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Lipid oxidation inhibition capacity of plant extracts and powders in a processed meat model system

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ABSTRACT

A meat model system was used for screening lipid oxidation inhibiting capacity of diverse horticultural plant materials. In the model, heme-containing sarcoplasmic proteins from the meat water-phase were homogenized with linoleic acid and thiobarbituric reactive substances (TBARS) were measured. 23 Plant materials were investigated at three high (50, 100, and 200 ppm) concentrations and five plant extracts were tested at three low (5, 10, and 20 ppm) concentrations over time. In the high concentration sets, summer savory freeze-dried powder, beetroot leaves extracted with 50% ethanol, and an olive polyphenol powder extracted from wastewater, inhibited oxidation the most effectively. After two weeks and at 200 ppm concentration, oxidation was reduced to 17.2%, 16.6% and 13.5% of the blank sample with no added antioxidants respectively. In the low concentration set, spray dried rhubarb juice inhibited oxidation the most after two weeks at 5 ppm where oxidation was reduced to 68.3% of the blank sample with no added antioxidants.

1. Introduction

Meat consumption is currently increasing globally (Godfray et al., 2018). This has environmental implications as well as several health risks, where colorectal cancer (CRC) has been the most disputed (IARC, 2015). Meat, however, is considered to be one of the most important sources of high-quality proteins in the human diet. Meat also provides B-group vitamins and essential minerals (Binnie, Barlow, Johnson, & Harrison, 2014), and is considered a healthy food component when consumed in moderate amounts. However, red meat contains heme and free iron, and more so than other types of fresh meat (Oostindjer et al., 2014). Heme iron from myoglobin or hemoglobin is one of the components commonly hypothesized to promote oxidation in meat, either as it is or when combined with nitrite in processed meat (Joosen et al., 2009), particularly when in presence of polyunsaturated fatty acids (PUFA) (Ishikawa, Tamaki, Ohata, Arihara, & Itoh, 2010). Lipid oxidation in meat products is known for decreasing shelf-life and nutritional value (Jiang & Xiong, 2016). Moreover, lipid oxidation secondary products, such as malondialdehyde (MDA) or 4-hydroxy-2-nonenal (4-HNE), are known to be cytotoxic and genotoxic (Kanner, 2007). Our project, "Sustainable plant ingredients for healthier meat products" (SUSMEATPRO) aims to evaluate the isolated health effect of inhibiting lipid oxidation in processed meat products by adding natural antioxidants. The present study constitutes the part of the project where lipid oxidation inhibiting capacity of several plant materials and extracts thereof is evaluated using an appropriate meat model.

Most available literature and data on plant polyphenols refer only to compounds that are easily extracted by aqueous-organic solvents, however, little attention is paid to the fraction of polyphenols that remain in the residues after fruit and berry processing. With the

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application of proper extraction technology, these polyphenols could be released from the food matrix and further used for other purposes. It has been confirmed that several agro-industrial side-streams and processing by-products are more abundant in polyphenols than the actual fruit or processing products (juice, jam, *etc.*) (Arranz, Silvan, & Saura-Calixto, 2010; Esparza-Martinez, Miranda-Lopez, Mata-Sanchez, & Guzman-Maldonado, 2016; Pérez-Jiménez, Díaz-Rubio, & Saura-Calixto, 2014). We have previously screened different plant materials *in vitro* for their antioxidant properties and their composition of phenols (Burri, Ekholm, Håkansson, Tornberg, & Rumpunen, 2017), where the importance of the composition for the antioxidants' *modus operandi* was emphasised. To corroborate the hypothesis that these antioxidants will inhibit lipid oxidation in meat products a meat model was considered appropriate for the present screening. Meat models have commonly been used to investigate lipid oxidation inhibition capacity of various antioxidants (Fasseas, Mountzouris, Tarantilis, Polissiou, & Zervas, 2008; Fernández, Romero, Doval, Sturla, & Judis, 2010; Hayes et al., 2009; Lee, Han, & Decker, 2002) where thiobarbituric reactive substances (TBARS) is commonly used as analytical method (Kumar, Yadav, Ahmad, & Narsaiah, 2015). The aim of this study was to develop a relevant meat model that could represent a processed meat product, and to investigate the lipid oxidation inhibiting capacity of a large amount of plant materials and extracts using this meat model.

2. Material and methods

2.1. Plant material and extracts

A total of 28 plant materials and extracts were obtained from project partners in Denmark, Estonia, Finland, Latvia and Sweden (Table 1), and were screened for their capacity to inhibit lipid oxidation in a processed meat model system. Samples were tested at either three high concentrations (50, 100, and 200 ppm) or three low concentrations (5, 10, and 20 ppm) based on their respective total phenol content, expressed as gallic acid equivalent (GAE) in mg mL^{-1} extract, in relation to the total meat model system volume. To allow handling of a large amount of samples they were divided into 4 sets where set 1–3 comprised samples that were tested at high concentrations and set 4 comprised samples tested at low concentrations. Horseradish (*Armoracia rusticana* P.Gaertn. & al.) and ramson (*Allium ursinum* L.) bulb formulated as non-extracted powders were tested at low concentrations because the amount of powder needed otherwise would have exceeded the maximum amount possible to use in the meat model. Similarly, spray dried rhubarb (*Rheum rhabarbarum* L.) juice, aronia (*Aronia* x *prunifolia* (Marshall) Rehder) and black currant (*Ribes nigrum* L.) extracts were also tested at low concentrations, due to suspected pro-oxidation effect previously noticed when screening samples *in vitro*.

Swedish phenol-rich extracts were prepared using 50% ethanol (aq) according to the protocol of Burri et al. (2017). Finnish phenol-rich extracts were prepared using 3 different methods. The bilberry (*Vaccinium myrtillus* L.) leaf extract was obtained using 50% ethanol (aq) with ultrasound-assisted extraction for 30 min. The bilberry leaf extract was then filtered and freeze-dried. The pressurised hot water extracted (PHWE) samples were obtained using a Dionex ASE 350 accelerated solvent extractor (Thermo Fischer Scientific Inc., Waltham, MA, USA). Extraction temperatures were 110 °C, 120 °C and 135 °C for sea buckthorn (*Hippophae rhamnoides* L.) leaves, bilberry leaves and blackcurrant juice press residues, respectively. The static extraction time was 1 min for all samples. After PHWE, the extracts were filtered and freeze-dried. The Scots pine (*Pinus sylvestris* L.) heartwood and Norway spruce (*Picea abies* [L.] Karst.) inner bark extracts were obtained from a two-step extraction using hexane and 95% ethanol (aq) according to the protocol of Laavola et al. (2015). Estonian phenol-rich extracts were prepared using a pilot-scale solid-liquid Naviglio extractor (Atlas Filtri, Limena, Italy) with 20% ethanol (aq). Extracted aronia fruit (*Aronia x prunifolia*) and black currant juice press residues samples were then concentrated to 50% before they were spray dried using a Mini Spray Dryer B-290 (Büchi Labortechnik, Flawil, Switzerland), with 25% maltodextrin. Extracts of rhubarb root and black currant leaves were also concentrated to 50% but were instead freeze-dried to a powder. Rhubarb juice was spray dried using 5% maltodextrin. Latvian phenol-rich extracts were prepared using 80% ethanol (aq) and water only according to the protocols of Gornas, Sne, Siger, and Seglina (2014) and Thomas and Thibault (2002). Danish samples were not extracted, only freeze dried and ground.

2.2. Chemicals

2-Thiobarbituric acid ≥98% (TBA), 1,1,3,3-tetramethoxypropane 99% (TMP), trichloroacetic acid ≥99.0% (TCA), linoleic acid 58–74% (GC) (CAS# 60-33-3) and ethanol 96% were obtained from Sigma-Aldrich Inc. St. Louis, MO, USA. Hydrochloric acid (40 mmol L^{-1}), and 85% ortho-phosphoric acid (H_3PO_4) were obtained from Merck KGaA, Darmstadt, Germany.

2.3. Analysis of total phenols

The concentrations of total phenols of the plant extracts were analysed using Folin Ciocalteu's reagent according to the protocol in Burri et al. (2017) and reported as gallic acid equivalent (GAE) in mg mL^{-1} extract.

2.4. Meat model system

Water phase sarcoplasmic proteins (SP) were extracted from 70 g of pork knuckle (*M. gastrocnemius*) using 700 mL of 0.15 M NaCl solution by homogenization with 13 mm extension arm diameter at 24′000 rpm for 30 s (Ultra-Turrax® T25, IKA). After homogenization, the solution was centrifuged at 1000*g* for 10 min. The supernatant containing SP was collected and the pellet was re-suspended with 300 mL of the 0.15 M NaCl solution to dissolve the remaining SP from the pellet, after which the aqueous phase was separated with centrifugation at 1000*g* for 10 min. The SP solutions were combined and the protein concentration was measured using FlashEA 1112 N/Protein analyzer (Thermo Fisher Scientific) to a concentration of 0.30%. The SP solution was then homogenized with linoleic acid into 20% oil emulsions (*w*/w). Next, three concentrations of 1 mL plant extracts were mixed with 10 g of the prepared emulsion, resulting in a total volume of 11 mL. Reference samples without plant extracts were made using distilled water only. Ramson bulb, horseradish, spray dried rhubarb juice, spray dried aronia and spray dried black currant extracts were added in 5, 10 and 20 ppm GAE, whereas all other samples were added in 50, 100 and 200 ppm GAE. The concentrations of the plant extracts were calculated based on the total phenol contents described in Table 1. Ethanol was evaporated from Swedish extracts prior to dilution with distilled water and addition to the emulsions. The other extracts (obtained as freeze dried powders) were directly diluted in distilled water in order to obtain the selected concentrations of total phenols. All test emulsions were made in triplicates. The emulsions were then heated at 72 °C for 20 min in order to release the iron from the myoglobin structure where the free iron among the SP was allowed to catalyse the lipid oxidation of the reactive linoleic acid. Extracts were added before heating and samples were then refrigerated at 4 °C overnight. After one, 7 and 14 days, the change in the content of thiobarbituric acid reactive substances (TBARS) was measured in both sample emulsions and emulsions with no added material or extract (blanks).

2.5. Thiobarbituric acid reactive substances - TBARS

The TBARS method was modified from Buege and Aust (1978) where the amount of TBA was doubled. Hence, 2.5 mL of a TBAR reagent containing 15% TCA, 0.75% TBA in 0.25 M HCl was added to

Table 1
Plant materials, countries of origin, extraction solutions, abbreviations, extraction methods, content of total phenols and set numbers used in this study.

Plant material	Latin name	Cultivar	Country	Extraction solution	Abbreviation	Extraction method	mg GAE mL^{-1} extract	Set no.
Black currant leaves	*Ribes nigrum* L.	'Ben Finlay'	Sweden	50% ethanol	BC	Burri et al. (2017)	4.8	1
Beetroot leaves	*Beta vulgaris subsp. vulgaris*	'Action'	Sweden	50% ethanol	BR	Burri et al. (2017)	1.0	1
Carrot leaves	*Daucus carota* L.	'Nairobi'	Sweden	50% ethanol	C	Burri et al. (2017)	1.0	1
Onion peel	*Allium cepa* L.	'Donna'	Sweden	50% ethanol	OP	Burri et al. (2017)	3.0	1
Onion skin	*Allium cepa* L.	'Donna'	Sweden	50% ethanol	OS	Burri et al. (2017)	3.0	1
Red currant leaves	*Ribes rubrum* L.	'Röda Versailler'	Sweden	50% ethanol	RC	Burri et al. (2017)	3.8	1
Sea buckthorn leaves	*Hippophae rhamnoides* L.	'Finskaja'	Sweden	50% ethanol	SBT	Burri et al. (2017)	8.8	1
Bilberry leaves	*Vaccinium myrtillus* L.	Native stands	Finland	50% ethanol	BB	See Section 2.3.	13.6	2
Bilberry leaves	*Vaccinium myrtillus* L.	Native stands	Finland	Pressurised hot water	BB PHWE	See Section 2.3.	11.6	2
Black currant juice press residues	*Ribes nigrum* L.	'Öjebyn'	Finland	Pressurised hot water	BC PHWE	See Section 2.3.	6.4	2
Japanese quince	*Chaenomeles japonica* (Thunb.) ex Spach	'Rondo'	Latvia	80% ethanol	JQ80	Thomas and Thibault (2002)	4.8	2
Japanese quince	*Chaenomeles japonica* (Thunb.) ex Spach	'Rondo'	Latvia	H2O	JQH2O	Modified from Thomas and Thibault (2002)	1.9	2
Lingonberries	*Vaccinium vitis-idaea* L.	Wild origin: China	Denmark	No extraction	LB	–	15.3	2
Pine heartwood	*Pinus sylestris* L.	–	Finland	Hexane and 95% ethanol (aq)	PHW	Laavola et al. (2015)	4.9	2
Red currant berries	*Ribes rubrum* L.	'Red Poll'	Denmark	No extraction	RCB	–	13.6	2
Sea buckthorn leaves	*Hippophae rhamnoides* L.	'Botnia Guldklimp'	Finland	Pressurised hot water	SBT PHWE	See Section 2.3.	7.0	2
Sea buckthorn leaves and sprouts	*Hippophae rhamnoides* L.	Mix of 'Botaņičeskaja Ļubiteļskaja' and 'Prozračnaja'	Latvia	80% ethanol	SBT80	Gornas et al. (2014)	13.2	2
Summer savory leaves	*Satureja hortensis* L.	Seed origin: Hild Samen	Denmark	No extraction	SS	–	12.0	2
Lyophilised black currant leaves	*Ribes nigrum* L.	'Pamjat Vavilova'	Estonia	20% ethanol	LBC	See Section 2.3.	10.1	3
Lyophilised rhubarb root	*Rheum rhabarbarum* L.	'Victoria'	Estonia	20% ethanol	LRR	See Section 2.3.	18.1	3
Olive polyphenols - Phenoliv	*Olea europaea* L.	Phenoliv™	Sweden	50% ethanol	OPP	Burri et al. (2017)	3.8	3
Sea buckthorn leaves and sprouts	*Hippophae rhamnoides* L.	Mix of 'Botaņičeskaja Ļubiteļskaja' and 'Prozračnaja'	Latvia	H2O	SBTH2O	Modified from Gornas et al. (2014)	9.2	3
Spruce inner bark	*Picea abies* (L.) Karst.	–	Finland	Hexane and 95% ethanol (aq)	SIB	Laavola et al. (2015)	14.1	3
Horseradish roots	*Armoracia rusticana P.Gaertn. & al.*	'Yugoslavien'	Denmark	No extraction	HR	–	1.2	4
Ramson bulb – resting phase	*Allium ursinum* L.	Wild origin: Risskov - Århus	Denmark	No extraction	RAB	–	0.7	4
Spray dried aronia juice press residues	*Aronia x prunifolia* (Marshall) Rehder	Seedling	Estonia	20% ethanol	SDA	See Section 2.3.	0.6	4
Spray dried black currant juice press residues	*Ribes nigrum* L.	'Pamjat Vavilova'	Estonia	20% ethanol	SDBC	See Section 2.3.	0.2	4
Spray dried rhubarb juice	*Rheum rhabarbarum* L.	'Sutton Seedless'	Estonia	–	SDRJ	See Section 2.3.	0.3	4

0.5 mL emulsion in a 15 mL falcon tube. The samples were then heated for 10 min in a 90 °C water bath before the absorbance was measured at 534 nm but also at 600 nm for turbidity measurement. The results of the TBARS were expressed as a percentage of lipid oxidation of the emulsion with no added material or extract (blank) of which there was a separate one for every set.

3. Statistical analyses

All statistical analyses were carried out using SPSS Statistics 25.0 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). A repeated measures General Linear Model (GLM) was performed on logarithmic values of lipid oxidation data in order to ensure normal distribution of samples (Skewness and Kurtosis with maximum values of ± 1.96 (Kim, 2013)). Post-Hoc tests were performed using the Tukey method. Estimated Marginal Means were extracted from the GLM to visualise the mean response of the factors; time and concentration, adjusted for the variables in the model. Moreover, a Pearson correlation analysis was conducted on the above mentioned data between samples tested at the same concentrations (50, 100 and 200 ppm, and 5, 10 and 20 ppm respectively). Moreover, Swedish samples with previous data on total phenol content (using Folin-Ciocalteu), ferric reducing ability of plasma (FRAP) and radical scavenging activity (using ABTS) (Burri et al., 2017) were analysed separately.

4. Results

4.1. TBARS – analytical methodology

The TBARS method was initially carried out on the emulsions according to the Buege and Aust (1978) protocol. However, these samples showed orange chromogens with strong absorbance at wavelength 453 nm and only weak absorbance at 534 nm where the malondialdehyde (MDA) reaction product is typically measured. Therefore, a TBA calibration curve was made where the amount of TBA working solution was gradually lowered in order to study the colour shift of the chromogens. Results showed that the lower the TBA concentration, the weaker the pink coloration and the higher the amount of orange chromogens. Therefore, we instead doubled the amount of TBA in the working solution to study whether the absorbance at 534 nm would further increase, which showed to be successful resulting in a more intense pink colour. This was then the TBA amount used in our study.

4.2. TBARS results

All samples were evaluated for the formation of TBAR substances in triplicate after three different storage times (1 day, 1 week and 2 weeks) and at different GAE concentrations (Supplementary Table 1). A repeated measures GLM-analysis was conducted, where the level of lipid oxidation at three storage times was the dependent factor of the analysis. Plant material and concentration showed to have a significant effect on the oxidation in all sets ($P < .001$), as did the interaction of them both in sets one to three ($P < .001$) but not in set four ($P = .506$) (Supplementary Tables 2 and 3). Plant material had the largest impact on lipid oxidation in all sets. The samples were analysed in three batches to be able to handle one batch per day (sets three and four were analysed for TBARS together but were analysed separately statistically due to concentration differences). The TBAR values of the blank emulsions (μM MDA/g emulsion) are shown in Table 2.

In the first set of samples, sea buckthorn leaves (SBT), onion (*Allium cepa* L.) skin (OS) and beetroot (*Beta vulgaris* subsp. *vulgaris*) leaves (BR) were the most efficient in inhibiting lipid oxidation, and increasingly so with higher concentration (200 ppm) and longer storage time (2 weeks) (Fig. 1). SBT reduced lipid oxidation to 31.2%, OS to 18.1%, and BR to 16.6% at 200 ppm GAE after 2 weeks of storage in comparison to the emulsion with no added samples.

Table 2
Average of lipid oxidation level in blank samples ($n = 3$) μM MDA/g emulsion.

μM MDA/g emulsion	1 day	1 week	2 weeks
Blank set 1	25.5	47.4	78.0
Blank set 2	11.9	29.6	62.8
Blank set 3 and 4	16.3	30.8	61.5

In the second set of samples, the summer savory (*Satureja hortensis* L.) powder (SS) and the pine heartwood extract (PHW) were the most powerful samples to inhibit lipid oxidation similarly to the samples in the first set (Fig. 2). However, sea buckthorn (SBT PHWE) and bilberry leaf samples (BB PHWE) extracted with pressurised hot water reached the greatest inhibition at 200 ppm already after 1 week. SS reduced oxidation to 17.2% and PHW to 35.4% at 200 ppm after 2 weeks of storage, while SBT PHWE reduced oxidation to 42.3% and BB PHWE to 33.6% at 200 ppm after 1 week of storage. Several samples showed to be pro-oxidative in this second set of samples: black currant juice press residues extracted with pressurised hot water (BC PHWE), bilberry leaves (BB), Japanese quince (*Chaenomeles japonica* (Thunb.) Spach) extracted with 80% ethanol and water (JQ80 and JQH2O), lingonberry (*Vaccinium vitis-idaea* L.) powder (LB) and red currant (*Ribes rubrum* L.) berry powder (RCB). The BB sample, however, inhibited oxidation at 200 ppm after 1 and 2 weeks of storage (to 73.8% and 74.1% respectively), as did the LB sample at 200 ppm after 2 weeks (81.2%).

In the third set of samples, the olive (*Olea europea*) polyphenol powder (OPP), spruce inner bark extract (SIB), sea buckthorn water extract (SBTH2O), lyophilised rhubarb root (LRR), and lyophilised black currant leaves (LBC) all inhibited oxidation most effectively at 200 ppm after 2 weeks (Fig. 3). OPP decreased oxidation to 13.5%, SIB to 19.0%, SBTH2O to 16.6%, and LRR to 23.2%.

In the fourth set of samples, all extracts were tested at lower concentrations (Fig. 4). The spray dried rhubarb juice samples (SDRJ) were the most effective, inhibiting oxidation to 68.3% at 5 ppm after 2 weeks. Ramson bulb (RAB) samples inhibited the oxidation after 2 weeks to 80.6% with 5 ppm, to 80.4% with 10 ppm and to 79.6% with 20 ppm of concentration. The horseradish sample (HR) also inhibited oxidation after 2 weeks at 5 ppm, lowering the oxidation to 80.6%.

4.2.1. Repeated measures GLM-analysis

In sets one to three, OPP showed to have the statistically strongest inhibiting capacity of lipid oxidation of all samples ($P < .001$) except when compared to the BR sample ($P = .151$) (Table 3). BR statistically differed from all samples except for OPP (see above) and SBT H2O ($P = .289$). All concentrations affected level of oxidation statistically ($P < .001$), where 200 ppm < 100 ppm < 50 ppm. In the fourth set, SDRJ was statistically stronger in reducing the lipid oxidation ($P < .001$) than the other samples (Table 4). The two lower concentrations (5 and 10 ppm) did not differ significantly ($P = .992$) but were however the strongest concentrations in inhibiting lipid oxidation.

4.2.2. Estimated marginal means

An overview of effects of factors is presented for all four data sets in Fig. 5a and b. The strongest effect, *i.e.* the strongest reduction of lipid oxidation in comparison to the control samples, was for all sets reached after 2 weeks of storage. In the sets one to three, 200 ppm was the most efficient concentration whereas in set four, 5 ppm and 10 ppm showed to be the most efficient concentrations. The positive interaction effect in sets one to three imply that the longer the storage time, the higher the oxidation, and a higher antioxidant concentration may modulate this increase of lipid oxidation. In set four however, the interaction effect was not statistically significant, which points towards that concentration and storage times are effective independently of each other (Fig. 5a, b).

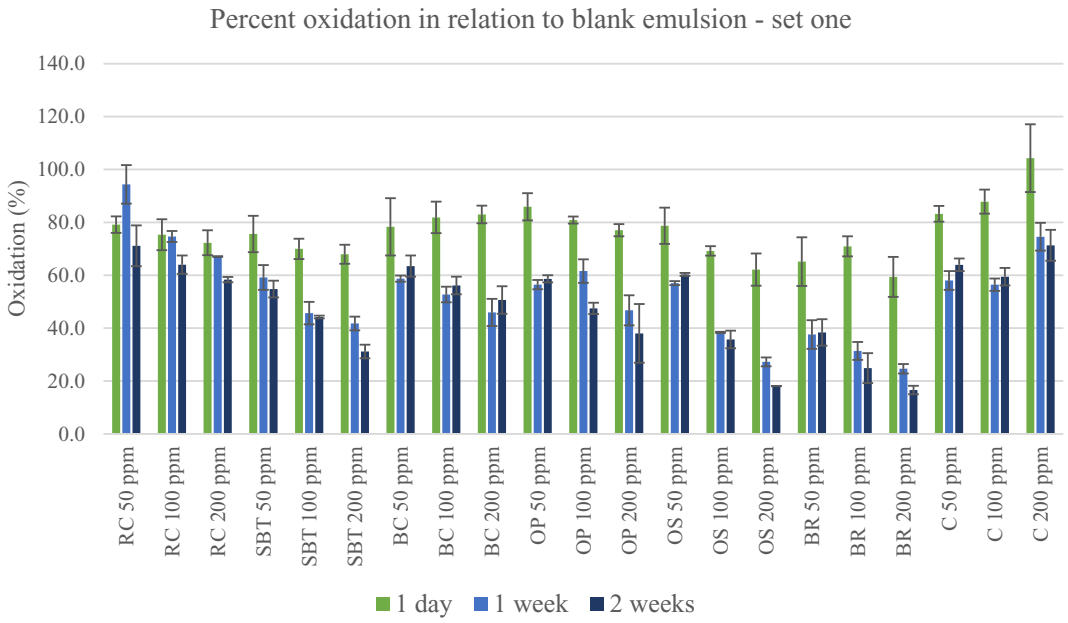

Fig. 1. Percent (%) lipid oxidation in first set of meat model system samples (replicates, n = 3). RC = Red currant leaves, SBT = Sea buckthorn leaves, BC = Black currant leaves, OP = Onion peel, OS = Onion skin, BR = Beetroot leaves, C = Carrot leaves.

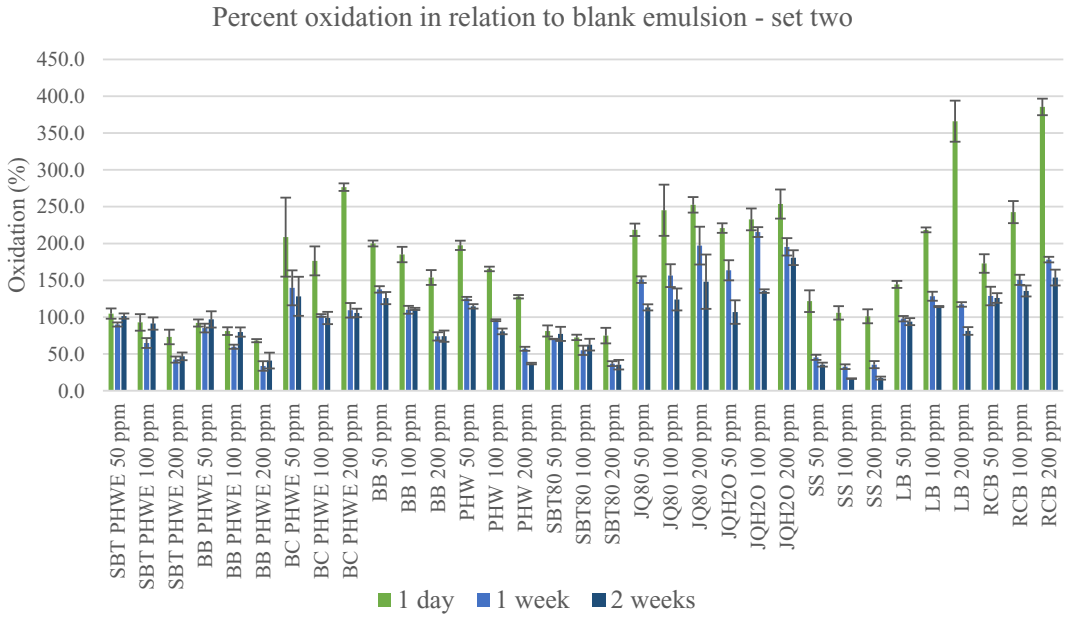

Fig. 2. Percent (%) lipid oxidation in second set of meat model system samples (replicates, *n* = 3). SBT PHWE = Sea buckthorn leaves pressurised hot water extracted, BB PHWE = Bilberry leaves pressurised hot water extracted, BC PHWE = Black currant juice press residues pressurised hot water extracted, BB = Bilberry leaves, PHW = Pine heartwood, SBT80 = Sea buckthorn 89% ethanol, JQ80 = Japanese quince 80% ethanol, JQH2O = Japanese quince water extracted, SS = Summer savory, LB = Lingonberry, RCB = Red currant berries.

4.3. Correlation analysis

There was a strong correlation (Pearson) between three methods measuring antioxidant properties in a previous study performed by Burri et al. (2017): FC-FRAP 0.956, FC-ABTS 0.974, FRAP-ABTS 0.994 ($P < .001$). The equations of the straight lines fitted to the data were FC-FRAP $y = 23.07\times - 15.90$ ($R^2 = 0.876$), for FC-ABTS $y = 22.286\times - 19.20$ ($R^2 = 0.903$), and for FRAP-ABTS $y = 0.86\times + 3.36$ ($R^2 = 0.820$). There was however no significant correlation between lipid oxidation inhibition capacity measured in the present study using the meat model system and the previous antioxidant measurements as well as content of total phenols (data not shown).

5. Discussion

The incorporation of plant antioxidants into readily oxidised meat products is increasing in popularity, but questions regarding factors such as; concentrations, plant species, meat product properties, storage times, chemical properties and effects of specific antioxidants, need further and thorough investigation (Jiang & Xiong, 2016). For screening of antioxidant effects of a great number of plant materials in different concentrations over time a standardised meat model system is useful. Previous studies on antioxidant capacity to inhibit lipid oxidation in

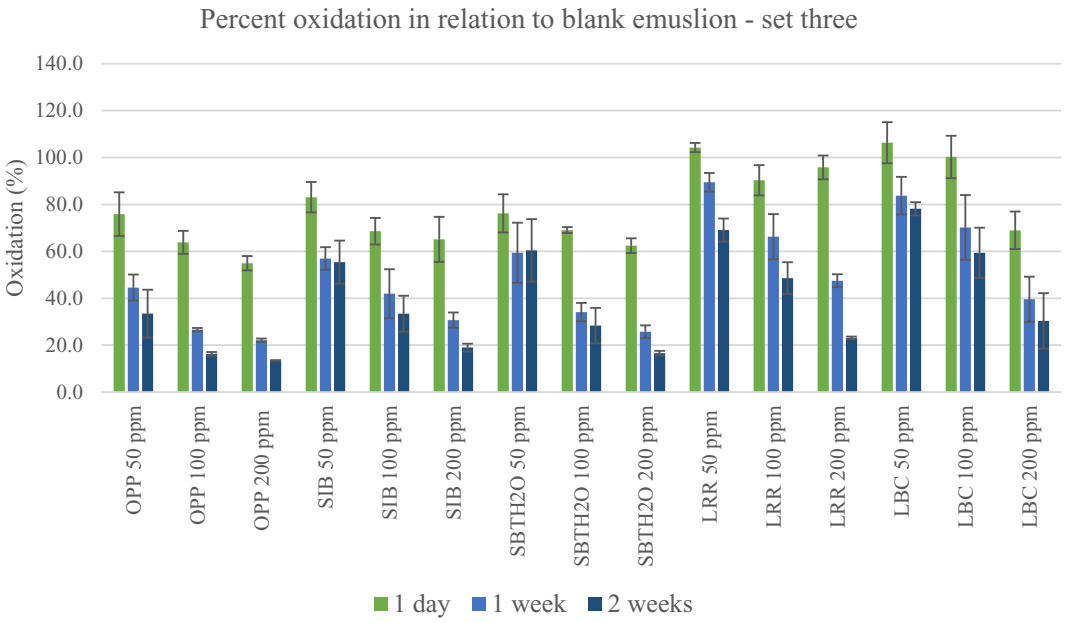

Fig. 3. Percent (%) lipid oxidation in third set of meat model system samples (replicates, n = 3). OPP = Olive polyphenols, SIB = Spruce inner bark, SBT H_2O Sea buckthorn water extracted, LRR = Lyophilised rhubarb root, LBC = Lyophilised black currant leaves.

muscle models have shown to be successful (Hayes et al., 2009; Lee et al., 2002) as has meat model emulsions of sarcoplasmic proteins and linoleic acid similar to those in our study (Hayes et al., 2009). However, in this study, the concentrations of antioxidants was lower than those of Hayes et al. (2009) where olive leaf and lutein was tested at 100, 200, and 300 ppm, ellagic acid at 300, 600, and 900 ppm, and sesamol was tested at 500, 1000, and 2000 ppm respectively. Moreover, our study focused on screening a large number of non-typical sources of antioxidants in a basic meat model containing the main components of importance to lipid oxidation in processed meat products (namely sarcoplasmic proteins and fat). The heme iron was made accessible by the heat treatment and catalysed the lipid oxidation reaction, as it would in an actual meat product since the heating conditions were similar to those used in the industry.

In our study most of the analysed plant materials inhibited oxidation with the exception of a few samples in set 2 (Fig. 2) and set 4 (Fig. 4), which instead had pro-oxidant effects.

Various antioxidants' ability to show pro-oxidant activity has widely been studied in previous research (Eghbaliferiz & Iranshahi, 2016; Rahal et al., 2014). The pro-oxidant effect of antioxidants highly depends on the mode of action, the concentration and the prevalence of transition metal ions, such as iron (Fe) (Eghbaliferiz & Iranshahi, 2016). Moreover, another plausible explanation for differences in phenol behaviour overall has been given by Radenkovs, Püssa, Juhnevica-Radenkova, Anton, and Seglina (2018) where they conclude that each polyphenol in the antioxidant acts independently and that the relative

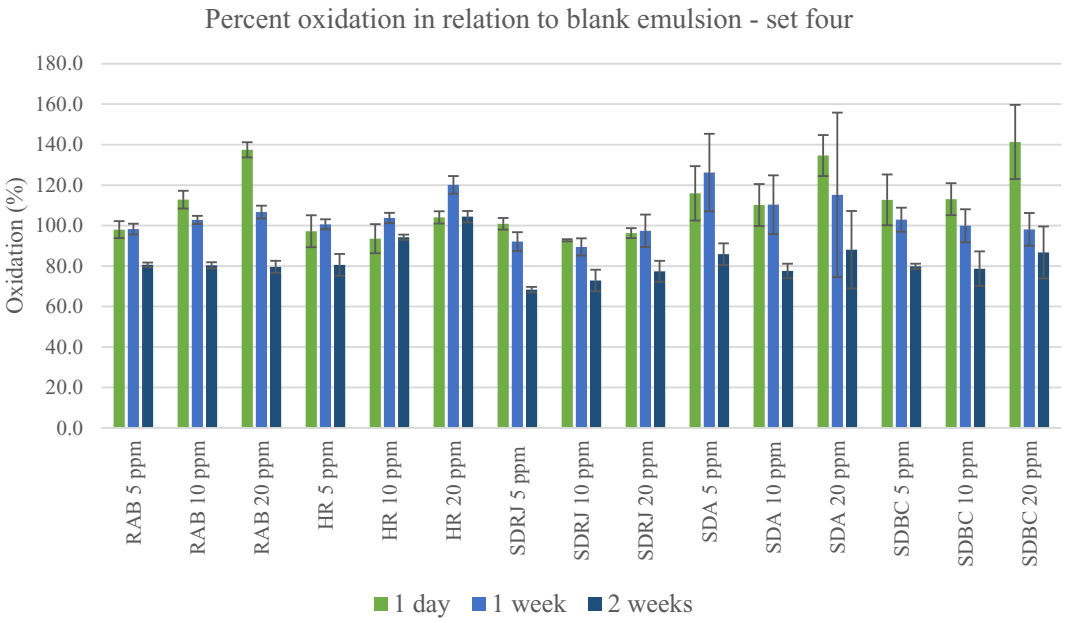

Fig. 4. Percent (%) lipid oxidation in fourth set of meat model system samples (replicates, n = 3). RAB = Ramson bulb, HR = Horseradish, SDRJ = Spray dried rhubarb juice, SDA = Spray dried aronia juice, SDBC = Spray dried black currant juice press residues.

Table 3
Tukey post-hoc table where OPP = olive polyphenols, BR = beetroot leaves, SBT H2O = water extracted sea buckthorn leaves, SS = summer savory leaves, OS = onion skin, SIB = spruce inner bark, SBT = sea buckthorn leaves, OP = onion peel, SBT80 = 80% ethanol extracted sea buckthorn leaves and sprouts, BC = black currant leaves, LRR = lyophilised rhubarb root, LBC = lyophilised black currant leaves, BB PHWE = bilberry leaves extracted with pressurised hot water, C = carrot leaves, RC = red currant leaves, SBT PHWE = sea buckthorn leaves extracted with pressurised hot water, PHW = pine heartwood, BB = bilberry leaves, LB = Lingonberries, BC PHWE = black currant juice press residues extracted with pressurised hot water, JQ80 = Japanese quince extracted with 80% ethanol, RCB = red currant berries, JQH2O = water-extracted Japanese quince.

Samples	N	Subset											
		1	2	3	4	5	6	7	8	9	10	11	12
OPP	9	1.5212											
BR	8	1.5774	1.5774										
SBT H2O	9		1.6287	1.6287									
SS	9			1.6470									
OS	9			1.6545									
SIB	9			1.6614	1.6614								
SBT	9				1.7199	1.7199							
OP	9					1.7720	1.7720						
SBT80	9					1.7792	1.7792						
BC	9						1.7918	1.7918					
LRR	9						1.8097	1.8097	1.8097				
LBC	9						1.8153	1.8153	1.8153	1.8153			
BB PHWE	9						1.8241	1.8241	1.8241	1.8241			
C	8							1.8479	1.8479	1.8479			
RC	9								1.8587	1.8587			
SBT PHWE	9									1.8744			
PHW	8										2.0222		
BB	9											2.0905	
LB	9											2.1298	
BC PHWE	9											2.1447	
JQ80	9												2.2331
RCB	9												2.2401
JQH2O	9												2.2626
P-value		0.125	0.251	0.945	0.086	0.075	0.227	0.125	0.334	0.078	1.000	0.170	0.981

Means for groups in homogeneous subsets are displayed based on observed means. The error term is Mean Square (Error) = 0.001.
a. Uses Harmonic Mean Sample Size = 8.856.
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c. Alpha = 0.05.

Table 4
Tukey post-hoc table where SDRJ = spray dried rhubarb juice, RAB = ramson bulb, HR = horseradish roots, SDBC = spray dried black currant juice press residues, SDA = spray dried aronia juice press residues. SDRJ significantly differed from the other samples in set 4 (*P*-value < .001).

Samples	N	Subset	
		1	2
SDRJ	9	1.9379	
RAB	9		1.9917
HR	9		1.9967
SDBC	9		1.9983
SDA	9		2.0195
P-value		1.000	0.293

Means for groups in homogeneous subsets are displayed based on observed means.
The error term is Mean Square(Error) = 0.001.
a. Uses Harmonic Mean Sample Size = 9.000.
b. Alpha = 0.05.

speed of the reaction depends predominantly on each compound's chemical structure rather than its concentration. This further reinforces the theories regarding the previously mentioned interplay complexity between antioxidant and matrix properties (Jiang & Xiong, 2016).We previously studied differing mode of actions of antioxidants by measuring their Ferric reducing ability of plasma (FRAP) and their radical scavenging capacity (using ABTS) and compared these to the total phenols content (using Folin-Ciocalteu) where we found that these properties were highly correlated (Burri et al., 2017). Moreover, a Principal Component Analysis (PCA) of the phenol composition together with phenol contents and antioxidant capacities allowed for visualization of which phenols correlated to which mode of action. In light of the previous results of Burri et al. (2017), it is reasonable to conclude that antioxidants may inhibit lipid oxidation differently depending on their composition and preferred mode of action.

The main effect plots from the GLM model showed that 1 day of storage had little to no effect on lipid oxidation levels for sets one to three, why the blue line, representing one day of storage (log10 values of percent oxidation), marks the highest values. The effect after 2 weeks of storage was largely noticed by a distinct lowering of the green line for all sets (Fig. 5a, b). The 200 ppm concentration inhibited lipid oxidation much > 100 and 50 ppm respectively in sets one to three, this can be seen most clearly after 1 and 2 weeks of storage. However, in the fourth sample set (Fig. 5b), the lower GAE concentrations, 5 and 10 ppm, were overall more effective than the higher one at 20 ppm which can be interpreted by an increase in oxidation values with higher concentrations.

Surprisingly, using data from our meat model system we found no correlations between lipid oxidation inhibition data with antioxidant data from previous studies (FC, FRAP and ABTS) conducted by Burri et al. (2017). It should be noted that the olive polyphenol powder (OPP) was analysed for its lipid oxidation inhibition capacity in set 3 and was thus not included in the correlation analysis regarding the previous antioxidant data (FC, FRAP and ABTS) and data from our set 1. However, including the OPP in the correlation analysis did not affect the results. Previous studies have found significant correlations between the radical scavenging activity (using DPPH) and inhibition of lipid oxidation of onion dry skin extracts (Nuutila, Puupponen-Pimiä, Aarni, & Oksman-Caldentey, 2003). However, it has also been proposed that radical scavenging capacity does not necessarily correlate with the

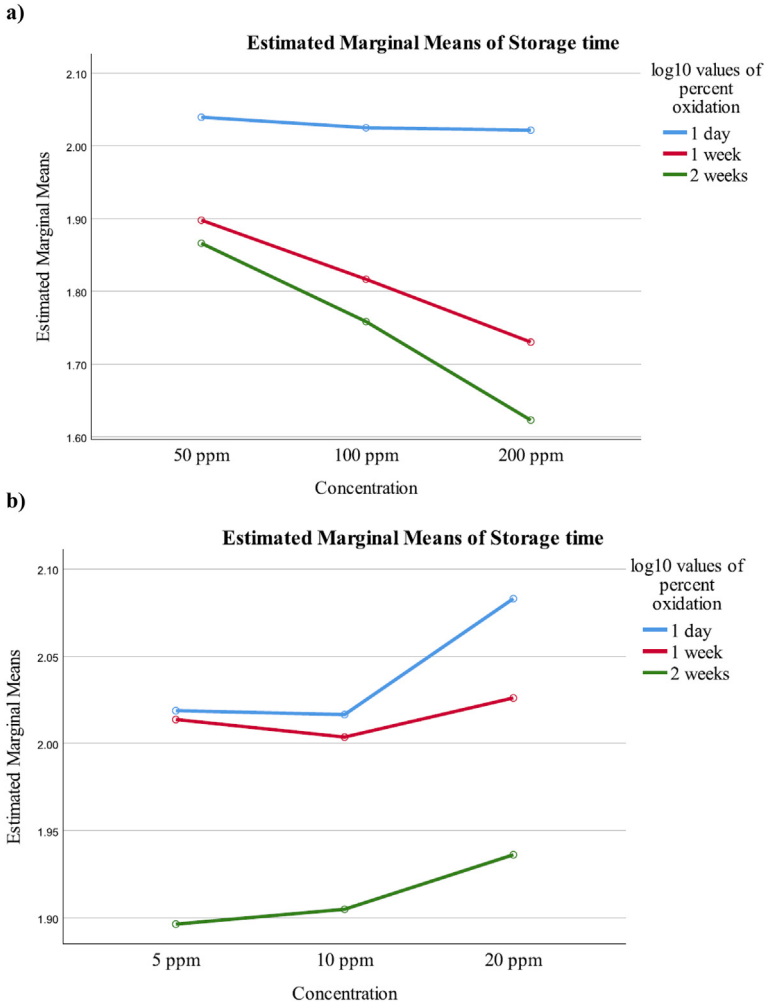

Fig. 5. a) Estimated marginal means (EMM) for the interactions between storage times and concentrations in set 1–3, and b) EMM for interactions between storage times and concentrations in set 4.

capacity to prevent lipid oxidation (Niki, 2010). A plausible explanation for not finding any significant correlation in this study might be that we analysed samples which differed greatly in composition and lipid oxidation inhibiting capacity, and thus possibly did not show results able to correlate with their radical scavenging capacity or ferric reducing ability of plasma.

Lipid oxidation typically occurs faster and earlier in oil-in-water emulsions than in bulk oil due to the increased contact between prooxidant compounds (such as Fe) and unsaturated fatty acids (Berton-Carabin, Ropers, & Genot, 2014). The lipid oxidation inhibiting ability of antioxidants is highly related to the interfaces formed in the emulsion (Frankel, Huang, Kanner, & German, 1994) and the solubility (hydrophilicity or hydrophobicity) of bioactive antioxidant compounds in different plant species. The distribution of the antioxidant compounds in the meat model, *i.e.* the access of the antioxidant compounds to the available lipids, could be essential for high activity. If lipid micelles were formed to some degree then hydrophilic antioxidants would not get access to all lipid surfaces and would result in less antioxidant action. Conversely, according to the polar paradox (Berton-Carabin et al., 2014), hydrophilic antioxidants may be more active at the interface due to their polarity in a relatively un-polar media, which is the case of the meat model emulsion. Moreover, the hydrophilic antioxidant compounds may act as metal chelating agents on the heme iron in the sarcoplasmic proteins, rather than being active at the interface, thereby counteracting lipid oxidation (Brewer, 2011). Since plant derived antioxidants contain a myriad of different phenolic compounds, they might act both at the interface and in the solution.

6. Conclusions

The aim of this study was to screen phenol-rich plant materials and extracts for their lipid oxidation inhibitory capacity in a relevant meat model system. The method showed to be effective for mapping antioxidant capacities in different concentrations over time but needs further validation in future studies where the effects of phenol-rich plant material and extracts will be studied in true meat products. Overall, the phenol-rich plant material and extracts successfully inhibited lipid oxidation with the highest efficacy at 200 ppm GAE and increasingly so over time where the summer savory powder, beetroot leaf extract and olive polyphenol samples inhibited oxidation down to 17.2%, 16.6% and 13.5% respectively compared to the blank sample.

7. Future perspectives

In further research, selected phenol-rich plant materials and extracts will be included in actual meat products to evaluate the lipid oxidation inhibitory capacity in the products *per se*.

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