In Vitro Iron Bioavailability and Antioxidant Activity of Raisins


ABSTRACT: Iron bioavailabilities and antioxidant activities of 3 generic raisin types—Golden Thompson, Dipped Thompson, and Sun-dried Thompson—were quantified and compared. Iron bioavailability was assessed with an in vitro digestion/Caco-2 cell culture model using cell ferritin formation as an index of iron bioavailability. Antioxidant activity was determined using the total oxyradical-scavenging capacity (TOSC) assay. Ferritin formation in Caco-2 cells was low for all 3 raisin types, indicating low iron bioavailability. Ethylenediaminetetraacetic acid (EDTA) enhanced iron bioavailability from raisins but ascorbic, citric, and tartaric acids showed no effect. Antioxidant activity was significantly higher in Golden Thompson than Dipped Thompson and Sun-dried Thompson, suggesting that enzymatic browning negatively affected antioxidant activity.

Keywords: raisins, iron, bioavailability, antioxidant activity, Caco-2 cell culture

Introduction

Owing to their perceived nutritive value and sweet taste, raisins have been a popular food for many centuries (Gunston 1983; Witherspoon 2000). Raisins have also been described as good sources of micronutrients including iron (Witherspoon 2000). Nonetheless, the bioavailability of raisin iron has not been thoroughly studied. The bioavailability of nonheme iron in foods may be greatly influenced by the presence of absorption enhancers or inhibitors in the same meal (Cook 1983; Hulten and others 1995). Potential enhancers of iron absorption in raisins include tartaric and malic acids (Gilloy and others 1983), although the effects of these 2 organic acids are not as strong as the promoting effects of ascorbic acid present in citrus fruit (Hazell and Johnson 1987). Raisins are also rich in phenolic compounds (Karadeniz and others 2000). These compounds (for example, tannins), on the contrary, are well known inhibitors of iron absorption due to the formation of complexes with iron in the gastro-intestinal lumen (Brune and others 1989). Studies on mushrooms showed that phenolic compounds inhibited in vitro iron bioavailability (DaDamio and Thompson 1992).

Phenolic compounds also act as substrates for enzymatic browning reactions in raisins. These reactions, catalyzed by polyphenol oxidase in grapes, give sun-dried raisins their typical dark reddish-brown color. Polyphenol oxidase is a generic term for the groups of enzymes that catalyze the oxidation of phenolic compounds to produce brown color on cut surfaces of fruits and vegetables (Whitaker and Lee 1995). Prevention of enzymatic browning is key to the production of light-colored raisins, such as the Golden Thompson Seedless raisins. The most common method of preventing enzymatic browning in raisin production is the addition of reducing agents, such as sulfur dioxide and sodium metabisulfite. These agents prevent browning by reducing enzymatically formed quinones back to dihydroxynones, or by reacting with quinone intermediates to form sulfones, which may irreversibly inhibit dihydroxynone oxidase (Walker 1995). Nonetheless, the effects of enzymatic browning reactions that take place during sun-drying of raisins on iron bioavailability are not known.

Despite a lack of known nutritional activity in fruits and vegetables, phenolic constituents present in grapes and wines are capable of scavenging free radicals, suggesting that phenolic compounds, when consumed in diets, could function as antioxidants (Sanchez-Moreno and others 1999). Epidemiological studies in the Netherlands showed that intakes of flavonoids (a category of phenolic compounds) and several related compounds were inversely associated with coronary heart disease (Hollman and others 1996). In another study, Karakaya and others (2001) ranked raisins among the highest in the total phenolic content and total antioxidant activity in solid fruit products. They also concluded that antioxidant activity was positively correlated with the phenolic content of a fruit.

Iron deficiency affects more than 30% of the world’s population, particularly women of reproductive age, infants, and children. Problems caused by iron deficiency constitute major sources of morbidity and are underlying factors of human suffering on a global scale (Gillespie 1998). The international research agenda advocates the utilization of agricultural practices and food-based systems as sustainable solutions to micronutrient malnutrition. Specifically, it calls for research on increasing the effectiveness of foods as sources of micronutrients and increasing the supply of micronutrient-rich foods as well as the micronutrient densities of foods (Combs and others 1996). Research into the iron content and bioavailability of raisins is directly in agreement with this international agenda. As an agricultural product and common component of many popular foods and desserts, raisins have the potential to significantly increase the concentration of dietary iron. Therefore, the 1st objective of this study was to quantify and compare the iron bioavailabilities of 3 common generic raisin types. In addition, there has been a rapidly increasing interest in identifying foods with health-related functional properties. Therefore, the 2nd objective of this study was to compare the antioxidant activities of raisins produced under different processing conditions.

Materials and Methods

Raisin samples

The iron bioavailabilities and antioxidant activities of 3 generic raisin types—Golden Thompson, Dipped Thompson, and Sun-dried Thompson—were studied. The raisin samples were a gift from Victor Packing Inc. (Madera, Calif., U.S.A.), which gathered these 3 types of raisins from hundreds of growers in San Joaquin Valley of California. All raisins were harvested in 2000. To eliminate batch-to-batch variations in raisin composition, 3 batches of each raisin type (at 3
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different packing dates) were mingled to give a representative raisin sample. Karadeniz and others (2000) have outlined the characteristics of the production processes for the 3 raisin types. Briefly, Sun-dried Thompson raisins were dried completely out of doors under the sun in the vineyard, whereas Dipped Thompson raisins were produced by 1st dipping the raisin grapes in hot water, followed by drying mechanically in a dehydration tunnel. For Golden Thompson, the raisin grapes were 1st treated with sulfur dioxide, which acted as a reducing agent, to preserve the golden color before they were mechanically dehydrated.

Determination of total iron content

An AOAC wet-ashing method (AOAC 1984), modified by Kosse and others (2001), was used to ash the raisin samples. Samples (0.5 g) were 1st placed in 25 mm × 150 mm (50 mL) Pyrex test tubes. One to two mL of concentrated HNO3 was dispensed into each tube, and the samples were allowed to partially-digest overnight in a fume hood at room temperature. The tubes were then placed in a digestion block inside a HC1O4-rated fume hood, and the temperature of the digestion block was raised to between 50 and 120 °C to dry the sample. Another 1 to 2 mL of HNO3 was then added, and the heating was continued until the second aliquot of HNO3 was completely evaporated. At this point, the temperature of the digestion block was raised to 150 °C. The HNO3 digestion step mentioned above would be repeated until the heated samples no longer gave off red brown fumes of nitrous oxide, and the samples were light brown to yellow in color. One half mL of a 50/50 (v/v) mixture of HNO3/HClO4 acids was then added to the samples. The temperature of the digestion block was increased to 180 °C, and the color of the samples was monitored for 1 h. When the digested samples became light yellow to colorless, the temperature of the digestion block was raised to 240 °C, and the samples were heated to dryness. The ashed samples were then re-dissolved in 0.25 mL of concentrated HCl and 10 mL of 5% HNO3, and the iron contents were analyzed by inductively coupled plasma atomic emission spectroscopy (ICAP-61E Thermal Jarrell Ash Trace Analyzer, Jarrell Ash Co., Franklin, Mass., U.S.A.).

Measurement of iron bioavailability

Bioavailability of iron from raisins was assessed with the in vitro digestion/Caco-2 cell culture model described by Glahn and others (1998b). Figure 1 shows a diagram of the in vitro digestion/Caco-2 cell culture model. Briefly, raisins were 1st blended into a puree in a buffer containing 140 mM NaCl and 5 mM KCl (at a concentration of 0.5 g raisin per 15 mL of buffer), adjusted to pH 2 with 0.1 M HCl, and incubated at 37 °C for 1 h in the presence of peptide. After the simulated intestinal digestion, the upper chamber was then adjusted to pH 7 with 0.1 M NaHCO3 and incubated at 37 °C for 2 more h in the presence of a mixture of pancreatic enzymes and bile salts to simulate intestinal digestion. This pancreatic-bile digestion took place in a small upper chamber positioned over a monolayer of cultured Caco-2 cells. Caco-2 cells (at passage 30-35) had been seeded at a density of 50000 cell/cm2. Previous studies indicated that the seeded cells reach confluence after 13 d (Glahn and others 1998b); hence, the experiment was conducted 13 d post seeding. The contents of the upper chamber were separated from the Caco-2 cell layer by a 15000 molecular weight cut-off dialysis membrane (Spectra/Por 2.1, Spectrum Medical, Garden, Calif., U.S.A.) that allowed iron released from the raisin samples to diffuse into the medium bathing the cells in the lower chamber. This soluble, low molecular weight iron would be taken up by the cells in proportion to its bioavailability.

After the simulated intestinal digestion, the upper chamber was removed, and the cells were further incubated at 37 °C for 24 h to allow ferritin to form. The growth medium was then removed and the cell monolayer was harvested from the bottom chamber by adding de-ionized water and placing in a sonicator to disrupt the cells. Caco-2 cells synthesize ferritin in response to increases in intracellular iron concentration; therefore, ferritin concentration in the cells was determined on an aliquot of the cell suspension using a radioimmuno assay (RAMCO, Houston, Texas, U.S.A.) and used as an index of iron uptake. The use of ferritin formation as a marker of cell iron uptake avoids the need for radio labeling of the raisin iron. This technique has been validated with numerous studies comparing iron bioavailability from various foods and iron supplements (Glahn and others 1998a, 1999, 2002).

To further study the effects of raisins on the bioavailability of fortification iron with different chelating agents, purees of Sun-dried Thompson raisins were fortified with 20 μM of ferrous sulfate plus either 200 μM of ascorbic acid, citric acid, ethylenediaminetetraacetic acid (EDTA), or tartaric acid. The bioavailability of iron from the fortified purees was assessed with the in vitro digestion/Caco-2 cell culture model aforementioned.

Measurement of antioxidant activity

Phytochemicals of the raisin samples were extracted using 80% (v/v) acetone in water (Eberhardt and others 2000). The total antioxidant activity of each raisin extract was then measured by the total oxyradical-scavenging capacity (TOSC) assay (Winston and others 1998). The TOSC assay is based on the partial inhibition by antioxidants of ethylene formation during the oxidative reaction between 2,2’-azobis-amidinopropane (ABAP) and α-keto-γ-methiolbutyric acid (KMBA). Antioxidant activities of raisin extracts were assessed at 4 different time points (15, 30, 45, and 60-min) to determine their TOSC values, and results were expressed as μmol vitamin C equivalents per g of raisins (Eberhardt and others 2000).

Since antioxidant activities may be correlated with the phenolic contents of foods, the total phenolic and flavonoid contents of the 3 raisin extracts were also determined using methods described by Singleton and others (1998) and Jia and others (1999) respectively. Total phenolic and flavonoid contents were expressed as μmol gallic acid equivalents and μmol catechin equivalents per g of raisins, respectively.

Statistical analysis

In vitro iron bioavailability and antioxidant activity measurements were replicated 6 times. All compositional analyses were replicated a minimum of 6 times. Data were analyzed by analysis of variance (ANOVA), and, when appropriate, means were separated by Fisher least significant difference (LSD) procedures.

Results and Discussion

Iron content

Table 1 shows the iron contents of the 3 raisin types. The concentrations of iron in Golden Thompson, Dipped Thompson, and Sun-dried Thompson raisins were 8.91, 9.38, and 11.18 μg/g, respectively, or approximately 6% of the Recommended Dietary Allowance for iron for adult male per quarter-cup serving. The iron content of Sun-dried Thompson was significantly higher than the other 2 raisin types. Since all the raisin samples originated from the same variety, that is, Thompson Seedless grapes, their average intrinsic iron contents should be roughly the same. Although variations in intrinsic raisin iron content could arise as a result of different growth locations, these variations were minimized by collecting samples of each raisin type from many different growers in the same geographical area, and by mingling collected raisins to give representative
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**Table 1—Total iron content, total phenolic content, and flavonoid content of the 3 raisin types (mean ± SEM)**

<table>
<thead>
<tr>
<th>Raisin type</th>
<th>Total iron content* (μmol)</th>
<th>Total phenolic content* (μmol gallic acid equivalents)</th>
<th>Flavonoid content* (μmol catechin equivalents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golden Thompson</td>
<td>0.160 ± 0.009a</td>
<td>33.8 ± 1.3a</td>
<td>6.42 ± 0.42a</td>
</tr>
<tr>
<td>Dipped Thompson</td>
<td>0.168 ± 0.010a</td>
<td>11.5 ± 1.1b</td>
<td>1.17 ± 0.12b</td>
</tr>
<tr>
<td>Sun-dried Thompson</td>
<td>0.200 ± 0.012b</td>
<td>10.8 ± 1.8b</td>
<td>0.91 ± 0.16b</td>
</tr>
</tbody>
</table>

*Means with different superscripts within the same column are significantly different (p < 0.05).*

samples. Hence, the higher iron content for the Sun-dried Thompson raisins could be due to contamination iron. Both Golden Thompson and Dipped Thompson raisins were mechanically dehydrated, whereas Sun-dried Thompson raisins were dried out of doors in the vineyard for up to 4 wk. It is possible that the higher iron content of Sun-dried Thompson raisins was the result of longer exposure to natural iron contaminants (for example, soil particles and dust) during the sun-drying process.

**Iron bioavailability**

Figure 2 shows the results of the iron bioavailability measurements assessed with the in vitro digestion/Caco-2 cell culture model. Intracellular ferritin formation in Caco-2 cells exposed to the raisin digests was used as an index of iron bioavailability, and the results were expressed as ng of ferritin per mg of cell protein.

Although no iron was added to the cell culture media, Caco-2 cells from the control showed a baseline ferritin level of 3 ng/mg. This was presumably a response to contamination iron present in the growth medium, which was found to contain 0.19 μg of iron per mL of medium. Ferritin levels were not significantly different between the control and the blank digest, indicating that the use of pepsin and pancreatin-bile mixture in the simulated peptic and intestinal digestion steps did not contaminate the Caco-2 cell culture model system. When the blank digest was fortified with an amount of iron (in the form of ferrous sulfate) approximately equivalent to the amount expected to be present in the raisin samples, ferritin level increased dramatically, verifying that the system was responding positively to bioavailable iron. Nonetheless, all 3 raisin types—Golden Thompson, Dipped Thompson, and Sun-dried Thompson—showed ferritin levels lower than the baseline level in the control. This suggested not only that iron from the raisins was not bioavailable, but also that raisins rendered some of the iron present in the growth medium unavailable to Caco-2 cells. These results suggest that raisins contain a high level of iron absorption inhibitors. Owing to the high total phenolic contents in raisins as shown in Table 1, it is not surprising that raisin iron has low bioavailability. For example, in the case of Sun-dried Thompson, which had the highest iron content and lowest total phenolic content among the raisin types, the mole ratio of phenolic compounds to iron was > 50.

To further study the inhibitory effects of raisins on iron bioavailability, we fortified the puree of Sun-dried Thompson raisins with iron and some common iron chelating agents and measured the resulting iron bioavailability. When the raisin puree was fortified with ferrous sulfate (20 μM) and fed to Caco-2 cells, there was no significant change in ferritin level as shown in Figure 3. The addition of 20 μM of ferrous sulfate plus 200 μM of ascorbic acid, a well-known iron absorption enhancer, did not increase the ferritin level, and neither did the addition of citric acid or tartaric acid. This lack of response in ferritin levels was a further indication of the high levels of iron absorption inhibitors present in raisins. Presumably, the effects of these inhibitors were so overwhelming that even uptake of the fortification iron by Caco-2 cells was inhibited. The addition of ascorbic acid, citric acid, or tartaric acid did not overcome these inhibitory effects.

When the raisin puree was fortified with 20 μM of ferrous sulfate plus 200 μM of EDTA, a 5-fold increase in ferritin level was observed.
Sensory and Nutritive Qualities of Food

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(3) EDTA is a strong metal chelator that can significantly increase the bioavailability of iron in the presence of iron absorption inhibitors (INACG 1993). It has been suggested that EDTA binds iron more strongly than citric acid (Hegenauer and others 1979), and Fe$^{3+}$ bound to an EDTA complex is not reducible by ascorbic acid at pH 2.6 to 6.0 (Hsieh and Hsieh 1997). Another study by South and Miller (1998) showed that both EDTA and ascorbic acid bind iron and prevent iron from forming complex with tannic acid, a hydrolyzable tannin that inhibits iron absorption, but EDTA is also capable of displacing iron from an iron/tannic acid complex. Whereas if iron forms complexes with tannic acid before exposure to ascorbic acid, ascorbic acid is not capable of displacing iron from the iron/tannic acid complex.

Several animal studies have shown that iron from NaFe(III)EDTA, a promising iron fortificant provisionally approved by the joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives for programmatic use (FAO/WHO 1993), is absorbed as well or better than iron from ferrous sulfate (Whittaker and Vanderween 1990; Dutra-de-Oliveira and others 1993). Human trials have also confirmed that iron bioavailability from NaFe(III)EDTA in diets containing significant amounts of iron (Whittaker and Lee 1995). Future research on identifying its absorption pathway and regulatory mechanism is warranted so as to determine if iron from EDTA complexes can be effectively down-regulated for individuals with adequate body iron stores.

Antioxidant activity

The TOSC value of Golden Thompson raisins was significantly higher than the other 2 raisin types, indicating much higher antioxidant activity of Golden Thompson raisins (Figure 4). A similar trend was observed in the total phenolic and flavonoid contents of raisins with the total phenolic and flavonoid contents of Golden Thompson raisins being about 3-fold higher than those of the other 2 raisin types (Table 1). These results suggest that enzymatic browning causes a decrease in antioxidant activity, as well as losses of phenolic compounds and flavonoids in raisins. These observations are consistent with the report by Karakaya and others (2001) that antioxidant activities of solid fruit products are positively correlated with their total phenolic contents.

Golden Thompson raisins used in this study were treated with sulfur dioxide in their production process to inhibit enzymatic browning. Enzymatic browning in grapes is catalyzed by catecholase (ortho-diphenol oxidase) enzymes, which convert o-diphenols to o-benzoquinones (Walker 1995). The o-benzoquinones are unstable and, in the presence of O$_2$, are further oxidized nonenzymatically and polymerized to form melanoids, the pigments responsible for the characteristic dark brown color of raisins. In fresh grapes, catecholase is physically compartmentalized from its substrates in the intact cell. In addition, the outer waxy layer of grape skins limits O$_2$ entry into the cell (Whitaker and Lee 1995). However, during raisin processing, these barriers are damaged as grapes lose their moisture and begin to contract. It has been shown that flavonoids with multiple hydroxyl group substitutions have higher antioxidant activities against peroxyl radicals than flavonoids without hydroxyl group substitutions (Cao and others 1997). In addition, the 2 ortho-hydroxyl groups (the 2 groups present in o-diphenols) are particularly important to the peroxyl radical-absorbing capacity of a flavonoid (Cao and others 1997). Hence, it is reasonable to speculate that Golden Thompson raisins retain more of these hydroxyl groups in their phenolic compounds due to the inhibition of catecholase by sulfur dioxide treatment, and so demonstrate higher antioxidant activity.
activity than the other 2 raisin types. Conversely, the lower antioxidant activities in Dipped and Sun-dried Thompson raisins suggest that enzymatic browning causes a decrease in antioxidant activity.

**Conclusions**

Our results show that the bioavailability of iron is low in all 3 generic raisin types. Given that the concentration of total phenolic compounds (in μmol gallic acid equivalents) is at least 50 times higher than the concentration of iron present in raisins, it is not surprising that raisin iron is poorly bioavailable, presumably due to the well-known inhibitory effects of phenolic compounds. In addition, under such a high concentration of phenolic compounds, of all chelating agents (enhancers) we tested, only EDTA appears to be effective in enhancing the bioavailability of raisin iron. Golden Thompson raisins, treated with sulfur dioxide in their production process to inhibit enzymatic browning, have much higher total phenolic content, hence higher antioxidant activity, than Dipped and Sun-dried Thompson raisins. The lower antioxidant activities in the latter 2 raisin types suggest that enzymatic browning causes a decrease in antioxidant activity.

**References**


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