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ORIGINAL PAPER

Antioxidant Activity of Potato Peel Extracts in a Fish-Rapeseed Oil Mixture and in Oil-in-Water Emulsions

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Abstract The objectives of the present work were (a) to extract the phenolic fraction from the peels of two Danish varieties of potatoes, viz. Sava and Bintje, and examine their antioxidant capacity in in-vitro systems (b) to evaluate the effect of these extracts on the storage stability of a fish-rapeseed oil mixture and oil-in-water emulsions. Multiple antioxidant activity of the potato peel extracts was evident from in-vitro systems as they showed strong reducing power, radical scavenging ability, ferrous ion chelating activity and prevented oxidation in a liposome model system. The Sava variety, which showed strong antioxidant activity in in-vitro systems, was tested in oil and oil-in-water emulsions. Ethanolic extracts of Sava (≥1,600 mg/kg) prevented lipid oxidation in emulsions and in oil. Water extracts showed no antioxidant activity in oil whereas it showed pro-oxidant activity in emulsions. Thus, the results of the present study show the possibility of utilizing waste potato peel as a promising source of natural antioxidants for retarding lipid oxidation.

Keywords Potato peel extract · Antioxidant · Fish-rapeseed oil · Oil-in-water emulsion

Introduction

Lipid oxidation is one of the most important quality deteriorating processes in lipid bearing foods and leads to great economic losses in the food industry. Addition of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and tertiary butyl hydroquinone (TBHQ) can control lipid oxidation in foods [1]. However, the use of synthetic antioxidants such as BHT has been restricted because of their health risks and toxicity [2]. Therefore, the importance of replacing synthetic antioxidants with natural ingredients has increased greatly. Several natural plant materials such as black pepper, rosemary and other oriental herbs have been reported to retard lipid oxidation effectively [3, 4]. Lipid oxidation is a particular problem in foods enriched with the healthy *n*-3 polyunsaturated fatty acids. Therefore, there is a strong interest in identifying new natural antioxidants that can prevent formation of unpleasant fishy odors and flavors in these foods.

Potatoes (*Solanum tuberosum*) are one of the most commonly consumed vegetables throughout the world. The global consumption of potatoes as food is shifting from fresh potatoes to value added processed products such as French fries, chips and puree. Peels are the major by-product of potato processing industries, which represent a major waste disposal problem for the industry concerned. Up-grading of this by-product to value-added products is therefore of interest to the potato industry. Potato peel extracts have shown to be a good source of dietary fiber [5] and rich in phenolic acids especially of chlorogenic, gallic, protocatechuic and caffeic acids [6]. Antioxidative compounds extracted from potato peel may therefore be potential value added products that can be utilized for the feed, food and health care industries. The antioxidant property of potato peel extract from some Asian varieties (Kurfi Chandramukhi) has been reported in muscle foods and in soybean oil [7, 8], but its use as an antioxidant in fish oil and fish oil enriched products has not yet been studied.

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Moreover, no studies have been made with the Sava and Bintje varieties, which are important in the Nordic countries. The overall objective of this study was therefore to provide more knowledge about the potential for using potato peel extracts as a source of effective antioxidants in food systems, particularly those enriched with *n*-3 PUFA. The specific aims were to compare two different potato varieties (Sava and Bintje) and two different extraction methods (water and ethanol) with respect to (1) the composition of phenolic compounds in the resulting potato peel extracts, (2) the antioxidant capacity of the extracts in in-vitro systems (DPPH radical scavenging ability, iron chelating, reducing power and prevention of oxidation in a liposome model system); and (3) to evaluate the effectiveness of the extracts in retarding lipid peroxidation in fish-rapeseed oil mixture and in oil-in-water emulsion. Lipid oxidation was assessed by determination of peroxide value, volatiles, tocopherol concentration and sensory evaluation. A final objective was to combine the compositional data of the different extracts with information of their antioxidant properties obtained from the in-vitro systems and their efficacy in food systems in order to understand the mechanisms behind the possible antioxidant effects of the extracts.

Materials and Methods

Chemicals

Two Danish varieties of potatoes (*Solanum tuberosum*), Sava and Bintje were purchased from a local market. Cod liver oil:rapeseed oil (1:1) was kindly donated by Maritex A/S (Sortland, Norway). The fatty acid composition (unsaturated only) of oil used was 16:1, 4.1%; 18:1, 36.6%; 18:2, 10.2%; 18:3, 4.5%; 18:4, 1.1%; 20:1, 6.5%; 20:5, 4.7%; 22:1, 3.12%; 22:5, 0.62%; 22:6, 6.2% and saturated fatty acid content of 10.8%. The peroxide value (PV) and anisidine value (AV) of the oil used were 0.07 ± 0.03 meq/kg and 0.47 ± 0.04, respectively. L-α phosphatidyl choline, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), thiobarbituric acid, ascorbic acid, Ethylene diamine tetra acetic acid (EDTA), and external standards for identification of volatile oxidation products were obtained from Sigma-Aldrich (Steinheim, Germany). Chloroform and methanol were of HPLC grade from Lab-Scan (Dublin, Ireland). All the other reagents were of analytical grade obtained from Merck (Darmstadt, Germany).

Preparation of Potato Peel Extract

Potato tubers were washed with tap water and peeled manually using a kitchen vegetable peeler (average yield of potato peel was 12%). The peels were dried in a hot air oven at 55 °C for 72 h and powdered by using a kitchen blender. The material that passed through an 80-mesh sieve was retained for use. For the preparation of ethanolic extract, 5 g of powdered peel was extracted with 50 ml of 96% ethanol at 5 °C overnight and centrifuged at 2,800 rpm for 10 min. The supernatant was collected in a separate bottle and the residue was re-extracted three times under the same conditions as mentioned above. The combined filtrate was evaporated in a rotary evaporator (BUCHI, Switzerland) below 40 °C. The extract obtained after evaporation of organic solvent was used as natural antioxidant. In the case of water extract, 5 g of powdered peel was extracted with 100 ml of distilled water using the same procedure as mentioned above except that the combined extracts were freeze dried instead of evaporation. These extracts were kept at −80 °C until analysis. The extracts were redissolved in water and used for the analysis.

Determination of Total Phenolics

Total phenolic content in the extracts was determined by the method described by Singleton and Rossi [9]. An aliquot (100 µl) of extract was mixed with 0.75 ml of Folin-Ciocalteu reagent (1:10 diluted) and allowed to stand at room temperature for 5 min. Sodium bicarbonate (6%, 0.75 ml) was added to the mixture and incubated at room temperature for 90 min. The absorbance was measured at 725 nm using a spectrophotometer (Shimadzu UV mini 1240, Duisburg, Germany). A standard curve was plotted using different concentrations of gallic acid and the amount of total phenolics was calculated as gallic acid equivalents in mg/100 g of dried potato peel.

Identification of Phenolic Acids by HPLC

Identification of phenolic acid was done by a modified method of Onyeneho and Hettiarachchy [6]. The extract was passed through a 0.45 µm filter (Millipore, Westboro, MA) before being injected into the HPLC. Reverse phase HPLC was performed with an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector (Agilent G13158). The column used was ZORBAX Eclipse® XDB C8 analytical column (150 mm × 4.6 mm) (Agilent, USA) with a 5 µm packing material. Elution was performed with an isocratic mixture of methanol and 10 mM ammonium acetate buffer, pH 5.4 (12:88 v/v) at a flow of 1 ml/min. Detection was done using a diode array detector with reference wavelength of 280 nm. Retention times and peak areas were monitored and computed automatically by Chem32 integrator (Agilent, USA). Individual phenolic acids were identified by the retention time of sample chromatographic peaks

being compared with those of authentic standards using the same HPLC operating conditions and also by the extract being spiked with the standard components.

Screening of the Extracts for Antioxidant Activity

The water and ethanolic extracts of potato peel were screened for antioxidant activity by employing four in-vitro systems namely 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, metal chelating activity and ability to prevent lipid oxidation in liposome model systems.

Scavenging Effect on DPPH Free Radical

The scavenging effect on α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical was measured by the method of Shimada et al. [10] with some modification. DPPH solution (1.5 ml, 0.1 mM in 95% ethanol) was mixed with 1.5 ml of extract (at a final concentration of 800, 1,600, 2,400, 3,200 and 4,000 μg/ml). The mixture was shaken and left for 30 min at room temperature. Absorbance of the resulting solution was measured at 517 nm using a spectrophotometer (Shimadzu UV mini 1240, Duisburg, Germany). For the blank, 1.5 ml distilled water was used instead of the sample and sample control was also made for each fraction by mixing 1.5 ml of sample with 1.5 ml of 95% ethanol. Radical scavenging capacity was calculated as follows.

$$\text{DPPH radical scavenging capacity}(\%) = 1 - \frac{(\text{Abs. of sample} - \text{Abs. of sample control})}{\text{Abs. Blank}} \times 100$$

Iron (Fe^{2+}) Chelating Activity

The Fe^{2+} chelating activity of the extracts was estimated by the modified method of Dinis et al. [11]. To 1 ml of the extract (800, 1,600, 2,400, 3,200 and 4,000 μg/ml) was added 2.7 ml of deionized water and 0.1 ml of 2 mM ferrous chloride. After 3 min, the reaction was inhibited by the addition of 5 mM ferrozine (0.2 ml). The mixture was shaken vigorously and left at room temperature for 10 min. Absorbance of the resulting solution was measured at 562 nm using a spectrophotometer (Shimadzu UV mini 1240, Duisburg, Germany). A blank was run in the same way by using distilled water instead of a sample. Sample control was made for each extract without adding ferrozine. The chelating capacity was calculated as follows.

$$\text{Iron chelating activity}(\%) = 1 - \frac{(\text{Abs. of sample} - \text{Abs. of sample control})}{\text{Abs. of Blank}} \times 100$$

Reducing Power

The reducing power was measured according to the method of Oyaizu [12] with some modifications. To 1 ml of extract (800, 1,600, 2,400, 3,200 and 4,000 μg/ml) was added 1 ml 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and 1 ml of 10% TCA was added into this reaction mixture. An aliquot of 2 ml from the incubation mixture was mixed with 2 ml of distilled water and 0.4 ml of 0.1% ferric chloride in test tubes. After 10 min the solution was measured at 700 nm using a spectrophotometer (Shimadzu UV mini 1240, Duisburg, Germany). Increased absorbance (A_{700}) of the reaction mixture indicated increased reducing power.

Inhibition of Lipid Peroxidation in a Liposome Model System

Liposomes were prepared from soybean phosphatidyl choline according to the method of Vikbjerg et al. [13] with some modification. Phosphatidyl choline (50 mg) was weighed into a screw cap vial containing 5 mL of chloroform to dissolve the lipid. The solvent was evaporated under a stream of nitrogen to get a uniform thin lipid film. MLV (Multilamellar vesicles) and LUV (large unilamellar vesicles) were subsequently prepared from this dried lipid film. MLV were prepared by dispensing the lipid film in 10 ml histidine buffer (5 mM Histidine, containing 0.12 M KCl, pH 6.8) and were kept at 37 °C for 1 h in order to ensure complete hydration. During this period, the lipid suspensions were vortexed every 15 min. LUV were subsequently prepared by extrusion of MLV through two stacked 100-nm pore size polycarbonate filters (Avanti Polar Lipids Inc., Alabaster, AL, USA) 10 times. Phospholipid concentrations in the liposome preparation was determined by measuring phosphate according to Fiske and Subbarow [14] as inorganic phosphorus liberated after Bartlette's perchloric acid digestion [15].

Lipid oxidation was performed in a model system containing 0.1 mg of phosphatidyl choline liposomes per ml of Phosphate Buffered Saline (PBS) (3.4 mM Na_2HPO_4–NaH_2PO_4, 0.15 M NaCl, pH 7.0) and extracts at a final concentration of 800, 1,600, 2,400, 3,200 and 4,000 μg/mL. Lipid oxidation was initiated by iron redox cycling using 50 μM $FeCl_3$ and 100 μM ascorbate. The order of addition was buffer, extracts, liposome, ferric chloride and ascorbic acid. The reactants were mixed by vortexing for 2 s and incubated at 37 °C in a water bath for 1 h. The liposome assay solution with distilled water instead of sample was used as control. Lipid oxidation was measured by determining the concentrations of thiobarbituric acid

reactive substance (TBARS) formed according to the method of Buege and Aust [16]. Aliquots (0.25 ml) of liposome suspensions were sampled into test tubes and made up to 1 ml by adding distilled water (0.75 ml). Then, 2 ml of TBA reagent (3.75 g/l TBA; 150 g/l trichloroacetic acid; HCL 0.25 mol/l; 0.1 g/l BHT) was added. The tubes were closed and heated in a boiling water bath for 15 min, immediately cooled and centrifuged (1,500×*g*; 10 min). A reagent blank was prepared in the same manner as mentioned above with distilled water instead of sample. The absorbance at 532 nm of the supernatant was read against a blank.

Effect of Potato Peel Extract in Bulk Fish-Rapeseed Oil

As the Sava variety showed good antioxidant activity in most of the in-vitro systems, it was selected for testing in real systems. Fish-rapeseed oil was used as the substrate for oxidation studies. Oil samples containing 800, 1600 or 2400 mg/kg of either water or ethanolic extracts of the Sava variety of potatoes were prepared. Each 250 ml oil sample was placed in a 500 ml Pyrex bottle, the freeze dried extracts were dissolved in methanol and added to oil. The solvent was removed under nitrogen by keeping the flasks at 55 °C in opened position for 1 h. Synthetic antioxidant BHT was mixed in oil for a comparative study at its legal limit of 200 mg/kg [17]. All the oil samples of each treatment were prepared in duplicate and were kept on a magnetic stirrer at 55 °C for 3 days in the dark. Control samples of oil without antioxidant were also placed under identical conditions. Oil samples (50 ml) of each treatment were withdrawn each day starting from day zero to assess the antioxidant activity of potato peel extract. The samples for chemical analysis were transferred to separate brown glass bottles, flushed with nitrogen and stored at −80 °C until analyzed. The criteria used for evaluating the antioxidant activity were peroxide value, anisidine value, tocopherol concentration and sensory evaluation.

Antioxidant Effect of Potato Peel Extract in 5% Oil-in-Water Emulsion

A 5% oil-in-water emulsion was prepared with 1% citrem as an emulsifier. In brief: 5 g of citrem and 25 g of fish-rapeseed oil were weighed into a glass beaker and mixed together by a magnetic stirrer. Buffer (470 ml Imidazole-Acetate, 10 mM, pH 7) was measured into a 1 L beaker and the antioxidants (either water or ethanolic extracts of Sava variety of potato at different final concentrations 1,600, 2,400 and 4,800 mg/kg) was dissolved into the buffer. A pre-homogenization was done for 3 min using an Ultra Turrax (T1500, Ystral, Dottingen, Germany) by adding the oil/citrem mix slowly over 1 min and then mixing was continued for a further 2 min. After the pre-homogenization the emulsion was prepared by using a high pressure homogenizer (Total pressure of 800 bar, Panda 2 K Homogeniser from Niro Soavi S.p.A, Parma, Italy). A control without antioxidant and an emulsion with 200 mg/kg of BHT were made for comparison. After making the emulsions, 400 ml each of the emulsions were poured into 500 ml sterile blue capped bottles in duplicate and Fe solution ($FeSO_4$ 100 μM) was added in order to induce oxidation. Bottles were kept on a magnetic stirring plate at 20 °C for 3 days in the dark. The sampling was done from the same bottle after 0, 12, 24, 36, 48 and 60 h. The samples for chemical analysis were transferred to separate brown glass bottles, flushed with nitrogen and stored at −80 °C until analyses. PV, volatiles, tocopherols and sensory evaluation were used to assess antioxidant activity.

Analysis of Peroxide Value (PV) and Anisidine Value (AV)

Lipids from the emulsions were extracted by chloroform:methanol (1:1 v/v) as described by Bligh and Dyer [18]. PV was measured directly on the oil or on Bligh and Dyer extract from emulsions according to the method described by the international IDF standards [19]. Anisidine value of the oil samples were measured by the method of AOCS [20].

Dynamic Headspace Analysis of Volatile Secondary Oxidation Products in Emulsion

Volatile secondary oxidation products from 4 g of emulsion were collected by purging the emulsion with nitrogen (150 ml/min) for 30 min at 45 °C, using 4-methyl-1-pentanol as the internal standard, and trapped on Tenax GR tubes (Perkin-Elmer, CN, USA) packed with 225 mg Tenax GR (60-80 mesh, Varian, Middelburg, Netherlands). The volatiles were desorbed (200 °C) from the trap in an automatic thermal desorber (ATD-400, Perkin-Elmer, Norwalk, CT) and cryofocused on a Tenax GR cold trap. Volatiles were separated by gas chromatography (HP 5890 IIA, Hewlett-Packard, Palo Alto, CA) as described by Timm et al. [21] and analysed by mass spectrometry (HP 5972 mass selective detector). The oven temperature program was: 45 °C held for 5 min, 1.5 °C/min to 55 °C, 2.5 °C/min to 90 °C, 12 °C/min to 220 °C and finally held at 220 °C for 4 min. The individual compounds were identified by both MS-library searches (Weley 138 K, John Wiley and Sons, Hewlett-Packard) and by authentic external standards. The individual compounds were quantified through calibration curves made by adding 1 μl of standards to Tenax GR tubes directly. The external standards used were 1-penten-3-ol, 2-penten-3-ol, heptanal,

hexenal, 2-heptenal, 4-heptenal, 2-hexenal, 2,4-heptadienal, 1-penten-3-on, 2,6-nonadienal at a concentration of 0.01, 0.05, 0.1, 0.25, 0.50 mg/g ethanol. The limit of detection and the limit of quantification were determined at a signal-to-noise ratio of 2 and 5, respectively, for each compound at a given condition as mentioned above.

Determination of Tocopherol Content

Tocopherol content was determined using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA), equipped with a fluorescence detector. About 50 mg of oil samples (in the case of emulsion, 2 g of the chloroform extract from the Bligh and Dyer evaporated under nitrogen) was dissolved in 10 mL *n*-heptane and from these 2 ml samples were put into separate vials before injection of an aliquot (40 µl) on a Spherisorb s5w column (250 mm × 4.6 mm) (Phase Separation Ltd, Deeside, UK). Elution was performed with an isocratic mixture of *n*-heptane/2-propanol (100: 0.4; v/v) at a flow of 1 ml/min. Detection was done using a fluorescence detector with excitation at 290 nm and emission at 330 nm and according to AOCS (1994) [22]. Results were expressed in µg tocopherol per g of lipid.

Sensory Evaluation

The sensory evaluation was performed by an expert panel composed of 4 persons. The emulsions and oils were evaluated for odor with the descriptors fishy/rancid and others. Samples were evaluated on a continuous intensity scale ranging from zero intensity to a maximum intensity of 9. The panel members assessed the samples on an individual basis. Subsequently the panel agreed on an average score for each descriptor. Samples of 50 ml were served in randomized order after incubation for one hour at 10 °C (emulsions) or 50 °C (oils).

Statistical Analyses

The data for total and individual phenolic compounds were subjected to one way analysis of variance, and all the other data obtained were analyzed by two-way analysis of variance. The statistical comparisons among the samples were performed with Bonferroni multiple comparison test by using a statistical package program Graphpad prism 4 (Graphpad Software Inc., San Diego, USA). A p value <0.05 was considered as statistically significant. Furthermore, PV, volatiles and tocopherol data of emulsions were subjected to principal component analysis using Unscrambler version 7.6 SR-1 (Camo, Oslo, Norway) [23]. All variables were weighted (1/standard deviation) and the models were validated using full cross validation.

Results and Discussion

Total Phenolics

Sava peels contained more polyphenols than Bintje peels (Fig. 1). The ethanolic extract of Sava contained a significantly ($p < 0.001$) higher quantity of polyphenols when compared to other extracts. There was no significant ($p < 0.001$) difference between the ethanolic extracts of Bintje and water extracts of Sava. The water extracts of Bintje had the lowest phenolic content. The total phenolic content of ethanolic extract of peels in the present study was two to three times higher than the one reported for the Asian variety of Kurfi chandramukhi peel ethanolic extract (70.8 mg of catechin equivalents/100 g of potato peel) [7]. This is because phenolic content of the potatoes varies with variety, color, geographical origin, season and storage [24–27]. The selection of solvent and the extraction methods also affect the concentration of total phenols. Rodriguez de Sotillo et al. [28] reported the total phenolic content in aqueous extract of potato peel as 32.2 mg/100 g and methanolic extract as 41.7 mg/100 g. Thus, our results agree with this finding by having a higher quantity of phenolics in ethanolic extract compared with the water extract.

Identification and Quantification of Phenolic Acids

The major phenolic acids in the extracts were identified by HPLC (Fig. 2). Water extracts showed significantly ($p < 0.001$) higher levels of gallic and protocatechuic acids

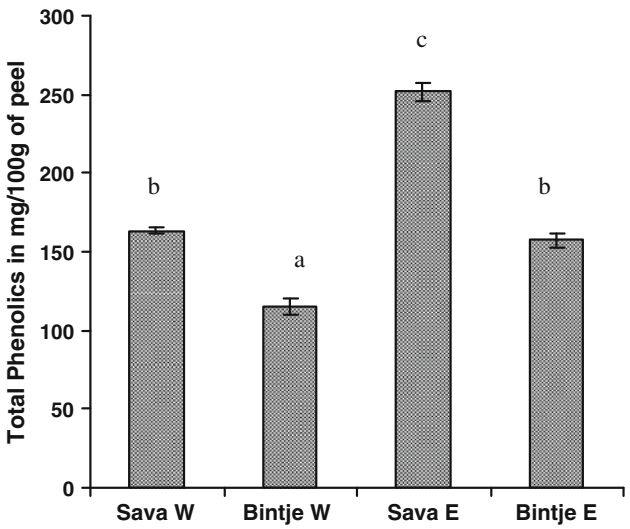

Fig. 1 Total phenolic content of ethanolic and water extracts of Sava and Bintje potato peels. Results are average of triplicate determinations ± standard deviation. Samples followed by the same letter are not significantly different in Tukey's test using 0.05 level of significance. *W* water extracts, *E* ethanolic extracts

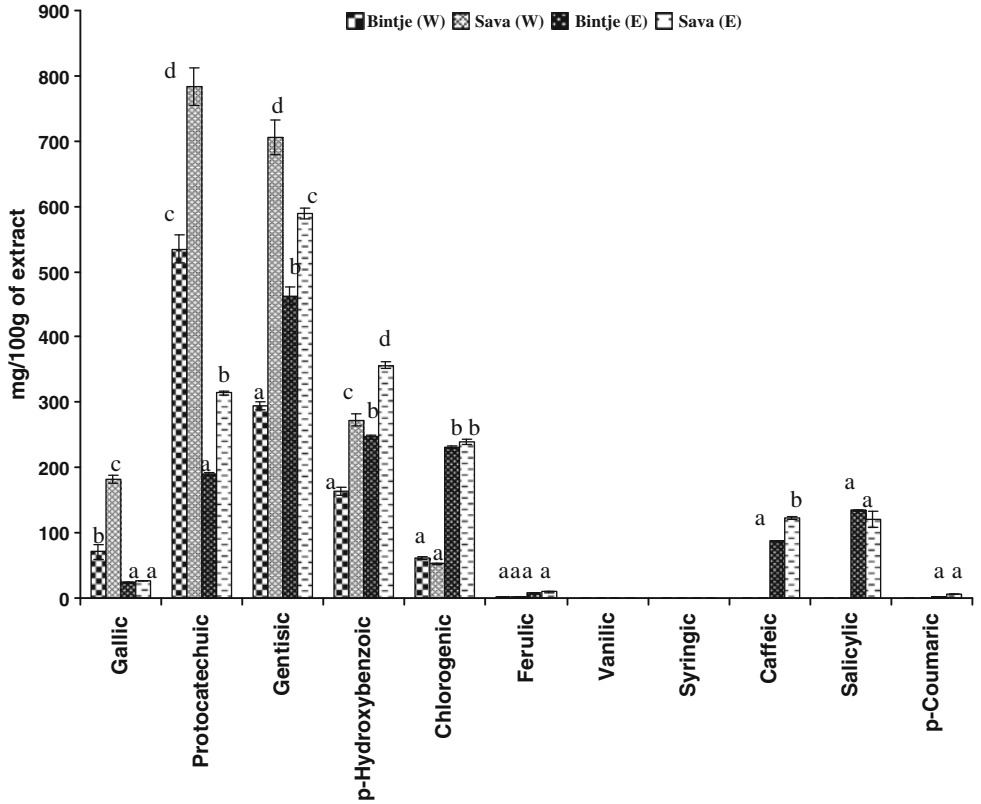

Fig. 2 The major phenolic acids in the extracts of Sava and Bintje potato peels identified by HPLC. Results are average of triplicate determinations ± standard deviation. Samples followed by the same letter are not significantly different in Tukey's test using a 0.05-level of significance. *W* water extracts, *E* ethanolic extracts

when compared to the corresponding ethanolic extract, while ethanolic extracts showed higher levels of *p*-hydroxy benzoic acid and chlorogenic acids. Some of the earlier studies have reported chlorogenic acid as the major constituent in potato peels [7, 28], but these Danish varieties of potatoes contained protocatechuic and gentisic acid as major constituents followed by *p*-hydroxybenzoic and chlorogenic acid. Coumaric acid was found in trace amounts in Sava ethanolic extract and was absent in Bintje ethanolic extracts, while vanillic and syringic acids were absent in both water and ethanolic extracts of both varieties. Only ethanolic extracts contained ferulic, caffeic and salicylic acids. One explanation for this is the difference in solubility of these phenolic compounds in water and ethanol. Unlike the study by Rodriguez de Sotillo et al. [28], we did not find caffeic acid in the water extracts. Mota et al. [29] found that the solubility of salicylic, ferulic and caffeic acid in water increased with increased temperature. As the temperature of extraction was low in the present study, this might have decreased the ability of water to extract these compounds. Another possible explanation for the lack of extraction of these phenolic compounds in water might be the formation of an insoluble complex with some water soluble proteins, which may be present in the water extract. As the proteins are not extracted by ethanolic extraction there is no chance of such type of complex formation and the phenolic acids will thus be available in their free form in the extracts. Masking of the antioxidant capacity by the interaction of phenolic compounds with protein has already been reported [30].

Antioxidant Activity in in-Vitro Systems

The Bintje variety showed significantly ($p < 0.001$) higher DPPH radical scavenging than Sava at most concentrations (Fig. 3a). Ethanolic extracts showed higher (80–90%) DPPH radical scavenging activity than water extracts in all the concentrations tested. In contrast, water extracts of both Sava and Bintje showed concentration dependency in their radical scavenging activity. At 800 and 1,600 μg/ml water extracts showed low (9 and 25%) scavenging activity with no significant difference ($p < 0.001$) between Sava and Bintje. From 2,400 μg/ml onwards water extracts showed a significant ($p < 0.001$) increase in radical scavenging activity. Nevertheless, irrespective of the concentration the order of scavenging activity was Bintje ethanolic > Sava ethanolic > Bintje water > Sava water. Earlier, Singh and Rajini [31] also reported a concentration-dependent

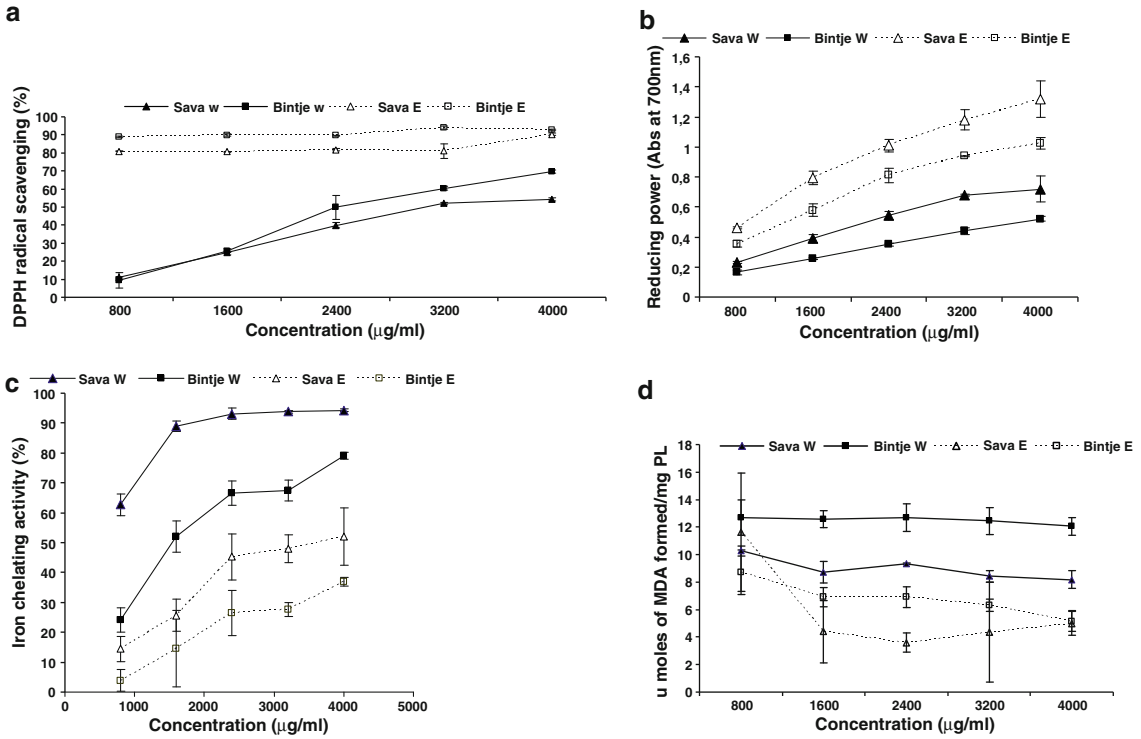

Fig. 3 **a** The DPPH radical scavenging capacity **b** reducing power **c** Fe^{2+} chelating capacity **d** antioxidant activity in liposome model system of water (*W*) and ethanolic (*E*) extracts of Sava and Bintje potato peels. Results are average of triplicate determination ± standard deviation

increase in DPPH scavenging effect of water extracts of potato peel. Phenolic compounds are generally very active in scavenging DPPH free radicals due to their fast electron transfer process whilst hydrogen atom abstraction becomes a marginal reaction path [32]. A linear correlation between radical scavenging ability and polyphenolic content has been reported in an extensive range of fruits [33]. The higher DPPH radical scavenging activity of the ethanolic extract compared to that of the water extract might be due to a high content of total phenolic acids and/or due to the presence of caffeic acid and ferulic acid in addition to other phenolic compounds present in water extracts. A high radical scavenging activity of caffeic acid and ferulic acid has already been reported [34, 35].

The reducing power can be explained as an index of secondary antioxidative activity. Reducing properties are associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [36]. It is clear that the reducing power (as indicated by the absorbance at 700 nm) was a function of concentration (Fig. 3b). In general, Sava extracts showed a higher reducing power than Bintje and similar to the findings for DPPH scavenging activity, ethanolic extracts were better than water extracts. The order of the reducing power was Sava ethanolic > Bintje ethanolic > Sava water > Bintje water. Several authors have observed a direct correlation between antioxidant activities and reducing power of plant extracts including potato peels [7, 31]. A number of phenolic compounds such as caffeic acid, ferulic acid and chlorogenic acid are reported to be good reducing agents [34, 35]. The higher reducing power of the ethanolic extract in the present study might be particularly due to the presence of caffeic acid and ferulic acid. Gulcin [34] reported that the reducing power of caffeic acid was higher than that of BHA, BHT, α-tocopherol and Trolox.

Transition metals, such as Fe^{2+} and Cu^{2+} can catalyze the generation of reactive oxygen species such as hydroxyl radical (·OH) and superoxide anion (O_2^-) [37]. Especially, Fe^{2+} generates ·OH by the Fenton reaction, by which the lipid peroxidation chain reaction is accelerated. Moreover, Fe^{2+} catalyzes the breakdown of lipid peroxides, which leads to the formation of volatile oxidation products responsible for off flavor formation. Therefore, the chelation of metal ions often reduces lipid oxidation. The Fe^{2+} chelating activity was found to be concentration dependent and increased with increase in concentration (Fig. 3c). The order of chelating activity was Sava water extract > Bintje water > Sava ethanolic > Bintje ethanolic. Thus, in contrast to the findings for DPPH and reducing power water extracts showed significantly ($p < 0.001$) higher Fe^{2+} chelating activity when compared to ethanolic extracts. Moreover, Sava was better than Bintje. The Fe^{2+} chelating activity of these extracts might be due to specific phenolic

compounds, which have been reported to be good chelating agents [33]. The catechol derivatives such as protocatechuic acid and catechin have been reported to be strong metal chelators [38]. The high metal chelating activity of water extracts may thus be due to their very high content of protocatechuic acid.

Antioxidant Activity in a Liposome Model System

A liposome system allows the lipid-soluble components to be present in the lipid phase without the presence of a co-solvent, while the water soluble antioxidants can be added to the aqueous phase of the liposome. Fig. 3d depicts the effect of different concentrations of extracts on phosphatidyl choline liposome oxidation, induced by ferric/ascorbic acid. After 1 h of incubation, the TBARS assays revealed that the control without adding any antioxidant showed the highest degree of oxidation with 14.23 μmol of malondialdehyde (MDA) formed/mg phospholipid (Data not shown). The ethanolic extracts were significantly ($p < 0.001$) better at preventing lipid oxidation compared to water extracts and Sava was better than Bintje. In spite of the higher Fe^{2+} chelating effect, water extracts showed less inhibitory effect on the Fe^{2+}-induced lipid peroxidation in the liposome model system. The order of effectiveness in preventing lipid oxidation was Sava ethanolic > Bintje ethanolic > Sava water > Bintje water. In the present study, the protective effect of different extracts on lipid oxidation in the liposome system correlates well with total phenolic content. Robert and Gordon [39] found that the water soluble extracts of some fruits and vegetables increased the lag phase linearly with total phenol concentration in liposomes. The fact that water extracts were less efficient than ethanolic extract in our study could be explained by the difference in radical initiator selected for inducing lipid peroxidation in liposome model system. Robert and Gordon [39] used 2.2′azobis (2-amidopropane) dihydrochloride (AAPH) as a radical initiator with EDTA to chelate all metal ions. In our study Fe^{2+} induced lipid peroxidation was used and the results could originate from the fact that, water-soluble antioxidants including ascorbic acid and gallic acid can become pro-oxidant in the presence of metal ions, depending on the antioxidant:metal ratio [40]. Thus, the lower efficiency of water extracts might be due to the lack of some of the phenolic compounds present in the ethanolic extracts such as caffeic acid, salicylic acid which have been shown to have very good antioxidant properties [41] and/or the presence of other water soluble substance such as ascorbic acid and metals.

Most of the in-vitro antioxidant tests proved that Sava was better than Bintje. Ethanolic extracts showed high DPPH radical scavenging, reducing power and ability to prevent lipid oxidation in the liposomal model system, whereas water extracts showed higher Fe^{2+} chelating activity than ethanolic extracts. On the basis of these findings both water and ethanolic extracts of Sava were selected for further testing in bulk oil and 5% oil-in-water emulsions.

Antioxidant Activity of Potato Peel Extracts in Bulk Oil

On the first day of storage there was no significant ($p < 0.001$) difference in the PV and AV values between different treatments groups (Table 1). PV ranged between 0.41 and 0.96 meq/kg oil and AV between 0.40 and 1.25. However, at the end of the storage period both PV and AV showed significant ($p < 0.001$) increase in all treatment groups. Ethanolic extract 2,400 mg/kg was most effective in retarding lipid peroxidation in bulk oil, as this sample

Table 1 Effect of different potato peel extracts on peroxide value and Anisidine value of fish-rapeseed oil stored at 55 °C

Storage period	Peroxide value			Anisidine value		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Control	$0.78 \pm 0.24^{a,v}$	$7.09 \pm 2.41^{a,v,w}$	$23.63 \pm 4.60^{b,w}$	$1.18 \pm 0.98^{a,v}$	$3.09 \pm 1.51^{a,v}$	$24.07 \pm 9.09^{b,w}$
BHT200	$0.67 \pm 0.18^{a,v}$	$4.59 \pm 2.65^{a,v}$	$18.87 \pm 3.74^{b,w}$	$1.25 \pm 1.01^{a,v}$	$4.24 \pm 3.11^{a,v}$	$22.02 \pm 9.36^{b,w}$
E800	$0.96 \pm 0.22^{a,v}$	$15.94 \pm 4.60^{b,w}$	$23.46 \pm 2.87^{b,w}$	$0.87 \pm 0.99^{a,v}$	$8.57 \pm 5.73^{a,v}$	$44.43 \pm 8.81^{b,x}$
E1600	$0.52 \pm 0.17^{a,v}$	$1.77 \pm 0.67^{a,v}$	$18.55 \pm 4.47^{b,w}$	$1.07 \pm 1.06^{a,v}$	$1.32 \pm 0.53^{a,v}$	$11.47 \pm 7.57^{a,v,w}$
E2400	$0.50 \pm 0.05^{a,v}$	$0.95 \pm 0.05^{a,v}$	$3.29 \pm 0.97^{a,v}$	$1.12 \pm 0.90^{a,v}$	$1.18 \pm 0.79^{a,v}$	$2.44 \pm 0.38^{a,v}$
W800	$0.52 \pm 0.04^{a,v}$	$11.76 \pm 4.82^{b,w}$	$21.76 \pm 3.57^{c,w}$	$0.40 \pm 0.27^{a,v}$	$5.35 \pm 3.02^{a,v}$	$38.95 \pm 1.46^{b,x}$
W1600	$0.41 \pm 0.10^{a,v}$	$4.01 \pm 0.45^{a,v}$	$23.58 \pm 1.89^{b,w}$	$1.01 \pm 1.09^{a,v}$	$1.80 \pm 1.12^{a,v}$	$20.86 \pm 5.15^{b,w}$
W2400	$0.66 \pm 0.14^{a,v}$	$16.71 \pm 2.43^{b,w}$	$25.22 \pm 3.97^{b,w}$	$1.04 \pm 0.33^{a,v}$	$10.84 \pm 1.85^{a,v}$	$47.31 \pm 0.81^{b,x}$

Results are averages of triplicate determinations on the duplicate samples ± standard deviation

Samples followed by the same letter are not significantly different in the Bonferroni post test using a 0.05-level of significance

[a,b] Storage time, [v,w,x]Treatments

W water extracts, *E* ethanolic extracts

had the lowest PV and AV values throughout the storage at 55 °C. At the end of storage, the PV and AV in the E2400 sample was found to be 5.7 and 9 times lower than those of the BHT samples, respectively. The ethanolic extract 1,600 mg/kg was found to be as effective as BHT in lowering PV and resulted in significantly ($p < 0.001$) lower AV than BHT. Water extract showed no significant difference ($p < 0.001$) compared to control for PV values and had generally significantly ($p < 0.001$) higher AV values than controls demonstrating no antioxidant effect or even pro-oxidant effects in bulk oil.

Table 2 summarizes the changes in different tocopherol homologues in oil during storage at 55 °C. The changes in levels of α- and γ-tocopherols were more prominent than the changes in levels of β-tocopherol. During the first day of storage there was no significant ($p < 0.001$) difference in the levels of all the tocopherol homologues between the different treatment groups. However, as the storage progressed a significant ($p < 0.001$) decrease was noticed in the levels of all tocopherols in different treatment groups except 2,400 mg/kg ethanolic extract, which showed no significant ($p < 0.001$) reduction in different tocopherols throughout the storage period. The ethanolic extract 1,600 mg/kg was more effective in preventing loss of α-, β- and γ-tocopherols than BHT, whereas ethanolic extract 800 mg/kg and water extract at 800 and 2,400 mg/kg showed lower levels to complete absence of α-, β- and γ-tocopherols compared to control at the end of the storage period.

Sensory analysis data (Table 3) also fits well with PV, AV and tocopherol data. Fishy/rancid odor increased during storage. At the end of storage fishy/rancid odor was lowest and similar in BHT, E1600 and E2400 mg/kg. Generally fishy/rancid intensities were higher in oil with added water extracts compared to oil with added ethanolic extracts.

From the results, it is evident that an ethanolic extract 2,400 mg/kg of Sava was more effective than synthetic antioxidant BHT in preventing lipid oxidation in bulk oil and an ethanolic extract 1,600 mg/kg was as effective as BHT. Water extract did not show any preventive effects against lipid oxidation in bulk oil rather it seemed to promote oxidation particularly when added in higher concentration. Our findings are consistent with the results of Zia-ur-Rehman et al. [8] who found that the antioxidant activity of 2,400 ppm of potato peel extract was comparable to BHT after a 60-day storage of soybean oil at 25 and 45 °C. The higher antioxidant activity of the ethanolic extracts in oil might be due to the tocopherol sparing effect and/or due to the free radical scavenging effect of the phenolic compounds present in ethanolic extracts. Caffeic acid, catechin or epicatechin has been reported to act synergistically with α-tocopherol, extending its antioxidant

Table 2 Effect of different potato peel extracts on α-, β-, and γ-tocopherol in fish-rapeseed oil stored at 55 °C

	α-Tocopherol			β-Tocopherol			γ-Tocopherol		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
Control	$227.00 \pm 28.8^{b,v}$	$206.32 \pm 3.5^{b,w}$	$29.51 \pm 25.4^{a,v}$	$19.62 \pm 2.9^{b,v}$	$19.33 \pm 4.8^{a,b,v}$	$10.76 \pm 0.4^{a,w}$	$170.43 \pm 19.1^{b,v}$	$162.75 \pm 11.6^{b,v}$	$103.68 \pm 11.6^{a,w,x}$
BHT200	$200.32 \pm 1.7^{b,v}$	$165.54 \pm 28.3^{b,v}$	$64.58 \pm 72.6^{a,w}$	$18.42 \pm 0.1^{a,v}$	$16.79 \pm 8.2^{a,v}$	$12.66 \pm 7.5^{a,x}$	$141.98 \pm 3.1^{a,v}$	$135.58 \pm 33.4^{a,v}$	$101.23 \pm 60.8^{a,w}$
E800	$185.16 \pm 5.9^{b,v}$	$157.33 \pm 61.7^{b,v}$	$0^{a,v}$	$21.56 \pm 2.3^{b,v}$	$20.36 \pm 5.5^{b,v}$	$1.75 \pm 0.8^{a,v}$	$160.53 \pm 5.3^{b,v}$	$153.67 \pm 16.6^{b,v}$	$17.75 \pm 12.1^{a,v}$
E1600	$225.23 \pm 17.6^{b,v}$	$222.37 \pm 0.8^{b,w}$	$130.02 \pm 25.3^{a,w}$	$26.25 \pm 1.4^{a,v}$	$23.54 \pm 0.3^{a,v,w}$	$17.69 \pm 6.9^{a,x}$	$169.43 \pm 0.6^{a,v}$	$173.71 \pm 2.6^{a,v}$	$136.74 \pm 36.4^{a,x}$
E2400	$237.53 \pm 25.6^{a,v}$	$231.69 \pm 24.97^{a,w}$	$195.82 \pm 10.6^{a,x}$	$26.14 \pm 3.9^{a,v}$	$25.99 \pm 1.2^{a,w}$	$22.34 \pm 0.8^{a,x}$	$171.25 \pm 13.9^{a,v}$	$176.32 \pm 1.5^{a,v}$	$160.13 \pm 20.2^{a,x}$
W800	$185.83 \pm 7.5^{b,v}$	$190.79 \pm 24.9^{b,v,w}$	$0^{a,v}$	$21.20 \pm 2.8^{b,v}$	$19.85 \pm 1.8^{b,v}$	$7.90 \pm 1.5^{a,v,w}$	$140.01 \pm 8.9^{b,v}$	$138.95 \pm 4.3^{b,v}$	$79.51 \pm 8.9^{a,w}$
W1600	$192.94 \pm 2.9^{b,v}$	$205.79 \pm 56.2^{b,w}$	$45.88 \pm 64.8^{a,v}$	$22.88 \pm 3.4^{b,v}$	$19.18 \pm 1.1^{a,b,v}$	$10.06 \pm 4.2^{a,w,x}$	$147.65 \pm 7.1^{b,v}$	$136.52 \pm 45.5^{b,v}$	$77.01 \pm 50.5^{a,w}$
W2400	$193.56 \pm 0.6^{b,v}$	$139.33 \pm 23.8^{b,v}$	$0^{a,v}$	$20.60 \pm 0.5^{b,v}$	$21.59 \pm 0.5^{b,v}$	$0^{a,v}$	$148.51 \pm 4.6^{b,v}$	$147.99 \pm 5.2^{b,v}$	$54.42 \pm 9.2^{a,v,w}$

Results are averages of triplicate determinations on the same samples ± standard deviation. Samples followed by the same letter are not significantly different in the Bonferroni post test using a 0.05-level of significance

^{a,b} Storage time, ^{v,w,x}Treatments

W water extracts, *E* ethanolic extracts

Table 3 The average scores for odour (attributes were fishy/rancid and others) for oil treated with different concentration of potato peel extracts

Treatment groups	Fishy/Rancid	Others (description)
Day 1		
Control	1	3 (Fresh/fruity)
BHT200	0–1	2 (sweet/fruity)
E 800	1	4 (Hay/sweet/cake)
E1600	1	4 (Hay/synthetic/cookie)
E2400	1	5 (Sweet/cookie)
W800	1	3 (Hay/sweet)
W1600	1	3 (Hay/sweet)
W2400	1	3 (Hay/sweet/cake)
Day 3		
Control	4	2 (Synthetic)
BHT200	2	2 (sweet/fruity)
E 800	5	2 (Hay)
E1600	2	2 (Hay)
E2400	1	2 (Sweet)
W800	4	3 (Sweet/hay)
W1600	3	3 (Sweet/hay)
W2400	6	2 (Synthetic)

W water extracts, *E* ethanolic extracts

capacity by recycling α-tocopherol from the α-tocopherol radical [42, 43].

Antioxidant Activity of Potato Peel Extracts in a 5% Oil-in-Water Emulsion

PV in all the emulsions containing fish oil increased significantly during storage (Table 4). At day 0, there was no significant ($p > 0.001$) differences in PV values between the groups except for water extracts 2,400 and 4,800 mg/kg which showed significantly ($p < 0.001$) higher values of PV. This shows that even in the preparation step itself these two samples oxidized. As the storage progressed, PV showed a gradual increase in all the fish oil emulsions and the PV of the control reached 21.9 meq/kg at the end of the storage period. Emulsions containing ethanolic extract 1,600, 2,400 and 4,800 mg/kg showed similar low PV levels as emulsions containing BHT (4–7 meq/kg). Addition of water extracts 2,400 and 4,800 mg/kg resulted in significantly ($p < 0.001$) higher values of PV even when compared to control without any added antioxidants, whereas water extracts 1,600 mg/kg showed no difference from the control [not significant ($p > 0.001$)].

The concentration of volatile secondary oxidation products such as 2-pentenal, 2,4-heptadienal, 1-pentene-3-one, 1-pentene-3-ol formed from ω-3 fatty acids, hexanal formed from ω-6 fatty acids and nonanal formed from ω-9 fatty acids were determined. The development of the volatiles showed two different patterns which are shown in Fig. 4a and b. Initially, there were no significant ($p > 0.001$) differences between samples for the different volatiles and their level increased gradually during the storage period. At the end of the storage period, the concentration of 2,4-heptadienal, 2-pentenal, 1-pentene-3-one,1-pentene-3-ol showed similar trends as found for PV as exemplified by 2-pentenal in Fig. 4a. Thus, all the ethanolic extracts and BHT showed significantly ($p < 0.001$) lower levels of these volatiles than all the other samples (Fig. 4a). The lowest values were found for ethanolic extracts 2,400 and 4,800 mg/kg. In contrast, ethanolic extracts showed significantly ($p < 0.001$) higher levels of hexanal and nonanal when compared to water extracts (except 2,400 mg/kg) which showed lower values of these volatiles than the control as exemplified by nonanal in Fig. 4b.

The different tocopherol homologues like α-, γ- and δ-tocopherols in emulsion showed similar trends as

Table 4 Effect of different potato peel extracts on peroxide value in 5% oil-in-water emulsion

Storage time	0 h	12 h	24 h	36 h	48 h	60 h
Control	$1.36 \pm 0.06^{a,v}$	$12.78 \pm 2.59^{b,w}$	$15.85 \pm 3.52^{b,c,w,x}$	$20.25 \pm 1.48^{c,x}$	$20.12 \pm 0.46^{c,x}$	$21.95 \pm 2.89^{c,w,x}$
BHT200	$1.52 \pm 0.08^{a,v}$	$3.88 \pm 0.13^{a,v}$	$3.65 \pm 0.37^{a,v}$	$4.78 \pm 0.36^{a,v}$	$5.14 \pm 1.29^{a,b,v}$	$6.87 \pm 0.56^{b,v}$
E1600	$1.37 \pm 0.11^{a,v}$	$2.59 \pm 0.40^{a,v}$	$3.52 \pm 1.13^{a,v}$	$3.73 \pm 1.06^{a,v}$	$6.55 \pm 6.47^{b,v}$	$6.52 \pm 0.81^{a,b,v}$
E2400	$2.29 \pm 0.27^{a,v}$	$3.29 \pm 0.99^{a,v}$	$3.60 \pm 0.91^{a,v}$	$2.87 \pm 0.18^{a,v}$	$5.15 \pm 0.52^{b,v}$	$6.10 \pm 0.33^{a,v}$
E4800	$1.98 \pm 0.37^{a,v}$	$1.69 \pm 0.14^{a,v}$	$2.76 \pm 0.88^{a,v}$	$2.09 \pm 0.16^{a,v}$	$3.73 \pm 0.17^{a,v}$	$4.09 \pm 1.06^{c,v}$
W1600	$1.35 \pm 0.04^{a,v}$	$12.14 \pm 1.06^{b,w}$	$11.91 \pm 1.42^{b,w}$	$12.37 \pm 2.80^{b,w}$	$13.29 \pm 0.46^{b,c,w}$	$17.71 \pm 3.24^{c,w}$
W2400	$15.05 \pm 0.46^{a,b,w}$	$13.52 \pm 1.42^{a,w}$	$16.49 \pm 3.07^{b,x}$	$17.49 \pm 7.41^{a,w}$	$15.05 \pm 1.62^{a,w}$	$26.39 \pm 5.21^{c,x}$
W4800	$15.99 \pm 0.42^{a,w}$	$19.28 \pm 1.72^{a,x}$	$21.51 \pm 3.60^{b,y}$	$19.77 \pm 3.13^{a,b,x}$	$24.77 \pm 1.12^{b,x}$	$39.63 \pm 4.06^{c,y}$

Results are averages of triplicate determinations on the duplicate samples ± standard deviation

Samples followed by the same letter are not significantly different in the Bonferroni post test using a 0.05-level of significance

^{a,b,c} Storage time; ^{v,w,x,y}Treatments

W water extracts, *E* ethanolic extracts

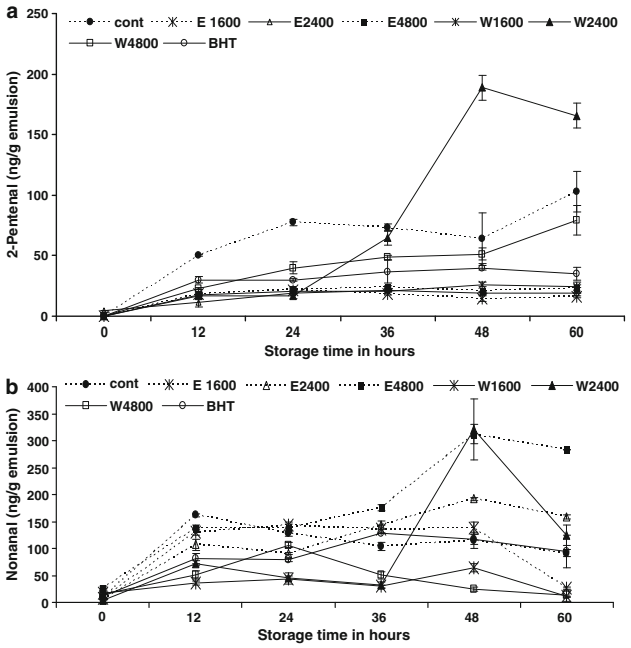

Fig. 4 Concentration of **a** 2-pentanal and **b** Nonanal in 5% oil in water emulsions treated with different concentrations of water (*W*) and ethanolic (*E*) extracts of potato peel. Results are averages of triplicate determinations on the same samples ± standard deviation

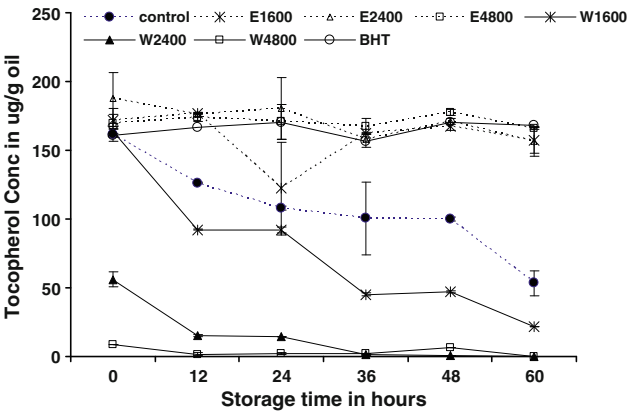

Fig. 5 The α-tocopherol content (μg/g oil) in emulsions containing different concentration of water (*W*) and ethanolic (*E*) extracts of potato peel. Results are average of triplicate determination on the same samples ± standard deviation

exemplified by α-tocopherol in Fig. 5. Initially, there was no significant ($p > 0.001$) difference between the concentrations of the tocopherol homologues in different emulsions except water extracts, 2,400 and 4,800 mg/kg, which showed significantly ($p < 0.001$) lower levels of all the tocopherol homologues from the beginning of the storage period (Fig. 5). This correlates well with PV as these samples showed very high levels of oxidation already at the beginning of the storage (Table 4). During storage there were no significant ($p > 0.001$) changes in the different tocopherol homologues for any of the concentrations of ethanolic extracts tested nor for BHT. Contrary to this, there was a significant ($p < 0.001$) reduction in different tocopherol homologues in control and water extracts. The emulsions containing 2,400 and 4,800 mg/kg of water extracts had completely lost all of the different tocopherol homologues at the end of the storage period.

The antioxidant potential of plant extracts is a result of not only the presence of the active phenolic compounds but also of other components present in the system/emulsion. In this study, samples with the water extract had low tocopherol levels (particularly at a high concentration of the extract) in the emulsion from the beginning of the storage period and tocopherols were totally absent at the end of storage period. This may suggest that some of the phenolic compounds present in the water extract in high quantity may react with α-tocopherol. Earlier, Peyrat-Maillard et al. [44] observed interaction between phenolic antioxidants and α-tocopherol in linoleic acid emulsions, where an antagonistic effect has been found in the presence of tocopherols. Also a study by Samotyja and Malecka [45] showed that black currant and rosemary extracts performed better in stripped substrates as they may interfere with native tocopherols present in rapeseed oil and show different activity according to the presence of water. However, a strong synergistic effect was observed by Wada and Fang [46] between α-tocopherol (0.02%) and rosemary extract (0.02%) in sardine oil at 30 °C. More research is required to verify the hypothesis about the influence of native tocopherol present in oils on the antioxidant activity of different plant extracts added.

Since the results of the volatiles and tocopherol analysis were relatively complex, a principal component analysis (PCA) was made on all the chemical data (Fig. 6) to give a better overview. In the PCA, principal component (PC) 1 and 2 explained 54 and 17% of the variation in the data, respectively. In the scores plot (Fig. 6a), all the ethanolic extracts were located to the far right together with BHT. The water extracts were located to the left with the highest concentrations further to the left and with W2400 in the top left corner. The control was located in the bottom of the plot close to the PC2 axis. Hence, PC1 mainly explained the difference between water and ethanolic extracts, whereas PC2 explained differences between the control and W2400. The location of W1600 and the control close to the PC2 axis suggests that these two samples have properties inbetween the ethanolic extracts/BHT and the samples with water extracts 2,400 and 4,800 for the parameters that are explained by PC1 in the loadings plot (Fig. 6b). From the loadings plot it is clear that PC1 mainly explained the variation in the tocopherol levels, PV and volatiles such as 1-pentene-3-ol, 2- pentenal and most of the heptadienal. Thus, PV and these volatiles were located

to the left confirming that their concentration was highest in water extract 2,400 and 4,800 samples and lowest in all samples with ethanolic extracts or BHT. The tocopherol variables were located to the right confirming that highest concentrations throughout storage were found in the samples with ethanolic extracts. The loadings plots also show that there was no clear pattern for volatiles 1-pentene-3-one, hexanal and nonanal.

The average values of the sensory scores are given in Table 5. The sensory analysis data for the emulsion does not correlate well with PV and other oxidation parameters. In general, scores for fishy/rancid off flavors for the different emulsions samples were low. The results suggest that there was a slight increase in the fishy/rancid odour for water extracts 4,800 mg/kg and control samples without any antioxidants added on storage. Even though PV and other oxidation parameters where high for water extracts 1,600 and 2,400 mg/kg, the sensory panel could not detect any clear rancid odour for these samples.

Thus, it is evident from all the results from storage of emulsions that all the tested concentrations of ethanolic extracts were effective in preventing lipid oxidation in emulsions. Water extracts in high concentrations (2,400 and 4,800 mg/kg) showed pro-oxidative effect in iron-induced

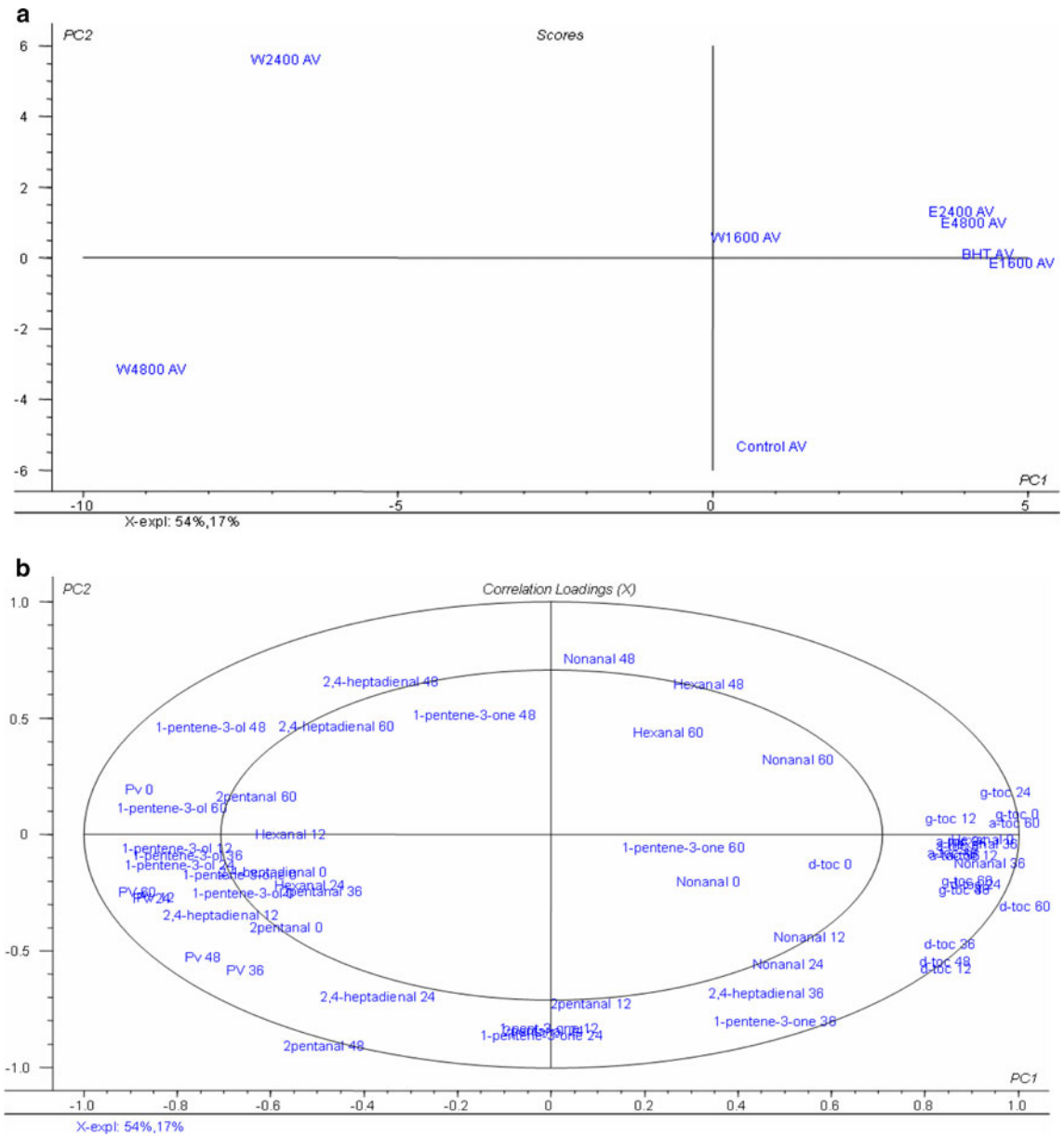

Fig. 6 **a** Principal component Analysis (*PCA*) score plot **b** PCA correlation loadings plot of 5% oil in water emulsion. The objects selected were different concentration (0, 1,600, 2,400 and 4,800 mg/kg) of the potato extracts and variables selected were PV, volatile secondary oxidation products and tocopherols at different storage time (0, 12, 24, 36, 48, 60 h)

Table 5 The average scores for odour (Attributes were fishy/rancid and others) for 5% oil in water emulsion treated with different concentration of potato peel extracts

Treatment groups	Fishy/Rancid	Others (description)
0 h		
Control	2	1 (Sour/fermented)
BHT200	0–1	1 (Sour/fermented)
E 1600	0–1	1–2 (Fresh/raw potato)
E 2400	0	2 (Fresh/raw potato)
E4800	0–1	2 (Smoked potato)
W 1600	0–1	2 (Smoked potato)
W 2400	0–1	2 (Fresh/raw potato)
W 4800	0–1	4 (Smoked potato)
60 h		
Control	3	1 (Sour/fermented)
BHT200	0–1	1–2 (Sour/fermented)
E 1600	0	3 (Fresh/raw potato)
E 2400	0–1	2 (Fresh/raw potato)
E 4800	0–1	3 (Smoked/raw potato)
W 1600	1	3 (Smoked potato)
W 2400	0–1	4 (Smoked potato)
W 4800	3	5 (Smoked potato)

W water extracts, *E* ethanolic extracts

oxidation in emulsions despite their higher Fe^{2+} chelating activity. This might be due to the difference in hydrophobicity/hydrophilicity of the phenolic compounds and thereby their localization in the emulsion system and/or due to the absence of certain phenolic acids such as caffeic acid and ferulic acid in water extracts, which have been shown to be good antioxidants in some studies [41], whereas other studies have indicated a poor effect of caffeic acid in the presence of iron [47]. The pro-oxidant activity of the water extract increased with increases in concentration. This might be due to the presence of a high content of polar phenolic compounds such as protocatechuic acid, which assumedly will be present in the aqueous phase in close proximity to the transition metal ions. Furthermore, polar phenolic compounds such as catechins have been shown to be pro-oxidative rather than antioxidant in the presence of transition metal ions, particularly iron [48]. Even though the ethanolic extracts prevented lipid peroxidation, they showed higher levels of hexanal and nonanal compared to water extracts. A similar effect was reported by Cuvelier et al. [49], who found a protective effect of α-tocopherol towards conjugated hydroperoxides and a simultaneous lack of protection against hexanal formation in sunflower oil triacylglycerol (TAG).

Though water extracts showed a higher Fe^{2+} chelating activity, they failed to prevent Fe^{2+}-induced oxidation in emulsions. In this study, citrem which is able to chelate metal ions, was used as an emulsifier [47]. As some of the added Fe^{2+} may have been chelated by citrem, the radical scavenging property may have become more important than the Fe^{2+} chelating activity in this system.

In conclusion, the ethanolic extracts of potato peel from the Sava variety exhibited strong antioxidant activity both in in-vitro antioxidant assays and also in bulk oil and oil-in-water emulsions and was better than/comparable to the synthetic antioxidant BHT. This might be due to the combined effect of radical scavenging, iron chelating, reducing properties and/or by the tocopherol sparing effect of different phenolic compounds present in ethanolic extracts. The poor antioxidant activity of water extracts might be due to low levels of total phenolics and absence of certain phenolic acids such as caffeic, salicylic and coumaric acid. Taken together, these data indicate that ethanolic extracts from potatoes may potentially be used as replacements for synthetic antioxidants in food systems, but further studies are necessary to characterize the compounds present in the extract and to investigate if they have any positive or negative health effects.

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References

1. Sobedio JL, Kaitaramita J, Grandgiral A, Malkkl Y (1991) Quality assessment of industrial pre-fried french fries. J Am Oil Chem Soc 68:299–302
2. Linderschmidt R, Trylka A, Goad M, Witschi H (1986) The effects of dietary butylated hydroxytoluene on liver and colon tumor development in mice. Toxicol 38:151–160
3. Kim SY, Kim JH, Kim SK, Oh MJ, Jung MY (1994) Antioxidant activities of selected oriental herb extracts. J Am Oil Chem Soc 71:633–640
4. Frankel EN, Aeschbach SW, Prior RE (1996) Antioxidant activity of a Rosemary extract and its constituents, Carnosic acid, Carnosol, and Rosmarinic acid, in bulk oil and oil-in-water emulsion. J Agric Food Chem 44:131–135
5. Toma RB, Orr PH, D'Appolonia, Dintzis FR, Tabekhia MM (1979) Physical and chemical properties of potato peel as a source of dietary fiber in bread. J Food Sci 44:1403–1407
6. Onyeneho SN, Hettiarachchy NS (1993) Antioxidant activity, fatty acid and phenolic acid composition of potato peels. J Sci Food Agric 62:345–350
7. Kanatt SR, Chander R, Radhakrishna P, Sharma A (2005) Potato peel extract-a natural antioxidant for retarding lipid peroxidation in radiation processed lamb meat. J Agric Food Chem 53:1499–1504
8. Zia-ur-rehman, Habib F, Shah WH (2004) Utilization of potato peels extract as a natural antioxidant in soy bean oil. Food Chem 85:215–220
9. Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic 16:144–158

10. Shimada K, Fujikawa K, Yahara K, Namkamura T (1992) Antioxidative properties of Xanthan on the autooxidation of soyabean oil in cyclodextrin emulsion. J Agric Food Chem 40:945–948
11. Dinis TCP, Madeira VMC, Almeida LM (1994) Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys 315:161–169
12. Oyaizu M (1986) Studies on products of browning reactions: antioxidative activities of browning products of browning reaction prepared from glucosamine. Jpn J Nutr 44:307–315
13. Vikbjerg AF, Andresen TL, Jørgensen K, Mu H, Xu X (2007) Oxidative stability of liposomes composed of docosahexaenoic acid-containing phospholipids. J Am Oil Chem Soc 84:631–637
14. Fiske CH, Subbarow Y (1925) The colorimetric determination of phosphorus. J Biol Chem 66:375–400
15. Bartlette GR (1959) Phosphorus assay in column chromatography. J Biol Chem 234:466–468
16. Buege JA, Aust SD (1978) Microsomal lipid peroxidation. In: Fleisher S, Packer L (eds) Methods in enzymology, biomembranes. part C: biological oxidations, vol 52. Academic Press, London, pp 302–310
17. Duh PD, Yen GC (1997) Antioxidant efficacy of methanolic extracts of peanut hulls in soyabean and peanut oils. J Am Oil Chem Soc 74:745–748
18. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:900–917
19. International IDF standards (1991) Section 74A: 1991. International Dairy Federation, IDF-square Vergot 41. Brussels, Belgium
20. AOCS (1994) Official method Cd 18–90: p-Anisidine value. Am Oil Chem Soc, Champaign
21. Timm M, Xuebing X, Nielsen NS, Jacobsen C (2003) Oxidative stability of milk drinks containing structures lipids produced from sunflower oil and caprylic acid. Eur J Lipid Sci Technol 105:459–470
22. CS AO (1992) official method Ce 8-89: determination of tocopherols and tocotrienols in vegetable oils and fats by HPLC. AOCS, Champaign
23. Martens H, Martens M (2001) Multivariate analysis of quality, an introduction. Wiley, UK
24. Al-Saikhan MS, Howard LR, Miller JC (1995) Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum* L). J Food Sci 60:341–343
25. Percival GC, Baird L (2000) Influence of storage upon light-induced chlorogenic acid Accumulation in potato tubers (*Solanum tuberosum* L.). J Agric Food Chem 48:2476–2482
26. Reyes LF, Miller JC, Cisneros-Zevallos L (2004) Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. Am J Potato Res 81:187–193
27. Mendez CDV, Delgado MAR, Rodriguez EMR, Romero CD (2004) Content of free compounds in cultivars of potatoes harvested in Tenerife (Canary Islands). J Agric Food Chem 52:1323–1327
28. Rodriguez de Sotillo D, Hadley M, Holm ET (1994) Phenolics in aqueous potato peel extract: extraction, identification, and degradation. J Food Sci 59:649–651
29. Mota FL, Queimada AJ, Pinho SP, Macedo EA (2008) Aqueous solubility of some natural phenolic compounds. Ind Eng Chem Res 47:5182–5189
30. Arts MJTJ, Haenen GRMM, Voss HP, Bast A (2001) Masking of antioxidant capacity by the interaction of flavonoids with protein. Food Chem Toxicol 39:787–791
31. Singh N, Rajini PS (2004) Free radical scavenging activity of an aqueous extract of potato peel. Food Chem 85:611–616
32. Foti MC, Daquino C, Geraci C (2004) Electron-Transfer reaction of cinnamic acids and their methyl esters with the DPPH radical in alcoholic solutions. J Org Chem 69:2309–2314
33. Robards K, Prenzler P, Tucker P, Swatsitang G, Glover W (1999) Phenolic compounds and their roles in oxidation process in fruits. Food Chem 66:401–436
34. Gulcin I (2006) Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). Toxicol 217:213–220
35. Graf E (1992) Antioxidant potential of Ferulic acid. Free Rad Biol Med 13:43–448
36. Gordon MH (1990) The mechanism of antioxidant action in vitro. In: Hudson BJF (ed) Food antioxidants. Elsevier, London, pp 1–18
37. Stohs SJ, Bagachi D (1995) Oxidative mechanisms in the toxicity of metal ions. Free Rad Biol Med 18:321–336
38. Ueda J, Saito N, Shimazu Y, Ozawa T (1996) Comparison of scavenging abilities of antioxidants against hydroxyl radicals. Arch Biochem Biophys 333:377–384
39. Roberts WG, Gordon MH (2003) Determination of the total antioxidant activity of fruits and vegetables by a liposome Assay. J Agric Food Chem 51:1486–1493
40. Yen GC, Duh PD, Tsai HL (2002) Antioxidant and pro-oxidant properties of ascorbic acid and gallic acid. Food Chem 79:307–313
41. Maqsood S, Benjakul S (2009) Comparative studies of four different phenolic compounds on in vitro antioxidant activity and the preventive effect on lipid oxidation of fish oil emulsion and fish mince. Food Chem. doi:10.1016/J.foodchem.2009.06.004
42. Laranjinha JO, Vieira O, Madeira V, Almeida L (1995) Two related phenolic antioxidants with opposite effects on vitamin E Content in low density lipoproteins oxidized by ferrylmyoglobin: consumption vs regeneration. Arch Biochem Biophys 323:373–381
43. Liao K, Yin M (2000) Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: importance of the partition coefficient. J Agric Food Chem 48:2266–2270
44. Peyrat-Maillard MN, Cuvelier ME, Berset C (2003) Antioxidant activity of phenolic compounds in 2,2′azobis (2-amidinopropane) dihydrochloride (AAPH) induced oxidation: synergistic and antagonistic effect. J Am Oil Chem Soc 80:1007–1012
45. Samotyja U, Małecka M (2007) Effects of blackcurrant seeds and rosemary extracts on oxidative stability of bulk and emulsified lipid substrates. Food Chem 104:317–323
46. Wada S, Fang X (1992) The synergistic antioxidant effect of rosemary extract and α-tocopherol in sardine oil model system and frozen-crushed fish meat. J Food Process Pre 16:263–274
47. Sørensen AM, Haahr A, Becker EM, Skibsted LH, Bergenståhl B, Nilsson L, Jacobsen C (2008) Interaction between iron, phenolic compounds, emulsifiers, and pH in omega-3-enriched oil-in-water emulsions. J Agric Food Chem 56:1740–1750
48. Retsky KL, Freeman MW, Frei B (1993) Ascorbic acid oxidation products (s) protect human low density lipoproteins against atherogenic modification: anti- rather than pro-oxidant activity of vitamin C in the presence of transition metal ions. J Biological Chem 268:1304–1309
49. Cuvelier ME, Lagunes-Galvez L, Berset C (2003) Do antioxidants improve the oxidative stability of oil-in-water emulsions. J Am Oil Chem Soc 80:1101–1105