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Comparison of solvent and microwave extracts of cranberry press cake on the inhibition of lipid oxidation in mechanically separated turkey

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Abstract

Cranberry press cake, an under utilized by-product of the cranberry processing industry is a potential source of food antioxidants. The objective of this research were (1) to prepare extracts from cranberry press cake using solvent extraction (SE) and microwave assisted solvent extraction (MASE), and (2) to test the ability of these extracts to inhibit lipid oxidation in mechanically separated turkey (MST). Water, ethanol and acetone were used as extraction solvents. Heating press cake prior to extraction with 70% ethanol increased antioxidant efficacy compared to extracting unheated press cake. Water extracts were least effective in inhibiting lipid oxidation. The most effective extracts were obtained by SE with 100% acetone or MASE with 100% ethanol. A poor correlation of 0.69 was obtained between the total phenols in the extracts and their ability to inhibit thiobarbituric acid reactive substances (TBARS) formation in MST. The correlation coefficient between the amount of quercetin in the extracts and the number of days of TBARS inhibition in MST was 0.87. This indicates that although quercetin may be good inhibitor of lipid oxidation, polyphenols other than quercetin are likely have a role in the inhibition of TBARS in MST. For a similar yield of the extracts, MASE extract using 100% ethanol was a better inhibitor than 100% ethanol SE extract of lipid oxidation in MST. In terms of choice of solvent, based on their flammability and toxicity, MASE with 100% ethanol would be a more likely a choice over SE with 100% acetone, for inhibiting oxidation in MST.

Keywords: Cranberry press cake; Solvent extraction; Microwave assisted extraction; Antioxidative behavior; Mechanically separated turkey

1. Introduction

Lipid oxidation is a major cause of quality deterioration in muscle foods (Ladikos & Lougovois, 1990). Oxidation could be retarded by an exogenous addition of antioxidants to the foods systems (Dziezak, 1986). Due to safety and toxicity concerns related to the use of synthetic antioxidants (Madhavi & Salunkhe, 1996) in foods, natural antioxidants are being increasingly used in the meat industry. Cranberry press cake, an under utilized byproduct of cranberry industry contains several phenolic compounds, which could be used as a potential food antioxidants (Zheng & Shetty, 2000). Cranberry press cake is a mixture of cranberry skin and seeds and is obtained after pressing/removing the juice from cranberries.

Several extraction techniques and solvents are used for obtaining antioxidant extracts from plant origins. Extraction techniques include solvent extraction (SE) (Chen, Shi, & Ho, 1992), microwave assisted solvent

Abbreviations: SE, solvent extraction; MASE, microwave assisted solvent extraction; MST, mechanically separated Turkey; TBARS, thiobarbituric acid reactive substances.

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extraction (MASE) (Kaufmann & Christen, 2002), soxhlet extraction (Bicchi, Binello, & Rubiolo, 2000) and supercritical fluid extraction (Bicchi et al., 2000). Among these, MASE is a relatively new method used for the extraction of natural products (Ganzler, Salgo, & Valko, 1986). Pan, Niu, and Liu (2003) had earlier shown that MASE was more effective than conventional extraction methods in the extraction of tea polyphenols and tea caffeine. Hong et al. (Hong, Yaylayan, Raghavan, Pare, & Belanger, 2001) used MASE to optimize the extraction of phenolic compounds from grape seeds.

Due to the low economic value of cranberry press cake (Zheng & Shetty, 2000), a preparation of food grade antioxidant extract from cranberry press cake would increase the economic value of cranberries. As MASE has been shown to be effective for the preparation of antioxidant extracts from grape seeds and tea leaves (Hong et al., 2001; Pan et al., 2003), we explored the possibility of preparing antioxidant extracts from cranberry press cake using MASE. The press cake extracts were tested for their ability to inhibit lipid oxidation in mechanically separated turkey (MST). MST was chosen due to its growing popularity as a food product as well as due to its susceptibility to lipid oxidation (Dawson & Gartner, 1983; Mielnik, Aaby, Rolfsen, Ellekjaer, & Nilsson, 2002; Wilson, Pearson, & Shorland, 1976). In order to determine whether MASE is an efficient method for preparing press cake extracts, we compared the yields and potency of press cake extracts prepared using MASE with that prepared using SE.

2. Materials and methods

2.1. Materials

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 "zipper" seal polyethylene bags with around 300 g in each bag and stored at −20 °C until use. Mechanically separated turkey (MST) prepared from freshly processed turkeys was shipped from Newberry, SC to the Kraft–Oscar Mayer (Madison, WI) by refrigerated trucking. The MST was then immediately vacuum packaged and stored at −80 °C until use. MARSXpress (model no: 907500), used for microwave assisted solvent extraction (MASE) was loaned by CEM Corporation (Matthews, NC). Distilled water was collected using Milli-Q plus (Millipore, Billerica, MA). Zipper seal polyethylene bags (10 × 15 cm) and Fisher Isotemp magnetic stirrer were purchased from Fisher Scientific Co. (Pittsburg, PA). Vacuum pouch 3 mil standard barrier (17.5 × 20 cm) was purchased from Koch Supplies (Chicago, IL). Chemicals and solvents were purchased from Sigma Chemical Co. (St. Louis, MO). All reagents were of ACS grade.

2.2. Extraction of cranberry press cake

2.2.1. Effect of heat on cranberry press cake

The effect of heating cranberry press cake on (i) the total phenolic content and (ii) the ability of the extract to inhibit lipid oxidation in MST, was studied. Cranberry press cake was heated for 4 h at 100 °C and cooled at room temperature. The heated and unheated press cakes were extracted using ethanol:water (7:3). Fifty grams of the cranberry press cake was blended with 500 mL of ethanol:water (7:3) solvent in a Waring commercial blender for 1 min. The mixture was then stirred for 2 h using a Fisher Isotemp magnetic stirrer and filtered using a Whatman filter paper no. 4 (Whatman Inc., Florham Park, NJ). The filtrate was evaporated using a Buchi rotavapor under vacuum at 30–35 °C until the volume of the filtrate has concentrated to less than 50 mL. The evaporated filtrate was frozen at −80 °C, freeze-dried and stored at −80 °C.

2.2.2. Microwave and solvent extraction of cranberry press cake

Cranberry press cake was dried in an oven at 100 °C for 1 h. The dried press cake was powdered using a Hamilton Beach grinder (model 80354, Proctor Silex Inc., Washington, NC) at fine grind setting. The powdered cranberry press cake was used for the preparation of antioxidant extracts using SE and MASE. The solvents used for extraction were, ethanol, acetone, water, ethanol:water (1:1) and acetone:water (1:1).

Twenty one grams of the powdered cranberry press cake was blended with 105 mL of the solvent 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 and then centrifuged at 8000*g* for 15 min at 4 °C using a Sorvall RC-5C Plus centrifuge (Kendro Laboratory Products, Asheville, NC). The supernatant was used for the preparation of the antioxidant extracts.

When organic solvents were used for extraction, clear supernatants were obtained. The supernatant was evaporated using a Buchi rotavapor, model R200 (BÜCHI Labortechnik AG, Switzerland) under vacuum at 30–35 °C until the volume of the supernatant has been concentrated to less than 50 mL (which is equal to the amount of water used for preparing 50% ethanol or 50% acetone). When 100% ethanol or 100% acetone was used as the extraction solvent, the supernatant was mixed with 50 mL water and evaporated in a rotavapor under vacuum at 30–35 °C, until the volume has been concentrated to less than 50 mL. The evaporated supernatant was frozen at −80 °C. The frozen mixture was freeze-dried using a freeze drier, model 52647 (Labconco Corp., Kansas City, MO) and used for antioxidant studies.

When water was used for extraction, a turbid supernatant was obtained. As the turbid supernatant could not be freeze dried, turbidity causing compounds were precipi-

tated using sodium chloride. Sodium chloride was dissolved in the aqueous supernatant at a concentration of 200 mM. Methanol was then added at a volume equal to the volume of the aqueous supernatant. The entire mixture was centrifuged at 8000*g* for 15 min at 4 °C using a Sorvall RC-5C Plus centrifuge. The clear supernatant obtained was evaporated in a rotavapor at 30–35 °C until the solvent could no longer be removed and then frozen at −80 °C. The frozen mixture was freeze-dried and used as the water extract.

The MARSXpress that was used for MASE had six extraction vessels. In each extraction vessel, 3.5 g of the cranberry press cake powder was added along with 20 mL of the solvent. The temperature of MARSXpress was raised to 125 °C over 10 min and held at 125 °C for 10 min. The temperature of the extracted mixture was brought back to room temperature and centrifuged at 8000*g* for 15 min at 4 °C using a Sorvall RC-5C Plus centrifuge. The supernatant was collected and antioxidant extracts were prepared using methods similar to that used for the solvent extraction of press cake powder.

2.3. Addition of extract to MST

Ethanol was used as the carrier solvent for adding the antioxidant extract to MST. 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.

MST was thawed overnight at 10 °C. The vacuum bag containing the MST was then cut open on one side and MST was mixed by hand within the vacuum bag by pressing the sides of the bag. Antioxidant extract was then added to MST at 0.15% of the muscle weight, mixed with a metal spatula and transferred into ''zipper'' sealed polyethylene bags (10 × 15 cm). The 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 °C.

2.4. Determination of lipid hydroperoxides

One-hundred milligrams 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 vortexed and filtered through a Whatman filter paper no. 4. The filtrate was removed and mixed with 3 mL of 0.5 M NaCl. The mixture was vortexed and centrifuged at 3600*g* for 5 min in an IEC Clinical tabletop centrifuge (International Equipment Co., Needham Hts, MA) to separate the sample into two phases. The chloroform phase was separated and its volume was made to 10 mL using chloroform/methanol (1:1). Ammonium thiocyanate (3.94 M) and ferrous iron solution (prepared by adding equal amounts of 0.132 M $BaCl_2$ and 0.144 M $FeSO_4$) were prepared as in Shantha and Decker (1994). 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.

2.5. Analysis of thiobarbituric acid reactive substances (TBARS)

The method of Lemon (1975) was modified according to the following procedure for measuring the thiobarbituric acid reactive substances (TBARS). TBARS was measured by extracting the MST samples with trichloroacetic acid solution. One gram of the sample was extracted using 6 mL of 7.5% trichloroacetic acid solution by vortexing (Fisher Vortex – Genie 2, Scientific Industries Inc., Bohemia, NY) the mixture 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 2-thiobarbituric acid solution and heated in a boiling water bath for 40 min. The color developed was spectrophotometrically measured at 530 nm. A standard curve was plotted using tetraethoxypropane. Malonaldehyde concentration was calculated using an extinction coefficient of $1.6 \times 10^5\ M^{-1}cm^{-1}$. All the experiments were done in duplicate.

2.6. Determination of quercetin equivalents

The total phenolic content of the cranberry press cake extracts was determined using Folin–Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventos, 1999; Singleton & Rossi, 1965) and expressed as quercetin equivalents. Quercetin equivalent was determined by dissolving 10 mg of the extract in 1 mL of ethanol and 0.1 mL of 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 developed was measured at an absorbance wavelength of 760 nm. Quercetin dissolved in ethanol was used as a standard. A standard stock solution of 0.25 g quercetin in 100 mL ethanol was prepared. Standard plot was made using 0–25 μL of the stock solution with 2.5 mL Folin–Ciocalteu reagent and 2 mL sodium carbonate.

2.7. Determination of quercetin using reverse phase high-performance liquid chromatography (RP-HPLC)

The antioxidant extracts prepared using SE and MASE were analyzed using RP-HPLC (Agilent 1100 series) (Porter, Krueger, Wiebe, Cunningham, & Reed, 2001). Samples for HPLC analyses were prepared by dissolving 5 mg extracts in 1 mL of methanol and water (1:1 ratio).

Fifty microliters fractions were injected onto a C-18 column (Discovery® HS, 120 Å, 5 μm, 25 cm × 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.

2.8. Statistics

A general linear model procedure of the SAS software (Statistical Analysis Software, SAS Americas, Cary, NC) was used to evaluate data from storage studies using data from replicated experiments. Analysis of variance was employed to examine the difference among treatments. Correlations were calculated by simple regression analysis.

3. Results

3.1. Effect of heat on the antioxidative potential of cranberry press cake extracts

The effect of heating cranberry press cake on the total phenolic content of the press cake extract and the ability of the extract to inhibit lipid oxidation in MST were studied. Cranberry press cake was heated in an oven at 100 °C for 4 h before extraction with ethanol:water (7:3) mixture for the preparation of the extract. The total phenolic content and the antioxidant activity of the heat-treated extracts were compared with that of the unheated extract. The total phenolics were expressed as quercetin equivalents (Table 1) and the antioxidant activity was expressed as the ability of the extracts to inhibit TBARS formation in MST (Fig. 1). The powdered extracts were dissolved in the carrier solvent, ethanol, and added at a level of 0.15% extract and 1% carrier solvent based on the muscle weight. A mixture of MST and ethanol was used as a control for oxidation studies. Lipid oxidation in MST, measured as TBARS, was significantly ($p < 0.01$) inhibited by both heated-treated and unheated press cake extracts compared to the control (Fig. 1). Heating the press cake prior to the preparation of extracts significantly ($p < 0.01$) improved the ability of the extracts to inhibit TBARS formation. However, there was no significant difference ($p > 0.01$) between the quercetin equivalents (Table 1) of the heated and unheated extracts.

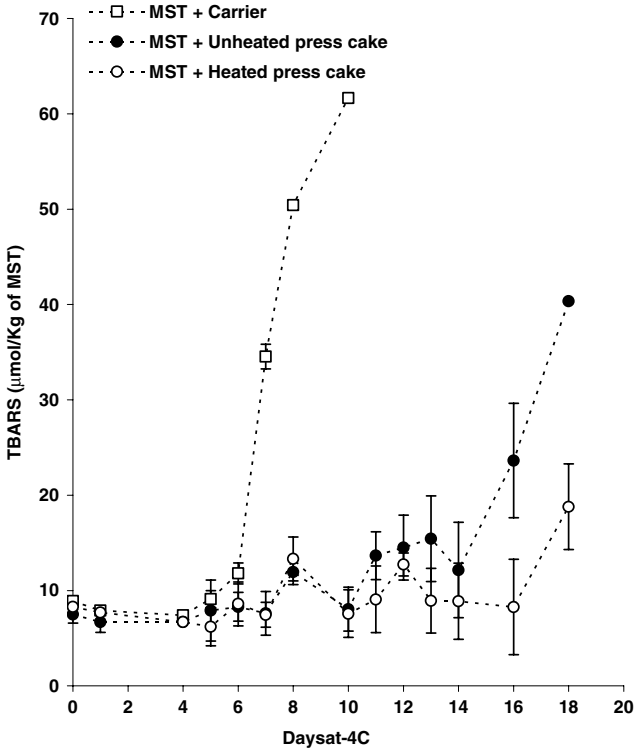

Fig. 1. Effect of heating press cake prior to ethanol:water (70:30) extraction on TBARS values in mechanically separated turkey (MST) during −4 °C storage. Freeze dried extracts were added to MST at 0.15% of muscle weight. Ethanol was used as carrier solvent at 1% of muscle weight. MST + carrier ethanol (□); MST + unheated press cake (●); MST + heated press cake (○).

Table 1
Effect of heating press cake prior to extraction on the quercetin equivalents of the extracts[A]

State of press cake prior to extraction	Extraction solvent	Quercetin equivalents (mmol/g of extract)
Unheated	70% ethanol/30% water	0.24 ± 0.03^{b}
Heated	70% ethanol/30% water	0.20 ± 0.03^{b}

Columns with different alphabets are significantly different ($p < 0.01$).
[A] $n = 3$.

3.2. Effect of extraction method on the yield, quercetin equivalents and the total amount of quercetin in cranberry press cake extracts

The effect of MASE and SE on (a) the yield of press cake extracts (b) the quercetin equivalents of the extracts and (c) the total amount of quercetin present in the extracts, were compared. Five different extraction solvents were used: 100% water, 100% ethanol, 100% acetone, 50% ethanol (1:1 ethanol and water) and 50% acetone (1:1 acetone and water). The yield of the extracts was calculated based on the original weight of cranberry press cake. The total phenolic content of the extracts was calculated based on quercetin equivalents. The total amount of quercetin was determined using reverse phase-HPLC.

When MASE was used, the use of water in organic extraction solvents, i.e., 50% acetone or 50% ethanol, significantly ($p < 0.01$) increased the yield of the extracts compared to the use of 100% organic solvents, i.e., 100% ethanol or 100% acetone (Fig. 2). When SE was used, there

was no significant difference ($p > 0.01$) among the yield of the extracts for various organic solvents. The yield of the extracts obtained for 50% ethanol or 50% acetone was higher for MASE than for SE, while the yields for 100% ethanol or 100% acetone were similar for both MASE and SE. For 50% ethanol and 50% acetone, the yields for MASE were 7.7% and 3.7%, respectively, and the corresponding yields for SE were 2.3% and 2.5%, respectively. Among the five solvents, water gave the highest extract yield of 17.6% using MASE and 9.9% using SE.

When MASE was used, the quercetin equivalent of the extracts was dependent on the amount of water present in the extraction solvent (Fig. 3). Decreasing the amount of water in the extraction solvent significantly ($p < 0.01$) increased the quercetin equivalent of the resultant extract. When 100% water was used, the total phenolic content of the extract was around 0.02 mmol quercetin equivalents/g of extract compared to 0.12 mmol with 50% ethanol, 0.30 mmol with 100% ethanol and 0.27 mmol with 50% acetone and 0.53 mmol/g of extract with 100% acetone. When SE was used, the quercetin equivalents of the extracts were similar for 100% and 50% ethanol but not for 100% and 50% acetone. The phenolic content for 100% acetone and 50% acetone extracts were 0.4 and 0.23 mmol/g extract, respectively.

The amount of quercetin measured in the water extracts were 9.4 μg for MASE and 15.3 μg for SE/g of extract (Table 2). When organic solvents like ethanol or acetone were used for extraction, there was a significant increase ($p < 0.01$) in the amount of quercetin compared to water as the extraction solvent. Between the two extraction methods, SE and MASE, SE extracted significantly ($p < 0.01$) more quercetin/g of extract compared to MASE. With 100% ethanol as the extraction solvent, SE extracted 1537 μg of quercetin and MASE extracted 1272 μg of quercetin/g of extract. The amount of quercetin extracted using 100% acetone were 1530 μg for SE and 960 μg for MASE/g of extract.

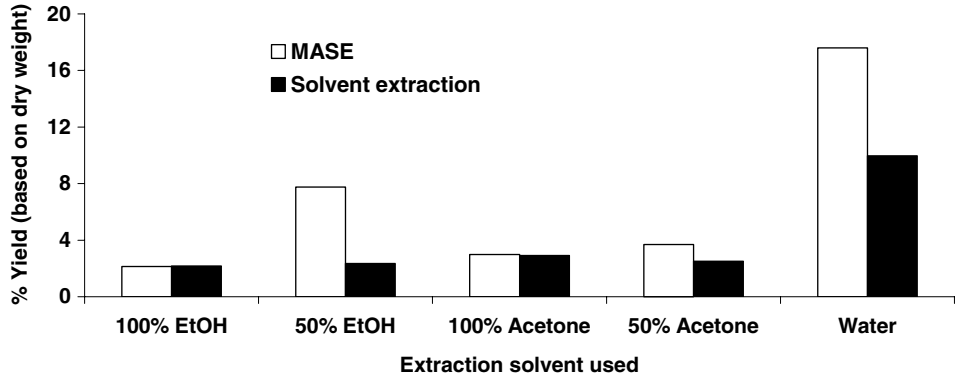

Fig. 2. Percentage yield of extracts based on powder weight of press cake: microwave assisted solvent extraction (MASE) vs. solvent extraction. MASE at 125 °C (10 min ramp, 10 min hold). 50% Ethanol = (50:50) ethanol and water; 50% acetone = (50:50) acetone and water.

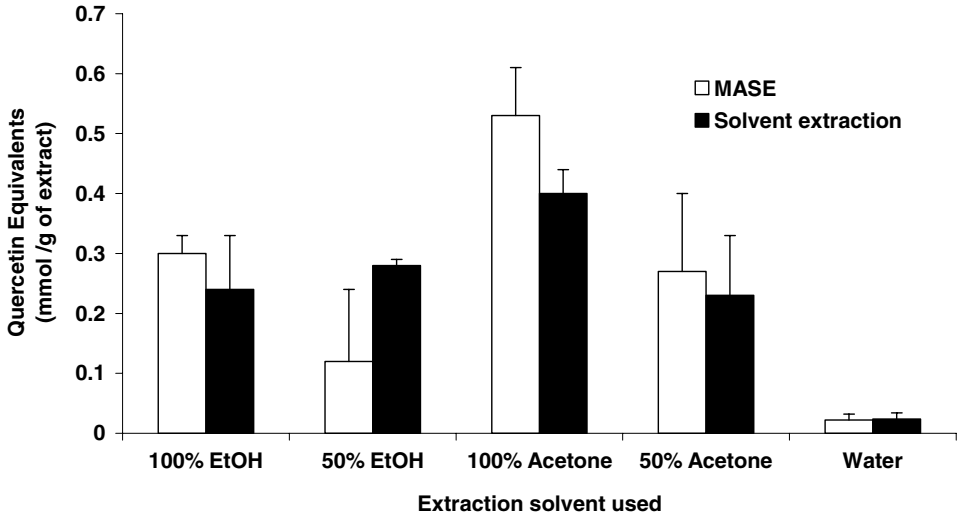

Fig. 3. Quercetin equivalents of powdered cranberry press cake extracts: solvent extraction vs. MASE ($n = 2$). MASE: microwave assisted extraction at 125 °C (10 min ramp, 10 min hold). 50% Ethanol = (50:50) ethanol and water; 50% acetone = (50:50) acetone and water.

3.3. Effect of press cake extracts obtained by SE and MASE on lipid oxidation in MST

Two different extraction methods, SE and MASE, and five different solvents, 100% acetone, 100% ethanol, 100% water, 50:50 ethanol:water mixture and 50:50 acetone:water mixture, were used for preparing extracts from cranberry press cake. The ability of the extracts to inhibit lipid oxidation was tested in MST. Oxidation was monitored by the measurement of TBARS and lipid peroxides. Extracts were added to MST at 0.15% of the muscle weight. Ethanol was used as the antioxidant carrier at 1% of the muscle weight. A mixture of MST and carrier ethanol was used as the control.

When acetone, ethanol or water was used at 100% level for extraction, the decreasing order of TBARS inhibition for SE extracts (Fig. 4a) were 100% acetone > 100% ethanol > water and for MASE extracts (Fig. 4b), the order was 100% ethanol > 100% acetone > water. The water extracts had no significant inhibitory effect ($p > 0.01$) compared to the control. The decreasing order of antioxidant activity of various extracts prepared using MASE and SE, in terms of TBARS measurement were, MASE 100% ethanol, SE 100% acetone > SE 100% ethanol, MASE 100% acetone, MASE 50% acetone > SE 50% ethanol, SE 50% acetone, MASE 50% ethanol, > SE water, MASE water (Figs. 4a–4c). When oxidation of MST was measured in terms of peroxide values (Figs. 5a,5b), the organic solvent extracts, i.e., 100% ethanol or 100% acetone extracts, significantly ($p < 0.01$) inhibited the oxidation of MST compared to control whereas, the water extracts did not have any significant inhibition.

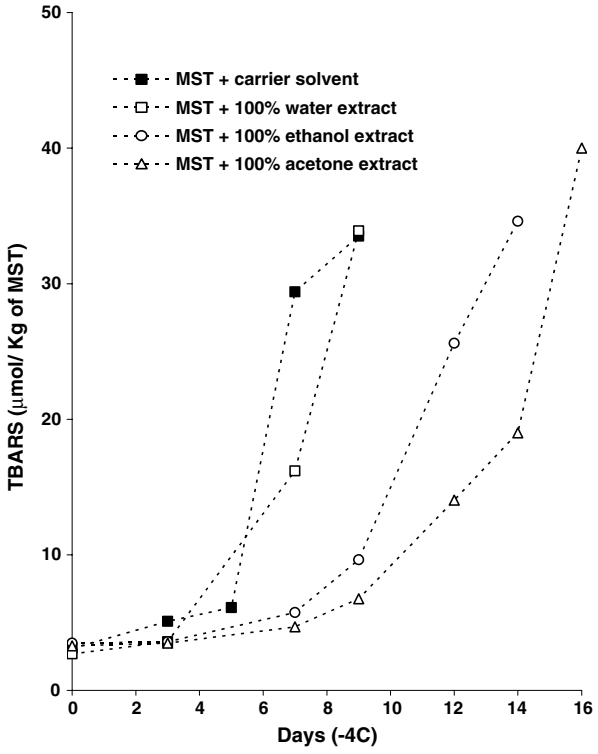

Fig. 4a. Effect of solvent extracts on TBARS value in mechanically separated turkey. Extracts were added at 0.15% of the muscle weight. MST + carrier ethanol added at 1% of muscle weight (■); MST + water extract (□); MST + 100% ethanol extract (○); MST + 100% acetone (△).

4. Discussion

The safety and potential carcinogenicity of synthetic antioxidants used in food has been a widely debated issue (Shahidi, 2000; Verhagen, Schilderman, & Kleinjans, 1991; Williams, Iatropoulos, & Whysner, 1999). Hence, natural antioxidants are increasingly being preferred in food systems. The low economic value of cranberry press cake and the presence of phenolics (Zheng & Shetty, 2000) makes the press cake suitable for the preparation of food grade antioxidant extracts. Microwave assisted extraction (MASE) is a relatively new method used for the preparation of antioxidant extracts from natural products (Hong et al., 2001; Huang & Zhang, 2004; Kaufmann & Christen, 2002; Pan et al., 2003). In this work, we wanted to determine the viability of using MASE as an extraction method for preparing antioxidant extracts from cranberry press cake and the ability of these extracts to inhibit lipid oxidation in MST. We also wanted to compare the antioxidant efficacy of extracts prepared using MASE with that of SE.

MASE process involves the application of heat during extraction. Hence, we wanted to determine first, the effect of heat on the antioxidant properties of cranberry press cake extracts. When the press cake was subjected to a heat treatment before extraction, the resultant extract showed

Table 2
Amount of quercetin in solvent extracts and microwave assisted solvent extracts (MASE)[a]

Type of extract	Amount of quercetin (μg)/g of extract	Total absorbance of flavonols at 360 nm (mAu)	Quercetin as % of total absorbance (%)
100% Water extract	15.3	74	17.5
100% MASE water extract	9.42	209	3.8
100% Ethanol extract	1537	17,813	7.3
100% MASE ethanol extract	1272	15,471	6.9
100% Acetone extract	1530	16,623	7.8
100% MASE acetone extract	960	13,125	6.2

[a] $n = 2$. mAu = milli ampere units. Amount of quercetin was determined using a (binary pump) reversed phase-HPLC. Methanol and water were used as mobile phases.

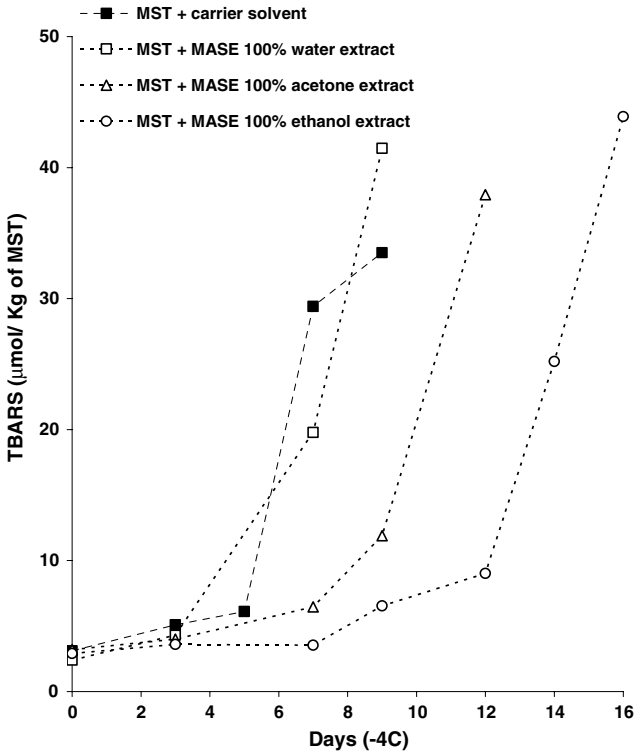

Fig. 4b. Effect of microwave assisted solvent extracts (MASE) on TBARS value in mechanically separated turkey. Extracts were added at 0.15% of the muscle weight. MST + carrier ethanol added at 1% of muscle weight (■); MST + MASE extract with 100% water (□); MST + MASE extract with 100% ethanol (○); MST + MASE extract with 100% acetone (△).

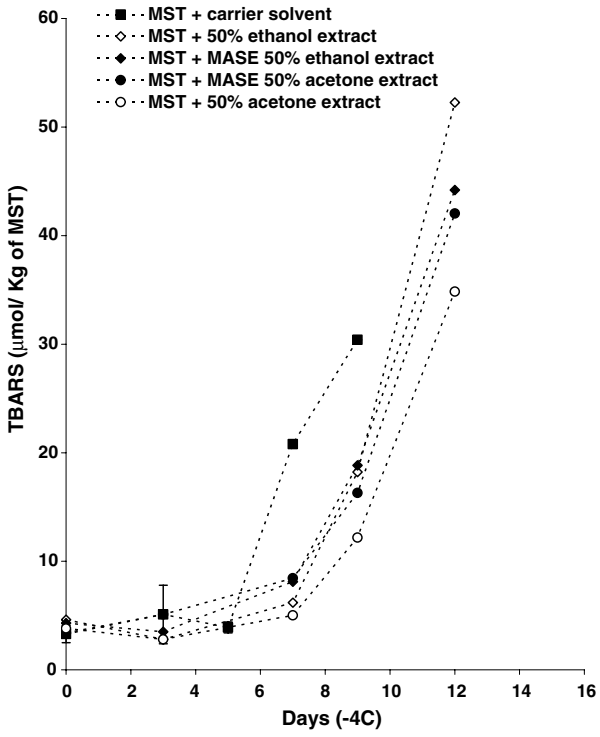

Fig. 4c. Effect of solvent and microwave assisted solvent extracts (MASE) on TBARS value in mechanically separated turkey. Extracts were added at 0.15% of the muscle weight. MST + carrier ethanol added at 1% of muscle weight (■); MST + (50:50) ethanol: water extract (◇); MST + (50:50) ethanol: water MASE extract (◆); MST + (50:50) acetone: water MASE extract (●); MST + (50:50) acetone: water MASE extract (○).

an improved ability (Fig. 1) compared to the unheated extract, to inhibit lipid oxidation in MST. Exposing the press cake to heat might have rendered the cell wall permeable, which could have enabled the extraction solvent to solubilize and extract antioxidant compounds from the press cake extract. However, there was no significant difference ($p > 0.01$) in the total amount of phenolic compounds measured in terms of quercetin equivalents between the heated and unheated press cake extracts (Table 1). One explanation for the observed discrepancy between quercetin equivalents and the antioxidant ability of the extracts could be that the unheated press cake extracts may have a lesser amount of desirable compounds that could inhibit lipid oxidation in MST.

Having determined that high temperature by itself was not detrimental to the antioxidant potency of cranberry press cake extracts, we used MASE for the preparation of press cake extracts. We also compared the yield, quercetin equivalents, total amount of quercetin and the antioxidant ability of the extracts prepared using MASE with that of SE. Among all the extraction solvents used, 100% water gave the highest yield of press cake extracts (around 17% for MASE and 10% for SE) (Fig. 2) but the extracts had the lowest total polyphenolic content (Fig. 3) and total amount of quercetin (Table 2). The low phenolic content of the water extracts was reflected in their inability to inhibit the formation of TBARS in MST. The low antioxidant activity of water extracts is probably due to the poor ability of water to extract polyphenols from the cranberry press cake.

As the presence of water in the organic extraction solvents decreased the total polyphenolic content of the extracts (Fig. 3), we focused our attention on the extracts prepared using 100% organic solvents. When 100% ethanol was used for MASE and SE, there was no significant difference ($p > 0.01$) within the yield of the extracts (Fig. 2) or within the total phenolic contents of the extracts (Fig. 3). However, the amount of quercetin in the SE extract was higher that of MASE extract (Table 2).

When 100% acetone was the extraction solvent, the yield of MASE and SE extracts were similar but the total phenolic content of MASE was higher than that of SE extract. When the amount of quercetin in the 100% acetone extracts was compared, SE had a higher amount of quercetin than MASE extract. Quercetin (Table 2) is one of the component polyphenols present in the press cake extract which contributes to the overall quercetin equivalent. For the MASE and SE extracts prepared using 100% ethanol or 100% acetone, a negative correlation ($r = -0.73$) was obtained between the quercetin equivalents and the total amount of quercetin. The negative correlation is likely due to the contribution of polyphenols other than quercetin, to the total quercetin equivalents.

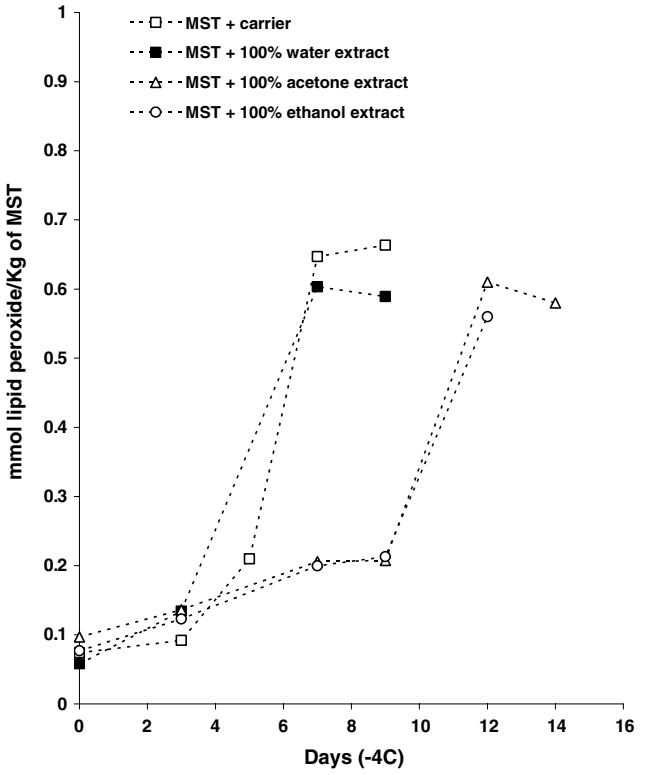

Fig. 5a. Effect of solvent extracts on the peroxide value in mechanically separated turkey. Extracts were added at 0.15% of the muscle weight. MST + 100% water extract (■); MST + carrier ethanol added at 1% of muscle weight (□); MST + 100% ethanol extract (○); MST + 100% acetone (△).

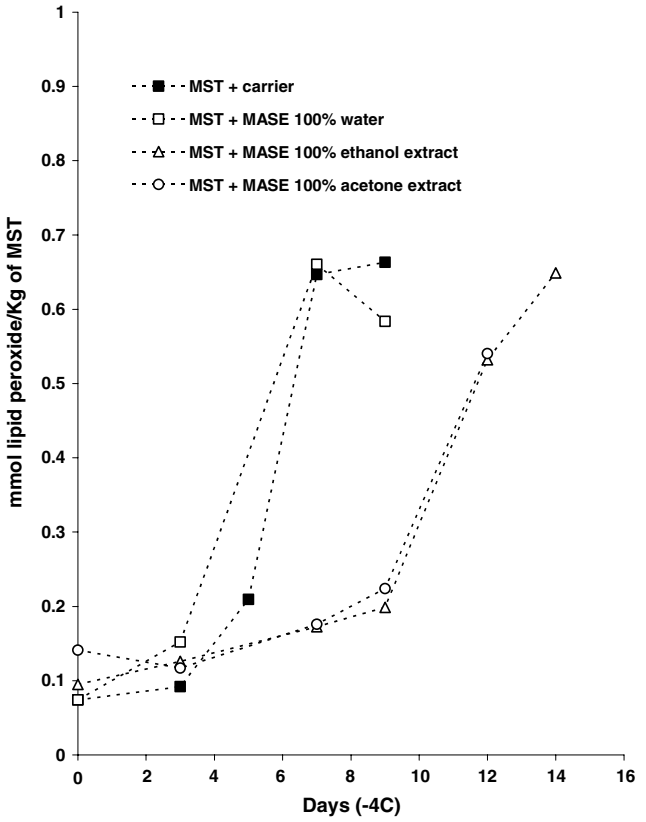

Fig. 5b. Effect of microwave assisted solvent extracts (MASE) on TBARS value in mechanically separated turkey. Extracts were added at 0.15% of the muscle weight. MST + carrier ethanol added at 1% of muscle weight (■); MST + MASE extract with 100% water (□); MST + MASE extract with 100% acetone (○); MST + MASE extract with 100% ethanol (△).

When the antioxidant activity of the extracts were measured by their ability to inhibit TBARS formation in MST, MASE extract prepared using 100% ethanol was more effective than a corresponding extract prepared using SE and the SE extract prepared using 100% acetone was more effective than a corresponding extract prepared using MASE (Figs. 4a and 4b). The correlation coefficient between the total phenolic content (Fig. 3) and the number of days of TBARS inhibition (Figs. 4a and 4b) was 0.69. This shows that quercetin equivalents of the press cake extracts could not be used as a direct measure of the ability of extracts to inhibit lipid oxidation in MST. The correlation coefficient between the amount of quercetin (Table 2) and the number of days of TBARS inhibition (Figs. 4a and 4b) was 0.87. This may be due to the contribution of polyphenols other than quercetin to the inhibition of TBARS in MST.

5. Conclusion

MASE extracts were similar to SE extracts in their ability to inhibit TBARS formation in MST for the extraction solvents, 50% ethanol, 50% acetone or 100% water. When 100% ethanol was used as the extraction solvent, MASE extract was more antioxidative than SE and for 100% acetone, the antioxidative effect of the extracts were reversed. There was a poor correlation between the quercetin equivalents of the extracts and their ability to inhibit TBARS formation. The correlation coefficient between the total amount of quercetin and the number of days of TBARS inhibition was 0.87, indicating that quercetin may be a potent inhibitor of lipid oxidation but also that polyphenols other than quercetin likely have a role in the inhibition of TBARS in MST. Irrespective of the method of extraction, 100% water extracts were least antioxidative compared to the organic solvent extracts. Among the two extraction methods, MASE and SE, and two solvents of extraction, ethanol and acetone, MASE with 100% ethanol could be used as an effective method for preparing antioxidant extracts. For a similar yield of the extracts, MASE extract using 100% ethanol was a better inhibitor of lipid oxidation in MST than a corresponding SE extract. As a solvent of extraction, ethanol is less volatile and toxic than acetone and other organic solvents. Hence, in terms of choice of solvents, MASE with 100% ethanol would likely be preferred over SE with 100% acetone.

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