Avocado as a functional ingredient in porcine patties: effect on protein carbonylation

Utrera M.[1], Rodríguez-Carpena J.G.[2], Morcuende D.[1] and Estévez M.[1]

[1] University of Extremadura / Department of Animal Production and Food Science (Food Technology Section) / Cáceres, Spain.
[2] Autonomous University of Nayarit / Department of Food Technology from the Faculty of Veterinary / Tepic, Mexico.

Abstract— **Protein carbonylation is known to affect the nutritional and technological properties of muscle foods. The effects of the addition of avocado oil as a back-fat replacer (50%) (AVOCADO vs. PORCINE) and a phenolic-rich avocado extract (TREATED vs. CONTROL) on the formation of specific protein carbonyls –α-aminoadipic and γ-glutamic semialdehydes (AAS and GGS, respectively)- in porcine patties, was studied. Both semialdehydes were detected and quantified as p-amino benzoic acid (ABA)- derivatized forms using an HPLC-FLD method. The total amount of protein carbonyls was also assessed by using the dinitrophenylhydrazine (DNPH) method. The amounts of both semialdehydes as well as the total amount of protein carbonyls, increased significantly upon cooking and during the following chilled storage. AAS and GGS were found to account up to 70% of the total protein carbonyls in the meat samples. Cooked and cooked & chilled AVOCADO patties had significantly ($p<0.05$) lower amounts of protein carbonyls than PORCINE patties. The addition of the avocado extract also inhibited the formation of AAS and GGS in cooked and cooked & chilled PORCINE patties. The inhibition of protein carbonylation may respond to the protecting effect of bioactive compounds (phenolic compounds, tocopherols and chlorophylls) in the avocado materials. Apparently, the combination of both strategies (back-fat replacement and addition and addition of avocado extract) does not lead to an enhanced advantage. Results from the present study highlight the remarkable technological applications of avocado materials as natural food additives in the design of healthy meat products.**

Keywords— **avocado, α-Aminoadipic semialdehyde, γ-Glutamic semialdehyde.**

I. Introduction

Avocado (*Persea americana* Mill.) is a tropical fruit with great economic importance in Latin America, principally Mexico and the Caribbean countries, the European Union and USA [1]. Consumption of avocado is mainly regarded to the fresh fruit and its oil due to the difficulty of preserving the fruit during long periods. Recent approaches aimed to assess the potential usage of avocado in food processing. Studies on the phenolic content and antioxidant capacity of pulp and residues from avocado fruit *in vitro* and in real food products [2, 3] have highlighted the nutritional and technological benefits of including avocado oil as back-fat replacers in porcine patties [3]. Furthermore, phenolic compounds extracted from the peel and the seed of the avocado fruit have been proved to inhibit lipid oxidation and color deterioration in raw and cooked porcine patties during chilled storage [2, 3]. Whereas the impact of avocado by-products on the occurrence and extent of protein oxidation has also been evaluated, inconclusive results have been obtained [3].

Protein oxidation has been recognized to affect color, texture and nutritional value of muscle foods [4]. However, little is known about the oxidation routes of particular amino acid residues and their impact on meat quality traits. It is, hence, of interest to detect and quantify protein oxidation products and their potential impact on the quality of muscle foods. One of the most remarkable changes in oxidized proteins is the carbonyl gain [4] which is due to an irreversible and non-enzymatic modification of proteins that involves the formation of carbonyls moieties induced by oxidative stress and other mechanisms [4]. The method commonly applied for the detection of protein carbonyls involves the derivatization of carbonyl moieties with dinitrophenylhydrazine (DNPH) [4]. However, this method has various drawbacks, including that lipid-derived carbonyls are also accounted, leading to unreliable results on the actual extent of protein oxidation [5, 6]. The detection of particular protein carbonyls has been proposed as an alternative and more specific procedure to assess protein carbonylation [6]. α-Aminoadipic semialdehyde (AAS), main oxidation product from lysine, and γ-glutamic semialdehyde (GGS), oxidative degradation form of arginine and proline, have been reported to be the most abundant carbonyls in oxidized plasma and liver proteins [7].

These compounds were firstly detected in food proteins by liquid chromatography coupled to mass spectrometry [6]. In a recent attempt to develop a specific and routine method for the analysis of these compounds in meat systems, Utrera *et al*. developed a procedure based on the separation, identification and quantification of p-amino benzoic acid (ABA)-derivatised forms of AAS and GGS by using fluorescent HPLC [8]. The present paper employs the aforementioned procedure to evaluate the effect of avocado oil and avocado peel extract on the formation of both semialdehydes in burger patties subjected to cooking and chilling procedures.

II. Materials and methods

A. Materials

All chemicals were supplied from Panreac (Panreac Química, S. A., Barcelona, Spain), Merck (Merk, Darmstadt, Germany) and Sigma Chemicals (Sigma–Aldrich, Steinheim, Germany). Porcine *longissimus dorsi* muscle and pork back-fat were purchased in a local butchery in Cáceres (Spain). "Hass" avocado fruit was bought from a local supermarket in Madrid (Spain) and avocado oil was acquired in a supermarket in Mexico City (Mexico).

B. Methods

Antioxidant Extracts Peel extracts were obtained from 10 g of peel from "Hass" avocado treated twice with 30 mL of acetone/water (70:30 v/v) evaporated and redissolved in 50 g distilled water [3].

Manufacture of porcine patties Depending on the partial replacement of porcine back-fat by avocado oil [PORCINE (P) *vs*. AVOCADO (A)] and the addition of avocado peel extract [CONTROL (C) *vs*. TREATED (T)], four types of burger patties were prepared, namely, PC, PT, AC and AT. Ingredients per kg of porcine patty (P) were as follows: 700 g porcine *longissimus dorsi* muscle, 180 g distilled water, 100 g pork back-fat and 20 g NaCl. Patties were manufactured according to the procedure described by Ganhão, et al [9]. For the elaboration of A-patties 50 g of pork back-fat was replaced by 50 g of avocado oil per kg of burger patty. For the manufacture of T-patties 50 g of the distilled water was replaced by 50 g of a water solution extract. Eighteen patties per type were produced in two independent manufacturing processes (9 patties per type each time). Depending on the processing treatment applied, the eighteen patties were divided into three different subgroups: Raw (R), Cooked (CO) and Cooked & Chilled (CC) burger patties (n=6 per type of burger patties and processing treatment). R-patties were frozen (-80 ºC) the day of manufacture until the analytical experiments. CO-patties were cooked at 170 ºC for 18 min in a forced-air oven and allowed to cool down at room temperature. CC-patties were, upon cooking, stored for 15 days at +5 ºC under white fluorescent light. CO and CC-samples were also frozen (-80 ºC) until required for analysis.

HPLC-FLD analysis of AAS and GGS Samples (5 mg protein) were derivatized with 50 mM ABA and subsequently hydrolyzed with 6N HCl. Hydrosylates were dried *in vacuo*, reconstituted with 200 µL milli-Q water and filtered through PVDF syringe filter. Samples were injected in a HPLC as described by Utrera *et al* [8]. Peaks areas corresponding to AAS-ABA and GGS-ABA were plotted against an ABA standard curve. Results are expressed as nmol of carbonyl compound per mg of protein.

Determination of Total Protein Carbonyls by the DNPH method Total protein carbonyls were quantified in burger patties according to the DNPH method described by Ganhão, *et al* [8]. Protein concentration was calculated from absorption at 280 nm using BSA as standard. The amount of carbonyls was expressed as nmol of carbonyl per mg of protein using an absorption coefficient of 21.0 nM^{-1} x cm^{-1} at 370 nm for protein hydrazones.

C. Statistical analysis

Eighteen burger patties per batch were prepared in two independent manufacturing processes (nine patties per treatment -PC, PT, AC & AT- each time) and used as experimental units for each technological process –R, CO, & CC- (n=6). Data were analyzed by Analyses of variance (ANOVA) using a two-way model and Tukey tests at 95% confidence level by SPSS (v. 15).

III. Results and discussion

The technological process applied to the burger patties caused successive increments in the amount of protein carbonyls in all groups of samples in the following order: R < CO < CC (Table 1). These results prove that amino acids from muscle proteins, namely lysine, arginine and/or proline, are oxidatively modified during meat cooking and the subsequent chilled storage to yield AAS and GGS. These compounds are formed as a result of the oxidative deamination of the side chains from the aforementioned basic amino acids in the presence of reactive oxygen species (ROS) and transition metals such as iron [4].

In addition, the present paper provides, for the first time, quantitative data on the amount of AAS and GGS in meat systems. AAS was more abundant than GGS. The total amount of protein carbonyls as quantified by the DNPH method was noticeably higher than the sum of AAS and GGS (Table 1). Both semialdehydes accounted for 17-41% of the total protein carbonyls, these data are in agreement with previous reports on biological systems [7], which stated that AAS and GGS comprised between 23-60% of the total protein carbonyls. As mentioned, the quantification of protein carbonyls by the DNPH method may lead to an overestimation of the total amount of protein carbonyls since lipid oxidation products could also react with the DNPH and hence, be accounted. In addition, other protein oxidation mechanisms such as peptide-backbone fragmentations lead to the formation of protein carbonyls others than AAS and GGS. Unlike the DNPH method, the detection of specific protein carbonyls provides reliable information on the extent of protein carbonylation occurred through specific oxidation mechanisms and pathways.

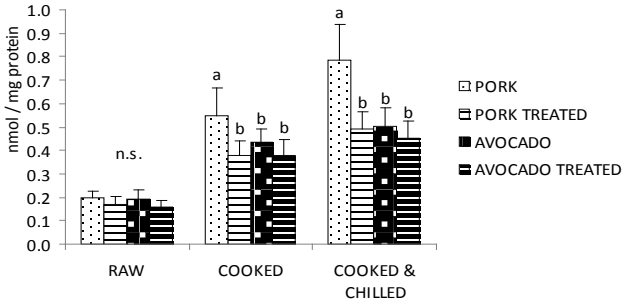
Table 1. Amounts of protein carbonyls in raw, cooked and cooked & chilled patties

P-Patties						
	Raw		Cooked		Cooked & Chilled	
	PC	PT	PC	PT	PC	PT
Total Carbonyls[A]	$1.59^{a}\pm$ 0.37	$1.31^{a}\pm$ 0.34	$1.87^{a}\pm$ 0.33	$1.69^{a}\pm$ 0.26	$3.42^{a}\pm$ 0.65	$3.19^{a}\pm$ 0.49
Total Carbonyls[B]	$0.29^{a}\pm$ 0.05	$0.27^{a}\pm$ 0.03	$0.77^{a}\pm$ 0.19	$0.56^{b}\pm$ 0.05	$1.21^{a}\pm$ 0.16	$0.79^{b}\pm$ 0.07
A-Patties						
	Raw		Cooked		Cooked & Chilled	
	AC	AT	AC	AT	AC	AT
Total Carbonyls[A]	$1.45^{a}\pm$ 0.31	$1.16^{a}\pm$ 0.45	$1.70^{a}\pm$ 0.35	$1.70^{a}\pm$ 0.34	$3.56^{a}\pm$ 0.83	$2.77^{b}\pm$ 0.39
Total Carbonyls[B]	$0.30^{a}\pm$ 0.06	$0.25^{a}\pm$ 0.04	$0.61^{a}\pm$ 0.09	$0.55^{a}\pm$ 0.10	$0.75^{a}\pm$ 0.10	$0.73^{a}\pm$ 0.15

Data are expressed as means ± standard deviation. Means with different superscript ($^{a-b}$) within a row and from same technological processes are significantly different ($p<0.05$). [A]Total amount of protein hydrazones quantified by the DNPH method. [B]Sum of GGS and AAS (nmol/mg protein).

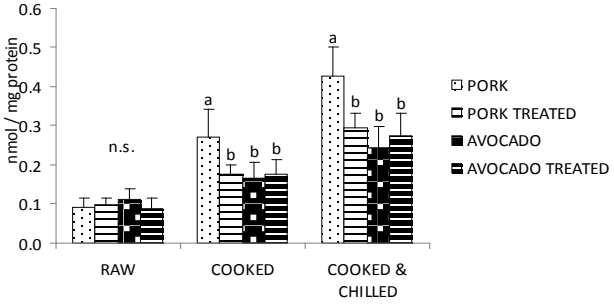
The initial amounts of AAS and GGS in R porcine patties are compatible with quantitative data provided by other authors in plasma proteins and several animal tissues [7]. The high temperatures reached during cooking likely enhanced the formation of ROS and, hence, the formation of both protein semialdehydes. Interestingly, cooking also increased the susceptibility of meat proteins to undergo further carbonylation as the increase of protein carbonyls during the following chilled storage was even more intense. This effect could be attributed to the disruption of cellular compartmentalization and exposure of membrane lipids creating a pro-oxidative environment that would eventually affect meat proteins. Furthermore, the release of catalytic free iron from myoglobin occurred during meat cooking has been reported to promote the oxidative reactions in protein and amino acids leading to the formation of semialdehydes [4].

The amount of both semialdehydes was significantly lower in AC-patties than in the PC-counterparts (Fig. 1 and 2). The present results show that the back-fat replacement by avocado oil inhibited the formation of AAS and GGS on CO and CC patties. It is remarkable that these differences were not detected by the DNPH method what highlights the higher specificity and sensitivity of the HPLC-FLD method for the detection of particular protein carbonyls. Natural components of avocado oil such as tocopherols, chlorophylls and polyphenols are known to have high antioxidant potential [1, 3]. It is plausible to consider that the addition of avocado oil to patties contributed to inhibit protein carbonylation through the antioxidant activity of those antioxidant compounds.



Different letters on top of the bars denote significant ($p<0.05$) differences between group of samples; n.s.: no significant differences between groups.

Figure 1. Amount of AAS in raw, cooked, and cooked & chilled burger patties with added peel avocado extract and/or avocado oil.



Different letters on top of the bars denote significant ($p<0.05$) differences between group of samples; n.s.: no significant differences between groups.

Figure 2. Amount of GGS in raw, cooked, and cooked & chilled burger patties with added peel avocado extract and/or avocado oil.

In fact, α-tocopherol and several avocado phenolics have been reported as efficient inhibitors of carbonylation in myofibrillar proteins [1, 3]. Avocado oil also increases the amount of unsaturated fatty acids which could be thought to increase the oxidative instability of the AC-patties. However, the antioxidant compounds from the avocado oil certainly compensated the potential negative impact of the increased degree of fat unsaturation. Adding the phenolic-rich avocado extracts also had a protecting effect against the carbonylation occurred during cooking and chilling of PC-patties. Results are in concordance with other studies where the same effect was observed by adding wild fruits and berries extracts porcine patties subjected to similar cooking and chilling procedures [9]. The antioxidant effect of the avocado extract could be ascribed to the large amount and good variety of phenolics compounds detected in the avocado peel, including catechins, procyanidins and phenolic acids [3]. Phenolic compounds behave as antioxidants, owing to their ability to act as radical

scavengers and metal chelators due to the reactivity of the phenol moiety (hydroxyl substituent on the aromatic ring) [1]. These mechanisms have also been reported to explain the protecting effect of phenolic compounds on myofibrillar proteins [10]. Several ROS such as the hydroxyl radical can initiate the oxidation of susceptible amino acids while transition metals such as iron trigger the eventual deamination of the amino acid side chain to yield the corresponding semialdehyde. Avocado phenolics could act at both levels i) scavenging the radical species and/or ii) chelating non-hem iron, hence, inhibiting its pro-oxidant action on proteins. In contrast to the results on P-patties, no significant effect of the avocado extract was detected on the formation of AAS and GGS in A-patties. These results would prevent from using both strategies (fat replacement and avocado extract) at the same time as does not lead to an enhanced antioxidant effect.

IV. Conclusion

The quantification of AAS and GGS by specific methodologies as HPLC-FLD enables the acquisition of an accurate protein oxidation index in meat and meat products. Using avocado oil as back-fat replacer and avocado peel extracts in burger patties lead to products with higher oxidative stability and enhanced nutritional and technological properties.

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