water phases were analyzed. To analyze the chloroform phase, it was first evaporated and lipids were redissolved in methanol:butanol (1:1v/v) before injecting into the HPLC system. A linear gradient of 0–100% B from 0 to 40 min was carried out at a flow rate of 1 ml/min. Multiwavelength detection was monitored at 280, 320, 360 and 520 nm. Quercetin and quercetin-β-D-glucoside were used as standards.

2.3. Addition of flavonols to MST

MST was vacuum packaged in 500 g portions and stored at −80 °C until use. The frozen packs were then thawed for 2 h at room temperature (without breaking the vacuum seal) before using them for treatments. Quercetin and quercetin-β-D-glucoside were added at 100 μmol/kg MST and thoroughly mixed with a spatula to ensure uniform distribution. All samples were stored in zip-lock polyethylene bags at −4 °C during the study period. The carrier solvent was 100% ethanol and was used at 1% of the final sample weight. A control sample containing 1% ethanol without the flavonols was also prepared for the respective trials.

2.4. Isolation of crude membranes from MST

The method described by Raghavan and Hultin (2005) was used for the isolation of crude membranes from MST. Ten grams of muscle was mixed with four volumes of cold 0.1 M HEPES buffer containing 0.2% (w/v) sodium ascorbate (pH 7.5) and homogenized at speed 6 for 40 s using a Kinematica Polytron PT 10–35 homogenizer (Brinkmann Instruments, Westburg, NY). The pH of the homogenate was adjusted to 7.5 and was centrifuged at 10,000*g* for 20 min at a sample temperature of 7–9 °C using a Beckman