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# Potato peel extract as a natural antioxidant in chilled storage of minced horse mackerel (*Trachurus trachurus*): Effect on lipid and protein oxidation

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ABSTRACT

The present work was undertaken to examine the utilisation of potato peel, a waste material, as a source of natural antioxidants for retarding lipid and protein oxidation in minced mackerel. Mackerel mince with two different concentrations (2.4 or 4.8 g/kg) of water or ethanol extracts of potato peel and a control with no added extracts were prepared. The samples were stored at 5 °C for 96 h and the sampling was done at time points 0, 24, 48 and 96 h. The ethanol extracts, which contained high amounts of phenolic compounds, was found to be very effective in retarding lipid and protein oxidation as it resulted in low levels of peroxide value, volatiles, carbonyl compounds and protected against the loss of α-tocopherol and tryptophan and tyrosine residues. Water extracts was less efficient especially at higher concentrations, which might be due to lower phenolic content or due to the pro-oxidative nature of some of the phenolic acids/co-extracted compounds.



## 1. Introduction

Fatty fish species are considered to be of great nutritional value due to higher levels of long chain omega-3 polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3). Epidemiological studies suggest that a high intake of these fatty acids are associated with low risk of cardiovascular disease, different types of cancer, diabetes and mental illness (Connor, 2000). Despite these obvious benefits, one major obstacle in using fatty fish is that they are highly susceptible to oxidation due to the presence of polyunsaturated fatty acids. Lipid oxidation will lead to the development of unpleasant odour, rancid taste and discoloration (Frankel, 2005). Moreover, the compounds resulting from lipid oxidation can modify proteins and amino acids of nutritional interest and decrease the protein functionality due to protein denaturation (Pokorny, Elzeany, Luan, & Janicek, 1976). One way of preventing lipid oxidation is by the addition of antioxidants. Addition of synthetic antioxidants has been restricted because of their health risks and toxicity (Linderschmidt, Trylka, Goad, & Witschi, 1986). Hence, the use of natural antioxidants is emerging as an effective methodology for controlling rancidity and limiting its deleterious consequences (Frankel, 1998).

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 been shown to be a good source of dietary fibre (Toma, Orr, D'Appolonia, Dintzis & Tabekhia, 1979) and rich in phenolic acids especially of chlorogenic, gallic, protocatechuic and caffeic acids (Habeebullah Nielsen, & Jacobsen, 2010). The antioxidant property of potato peel extract from some Asian varieties (Kurfi Chandramuki) has been reported in muscle foods and in soybean oil (Kanatt, Chander, Radhakrishna, & Sharma, 2005; Zia-ur-rehman, Habib, & Shah, 2004).

In our earlier study we found that extracts of peels from Sava variety of potatoes were highly efficient in reducing lipid peroxidation both in fish oil and in oil-in-water emulsions (Habeebullah et al., 2010). However, the use of potato extracts as an antioxidant in fish muscle, which is a complex mixture of proteins, lipids, pro-oxidants such as haemoglobins, iron and antioxidants such as alpha tocopherols, ascorbic acid has not yet been studied. The overall objective of this study was therefore to provide more knowledge about the potential of potato peel extracts as a source of effective antioxidants in horse mackerel mince, a fatty fish particularly high in n-3 PUFA. The specific aim was to evaluate the effectiveness of potato peel extracts in retarding both lipid and protein oxidation in minced horse mackerel. Lipid oxidation was assessed by determination of peroxide value, volatiles and tocopherol concentration. The changes in protein components were assessed by determination of protein solubility, loss of sulphydryl group, protein

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carbonyls and loss of tryptophan and tyrosine fluorescence. The colour changes to the mince due to protein and lipid oxidation was also determined.

## 2. Materials and methods

### 2.1. Chemicals

Chemicals and external standards for identification of volatile oxidation products and phenolic compounds 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).

### 2.2. Preparation of potato peel extract

The Sava variety of potato (*S. tuberosum*), were purchased from potato farmer (NordJylland, Denmark) in September 2009. 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 45 °C for 72 h and powdered by a kitchen blender. The material that passed through an 80 mesh sieve was retained for use. For the preparation of ethanol extract, 5 g of powdered peel was extracted with 50 ml of 96% ethanol at room temperature overnight and centrifuged at 2800 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 were extracted overnight with 100 ml of distilled water at room temperature and centrifuged at 2800 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 extracts were freeze dried instead of evaporation. These extracts were kept at −80 °C until analysis.

### 2.3. Determination of total and individual phenolic compounds

Total phenolic content in the extracts was determined by using Folin–Ciocalteu reagent as per the method described by Singleton and Rossi (1965). 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 individual phenolic acid was done as per the procedure described in our previous study (Habeebullah et al., 2010) using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA), equipped with a diode array detector (Agilent G13158). The column used was a ZORBAX Eclipse® XDB C8 analytical column (150 mm × 4.6 mm) (Agilent, USA) with a 5 μm packing material. The 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. The 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.

### 2.4. Minced mackerel storage experiment

Horse mackerel (*Trachurus trachurus*) was kindly donated by Sæby Fish Industry (Sæby, Denmark). The fish were beheaded and eviscerated immediately after catch and frozen at −30 °C until arrival at laboratory. Then, they were stored in plastic bags under vacuum in a −40 °C freezer until processing, which was carried out within 1 month. All processing steps were performed in a 2 °C temperature-controlled room. Horse mackerel (10 kg) were defrosted, filleted and hand skinned manually. 600 g each of fillets was minced by using a kitchen blender after adding a final concentration of 2.4 and 4.8 g/kg water extract (WE) and ethanol extracts (EEs) of potato peel. Mince without addition of any extract was made in a similar manner and was used as a control. To ensure even distribution of the extracts throughout the mince, the extracts were dissolved in 5 ml of distilled water and added to the mince. In the control sample 5 ml of distilled water without extract was added. Each of the five samples (Control, 2.4 WE, 4.8 WE, 2.4 EE and 4.8 EE) obtained were further divided into portions of 150 g and placed in aluminium boxes (14.5 × 11.5 cm) closed with a cardboard and aluminium cap. The boxes were stored at 5 °C for 96 h. At each time point (T0, T24, T48 and T96) the content of the boxes was vacuum packed in plastic bags and stored at −80 °C until further analysis. At T0, samples were vacuum packed in plastic bags and stored at −80 °C immediately after mincing.

#### 2.4.1. Water content, pH and colour

The water content was determined by difference in weight after drying approximately 3 g of sample at 115 °C for 18 h according to the AOAC (1995). Analyses were performed in triplicate and results were expressed as g water per 100 g wet sample. The pH of the samples was measured using a Metrohm 780 pH meter (Switzerland) with probe number 6.0226.100. The colour measurements were done with Minolta Chromameter (Cr-200, Minolta, Osaka, Japan). The colour was measured in three different places on the sample and each measurement consisting of 3 readings. The average of triplicate measurement of $L^*$, $a^*$, and $b^*$ were calculated.

#### 2.4.2. Determination of total lipid content and peroxide value (PV)

Total lipids were extracted in triplicate from 10 g of sample with methanol/chloroform (1:1, v/v) according to the method of Bligh and Dyer (1959). The lipid content was determined gravimetrically and the results were expressed as g lipid/100 g wet sample. PV was measured directly on the Bligh and Dyer extract according to the method described by the International IDF Standards (1991).

#### 2.4.3. Estimation of fatty acid composition and free fatty acids

Fatty acids in the Bligh and Dyer extract were trans-esterified to methyl esters (FAMEs) using a base-catalysed transesterification followed by a Borontrifluoride-catalysed esterification according to AOCS (1998, Official method). The FAMEs (1.5 μl) were injected into an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler and a flame ionisation detector. The separation was carried out on an DB-WAX fused silica capillary column (10 m × 0.1 mm, 0.1 μm; Agilent Technologies, Palo Alto, CA, USA) using helium as carrier gas. Methyl esters were identified by comparison of the retention times of authentic standards and fatty acid compositions were expressed as area percentage of total fatty acids.

The free fatty acids (FFAs) contents were determined by titration (0.1 M, NaOH) of the Bligh and Dyer extract (10 g) after adding ethanol (15 ml) and using phenolphthalein as an indicator. FFA content was calculated in % oleic acids (AOCS, 1998).

*2.4.4. Determination of tocopherol content*

Tocopherol content was determined on the Bligh and Dyer lipid extracts by HPLC using an Agilent 1100 series HPLC (Agilent Technologies, Palo Alto, CA, USA), equipped with a fluorescence detector according to AOCS Official method (1992). Results are expressed in μg tocopherol per g mince.

*2.4.5. Determination of volatile secondary oxidation products*

The volatile compounds were determined by dynamic headspace analysis according to Eymard, Baron, and Jacobsen (2009). The volatile compounds in the samples were collected on Tenax GR traps (Chromapack, Bergen op Zoom, The Netherlands) by purging with nitrogen (flow rate of 340 ml/min) at 37 °C in a water bath for 30 min. A Perkin–Elmer (Norwalk, CT) ATD-400 automatic thermal desorber system was used for thermally desorbing the collected volatiles from the Tenax traps using Helium as a carrier gas. The transfer line of the ATD was connected to a Hewlett–Packard (Palo Alto, CA) 5890 IIA gas chromatograph equipped with a HP 5972A mass-selective detector. A DB 1701 column (30 × 0.25 mm, 1.0 μm; J1 W Scientific, Folsom CA, USA) with a flow of 1.3 ml of helium/min and the following temperature program was used: 35 °C for 5 min, 35 °C to 90 °C at 3 °C/min, 90 °C to 240 °C at 10 °C/min, and finally hold at 240 °C for 4 min. The GC–MS transfer line temperature was kept at 280 °C. The mass-selective detector used ionisation at 70 eV in EI mode and 50 μA emission. The scans were performed in the mass range of 30–350 atomic mass unit with a repetition rate 2.2 scans/s. The compounds were identified by MS library searches and by comparing retention time and spectra with MS runs of 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.

*2.4.6. Determination of protein solubility*

Samples (0.5 g) were homogenised in 10 ml of 0.6 M KCl in 50 mM pH 7.4 tris–HCl buffer with a Polytron PT1200CL (Kinematica AG, Lucerne, Switzerland) for 1 min. The homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C (Sorvall RC 5B Plus, SS24 rotor, Dupont, Norwalk, CT, USA). The supernatant was diluted ten fold with 0.6 M KCl and protein determination was performed using the BCA kit (Pierce, Rockford, IL, USA). The solubility was expressed in mg of soluble protein/g of mince.

*2.4.7. Determination of sulphydryl groups*

The total sulphydryl contents were determined using 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) according to Ellman's method (1959) with some modifications. 0.5 g sample was homogenised in 10 ml of 0.05 M phosphate buffer pH 7.2 with a Polytron PT1200CL (Kinematica AG, Lucerne, Switzerland) for 30 s. Subsequently, 1 ml of the homogenate was mixed with 9 ml of 0.05 M phosphate buffer pH 7.2 containing 0.6 M NaCl, 6 mM ethylenediaminetetraacetic acid (EDTA), and 8 M urea. The mixture was centrifuged 10 min at 10,000 rpm at 5 °C (Sorvall RC 5B Plus, SM34 rotor, Dupont, Norwalk, CT, USA). To 3 ml of the supernatant 0.04 ml of 0.01 M DTNB solution in 0.05 M sodium acetate were added and incubated at 40 °C for 15 min. A blank was prepared replacing the homogenate with 0.05 M phosphate buffer pH 7.2 containing 0.6 M NaCl, 6 mM (EDTA) and 8 M urea. The absorbance was measured at 412 nm (Shimadzu UV 160A, Kyoto, Japan) and the SH content was calculated using a molar extinction coefficient of 13,600/M/cm. Results were expressed in micromoles of SH per g of mince.

*2.4.8. Determination of protein carbonyls*

Protein carbonyls were measured as described by Levine, Williams, Stadtman, and Shacter (1994). A sample of mince (0.5 g) was homogenised in 10 ml tris-buffer (pH: 7.4, 50 mM, 1 mM EDTA) containing 0.01% BHT and 200 μl of the homogenate were precipitated with 50 μl of TCA (100%). After centrifugation (12,000*g*, 3 min) the pellet was incubated with 500 μl of 10 mM dinitrophenylhydrazine (DNPH) in 2 M HCl, in the dark for 1 h. For each sample a blank incubated in 2 M HCl and without added DNPH was run in parallel. The samples were precipitated with 50 μl TCA (100%), centrifuged (12,000*g*, 3 min) and the pellets were washed three times with 1 ml ethanol/ethyl acetate 1:1 (v/v). The pellet was re-dissolved in 1 ml of 6 M guanidine chloride in 20 mM $KH_2PO_4$. The carbonyl content was determined by measuring the absorbance at 370 and 280 nm against guanidine as blank. Results are expressed in nmoles carbonyl per mg of protein.

*2.4.9. Measurement of tryptophan and tyrosine fluorescence*

The natural fluorescence of tryptophan and tyrosine was assessed by fluorescence spectroscopy. 1 g of mince was homogenised in 10 ml (1:10 w/v) of 20 mM sodium phosphate buffer (pH 6.5) containing 0.6 M NaCl using a Polytron PT1200CL (Kinematica AG, Lucerne, Switzerland) for 30 s. The homogenates were filtered through gauze to remove insoluble materials and 250 μl of the filtered homogenate was transferred to microplates. Emission spectra of tryptophan were recorded from 300 to 500 nm with excitation wavelength established at 280 nm by using SPECTRAmax GEMINI microplate spectrofluorometer. The emission spectra of tyrosine were recorded from 300 to 500 nm with excitation wavelength established at 270 nm. The excitation and emission slit widths were set to 10 nm, and the data were collected at 500 nm/min. Tryptophan fluorescence was taken as fluorescence intensity units emitted at 350 nm and tyrosine as fluorescence intensity emitted at 310 nm. The % loss of fluorescence was calculated as (F0–F96)/F0 × 100, where F0 is the fluorescence intensity at Time 0 and F96 is fluorescence intensity emitted at 96 h.

*2.4.10. Preliminary sensory evaluation*

A preliminary sensory evaluation was performed by an expert panel composed of three persons. The mince was evaluated for odour 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. Therefore, no statistical analysis could be made on these data. Samples of 20 g were served in randomized order after incubation for 1 h at 5 °C.

*2.5. Statistical analyses*

The data for the total and individual phenolic compounds were subjected to one way analysis of variance, and all the other data obtained were analysed by two-way analysis of variance. The statistical comparisons among the samples were performed with *Bonferroni* multiple comparison test using a statistical package program Graphpad prism 4 (Graphpad Softwarer Inc., San Diego, USA). A $p$-value < 0.05 was considered as statistically significant.

## 3. Results and discussion

*3.1. Total phenolics and individual phenolics*

Phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators and hence the total amount of phenolics as well as the content of individual

phenolic acids in potato peel extracts was determined. The ethanol extract contained a significantly ($P < 0.001$) higher quantity of total phenolics (68.7 ± 5.7 mg/100 g potato peel) when compared to water extracts (26.1 ± 0.5 mg/100 g potato peel). This finding is in agreement with our earlier study (Habeebullah et al., 2010). The higher content of phenolic compounds in the ethanol extract might be due to the ability of ethanol to release phenolic compounds attached to proteins, but could also be due to higher solubility of certain phenolics in ethanol than in water.

The major phenolic acids in the extracts were identified by HPLC (Table 1). Both water and ethanol extracts contained high levels of protocatechuic acid. Water extracts were shown to contain significantly ($P < 0.001$) higher levels of gallic acids when compared to the corresponding ethanol extracts, while ethanol extracts showed higher levels of gentisic and caffeic acids. P-hydroxy benzoic acid and ferulic acids was present in only the water extracts, while salicylic and syringic acid was present only in ethanol extracts. The difference in the composition of the two extracts is due to the difference in solubility of these phenolic compounds in water and ethanol. Unlike in our previous study (Habeebullah et al., 2010), we were not able to detect p-hydroxy benzoic acid and ferulic acid in ethanol extracts, but they were both present in the water extracts. Moreover, vanillic and syringic acid, which were completely absent in both extracts in the previous study, were found in either both extracts or in the ethanol extracts, respectively, in the present study. The solubility of phenolic compounds has been reported to increase with increase in temperature (Mota, Queimada, Pinho, & Macedo, 2008). Therefore, one of the possible explanation for this discrepancy between the results of our two studies may be the higher temperature of extraction, which was used in the present study (room temperature) compared to the previous study (5 °C). Another explanation may be that the extracts were prepared from different batches of potatoes. The phenolic content of the potatoes was reported to vary with variety, colour, geographical origin, season and storage (Al-Saikhan, Howard, & Miller, 1995; Mendez, Delgado, Rodriguez, & Romero, 2004; Percival & Baird, 2000; Reyes, Miller, & Cisneros-Zevallos, 2004).

### 3.2. General composition of mince

Moisture and fat contents of the mince were found to be in the range of 61–63, and 21–24 g/100 g respectively (Table 2). The pH value was approximately 6.1 in all minces. The fatty acid composition of the total lipids of mackerel is given in Table 2. The proportion of saturated fatty acids in the lipid fraction was about 20% of the total fatty acids, whereas the mono and polyunsaturated fatty acids constituted 43.10–44.31% and 35.19–36.34% of the total fatty acids, respectively. As expected, the fatty acid composition was similar in all samples and did not show much change during the experiment so only the values at T0 are shown.

**Table 1**
The major phenolic acids in the extracts of potato peel (mg/g extract). Values are mean ± SD ($n = 3$). The letters a, b indicates significant difference between water and ethanol extracts.

| Phenolic acids | Water extract (WE) | Ethanolic extract (EE) |
|---|---|---|
| Gallic acid | 11.38 ± 0.25[b] | 0.90 ± 0.01[a] |
| Protocatechuic acid | 6.82 ± 0.51[a] | 7.38 ± 0.04[a] |
| Gentisic acid | 3.66 ± 1.29[a] | 15.42 ± 0.10[b] |
| P-hydroxy benzoic acid | 4.76 ± 0.29 | – |
| Chlorogenic acid | 0.56 ± 0.02[a] | 0.43 ± 0.12[a] |
| Vanilic acid | 0.45 ± 0.09[a] | 1.10 ± 0.08[a] |
| Syringic acid | – | 0.64 ± 0.06 |
| Caffeic acid | 0.11 ± 0.01[a] | 2.79 ± 2.01[b] |
| Salicylic acid | – | 0.11 ± 0.04 |
| P-coumaric acid | 0.05 ± 0.01[a] | 0.58 ± 0.09[a] |
| Ferulic acid | 0.09 ± 0.04 | – |

### 3.3. Lipid oxidation during storage at 5 °C

The peroxide value (PV) of different treatment groups are shown in Fig. 1a. At T0, there was no significant ($P > 0.05$) differences in PV values between the groups. As the storage progressed, the PV showed a gradual increase in the control and in samples with water extracts. The PV of the control reached 3.45 meq/kg at the end of the storage period. The mince with 4.8 g/kg and 2.4 g/kg water extracts showed no significant increase in PV up to 24 h and 48 h, respectively. However, thereafter, there was a significant ($P < 0.001$) increase in the PV values in both treatment groups and the PV reached 5.78 meq/kg at the end of storage period in 4.8 g/kg water extracts, which was significantly ($p < 0.001$) higher than the PV in control. The mince with 2.4 or 4.8 g/kg ethanol extracts showed greater resistance towards oxidation by having low PV throughout the storage period and at the end of the storage period the PV was 0.58 and 0.41 meq/kg, respectively.

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. All of the volatiles showed similar tendency and only 2 of them: 1-pentene-3-ol and 2,4-heptadienal are presented in Fig. 1b and c. Initially, there were no significant ($P > 0.05$) differences between samples for the different volatiles. However, during storage their level increased gradually in the control and in minces with water extracts. Thus, the ethanol extracts showed significantly ($P < 0.001$) lower levels of volatiles than all the other samples (Fig. 1b and c). Unlike the findings for PV, the volatiles in the water extracts were also significantly ($P < 0.001$) lower than the controls at the end of the storage period.

The content of α-tocopherol in mince is shown in Fig. 1d. Initially, there was no significant ($p > 0.05$) difference between the concentrations of α-tocopherol in different treatment groups. During storage there were no significant ($P > 0.05$) changes in α-tocopherol for any of the minces containing ethanol extracts. Contrary to this, there was a significant ($P < 0.001$) reduction in α-tocopherol in the control and in minces added with water extracts. The mince containing 4.8 g/kg of water extracts showed the lowest α-tocopherol concentration at the end of the storage period.

From these results it is clear that the ethanol extracts were very effective in retarding lipid oxidation of fish mince. Water extracts had the same effect in retarding lipid peroxidation as far as volatiles are concerned but when taking PV and tocopherols into consideration, water extracts only exerted an antioxidative effect when added in a concentration of 2.4 g/kg. When added in a concentration of 4.8 g/kg the water extract had a pro-oxidant tendency. The overall order of oxidative stability was 4.8 EE > 2.4 EE > 2.4 WE > control > 4.8 WE.

These results are consistent with our earlier study performed on both homogenous and heterogeneous systems, namely bulk and emulsified oils (Habeebullah et al., 2010). In that study it was also found that ethanol extracts had a higher antioxidant activity when compared to water extracts. The present results also in accordance with reports of earlier studies. Kanatt et al. (2005) reported that ethanolic potato extracts were effective in retarding lipid oxidation in lamb meat. The freeze-dried extract of potato peel was reported to be effective in controlling lipid oxidation and colour changes during cold storage in beef patties, although to a lesser extent as compared to ginger rhizomes and fenugreek seeds (Mansour & Khalil, 2000). However, this is the first time that the ability of ethanolic potato extracts to prevent lipid oxidation in a more oxidation sensitive system like mackerel mince has been documented.

**Table 2**
Water content, pH, fat content and fatty acid composition of different treatment group at T0. Values are mean ± SD ($n$ = 3).

| | Control | 2.4 WE | 4.8 WE | 2.4 EE | 4.8 EE |
|---|---|---|---|---|---|
| Water content (g/100 g) | 61.9 ± 0.1 | 62.4 ± 0.7 | 61.1 ± 0.9 | 63.1 ± 0.1 | 62.5 ± 0.4 |
| pH | 6.07 | 6.07 | 6.07 | 6.05 | 6.07 |
| Fat content (g/100 g) | 22.9 ± 2.3 | 24.7 ± 0.7 | 23.5 ± 4.4 | 22.5 ± 2.3 | 21.4 ± 0.7 |
| Fatty acid composition (%) | | | | | |
| 13:00 | 0.58 | 0.45 | 0.47 | 0.43 | 0.51 |
| 14:00 | 6.04 | 5.99 | 5.99 | 6.27 | 6.23 |
| 16:00 | 11.89 | 11.76 | 11.79 | 11.83 | 11.91 |
| 18:00 | 2.13 | 2.12 | 2.13 | 2.08 | 2.11 |
| Σsaturated | 20.64 | 20.32 | 20.38 | 20.61 | 20.76 |
| 16:1 (n-7) | 3.55 | 3.52 | 3.54 | 3.63 | 3.57 |
| 18:1 (n-9) | 9.05 | 9.01 | 9.03 | 8.96 | 9.03 |
| 18:1 (n-7) | 1.71 | 1.69 | 1.70 | 1.68 | 1.69 |
| 20:1 (n-9) | 10.56 | 10.60 | 10.57 | 10.29 | 10.46 |
| 20:1 (n-7) | 0.17 | 0.17 | 0.00 | 0.09 | 0.16 |
| 22:1 (n-11) | 17.31 | 17.50 | 17.78 | 16.72 | 17.37 |
| 22:1 (n-9) | 0.84 | 0.77 | 0.36 | 0.73 | 0.38 |
| 24:1 (n-9) | 1.03 | 1.05 | 1.03 | 1.00 | 1.02 |
| Σmono unsaturated | 44.22 | 44.31 | 44.11 | 43.1 | 43.68 |
| 18:2 (n-6) | 1.50 | 1.52 | 1.53 | 1.57 | 1.57 |
| 18:3 (n-3) | 1.65 | 1.66 | 1.65 | 1.71 | 1.71 |
| 18:4 (n-3) | 4.25 | 4.24 | 4.24 | 4.47 | 4.41 |
| 20:4 (n-3) | 1.02 | 1.02 | 1.02 | 1.04 | 1.03 |
| 20:5 (n-3) | 6.02 | 6.00 | 6.02 | 6.21 | 6.08 |
| 22:5 (n-3) | 1.15 | 1.15 | 1.15 | 1.15 | 1.14 |
| 22:6 (n-3) | 11.20 | 11.23 | 11.25 | 11.35 | 11.29 |
| Others | 8.40 | 8.61 | 8.79 | 8.84 | 8.37 |
| ΣPoly unsaturated | 35.19 | 35.43 | 35.65 | 36.34 | 35.8 |

*NoteS*: EE – ethanol extract; WE – water extract.

The above results show that a high inhibition of lipid oxidation in mackerel muscle correlates well with high total phenolics in the ethanol extracts. In addition to the total phenolics, individual phenolic compounds in the extracts also play an important role for their antioxidative activity. The ethanol extracts contained significantly higher levels of caffeic and gentisic acids and trace levels of syringic and salicyclic acid when compared to water extracts. The lower efficacy of water extracts in retarding lipid oxidation may either due to the pro-oxidative effect of some of co-extracted compounds such as ascorbic acid or some phenolic compounds, which are present in higher quantities in water extracts. Water extracts contained significantly ($P < 0.001$) higher levels of gallic acid when compared to ethanol extracts. In a multiphase system, such as fish mince the antioxidant activity is affected by several factors including concentration of antioxidants, partitioning of antioxidants, and interactions with other compounds such as transition metals. Rice-Evans, Miller, and Paganga (1997) has reported that gallic acid and epigallocatechin are preferentially localised in the aqueous phase and thus having less access to the lipid peroxyl radicals. This might be one of the reasons for the lower efficacy of water extracts in preventing lipid oxidation compared to ethanol extracts. However, Medina, Gallardo, Gonzalez, Lois, and Hedges (2007) have shown that caffeic acid in spite of its localisation in aqueous phase was more effective in retarding lipid peroxidation in minced horse mackerel than other hydroxyl cinnamic and benzoic acids. They also suggested that the high antioxidant efficacy was related to the excellent reducing properties of caffeic acid rather than its metal chelating capacity.

Free fatty acids (FFAs) were measured as an estimation of lipolysis and are shown in Table 3. FFA production followed a pattern different from oxidation parameters (PV and volatiles). The samples containing extracts showed higher FFA values when compared to the control with no added antioxidants. At T0 the control showed lowest FFA and there was no significant difference ($P > 0.05$) between samples with the water or ethanol extracts. As the storage progressed, FFA showed a significant increase ($P < 0.05$) in all the samples. However, FFA values were still low after 96 h (1.6–1.9%). At T96, there was no significant difference ($P > 0.05$) between the samples with water or ethanol extracts, but the control showed significantly ($P < 0.05$) lower levels of FFA than the treatment groups. This pattern is not totally unusual, as an earlier study by Boyd, Green, Giesbrecht, and King (1993) also found an increase in FFA, which did not correspond to increased oxidation patterns. This study was carried out on frozen cooked fish flakes added tert-Butyl hydroquinone and rosmary extract.

### 3.4. Protein alterations during storage at 5 °C

Lipid and protein oxidation are catalysed by the same compounds. These processes can develop independently or in parallel (Karel, 1973; Karel, Schiach, & Roy, 1975). Moreover, the two processes can interact with each other. It is thus well known that radicals, hydroperoxides and secondary compounds resulting from lipid oxidation react with protein leading to the loss of protein functionality (Gardner, 1979). Aldehydes formed during lipid oxidation can interact with amino groups of protein to form Schiff base products (Zamora, Alaiz, & Hidalgo, 1999). The impact of protein oxidation on protein functionality and muscle food quality has recently received more attention (Baron, Kjærsgård, Jessen, & Jacobsen, 2007).

The formation of protein carbonyl compounds is one of the most important changes taking place during the oxidation of proteins. Some major amino acids, such as lysine, histidine, proline and arginine yield carbonyl compounds and therefore the concentration of such compounds is a meaningful indicator of the oxidative status of muscle proteins (Dalle-Donne, Rossi, Giustarini, Milzani, & Colombo, 2003). The protein carbonyls of different treatment groups are shown in Fig. 2a. At T0, there was no significant ($P > 0.05$) difference in protein carbonyl content between the treatment groups. As storage progressed protein oxidation was evident by a significant ($P < 0.05$) increase in the protein carbonyl content. At the end of the storage period, control showed the highest and

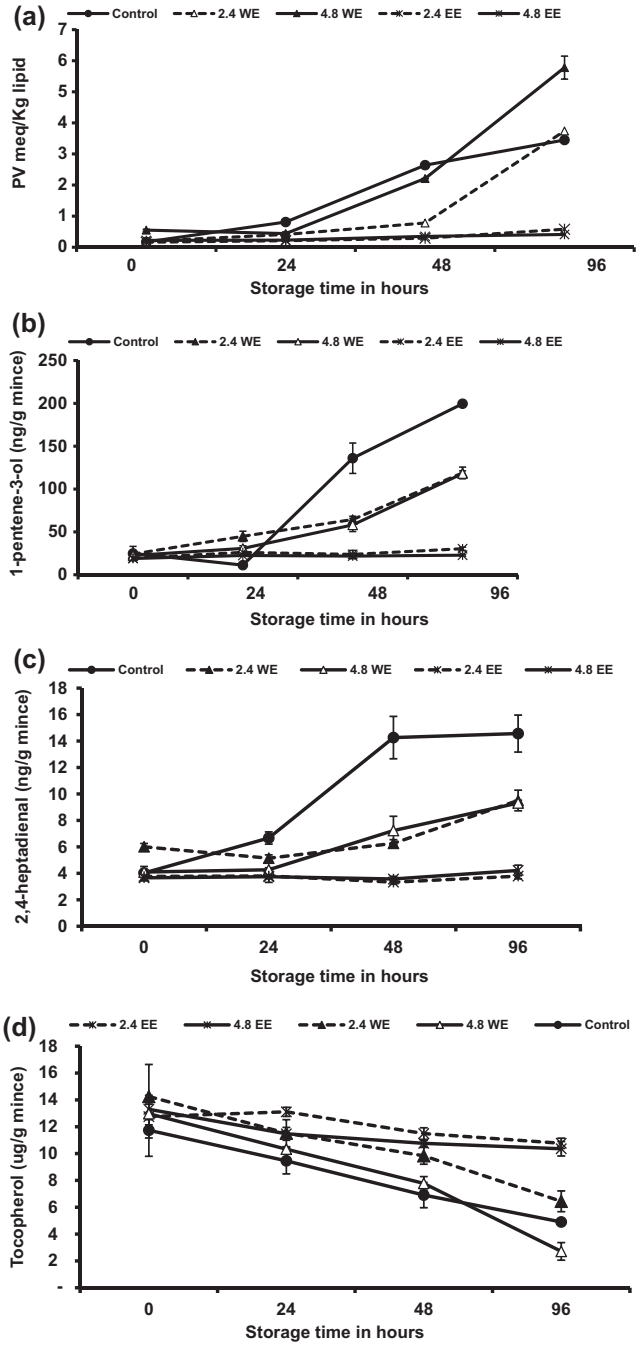


**Fig. 1.** Lipid oxidation parameters during storage of minced mackeral at 5 °C. (a) Peroxide values (PV); (b) concentration of 1-penten-3-ol; (c) concentration of 2, 4, heptadienal; (d) the α-tocopherol content (μg/g mince) in mince treated with different concentrations of water (WE) and ethanol (EE) extracts of potato peel. Values are mean ± SD ($n$ = 3).

**Table 3**
Free fatty acid (FFA) content, protein solubility, thiol group and colour measurements of different treatment groups of mince. Values are mean ± SD ($n$ = 3).

| Parameters/codes | Storage time in hours | |
|---|---|---|
| | 0 | 96 |
| **FFA** (% oleic acids) | | |
| Control | 0.87 ± 0.07$^{a,x}$ | 1.55 ± 0.02$^{b,x}$ |
| 2.4 EE | 1.01 ± 0.01$^{a,x,y}$ | 1.73 ± 0.01$^{b,y}$ |
| 4.8 EE | 1.01 ± 0.01$^{a,x,y}$ | 1.85 ± 0.05$^{b,y}$ |
| 2.4 WE | 1.09 ± 0.06$^{a,y}$ | 1.85 ± 0.11$^{b,y}$ |
| 4.8 WE | 1.1 ± 0.03$^{a,y}$ | 1.86 ± 0.02$^{b,y}$ |
| Protein solubility (mg/g mince) | | |
| Control | 39.83 ± 1.7$^{b,x,y}$ | 34.91 ± 0.6$^{a,x}$ |
| 2.4 EE | 37.65 ± 3.4$^{b,x}$ | 33.96 ± 0.7$^{a,x}$ |
| 4.8 EE | 41.91 ± 0.9$^{b,y}$ | 37.11 ± 0.6$^{a,y}$ |
| 2.4 WE | 37.28 ± 1.8$^{b,x}$ | 33.89 ± 0.9$^{a,x}$ |
| 4.8 WE | 38.55 ± 2.3$^{b,x}$ | 35.11 ± 1.0$^{a,x,y}$ |
| Thiol groups (μmol/g mince) | | |
| Control | 28.58 ± 0.9$^{b,x}$ | 26.03 ± 0.9$^{a,x}$ |
| 2.4 EE | 31.40 ± 1.6$^{b,y}$ | 27.32 ± 0.9$^{a,x,y}$ |
| 4.8 EE | 30.02 ± 1.6$^{a,x,y}$ | 28.37 ± 1.5$^{a,y}$ |
| 2.4 WE | 31.37 ± 1.5$^{b,y}$ | 27.53 ± 0.8$^{a,x,y}$ |
| 4.8 WE | 28.55 ± 1.4$^{b,x}$ | 26.12 ± 0.9$^{a,x}$ |
| $L^*$ Values | | |
| Control | 59.8 ± 0.9$^{a,y}$ | 62.1 ± 2.7$^{a,z}$ |
| 2.4 EE | 59.1 ± 1.4$^{a,y}$ | 59.1 ± 1.7$^{a,y}$ |
| 4.8 EE | 58.5 ± 0.5$^{a,y}$ | 58.9 ± 1.1$^{a,y}$ |
| 2.4 WE | 56.1 ± 1.3$^{a,x,y}$ | 55.7 ± 0.1$^{a,x}$ |
| 4.8 WE | 55.4 ± 1.5$^{a,x}$ | 54.4 ± 1.2$^{a,x}$ |
| $a^*$ Values | | |
| Control | 4.9 ± 0.1$^{b,\ y}$ | 1.8 ± 0.1$^{a,x}$ |
| 2.4 EE | 3.8 ± 0.7$^{b,x}$ | 2.2 ± 0.1$^{a,x,y}$ |
| 4.8 EE | 4.1 ± 0.7$^{b,x}$ | 2.3 ± 0.1$^{a,\ x,y}$ |
| 2.4 WE | 5.5 ± 0.1$^{b,y}$ | 3.1 ± 0.2$^{a,y}$ |
| 4.8 WE | 4.9 ± 0.2$^{b,y}$ | 2.9 ± 0.3$^{a,y}$ |
| $b^*$ Values | | |
| Control | 10.3 ± 0.2$^{a,x}$ | 12.2 ± 0.9$^{b,x,y}$ |
| 2.4 EE | 10.8 ± 0.6$^{a,x}$ | 10.9 ± 0.3$^{a,x}$ |
| 4.8 EE | 11.1 ± 1.3$^{a,y}$ | 10.6 ± 0.6$^{a,x}$ |
| 2.4 WE | 10.7 ± 0.5$^{a,x}$ | 13.1 ± 0.5$^{b,y}$ |
| 4.8 WE | 11.7 ± 0.4$^{a,y}$ | 13.9 ± 0.9$^{b,y}$ |

*Notes*: EE – ethanol extract; WE – water extract.

the mince with 2.4 and 4.8 g/kg of ethanol extracts showed lowest levels of carbonyl content. The order of protection offered against the formation of carbonyl compounds was 4.8 EE > 2.4 EE > 2.4 WE > 4.8 WE > control. These results are consistent with volatiles oxidation products suggesting a possible relationship between the protein carbonyl formation and volatiles. The formation of protein carbonyl compounds have been reported to be the second sign of protein oxidation due to proteins interacting with secondary lipid oxidation products (Hidalgo & Kinsella, 1989). The lowest carbonyl contents in mince with 2.4 and 4.8 g/kg ethanol extracts suggests a protective role of this extract against oxidation of proteins. Certain phenolic acids (Vuorela et al., 2005), phenolic diterpenes (Estévez, Ventanas, & Cava, 2005), flavanols (Salminen, Estévez, Kivikari, & Heinonen, 2006) and tea catechins (Rababah et al., 2004) have been found to inhibit formation of protein carbonyls in meat products as measured by DNPH method. Likewise, in our study also the phenolic rich extracts of potato peel prevented the formation of protein carbonyls and thereby protein oxidation. Phenolic compounds have been suggested to inhibit the oxidation of proteins either by retarding the lipid oxidative reactions or by binding to the proteins or by forming complex with them (Siebert, Troukhanova, & Lynn, 1996).

The presence of the aromatic ring in tryptophan and tyrosine residues are responsible for the natural fluorescence emitted by these amino acids at 350 nm and 310 nm when excited at 280 and 270 nm respectively. Tryptophan and tyrosine fluorescence has been shown to have good correlations with their concentration and degradation. Moreover, bityrosine production upon oxidation by radicals has been shown to decrease the fluorescence (Davies, Delsignore, & Lin, 1987). Thus, the changes in intrinsic fluorescence can be used to monitor structural changes in the proteins. In the present study, the fluorescence spectrum of tyrosine resembled that of tryptophan, but the fluorescence intensity of tyrosine was lower than that of tryptophan when excited at 270 nm and 280 nm respectively (data not shown). This may be due to the fact that tyrosine is a weaker emitter than tryptophan. Moreover, in folded protein, the intrinsic fluorescence of these amino acids has

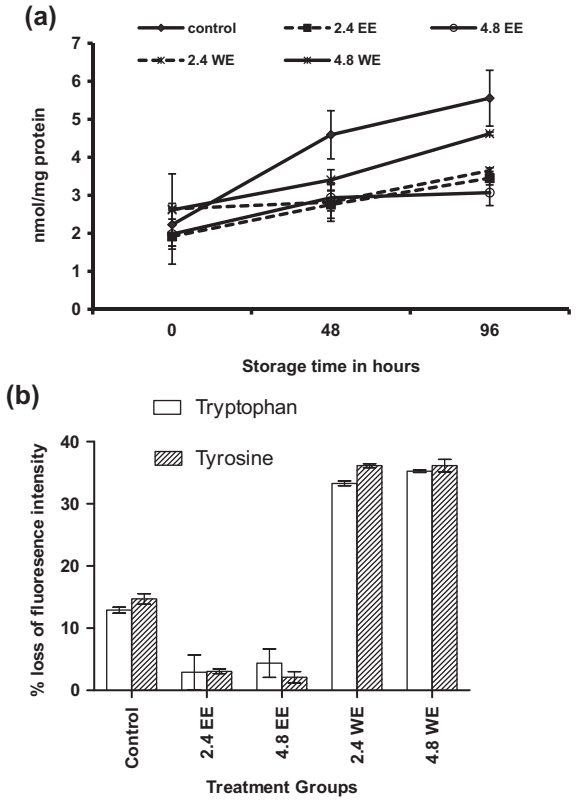


**Fig. 2.** Protein oxidation parameters during storage of minced mackeral at 5 °C. (a) Progress in carbonyl contents; (b) percent loss of tryptophan and tyrosine fluorescence in minced mackeral treated with different concentrations of water (WE) and ethanol (EE) extracts of potato peel. Values are mean ± SD ($n$ = 3).

been shown to differ depending upon location (buried/surface) or by neighbouring amino acids (Lakowicz, 2006). The fluorescence of tryptophan and tyrosine residues was measured and is presented as % loss of fluorescence intensity (Fig. 2b). The mince with ethanol extracts showed significantly ($P < 0.001$) higher protection against the loss of tryptophan and tyrosine fluorescence while the mince containing the water extracts showed significantly ($P < 0.001$) lower protection against tryptophan and tyrosine loss when compared to control. This result is in agreement with PV where water extracts showed higher PV than the controls. This finding is also in agreement with earlier studies showing that the loss of tryptophan fluorescence is an early event of protein oxidation and follows the primary oxidation products (Giessauf, Steiner, & Esterbauer, 1995; Rampon, Lethuaut, Mouhous-Riou, & Genot, 2001). The decrease in the intrinsic fluorescence of tryptophan and tyrosine can be explained by physicochemical changes in proteins, including those derived from oxidative stress. The significantly higher percent inhibition exhibited by ethanol extracts against tryptophan and tyrosine loss could be related to the lower levels of lipid oxidation which is attributed to high concentration of phenolic compounds including caffeic acid in this extract. Among the hydroxycinnamic acids, caffeic and chlorogenic acids were reported to have more profound antioxidant effect towards the oxidation of tryptophan (Salminen & Heinonen, 2008). Plant phenolics are known as redox–active compound that display antioxidant and pro-oxidant actions depending on their concentrations and the presence of other redox compounds (Cao, Sofic, & Prior, 1997). Hence, the overall effect (antioxidant or pro-oxidant) of plant phenolics is largely determined by the balance between the antioxidant and pro-oxidant actions. The higher tryptophan and tyrosine loss of minces containing water extracts is in agreement with the PV measurement and may be is due to higher lipid oxidation and/or pro-oxidative action of certain phenolics and co-extracted compounds on proteins.

The alteration of protein extractability is a useful factor which may be used to determine the textural quality of fish muscle, as protein aggregation is accompanied by a significant decrease in their solubility (Badii & Howell, 2002). In the present study, protein solubility decreased significantly ($P < 0.05$) in all the groups as the storage progressed (Table 3) indicating the formation of protein aggregates. There was no clear effect of any of the extracts on the decrease in protein solubility. A similar finding was observed by Medina, González, Iglesias, and Hedges (2009). They found that the hydrocynnamic acids added showed no protection against loss of solubility in frozen minced horse mackerel. The results of sulphydryl group determinations also correlated well with solubility data. Thus, significant decrease on storage in all the samples except mince containing 4.8 g/kg ethanol extract was observed (Table 3). A decrease in total sulphydryl group content is due to the formation of disulphide bonds through oxidation of sulphydryl groups or disulphide interchanges (Hayakawa & Nakai, 1985; Lanier, 2000). The formation of disulphide bond which results in the aggregation of proteins might have contributed to low solubility of proteins. Lower loss of sulphydryl groups in mince containing 4.8 g/kg ethanol extract might be due to lower oxidation. The similar reduction in protein solubility and sulphydryl groups in controls and samples treated with extracts suggest that none of the treatments have been effective in reducing the rate of solubility and sulphydryl group loss. Taken together it also suggests that there is not a direct relationship between lipid oxidation and protein aggregation in chilled storage of minced horse mackerel.

### 3.5. Colour

Colour attributes of the mince were expressed as Lightness (L), redness (a) and yellowness (b) and are shown in Table 3. There was no significant ($P > 0.05$) changes in lightness over time in the different treatment groups. However, there was a significant reduction ($P < 0.05$) in redness in all the groups and the control was shown to have to have lowest redness at the end of storage time. The reduction in redness was thus comparatively lower for the treated groups. This indicates that the mince treated with extracts prevented the oxidation of the heme proteins, haemoglobin and myoglobin, which are red in their reduced from and brown in their oxidised ferric form. Earlier Tesoriere, Butera, Gentile, and Livrea (2007) found that the phenolic extracts from capers effectively prevented the activation of myoglobin to its hypervalent state ferryl myoglobin, indicating a potential interaction between some phenolic compounds and heme protein redox reactions. Therefore, the interaction between heme proteins, in their different oxidative states, and phenolic compounds needs further attention.

There was no significant change ($P > 0.05$) in yellowness in mince with ethanol extracts, but the control and the mince with water extracts showed a significant ($P < 0.05$) increase in the yellowness on storage. The increase in yellowness in control and water extracts can be due to increased lipid oxidation, and these data also correlated well with PV values. The aldehydes produced from lipid oxidation are able to modify very rapidly some amino acid residues, such as lysine producing pyrroles that, by means of a polymerisation reaction, are responsible for the colour changes (Zamora et al., 1999). Although the colour changes associated with lipid oxidation is widely reported, the colour changes in the present study is difficult to compare with lipid or protein oxidation as the extracts itself has some colour (yellow for ethanol extracts and black for water extracts) and may have some influence in the colour. The colour perceived by the sensory analysis is given in Table 4.

**Table 4**
The average sensory scores for odour (attributes were fishy/rancid and others) and colour for the control and treated minced mackerel.

| Treatment groups | Fishy | Rancid | Others (description) | Colour |
|---|---|---|---|---|
| 0 h | | | | |
| Control | 2 | 1 | 0 | Reddish brown |
| 2.4 EE | 3 | 1 | 1–3 (green, potato) | Yellowish brown |
| 4.8 EE | 2 | 1 | 1 (rubbery, green, potato) | Yellowish brown |
| 2.4 WE | 4 | 4 | 2 (rubbery) | Dark reddish brown |
| 4.8 WE | 3 | 3 | 2 (rubbery) | Dark reddish brown |
| 96 h | | | | |
| Control | 5 | 5 | 1 (rubbery) | Pale brown |
| 2.4 EE | 5 | 4 | 2 (rubbery) | Brown |
| 4.8 EE | 3 | 2 | 1 (rubbery, green, burnt) | Brown |
| 2.4 WE | 4 | 5 | 2–3 (rubbery) | Dark brown |
| 4.8 WE | 3 | 3–4 | 1 (rubbery) | Dark brown |

*Notes*: EE – ethanol extract; WE – water extract.

### 3.6. Sensory analysis

The average values of the sensory scores are given in Table 4. In general, scores for fishy/rancid off flavours for the different mince samples were low and there was a slight increase in the fishy and rancid odours for all the mince samples upon storage. The sensory analysis data for the mince do not correlate well with PV and other oxidation parameters. Even though ethanol extracts 2.4 g/kg showed very low PV and other oxidation parameters the sensory scores for fishy/rancid was found to be higher. The opposite is the case for the mince containing 4.8 g/kg water extract where PV and other oxidation parameters where high but the sensory panel could not detect any clear rancid odour for these samples. In the case of 4.8 g/kg ethanol extract, the sensory score was in agreement with PV and other oxidation parameters. Nevertheless, the sensory data also indicate that extracts from potato peel potentially may reduce oxidative flavour deterioration in fish mince and this deserves further investigation by a larger sensory panel than that applied in the present study.

## 4. Conclusion

The results of the present study indicated that lipid and protein oxidation developed simultaneously in the different minces and it seemed that the reactions were linked. The addition of the ethanol extracts of potato peel at 2.4 and 4.8 g/kg provided best protection towards lipid and protein oxidation in minced horse mackerel. The high efficacy of the ethanol extracts correlated with higher total phenolics and caffeic acid content in these extracts. The ethanol extracts were also shown to reduce tocopherol loss, indicating a tocopherol sparing property of the phenolic compounds. The water extracts showed lower efficacy and/or pro-oxidative effects especially when added in high concentration. This might be due to low phenolic contents and/or pro-oxidative action of certain phenolics and co-extracted compounds. There was no evidence to suggest that extracts reduced protein solubility or loss of sulphydryl groups. Protein aggregation, as monitored by losses in solubility, was not decreased by the addition of extracts. Thus the results of the present study suggest that ethanol extracts of potato peel can be employed as natural antioxidant to prevent lipid and protein oxidation of fish fillets/mince in chilled storage of mackerel. However, further studies are necessary to characterise the compounds present in the extract and to investigate if they have any positive or negative health effects.

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