



Effects of Rose Hip (*Rosa canina* L.) Extract as a Natural Ingredient on the Nutritional Composition, Oxidative Stability and Sensory Attributes of Raw and Cooked Pork Patties from Majorcan Black Pig Breed under Retail Conditions

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Abstract

This research evaluated the effect of rose hip (RC, *Rosa Canina* L.) extract as a natural antioxidant ingredient on the nutritional composition and sensory attributes of Majorcan Black Pig patties. Patties were elaborated with 1.5 and 3.0g of RC and with or without 0.03g of ascorbic acid. The results suggested that the patties with 15% fat (lower fat, higher α -tocopherol), would be healthier than the ones with 23% fat. The patties containing RC had similar amounts of vitamins C and E, which differed significantly from control. The results indicated that RC could reduce the patty lipid oxidation together with ascorbic acid and this could be a good alternative for food industry. The rose hip.

Flavour did not show significant differences and its scores were low, suggesting that rose hip extract did not influence negatively the taste of the patties as well as their texture. However, future research is needed to test different concentrations of the extract.

Keywords: Majorcan Black Pig Patties; Rose Hip Extract (*Rosa Canina* L.); Innovative Meat Products; Vitamin C, E; Warmed-Over Flavour; Volatile

Introduction

European Union is supporting measures that stimulate enhanced added value-products to conserve local and threatened livestock breeds [1]. Majorcan Black Pig (MBP) is an endangered native breed from Mallorca Island [2,3] linked to the local economy and cultural heritage of the region [4,5]. The nutritive value of MBP meat is based on the fatty acid profile, who differed from intensive pig meat production (mainly due to higher monounsaturated (MUFA), 49.7% and lower polyunsaturated (PUFA), 11% fatty acids percentages) [6]. The demand for MBP fat is important for the efficiency of the system, as it is used for elaborating the *Sobrasada*, a traditional type of sausage, and the *Ensaïmada de Mallorca*, a traditional local cake.

Nowadays, consumer perceptions towards healthier meat products are associated with how meat is produced and processed, its nutritional composition, sensory properties and social aspects [7]. According to this, the meat industry has been using three main ways to improve the healthiness of meat products: (i) reducing fat and improving the lipid profile, (ii) reducing salt content, (iii) reducing nitrites, nitrates and the replacement of synthetic antioxidants by natural ones [8]. Meat and meat products (raw and cooked) are susceptible to oxidative changes during processing and storage affecting lipids, proteins and the myoglobin iron state [9]. The oxidation of muscle lipids involves the degradation of PUFA, generation of malondialdehyde (MDA) and lipid-derived volatiles

leading to the sensory and nutritional deterioration of meat products [10,11]. Consequently, oxidation leads to rancid odour and off-flavour ("warmed-over" flavour, WOF), discolouration, and loss of nutrient value [12-14].

Concerning WOF and warmer-over odour, it is the result of the oxidation of membrane phospholipids, a process triggered by haemoproteins and other iron during cooking [15], which develops within 4 to 48 h of refrigeration and reheating [16,17]. Some volatile compounds are directly associated with the development of WOF in meat subjected to refrigerated storage [18-20], such as the hexanal which was reported to be the most sensitive indicator for lipid oxidation (Ahn, *et al.* 1998). However, other lipid-derived volatiles such as heptanal, octanal and nonanal should also be taken into account due to their low odour threshold values [22,23]. Hexanal and heptanal are degradation products from long-chain polyunsaturated n-6 fatty acids, mainly linoleic acid, while nonanal, octanal as well as heptanal arise from the oxidation of monounsaturated n-9 fatty acids, e.g., from oleic acid [24].

Antioxidants can be used to minimize the development of WOF and to improve the shelf life of meat products [19], in particular, α -tocopherol and ascorbic acid [14,25]. More recently, Rose hip (*Rosa canina* L, RC), as a source of natural antioxidants, has been seen to delay the onset of rancid flavours and to stabilize the colour, improve the sensory, nutritional quality and shelf life of meat and meat products due to its antioxidant properties [26-28].

In addition, as RC fruits contain from 65.75 to 136.14 mg of ascorbic acid/100g dry weight [29], which resulted in less sodium ascorbate added during manufacturing of frankfurters [30]. Previous studies reported that the addition of 5g/Kg and 30g/Kg of RC extracts to porcine patties [31] and 5g/Kg of RC to frankfurters [30], have resulted in delaying lipid and protein oxidation with no apparent drawbacks. Food manufacturers may focus on "Clean Label" reformulations mainly because consumers are much more concerned about the heavy use of artificial ingredients, additives or colorants [32].

Rose hip (RC) can be found in the Mediterranean countries [29,33]. Currently, small scale industrial production of RC under the trademark of GRATACOOL™ takes place in the region of *La Cerdanya* (located in the Pyrenees) which is dispatched to local and premium shops.

The present study aimed at producing an innovative healthier product from MBP meat, patties, by enriching them with a Mediterranean berry (rose hip-*Rosa Canina* L), as a possible alternative to ascorbic acid (E 300). Furthermore, this research aimed to assess if rose hip contributed to reduce or mask the off-odours and off-flavours developed in patties previously oxidized under retail display conditions.

Material and Methods

Rose hip (*Rosa canina* L)

Rose hip extract was obtained from GRATACOOL™ (Bellver de Cerdanya, Catalonia). RC extract was 100% fresh; it was picked and processed manually to obtain the fresh pulp - free of most of the peel, which was submitted to a pressing process to obtain the paste. All fruit was used to get rose hip extract, with about 40% pulp extract and 60% residues. When obtained, the rose hip extract was frozen (-20°C) until used to carry out the experiments.

Manufacture, packaging and display of Majorcan black pig patties

The experimental MBP patties were manufactured in a pilot plant at Institute of Agrifood Research and Technology (IRTA, Monells, Girona), following a traditional receipt from a local butcher in order to have the sensory characteristics of this product. Two batches of minced meat containing two different fat content (15 and 23% fat) were elaborated using the shoulders and backs, each 39 kg weight, in two separate trials: The shoulders and backs were frozen at -17°C, 72 hours after slaughter (Can Company, Majorca). They were shipped frozen to the pilot plant and received at -10°C, then defrosted to -3°C for 3 days in a refrigeration chamber, and the day of the trial, the pieces were ground using a Guillotine (TecMaq SA, Barcelona) with 4 mmdiameter-cutting disks at a core temperature of -3°C. Fat content was removed from the MBP meat with a knife to obtain the adequate batch fat content (15% or 23%), which was measured by Near-Infrared Transmittance (FoodScan™ analyzer, Type 78810, FOSS, Hillerød, Denmark) [34].

Five different treatments of patties were prepared per batch differing in the added amount of RC extract and ascorbic acid (E-300, AA; Collelldevall). Also, 0.3g sodium metabisulphite (E-223, Collelldevall), 14.5g sodium chloride and 100 mL of pasteurized egg per kg of meat were added to each batch. According to the literature, the ascorbic acid content of Dog Rose ranges between 23.7

and 27.5g/kg fresh matter [29,36]. Assuming that a major part of ascorbic acid can be extracted from the fresh matter, about 0.355 and 0.750g ascorbic acid per kg patties could be present in the 15 RC or 30 RC patties, respectively. These amounts could be comparable to what is usually added in commercial patties as ascorbic acid (E300), metabisulfite of potassium (E224) and octyl gallate (E311).

The RC extract and ascorbic acid amounts were mixed according to each treatment: T₁ - 0.25g ascorbic acid; T₂- 15g rose hip; T₃ - 30g rose hip; T₄ - 15g rose hip, 0.25g ascorbic acid; T₅ - 30g rose hip, 0.25g ascorbic acid (See Figure 1). As described above, in one trial the patties contained 15% of fat and in the other 23% fat.

MBP patties (100g) were prepared in moulds using a conventional burger maker to give average ellipse dimensions of 8.5 (ma-

ior axis) and 8 cm (minor axis) and 1.4 cm of thickness. Patties for each time display (1, 3 and 6 days) and treatment (Control (T₁) vs T₂, T₃, T₄ and T₅) were wrapped with an oxygen-permeable film, dispensed in polypropylene trays and subsequently stored in a display case (EURO 334 RV, ISA, Perugia, Italy) (BASTIA UMBRA, PG, Italy) with an internal average temperature of 4 ± 1 °C, 12h of an 800 lux commercial light, and 12 h of darkness each day to simulate commercial conditions.

Figure 1 Experimental design of MBP patties (100g). A.A.: ascorbic acid, RC: rose hip. Treatments: T₁: (0.03g ascorbic acid); T₂: (1.5g RC); T₃: (3.0g RC); T₄: (0.03g ascorbic acid + 1.5g RC); T₅: (0.03g ascorbic acid + 3.0g RC). Time: t₁ (1day), t₃ (3 days), t₆ (6 days). Storage time after cooking: patties (tasted after cooking, t₁:0 days) and patties (tasted after cooking and storage under 2 °C for 4 days and reheated, t₄).

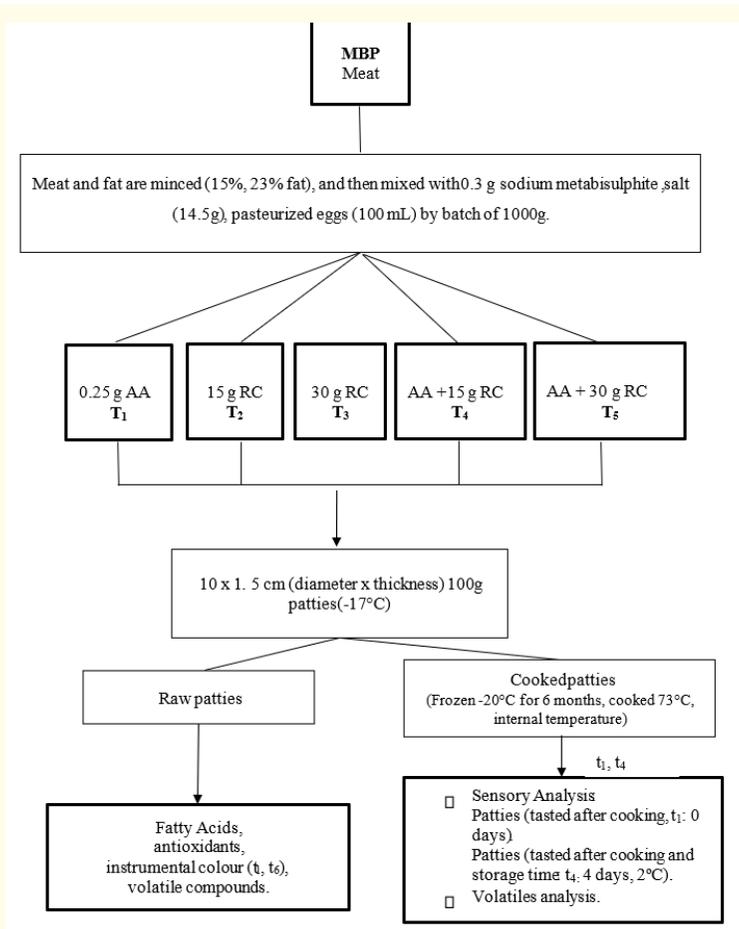


Figure 1: Experimental design of MBP patties (100g). A.A.: ascorbic acid, RC: rose hip. Treatments: T₁: (0.03g ascorbic acid); T₂: (1.5g RC); T₃: (3.0g RC); T₄: (0.03g ascorbic acid + 1.5g RC); T₅: (0.03g ascorbic acid + 3.0g RC). Time: t₁ (1day), t₃ (3 days), t₆ (6 days). Storage time after cooking: patties (tasted after cooking, t₁:0 days) and patties (tasted after cooking and storage under 2 °C for 4 days and reheated, t₄).

Majorcan black pig meat composition and fatty acid profile

Composition (moisture, protein and fat) was determined from the initial ground MBP meat in the mixture used for the preparation of the patties in the traditional way, before the addition of salt and other ingredients. Ground MBP meat composition was determined using a FoodScan™ analyzer (Type 78810, FOSS, Hillerød, Denmark) [34]. Subsequently, all samples except those used for the display study, were vacuum packed in aluminium bags and stored at -20°C for further analysis at IRTA, Monells, Girona.

Fatty Acids were extracted from MBP patties using chloroform-methanol 2:1 [36]. The solution was treated in a settling funnel containing a saturated NaCl solution (40% by volume relative to the organic phase). Once the two phases were well separated, the internal pattern was added. Concentrated methyl esters of fatty acids were obtained from KOH-methanol (2 N) and 14% BF₃ in methanol (Sigma). The reaction took place at 80 ° C for 1 h. Fatty acid methyl esters were extracted with hexane after the addition of water saturated with NaCl [37]. Fatty acid composition was determined by gas chromatography (GC) with flame ionization detector in Split mode (Agilent Technologies 6890N, Network GC System, China). The column used was a polar stationary phase (cyano propylphenyl-methyl polysiloxane) model DB-23: 60 m × ID, 0.25 mm ID, 0.25-µm film thickness (Agilent Technologies, Inc., USA). The carrier gas was Nitrogen at a flow rate of 45 ml/min. The oven temperature was maintained at 50°C for 2 min, increasing to 210 at a rate of 4°C/min. Injector and detector temperatures were 300 ° C. The fatty acid profile of meat was quantified using tripentadecanoin (MKBV 2973V, Sigma-Aldrich Ltd., USA) as internal standard. Methyl esters were identified using Sigma Chemical Co. Standard (Lipid Standard: Fatty Acid Methyl Ester Mixture # 189-19).

Antioxidants analysis

β-carotene, coenzyme Q and tocopherol analysis in MBP patties with the rose hip (Rosa canina L).

Two grams of sample (± 0.05) were accurately weighed, and 4 ml 0.15 M sodium chloride were added, sonicated for 10 minutes and mixed with 4 ml of ethanol (0.1% BHT). Ten ml of hexane were added and then the samples were centrifuged at 3120g for 5 min at 4°C in a 4200 centrifuge (Kubota Corporation, Japan). The organic phase was separated and evaporated to dryness with nitrogen, reconstituted with 200 µL of 2- propanol and 800 µL of acetonitrile

and filtered through a PTFE filter (0.2 µm porosity). The filtrate was analysed by HPLC -DAD-MS. Three replicates were done for each sample.

Chromatographic conditions are summarized in table S 1 (supplementary). Three replicates were taken for each sample. The whole procedure was carried out quickly to minimize exposure to light and oxygen. Several standard solutions of varying concentrations, 0.05 µg/g to 2 µg/g, from a mixed stock solution of α-tocopherol and β-carotene and 0.1 µg/g to 5 µg/g from a stock solution of Coenzyme - Q was prepared in ethanol to determine a suitable working range for routine analysis.

Vitamin C analysis

The extraction of Vitamin C was carried out following the method of O. Parbhunath [38]. Briefly, 200 mg of sample were mixed with 25 mL of 4.5% metaphosphoric acid solution (Sigma Aldrich Chemie, Sant Quentin Fallavier, France). Solutions were centrifuged at 3000g for 10 min (Kontron Centrikon T-2000, Italy). Supernatants were filtered through ACRODISC, 13 mm mini spike 0.2 µm GHP filters and transfer to amber vials for analysis.

Identification and quantification of Total L-ascorbic acid by HPLC with UV detection

Chromatographic conditions are summarized in table s1 (supplementary). Quantification of

Vitamin C (as the sum of AA and dehydroascorbic acid, DHAA) was done with an external calibration of L-ascorbic acid (L-AA) (Sigma Aldrich Chemie, Sant Quentin Fallavier, France) at concentrations ranging from 1 to 200 ppm.

Instrumental colour analysis during retail conditions display

Initial colour measurements (day 1 of the display) were taken after 30 min of bloom time from patty preparation and packaging. The instrumental colour of each batch of patties was measured at three points on the surface, obtaining the mean value, at time 0 and 6 days of the display, using a portable spectrophotometer MINOLTA CM-600d, (Konica Minolta INC, Japan), with illuminant D65, 10° standard observer and 8 mm opening size. Results were reported as the average of three consecutive measurements from random locations of each patty.

HPLC parameters	β -carotene, Tocopherol, Coenzyme Q			Vitamin C
Chromatographic system	Waters 1525 HPLC system (Waters, Milford, MA, USA)			Agilent 1100 series HPLC system (Agilent Technologies, USA)
Detector/s	Diode array detector (Water 2996, Waters, Milford, MA, USA)			UV detector
	Fluorescence detector (Waters 2475, Waters, Milford, MA, USA).			
Column	Luna® 5 μ m C18 (2) 100 Å, LC Column 250 x 4.6 mm (Phenomenex, Spain).			RP C18 column (1.8 μ m x 1.0 x 150 mm) (Waters, USA)
Column Temperature	Room temperature			Room temperature
Mobile Phase	A = Acetonitrile			A: Milli- Q water/ acetonitrile/ formic acid
	B = 20% Acetonitrile:Tetrahydrofuran			99: 0.9: 0.1, v/v/v, pH 2.6
Elution profile	time	%A	%B	Isocratic
	0	100	0	
	3	90	10	
	12	0	100	
	12.1	100	0	
Flow rate	1 mL/min			1 mL/min
Injection volume (μ L)	10 μ L			30 μ L
DAD	β -carotene: 445 nm			245 nm
	Coenzyme Q: 275 nm			
Fluorescence	Tocopherol	Excitation: 280 nm		
		Emmision: 330 nm		

Table S1: Instrument conditions for vitamin analysis.

Volatile compounds analysis of raw and cooked patties

Lipid oxidation was assessed by determining the lipid-derived volatiles pentanal, hexanal, heptanal, octanal and nonanal according to M. Estévez [39]. Two batches of MBP patties were prepared, i) one batch of raw patties of five treatments (Figure 1), and ii) the second batch of cooked patties under refrigerated conditions (2°C by 4 days) of five treatments.

Solid-phase micro extraction for lipid-derived volatile compounds

Two grams of the homogenized patty was mixed with 1800 μ l of saturated sodium chloride solution placed in a 20 ml vial. The vial was then placed in the water bath at 37° C for 30 min.

Then, the SPME fibre assembly (divinylbenzene/carboxen/polydimethylsiloxane, 50/30 μ mSupelco, Bellefonte, U.S.A.) was exposed to the headspace while the sample equilibrated during 30 min immersed in water at 37° C. Three replicates were prepared for each sample.

Gas chromatography-mass spectrometry (GC-MS) analysis of SPME extracts

Analyses were performed on a 7890-5975gC-MS system coupled to a mass selective detector (triple-axis Detector, Agilent 5975C). Volatiles were separated using a column (DB-5. 30m, 0.25 mm id, 0.25 μ l film thickness; Agilent 122-5532UI). The flow rate was 2mL/min.

The split less injection was used, and the injection volume was 2 μ L. The injection temperature was 250 °C. Operation parameters for mass spectra registration were as follows: mass range, 29400. EL mode (1294 eV), carrier gas, helium; interface temperature, 300 °C; source temperature, 200 °C; temperature program, 35 °C for 3 min followed by a ramp rate of 4 °C/min to 250 °C, held for 5 min.

Experimental linear retention indices (LRI) were calculated about the retention times of a series of standard/alkanes, run under the same GC/MS conditions. Identifications of compounds were based on the correlation of MS data with spectra in the NIST/Adams MS library and by comparison of experimental LRI values with LRI data published for authentic compounds. Where both MS and LRI data was consistent with those in the literature and obtained for authentic compounds, identifications were considered to be positive. When MS data agreed but no reference LRI data were available, identifications were considered to be tentative.

Volatile compounds were identified by comparison of each mass spectrum with spectra from authentic compounds analysed in Flavour chemistry laboratory of University of Reading, spectra from NIST/Adams Mass Spectral database (Version 2.0 a, 2002). To confirm the identification, the linear retention index (LRI) was calculated for each volatile, using the retention times of a homologous series of C5-C22 n-alkanes and by comparing the LRI with those of authentic compounds analysed under similar conditions [40]. The results were provided in arbitrary area units (AAU $\times 10^3$).

Sensory analysis

The sensory evaluation of the samples (patties with 23% and 15% fat) was carried out by eight selected and trained assessors [41,42]. Before the sensory analysis, two groups of patties for each treatment were considered. In one group, samples were warmed up in a double preheated hot-plate grill and they were cooked at 73 °C for 3 min (0 days of storage); the second group, were left under refrigeration conditions of 2 °C for 4 days (storage time), to generate the oxidation of the patties. On the day of the sensory test, this group of patties was reheated (73 °C for 3 min) before the test.

Each patty was cut into eight pieces of 3 x 2.7 x 1.5 cm serving samples. The generation of the descriptors of patties was carried out by open discussion in three previous sessions to the test, looking for traditional characteristics in flavour and texture. Then

the description of each descriptor was agreed as well as the scoring scale. The selected descriptors to describe the samples were: i) odour: Intensity, warmed-over odour or rancid, rose hip odour, ii) flavour: saltiness, WOF (warmed-over flavour) or rancid flavour, rose hip, acid, greasy, metallic/liver and iii) texture: juiciness, grainy, crumbliness and oiliness.

Samples were coded with three-digit random numbers and were presented to the assessors in ten different sessions balancing the first-order and the carry-over effects [43]. A 0-10 scoring scale was used for analysis, where 0 indicates the absence of the descriptor and 10 indicates the higher intensity of each descriptor.

Data analysis

Chemical and instrumental data analyses were performed using the SAS 9.4 software (SAS Institute Inc., Cary, NC, USA, 2012). The model included the treatment, the fat content and their interaction as fixed effects. Least-squares means (LSMeans) were separated by Tukey's test ($p < 0.05$). Statistical analysis was performed with Proc GLM -SAS 9.4 software.

Concerning antioxidants, colour parameters (L^* , a^* , b^*) and volatile compounds data, in order to improve the normality and homogeneity of variance of data set (both requirements for linear model application), values were transformed using natural logarithmic transformation, and results were back-transformed for presentation. In the case of colour parameters model included the treatment, fat and time (0 and 6 days) and their interactions as fixed effects. The volatile compounds model incorporated treatment, fat and their interaction as fixed effects. The estimation method was a restricted maximum likelihood and a Residual approximation was used to determine degrees of freedom. Statistical analysis of volatiles was executed with Proc GLIMMIX- SAS 9.4 software.

For the sensory scores, data were subjected to analysis of variance (ANOVA), including oxidation effect in the statistical model, using the XLSTAT 2014 software (Addinsoft, Paris, France). Panelists and sessions were not taken into account for the model.

Results and Discussion

Ground MBP composition

The contents of fat, protein and moisture were 23, 17 and 60%, respectively in-ground MBP meat of batch 1 whereas those of batch

2 were 15, 18 and 66%, respectively. Both batches were used for the preparation of experimental patties as designed.

Effect of rose hip and fat percentage of patties on their fatty acid composition

In this study, treatments enriched with rose hip (1.5 and 3% w/w) had no significant effect on the fatty acid (FA) composition of MBP patties and because of this, the results are not presented in a Table. Conversely, a significant effect of the fat content on the main fatty acids was found (Table 1); oleic acid content was significantly higher in patties with 23% fat (47.55%) in comparison to patties with 15% fat (46.54%). The properties of technological meat quality influenced by fatty acids are fat tissue firmness (hardness), shelf life (lipid and pigment oxidation) and flavour [44].

The results of this study in patties differed from those of J. Wood [44]: this autochthonous breed had higher levels of oleic acid (47%) and lower levels of linoleic and linolenic acids (6% and 0.4%, respectively), which could make the meat of MBP less susceptible to lipid oxidation.

Our results differed also from those obtained by J. Gonzalez [6] on MBP, since in the present research less percentage of linoleic acid and higher percentage of oleic acid were found. In addition, an increase in the ratio of n-6: n-3 from 10.30 to 18.06 was observed, which could be due to the fact that the patties contained a mixture of back fat, inter and intramuscular fat. Despite this, the n-6: n-3 fatty acids ratio found in MBP fat was still lower than those found in other pig breeds (intensive production) as reported by previous studies, who showed values around 23 (*Longissimus thoracis* intramuscular fat of York-sired pigs) [45].

Fatty acids (%)	Structure	Fat		P value	RMSE
		23%	15%		
Palmitic	C16:0	23.82 ^a	22.92 ^b	< 0.001	0.434
Stearic	C18:0	11.16	11.10	ns	0.595
Oleic	C18: 1 (n-9)	47.55 ^a	46.54 ^b	< 0.001	0.844
Linoleic	C18: 2 (n -6)	6.19 ^b	7.05 ^a	< 0.001	0.239
Linolenic	C18:3 (n-3)	0.36 ^b	0.38 ^a	< 0.001	0.019
	n-6 /n-3	18.09 ^b	20.53 ^a	< 0.001	0.987
	PUFA/ SFA	0.19 ^b	0.24 ^a	< 0.001	0.013

Table 1: Least-squares means of the main fatty acid profile (%) and nutritional ratios from MBP patties (raw) with 15% and 23% fat.

Values are the LSMEAN of triplicate analysis (patty: 100g). Expressed as % Fat: 23% and 15% fat.

RMSE: Root mean square error. Different letters (a, b) within the same row indicates significant differences between treatments. ns: not significant.

Effect of treatment and fat content on the antioxidant composition

Table 2 shows the effects of treatment (T) and fat (F) content on the quantity of antioxidants detected in the MBP patties (β -carotene, coenzyme Q and α-tocopherol), as well as the interaction (T*F). No significant differences were found between treatments for α-tocopherol, β-carotene and coenzyme Q. The treatments (T₂ to T₅) had similar amounts of these antioxidants, which differed significantly from T₁, with no rose hip added and only ascorbic acid, where no detectable concentrations of these compounds

were found. These results may have been influenced by different factors, such as the origin of the rose hip, treatment of the sample, temperature and oxygen, since these antioxidants are thermolabile and photosensitive [46]. Significant variations in organic acids, phenolics, water soluble vitamins, and minerals of RC have been reported over the years by various researchers [47].

Regarding Vitamin C, significant differences were observed between treatments. T₁ (control) patties contained ascorbic acid additive (E 300), T₄ and T₅ patties contained two sources of this vita-

min, commercial ascorbic acid and RC extract and T₂ and T₃ patties contained only RC extract as a natural source of this antioxidant. Regardless of the fat content, significantly higher concentrations of AA were found in T₁, T₄, and T₅ compared to T₂ and T₃, as expected. The ascorbic acid from RC extract gave an added value to the nutritional composition of the patties (T₂ and T₃). Apart from slightly contributing to reduce the use of ascorbic acid in their formulation in relation to the control patties, as the rose hip extract provided small amounts of other natural antioxidants together with ascorbic acid, the patties were enriched with a pool of different antioxidants. Previous studies have showed that RC was suitable for use as a functional ingredient in raw pork burgers patties (250g of water solution of *Rosa canina* L, [31]), beef patties (50g *Rosa canina* L water extract [48]), and porcine frankfurters (5g/kg and 30g/kg *Rosa canina* L [30]) because of its content in ascorbic acid and its health benefits [46]. RC extract was very useful in processed meats, more specifically in pig burgers, since it enhanced oxidative stability, colour, texture and delayed lipid and protein oxidation [31].

Concerning the effect of the patties fat content on these variables, only significant differences (p < 0.001) were observed for

α-tocopherol. The patties with 15% fat had a higher content of α-tocopherol (1.82 mg/kg) compared to the patties with 23% fat (1.20 mg/kg). These results can be explained as a chemical reaction among tocopherol with the number of lipid radicals available. Previous studies reported that lipid oxidation depends on the numbers of available lipid radicals that react with molecular oxygen [49]. The chain reaction propagates itself and it ends when an inactive substance is formed such as α-tocopherol (vitamin E) [49]. This result suggested that the patties with 15% fat would be healthier because of their lower fat percentage and because they contained higher concentrations of natural antioxidants, especially α-tocopherol. So, although the amount of the vitamins were relatively small, it could be said that these patties were enriched and that this could also positively influence their shelf life. Concerning this, previous studies showed that while vitamin E had a negative correlation with hexanal displayed its antioxidative effect and thus, its ability to preserve sensory fresh meat flavour/odour [16]. Also, RC extract could contribute with small doses of vitamins E and C to the nutritional value of the meat products.

Antioxidants	Treatment*Fat					P value			RMSE
	T ₁	T ₂	T ₃	T ₄	T ₅	T	F	T*F	
β-carotene									
23%Fat	nd	0.1	0.05	0.04	0.09	0.571	0.129	0.105	0.634
15% Fat	nd	0.05	0.11	0.15	0.14				
Coenzyme Q									
23%Fat	nd	1.73	1.89	1.6	1.11	0.239	0.263	0.846	0.329
15% Fat	nd	1.79	2.03	1.90	1.57				
α- Tocopherol									
23%Fat	nd	1.4 _y	1.34 _y	1.52 _y	1.69 _y	0.891	< .0001	0.115	0.157
15% Fat	nd	2.44 _x	2.3 _x	2.36 _x	2 _x				
Vitamin C									
23%Fat	180.6 ^b	11.7 ^d _y	41.9 ^c	190.2 ^{ab}	245 ^a	<.0001	0.004	< .0001	0.1546
15% Fat	215.6 ^a	27.3 ^d _x	45.3 ^c	196.9 ^a	196.3 ^a				

Table 2: Least-squares means of natural antioxidants from MBP patties (raw) with 15 % and 23% fat enriched with rose hip (RC, *Rosa canina* L.).

Values are the LSMEAN of triplicate analysis (patty: 100g). Expressed as (mg/kg).

Treatments: T₁ : (0.03 g ascorbic acid, AA); T₂ : (1.5 g RC); T₃ : (3.0g RC); T₄ : (0.03g AA +1.5g RC); T₅ : (0.03 g AA + 3.0g RC). RMSE: Root means square error. Different letters (a, b, c, d) within the same row indicate significant differences between treatments. Different letters (x, y) within the same column indicate significant differences between percent fat. nd: no detectable.

Instrumental colour analysis of MBP patties during retail conditions display

There were no significant differences ($p > 0.05$) of the main effects (fat, time and treatment). The significant interactions were Fat * time for Lightness (L^*), and Treatment * time for redness (a^*). In the case of yellowness (b^*), there were significant differences in the interaction Treatment 346 *time (Table 3).

Meat ground from this study can be considered dark and red, as it was shown by the L^* and a^* values (47.9 and 6.9, respectively) at 6 days. These values were similar to those obtained in Iberian 349 pigs or crosses of them [50,51]. L^* value among MBP patties with 23% and 15% of fat, presented significant ($p > 0.05$) differences (51 and 43, respectively) at 0 days and (48 and 44) at 6 days, respectively with the highest values attributed to the patties with the highest fat content (Table 3). The value of L^* and a^* reflected the physical state of the meat, the structure of the muscle fibres and the amount of light [52].

The high values of a^* might be explained because this colour component is related to the muscle pigment content, which increases during the animals' life; therefore animals slaughtered at heavy weights are more prone to produce more intense red meat [53], as it is the case of MBP (slaughter weight around 150 kg) [54]. The changes in the a^* and the oxymyoglobin values appear to be driven by lipid oxidation [53,54].

Regarding b^* value, there were no significant differences between treatments and the amount of fat. These results may indicate that the chemical state of the MBP meat pigment (b^*) did not change. Previous authors reported that the relationship between lipid oxidation and the pigment that gives meat colour, myoglobin, is proportional [57]. Also reported that a rapid reduction of meat redness is mainly due to lipid oxidation, while the antioxidants could retard such a decrease [58].

Treatment *Fat (T*F)						Treatment * time (T*t)						Fat*time (F*t)			P value						
L*	T ₁	T ₂	T ₃	T ₄	T ₅	L*	T ₁	T ₂	T ₃	T ₄	T ₅	L*	22% F	15%F	T	F	t	T*F	T*t	F*t	RMSE
22% F	47	50	49	50	49	0 days	46	47	46	47	46	0 days	50 ^a _x	43 ^b	0.84	<.001	0.66	0.63	0.9	0.01	0.07
15% F	44	43	44	43	43	6 days	46	47	47	46	45	6 days	48 ^a _y	44 ^b							
a*						a*						a*			0.06	0.19	<.001	0.06	0.003	0.57	0.12
22% F	8 ^b _y	10 ^a	10 ^a	9 ^a _y	10 ^a	0 days	12 _x	12 _x	13 _x	12 _x	11 _x	0 days	12 _x	12 _x							
15% F	10 ^a _x	9 ^b	10 ^a	11 ^a _x	10 ^a	6 days	7 ^{bc} _y	7 ^c _y	7 ^{abc} _y	9 ^a _y	8 ^{ab} _y	6 days	7 _y	8 _y							
b*						b*						b*			0.25	0.36	0.06	0.17	0.02	0.06	0.08
22% F	14	15	16	15	14	0 days	16 _x	15	16	14	14	0 days	15 ^a _x	15 ^b							
15% F	15	14	15	15	14	6 days	13 ^b _y	14 ^a	15 ^a	16 ^a	14 ^a	6 days	14 _y	15							

Table 3: Least-squares means of lightness (L^*), redness (a^*) and yellowness (b^*) in MBP patties (raw) enriched with rose hip (RC, *Rosa canina* L) for 0- and 6-days storage under retail display conditions in the main effects.

Values are LSMEAN (n=3, patty: 100g): L^* (lightness), a^* (redness), b^* (yellowness). Fat: 23% and 15% fat, time: 0 days, 6 days. Treatment (T): T₁:0.03 g ascorbic acid, AA; T₂: 1.5 g RC; T₃: 3 g RC; T₄: 0.03 g AA +1.5 g RC; T₅: 0.03 g AA + 3.02 g RC. RMSE: root means square error. Different letters (a, b) within the same row indicate significant differences between treatments or percentage of fat; and different letters (x, y) within the same column indicate significant differences between percentage fat or between time zero and six days; $p > 0.05$: not significant.

Volatile compounds of raw and cooked MBP patties as part of the oxidation process

The ANOVA test results of the volatile compounds derived from the lipid oxidation of the raw and cooked patties are summarized in Tables 4 and 5, respectively. The interaction among Treatment and fat was significant ($p < 0.05$).

In raw MBP patties (frozen at -20°C for 6 months) the following four volatile lipidaldehydes were identified: pentanal, hexanal, heptanal and nonanal; each of them presented significant differences in the interaction between treatment and fat ($T * F$, $p < 0.05$) (Table 4). In general, these aldehydes were found in every treatment. But, only the treatments enriched with rosehip and ascorbic acid (T_4 and T_5) as well as the control (T_1) in 15% fat (heptanal, nonanal) presented greater antioxidant power since the areas of these four volatile compounds were smaller than in the other treatments.

In the case of cooked (78°C for 4 min) and oxidized (2°C for 4 days and reheated) MBP patties, octanal was also identified. There were significant differences in the interaction between the treatment and the fat percentage ($p < 0.0001$) for all five lipid-derived volatile compound (Table 5). It was also observed that the treatments enriched with ascorbic acid and rosehip extract (T_4 and T_5) have greater anti-oxidizing power since the amounts of all the volatile compounds formed were smaller. Although, in the treatment (T_3) area values were even smaller than T_4 for pentanal, hexanal, heptanal, octanal at patties with 23% fat in comparison to the relation of the T_3 of patties with 15% Fat. Concerning the fat effect, the presence of these lipid-derived volatile compounds seemed to be correlated with the amount of fat present in the patties, significantly higher in the treatments with 23% fat, compared to the treatments with 15% fat, thus indicating that patties with a higher amount of fat were more sensitive to lipid oxidation. Moreover, the areas of the volatiles in T_3 with 23% fat were lower than T_4 and T_5 areas. These results can be explained in relation to the content of the 6% of linoleic acid and 47% of oleic acid of those batches of MBP patties (Table 1). The content of these fatty acids influenced the lipid degradation. In the case of batch of patties with 15% fat, those patties had less percentage of oleic acid (46%) and higher percentage of linoleic acid (7%) that the ones of 23% fat (Table 1).

In this sense, the presence of hexanal is reported as the most sensitive indicator of lipid oxidation [59]. But, the other lipid-derived volatile compounds such as heptanal, octanal and nonanal are also taken into account due to their low odour threshold [24]. Moreover, hexanal and heptanal are degradation products from long-chain polyunsaturated n-6 fatty acids, mainly linoleic acid, while nonanal, octanal as well as heptanal arise from the oxidation of monounsaturated n-9 fatty acids, e.g., from oleic acid [24]. Long-chain polyunsaturated fatty acids are known to be less stable towards oxidation compared to monounsaturated fatty acids, which could be a reason that MBP patties had high hexanal area values. In general, i) no dose of RC tested in this study had acted as an efficient antioxidant when used alone in the formulation of treatments T_2 and T_3 (Table 4 and 5). This may be that the effect of certain potential antioxidants may vary considerably depending on a complex interaction between various factors, involving the type and concentration of active components and the nature of the food system [60]. In addition, ascorbic acid may act as a prooxidant in specific conditions, most likely due to the strong reducing power and weak metalchelating ability [61]. Various other studies have reported pro/oxidant effects of ascorbic acid, for lipid or protein oxidation in pork [62], frankfurters [63] and chicken [64]. But, in the case of patties under oxidative conditions, ii) the treatments enriched with a mixed of ascorbic acid and rose hip extract showed clear antioxidant activities compared to the control (Table 5).

It can be concluded that neither AA nor RC extract on their own were as effective as the combination of AA and RC extract in reducing the formation of lipid-derived volatiles. However, this could be a good alternative for meat product industry, because with this combination of antioxidants such as ascorbic acid and rose hip, the product can be declared as ECO or "Clean Label".

Sensory analysis of cooked MBP patties under and without oxidation conditions

Table 6 summarises the mean panel scores of the sensory attributes for the MBP patties (T_1 , T_2 , T_3 , T_4 and T_5), storage time after cooking (0 days, under 2°C by 4 days) and two fat levels. The most relevant differences were the effect of the Treatment and storage

	Treatment					P value			
	T ₁	T ₂	T ₃	T ₄	T ₅	Treatment	Fat	T*F	RMSE
Pentanal									
23%Fat	31	16 _y	18	5 _y	8	0.089	0.021	0.026	0.641
15% Fat	11	50 _x	30	24 _x	18				
Hexanal									
23%Fat	526 ^a	402 ^{ab} _y	397 ^{ab}	173 ^{bc}	109 ^c _y	0.005	0.114	0.032	0.527
15% Fat	267 ^b	990 ^a _x	355 ^b	213 ^b	391 ^b _x				
Heptanal									
23%Fat	21 ^a _x	27 ^a	24 ^a	14 ^{ab}	11 ^b	0.001	0.289	0.023	0.384
15% Fat	7 ^d _y	23 ^{ab}	34 ^a	11 ^{cd}	16 ^{bc}				
Nonanal									
23%Fat	24 ^c _x	126 ^a _x	55 ^b	53 ^b	33 ^{bc}	<.0001	0.003	0.040	0.424
15% Fat	7 ^b _y	38 ^a _y	53 ^a	40 ^a	35 ^a				

Table 4. Volatile compounds (AAU*) from lipid oxidation of MBP raw patties with 23 and 15 % fat enriched with rose hip extract (RC, *Rosa canina* L).

*Arbitrary area units: AAU x10³. Treatment (T): T₁: 0.03 g ascorbic acid, AA; T₂: 1.5 g RC; T₃: 3 g RC; T₄: 0.03 g AA +1.5 g RC; T₅: 0.03 g AA +3.02 g RC. Fat: 22% and 15% Fat. RMSE: root mean square error. Different letters (a, b) in the same row indicate significant differences between Treatments (p < 0.05); and different letters (x, y) in the same column indicate significant differences between the percentage of fat (p < 0.05).

Volatile compounds	Treatment					P value			
	T ₁	T ₂	T ₃	T ₄	T ₅	Treatment	Fat	T*F	RMSE
Pentanal									
23%Fat	77 ^a	65 ^{ab} _x	33 ^c	54 ^b _x	17 ^d _x	<.0001	<.0001	<.0001	0.193
15% Fat	64 ^a	11 ^c _y	28 ^b	12 ^c _y	7 ^d _y				
Hexanal									
23%Fat	1351 ^a _x	1700 ^a _x	428 ^b	569 ^b _x	162 ^c _x	<.0001	<.0001	<.0001	0.228
15% Fat	883 ^a _y	116 ^c _y	458 ^b	72 ^d _y	25 ^e _y				
Heptanal									
23%Fat	65 ^a _x	62 ^a _x	22 ^b _x	25 ^b _x	12 ^c _x	<.0001	<.0001	0.0006	0.236
15% Fat	36 ^a _y	10 ^{bc} _y	14 ^b _y	8 ^{cd} _y	6 ^d _y				
Octanal									
23%Fat	27 ^a _x	20 ^a _x	4 ^c	8 ^b _x	2 ^c	<.0001	<.0001	<.0001	0.294
15% Fat	14 ^a _y	2 ^c _y	3 ^{bc}	1 ^d _y	4 ^b				
Nonanal									
23%Fat	87 ^a _x	66 ^a _x	63 ^a _x	23 ^b _x	13 ^b	0.0006	<.0001	0.0174	0.453
15% Fat	21 _y	14 _y	17 _y	8 _y	17				

Table 5: Volatile compounds (AAU*) from lipid oxidation of cooked-oxidized MBP patties with 23 and 15 % fat (under 2^oC by 4 days) enriched with rose hip extract (RC, *Rosa canina* L).

*Arbitrary area units: AAU x 10³. Treatment (T): T₁: 0.03g ascorbic acid, AA; T₂: 1.5g RC; T₃: 3g RC; T₄: 0.03g AA +1.5g RC; T₅: 0.03g AA +3.02g RC. Fat: 22% and 15%. RMSE: root mean square error. Different letters (a, b, c) within the same row indicate significant differences between treatments (p < 0.05); and different letters (x, y) within the same column indicate significant differences between percentage of fat (p < 0.05).

time after cooking which are detailed below. No significant differences were found between their interactions.

Treatment effect

Odour parameters such as intensity, warmed-over odour or rancid showed significant higher scores in the control treatment (T_1) with respect to the rest of the treatments. These results suggest that the rose hip could mask the rancid odour in the patties, highlighting the potential of rose hip for this application. Concerning WO odour, previous authors showed that RC extracts controlled the lipid oxidation under refrigerated storage (150 days) in cooked ham, which is due to the presence of ascorbate (vitamin C) as a natural antioxidant of rose hip [65].

Flavour attributes such as saltiness and greasy of patties were not affected by the formulation of each treatment; WOF flavour had the same behaviour as the odour attributes of MBP patties, and acid and metallic flavour had significant differences but were not relevant because the scores were very low. These results suggested that WOF can be masked by rose hip extract, and as consequence, our study suggests that RC could reduce the off-flavours in oxidized-cooked MBP patties. These results are also showed in frankfurters during 60 days of chilled storage [30].

Concerning texture descriptors, there were no significant differences among the scores of control treatment (T_1) and the treatments enriched with rose hip. That meant that the addition of different amounts of RC did not affect the texture of the patties. Similar results were reported by other authors, who showed that the addition of RC improved the colour of cooked burger patties with no apparent drawbacks in textural properties [66]. However other studies reported that RC changed slightly the texture and it also contributed to the pink colour formation of the frankfurters [30,48].

Storage time after cooking and level of fat effect

Two groups of patties were evaluated: i) patties were cooked (at 73°C for 3 min) just before the sensory test and ii) patties were cooked and then were left under refrigeration conditions (2°C for 4 days), causing the oxidation of the patties. On the day of sensory test, this last group of patties were reheated before the test session. Regarding the oxidation effect, significant differences were found

in the intensity and WO odour among the patties that were under oxidative conditions and patties that were not. Although there were significant differences in the smell of WO, the scores were low in the two groups of patties. Additionally, the rose hip odour received low scores by panelists in the two types of cooked patties under storage conditions, with no significant differences between them, thus suggesting an effect of RC to prevent off-odours.

Regarding flavour, significant differences were observed in WOF and metallic attributes. The WOF had similar behaviour as the WO odour, although this parameter showed significant differences, the scores were low in both patties under and without storage time. The rose hip flavour did not show significant differences and its scores were low in the two kinds of patties, suggesting that rose hip extract did not influence negatively the taste of the patties. Moreover, RC extract could be used as a functional ingredient, it even could enhance the shelf life of meat products, and also as an ingredient to control or mask the WOF development in meat products sensitive to lipid oxidation during refrigerated storage.

According to this, R. Ganhão [10] reported that using RC extract as an ingredient in patties may be an efficient strategy to enhance the nutritional value, safety and sensory parameters of meat products. E. Saldaña [8] reported that the main effect of the use of these natural antioxidants of different sources is to prevent lipid oxidation and consequently the development of rancid odour, off-flavours and meat discolouration due to myoglobin oxidation. In addition, R. Pegg and F. Shahidi [67] indicated that those flavours are considered undesirable flavours in meat patties, which have always been a problem in lipid-rich foods. Prevention of WOF may be achieved to some degree by the incorporation of antioxidants and chelators into foods [67], such as the use of RC in this study. On the other hand, E. Saldaña [8] reported that depending on the origin and concentration of the natural antioxidant, it can interfere with the colour, odour and taste of the meat product, decreasing the sensory liking. This negative effect of natural antioxidants in sensory properties has been widely reported by several studies, such as chitosan and propolis [68], oregano extract [69] or olive extract [70] on different meat matrices.

Concerning metallic (Liver) flavour, the patties under oxidation conditions had significantly lower scores than those not oxidized,

probably because of the influence from WOF. Previous studies have indicated that WOF is associated with the auto/oxidation of poly-unsaturated fatty acids, and that iron, is an important catalyst for the reaction [21,71-74].

Regarding the texture, the trained panellists did not find significant differences between the patties under oxidation conditions and those that did not suffer oxidation. However, M. Utrera and E. Vossen [30,46] showed that RC modified slightly the texture of the frankfurters, so these apparent contradictory results can indicate that the matrix is influencing the result. For this reason, using RC as a natural antioxidant in patties, may be an advantage, as it has not influenced the quality and texture of the product.

Fat effect

Regarding the fat effect, though there were significant differences in some flavour and texture parameters in the two types of patties, the scores given by the panellists were low. This means that the sensory quality of the burgers was not affected.

Overall, the results of the sensory analysis and the composition of volatile compounds derived from lipid oxidation have showed that RC may help to prevent oxidation and mask off-flavours in the cooked patties.

Sensory descriptors	Treatment						Storage time after cooking (Oxidation)			Fat			RMSE
	T1	T2	T3	T4	T5	P value	0 days	4 days	P value	23%	15%	P value	
Odour													
Intensity	5.0 ^a	4.6 ^{ab}	4.4 ^b	4.6 ^b	4.5 ^b	< 0.001	4.5 ^b	4.8 ^a	< 0.01	4.6	4.6	ns	0.96
WO/ rancid	2.5 ^a	1.4 ^b	1.0 ^b	1.3 ^b	0.8 ^b	< 0.001	0.7 ^b	2.1 ^a	< 0.001	1.5	1.3	ns	1.50
Rose hip	0.3 ^b	0.5 ^{ab}	0.7 ^a	0.6 ^{ab}	0.8 ^a	< 0.001	0.6	0.5	ns	0.6	0.5	ns	0.94
Flavour													
Saltiness	3.2	3.2	3.1	3.2	3.4	ns	3.2	3.2	ns	3.3	3.2	ns	0.93
WOF/rancid	3.0 ^a	1.8 ^b	1.2 ^{bc}	1.3 ^{bc}	0.8 ^c	< 0.001	0.7 ^b	2.5 ^a	< 0.001	1.8 ^a	1.3 ^b	< 0.01	1.57
Rose hip	0.5 ^b	0.7 ^b	1.5 ^a	0.9 ^{ab}	1.4 ^a	< 0.001	1.0	1.0	ns	1.0	1.0	ns	1.23
Acid	1.2 ^{ab}	1.2 ^{ab}	0.9 ^b	1.2 ^{ab}	1.3 ^a	0.05	1.2	1.1	ns	1.1	1.2	ns	0.91
Greasy	2.3	2.4	2.2	2.3	2.2	ns	2.2	2.3	ns	2.5 ^a	2.1 ^b	< 0.01	1.08
Metallic /Liver	2.0 ^a	1.5 ^{ab}	1.2 ^b	1.7 ^{ab}	1.6 ^{ab}	< 0.001	2.0 ^a	1.2 ^b	< 0.001	1.4 ^b	1.8 ^a	< 0.05	1.17
Texture													
Juiciness	3.8	3.9	3.7	3.8	3.6	ns	3.7	3.8	ns	4.1 ^a	3.5 ^b	< 0.001	0.94
Grainy	3.5	3.5	3.5	3.5	3.5	ns	3.5	3.5	ns	3.4 ^b	3.7 ^a	< 0.05	0.95
Crumbliness	4.3	4.5	4.3	4.5	4.5	ns	4.4	4.5	ns	4.4	4.5	ns	0.73
Oiliness	2.9	2.8	2.7	2.8	2.4	ns	2.6 ^b	2.8 ^a	< 0.05	3 ^a	2.4 ^b	< 0.001	1.00

Table 6: Effect of the addition of RC (Treatments: T₁ (control), T₂, T₃, T₄ and T₅), and storage time after cooking (0 days and under 2°C for 4 days), in the mean values of sensory scores of the cooked MBP patties.

Values are Mean (n = 80, patty: 100g) in the same row with uncommon letters (a, b, c) are significantly different, ns: not significant. RMSE: root means square error. Treatment (T): T₁: 0.03g ascorbic acid, AA; T₂: 1.5g RC; T₃: 3g RC; T₄: 0.03g AA + 1.5g RC; T₅: 0.03g AA + 3.02g RC. Storage time after cooking (Oxidation): (0 days: means patties cooked before tasting (no oxidation); 4 days: means patties cooked, stored at 2 °C for 4 days, reheated before tasting (with oxidation). Fat (F): 23 % and 15% Fat. Scoring scale (0-10): 0 indicates the absence of the descriptor and 10 indicates the higher intensity of each descriptor.

Conclusion

The rose hip it's a local product from the Mediterranean that grows at different heights on the mountains. Enrichment of MBP patties with (1.5 and 3% w/w) rose hip extract did not affect their fatty acid composition.

The addition of Rose hip extract enriched them mainly with vitamin C, although the studied concentrations did not provide the expected amount of these antioxidants (under the principle Rose hip, a natural source of vitamins). In this sense, future research should test different concentrations of the extract and its influence on sensory traits. The degree of naturalness of the extract could be an alternative to the use of ascorbic acid to the pre-cooked and frozen food industry that is focusing on clean label reformulations.

The higher amount of fat of the patties, the lower was the concentration of α -tocopherol. This result suggested that the patties with 15% fat would be healthier because of their lower fat percentage and because they contained higher concentrations of natural antioxidants, especially α -tocopherol.

The rose hip had a slight effect on the patties colour, but it had greater effect in their sensory attributes. In the patties submitted to oxidation conditions before the sensory test, the rose hip extract together with ascorbic acid minimized WOF odour and flavour. Thus, the use of rose hip (*Rosa Canina* L) extract can be considered as a potential method to enrich pig patties with natural antioxidants, because provoke retard colour change, reduce off-flavours and did not have a negative effect on the texture.

The enrichment of meat products with natural antioxidants from the vegetal food group, as well as the reduction of fatness in patties, is a way on innovation in these agri-food local systems [5]. It is also a way to improve nutritional properties of the patties and a system to exploit little-known natural resources of the territory and therefore help to its sustainability. This principle is in accordance with the recommendations of OMS-Sustainable development goals (SDS).

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