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RESEARCH ARTICLE (Open Access)

Lipid oxidation degree and antioxidant activity of several polyphenolic extracts in porcine meat during storage

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Abstract:

Extracts of vegetable origin are used widely nowdays in the food industry in the role of antioxidants, especially in the meat processing industry and in the industry of its byproducts. Subject of this study have been porcine meat samples, which have been subjected to polyphenolic extracts, such as those from: tea, rosemary and oregano conserved in a timeframe of 1, 4, 7 and 10 days. TBA (thiobarbituric acid) assay show that polyphenolic extracts tend to increase oxidative endurance of meat sample, while DPPH assay shows an increased level of antioxidant activity. Lipids oxidation degree and antioxidant activity of the samples of porcine meat treated with rosemary, oregano and tea polyphenolic extracts is lower than the control samples either in treated or not treated in 85°C samples. The samples which have been subjected to tea polyphenolic extract show a lower lipid oxidation degree and a higher antioxidant activity compared not only to control samples, but also to the samples treated with other polyphenolic extracts. Lipid oxidation degree and antioxidant activity result are greater in temperature treated samples compared to those in raw state.

Keywords: ***oxidation, antioxidant activity, polyphenol, TBA, DPPH, porcine meat.***

1. Introduction

Lipid oxidation in meat is one of the reasons for quality degradation during storage. This process is associated with the presence of free radicals that lead to the production of aldehydes responsible for the development of rancid flavours and changes in meat colour [**4**]. The complex mechanism by which the oxidation takes place, also affects proteine function . This may lead to loss of proteine solubility, loss of colour and reduced nutritional value. Vitamins are also oxidised during this process and, for this reason, vitamin E (α-tocopherol) is often used as an antioxidant [**3**]. Vitamins A, β-carotene and ascorbic acid are also susceptible to oxidation. Vitamin oxidation may protect the fatty acids; however, the nutritional value of meat is negatively affected as a result of a general reduction in the avaibility of vitamins A, D, E and C [**11**].

Literature shows that an assessment of the antioxidant activity of 22 herbs, such as oregano, sage, thyme, cinnamon, basil, black and white pepper, incorporated (as liquid extract) at levels ranging from 0.2% to 2.5% w/w, on homogenized samples of porcine and bovine meat, revealed that lipid oxidation was prevented by all the extracts.

It is possible that animal nutrition can serve as a route to pass antioxidant activity from the diet to the meat. This has been confirmed in experiments conducted in broilers and turkeys with dietary oregano essential oil and α-tocopherol acetate included in feed at concentrations ranging from 100 to 200 mg/kg feed. Meat processing and storage, prior to consumption can have a significant effect on meat quality. The aim of this work was to assess the antioxidant activity of three popular herbs (namely rosemary oregano and tea) in porcine meat samples upon storage at 4 °C, in the raw or cooked state, over a 10 day period using the TBA (thiobarbituric acid) and DPPH assays. A purpose of this study was also to evaluate the total poliphenolic, anthocyanins and flavonoids content in the poliphenolic extracts of the three herbs.

2. Material and Methods

2.1. Extracts preparation

Minced fresh leaves of rosemary, oregano and tea acquired from the local market were extracted with distilled water at room temperature overnight. The extracts were filtered and stored at 4 °C. The extracts

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were used as liquid on porcine meat samples. No extract was added to the control samples.

2.2. Samples preparation

Fresh porcine meat (*Biceps femoris*) was obtained from the local market and visible fat was removed. The meat was then divided into four groups and was homogenized with 200mg/kg of rosemary, oregano and tea extracts in a multi-functional food blender. No extract was added to the control samples. All samples were packed in polyethylene film from a local market and stored at 4 °C in darkness for 24 h, than each sample was split into two. One portion remained in the raw state and the other was cooked at 85 °C in an oven for 30 min [**2**]. Both raw and cooked samples were assayed for lipid oxidation and antioxidant activity as described below at 1, 4, 7 and 10 days of storage at 4 °C in darkness, in a polyethylene film.

2.3. Determination of total phenolic content in the extracts (TPC)

Total phenolic content (TPC) of rosemary, oregano and tea extracts were determined using the Folin–Ciocalteau assay [**13**]. In brief an aliquot of 10 and 25 µl of extract was added to a 15 ml volumetric flask containing 5 ml distilled water. Then 1 ml of ethylic alcohol was added followed by an addition of 0.5 ml of Folin-Ciocalteau reagent. The content was then mixed and after 3 minutes was added 1 ml Na_2CO_3 (5g/l) and then the mixture was vortexed. After keeping the samples at room temperature for 1 hour their absorbance was measured at 725 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (0-100 mg/l). The concentration of total phenolic content is expressed as the gallic acid equivalent (GAE) per 100 ml of extract.

2.4. Determination of total anthocyanins content in the extracts (TAC)

The determination of the total anthocyanins content (TAC) was realized by the method proposed by Di Stefano [**1**]. The samples were diluted with a solution consisting of 70/30/1 (v/v/v) ethanol/water/HCl (concentrated) and the absorbance was measured at 540 nm. Due to the lack of a malvidine-3-glucoside standard, the total anthocyanic content were expressed as malvidine-3-glucoside equivalents and were calculated using the following equation proposed by Di Stefano.

$$TA_{540nm}\ (mg/l) = A_{540nm}16.7d$$

where A_{540nm} is the absorbance at 540 nm and d is the dilution.

2.5. Determination of total flavonoids content in the extracts (TFC)

Total flavonoid content (TFC) was evaluated according to a colorimetric assay with aluminum chloride. An 1 ml aliquot of the extract sample was added to a 15 ml volumetric flask containing 4 ml of distilled water, followed by the addition of 0.3 ml of solution of $NaNO_2$ (0.5 g/l). After 5 minutes, 0.3 ml of a 1 g/l solution of AlCl3 was added and 2 ml of NaOH (1 mol/l) was added to the mixture 6 minutes later. The total volume was made up to 10 ml with distilled water, the solution was mixed and the absorbance was measured at 510 nm against water as blank. The results were expressed as absorbance values.

2.6. TBA assay

In brief, 0.03 g of sample was mixed with 0.6 ml deionised H_2O, 0.9 ml of phosphoric acid (pH 2.0) and 0.9 ml 0.8% (w/w) of thiobarbituric acid (TBA) in 1.1% (w/w) sodium dodecylsulfate (SDS) in a test tube, and then vortexed and heated at 100 °C for 60 min in a water bath. After cooling, butan-1-ol (3 ml) was added and the solution mixed. Samples were then centrifuged at 8960×g for 10 min. The absorbance of the upper layer was determined at 532 nm. Butan-1-ol was used as blank. The results were expressed as TBA values.

2.7. DPPH assay

The hydrogen atom or electron-donating ability of the meat samples was measured from the bleaching of a purple-colored methanolic solution of DPPH [**5**]. Free radical scavenging activity was evaluated by the DPPH assay using the method of Tepe et al. [**12**]. Samples (0.03 g) were constantly mixed with 3 ml of 0.004% DPPH in methanol in a test tube at room temperature for 30 min. The samples were centrifuged at 1430×g for 10 min. Absorbance of the supernatants was measured at 517 nm. Results were expressed as absorbance and decreasing values indicated increasing antioxidant activity.

3. Results and Discussion

The data in Table 1 show that rosemary, oregano and tea extracts exhibited different concentrations of total phenols, which can be responsible for the differences noted between the TBA and DPPH data.

The flavonoids total content (TFC) showed that oregano extracts are richer in flavonoids than tea and rosemary. Furthermore, oregano extracts showed higher levels of anthocyanins content (TAC) than tea and rosemary, but the differences were lower than TFC comparisons.

Table1. Total phenolic, anthocyanins and flavonoids content in rosemary, oregano and tea extracts

Sample	*TPC (mg/100ml)*	*TAC (mg/100ml)*	*ABS TFC*
Rosemary	434.24	137.5	0.592
Oregano	480.54	272.2	0.909
Tea	541.37	242.4	0.622

Lipid oxidation is characterized by the formation of conjugated dienes, hydroperoxides and aldehydes (**8**). Aldehydic products of lipid oxidation, especially MDA (malondialdehyde) can be estimated by the reaction with TBA (thiobarbituric acid) and the TBA value is routinely used as an index of lipid oxidation in meat products [**10**].

The data in Table 2 and Figure 1 show that the TBA value was decreased in all samples after rosemary, oregano and tea extracts addition, but tea was more effective against lipid oxidation.

Rosemary, oregano and tea treatments results showed decreased oxidation levels than those found in both controls stored at 4 °C. The DPPH assay results of tea extracts showed that probably tea has the greatest protective role in comparison to the other herbs. The data of the TBA assay were supported by the results of the DPPH method. In all samples during the storage, both lipid oxidation and antioxidation activity increased until the day 7 and then they decreased drastically in day 10.

Table 2. TBA data for the lipid oxidation and DPPH data for antioxidant activity of porcine meat, raw and cooked, treated with rosemary, oregano and tea poliphenolic extracts

Sample	*TBA values*				*DPPH values*			
	Day1	**Day 4**	**Day 7**	**Day 10**	**Day1**	**Day 4**	**Day 7**	**Day 10**
RC	0.042	0.652	1.094	0.234	0.697	0.809	1.134	0.707
CC	0.055	0.824	1.013	0.170	0.721	0.885	1.100	0.768
RR	0.039	0.559	0.838	0.163	0.669	0.527	1.086	0.666
CR	0.047	0.770	1.386	0.133	0.508	0.599	1.061	0.692
RO	0.014	0.527	0.689	0.125	0.512	0.764	0.846	0.468
CO	0.041	0.761	0.982	0.137	0.596	0.815	1.121	0.605
RT	0.025	0.622	1.116	0.200	0.330	0.558	0.994	0.475
CT	0.045	0.700	0.876	0.247	0.322	0.684	0,905	0.510

RC= raw control; **CC**= cooked control; **RR** = raw + rosemary; **CR** cooked + rosemary; **RO** = raw + oregano; **CO** cooked + oregano; **RT** = raw + tea; **CT** = cooked + tea

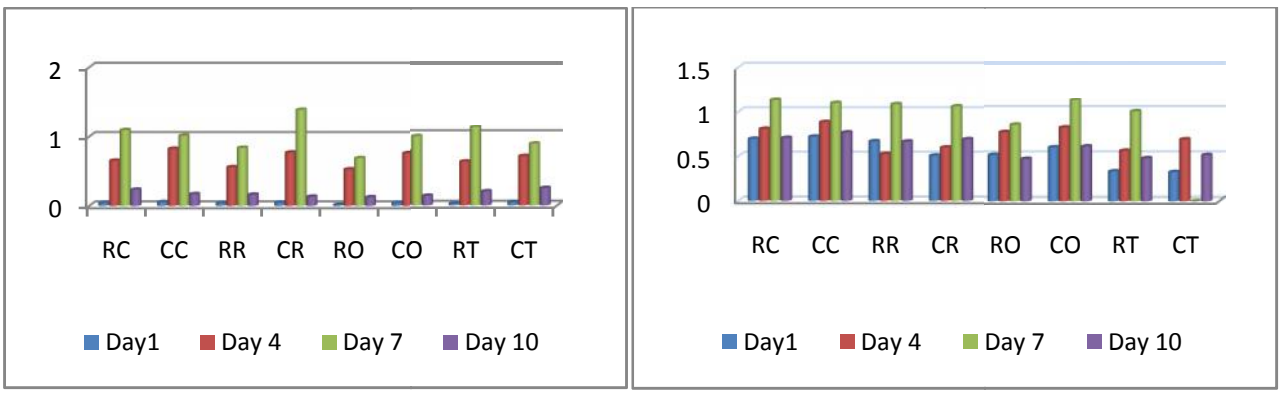

Figure 1. Graphical presentation of TBA and DPPH data for the lipid oxidation and the antioxidant activity of porcine meat, raw and cooked, treated with polyphenolic extracts during storage.

The results of this study using three different treatments (rosemary, oregano and tea), gave generally higher antioxidant activity compared to the control. The treated samples resulted in lower oxidation of porcine meat upon storage at 4°C, with the tea treatments being the most potent. Generally, our results were in agreement with other research studies that had investigated the effects of tea in meat protection from oxidation through feeding.

Conversely this data showed that tea and oregano treatment gave the greatest antioxidant activity perhaps because of the higher total phenolic content. The results further demonstrate that the rosemary, oregano and tea extracts contain different types of phenolic compounds.

Regarding the TPC extracts, the results agree with litterature that suggested a relationship between antioxidant activity and phenolic compound content [**6,7**].

4. Conclusions

In all samples during the storage, both lipid oxidation and antioxidation activity increased until the day 7 and then they decreased drastically in day 10.

The results of this study using three different treatments (rosemary, oregano and tea), gave generally higher antioxidant activity compared to the control. The treated samples resulted in lower oxidation of bovine meat upon storage at 4°C, with the tea treatments being the most potent.

The TBA assay can be effectively used to investigate oxidation occurring during cooking and storage of meat, because the lipids are dramatically affected. Extracts from rosemary, oregano and tea are effective antioxidants and the tea extracts were more effective in inhibiting meat oxidation.

The addition of antioxidant plants in animal food may be a way to enrich the antioxidant content of the meat and to prevent the oxidation of lipids before its consumption.

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