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Mediterranean Berries as Inhibitors of Lipid Oxidation in Porcine Burger Patties Subjected to Cooking and Chilled Storage

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Abstract

The efficiency of extracts from *Arbutus unedo* L. (AU), *Crataegus monogyna* L. (CM), *Rosa canina* L. (RC), and *Rubus ulmifolius* Schott. (RU) to inhibit lipid oxidation in raw, cooked and cooked and chilled (2°C/12 d) porcine burger patties, was investigated. The modification of the fatty acid profile during processing treatments (cooking and chilling), the quantitative measurements of thiobarbituric acid reactive substances (TBA-RS), and lipid-derived volatiles, were used as indicators of lipid oxidation. Polyunsaturated fatty acids (PUFA) gradually decreased during cooking and the subsequent storage of cooked burger patties with this decrease being significantly greater (P<0.05) in control patties than in those with added berry extracts. In accordance, the control patties showed significantly higher TBA-RS numbers and counts of lipid-derived volatiles in all treatments when compared to the berry-added counterparts (P<0.05). Results from the present work show, for the first time, that extracts from *A. unedo*, *C. monogyna*, *R. canina*, and *R. ulmifolius* are promising antioxidants which could enhance the nutritional, safety and sensory properties of porcine burger patties.

Key words: berries, lipid oxidation, meat pattiesm TBA-RSm polyunsaturated fatty acids, lipid-derived volatiles

INTRODUCTION

The importance of meat and meat products in human's health has been documented extensively (Higgs 2000; Valsta *et al*. 2005). It is undeniable; however, the bad image of meat products amongst consumers who associate meat consumption with coronary-heart diseases (CHD) and cancer (Verbeke *et al*. 1999; Ferguson 2010). This relationship could be partly explained by the high oxidation rates in particular meat products and the impact of the resulting oxidation products in consumer's health (Verbeke *et al*. 1999). Lipid oxidation occurs during handling, processing and storage of meat and meat products leading to loss of quality and acceptability (St. Angelo *et al*. 1990; Estévez *et al*. 2007). The oxidation of muscle lipids involves the degradation of polyunsaturated fatty acids (PUFA) and generation of residual products, such as malondialdehyde (MDA) and lipid-derived volatiles leading to sensory and nutritional deterioration of muscle foods (Shahidi and Pegg 1994; Morrissey *et al*. 1998). Some volatiles, particularly hexanal are mainly associated with the development of lipid oxidation in cooked meat under refrigerated storage (St. Angelo *et al*. 1987). This volatile aldehyde, amongst some others, may contribute to the development of oxidative rancidity known as warmed-over flavor (WOF) in meat subjected to refrigerated storage (Ladikos and Lougovois 1990; Ahn *et al*. 2002; Grün *et al*. 2006). Second-

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ary oxidative compounds are not only responsible for quality deterioration of precooked meat, but ingested oxidative products can also be a risk for human health (Aikawa and Chikuni 1988). Particular meat products consumed world-wide, such as burger patties, are considerably susceptible to oxidation as mincing, cooking and the addition of salt, promote the formation of reactive oxygen species (ROS) and hence, the occurrence and intensity of the oxidative reactions (Ladikos and Lougovois 1990).

Enhanced oxidative stability is needed for maintaining the safety and quality of precooked meats (Buckley *et al*. 1995). In order to inhibit oxidative reactions, natural antioxidants are employed in meat products because of their potential health benefits and safety. There is, therefore, a growing interest in the identification of novel, natural antioxidants for developing functional muscle foods with enhanced nutritional and health properties. The antioxidant activity of plant/fruit extracts is of particular interest both because of their beneficial physiological activity on human cells, and their potential to replace synthetic antioxidants, used in foodstuffs (Andersen *et al*. 2003; Heinonen 2007). Numerous studies have documented the effectiveness of antioxidative components in many natural plant and fruits extracts contain primarily phenolic compounds, which are potent antioxidants (St. Angelo *et al*. 1990; Kahkonen *et al*. 1999; Estévez *et al*. 2007). Whereas the antioxidant potential of berries from Scandinavian forests against muscle lipid oxidation have been profusely documented (Vuorela *et al*. 2005), the potential use of certain Mediterranean berries such as *Arbutus unedo* L. (AU), *Crataegus monogyna* L. (CM), *Rosa canina* L. (RC), and *Rubus ulmifolius* Schott. (RU), is ignored. In a recent study these berries were reported to display potent antioxidant activity *in vitro* assays against 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radicals (Ganhão *et al*. 2008). However, there is still a lack of information regarding their effects on the oxidative stability of meat subjected to cooking and subsequent refrigerated storage.

The aim of this study was to determine the effect of the addition of extracts from Mediterranean berries (RC, CM, RU, and AU) on the oxidative stability of raw, cooked and cooked and chilled pork burger patties. In addition, the analysis of the oxidative stability of a meat system taking into account the fatty acid profile and the generation of TBA-RS and lipid-derived volatiles at the same time is an interesting challenge since they are usually studied separately.

RESULTS AND DISCUSSION

Chemical composition of raw and cooked burger patties

The proximate composition (moisture, fat, protein and ash contents) of raw and cooked pork burgers patties with added berry extracts and Q is shown in Table 1. As expected the addition of berry extracts did not affect the chemical composition of burger patties as no significant differences were found amongst batches (P>0.05). The cooking and storage losses ranged from 21.7 to 23.6% and from 2.4 to 4.4%, respectively (Table 1). Once more, the loss of weight after cooking and chill storage of burger patties was not affected by the addition of berry extracts.

Table 1 Chemical composition of raw and cooked burger patties and cooking and storage losses of cooked burger patties with added berry extracts and quercetin

	CT	RC	CM	RU	AU	Q	SEM
Composition[1)]							
Raw							
Moisture	76.67	77.08	77.04	76.50	76.92	76.64	0.08
Fat	2.05	2.02	2.05	1.94	2.08	2.22	0.04
Ash	1.60	1.56	2.23	1.56	2.27	1.84	0.09
Protein	18.30	18.60	18.70	18.60	18.63	18.10	0.07
Cooked							
Moisture	70.84	72.46	70.63	70.67	70.48	70.93	0.19
Fat	2.19	1.86	2.19	1.97	1.96	2.53	0.06
Ash	3.18	3.36	3.33	2.91	3.37	3.46	0.07
Protein	23.03	23.38	23.70	23.01	23.50	22.53	0.02
Losses[2)]							
Cooking loss	22.06	23.59	22.71	21.73	22.73	22.24	0.30
Storage loss	3.23	2.85	3.09	2.95	2.37	4.37	0.18

[1)] Expressed as g 100 g^{-1} burger patty.

[2)] Expressed as percentage.

CT, negative; RC, *Rosa canina* L.; CM, *Crataegus monogyna* L.; RU, *Rosa canina* L.; AU, *Arbutus unedo* L.; Q, positive control; SEM, standard error of the mean. The same as below.

Fatty acid composition of burger patties

The fatty acid profile of raw, cooked and cooked and chilled burger patties is shown in Table 2. As expected, all raw patties had similar percentages of

fatty acids (P>0.05) as the fatty acid profile of burger patties reflected that of the raw material used for their production and the addition of berry extracts had no impact at all. Monounsaturated fatty acids (MUFA) showed the highest percents (46.95-47.69%), with oleic acid (C18:1n-9) being the most abundant fatty acid, followed by saturated fatty acids (SFA) (37.38-37.82%) and by polyunsaturated fatty acids (PUFA) (14.93-15.27%). The predominant fatty acids amongst SFA were palmitic (C16:0) and stearic acids (C18:0) whereas the most abundant PUFA, was linoleic acid (C18:2n-6). These results are agreement with others works, which described similar fatty acid profiles in porcine meat (Estévez *et al*. 2004). The lack of effect of added berry extracts on the fatty acid profile of burger patties is explained by the chemical composition of these berries, which had a small fat content (Ganhão *et al*. 2008).

The technological processes applied to burger patties, namely cooking and chill storage, influenced their fatty acid profiles, with the PUFA being the most affected fatty acids. During cooking, control patties suffered a slight but significant (P<0.05) decrease of PUFA (1%) and long chain PUFA (5.8%) whereas no significant changes were observed in the patties with added berry extracts. Cooking did not affect (P>0.05) the fatty acid percents amongst SFA and MUFA. As a result of this changes, control cooked patties had significantly lower percentages of PUFA than cooked patties with added berry extracts (P<0.05). The study of the oxidative deterioration of a meat product as assessed by PUFA degradation is of high importance because PUFA are preferentially affected by oxidative reactions due to the presence of double bonds in the hydrocarbon chain, leading to generation of unpleasant odours and reducing nutritional value of meat products (Gray *et al*. 1996; Morrissey *et al*. 1998). The results from the present study reflect that the high temperatures reached during patties cooking enhanced the oxidative decomposition of PUFA. It is plausible to consider that the formation of hydroperoxides and acyl radicals during thermal decomposition of unsaturated fatty acids was efficiently inhibited by antioxidant compounds from berry extracts.

In cooked and chilled burger patties, a similar trend was observed. The refrigerated storage caused an additional loss of PUFA from cooked burger patties. In fact, the loss of PUFA (5.9%) during chill storage of control cooked patties was more intense than that observed during cooking. The fatty acid profile of burger patties with added berry extracts remained invariable during cooking and the subsequent chill storage. The acceleration of lipid oxidation in burger patties following cooking, reflected in the significant loss of PUFA, could been attributed to heat-induced changes in muscle components, including disruption of cellular compartmentalization and exposure of membrane lipids to a pro-oxidative environment, release of catalytic free iron from myoglobin (Kristensen and Purslow 2001), and thermal inactivation of antioxidant enzymes (Lee *et al*. 1996). Furthermore, PUFA are mainly placed on the phospholipids located in cellular membranes where the oxidative reactions commence because of the proximity to cellular prooxidants such as metalloproteins (Pikul *et al*. 1984; Morrissey *et al*. 1998). The modification of the fatty acid profile of burger patties as a result of oxidative reactions would have a relevant impact on the nutritional value of these meat products. According to the results from the present study, cooking and chilling storage of burger patties involves a significant loss of n-6 and n-3 essential fatty acids such as linoleic (C18:2n-6), linolenic (C18:3n-3), arachidonic (C20:4n-6) and docosapentanoic acid (DPA, C22:5n-3). Whereas long chain PUFA are minority fatty acids in meat products, considerable attention has been paid to these fatty acids owing to their role in the development of coronary heart diseases (CHD) (Okuyama and Ikemoto 1999). Additionally, the loss of PUFA during refrigerated storage of cooked meat leads to the formation of abnormal flavours, named as WOF (warmed-over-flavour) (St. Angelo *et al*. 1987; Shahidi and Pegg 1994; Morrissey *et al*. 1998). Results obtained in the present work suggest that the addition of berry extracts in burger patties protected PUFA and long chain PUFA from oxidative degradation during cooking and the subsequent refrigerated storage. Phenolic compounds are natural components of the tested berries and very likely, main responsible of the antioxidant action observed against PUFA degradation. *Rose* spp. are known to contain considerably high levels of ascorbic acid and glucoside proanthocyanidins such as cyanidin-3-glucoside (Hellström

Table 2 Fatty acid profile of raw, cooked and cooked and chilled burger patties with added berry extracts and quercetin[1)]

Fruit treatment[2)]	CT			RC			CM			RU			AU			Q			*P* value[3)]			
	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Fruit (F)	Treatment (T)	F×T	SEM
C10:0	0.27	0.28	0.28	0.27	0.29	0.29	0.29	0.29	0.31	0.27	0.29	0.28	0.26	0.30	0.27	0.26	0.27	0.28	0.380	0.125	0.984	0.004
C12:0	0.14	0.15	0.14	0.16	0.17	0.16	0.15	0.15	0.16	0.14	0.15	0.15	0.14	0.16	0.15	0.14	0.14	0.14	0.000	0.023	0.867	0.002
C14:0	1.20	1.24	1.17	1.22	1.19	1.17	1.24	1.19	1.26	1.17	1.17	1.19	1.16	1.19	1.18	1.17	1.16	1.14	0.003	0.815	0.246	0.006
C15:0	0.09	0.09	0.09	0.11	0.10	0.09	0.09	0.09	0.10	0.09	0.09	0.09	0.09	0.09	0.09	0.10	0.10	0.09	0.001	0.196	0.032	0.001
C16:0	23.38	23.67	23.54	23.91	23.13	23.33	23.78	23.27	23.98	23.60	23.27	23.42	23.36	23.11	23.53	23.65	23.32	23.10	0.603	0.115	0.492	0.061
C16:1n-7	3.00	3.05	3.04	3.02	2.94	2.98	3.04	2.95	3.08	3.01	2.94	2.99	2.91	2.92	2.93	2.88	2.83	2.82	0.000	0.156	0.500	0.012
C17:0	0.45	0.47	0.46	0.50	0.49	0.48	0.47	0.48	0.48	0.46	0.47	0.46	0.48	0.48	0.47	0.52	0.53	0.52	0.000	0.675	0.994	0.003
C17:1n-7	0.33	0.35	0.34	0.36	0.35	0.35	0.35	0.35	0.36	0.34	0.35	0.34	0.34	0.35	0.34	0.36	0.37	0.37	0.001	0.765	0.903	0.002
C18:0	10.96	10.83	11.02	10.70	10.78	10.81	10.80	10.98	10.86	10.86	10.92	10.90	11.04	10.91	10.99	11.06	11.17	11.14	0.000	0.553	0.675	0.022
C18:1n-9	38.43	38.33	39.05	37.86	37.96	38.08	37.97	38.07	38.50	38.01	37.86	38.10	38.13	38.20	38.14	37.94	37.80	38.15	0.011	0.054	0.914	0.059
C18:1n-7	4.77	4.76	4.87	4.73	4.83	4.82	4.76	4.78	4.77	4.76	4.76	4.77	4.74	4.79	4.69	4.61	4.64	4.64	0.000	0.140	0.220	0.011
C18:2n-6	10.90	10.94	10.34	10.81	11.09	10.91	10.77	10.88	10.31	11.00	11.23	10.95	10.92	10.97	10.92	11.24	11.36	11.36	0.001	0.035	0.629	0.050
C18:3n-3	0.61	0.61	0.58	0.60	0.61	0.60	0.62	0.61	0.59	0.63	0.63	0.62	0.65	0.65	0.65	0.64	0.63	0.64	0.002	0.559	0.948	0.004
C20:0	0.43	0.43	0.45	0.45	0.48	0.46	0.45	0.47	0.46	0.43	0.44	0.44	0.47	0.46	0.46	0.43	0.44	0.45	0.009	0.389	0.860	0.003
C20:1n-9	1.11	1.08	1.12	1.11	1.13	1.13	1.12	1.11	1.10	1.07	1.08	1.08	1.12	1.14	1.09	1.11	1.13	1.14	0.184	0.911	0.916	0.006
C20:2n-6	0.66	0.66	0.65	0.62	0.66	0.65	0.65	0.66	0.63	0.63	0.66	0.64	0.64	0.66	0.65	0.67	0.69	0.70	0.041	0.174	0.911	0.004
C20:3n-3	0.47	0.45	0.41	0.52	0.56	0.54	0.51	0.54	0.46	0.51	0.53	0.52	0.52	0.53	0.50	0.48	0.51	0.49	0.000	0.073	0.796	0.006
C20:4n-6	1.61	1.50	1.30	1.81	1.94	1.88	1.75	1.85	1.51	1.79	1.88	1.82	1.80	1.83	1.72	1.61	1.74	1.63	0.000	0.059	0.681	0.028
C21:0	0.17	0.17	0.16	0.15	0.16	0.16	0.16	0.17	0.16	0.16	0.16	0.16	0.16	0.17	0.16	0.17	0.17	0.17	0.080	0.273	0.914	0.001
C20:5n-3	0.08	0.08	0.09	0.08	0.09	0.09	0.09	0.09	0.08	0.08	0.09	0.09	0.08	0.08	0.08	0.07	0.08	0.07	0.004	0.501	0.259	0.001
C22:0	0.01	0.02	0.02	0.04	0.03	0.03	0.02	0.03	0.02	0.02	0.03	0.04	0.04	0.03	0.04	0.03	0.03	0.03	0.139	0.601	0.827	0.002
C22:1n-9	0.05	0.06	0.11	0.05	0.04	0.06	0.02	0.06	0.07	0.07	0.06	0.05	0.05	0.06	0.05	0.05	0.05	0.05	0.004	0.004	0.001	0.003
C22:5n-3	0.46	0.41	0.38	0.47	0.51	0.49	0.47	0.48	0.41	0.47	0.48	0.48	0.47	0.48	0.46	0.45	0.46	0.45	0.001	0.080	0.274	0.006
C24:0	0.29	0.27	0.23	0.30	0.33	0.31	0.30	0.32	0.26	0.30	0.31	0.30	0.30	0.31	0.29	0.27	0.29	0.28	0.002	0.032	0.303	0.004
C22:6n-3	0.14	0.12	0.13	0.13	0.13	0.12	0.12	0.13	0.11	0.13	0.13	0.12	0.13	0.12	0.13	0.11	0.11	0.12	0.691	0.873	0.896	0.003
SFA	37.38	37.60	37.58	37.82	37.15	37.29	37.77	37.46	38.04	37.51	37.32	37.44	37.50	37.21	37.64	37.79	37.61	37.36	0.434	0.192	0.499	0.053
MUFA	47.69	47.63	48.53	47.14	47.26	47.42	47.26	47.32	47.86	47.26	47.04	47.33	47.29	47.46	47.25	46.95	46.81	47.17	0.002	0.053	0.774	0.071
PUFA	14.93	14.77	13.90	15.05	15.60	15.29	14.98	15.23	14.10	15.23	15.64	15.24	15.21	15.33	15.11	15.27	15.58	15.47	0.001	0.012	0.348	0.079
LC PUFA	3.42	3.22	2.97	3.64	3.89	3.77	3.59	3.74	3.20	3.61	3.77	3.67	3.64	3.70	3.54	3.39	3.58	3.47	0.001	0.068	0.596	0.041

[1)] Means in percent of methyl esters from total analyzed.

[2)] SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC PUFA, long chain polyunsaturated fatty acids.

[3)] *P* values for the studied factors: F, fruit; T, treatment; F×T, interaction fruit×treatment. The same as below.

et al. 2009), CM contain a large variety of phenolics including proanthocyanidins, anthocyanins, catechins and chlorogenic acid (Bahorun *et al*. 2004) and RU and AU also contain large amounts of phenolic acids and glucoside flavonols (Dall'Acqua *et al*. 2008). Berry phenolics could have protected PUFA by scavenging initiators of the oxidation process, namely ROS or by chelating transitions metals such as iron. Both antioxidant mechanisms have been ascribed to the phenolic components of the berries employed in this study (Rice-Evans *et al*. 1997).

TBA-RS numbers

The effect of the addition of berry extracts on TBA-RS formed in raw, cooked and cooked and chilled burger patties is shown in Table 3. Significant effects (P<0.05) were found for the addition of berry extracts, the technological treatment and the interaction between berry×treatment. In general, TBA-RS numbers increased during cooking and the following refrigerated storage with the control patties being particularly affected. In fact, the highest TBA-RS numbers were found in control burger patties (P<0.05). Regardless of the treatment applied, burger patties with added berries (RC, CM, RU, AU) and quercetin showed significantly lower TBA-RS numbers than the control burger patties. In agreement with the loss of PUFA previously reported, the increase of TBA-RS during cooking of control patties (41%) was not as intense as the increase of TBA-RS observed during the subsequent chill storage (144%). The timely consistency between both measurements reflects that the oxidative degradation of PUFA during cooking and storage of cooked patties yielded MDA and other TBA-RS. According to these results, the cooking process moderately promoted lipid oxidation whereas 12 d of refrigerated storage had a greater impact on the development of lipid oxidation in cooked burger patties. As aforementioned, the acceleration of lipid oxidation during storage may be caused by a number of factors including the physico-chemical changes occurred during cooking which enhances the susceptibility of meat to undergo oxidative reactions. Iron is one of the main promoters of oxidative rancidity in meat and non-heme iron has been reported to be the active catalyst form in cooked meats because heating destroys heme iron and increase the level of non-heme iron during storage (Sato *et al*. 1973; Igene and Pearson 1979; Estévez and Cava 2004). The accumulation of MDA and other TBA-RS in muscle foods involves a straight loss of quality as most of these compounds contribute to the deterioration of colour and flavor of meat products (Morrissey *et al*. 1998).

Table 3 TBA-RS (mg MDA kg^{-1} patty) and volatile compounds (AAU) of raw, cooked and cooked and chilled burger patties with added berry extracts and quercetin

Fruit treatment	CT			RC			CM			RU			AU			Q			*P* value			
	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	Raw	Cooked	Cooked+ chilled	F	T	F×T	SEM
TBA-RS	0.312	0.439	1.071	0.067	0.082	0.143	0.104	0.095	0.181	0.083	0.085	0.082	0.071	0.075	0.113	0.095	0.129	0.205	0.000	0.000	0.000	0.031
Pentanal	3.4	95.6	101.6	0.1	10.5	23.3	0.0	8.7	29.9	nd	1.8	4.2	0.0	3.7	9.2	nd	1.7	4.7	0.000	0.000	0.000	3.622
Hexanal	251.1	1 081.3	1 279.7	10.9	150.7	356.5	8.4	142.5	467.0	9.7	29.3	57.3	5.3	66.5	148.2	19.8	37.4	78.5	0.000	0.000	0.000	42.966
Heptanal	6.1	44.7	55.3	1.7	8.9	26.7	0.5	10.6	35.3	0.3	3.8	7.0	0.3	7.1	11.4	0.3	4.3	9.0	0.000	0.000	0.000	1.925
(E) 2-Heptenal	3.3	2.4	2.7	nd	nd	nd	nd	nd	0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.000	0.050	0.001	0.126
Octanal	7.2	49.7	70.9	5.2	18.0	39.2	1.9	17.7	52.1	1.6	7.7	9.9	1.6	12.9	15.9	2.1	10.0	11.8	0.000	0.000	0.000	2.388
(E) 2-Octenal	4.4	3.1	4.9	nd	0.1	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.000	0.001	0.000	0.187
Nonanal	39.4	102.4	153.0	21.8	37.3	73.9	8.5	32.6	77.8	6.6	23.1	38.3	5.9	27.2	36.8	8.8	22.1	30.3	0.000	0.000	0.000	4.539
2-Nonenal	1.2	0.9	1.7	0.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.000	0.010	0.000	0.060
Decanal	2.0	4.6	5.4	3.3	4.8	5.4	2.1	4.1	5.0	1.2	4.0	3.9	1.2	3.9	4.1	1.3	3.6	3.6	0.008	0.000	0.977	0.196
(EE) 2,4-Nonadienal	0.4	0.2	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.000	0.262	0.132	0.018

nd, no detected.

In addition, some of them, such as MDA, have been recently highlighted as compounds with mutagenic and toxic potential (Del Rio *et al*. 2005). The intense oxidative reactions occurred during chilling storage of cooked meats challenge meat technologists to develop antioxidant strategies for muscle foods retailed as refrigerated pre-cooked meats.

The results obtained in the present study indicates that lipid oxidation was effectively retarded by RC, CM, RU, AU and Q in raw, cooked and cooked and chilled burger patties. Whereas the TBA-RS numbers were similar amongst treated patties (P>0.05), each batch behaved differently when patties were subjected the technological treatments. The TBA-RS numbers remained at low levels during cooking and chilling storage in burger patties containing RU while patties containing RC, AU and CM suffered a significant increase of TBA-RS (P<0.05) during chill storage of cooked patties. On the other hand, TBA-RS significantly increased in patties with added Q after cooking and the subsequent refrigerated storage (P<0.05). The ability of RC, CM, RU and AU (containing 1 175, 2 068, 495, and 428 mg of gallic acid equivalents 100 g^{-1} berry fresh matter, respectively; Ganhão *et al*. 2008) to inhibit TBA-RS formation derives from the presence of high amount of polyphenols with intense antioxidant activity. Since a number of phenolic compounds are present in these berries, the antioxidant potential demonstrated in the present study may also be due to the additive and synergistic effects of the individual compounds present. These results are in agreement with those obtained by many authors, that reported the effectiveness of natural antioxidants in controlling lipid oxidation in meat products (Fernández-López *et al*. 2003; Ahn *et al*. 2007; Estévez *et al*. 2007; Juntachote *et al*. 2007). In fact, in cooked and cooked and chilled burgers patties, TBA-RS numbers in burger patties with added Q were higher than those found in patties with added berries, although these differences were not significant (P>0.05). Taking into consideration the serious consequences of TBA-RS formation in muscle foods, the addition of these berry extracts could enhance the quality of cooked and chilled burger patties through the inhibition of oxidative reactions.

Volatile compounds

The analysis of volatiles in meat and meat products has reached high importance because of the interesting diversity of information provided by this type of analysis. In addition to the assessment of lipid oxidation through the analysis of lipid-derived volatiles, the volatile components of a muscle food reflect the appropriateness of the processing and/or storage and its aromatic profile (Estévez *et al*. 2003, 2004). The volatile compounds generated in porcine burger patties with added berry extracts after three different treatments (raw, cooked, and cooked and chilled) were analyzed. From the total volatile compounds isolated from the headspace of porcine burger patties, ten major lipid-derived aldehydes are shown in Table 3. Aldehydes are the most prominent volatiles produced during lipid oxidation and have been used to successfully follow lipid oxidation in meat or meat products and contribute to the overall off-flavours of oxidized meat (Yasuhara and Shibamoto 1990; Im *et al*. 2004). As for TBA-RS, there were significant effects of the berry, the processing treatment and the interaction of these two factors on the formation of most volatiles (P<0.05). Hexanal, nonanal and pentanal were the most abundant volatiles detected in all burgers patties whereas octanal and heptanal showed intermediates values (P<0.05). Amongst these straight-chain saturated aldehydes, hexanal, which is formed from the oxidation of n-6 unsaturated fatty acids (i.e., linoleic acid), indicates muscle lipid oxidation more effectively than any other volatile compound (Dupuy *et al*. 1987; Ajuyah *et al*. 1993; Shahidi and Pegg 1994). Some other minor volatiles such as (E) 2-heptenal, (E) 2-octenal, (E) 2-nonenal and (EE) 2,4-nonadienal are likely derived from the oxidative decomposition of PUFA, display low olfactory thresholds and are related to intense rancidity perception (Dupuy *et al*. 1987).

In accordance with the TBA-RS, the counts of most aldehydes such as pentanal, hexanal, heptanal, and nonanal significantly increased during cooking which denotes the enhancement of the oxidative reactions by high temperatures. In contrast with the TBA-RS, however, the increase of hexanal during cooking in control patties was more intense (431%) than that observed during chill storage of cooked patties (118%).

Whereas TBA-RS accumulate in patties during refrigerated storage, it is plausible to consider that volatiles generated during cooking and the subsequent storage, are released from the product. In addition, the analysis of volatiles using SPME is dependent on the absorption/desorption process of particular volatiles and that is, in turn, affected by the chemical properties of the volatiles and the physico-chemical properties of the food matrix (Kataoka *et al*. 2000). The cooking procedure may have influenced this desorption process and hence, the analysis of volatiles using the SPME. Therefore, the amount of hexanal isolated from the headspace of cooked and chilled patties might not precisely reflect the actual oxidation state of burger patties. Nevertheless, the overall results from the volatile compounds, and particularly hexanal, were highly consistent with those obtained from the TBA test. Raw control patties had significantly higher amounts of all volatiles, including hexanal compared to the berry-added counterparts. The oxidation occurred during the relatively mild processing applied to raw patties (blending, mincing, shaping, and so on was effectively diminished by fruit extracts. No significant differences (P>0.05) were detected amongst burger patties with added berry extracts, with the exception of AU and RU that had smaller amounts of nonanal than RC (P<0.05). The counts of most aldehydes such as pentanal, hexanal, heptanal, and nonanal significantly increased during cooking as a likely result of high temperatures. Cooked burger patties treated with berry extracts showed smaller amounts of volatiles than the control group. In agreement with the TBA-RS, cooking and chilling was the treatment with the greatest impact on the formation of lipid-derived volatiles. The amount of every single volatile aldehyde significantly (P<0.05) increased in all batches during chill storage of cooked patties. However, this increase was significantly higher in control burgers patties than in the berry-added counterparts. Amongst cooked and chilled patties, those with added RU and AU had the lower amounts of aldehydes such as hexanal, pentanal, heptanal, nonanal and octanal. Cooked and chilled patties with added RC and CM had intermediates amounts of these aldehydes. Compared to cooked and chilled patties with added berries, the control counterparts had from 3- to 10-fold times higher hexanal contents. There were also some minor volatile compounds that were only detected in the headspace of control patties, such as (E) 2-heptenal, (E) 2-octenal, (E) 2-nonenal, and (EE) 2,4-nonadienal. These compounds display very low odour threshold values (0.2 ppb) as compared to the aforementioned hexanal (58 ppb) (Devos *et al*. 1990) and therefore, may have a greater impact on odour. These minor compounds have been associated with the oxidative deterioration of PUFA and have been linked to unpleasant odours notes in cooked meat products. (EE) 2,4-Nonadienal, have been linked to unpleasant characteristics in cooked meat, with "rancid" odours. (E) 2-Nonenal derive from the oxidation of linoleic acid and has been related to "cardboard" like odour (Im *et al*. 2004). (E) 2-Octenal is also generated from PUFA decomposition and contributes with "tallowy" and "stale" notes (Im *et al*. 2004). A more intense degradation of PUFA in the control burger patties could explain the higher content of volatile compounds derived from those fatty acids. These volatile compounds had been related to strong lipid degradation due to the development of high oxidation phenomena (Estévez *et al*. 2003) and were probably related to WOF perception (Shahidi 1994; Ahn *et al*. 2002). WOF is mainly believed to be the result of oxidation of membrane phospholipids, a process triggered by hemoproteins and other iron species during cooking (Ingene and Pearson 1979; St. Angelo *et al*. 1987; Estévez *et al*. 2006). These results suggest a more intense development of a warmed over flavor during refrigeration of cooked burger patties than in patties with added berry extracts (Frankel 1984; Estévez *et al*. 2004; Im *et al*. 2004).

In fact, the addition of RC, CM, RU and AU extracts, significantly reduced the total amount of lipid-derived volatiles isolated from burger patties and inhibited the formation of some minor volatiles responsible of off-flavours. In this sense, the addition of these natural antioxidants might have reduced patties deterioration through the inhibition of the generation of certain lipid-derived volatiles and consequently the development of WOF and rancid aromatic notes. These results are consistent with those obtained from the TBA test. TBA-RS numbers and hexanal content correlated well over all treatments (R^2=0.90; P<0.05) and agree with those previously

reported (Ahn *et al*. 2002; Rey *et al*. 2005; Estévez *et al*. 2007; Juntachote *et al*. 2007).

CONCLUSION

The analysis of PUFA degradation together with the formation of TBA-RS and lipid-derived volatiles provides a precise and complete assessment of the intensity and consequences of lipid oxidation in burger patties subjected to cooking and chill storage. The results of the present study show for the first time in a cooked meat system that *A. unedo*, *C. monogyna*, *R. canina* and *R. ulmifolius* are effective antioxidants. The addition of extracts from these berries in porcine burger patties protects PUFA from oxidative degradation and inhibits the formation of TBA-RS and volatiles compounds. Using these fruit extracts as ingredients in burger patties may be an efficient strategy to enhance the nutritional value, safety and sensory traits of these meat products.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used for the present work were AAS grade and purchased from Panreac (Panreac Química, S. A., Barcelona, Spain), Merck (Merck, Darmstadt, Germany) and Sigma Chemicals (Sigma-Aldrich, Steinheim, Germany).

Berries

Samples of strawberry tree (*Arbutus unedo* L., AU), common hawthorn (*Crataegus monogyna* L., CM), dog rose (*Rosa canina* L., RC) and elm-leaf blackberry (*Rubus ulmifolius* Schott., RU) cultivars were collected at the stage of full ripeness in Cáceres Region, Spain (altitude=450 m) during the summer and autumn of 2007. After hand-harvest, the samples were immediately transferred to the laboratory, cleaned and sorted to eliminate damaged and shrivelled fruits and then frozen at -80°C.

Extraction of berry phenolics

Berries (30 g), including peel and pulp, were cut into pieces while the seeds were carefully removed. Berries were grounded, dispensed in a falcon tube and homogenized with 10 volumes (w/v) of absolute ethanol. The homogenates were centrifuged at 4 000 r min^{-1} for 10 min at 6°C. The supernatants were collected and the residue was re-extracted once more following the procedure previously described. The two supernatants were combined, evaporated using rotaevaporator and redissolved using 250 g of distilled water. Water solutions from each berry were prepared and stored in refrigeration until used for the manufacture of porcine burgers (less than 24 h) as described below. No insoluble fragments or residues were observed in the water solutions.

Manufacture of porcine burgers patties

The experimental burgers patties were prepared in a pilot plant. Six batches of porcine burger patties were prepared depending on the addition of different berry extracts (AU, CM, RC, RU) including negative (no added extract, CT) and positive control (added quercetin; 230 mg kg^{-1}, Q) groups. In the basic formulation, the ingredients per kg of patty were as follows: 725 g meat (porcine *longissimus dorsi* muscle), 250 g distilled water, and 25 g sodium chloride. In the formulation of the treated patties, the 250 g of distilled water were replaced by 250 g of a water solution containing the corresponding fruit extract or the quercetin. All ingredients were minced in cutter until a homogeneous raw batter was obtained. The meat temperature during processing did not exceed 12°C. Twelve burger patties per batch were prepared in two independent manufacturing processes (six patties per batch each time) in order to assure the repeatability of the experiment and the reliability of the data. Burger patties were formed using a conventional burger-maker (100 g/patty), to give average dimensions of 10 cm diameter and 1 cm thickness. Eight patties per batch were placed on trays and cooked at 170°C (temperature at the core controlled by thermometer) for 18 min in a forced-air oven. Preliminary cooking trials were performed to establish the cooking conditions required to achieve a meat core temperature of 73°C. Four cooked burger patties per batch were dispensed in polypropylene trays, wrapped with PVC film and subsequently stored for 12 d at +2°C in a refrigerator under white fluorescent light (620 lux), simulating retail display conditions. Therefore, all types of burger patties were divided into three groups depending on the processing applied: raw patties (n=4 per batch), cooked patties (n=4 per batch) and cooked and chilled patties (n=4 per batch). All burger patties were analyzed for their fatty acid composition, TBA-RS and volatile compounds. All types of samples were frozen (-80°C) until analytical experiments were carried out (less than 2 wk).

Cooking and storage losses

The cooking loss of burger patties was calculated as follows: Cooking loss (%)=$[(W_b-W_a)/W_b]\times100$, where W_b and W_a are

the weights of the burger patties before and after cooking, respectively. Storage loss was calculated as the weight loss during refrigerated storage of cooked burger patties as follows: Storage loss (%)=$[(W_c-W_{c+c})/W_c]\times100$, where W_c and W_{c+c} are the weight of the cooked and cooked and chilled burger patties, respectively.

Proximate composition of burger patties

Moisture, ashes and total protein contents were determined using official methods (AOAC 2000). The method of Folch *et al*. (1957) was used for determining fat content in burger patties.

Fatty acid composition

Fatty acid methyl esters (FAMEs) were prepared by acidic esterification in the presence of sulfuric acid following the method described by Sandler and Karo (1992). FAMEs were analyzed by gas chromatography using a Hewlett-Packard HP-5890A gas chromatograph, equipped with an on-column injector and a flame ionization detector, using a polyethyleneglycol capillary column (Supelcowax-10, Supelco, Bellefonte, PA) (60 m×0.32 mm i.d.×0.25 μm film thickness). Gas chromatograph oven program temperature was as follows: initial temperature of 190°C, 2°C min^{-1} to 235°C; 15 min at this temperature and thereafter 6°C min^{-1} to 250°C, and then kept for an additional 20 min. Injector and detector temperatures were 250°C. Carrier gas was helium at a flow rate of 0.8 mL min^{-1}. Individual FAME peaks were identified by comparison of their retention times with those of standards (Sigma, St. Louis, MO). Tridecanoic acid was used as internal standard. Results were expressed as grams per 100 g of detected FAMEs.

TBA-RS numbers

Lipid oxidation was determined in burger patties by the thiobarbituric acid-reactive substances (TBA-RS) assay using the distillation method of Tarladgis *et al*. (1960) with some modifications as follows. Briefly, 12 g of each burger patty was dispensed in cone plastic tubes and homogenized with 35 mL of 3.86% perchloric acid, using an Omni-mixer homogenizer for 1 min. The homogenate blended was centrifuged (3 000 r min^{-1} for 3 min) and filtered through Whatman nº 54 filter paper into a 50 mL Erlenmeyer flask and washed with perchloric acid. The filtrate was adjusted to 50 mL by adding perchloric acid (3.86%) and then the samples were distilled and the first 50 mL of distillate collected. Next, 2 mL aliquot of the distillate was mixed with 2 mL of 0.02 mol L^{-1} TBA in perchloric acid (3.86%) in test tubes (duplicate). The test tubes were vigorously vortexed and these together with the tubes from the standard curve were incubated at room temperature (24°C) in the dark for 20 h, in order to develop the colour reaction. All tubes test were centrifuged (3 000 r min^{-1} for 2 min) and the absorbance was measured at 532 nm using a Hitachi U-2000 spectrophotometer against a blank containing 2 mL of distillate water and 2 mL of TBA reagent. The results from the samples were plotted against a standard curve prepared with known concentrations of tetraethoxypropane (TEP). The recovery value of TEP was 76%. The results were expressed as TBA-RS numbers, mg malonodialdehyde (MDA) kg^{-1} burger patties.

Volatile compounds

Volatile compounds were analysed from the headspace of raw, cooked and cooked and chilled samples by using the solid-phase micro extraction (SPME) and gas chromatography/mass spectrometry (GC/MS). The method developed by Estévez *et al*. (2004) was employed with minor modifications as follows: The SPME fibre, coated with divinylbenzene-carboxenpoly (dimethylxilosane) (DVB/CAR/PDMS) 50/30 μm, was preconditioned prior to analysis at 220°C during 45 min. One gram of minced sample was placed in a 4 mL SPME vial and sealed with a silicone septum. The sample was allowed to equilibrate during 30 min while immersed in water at 37°C. During the extraction, the SPME fibre was inserted through the septum and exposed to the headspace of the vial. After extraction, the SPME fibre was immediately transferred to the injector of the chromatograph (HP5890GC series II gas chromatograph) which was in splitless mode at 220°C. Volatiles were separated using a 5% phenyl-95% dimethyl polysiloxane column (Restek, USA) (30 m×0.25 mm i.d., 1.05 m).

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References

Ahn J, Grün I U, Fernando L N. 2002. Antioxidant properties of natural plant extracts containing polyphenolic compounds in cooked ground beef. *Journal of Food Science*, **67**, 1364-1369.

Ahn J, Grün I U, Mustapha A. 2007. Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiology*, **24**, 7-14.

Aikawa K, Chikuni K. 1988. Antimutagenic effect of volatile decomposition products from thermally oxidized

linoleate. *Mutation Research*, **208**, 163-166.

Ajuyah A O, Fenton T W, Hardin R T, Sim J S. 1993. Measuring lipid oxidation volatiles in meats. *Journal of Food Science*, **58**, 270-273.

Andersen M L, Lauridsen R K, Skibsted L H. 2003. Optimising the use of phenolic compounds in foods. In: Johnson I T, ed., *Phytochemical Functional Foods.* Woodhead Publishing, Limited, Cambridge. pp. 315-346.

AOAC. 2000. *Official Methods of Analysis*. 17th ed. Association of Official Analytical Chemists Gaithersburgh, Md.

Bahorun B, Luximon-Ramma T, Crozier A, Aruoma A. 2004. Total phenol, flavonoid, proanthocyanidins and vitamin C levels and antioxidant activities of Mauritian vegetables. *Journal of Science and Food Agricultural*, **84**, 1553-1561.

Buckley D J, Morrissey P A, Gray J I. 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. *Journal of Animal Science*, **73**, 3122-3130.

Dall'Acqua S, Cervellati R, Loi M C, Innocenti G. 2008. Evaluation of *in vitro* antioxidant properties of some traditional Sardinian medicinal plants: investigation of the high antioxidant capacity of *Rubus ulmifolius*. *Food Chemistry*, **106**, 745-749.

Del Rio D, Stewart A J, Pellegrini N. 2005. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. *Nutrition, Metabolism & Cardiovascular Diseases*, **15**, 316-328.

Devos M, Patte F, Rouault J, Laffort P, van Gemert L J. 1990. *Standarized Human Olfactory Thresholds*. IRL Press, Oxford. p. 165.

Dupuy H P, Bailey M E, St Angelo A J, Legendre M G, Vercelotti J R. 1987. Instrumental analysis of volatiles related to warmed-over flavour of cooked meat. In: St Angelo A J, Bailey M E, eds., *Warmed over Flavour of Meat*. Academic Press, Orlando, FL. pp. 165-191.

Estévez M, Cava R. 2004. Lipid and protein oxidation, release of iron from heme molecule and colour deterioration during refrigerated storage of liver pâté. *Meat Science*, **68**, 551-558.

Estévez M, Morcuende D, Cava R. 2006. Extensively reared Iberian pigs versus intensively reared white pigs for the manufacture of frankfurters. *Meat Science*, **72**, 356-364.

Estévez M, Morcuende D, Ventanas S, Cava R. 2003. Analysis of volatiles in meat from Iberian pigs and lean pigs after refrigeration and cooking by using SPME-GC-MS. *Journal of Agricultural and Food Chemistry*, **51**, 3429-3435.

Estévez M, Ramírez R, Ventanas S, Cava R. 2007. Sage and rosemary essential oils versus BHT for the inhibition of lipid oxidative reactions in liver pâté. *Lebensmittel-Wissenschaft und-Technologie*, **40**, 58-65.

Estévez M, Ventanas S, Cava R. 2007. Oxidation of lipids and proteins in frankfurters with different fatty acid compositions and tocopherol and phenolic contents. *Food Chemistry*, **100**, 55-63.

Estévez M, Ventanas S, Ramírez R, Cava R. 2004. Analysis of volatiles in porcine liver pâtés with added sage and rosemary essential oils by using SPME-GC-MS. *Journal of Agricultural and Food Chemistry*, **52**, 5168-5174.

Ferguson L R. 2010. Meat and cancer. *Meat Science*, **84**, 308-313.

Fernández-López J, Sevilla L, Sayas-Barberá M E, Navarro C, Marín F, Pérez-Alvarez J A. 2003. Evaluation of the antioxidant potential of hyssop (*Hyssopus officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) extract in cooked pork meat. *Journal of Food Science*, **68**, 660-664.

Folch J, Lees M, Sloane-Stanley G H. 1957. A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry*, **226**, 497-509.

Frankel E. 1984. Lipid oxidation: mechanism, products and biological significance. *Journal of the American Oil Chemists' Society*, **61**, 1908-1917.

Ganhão R, Estévez M, Morcuende D. 2008. Antioxidant potential of eight selected Mediterranean wild fruits. *Alimentaria*, **11**, 132-139.

Gray J I, Gomaa E A, Buckley D J. 1996. Oxidative quality and shelf life of meats. *Meat Science*, **43**, 111-123.

Grün I U, Ahn J, Clarke A D, Lorenzen C L. 2006. Reducing oxidation of meat. *Food Technology*, **60**, 36-43.

Heinonen M. 2007. Antioxidant activity and antimicrobial effect of berry phenolics: a finnish perspective – Review. *Molecular Nutrition & Food Research*, **51**, 684-691.

Hellström J K, Törrönen A R, Mattila M H. 2009. Proanthocyanidins in common food products of plant origin. *Journal of Agricultural and Food Chemistry*, **57**, 7899-7906.

Higgs J D. 2000. The changing nature of red meat: 20 years of improving nutritional quality. *Trends in Food Science and Technology*, **11**, 85-95.

Igene J O, Pearson A M. 1979. Role of phospholipids and triglycerides in warmed-over flavor development in meat model systems. *Journal of Food Science*, **44**, 1285-1290.

Im S, Hayakawa F, Kurata T. 2004. Identification and sensory evaluation of volatile compounds in oxidized porcine liver. *Journal of Agricultural and Food Chemistry*, **52**, 300-305.

Juntachote T, Berghofer E, Siebenhandl S, Bauer F. 2007. The effect of dried galangal powder and its ethanolic extracts on oxidative stability in cooked ground pork. *Lebensmittel-Wissenschaft und-Technologie*, **40**, 324-330.

Kahkonen M P, Hopia A I, Vuorela H J, Rauha J P, Pihlaja K, Kujala T S, Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, **47**, 3954-3962.

Kataoka H, Lord H L, Pawliszyn J. 2000. Applications of solid-phase microextraction in food analysis. *Journal of Chromatography* (A), **880**, 35-62.

Kristensen L, Purslow P P. 2001. The effect of processing temperature and addition of mono- and di-valent salts on the heme-nonheme-iron ratio in meat. *Food Chemistry*, **73**, 433-439.

Ladikos D, Lougovois V. 1990. Lipid oxidation in muscle foods: a review. *Food Chemistry*, **35**, 295-314.

Lee S K, Mei L, Decker E A. 1996. Lipid oxidation in cooked turkey as affected by added antioxidant enzymes. *Journal of Food Science*, **61**, 726-728.

Morrissey P A, Sheehy P J A, Galvin K, Kerry J P, Buckley D J. 1998. Lipid stability in meat and meat products. *Meat Science*, **49**, S73-S86.

Okuyama H, Ikemoto A. 1999. Needs to modify the fatty acids composition of meat for human health. In: *Proceedings of the 45th ICOMST*. vol. II, Yokohama, Japan. pp. 638-640.

Pikul J, Lesczynski D E, Kummerow F A. 1984. Relative role of phospholipids, triacylglycerols, and cholesterol esters on malonaldehyde formation in fat extracted from chicken meat. *Journal of Food Science*, **49**, 533-538.

Rey A I, Hopia A, Kivikari R, Kahkonen M. 2005. Use of natural food/plant extracts: cloudberry (*Rubus chamaemorus*), beetroot (*Beta vulgaris* "vulgaris") or willow herb (*Epilobium angustifolium*) to reduce lipid oxidation of cooked pork patties. *Lebensmittel-Wissenschaft und-Technologie*, **38**, 363-370.

Rice-Evans C A, Miller N J, Paganga G. 1997. Antioxidant properties of phenolic compounds. Review. *Trends in Plant Science*, **2**, 152-159.

Sandler S, Karo W. 1992. *Sourcebook of Advanced Organic Laboratory Preparations*. Academic Press, San Diego.

Sato K, Hegarty G R, Herring H K. 1973. The inhibition of warmed-over flavor in cooked meats. *Journal of Food Science*, **38**, 398-403.

Shahidi F. 1994. Flavor of meat and meat products-an overview. In: Shahidi F, ed., *Flavor Of Meat and Meat Products*. Chapman and Hall, Cornwall, UK. pp. 1-3.

Shahidi F, Pegg R B. 1994. Hexanal as an indicator of meat flavor deterioration. *Journal of Food Lipids*, **1**, 177-186.

St. Angelo A J, Vercellotti J R, Legendre M G, Vinnett C H, Kuan J W, James Jr C. 1987. Chemical and instrumental analyses of warmed-over flavor in beef. *Journal of Food Science*, **52**, 1163-1168.

St. Angelo A J, Crippen K L, Dupuy H P, James Jr C. 1990. Chemical and sensory studies of antioxidant-treated beef. *Journal of Food Science*, **55**, 1501-1505.

Tarladgis B G, Watts B M, Younathan M T, Dugan Jr L R. 1960. A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of American Oil Chemistry Society*, **37**, 44-48.

Valsta L M, Tapanainen H, Männistö S. 2005. Meat fats in nutrition. *Meat Science*, **70**, 525-530.

Verbeke W, van Oeckel M J, Warnants N, Viaene J, Boucqué C V. 1999. Consumer perceptions, facts and possibilities to improve acceptability of health and sensory characteristics of pork. *Meat Science*, **53**, 77-99.

Vuorela S, Salminen H, Makela M, Kivikari R, Karonen M, Heinonen M. 2005. Effect of plant phenolics on protein and lipid oxidation in cooked pork meat patties. *Journal of Agricultural and Food Chemistry*, **53**, 8492-8497.

Yasuhara A, Shibamoto T. 1990. Headspace volatiles from heated pork. *Food Chemistry*, **37**, 13-20.

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