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Assessment of the antioxidant effect of astaxanthin in fresh, frozen and cooked lamb patties

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ABSTRACT

Astaxanthin is a natural red carotene exerting a strong antioxidant action. The effect of this carotene on the oxidative stability of raw and cooked lamb patties was evaluated. Seven experimental treatments were included in this study depending on the antioxidants added, which are: no antioxidant added (control), 450 mg/kg of sodium metabisulphite, 500 mg/kg of sodium ascorbate, and 20 mg/kg, 40 mg/kg, 60 mg/kg and 80 mg/kg of astaxanthin. The raw patties were either refrigerated for up to 11 days or frozen for 3 months under aerobic conditions. Changes in thiobarbituric reactive substances (TBARS), instrumental colour, pH and Eh were determined in the refrigerated patties and TBARS in the frozen patties. Volatile compounds were determined in cooked patties and cholesterol oxides in both cooked and after cooking microwave reheated patties. The changes in TBARS of cooked patties during a four-day refrigerated storage were also studied. Compared to the control patties, the use of astaxanthin reduced the TBARS generation in a manner depending on the dose for both raw and cooked patties during storage ($P < 0.05$). Astaxanthin added at levels of 60 and/or 80 mg/kg showed a greater antioxidant effect than ascorbate and metabisulphite. The presence of astaxanthin, like that of ascorbate, decreased the oxysterols levels of cooked patties with regard to controls. The amount of volatiles released from the cooked patties was also reduced by astaxanthin. This effect was not observed for ascorbate or metabisulphite. Astaxanthin in lamb patties at levels of 60–80 mg/kg could improve raw and cooked lamb patty oxidative stability during refrigerated aerobic storage, protect their lipids against thermal degradation more than ascorbate and metabisulphite, and reduce oxysterols formation during cooking in a similar way to ascorbate.

1. Introduction

Lipid oxidation is considered as an inevitable, irreversible and complex process that occurs in all food matrices during processing, storage or distribution, and remains a major concern in terms of loss of sensory quality besides loss of nutritional and economic value (Estévez, 2017). Furthermore, during oxidation, several reactive species (RS) are generated, which are the main agents responsible for health disorders in the consumer (Bekhit, Hopkins, Fahri, & Ponnampalam, 2013). For example, in meat, as in other foods of animal origin, RS can react with cholesterol, originating the formation of at least 60 different oxidation products (Razzazi-Fazeli, Kleineisen, & Luf, 2000) which have been found to be mutagenic, cytotoxic and carcinogenic (Min et al., 2016). Furthermore, the presence of some of them in food has been associated with arteriosclerosis and neurodegenerative diseases (Poli, Biasi, & Leonarduzzi, 2013; Savage, Dutta, & Rodriguez-Estrada, 2002).

The use of antioxidant additives by the meat industry has proved to be a good strategy to delay or prevent oxidation processes (Liu, Xu, Dai, & Ni, 2015). Thus, ascorbic acid (or ascorbate) is a commonly used antioxidant additive in processed meat. This is a water-soluble molecule, used as an additive according to the principle of 'just enough', and is considered to have no toxic effect on consumers (Varvara et al., 2016). Moreover, sulphur dioxide, or its precursors such as metabisulphite, although less commonly used is allowed at maximum levels of 450 mg/kg in specific meat preparations such as burger meat (SANCO, 2017). The functions of this additive are to reduce microbial growth, protect against oxidation and enhance the red colour of meat (Mathenjwa, Hugo, Bothma, & Hugo, 2012; Ough & Were, 2005).

There is growing consumer demand for natural antioxidants over synthetic ones that have been associated with toxicological or anti-nutritional effects, such as sulphur dioxide, butylhydroxytoluene or butylhydroxyanisol (Shah, Bosco, & Mir, 2014). This has driven the use of natural materials or their extracts rich in antioxidant molecules as meat product ingredients, in order to delay oxidation processes, besides

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contributing to nutritional improvement (Kumar, Yadav, Ahmad, & Narsaiah, 2015; Shah et al., 2014; Villalobos et al., 2015).

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is a red xanthophyll carotenoid which is naturally found in crustaceans, salmonids and some types of bird feathers, yeasts and algae, *Haematococcus pluvialis* being the main source of this antioxidant for human consumption (Ambati, Moi, Ravi, & Aswathanarayana, 2014; Guerin, Huntley, & Olaizola, 2003; Kidd, 2011; Kobayashi et al., 1997). Interest in astaxanthin extracts in the nutritional supplement and food industries is growing (Higuera-Ciapara, Félix-Valenzuela, & Goycoolea, 2006). Several functions have been attributed to this carotene regarding its use as food ingredient, i.e. natural colorant and antioxidant, or as a nutritional supplement, i.e. antioxidant, anti-inflammatory and anti-diabetic activity in the organism (Ambati et al., 2014; Guerin et al., 2003; Higuera-Ciapara et al., 2006). It is well known that its antioxidant capacity is greater than that shown by other carotenoid compounds (Ambati et al., 2014; Naguib, 2000).

In spite of the increasing interest within the food industry in extending the use of astaxanthin, there are few studies about the effect of the presence of this compound on meat quality. To the best of our knowledge, it has been reported that feeding of pigs (Carr, Johnson, Brendemuhl, & Gonzalez, 2010) or chickens (Perenlei et al., 2014) with appropriate doses of this carotene can lead to an improvement in meat colour and oxidative stability. Furthermore, Abdelmalek et al. (2016) demonstrated an antioxidant effect of astaxanthin in marinated chicken steaks during refrigerated storage. No studies have been carried out, however, on the effect of astaxanthin on the quality of red meat and specifically that of lamb, which is widely consumed in European Mediterranean countries where light lamb meat is considered a luxury meat (Linares, Bórnez, & Vergara, 2007).

Taking all this into account, the aims of the present study were to evaluate the effect of the addition of different levels of a commercial astaxanthin powder on the oxidative lipid stability of raw lamb patties during refrigerated and frozen storage and during their heat treatment and refrigerated storage of cooked patties. For a better assessment of this effect, patties without antioxidants and patties with ascorbate and metabisulphite were included in the study.

2. Materials and methods

2.1. Patty manufacturing and sampling

The meat used in this experiment was obtained from the legs of 12 lambs that were reared under a conventional system consisting of suckling until 13.5 kg body weight followed by weaning and fattening on a complete pelleted diet until a target body weight of 27 kg was achieved, as described by Santos, Giráldez, Mateo, Frutos, and Andrés (2018). Legs were obtained from the cold carcasses at 24 h post mortem, and deboned. The flesh was then cut into approximately 3-cm^3 pieces and trimmed of any visible fat. The meat pieces from the 12 lambs were mixed together, vacuum packaged in three different packaging bags and frozen-stored for a period of 8–9 months at −18 °C. Afterwards, the meat was thawed for 24 h at 5 °C in order to prepare three batches of patties, each one with the meat from one packaging bag. The patty batches were prepared on three different days at the Food Processing Hall of the Department of Food Hygiene and Technology (University of León, León, Spain).

Each batch (weighing 3.5 kg) consisted of seven 500-g sub-batches of patties according to the type or amount of antioxidant added, i.e. antioxidant treatment, which were labelled as CON (no addition of antioxidant), SULP (addition of 450 mg of sodium metabisulphite per kg of patty mixture: 450 ppm; Panreac, Barcelona, Spain), ASC (500 ppm of sodium ascorbate; Panreac), AST20 (20 ppm of astaxanthin), AST40 (40 ppm of astaxanthin), AST60 (60 ppm of astaxanthin) and AST80 (80 ppm of astaxanthin). The astaxanthin added to the patties was part of a commercial dietary supplement (Astaxantina-Lider, Naturlider, Ciudad Real, Spain) containing astaxanthin (1%) extracted from *H. pluvialis*, and excipients: maltodextrin, magnesium stearate and silicon dioxide. The amount of supplement added to the corresponding patties was adjusted to the above-mentioned astaxanthin levels. The amount of sodium ascorbate used was in the range generally applied in processed meat (400–600 ppm; Feiner, 2006), that of metabisulphite was the maximum allowed level for burger meat (SANCO, 2017), and those of astaxanthin were such that the amount contained in a 100-g patty was close to the dose recommended for astaxanthin as a dietary supplement for humans, i.e. approximately 6 mg/day (Ambati et al., 2014). The antioxidant potential of the antioxidants used in the experiment was determined in the lab using 2,2-diphenyl-1-picrylhydrazyl (DPPH; Serpen, Gökmen, & Fogliano, 2012) and the results obtained for sodium metabisulphite, sodium ascorbate, and astaxanthin, which was previously extracted from the dietary supplement with ethyl acetate, were respectively 0.34, 4.07 and 0.86 mmol Trolox equivalents per g of substance.

The formulation of patties was as follows: 79.5% of lean minced leg lamb (using a butcher's mincer equipped with a 5-mm diameter sieve), 4% of potato starch, 15% of water and 1.5% of common salt. Moreover, depending on the treatment, the corresponding antioxidant was added to the patties. All the components were manually mixed for 2 min. Finally, five 100-g patties were formed from each sub-batch; out of those, three patties were used to evaluate the oxidative status and stability when raw refrigerated, one when raw frozen, and the other when cooked and reheated (Table 1). The raw patties were analysed for thiobarbituric reactive substances (TBARS), colour, Eh and pH on the preparation day and after 5 and 11 days of refrigerated storage (4 °C) on polystyrene foam trays wrapped with polyvinyl chloride cling film, using one patty per storage day. The frozen patties, which had been previously wrapped with polyvinyl chloride cling film and then stored for 90 days at −18 °C, were analysed just after thawing (1 day at 4 °C) for TBARS. For the cooked patties, a patty from each antioxidant treatment was first stored raw under refrigeration (4 °C) for 5 days, then cooked in a convection oven at 150 °C for 15 min until reaching a core temperature of 70 °C (± 2 °C), and subsequently divided into four quarters. One quarter was vacuum packaged and frozen at −18 °C for up to 3 weeks before analysis of TBARS, cholesterol oxides and volatile compounds. The other three quarters were stored under refrigeration (4 °C) on polystyrene trays covered with polyvinylchloride cling film for 2 (two quarters) and 4 days (one quarter). One of the quarters stored for 2 days, and the quarter stored for 4 days were immediately frozen under vacuum (up to 3 weeks) before analysis of TBARS. The other quarter stored for 2 days was first reheated in a domestic microwave (400 W) for 2 min and then frozen under vacuum (up to 3 weeks) before analysis of cholesterol oxides. Moreover, a digital photograph of a raw patty per treatment and batch was taken just after patty preparation. A photo of a patty from each treatment has been included in Fig. 1 in order to show the reader the effect of the used levels of astaxanthin on the colour of

Table 1
Analysis carried out in three batches of raw refrigerated, raw frozen, cooked and reheated patties and the patty storage duration before analysis.

	Oxidative status analysis	Storage day
Raw refrigerated (three patties)	TBARS, colour, pH and redox potential	0, 5 and 11[a]
Raw frozen (one patty)	TBARS	90[a]
Cooked (after five days of refrigerated storage; three quarter patty)	TBARS	0, 2 and 4[b]
	Cholesterol oxides and volatile compounds	0[b]
Cooked and microwave reheated after two days of refrigerated storage (one quarter patty)	Cholesterol oxides	2[b]

[a] Day of sampling after the elaboration of patties during refrigerated storage.
[b] Day of sampling after the cooking of the patty.