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Assessment of Freeze-Dried Hydrodistilled Extracts from Clove; Caraway and Coriander Herbs as Natural Preservatives for Butter Oil

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Abstract: Antioxidant activities of Freeze-Dried Hydrodistilled (FDH) extract of three herbs; clove, (*Syzygium aromaticum* L.); caraway (*Carum carvil* L.) and coriander (*Coriandum satvium* L.) was evaluated *in vitro* by iron reduction; iron chelation and inhibition of lipid peroxidation methods. Also, total phenols content and the extraction yield was determined. Furthermore, simple model of butter oil was designed for evaluate the produced extracts as natural preservatives. Acid value, peroxide value and thiobarbituric acid (TBA) test were determined for oxidation system of butter oil. Antioxidant activity of clove FDH extract was significantly higher than caraway and coriander extracts when evaluated in iron reduction; iron chelation and inhibition of lipid peroxidation methods. Based on acid value, peroxide value and thiobarbaturic acid test, different FDH extracts exhibited antioxidant effect specially at rate of 400 ppm from individual extracts with order of clove>coriander >caraway. Also, different correlation relationships were recorded in present study.

Key words: Antioxidant, freeze-dried, hydrodistilled, caraway, clove, coriander, natural preservatives, butter oil

INTRODUCTION

Food quality decreasing occurs during processing and storage is related to oxidative processes which cause degradation affects on lipids, carbohydrates and protein these are often catalyzed by e.g., ferrous or copper (Halliwell, 1997). Synthetic antioxidants such as BHA (butylated hydroxyl anisole) or BHT (butylated hydroxyl toluene) usually used to decelerate these processes. These antioxidants suffer from the disadvantage that they are volatile and easily decompose at high temperatures. Additionally, it is still unclear whether chronic consumption can lead to health risks (Martinez-Tome *et al*., 2001). Plant-derived food additives, especially polyphenolic compounds, have also been ascribed health-promoting properties, as for example in terms of prevention of chronic cardiovascular diseases (Harborne and Williams, 2000; Singh *et al*., 2008).

Such food additives are required to be odour free and tasteless. Many herbs and spices, usually used as an excellent source of phenolic compounds which have been reported to serve as natural food preservatives due to their good antioxidant activity (Rice-Evans *et al*., 1996; Zheng and Wang, 2001; Justesen and Knuthsen, 2001). However, herbs and spices usually contain essential oils which show antioxidant activity but also carry flavour. Thus, the preparation of hydrodistilled extracts is using to remove the intrinsic flavour from the plant material Furthermore, use of an aqueous solvent prevent solubility problems and this avoids harmful residues from organic solvents. Also, hydrodistilled extracts may use in the functionalization of foods and beverages as source of phenolic compounds which has a health-promoting properties (Harborne and Williams, 2000; Hinneburg *et al*., 2006; Ruberto *et al*., 2000; Teissedre and Waterhouse, 2000).

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Some studies concerned for evaluate antioxidant activity of FDH extracts of different herbs (Dorman *et al*., 2003; Hinneburg *et al*., 2006; El-Ghorab *et al*., 2008), but there is no studies about clove, caraway and coriander FDH extracts. In present study, FDH extracts were prepared from three aromatic plants; clove, (*Syzygium aromaticum* L.); caraway (*Carum carvil* L.) and coriander (*Coriandum satvium* L.). Also, total phenols content and extraction yields were determined and *in vitro* antioxidant activity (iron reduction; iron chelation and inhibition of lipid peroxidation) was studied in producing extracts. Furthermore, simple model of butter oil was designed for evaluate the produced extracts as natural preservatives.

MATERIALS AND METHODS

Materials

Dried plant materials were obtained from experimental station of Faculty of Pharmacy, Cairo University during 2008. Ultrapure water was prepared using a Millipore Milli-RO 12 plus system (Millipore Corp., Bedford, MA). All reagents and solvents were either of analytical or HPLC grade and were obtained from Sigma Chemical Co., (St. Louis, MO).

Preparation of Freeze-Dried Hydrodistilled Extracts (FDH Extract)

Hundred gram of ground plant material were suspended in 500 mL ultrapure water and hydrodistilled for 3 h in a hydrodistillation glass apparatus. This process was repeated for 2 h with fresh solvent and the combined aqueous extracts were then filtered, reduced in volume *in vacuo* (-45°C), freezedried and stored at -4°C. The extracts were dissolved in ultrapure water prior to use.

Determination of Total Phenols Content

The total phenols were estimated according to the Folin-Ciocalteu method (Singleton *et al*., 1999). To 100 μL sample were added 250 μL of undiluted Folin-Ciocalteu-reagent. After 1 min, 750 μL of 20% (w/v) aqueous Na_2CO_3 were added and the volume was made up to 5.0 mL with H_2O. The control contained all the reaction reagents except the extract. After 2 h of incubation at 25°C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. Total phenols were determined as gallic acid equivalents (mg gallic acid/g extract) and the values are presented as means of triplicate analysis.

Measurement of Iron (III) to Iron (II)-Reducing Activity

The ability of the extracts to reduce iron (III) was assessed by the method of Benjakul *et al*. (2005). Three milliliter aliquot of each extract, was dissolved in water, was mixed with 5 mL of phosphate buffer (0.2 M, pH 6.6) and 5 mL of a 1% potassium hexacyanoferrate $[K_3Fe(CN)_6]$ solution. After a 30 min incubation at 50°C, 5 mL of 10% trichloroacetic acid were added and the mixture was centrifuged for 10 min. Five milliliter aliquot of the upper layer was mixed with 5 mL of water and 1 mL of 0.1% aqueous $FeCl_3$ and the absorbance was recorded at 700 nm. Iron (III) reducing activity was determined as ascorbic acid equivalents (mmol ascorbic acid/g extract). The values are presented as the means of triplicate analysis.

Study the Inhibition of Linoleic Acid Peroxidation

The antioxidant activity was determined as the degree of inhibition on the hemoglobin-catalysed peroxidation of linoleic acid according to Kuo *et al*. (1999). Sample (20 μL) were added to 0.5 mL of 0.05 M phosphate buffer (pH 7.0), containing 0.14% Tween 20 and 4 mM linoleic acid and then equilibrated at 37°C for 3 min. The peroxidation of linoleic acid in the above reaction mixture was initiated by adding 30 μL of 0.035% hemoglobin (in water), followed by incubation at the same temperature for 10 min and stopped by adding 5 mL of 0.6% HCl (in ethanol). The hydroperoxide

formed was assayed according to a ferric thiocyanate method with mixing, in order, of 30% ammonium thiocyanate (0.2 mL) and 0.02 M ferrous chloride (0.1 mL). The absorbance (A_s) was measured at 480 nm after 5 min. The absorbance blank (A_0) was obtained without adding hemoglobin to the above reaction mixture; the absorbance of the control (A_{100}) was obtained by replacing the sample by buffer.

$$AA(\%) = \frac{1-(A_s - A_0)}{A_{100} - A_0} \times 100 \quad (1)$$

The antioxidant activities of the samples were calculated as trolox equivalents (mg trolox/g extract) according to Eq. 1. The values are presented as the means of triplicate analysis.

Measurement of Iron (II) Chelation Activity

The chelation of iron (II) ions by the different extracts was carried out as described by Carter (1971). Two hundred microlitres of each extract were added to 100 μL of 2.0 mM aqueous $FeCl_2$ and 900 μL methanol. The controls contained all the reaction reagents except the extract or positive control substance. After a 5 min incubation, the reaction was initiated by added 400 μL of 5.0 mM ferrozine. After a 10 min equilibrium period, absorbance at 562 nm was recorded. The iron chelation activities were calculated from the absorbance of the control (A_c) and of the sample (A_s) using Eq. 2 and expressed as Na_2EDTA equivalents (mg Na_2EDTA/g extract). The values are presented as the means of triplicate analysis.

$$\text{Inhibition}(\%) = \frac{A_c - A_s}{A_c} \times 100 \quad (2)$$

Preparation of Butter Oil

Fresh buffalo milk was pasteurized and separated into cream and skimmed milk using an Alfa Laval separator (AESC, Sweden). The cream was churned to obtain unsalted butter. The resultant butter was melted using moderate heat (55°C) and the clear butter oil layer was carefully decanted.

Chemical Analysis

Acid and peroxide values were determined according to the methods of the Association of Official Analytical Chemists (1980). The thiobarbituric acid (TBA) test was carried out according to the method described by Patton (1973).

Design of Oxidation System

The Butter Oil (BO) was distributed into eight portions (200 g each) in sterilized glass bottles. The first portion served as a control. BHT at rate of 200 ppm was added to the second portion. To the 3rd and 4th portions, FDH extract of clove was added at a rate of 200 and 400 ppm, respectively. To the 5th and 6th, FDH extract of caraway was added at a rate of 200 and 400 ppm, respectively. In the 7th and 8th, FDH extract of coriander was added at a rate of 200 and 400 ppm, respectively. Control and treated samples were placed in an incubator at 60±1°C to accelerate the auto-oxidation of butter oil, (Thampson, 1960). The experimental period was terminated when an objectional odour and high lipid rancidity values were obtained with the samples after 28 days.

Statistical Analysis

All statistical analysis were carried out using iner-STAT-a (Vargas, Mexico) as add-in for Microsoft Excel 2007. Analysis of variance (ANOVA) was followed by Tukeys' pairwise comparison test at a level of $p<0.05$ for the determination of significant differences between treatments and control.

RESULTS AND DISCUSSION

Extraction Yield and Total Phenols

Table 1 shows the extraction yields and total phenols, for the FDH extract of selected herbs. The extraction yields was 245; 210 and 130 mg g^{-1} for clove; coriander and caraway, respectively. Significant association found between the extraction yields and total phenols and the results from the different antioxidant assays. Phenolic substances which investigated by the Folin-Ciocalteu method (mg gallic acid/g extract) have been shown to be responsible for the antioxidant activity of plant materials. Phenolic substances were found in the order clove>coriander>caraway ($p<0.05$). The content of total phenolic shows a good correlation with the iron reduction assay ($R^2 = 0.8871$, $p<0.05$) and inhibition of lipid peroxidation assay ($R^2 = 0.7327$, $p<0.05$) while no correlation with the iron (III) to iron (II)-reducing assay or iron (II) chelation assay. The total phenols/extractable compounds ratio was 33.7% for clove; 28.6% for coriander and 18.4% for caraway.

Iron (III) to Iron (II)-Reducing Activity

Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Yildirim *et al*., 2001). The iron (III) to iron (II)-reducing activity is expressed as ascorbic acid equivalents (mmol ascorbic acid/g sample). The ascorbic acid equivalent in clove extract was significantly higher than for the other extracts ($p<0.05$). Ascorbic acid equivalents was 0.78 mmol ascorbic acid/g for clove extract; 0.32 mmol ascorbic acid/g for coriander extract and 0.13 mmol ascorbic acid/g for caraway extract.

Peroxidation of Linoleic Acid

In the hemoglobin-catalysed peroxidation of linoleic acid assay, linoleic acid served as a lipid model. Peroxidation was induced by hemoglobin and the damage was assayed following the thiocyanate and Trolox was used as reference substance (Kuo *et al*., 1999). Results indicated that clove was significantly better inhibitors of lipid peroxidation than the other extracts ($p<0.05$), Trolox equivalents ranked from 443±12.11 mg trolox/g extract for clove to 93±4.21 mg trolox/g extract for caraway. The antioxidant activity in this assay correlated well only with iron reduction ($R^2 = 0.8958$, $p<0.05$).

Iron (II) Chelation

Foods contaminated with transition metal ions which may be introduced by processing methods, these ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via., Fenton chemistry (Halliwell, 1997). These processes can be delayed by iron chelation and deactivation. Therefore, the ability of the extracts to chelate iron (II) ions was evaluated and expressed as mg Na_2EDTA/g extract. Clove show the significantly ($p<0.05$) best iron chelation (234±5.21 mg Na_2EDTA/g extract), followed by coriander (143±2.71 mg Na_2EDTA/g extract) and (87±1.43 mg Na_2EDTA/g extract) for caraway. No significant correlation was found between the iron chelation ability of the extracts and the other antioxidant activities.

Effect of FDH Extract of Different Plants on Lipid Rancidity

In the present study the simple model system consists of butter oil mixed with FDH extract of selected herbs were designed to study oxidative process during storage. BHT and BHA are added as

Table 1: Extraction yields and total phenols for hydrodistilled extracts

Herbs	Extraction yield (mg g^{-1})	Total phenols (mg GA g^{-1})
Clove	245	85.60±9.50
Coriander	210	60.21±6.40
Caraway	190	35.32±8.10

Table 2: Acid values for Butter Oil (BO) mixed with BHT and different FDH extract during storage

	Storage period (days)									
Treatments	0	3	6	10	14	18	22	24	28	Mean
Control	0.45	0.51	0.53	0.54	0.52	0.64	0.71	0.75	0.74	0.610
BO+BHT at 200 ppm	0.44	0.54	0.55	0.57	0.53	0.50	0.53	0.56	0.55	0.541
BO+clove FDH 200 ppm	0.48	0.50	0.54	0.51	0.52	0.54	0.51	0.53	0.50	0.526
BO+clove FDH 400 ppm	0.42	0.54	0.57	0.57	0.57	0.54	0.51	0.54	0.49	0.530
BO+coriander FDH 200 ppm	0.43	0.53	0.58	0.57	0.54	0.56	0.69	0.68	0.64	0.590
BO+coriander FDH 400 ppm	0.44	0.56	0.58	0.54	0.64	0.60	0.59	0.58	0.57	0.570
BO+caraway FDH 200 ppm	0.41	0.52	0.58	0.55	0.60	0.62	0.59	0.58	0.60	0.570
BO+caraway FDH 400 ppm	0.47	0.50	0.54	0.56	0.55	0.58	0.61	0.62	0.60	0.580

Acid value is expressed as mg KOH /1 g butter oil

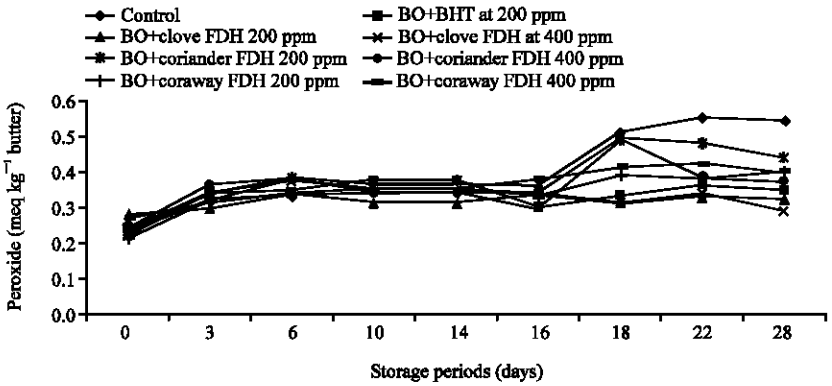

Fig. 1: Effect of different FDH extract on Butter Oil (BO) oxidative rancidity during storage

common effective synthetic antioxidants to food products in the range of 100-400 ppm (Allen and Hamiliton, 1999). Experiments were conducted using BHT at 200 ppm in order to compare the anti-oxidative activity of different FDH extract towards butter oil rancidity. A high storage temperature (60±1°C) was chosen to accelerate of BO oxidation due to a low degree of unsaturation of butter oil (Thampson, 1960). The experimental period was terminated when an objectional odour and high lipid rancidity values were obtained with the samples after 28 days.

Butter Oil System

Acid Values (AV) of butter oil (control) and mixed with different FDH extract at different rate and BHT during storage is shown in Table 2 and Fig. 1. The AV results indicate that different FDH extract exhibited antioxidant effect specially at rate of 400 ppm from different extracts with order of clove> coriander>caraway. Changes in the PV of butter oil mixed with different FDH extract at different rate and BHT during storage are shown in Fig. 1. The PV values of BO (control) and mixed with different extracts were very low at zero time and gradually increased with storage time. BHT produced significantly lower PV of butter oil when compared with other treatments. The addition of different extracts was effective as antioxidant when compared with the control experiment and in the same order; clove>coriander>caraway. The changes in the secondary oxidation products of BO are shown in Fig. 2, a result of the addition of different FDH extract based on the dada of TBA values. Addition of BHT at 200 ppm to butter oil caused very low increase in TBA values over time. On the other hand, the systems containing different FDH extract at different rate significantly linear reduce the formation of secondary oxidation products in comparison with the control experiment. The inhibitory effect of antioxidants against oxidation process in butter oil has been attributed to their donation of electrons or hydrogen atoms from phenolic hydroxyl groups to butter oil containing free

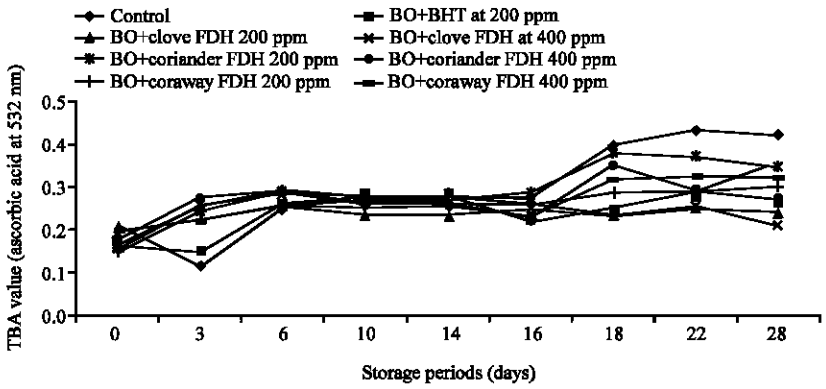

Fig. 2: Effect of different FDH extract on Butter Oil (BO) secondary oxidation products

radicals and to the formation of stable free radicals which do not initiate or propagate further oxidation of oils (Farag *et al*., 1993;Wollgast and Anklam, 2000). The good correlation between the results from total phenols analysis and the antioxidative assays has been earlier reported by Zheng and Wang (2001) and Dorman *et al*. (2003, 2004).

In complex systems, such as food and food preparations, various different mechanisms may contribute to oxidative processes, such as in Fenton reactions, where transition metal ions play a vital role, different reactive oxygen species might be generated and various target structures such as lipids, proteins and carbohydrates, can be affected. Therefore, it is important to characterize the extracts by a variety of antioxidant assays (Halliwell, 1997; El-Ghorab *et al*., 2008; Ollanketo *et al*., 2002).

CONCLUSION

The result of the present study showed that the different FDH extracts which contain highest amount of phenolic compounds, exhibited the greatest antioxidant activity. All of the extracts in this research exhibited different extent of antioxidant activity. The catalytic effectiveness of the added materials can be ranked according to inhibition power as BHT (200 ppm)>clove>coriander>caraway> control. Plant extracts might substitute synthetic food antioxidants, which may influence human health when consumed chronically (Martinez-Tome *et al*., 2001).

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