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Inhibition of lipid oxidation and dynamics of polyphenol content in mechanically deboned meat supplemented with sea buckthorn (*Hippophae rhamnoides*) berry residues

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Abstract

Mechanically deboned meat (MDM) contains about 10 times more polyunsaturated fatty acids (PUFAs) and also more hemoproteins than hand deboned meat (HDM) and is essentially more susceptible to both chemical and biochemical oxidation. The oxidation, leading to the formation of potentially mutagenic and carcinogenic derivatives of PUFAs, can be inhibited by berry extracts rich in antioxidant polyphenols. Using the 2-thiobarbituric acid reactive substances (TBARS) method, we have established that the ethanol slurry of the juice-free solid residue of sea buckthorn (*Hippophae rhamnoides* – SB) berries inhibits oxidation of unsaturated fatty acids, of both chicken and turkey MDM. The polyphenols, mainly flavonols, responsible for this inhibition, are comparatively stable during short-term cooking and 6-day storage of cooked SB-MDMs at +6 °C. About half of the polyphenols are lost, obviously oxidised, during the storage of the uncooked samples of turkey 2%SB-MDM at +6 °C. The loss of polyphenols is much smaller in the case of chicken MDM, which is characterised by an *in situ* lower content of fatty acids, including the polyunsaturated ones. The liquid chromatography–diode array detection–tandem mass spectrometry (LC–DAD–ESI-MS/MS) method was used for identification and ranking of the potent polyphenolic antioxidants in the berry residue.
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1. Introduction

Health-promoting functional foods, prepared on the basis of meat, are becoming more and more popular. Mechanically deboned (or recovered) meat (MDM) is economically substantiated as a raw material for preparation of these products. The main potential health problems accompanied with consumption of MDM are an elevated risk of bacterial intoxication and higher ingested doses of (per)oxidated, potentially mutagenic and carcinogenic fatty acids in comparison with use of hand deboned meat (HDM). Oxygen which is bound to the meat mass and enzymes as well as heme released due to extensive stress and aeration during mechanical grinding catalyse peroxidation of the polyunsaturated fatty acids (PUFA) and hence accelerate the oxidative deterioration of the MDM, especially of turkey origin (Dawson & Gartner, 1983). As a result of the inclusion of the bone marrow, there is also a higher content of cholesterol and phospholipids in MDM (Al-Najdawi & Abdullah, 2002) than in respective hand deboned meat (HDM). Polyphenols contained in plant supplements may help to reduce these adverse effects, contributing an extra antibacterial as well as antioxidant capacity to the MDM (Mielnik, Aaby, & Skrede, 2003).

A number of papers have been published reporting the results of the investigation of the effect of plant poly-

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phenols on lipid oxidation, measuring the change in concentration of 2-thiobarbituric acid reactive substances (TBARS), derived from PUFAs, during long-term storage at negative temperatures of either MDM (Mielnik et al., 2003; Raghavan, & Richards, 2006) or more often different kinds of HDM (Rey, Hopia, Kivikari, & Kahkonen, 2005; Sallam, Ishioroshi, & Samejima, 2004; Sebranek, Sewalt, Robbins, & Houser, 2005; Tang, Kerry, Sheehan, & Buckley, 2002). Very little data is available concerning the effect of plant polyphenol extracts on the quality of MDM, stored at refrigeration temperatures (Hassan & Fan, 2005). Several groups have been working with other meat products supplemented with plant extracts and stored at low positive temperatures. Inhibition of lipid oxidation, in cooked chicken slurries, by using several plant extracts containing polyphenols has been studied during 9 days at +4 °C, by Beltran, Pla, Yuste, and Mor-Mur (2004). The protective effect of ground rosemary leaves on minced, pressure-processed chicken meat was studied during 9 days at +5 °C by Bragagnolo, Danielsen, and Skibsted (2005). The same authors have recently studied the mechanism of this effect, after cooking of meat at different temperatures, by electron spin resonance (ESR) and electrochemical detection of oxygen consumption (Bragagnolo, Danielsen, & Skibsted, 2007).

The antioxidant and antibacterial activities of several plant extracts on beef meatballs, during 12 days at +8 °C have been studied by Fernandez-Lopez, Zhi, Aleson-Carbonell, Perez-Alvarez, and Kuri (2005). Mitsumoto and co-workers have studied the behaviour of raw beef and chicken patties during 7 days at +4 °C (Mitsumoto, O'Grady, Kerry, & Buckley, 2005). O'Sullivan and co-workers have studied the antioxidant potential of a number of natural ingredients in chicken patties during 9 days at +4 °C (O'Sullivan, Lynch, Lynch, Buckley, & Kerry, 2004). According to our knowledge, no data is available concerning the actual persistence of added polyphenols during cooking and storage, of either MDM or HDM compositions at any temperatures.

Sea buckthorn (SB – *Hippophae rhamnoides*) berries are a well-known source of polyphenols and vitamins and they contain large amounts of the potent antioxidant quercetin, as well as other flavonols in various glycosidic forms (Guliyev, Gul, & Yildrim, 2004; Rösch, Bergmann, Knorr, & Kroh, 2003).

The aim of the present contribution was to study the stability of chicken (MDCM) and turkey (MDTM) mechanically deboned meat, supplemented with 1, 2 or 4% of dried sea buckthorn berry powder, in the course of short-term cooking and 6-day storage at +6 °C. Special attention was paid to determination of the most important polyphenol antioxidants and to the establishment of their stability, during cooking and storage of the MDM compositions. The lipid oxidation in the mixtures was estimated by a TBARS-assay and the polyphenols were analysed by liquid chromatography–diode array detection–tandem mass spectrometry (LC–DAD–ESI-MS/MS).

2. Materials and methods

2.1. Raw materials and chemicals

MDCM was prepared in Tallegg AS, Estonia, from broiler skeleton, by a bone press Weiler Beehiw PSTD 06 and the MDTM was obtained from Atria OY, Finland, as frozen in 20 kg blocks. The blocks were sawed into portions of about 1 kg and stored at −40 °C until used. The HDCM (chicken breast) was obtained from Tallegg AS, Estonia. The solid juice-depleted residue of sea buckthorn (mixed cultivars) berries powder, dried at +40 °C, was obtained from Tervix OÜ, Estonia.

Solid phase extraction (SPE) cartridges Accubond II ODS-C18, 100 mg, 1 ml were purchased from Agilent Technologies, 2-thiobarbituric acid (TBA), *t*-butyl hydroxytoluene (BHT, min 99%) and fatty acid methyl ester standards were purchased from Sigma, perchloric acid (*puriss p.a.*) from Riedel de Haën, tetraethoxypropane (TEP, *purum*) and formic acid (*puriss p.a.*) from Fluka, methanol and acetonitrile (Ultra gradient grade) from Romil, hexane (Ultra-Residue analysed) and chloroform was purchased from Baker.

2.2. Fatty acid content of the meats studied

Fatty acids were extracted from the meat samples by the method of Folch, Lees, and Sloane-Stanley (1957) and quantified by gas chromatography (GC), according to Christie (1989). Briefly, 10 ml of chloroform:methanol (2:1) was added to the aliquot of a MDM or HDM (0.1 or 1.0 g) and homogenised. After addition of 2.0 ml of water, the chloroform layer was separated and the solvent evaporated, then 1 ml of toluene and 2 ml of 1% sulphuric acid in methanol were added. The mixture was left overnight at 50 °C, then 5 ml of 5% sodium chloride in water was added and the fatty acid methyl esters formed were extracted with hexane (2.5 ml) and quantified by GC using Agilent 6890N Gas Chromatograph, equipped with a flame ionisation detector. Separation of fatty acid methyl esters was performed at capillary fused silica column Carbowax 30 m × 0.25 mm at 180–210 °C. Peak identification was carried out by comparison with the authentic standards.

2.3. SB-MDM samples preparation

The solid residue of SB berries was, after removal of the juice by pressing, dried at 40 °C, stored in polyethylene bags at room temperature, in the absence of light, and milled shortly before usage. A 1 kg portion of frozen chicken or turkey MDM was thawed at +6 °C overnight and 2.4, 4.8 or 9.6 g of berry residue, macerated during 20 h in 15 ml ethanol, was added to 240 g of MDM by mixing during 1 min. The products were divided into two approximately equal parts, one of them was cooked for 3 min in a microwave oven at 800 W. The first analytical samples were taken immediately and both the raw and

cooked SB-MDM compositions were stored for 6 days at a temperature of +6 °C. The initial moisture content, 68.8 g/100 g, was reduced to 60.1 g/100 g in the course of cooking. Alteration of the moisture content during the following sample storage was insignificant. Reduction of the water content, during cooking, was taken into account when integrating the areas under the curves (Table 2), extracted ion chromatogram (EIC) peak relative heights (Table 3) and the construction of Figs. 2 and 4. Every experiment was repeated twice and two secondary samples were taken, from every sample, for polyphenol analysis.

2.4. Assay of thiobarbituric acid reactive substances (TBARS)

The modified extraction method of Esterbauer and Cheeseman (1990) was used. Ten grams of a MDM or HDCM composition were homogenised, during 1 min at 10,000 rpm in 40 ml of 4% perchloric acid, containing 90 mg/100 g BHT (0.5 ml 7.2% solution of BHT) to retard the oxidative processes. The mixture which was obtained was filtrated through a glassfibre filter. Five millilitres of the filtrate and 5 ml of 0.02 M 2-thiobarbituric acid solution were pipetted into the test tubes, which were closed with stoppers and heated using a water bath at 80 ± 0.2 °C, during 1 h. The tubes were cooled in a cold water bath for 1 min and the absorption of the solution was determined using a spectrophotometer Specord 200 (Analytik Jena, Germany), at $\lambda = 532$ nm. Respective quantities of malonic dialdehyde (mg/kg of sample) were calculated using calibration curves, obtained with standard compound 1,1,3,3-tetraethoxypropane (TEP). The analysis of every item was repeated twice.

2.5. Determination of polyphenol content by HPLC–DAD–ESI-MS/MS

Sample preparation (Weisburger et al., 2002): 2.00 ± 0.01 g of SB-MDM was extracted with 4 ml of methanol by shaking for 30 min and then centrifuged, at 20 °C, using a cooling centrifuge Eppendorf 5810R equipped with a swinging bucket rotor for 10 min at 978*g*. The supernatant was treated twice with 2 ml of hexane and the methanol layer was passed through a 100 mg reversed phase SPE column and kept at −40 °C until analysed.

For chromatographic analysis of the SB polyphenols, a tandem liquid chromatography–diode array–mass spectrometry (LC–DAD–ESI-MS/MS) method was developed. For separation of compounds, a Zorbax 300SB-C18 column (2.1 × 150 mm; 5 μm) was used, in a stepwise mobile phase gradient of 0.1% formic acid (solvent A) and acetonitrile (solvent B), at velocity 0.3 ml/min at 35 °C. The sample injection volume was 15 μl. For detection and quantitation of substances, the Agilent 1100 Series UV–Vis diode array detector (DAD) and LC/MSD Trap-XCT with an electrospray (ESI) interface were connected to an Agilent 1100 Series HPLC instrument, consisting of an autosampler, solvent membrane degasser, binary pump and column thermostat. The HPLC 2 D ChemStation Software with a ChemStation Spectral SW module was used for the process guidance. The conditions of MS/MS detection: m/z interval 50–1000 in negative ionisation mode; target mass – 400; number of fragmented ions – 2; maximal accumulation time – 100 ms; compound stability – 100%; drying gas (N_2 from generator) flow rate 10 l/min, gas temperature 350 °C; nebulizer pressure 30 psi, collision gas He pressure 6×10^{-6} mbar.

DAD was working at the interval 200–600 nm and the eluate optical density was continuously monitored at wavelengths 250 (phenolic acids), 280 (flavanols), 306 (*trans*-stilbenes) and 370 (flavonols) nm.

3. Results and discussion

3.1. Fatty acid composition of the original MDM-s

The MDMs studied contained, in comparison with HDM, up to 10 times higher quantities of PUFAs, mainly linoleic (*cis*,*cis*-9,12-octadecadienoic) acid (see Table 1). PUFAs are excellent substrates for lipid peroxidation because of the presence of active bis-allylic methylene groups in their molecules. The carbon–hydrogen bonds of these activated methylene units have lower bond dissociation energies, making these hydrogen atoms more easily abstractable in the radical reactions. The susceptibility of PUFAs toward peroxidation increases with an increase in the number of unsaturated sites in the hydrocarbon chain of the fatty acids. Although the main target of oxidation in MDMs is linoleic acid with two double bonds, oxidation of arachidonic (all-*cis*-5,8,11,14-eicosatetraenoic) acid containing four double bonds also makes a weighty contribution to the summary peroxidation process of PUFAs.

3.2. Thiobarbituric acid reactive substances (TBARS)

The dynamics of TBARS values (mg/kg), indicating to malonic dialdehyde (MDA) formation via oxidation of unsaturated fatty acids, during both microwaving and storage of MDCMs is illustrated in Fig. 1.

It can be stated that during cooking of MDCM a remarkable oxidation of fatty acids with formation of MDA already takes place. This oxidation is slightly

Table 1
Fatty acid composition of the meats studied (FAs, fatty acids; MUFAs, monounsaturated and PUFAs, polyunsaturated fatty acids)

Type of meat	MDCM	MDTM	HDCM
Total fat (g/100 g of meat)	14.7	20.3	1.4
% of various fatty acids			
Saturated FAs	29.7	31.2	28.4
MUFAs	48.6	47.2	42.5
PUFAs,	21.5	21.3	29.2
Linoleic acid	17.8	18.4	24.3
α-Linolenic acid	2.7	2.3	1.3
Arachidonic acid	0.4	0.3	1.8

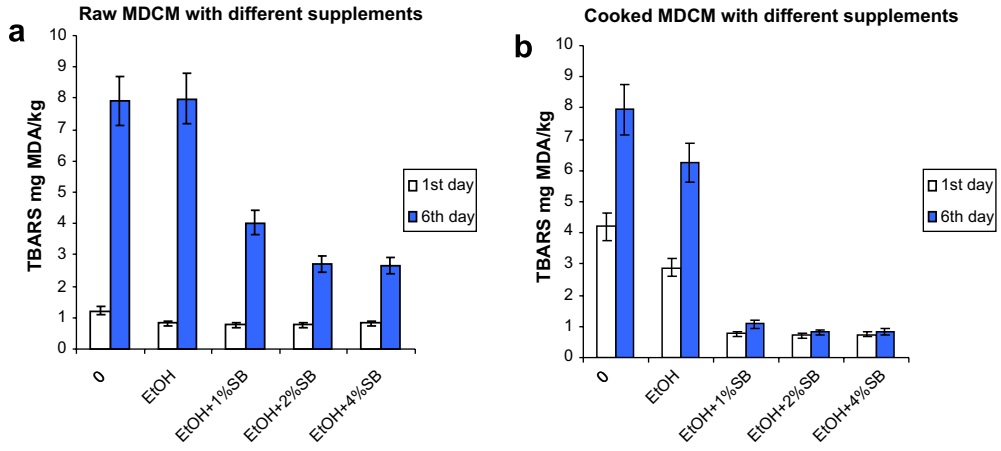

Fig. 1. Dynamics of TBARS values in the course of aging of raw and cooked MDCMs at +6 °C. EtOH = ethanol.

retarded by the added ethanol but completely stopped by the SB extracts, starting from the lowest studied level (1%) of the supplement (see Fig. 1b). Ethanol has no effect on the oxidation of the raw MDCM, this process is again remarkably, but in this case not entirely, inhibited by the SB supplements (Fig. 1a).

MDTM, used for the experiments, was although still officially utilisable and without any obvious signs of putrefaction, extensively and heterogenously oxidised, with TBARS numbers in the interval of 3–5 mg MDA/kg. Nevertheless, it was decided to use just this MDTM as a proper matrix to reveal whether organoleptically allowable, relatively low amounts of plant antioxidant that change neither the texture nor taste of the meat composition, can manage with a high initial oxidative potential of the lipid material.

3.3. Stability of SB polyphenols in the compositions

A functional food is capable of causing desired health effects only in the case of containing the health-promoting compounds in the final (ready-for-eating) product, after a definite storage. Sea buckthorn berries contain large amounts of flavonol glycosides, characterised by a specific UV absorption maximum around $\lambda = 370$ nm as the main group of polyphenols (Guliyev et al., 2004). Using tandem UV–Vis and MS/MS detection, 11 major polyphenols, all flavonol glucosides, were identified in the SB-MDM samples (see Fig. 2 and Table 3). Since the method used does not allow determination of the exact location of a glycosidic group in the aglycone molecule, the position numbers are indicated only for the glycosides identified earlier in sea buckthorn berries (Rösch et al., 2003). Example UV-chromatograms at 370 nm of the raw 2% SB-MDTM and SB-MDCM, before and after 6-day storage, are presented in Fig. 2. As an approximate measure of the total flavonol content, S_{370}, the area under the HPLC chromatogram (AUC) between the 7th and 19th minutes at wavelength 370 nm (see Fig. 2), which is the sum of the areas of peaks of individual flavonols, can be taken (see Table 2). An obvious correlation between the AUC numbers and the initial concentration of the SB in the respective SM-MDMs should be noted. Pearson's r value is −0.83 and −0.96 for the linear correlation between percentage (1, 2 and 4) of SB supplement in MDM and the $\Delta S_{370\,nm}$ values in the case of raw and cooked HDCM, respectively. It shows a sufficient quality of the sample preparation as well as the analytical process in total.

The numbers in Table 2 indicate that the total flavonol glycoside content in SB-MDMs is reduced in average by 9.5% during cooking and by 50.8% and 21.4% during storage of uncooked SB-MDTM and SB-MDM samples, respectively. The content of polyphenols is only slightly reduced in the course of storage of the cooked samples.

Various identified flavonol glucosides behave differently during the storage of uncooked SB-MDTM (see Fig. 3). Derivatives of isorhamnetin (except rhamnoside) and kaempferol are decidedly more persistent than quercetin derivatives (see Table 3 and Fig. 4).

It has been published that plant polyphenols, like quercetin, are rather stable during short-term cooking or microwaving (Liazid, Palma, Brigui, & Barroso, 2007; Lombard, Peffley, Geoffriau, Thompson, & Herring, 2005). Our results confirm these findings (see Tables 2 and 3).

A hypothesis can be set up that the different stability of various flavonol glycosides during storage is mostly caused by differences in their antioxidative capacities (free radical scavenging abilities). More potent catchers of free radicals must also have been more completely consumed during both microwaving and storage. Rösch with co-authors has shown that the antioxidant capacities of flavonol glycosides depend first of all on the availability of free OH-groups (*o*-diphenolic arrangement) in the positions 3′ and 4′ of the aglycone molecule, enabling formation during oxidation of *o*-quinonic moiety, stabilised by conjugation (Rösch et al., 2003). This situation is realised in the molecule of quercetin and its identified glycosides but not in the case of derivatives of isorhamnetin and kaempferol. The second site where the *o*-diphenolic moiety can be

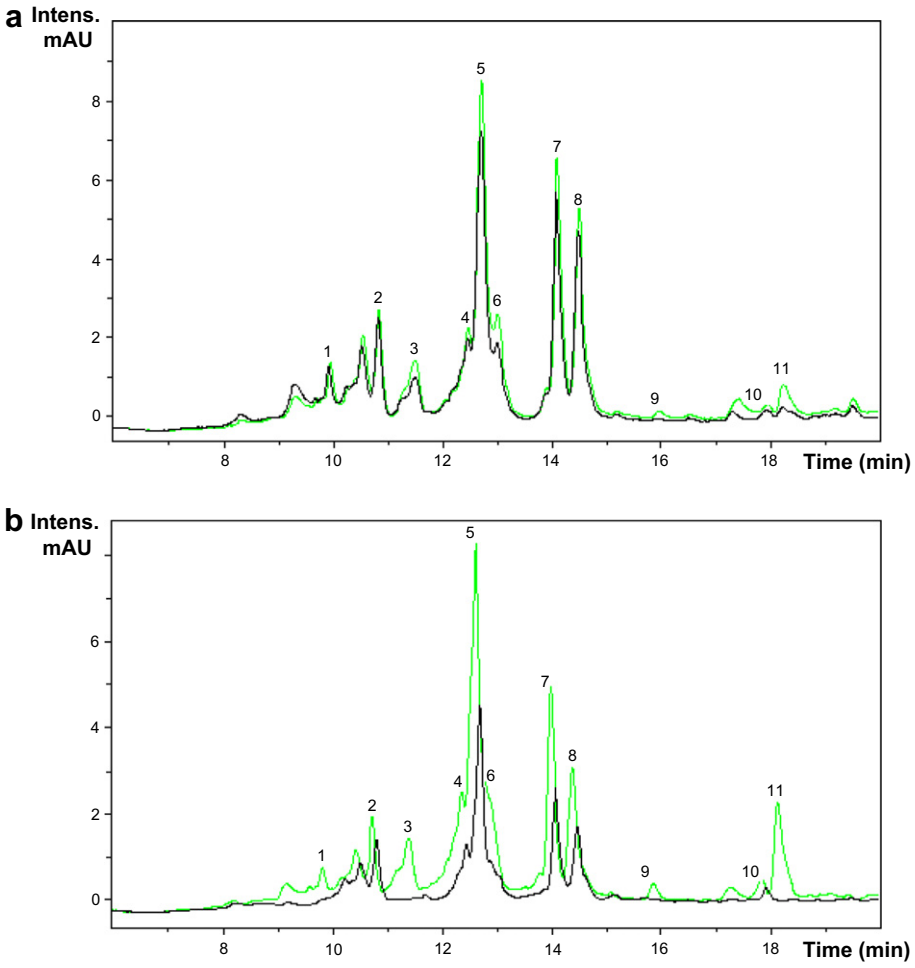

Fig. 2. HPLC–UV chromatogram of the extracts of raw 2% SB-MDCM (a) and SB-MDTM (b) at $\lambda = 370$ nm at 1st (light line) and 6th day (dark line) days of storage at +6 °C. For the names of polyphenols behind the peak numbers, see Table 3.

Table 2
Areas under the curves of HPLC–DAD chromatograms ($\lambda = 370$ nm) of raw and cooked SB-MDMs on the 1st day and after storage during 6 days

Type of meat	% of SB	Raw		Cooked	
		1st day	6th day	1st day	6th day
MDTM	1	242 ± 30	109 ± 18	227 ± 15	183 ± 10
	2	551 ± 42	231 ± 32	463 ± 33	396 ± 32
	4	1073 ± 88	651 ± 43	973 ± 40	884 ± 45
MDCM	1	291 ± 30	190 ± 29	239 ± 15	247 ± 17
	2	589 ± 44	500 ± 37	584 ± 32	474 ± 30
	4	1227 ± 75	1051 ± 55	1147 ± 50	983 ± 40
HDCM	1	316 ± 25	372 ± 30	[a]	[a]
	2	692 ± 48	669 ± 50	[a]	[a]

[a] Not estimated.

R_1	R_2	Compound
H	H	Kaempferol
OH	H	Quercetin
OMe	H	Isorhamnetin

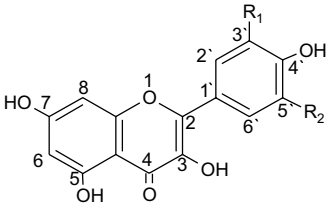

Fig. 3. Structure of flavonols.

formed during oxidation is around positions 3 and 4, provided that the OH-group in position 3 is free (non-substituted). This is the situation with quercetin-7-rhamnoside, that is indeed the most consumed during oxidation of both the MDTM and MDCM quercetin derivative. As it can be seen in Table 4, among isorhamnetin and kaempferol glycosides this situation is realised in the case of isorhamnetin-7-rhamnoside, which has the highest antioxidant capacity among isorhamnetin derivatives (Rösch et al., 2003) and which is also clearly the most depleted non-quercetin flavonol in the case of MDTM in our investigation. The generally smaller disappearance of flavonol derivatives during aging of cooked rather than uncooked MDMs demonstrates that the oxidation process of meat unsaturated fatty acids is a combination of both enzymatic (including pseudoenzymes like hemoproteins) and non-enzymatic reactions. These results are in harmony with the data obtained by Rösch and co-authors and prove our initial hypothesis.

Table 3
Mass data and relative heights of the EIC peaks of the main identified polyphenols determined in 2% SB-MDTM and SB-MDCM (amu – atom mass units)

Comp. no. in Fig. 2	Flavonol *O*-glucoside	$[M-H]^-$ amu	Main daughter ions (in the order of decrease of intensity)	EIC-peak relative height (%)[a]			
				Raw 6th day	Cooked, 1st day	Cooked, 6th day	Cooked 6th day/cooked 1st day[b]
MDTM, quercetin-							
1	-Diglucoside-rhamnoside	771	625; 301; 300; 446; 445; 463	0	97	68	70
3	-Glucoside-rhamnoside	609	447; 301; 463; 519	0	97	50	52
5	-3-Rutinoside	609	301; 271; 344; 255	0	108	40	37
6	-3-Glucoside	463	301; 306; 300; 151; 179	45	84	43	52
9	-7-Rhamnoside	447	301; 151; 427; 177; 285	0	119	0	0
MDTM, isorhamnetin-							
2	-Diglucoside-rhamnoside	785	639; 315; 610; 623; 733	66	92	90	98
4	-3-Glucoside-7-rhamnoside	623	477; 461; 315; 300	71	72	78	100
7	-3-Rutinoside	623	315; 300; 271; 255	68	90	82	91
8	-3-Glucoside	477	314; 315; 271; 285; 357	48	80	80	100
11	-7-Rhamnoside	461	315; 446; 300; 151; 287		100	0	0
MDTM, kaempferol-							
10	-Glucoside-rhamnoside	593	285; 447; 307	80	95	87	92
MDCM, quercetin-							
1	-Diglucoside-rhamnoside	771	625; 301; 300; 446; 445; 463	85	89	104	100
3	-Glucoside-rhamnoside	609	447; 301; 463; 519	90	83	80	96
5	-3-Rutinoside	609	301; 271; 344; 255	55	71	55	77
6	-3-Glucoside	463	301; 306; 300; 151; 179	57	77	74	96
9	-7-Rhamnoside	447	301; 151; 427; 177; 285	47	106	92	87
MDCM, isorhamnetin-							
2	-Diglucoside-rhamnoside	785	639; 315; 610; 623; 733	86	96	70	73
4	-3-Glucoside-7-rhamnoside	623	477; 461; 315; 300	96	78	79	100
7	-3-Rutinoside	623	315; 300; 271; 255	76	67	77	100
8	-3-Glucoside	477	314; 315; 271; 285; 357	84	92	68	74
11	-7-Rhamnoside	461	315; 446; 300; 151; 287	46	89	94	100
MDCM, kaempferol-							
10	-Glucoside-rhamnoside	593	285; 447; 307	97	122	121	100

[a] The height of respective polyphenol peak in the raw meat at the 1st day is taken as 100%.
[b] Per cent of the particular polyphenol preserved during storage of cooked MDM.

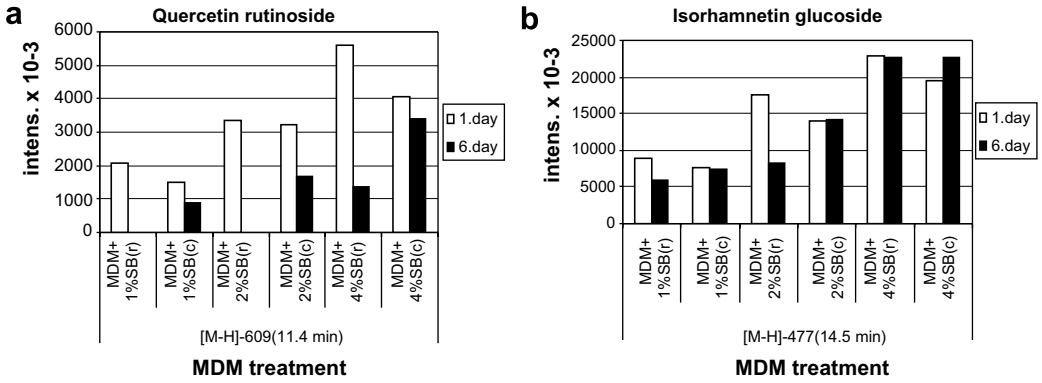

Fig. 4. Comparison of concentration dynamics of two selected flavonol glucosides in the course of 6-day storage of SB-MDTM (r – raw, c – cooked). Numbers on the *y*-axis are the intensities of the respective EIC peaks.

Table 4
Results of the correlation analysis

Parameter	ΔΔTBARS of MDCM	
	Raw	Cooked
SB supplement percentage	0.82*	0.71*
$\Delta S_{370\,nm}$	−0.75*	−0.80*
ΔC quercetin rutinoside	−0.05	0.26
ΔC isorhamnetin rutinoside	−0.69	−0.37
ΔC isorhamnetin glucoside	0.83*	−0.35

Pearson's critical *r* value at the significance level of 95% ($p = 0.05$) is 0.707. Statistically significant correlation coefficients at the significance level of 95% ($p = 0.05$) are marked with an asterisk.

In Fig. 4, the concentration dynamics of two polyphenols from Table 3 – quercetin rutinoside as an efficient oxidation inhibitor and isorhamnetin glucoside as a relatively poor oxidation inhibitor (antioxidant) are compared.

To assess the role of SB polyphenols in the inhibition of fatty acid oxidation, a correlation analysis between differences in changes in TBARS values (ΔΔTBARS), in the case of MDM without supplement, and at three different supplement concentrations and four parameters, reflecting the sea buckthorn composition, was performed (see Table 4). ΔΔTBARS corresponds to the part of ΔTBARS reduced by the SB supplement during MDM storage at +6 °C. For one of those parameters the decrease of total flavonol content expressed in the units of $\Delta S_{370\,nm}$ was chosen and for the other three changes in concentration (ΔC), of the three initially most abundant flavonols, was used.

Table 4 shows that the summary antioxidant effect of SB supplement is obviously a combination of effects of the various flavonol glycosides. There is a statistically relevant correlation between the decrease of the summary flavonol concentration, expressed in the units of $\Delta S_{370\,nm}$, and ΔΔTBARS in the case of both raw and cooked MDCM. None of the main three polyphenols has a statistically proved antioxidant effect on cooked MDCM but the ΔC value of isorhamnetin glucoside is satisfactorily correlated with ΔΔTBARS values, in the case of uncooked MDCM.

It has been shown that quercetin is oxidised to the dimer and trimer by heme-containing proteins like hemoglobin (Cherviakovsky et al., 2006). Analogically, isorhamnetin and its glycosides can be oxidatively oligomerised. Since MDMs have a sufficiently higher concentration of hemoproteins than HDM, it may happen also in the case of MDM. At the extracted ion (EIC) chromatograms of oxidised MDMs, ultralow peaks belonging to quercetin oxydimer ($[M-H]^- = 601$), isorhamnetin oxydimer ($[M-H]^- = 629$), and isorhamnetin rhamnoside oxydimer ($[M-H]^- = 921$) were recognised. These new compounds can still exert, although lower, antioxidant properties. In a chemically complicated matrix like meat, numerous other reaction pathways as well as formation of other so far unknown oxidation products is possible.

The concentration of 2% is probably optimal for the sea buckthorn supplement in both chicken and even in the highly oxidated turkey MDM. A lower content of polyphenols is not sufficient to guarantee complete inhibition of the fatty acid oxidation and leaving of part of the added antioxidant polyphenols still in the composition. A still higher content of the plant material may reduce the organoleptic properties of the patties made from the MDM.

Besides polyphenols, berries of SB contain tocopherols and carotenoids, that may have their own important role in the antioxidant capacity of the mixtures.

4. Conclusion

The significantly lower increase of TBARS values during cooking and storage of uncooked MDMs, in the presence of buckthorn berry residue, demonstrates inhibition of the oxidation of unsaturated fatty acids in the MDMs. The SB-residue almost stops the time-dependent oxidation of cooked MDMs. This result is a combined effect of the set of antioxidants. The total decrease of flavonol content, in SB-MDMs, is well correlated with the respective changes in the TBARS values both for uncooked and cooked meat samples.

Potent antioxidant flavonols of sea buckthorn are well preserved during storage of cooked 1%, 2% or 4% SB-MDMs, at +6 °C, but about a half has been lost, probably oxidised, in the uncooked samples of SB-MDTM during

storage at +6 °C. The loss of polyphenols is much smaller in the case of SB-MDCM, which contains less fatty acids and is initially less oxidated than MDTM. The cooking process itself has little effect on polyphenol integrity in the SB-MDMs.

Consequently, it is safe to say that the processing residue of sea buckthorn juice is a good functional supplement to MDM or HDM products, guaranteeing inhibition of the oxidation of fatty acids as well as enriching the meat products with plant-derived health-beneficial polyphenols. In addition, the optimal 2% supplement of berry powder does not deteriorate the organoleptic properties like taste, flavour or texture of the patties prepared from the poultry MDM.

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