Avocado Phenolics Inhibit the Oxidation of Cholesterol in Porcine Patties

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Abstract— **Cholesterol oxidation products (COPs) are commonly found in processed meat products and display cytotoxic, atherogenic, and carcinogenic effects. The outcome of the addition of phenolic-rich extracts from avocado peel on the formation of COPs (treated group; T) in porcine patties subjected to cooking and chill storage was studied. Seven COPs (7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, 20α-hydroxycholesterol, 25-hydroxycholesterol, cholestanetriol, 5,6β-epoxycholesterol and 5,6α-epoxycholesterol) were identified and quantified by a GC-MS method. 7-ketocholesterol was the most abundant with this compound being a common indicator of cholesterol oxidation in numerous animal source foods. The addition of avocado extracts to porcine patties significantly inhibited the formation of COPs during cooking. Cooked control (C)-patties were found to contain a larger variety and higher amounts of COPs than the T-counterparts. The formation of COPs sharply increased in cooked patties during the subsequent chilled storage. This increase was significantly higher in C-patties than in the T-patties. Interestingly, the amount of COPs in cooked and chilled T-patties were similar to those shown by cooked C-patties. The inhibitory effect displayed by the avocado extracts could be attributed to the efficient activity of avocado polyphenols as radical scavengers via hydrogen atom donation or as radical quenchers through electron donation and singlet oxygen quenching. In conclusion, the addition of avocado extracts is a successful strategy to produce meat products with enhanced healthy properties through the inhibition of COPs formation.**

Keywords— **Cholesterol oxidation products, avocado, phenolic compounds.**

I. Introduction

Cholesterol is a natural component of the lipid bilayer cell membrane in animal tissues. It consists of four fused rings and various functional groups which are susceptible to oxidation leading to the formation of a variety of cholesterol oxidation products (COPs) [1]. These compounds, also called oxycholesterols or oxysterols, contain an additional hydroxyl-, epoxide- or keto-group at the cholest-5-en structure, or a hydroxyl group at the side chain of the molecule [2]. Recently, cholesterol oxidation in foods has begun to attract attention because the adverse effects on health as have been well documented for being potentially cytotoxic, mutagenic, carcinogenic, and accelerators of the fatty streak lesion formation and promotion of atherosclerosis [3, 4].

In meats, the manufacture and/or processing conditions (heating, long-term storage or packing manner) promote the oxidation of unsaturated fatty acids through the development of free radicals and peroxides, that accelerate the formation of COPs [5, 6]. Efforts to prevent or to reduce cholesterol oxidation are directed to the addition of either synthetic or natural antioxidants to foods or as dietary supplementation for animals [7]. Certain antioxidants have been described as efficient not only against triglyceride oxidation, but also against the formation of oxycholesterols [8]. Few studies have focused on the antioxidant effect and mode of action of plant extracts against COPs formation in meat systems [9, 10, 11]. Plant phenolics are compounds of increasing interest among consumers and researchers owing to their efficacy as antioxidants as their role as natural compounds with beneficial biological effects. The aim of the present study was to evaluate the effect of the addition of phenolic-rich extracts from avocado peel as inhibitor of the formation of COPs in porcine patties subjected to cooking and chilling.

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). The gases used in GC–MS (helium) and evaporation of solvents (nitrogen) were supplied by Abelló Linde S.A. (Barcelona, Spain). Porcine *longissimus dorsi* muscle and porcine back-fat were purchased in a butchery in Cáceres (Spain) while 'Hass' avocado fruit were bought from a local supermarket in Madrid (Spain).

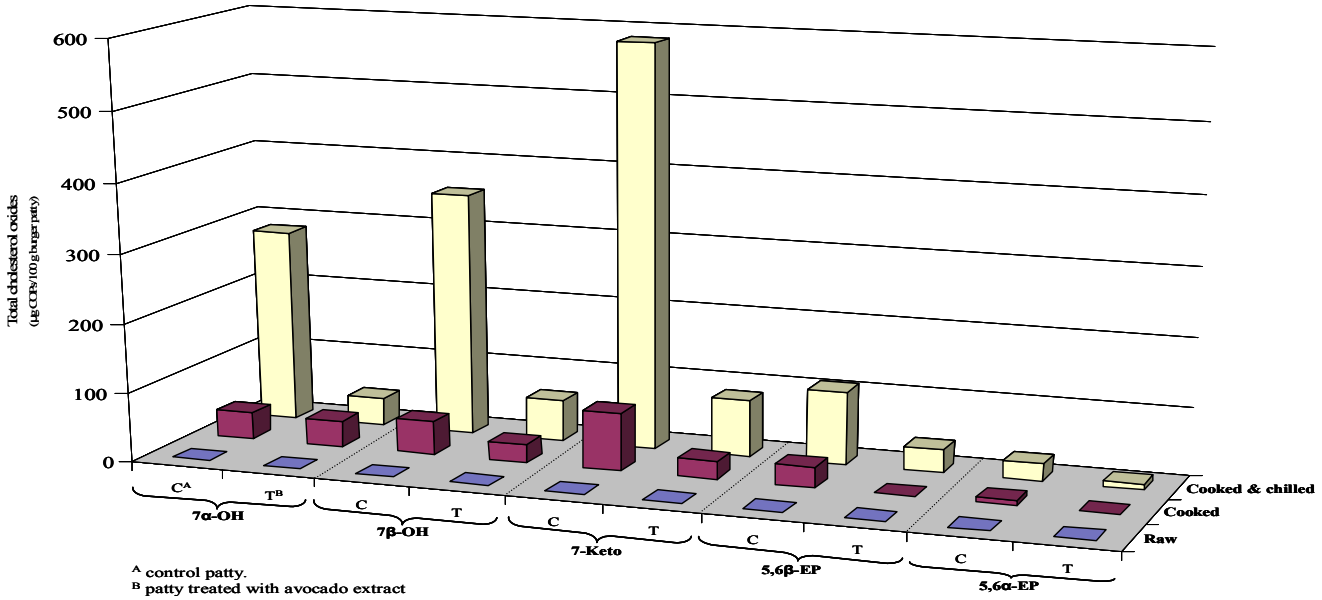


Fig. 1. Cholesterol oxides content of raw, cooked and cokeed & chilled porcine patties manufactured using peel avocado extracts

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 [12].

Manufacture of porcine patties: Ingredients per kg of porcine patty (Control patty; "C") were as follows: 700 g porcine *longissimus dorsi* muscle, 180 g distilled water, 100 g porcine back-fat and 20 g sodium chloride. Patties were elaborated following the process described by Ganhão, *et al.* [13]. Patties treated with extracts from 'Hass' avocado peel ("T") were produced by replacing in the aforementioned recipe 50 g of the distilled water by 50 g of a water solution extract. Eighteen patties per group were produced in two independent manufacturing processes (9 patties per group 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 group and processing treatment). R-patties were frozen (-80 ºC) the day of manufacture until the analytical experiments (less than four weeks). 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 (less than four weeks).

Isolation, identification and quantitative determination of COPs from porcine patties: Eight COPs were extracted from porcine patties. A column fractionation without previous cold saponification, was performed to determine free COPs and were identified by gas chromatography-mass spectrometry (GC-MS) according to the procedure described by Petrón *et al*. [14]. All samples were analyzed in selected ion monitoring (SIM) mode for quantification purposes of the compounds, in which the ions of m/z 7α-hydroxycholesterol (m/z 456.3), 7β-hydroxycholesterol (m/z 456.3), 7-ketocholesterol (m/z 472.3), 20α-hydroxycholesterol (m/z 201.2), 25-hydroxycholesterol (m/z 131.1), cholestanetriol (m/z 403.2), 5,6β-epoxycholesterol (m/z 445) and 5,6α-epoxycholesterol (m/z 366) were selected as the most characteristic of the oxides of cholesterol standards. The content of each COP was calculated using an external standard procedure.

C. Statistical analysis

Six burger patties per group and technological process were prepared and used as experimental units. Data from experiments were analyzed by Analyses of Variance (ANOVA) using a two-way model. When a significant effect ($p<0.05$) was detected, the comparative analyses between means were conducted using the Tukey test. Statistical analyses were performed by using SPSS (v. 15).

III. Results and Discussion

The concentration COPs in R, CO, and CC porcine patties are presented in Figure 1. The presence of cholesterol in meat and meat products enables that, under

particular processing and storage conditions, this susceptible molecule is oxidatively degraded to yield various COPs. No COPs were detected in R-samples while successive increases were found to occur upon cooking and the subsequent chilled storage. Among the total 8 COPs investigated, five of them, namely, 7α-hydroxycholesterol (7α-OH), 7β-hydroxycholesterol (7β-OH), 7-ketocholesterol (7-keto) 5,6α-epoxycholesterol (5,6α-EP) and 5,6β-epoxycholesterol (5,6β-EP) were detected and quantified in the present samples. In general, only three COPs (7α-OH, 7β-OH, and 7-keto) were consistently present in detectable quantities in all samples subjected to CO and CC processes. 7-Keto was, by far, the most abundant COP. This compound occurs in relatively high concentrations in many foods, and it has been proposed as an indicator of cholesterol oxidation [15, 16, 17]. In general, the patties containing the added avocado extract contained smaller concentration of COPs than the C-counterparts.

The cooking process induced the formation of 7α-OH, 7β-OH, 7-keto, 5,6β-EP and 5,6α-EP in porcine patties. Detectable amounts of the two latter were found only in cooked C-patties. Right after cooking, T-patties had significantly lower amounts of 7-keto than C-patties. In general the amounts of COPs reported in the present study are consistent with those found by Pie *et al.* [15] in cooked meats. In contrast, Broncano *et al.* [16] recently found considerably larger levels of COPs in meats cooked following several procedures (grilled, fried, microwaved and roasted). As expected, the highest concentrations of COPs were found in CC patties. The concentrations of COPs in CO/C-patties increased between 3- and 7-fold times after 15 days of chilled storage. The increment observed in CO/T-patties during the same period was noticeably more moderated (from 1 to 3-fold times the initial values). 7-Keto underwent the highest increase, with this compound being the most abundant in CO and CC patties (Figure 2).

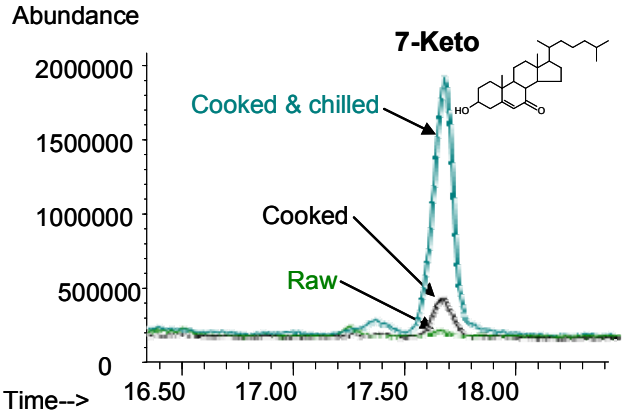
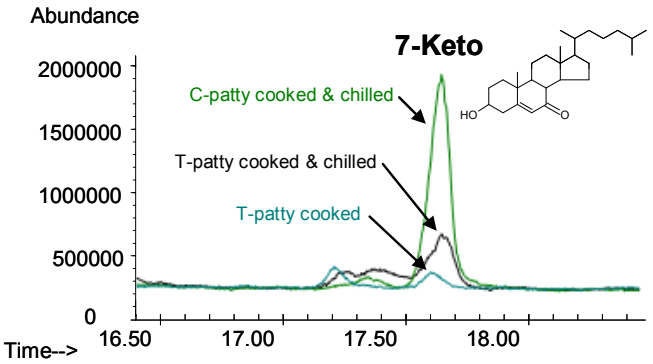


Figure 2. The influence of the technological treatment on 7-ketocholesterol formation in porcine patties.

These results show that the cooking procedure not only promoted the formation of COPs but also promoted the creation of an intense pro-oxidant environment in which cholesterol was readily oxidized during the subsequent chilling storage. According to the present results, cholesterol oxidation in cooked meats is not inhibited by low temperatures but actually highly promoted by radicals formed during the previous cooking. Whereas refrigeration during a limited period of time could delay cholesterol oxidation in certain food systems, the present results advice against using such technology for preserving cooked meats against COPs formation.

Adding the avocado extracts to porcine patties significantly inhibited the formation of COPs during cooking and the following chilled storage. In particular, avocado extracts significantly reduced the formation of 7-keto and avoided the formation of the harmful epoxycholesterols during cooking. The major benefit, however, was observed during the subsequent chilled storage. T-patties subjected to cooking and chilling had between 3 and 7-fold times lower amount of COPs than the C-counterparts (Figure 3). In fact the final level of COPs in CC patties treated with avocado extract was equivalent to



C-patty: control patty.
T-patty: patty treated with avocado peel extract.

Figure 3. Antioxidant action of the avocado phenolics against 7-ketocholesterol formation in porcine patties.

that displayed by freshly CO/C-patties. The antioxidant effect of the peel avocado extract could be ascribed to its high concentration of polyphenols. Avocado phenolics are able to scavenge fatty acyl peroxide radicals and inhibit lipid peroxidation, as demonstrated by Wang *et al.* [17] and Rodríguez-Carpena *et al.* [12]. The present results confirm the efficacy of these compounds to inhibit the formation of COPs during cooking and chilling of porcine patties, including the highly toxic 5,6-EPs.

IV. Conclusion

This study demonstrated that peel avocado extract contains active antioxidant compounds that prevent the rapid formation of oxidation products in porcine patties during cooking and chilled storage. The usage of phenolic-rich extracts could be used as an appropriate strategy to elaborate processed meat products with a reduced content of COPs. However, further research must be conducted to provide a better understanding of what compounds from the avocado extract are responsible for the inhibiting effects of cholesterol oxidation.

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