

## Partitioning and Inhibition of Lipid Oxidation in Mechanically Separated Turkey by Components of Cranberry Press Cake

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Extracts from cranberry press cakes were prepared either using ethanol or an ethyl acetate–acetone mixture. The press cake extracts were compared with extracts from cranberry juice powder (CJP), prepared using chloroform:methanol (1:1), for their ability to inhibit lipid oxidation in mechanically separated turkey (MST). Because of the susceptibility of muscle membrane lipids to oxidation, the ability of quercetin in the extracts to partition between the aqueous and the membrane phases was studied. Membrane suspensions were prepared from MST. Partitioning of quercetin was quantified using high-performance liquid chromatography. Oxidation was studied by measuring thiobarbituric acid reactive substances and lipid peroxides. The effectiveness of the extracts to inhibit lipid oxidation was CJP extract > ethyl acetate extract of press cake  $\geq$  ethanol extract of press cake. The amount of quercetin in the extracts and the amount of quercetin that partitioned into the membranes followed the same order. However, the total phenolic content of the extracts did not follow the same order as that of inhibitory power. The phenolic content of the extracts decreased, ethyl acetate extract > ethanol extract of press cake  $\geq$  chloroform extract of CJP. Irrespective of the extraction method, around 78% quercetin from the extracts partitioned into the membranes. It could be concluded that increasing the amount of quercetin in the press cake extracts increases the ability of the extracts to inhibit lipid oxidation in MST. Hence, a proper choice of solvents and extraction method, which would increase the amount of quercetin in the press cake extracts, might increase the antioxidant potential of the extracts and hence their economic value.

**KEYWORDS:** Antioxidant; cranberry; ethanol

### INTRODUCTION

Muscle foods are susceptible to oxidation, which leads to quality deterioration and a loss in the economic value of the muscle product (1). Antioxidants are generally used to inhibit or delay oxidation in food products. Antioxidants could be classified into two categories, natural and synthetic. Because of safety and carcinogenicity issues associated with synthetic antioxidants (2, 3), natural antioxidants are increasingly preferred in muscle food products. Polyphenols have often been used as inhibitors of lipid oxidation in food systems (4–6). Cranberries (*Vaccinium macrocarpon* Ait) are a rich source of phenolic compounds such as flavonols and anthocyanins (7). One of the products prepared from cranberries is cranberry juice. A major underutilized byproduct from cranberry juice production is cranberry press cake, containing seeds and skins. Cranberry press cake contains many phenolic compounds (8) and could be used as a potential source for preparing antioxidant extracts (9). A number of researchers have examined the role of polyphenols from cranberry fruits for their radical scavenging activities (10, 11). Some studies have also examined the role

of cranberry seeds for their antioxidant and radical scavenging properties (12). However, very few studies have examined the role of cranberry press cake as an antioxidant in a food system. The first objective of our research was to prepare antioxidant extracts from cranberry press cake using solvents of varying polarity and screen those extracts for their ability to inhibit the formation of thiobarbituric acid reactive substances (TBARS) in mechanically separated turkey (MST). In our studies (data not shown), we found that the chloroform extract of cranberry juice powder (CJP) was more effective in inhibiting lipid oxidation in MST as compared to extracts prepared using other solvents. The second objective of our research was to compare the ability of press cake extracts with the chloroform extract of CJP to inhibit TBARS and lipid peroxide formation in MST.

Among the various lipids present in muscle foods, membrane lipids are most susceptible to oxidation due to their polyunsaturation and large surface area (13). Directing antioxidants into the membranes could increase the shelf life of muscle foods (14, 15). In our study, we prepared a crude membrane suspension from MST and determined the ability of two press cake extracts (ethanol and ethyl acetate) and the CJP extract to partition into the membranes from an aqueous suspension. We

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attempted to correlate the TBARS inhibitory power of the extracts with the ability of quercetin, a component of the extracts, to partition into the membranes from an aqueous solution of the extracts.

## MATERIALS AND METHODS

**Materials.** Dried cranberry juice (Cranberry 90MX powder) was supplied by Ocean Spray Cranberries Ingredient Technologies Group (Middleboro, MA). Cranberry 90MX powder was stored at  $-20\text{ }^{\circ}\text{C}$  until use. Cranberry press cake was packaged with frozen gel packs in an insulated packaging material and was shipped by Ocean Spray Cranberries, Inc., from Tomah, WI, by overnight delivery. Upon arrival, the cranberry press cake was immediately repackaged in Zip Lock plastic bags with around 300 g in each bag and stored at  $-20\text{ }^{\circ}\text{C}$  until use. The maximum recommended temperature for storing cranberry fruits is around  $4\text{--}5\text{ }^{\circ}\text{C}$  (16). However, as no data were available on the optimum storage conditions for cranberry press cake, we chose a storage temperature of  $-20\text{ }^{\circ}\text{C}$ . MST prepared from freshly processed turkeys was shipped from Newberry, SC, to Kraft-Oscar Mayer (Madison, WI) by refrigerated trucking. The MST was then immediately vacuum packaged and stored at  $-80\text{ }^{\circ}\text{C}$  until use. Distilled water was collected using Milli-Q plus (Millipore, Billerica, MA). Zip Lock bags (10 cm  $\times$  15 cm) and vacuum pouch 3 mil standard barrier were purchased from Koch Supplies (Chicago, IL). Chemicals and solvents were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents were of ACS grade.

**Extraction of Cranberry 90MX Powder Using Chloroform:Methanol.** Fifty grams of cranberry 90MX powder was blended with 500 mL of a 1:1 chloroform:methanol mixture in a Waring commercial blender model 51BL32 (Waring Commercial, Torrington, CT) for 1 min. The mixture was stirred for 2 h using a Fisher Isotemp magnetic stirrer at room temperature. One hundred milliliters of 0.5% NaCl was added, and the mixture was transferred into a separatory funnel for phase separation. The chloroform layer was separated, mixed with 20 mL of water, and evaporated using a Büchi rotavapor, model R200 (Büchi Labortechnik AG, Switzerland) under vacuum at  $30\text{--}35\text{ }^{\circ}\text{C}$ , until the volume was concentrated to less than 20 mL. The concentrated extract was freeze-dried using a freeze drier, model 52647 (Labconco Corp., Kansas City, MO), and stored at  $-80\text{ }^{\circ}\text{C}$  for high-performance liquid chromatography (HPLC) and antioxidant studies.

**Preparation of Cranberry Press Cake Extracts.** Five different solvent mixtures were used for the extraction of cranberry press cake. The solvents were water (100%), hexane (100%), chloroform:methanol (50:50), ethanol:water (70:30), and a two-step extraction method using acetone:water (70:30) and ethyl acetate. Dielectric constants of the solvent mixtures were calculated based on the standard dielectric constant at  $25\text{ }^{\circ}\text{C}$  and using the formula,  $\epsilon$  (mixture) = (%A  $\epsilon_A$  + %B  $\epsilon_B$ )/100, where  $\epsilon_A$  and  $\epsilon_B$  are the dielectric constants of carrier solvents A and B while  $\epsilon$  (mixture) is the dielectric constant of the resultant mixture (17).

**Preparation of Press Cake Extracts Using Water, Hexane, or Ethanol:Water (70:30) Mixture.** Fifty grams of cranberry press cake was blended with 500 mL of hexane, water, or a mixture of (70:30) ethanol:water in a Waring commercial blender for 1 min. The mixture was stirred for 2 h at room temperature and filtered using Whatman filter paper no. 4 (Whatman Inc., Florham Park, NJ). The filtrate was evaporated using a Büchi rotavapor under vacuum at  $30\text{--}35\text{ }^{\circ}\text{C}$ , freeze-dried, and stored at  $-80\text{ }^{\circ}\text{C}$  until use.

**Preparation of Extract Using Acetone, Ethyl Acetate Mixture.** Fifty grams of cranberry press cake was mixed with 500 mL of acetone:water (70:30) mixture and subjected to extraction and filtration as mentioned above. The filtrate was concentrated by evaporation in a rotavapor under vacuum to around 150 mL and mixed with three volumes of ethyl acetate. The mixture was transferred into a separatory funnel, and the phases were allowed to separate overnight. The ethyl acetate phase was removed, evaporated under vacuum, freeze-dried, and stored at  $-80\text{ }^{\circ}\text{C}$  until use.

**Preparation of Extract Using Chloroform:Methanol (70:30).** Fifty grams of cranberry press cake was blended with 500 mL of chloroform:methanol (70:30) mixture in a Waring blender for 1 min. The mixture

was stirred for 2 h at room temperature, and a phase separation was achieved using 100 mL of 0.5 M NaCl. The chloroform layer was separated, evaporated using a Büchi rotavapor under vacuum, freeze-dried, and stored at  $-80\text{ }^{\circ}\text{C}$  until use.

**Addition of Extracts to MST for Oxidation Studies.** Cranberry antioxidant extracts were added to MST using ethanol as the carrier solvent. The amount of carrier solvent used was 1% of the muscle weight. In order to dissolve the antioxidant extract in ethanol, 1 N HCl was added to ethanol at a volume ratio of 1:6. When the ethanolic extract was added to MST, there was no significant decrease ( $p > 0.01$ ) in the pH of MST. The MST was thawed overnight at  $10\text{ }^{\circ}\text{C}$  and was mixed by hand within the bag by pressing the sides of the bag.

While screening the ability of ethanol and ethyl acetate extracts from press cake to inhibit lipid oxidation, the extracts were added to MST at 0.15% of the muscle weight. While comparing CJP extract with ethanol and ethyl acetate extracts from press cake, the extracts were added at 0.10% of the muscle weight. The extracts were mixed with MST using a metal spatula and transferred into Zip Lock bags (10 cm  $\times$  15 cm). The Zip Lock bags containing the sample were placed between two plastic sheets (0.30 cm thick) and pressed by another plastic sheet to evenly distribute the sample within the bag. The sample thickness was around 0.3 cm. The samples were stored at  $-4\text{ }^{\circ}\text{C}$ .

**Determination of Lipid Peroxides.** Lipid peroxides were used as a measure of lipid peroxidation. One-tenth of a gram of MST was taken in a disposable glass tube and homogenized for 1 min with 10 mL of chloroform:methanol (1:1) using a Polytron PT 10-35 homogenizer (Brinkmann Instruments, Westburg, NY). The mixture was mixed with 3 mL of 0.5 M NaCl, vortexed, and centrifuged for 5 min at 1000g in an IEC Clinical tabletop centrifuge (International Equipment Co., Needham Heights, MA) to separate the sample into two phases. Two milliliters of the chloroform phase was separated using a needle, and its volume was doubled using a mixture of chloroform:methanol (1:1). Ammonium thiocyanate and ferrous chloride were prepared as in Shantha and Decker (18). Twenty-five microliters of each reagent was added and vortexed for 10 s. The samples were incubated for 20 min at room temperature, and the absorbance was measured at 500 nm. A standard curve was prepared using cumene hydroperoxide. All experiments were done in duplicate.

**Analysis of TBARS.** The formation of TBARS was used as another measure of lipid oxidation. The method of Lemon (19) was modified according to the following procedure for measuring TBARS in MST. MST samples were extracted using trichloroacetic acid (TCA) solution by vortexing (Fisher Vortex-Genie 2, Scientific Industries Inc., Bohemia, NY) 1 g of MST with 6 mL of 7.5% TCA solution at high speed for 1 min. The samples were filtered through Whatman filter paper no. 4, and the filtrate was collected for TBARS determination. Two milliliters of the filtrate was mixed with 2 mL of 0.02 M TBA solution and heated in a boiling water bath for 40 min. The color that developed was spectrophotometrically measured at 530 nm. A standard curve was plotted using tetraethoxypropane. The malonaldehyde concentration was calculated using an extinction coefficient of  $1.6 \times 10^5\text{ M}^{-1}\text{ cm}^{-1}$ . All of the experiments were done in duplicate.

**Isolation of Crude Membrane from MST.** The method of Raghavan et al. (20) was used for the isolation of crude membrane samples. One hundred grams of MST was thawed overnight at  $10\text{ }^{\circ}\text{C}$  and mixed by hand by pressing the sides of the bag. For every 10 g sample of MST, four volumes of cold 0.1 M HEPES (N-[2-hydroxyethyl]piperazine-N-[2-ethanesulfonic acid]) buffer (pH 7.5) was added and homogenized at speed 4 for 40 s using a Kinematic Polytron PT 10-35 homogenizer (Brinkmann Instruments, Westburg, NY). The pH of the homogenate was adjusted to 7.5, and the homogenate was centrifuged at 10000g for 20 min at a sample temperature of  $7\text{--}9\text{ }^{\circ}\text{C}$  using a Beckman L5-65B ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA). The supernatant obtained was centrifuged at 130000g for 30 min to obtain the crude membrane as sediment.

**Determination of Quercetin Equivalents (QEs).** The total phenolic content of the cranberry press cake extracts was determined using the Folin-Ciocalteu method (21, 22) and expressed as QEs. The QE was determined by dissolving 10 mg of the extract in 1 mL of ethanol and 0.16 mL of 0.1 N HCl. Twenty-five microliters of the sample was vortexed with 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times)

and 2 mL of sodium carbonate (7.5%). The mixture was incubated at room temperature for 2 h. The color that developed was measured at an absorbance wavelength of 760 nm. Quercetin dissolved in ethanol was used as a standard.

#### Determination of Quercetin Using Reverse Phase (RP) HPLC.

The amounts of quercetin present in the cranberry press cake and juice extracts were analyzed using RP-HPLC (Agilent 1100 series) (23). The extracts were prepared in methanol and water (1:1 ratio). Each fraction (50  $\mu$ L) was injected onto a C-18 column (Discovery HS, 120  $\text{\AA}$ , 5  $\mu$ m, 25 cm  $\times$  0.46 cm). The fractions were eluted using 0.1% trifluoroacetic acid in water (solvent A) and 0.1% trifluoroacetic acid in methanol (solvent B). A step gradient program of 100% solvent A to 100% solvent B over 30 min under a linear gradient and isocratic for 10 min at 100% solvent B was used. The flow rate was maintained at 1 mL/min, and the eluent was monitored at 280, 320, 360, and 520 nm by a Waters 996 diode array detector. Quercetin (0.02–1 mg) dissolved in 1 mL of methanol and water mixture (1:1) was used as a standard.

#### Partitioning of Cranberry Extracts into the Crude Membrane.

A 5 mg sample of ethanol, ethyl acetate, or chloroform extract was dissolved in 2 mL of a water:methanol (1:1) mixture by vortexing. In order to aid the dissolution of the antioxidant extract in the water:methanol mixture, 0.05 N HCl was added at a volume ratio of 1:6. The amount of quercetin in the extracts was determined using HPLC. A crude membrane sample prepared from 10 g of MST was added to the water:methanol mixture of cranberry extract, hand homogenized for 2 min using a Potter–Elvehjem tissue grinder, and centrifuged at 13000g for 30 min. The amount of quercetin in the supernatant was determined using HPLC. The difference in the quercetin values between the supernatant and the extract was calculated as the amount of quercetin partitioned into the membranes.

**Statistics.** JMP Statistical Discovery Software (version 5.0) was used to evaluate data from storage studies. All studies were done in duplicate. Analysis of variance was employed to examine the difference among treatments. Tukey's honestly significant test was used to compare the differences among means between treatments. Correlations were calculated using simple regression analysis.

## RESULTS

**Screening Cranberry Press Cake Extracts for Their Ability To Inhibit TBARS.** Extracts from cranberry press cake were prepared using various solvent mixtures. In one case, solvents differing in their polarities (measured as dielectric constant,  $\epsilon$ ), such as hexane ( $\epsilon = 2.0$ ), 70% ethanol ( $\epsilon = 43.5$ ), or water ( $\epsilon = 80$ ), were used. In another case, a mixture of (1:1) chloroform and methanol or (1:3) acetone and ethyl acetate was used. Ethanol was used as a carrier solvent at the 1% level based on the weight of MST. Lipid oxidation in MST was measured by the formation of TBARS (Table 1). Among extraction solvents of different polarities, nonpolar hexane and polar water were less effective in TBARS inhibition as compared to ethanol, a solvent of intermediate polarity. However, water and hexane gave higher yields of extract as compared to ethanol. When ethyl acetate was used in a two-step extraction process, the press cake extract was most potent by inhibiting TBARS for 28 days. However, the yield of ethyl acetate extract was lowest among all of the extraction solvents at 0.37% of the dry weight of cranberry.

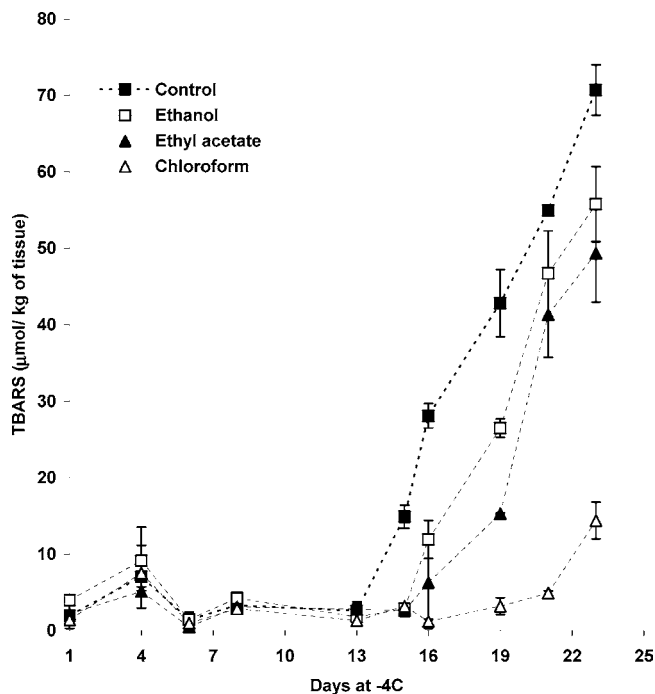
Among the press cake extracts, ethyl acetate and ethanol extracts were chosen for further examination. Ethyl acetate extract was chosen as it was most potent among all of the extracts in inhibiting TBARS. Ethanol extract was chosen both for potency as well as for the relatively low toxicity of ethanol (24).

**Comparison of Cranberry Press Cake and Juice Extracts for Their Ability To Inhibit Lipid Oxidation.** Cranberry press cake extracts prepared using ethanol or ethyl acetate were

**Table 1.** Yield and TBARS Inhibitory Power of Cranberry Press Cake Extracts

sample	dielectric constant ( $\epsilon$ ) <sup>a</sup>	% yield of the extract <sup>b</sup>	days of TBARS inhibition <sup>c</sup>
control	ND	ND	6 x
hexane	2.0	4.5	8.5 x
ethanol (70%)	43.5	1.6	14 y
water	80	9.5	6 x
chloroform:methanol	14.5	1.4	9 xy
ethyl acetate mixture	ND	0.37	28 z

<sup>a</sup> The dielectric constant was calculated based on the standard dielectric constant at 25 °C and using the formulas,  $\epsilon$  (mixture) = (%A  $\epsilon_A$  + %B  $\epsilon_B$ )/100, where  $\epsilon_A$  and  $\epsilon_B$  are the dielectric constants of carrier solvents A and B while  $\epsilon$  (mixture) is the dielectric constant of the resultant mixture (17). <sup>b</sup> The yield of the extract was calculated based on the dry weight of the press cake. <sup>c</sup> TBARS inhibition was measured as  $\mu$ mol malonaldehyde/kg of muscle tissue. The inhibitory power was calculated as the number of days required for a sample to reach a TBARS level of 10  $\mu$ mol/kg of tissue during  $-4$  °C storage. The extracts were added at 0.15% of the muscle weight. Treatments with similar letters are statistically similar ( $p > 0.05$ ). ND, not determined.

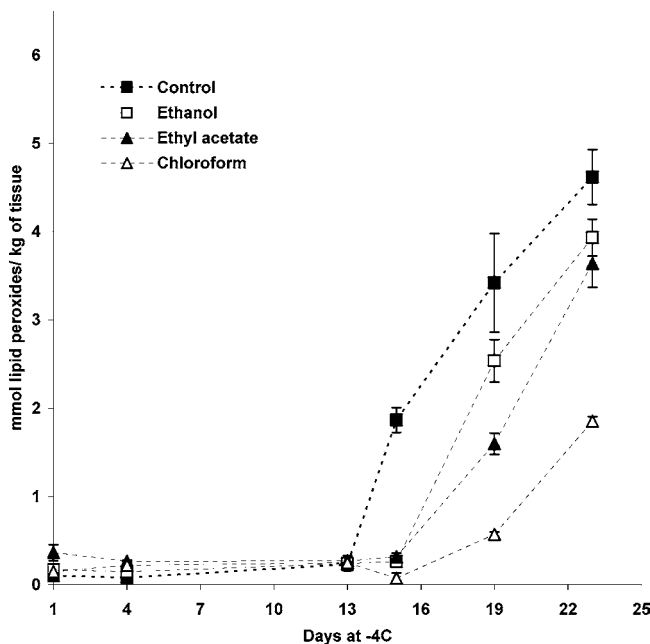


**Figure 1.** Comparison of press cake extracts obtained using solvents, ethanol ( $\square$ ) and ethyl acetate ( $\blacktriangle$ ), with cranberry juice extract obtained using chloroform ( $\triangle$ ) for their ability to inhibit TBARS. Extracts were added at 0.10% of the weight of MST using ethanol as the carrier solvent.

compared with CJP extract prepared using chloroform for their ability to inhibit lipid oxidation in MST. The antioxidative ability was measured by the ability of the extracts to inhibit TBARS formation (Figure 1) and the formation of lipid peroxides (Figure 2). The extracts were added to MST at 0.1% of muscle weight. Ethanol was used as an antioxidant carrier solvent at 1% of muscle weight.

The extracts added at 0.1% level significantly ( $p < 0.05$ ) inhibited oxidation in MST. Among the extracts, the order of inhibition of TBARS and peroxides at  $p < 0.05$  level was CJP extract  $>$  ethyl acetate extract from press cake  $\geq$  ethanol extract from press cake  $>$  control.

**Comparison of QEs and Quercetin in Cranberry Press Cake and CJP Extracts.** The total phenolic contents in the press cake and cranberry juice extracts were determined using



**Figure 2.** Comparison of press cake extracts obtained using solvents, ethanol (□) and ethyl acetate (▲), with cranberry juice extract obtained using chloroform (△) for their ability to inhibit the formation of lipid peroxides. Extracts were added at 0.10% of the weight of MST using ethanol as the carrier solvent.

**Table 2.** QEs and the Total Amount of Quercetin the Press Cake and Juice Extracts

sample	QEs (mmol/g powdered extract) <sup>a</sup>	quercetin (mg/g powdered extract) <sup>b</sup>
ethanol (70%), press cake	0.54 ± 0.06 a	0.62 ± 0.01 x
ethyl acetate, press cake	0.79 ± 0.03 b	1.83 ± 0.51 x
chloroform, juice	0.39 ± 0.01 a	27.2 ± 0.72 y

<sup>a</sup> QEs were determined by the Folin–Ciocalteu method (21, 22) as described in the Materials and Methods section. <sup>b</sup> The amount of quercetin was determined using reversed phase HPLC as described in the Materials and Methods section. Columns with similar letters are statistically similar ( $p > 0.05$ ).

the Folin–Ciocalteu method and expressed as equivalents of quercetin per g of extract (Table 2). Among the three extracts, the ethyl acetate extract prepared from press cake had the maximum amount of total phenolics at 0.79 mmol QE/g extract, while the chloroform extract from cranberry juice had the least amount of phenolics at 0.39 mmol QE/g extract. When the amount of quercetin in the extracts was determined using HPLC, the chloroform extract from cranberry juice had the highest amount of quercetin at 27 mg/g of extract while the ethanol extract had the least amount of quercetin at 0.6 mg/g of extract (Table 2).

**Partitioning of Quercetin from Cranberry Extracts into Aqueous Membrane Suspensions of MST.** In order to determine the partitioning efficiency of the components of cranberry extracts into the membranes of muscle tissue, we studied the partitioning of quercetin into muscle membranes in aqueous suspension. Quercetin was chosen as it is a phenolic component of both CJP extracts and cranberry press cake extracts. For this study, a model system comprised of membranes prepared from MST and a solution of antioxidant extract

in (1:1) methanol and water was used. Quercetin was quantified using HPLC. The amount of quercetin partitioned into the membranes was calculated by the difference between the amount of quercetin present in the methanol and water (1:1) solution, before and after the addition of membranes.

The amount of quercetin present in 5 mg of extract, dissolved in 2 mL of methanol and water (1:1), varied from 2.9  $\mu\text{g}$  in the ethanol extract of the press cake to around 162  $\mu\text{g}$  in the chloroform extract of CJP (Table 3). When membranes were homogenized with the extract, the amount of quercetin remaining in the water and methanol (1:1) mixture varied between 0.7  $\mu\text{g}$  in the ethanol extract to around 38  $\mu\text{g}$  in the chloroform extract of CJP. When the results were expressed on a percentage basis, the amount of quercetin that partitioned into the membranes was around 78% of the initial amount added to the membranes, irrespective of the extracts examined (Table 3). Among the different extracts, there was a significant difference ( $p < 0.05$ ) in the amount of quercetin partitioned into the membranes. When the chloroform extract of CJP was used, nearly 123  $\mu\text{g}$  of quercetin partitioned into the membranes, while for the ethanol extract, 2.3  $\mu\text{g}$  of quercetin partitioned into the membranes.

## DISCUSSION

Cranberries are a rich source of flavonols including glycosides of quercetin (~170 mg/kg cranberry), myricetin (~15 mg/kg), and kaempferol (~2 mg/mg) (25–27). During cranberry processing, water-soluble flavonols could separate into cranberry juice (28), making dried CJP a good source of antioxidants. One of the byproducts of cranberry processing is cranberry press cake. Our aim was to prepare antioxidant extracts from cranberry press cake and compare its efficacy to CJP in inhibiting lipid oxidation in MST. Cranberry press cake contains seeds, stalks, and skins. Cranberry seeds are a rich source of linoleic acid (~70%) with around 6% linolenic acid and 0.02%  $\beta$ - and  $\gamma$ -tocopherol based on lipid weight (29). Because of the presence of unstable unsaturated fatty acids in cranberry seeds, we refrained from using cranberry seed oil for inhibiting lipid oxidation in MST. Cranberry oil is usually prepared by cold pressing cranberry seeds in a mechanical press (30). In our studies, we used solvents of different polarities to prepare extracts from cranberry press cake. Mixing with solvents would exert lesser mechanical force on the press cake as compared to pressing cranberry press cake in a mechanical press. Hence, mixing with solvents would extract a lesser amount of cranberry oil. However, depending on the polarity of the extraction solvent, various components of cranberry press cake could be extracted.

The antioxidant efficacy of cranberry extracts was measured by their ability to inhibit TBARS and lipid peroxides in MST. At the 0.10–0.15% level, the extracts did not significantly affect the color and odor of MST (data not shown). Among the various press cake extracts, the water and hexane extracts (Table 1) were significantly less effective ( $p < 0.05$ ) than the ethanol extract in inhibiting TBARS formation in MST. Hexane, a nonpolar solvent ( $\epsilon = 2.0$ ), would primarily extract oil-soluble compounds while water, a polar solvent ( $\epsilon = 80.0$ ), would extract polar compounds. The results of Table 1 show that compounds of intermediate polarity were more effective in inhibiting lipid oxidation in MST as compared to compounds of extreme polarities.

Ethyl acetate and ethanol extracts showed significant ( $p < 0.05$ ) TBARS inhibitory power among the press cake extracts. When ethanol and ethyl acetate extracts of the press cake were compared with the chloroform extract of CJP (Figure 1 and

**Table 3.** Partitioning of Quercetin from Aqueous Solution into Membrane Suspensions

sample	extraction solvent	quercetin ( $\mu\text{g}$ ) in solution		quercetin in membranes ( $\mu\text{g}$ ) <sup>c</sup>	% quercetin in membranes <sup>d</sup>
		before membrane addition <sup>a</sup>	after membrane addition <sup>b</sup>		
press cake (5 mg)	70% ethanol	2.9 a	0.67	2.3 p	77.5 x
press cake (5 mg)	ethyl acetate mixture	10.9 b	2.0	8.9 q	81.5 x
dried juice (5 mg)	$\text{CHCl}_3$ : $\text{CH}_3\text{OH}$ mixture	162.1 c	38.7	123.4 r	76.1 x

<sup>a</sup> The amount of quercetin was determined in the solution before mixing and homogenizing the membranes. <sup>b</sup> The amount of quercetin was determined in the solution after sedimenting the homogenized membranes using centrifugation. <sup>c</sup> The amount of quercetin was partitioned into the membranes from the solution. <sup>d</sup> % Quercetin in the membranes based on the amount present in the solution. Columns with similar letters are statistically similar ( $p > 0.05$ ).

2), the decreasing order of antioxidant efficacy was chloroform extract > ethyl acetate extract  $\geq$  ethanol extract > control. This decreasing trend was reflected in the amount of quercetin present in the extracts ( $r = 0.92$ ) but not observed in the QEs ( $r = -0.44$ ) of the extracts (Table 2). QEs measure the chemical reducing capacity of phenolic components present in the cranberry relative to the reducing capacity of quercetin (21, 22). The measure of QEs will be affected not only by the number of phenolic groups but also by the position of phenolic groups within the molecule, the presence of amino acids, carotenoids, sugars, and vitamin C (31). Quercetin is one of the components of cranberry that contributes to the overall measure of QEs. The negative correlation between the QE of the extracts and the ability of extracts to inhibit lipid oxidation may indicate that the inhibitory action of cranberry extracts may be related more to the specific type of phenolic compounds than to the overall number of phenolic groups. This observation could be substantiated by a positive correlation between the quercetin and the TBARS/peroxide inhibitory activity of the extracts. Lee et al. (32) had earlier obtained similar results with quercetin as an inhibitor of lipid oxidation in MST. They found that 210  $\mu\text{mol}$  of quercetin could inhibit TBARS and lipid peroxide formation up to 90 and 99%, respectively, as compared to 36% TBARS inhibition and 25% lipid peroxide inhibition by 50  $\mu\text{mol}$  of quercetin stored for 12 days at 2 °C.

In muscle foods, membrane lipids are the substrates most susceptible to oxidation (13). Raghavan et al. (15, 20) had earlier shown that directing a lipid-soluble antioxidant,  $\delta$ -tocopherol, into the membranes of cod muscle would enhance the oxidative stability of the muscle, measured as TBARS. Hence, we wanted to determine the partitioning ability of the components of cranberry extracts into the membrane lipids and correlate this ability with the antioxidant efficacy of extracts. As cranberry extracts contain a number of phenolic compounds, we chose one of the phenolic compounds, quercetin, to measure the partitioning ability. Saija et al. (33) had earlier studied the relationship between the antioxidant efficiency of flavonoids and the membrane interactions. They concluded that an important requisite for the expression of flavonoid antioxidant activity would be the ability to interact with biomembranes. Movileanu et al. (34) proposed that at acidic pH quercetin would be embedded in planar lipid bilayers, while at physiological pH quercetin would interact with the polar head groups of the membranes. For partitioning studies, we used a model system comprised of a membrane suspension with cranberry extract. Using this model system (Table 3), we determined that the amount of quercetin that partitioned into the membranes was around 78% irrespective of the initial amount of quercetin present in the extracts. The amount of quercetin partitioned into the membranes increased significantly ( $p < 0.05$ ) in the order ethanol extract of press cake < ethyl acetate extract of press cake < chloroform extract of CJP. The results from these model

system studies show that the ability of CJP extract to inhibit oxidation could be due to the high content of quercetin and to the partitioning ability of quercetin into the membranes.

In conclusion, the ability of cranberry extracts to inhibit lipid oxidation depends on the type and amount of specific constituent phenolic compounds, like quercetin, rather than the total amount phenolics components, measured as QEs. Increasing the amount of quercetin in the press cake extracts could increase the ability of the extracts to inhibit lipid oxidation in MST. It could be concluded that a proper choice of solvents and extraction method, which would increase the amount of quercetin in press cake extracts, would increase the antioxidant potential of the extracts and hence their economic value.

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