Food Research International 65 (2014) 137–143

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effect of annatto powder and sodium erythorbate on lipid oxidation in pork loin during frozen storage

Bruno Chacon Figueirêdo, Isabela Jorge Trad, Lilian Regina Barros Mariutti, Neura Bragagnolo *

Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), 13083-862, Campinas-SP, Brazil

ARTICLE INFO

Article history:
Received 31 January 2014
Received in revised form 30 June 2014
Accepted 20 July 2014
Available online 28 July 2014

Keywords:
Annatto
Lipid oxidation
Antioxidants
Pork meat
Cholesterol oxides

ABSTRACT

There are few reports about the antioxidant properties of annatto (*Bixa orellana*), especially considering their antioxidant action in food. In this study we evaluated the antioxidant effect of annatto powder and sodium erythorbate on lipid oxidation in pork loin patties by measuring the secondary products of lipid oxidation, the changes in the fatty acid composition, cholesterol oxidation, and the extent of bixin degradation during storage at −18 °C for 120 days and subsequent thermal treatment. Sodium erythorbate (0.1%) and annatto (0.05%), used alone or in combination, were able to reduce the formation of thiobarbituric acid reactive substances and cholesterol oxidation after thermal treatment when compared with pork patties without antioxidant (control). Bixin degradation was less pronounced in the patties containing annatto (0.05%) + sodium erythorbate (0.1%), suggesting that sodium erythorbate protected this carotenoid from degradation. The addition of annatto, sodium erythorbate or annatto + sodium erythorbate did not have any impact on SFA, MUFA and PUFA oxidation regardless thermal treatment or storage. The cholesterol contents did not change during storage; however, there was a significant loss of 15% due to thermal treatment.

1. Introduction

Bixin (methyl (9-*cis*)-hydrogen-6,6′-diapo-Ψ,Ψ-carotenedioate) is the main carotenoid responsible for the orange-red colour in the seeds and extracts of annatto representing approximately 80% of the total carotenoids (Preston & Rickard, 1980). This pigment is extracted from *Bixa orellana* L. (annatto), a plant native to tropical America, which accumulates in its seeds and leaves several compounds possessing antioxidant capacity, such as carotenoid derivatives, terpenoids, tocotrienols and phenolic compounds, specifically hypolaetin and a caffeoyl acid derivative (Chisté, Yamashita, Gozzo & Mercadante, 2011; Rodrigues et al., 2007).

There are few reports about the antioxidant properties of annatto, especially considering its antioxidant action in food. Annatto extracts are able to scavenge reactive species of biological relevance, including peroxyl radicals, *in vitro* (Chisté, Mercadante, Gomes, Fernandes, Lima, & Bragagnolo, 2011). Bixin and its derivatives also exert protective effects against tunicamycin induced retinal damage and prevent retinal degeneration induced by endoplasmic reticulum stress *in vitro* and *in vivo* (Tsuruma et al., 2012).

Lipid oxidation is a major cause of food deterioration being responsible for the generation of undesirable flavors, decrease of shelf life, loss of nutritional value and formation of harmful compounds to human health, such as cholesterol oxides. Pork is the most produced and consumed meat in the world. Estimates for 2012 indicated that the world production of pork meat would be 110.8 million ton, while the production of bovine, poultry and ovine meat would be 66.8, 104.5 and 13.9 million ton, respectively (FAO, 2012). Pork meat has about 60% of unsaturated fatty acids (Bragagnolo & Rodriguez-Amaya, 2002), which makes it prone to lipid oxidation, especially when processed. The addition of antioxidants to pork meat is one of the ways to prevent or delay the lipid oxidation.

The addition of colorifico, a mixture of corn flour and annatto powder, resulted in a delay of lipid oxidation in grilled chicken meat during storage at −18 °C up to 30 days (Castro, Mariutti, & Bragagnolo, 2011). In raw fish patties, the addition of annatto powder was efficient in the control of lipid oxidation by preserving the essential fatty acids during storage, especially when it was added in combination with coriander;

Abbreviations: 19-OH, 19-hydroxycholesterol; 20α-OH, 20α-hydroxycholesterol; 22*R*-OH, 22*R*-hydroxycholesterol; 22*S*-OH, 22*S*-hydroxycholesterol; 24-OH, 24*S*-hydroxycholesterol; 25-OH, 25-hydroxycholesterol; 5,6α-EP, 5,6α-epoxycholesterol; 5,6β-EP, 5,6β-epoxycholesterol; 7-keto, 7-ketocholesterol; 7α-OH, 7α-hydroxycholesterol; 7β-OH, 7β-hydroxycholesterol; COP, cholesterol oxidation products; EDTA, ethylenediamine tetraacetic acid; FAME, fatty acids methyl esters; KOH, potassium hydroxide; LOD, limit of detection; LOQ, limit of quantification; MA, malonaldehyde; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TBARS, thiobarbituric acid reactive substances; TEP, 1,1,3,3-tetraethoxypropane.

* Corresponding author. Tel.: +55 19 35212162; fax: +55 19 35212153.
E-mail address: neurabragagnolo@gmail.com (N. Bragagnolo).

http://dx.doi.org/10.1016/j.foodres.2014.07.016
0963-9969/

however, no effect was observed when the patties were cooked (Sancho, Lima, Costa, Mariutti, & Bragagnolo, 2011).

Sodium erythorbate is a stereoisomer of sodium ascorbate which is capable of preventing lipid oxidation by quenching singlet oxygen, donating hydrogen atoms and as a reducing agent (Reische, Lillard, & Eitenmiller, 2002).

The aims of the present study were to evaluate the antioxidant effect of annatto powder on lipid oxidation in pork loin patties in comparison with that of sodium erythorbate and to verify the interaction between these compounds. In this sense, we measured the secondary products of lipid oxidation, the changes in the fatty acid composition, cholesterol oxidation, and the extent of bixin degradation in pork loin patties stored in the dark at −18 °C for 120 days and subsequent thermal treatment.

2. Materials and methods

2.1. Chemicals

A mixture of fatty acid methyl ester (FAME) standards from 4:0 to 24:0 (FAME Mix C4–C24) was acquired from Supelco (Bellefonte, PA, USA) and methyl docosatetraenoate (22:4n-6 FAME) and methyl tricosanoate (23:0 FAME) from Sigma-Aldrich (St Louis, MO, USA). The standards of cholesterol (5-cholesten-3β-ol), 19-hydroxycholesterol (19-OH), 20α-hydroxycholesterol (20α-OH), 22*S*-hydroxycholesterol (22*S*-OH), 25-hydroxycholesterol (25-OH), 5,6α-epoxycholesterol (5,6α-EP), 5,6β-epoxycholesterol (5,6β-EP) and 7-ketocholesterol (7-keto) were acquired from Sigma-Aldrich. The 22*R*-hydroxycholesterol (22*R*-OH), 24*S*-hydroxycholesterol (24-OH), 7β-hydroxycholesterol (7β-OH) and 7α-hydroxycholesterol (7α-OH) were purchased from Steraloids (USA). The purity of the standards ranged from 95% to 98%. Hexane HPLC grade (minimum 63% of n-hexane) was acquired from Burdick and Jackson (Muskegon, MI, USA), chloroform and propyl gallate from Sigma-Aldrich, trichloroacetic acid, ethylenediamine tetraacetic acid (EDTA) and 1,1,3,3-tetraethoxypropane (TEP) from Merck (Darmstadt, Germany), and thiobarbituric acid from Acros Organics (Fair Lawn, NJ, USA). The sodium erythorbate monohydrate (purity 97%) was acquired from Sigma-Aldrich.

2.2. Sample preparation

The annatto seeds (200 g) were acquired in Campinas, São Paulo, Brazil. The seeds were ground (CM-180, Eastern Electric, Asbury, NJ, USA), the powder was passed through a 100 mesh sieve and immediately added to the samples. The bixin content of the annatto seeds was 14 ± 2 mg/g (Chisté, Mercadante, et al., 2011).

For the optimization and validation of the saponification and extraction conditions, 2 kg of pork loin without external fat and connective tissues was minced using a food processor. After homogenization, the samples were divided in two parts. One part was used to assess the cholesterol content of the pork meat and the presence of cholesterol oxides and the other part was used to validate the method. Pork patties (60 ± 1 g) were molded, identified, packed in low density polyethylene film (6 μm thick) covered by an aluminum foil and stored at −18 °C until the analysis.

For the storage experiment, approximately 12 kg of pork loin were acquired in a local market (Campinas, São Paulo, Brazil). The apparent fat and connective tissues were removed. The meat was minced in a food processor (Walita, São Paulo, SP, Brazil) until a homogenous mass was obtained. The minced meat was divided into four fractions. One fraction served as control without addition of antioxidants, and to each one of the other three fractions 0.05% annatto, 0.1% sodium erythorbate and 0.05% annatto + 0.1% sodium erythorbate were added. Pork patties (60 ± 1 g, total of 40 patties for each fraction) were molded, packed in low density polyethylene film (6 μm thick) covered by an aluminum foil and stored at −18 °C for 120 days. At days 0, 15, 45, 60, 75, 90 and 120 of storage two patties of each fraction were thawed at 5 °C for 24 min and one of each fraction was grilled at 165 °C (core temperature 70 °C) for 4 min each side. The internal temperature was monitored using a calibrated thermometer (Traceable Long-Stem, Friendswood, TX, USA). After thawing or grilling, the patties were homogenized in a mortar and aliquots were taken for analyzes. Moisture and TBARS were carried out at days 0, 15, 45, 60, 75, 90 and 120; bixin was determined at days 0, 15, 30, 45, 60, 75, 90 and 120; cholesterol and cholesterol oxides were determined at days 0, 30, 60, 90 and 120 and the fatty acid composition was assayed at days 0 and 120. All analyzes were carried out in triplicate.

2.3. Analyses

Moisture content was determined according to AOAC (1997). Bixin content was spectrophotometrically determined (Castro et al., 2011) only in the grilled samples containing annatto.

2.3.1. Cholesterol and cholesterol oxides

The method previously developed by Mariutti, Nogueira, and Bragagnolo (2008) for chicken meat was optimized to analyze pork meat by means of response surface methodology (Box, Hunter, & Hunter, 1978). First, a factorial screening design 2^{6-2} (plus three central points) was carried out to verify the simultaneous effects of six independent variables (Table 1) on cholesterol and cholesterol oxidation products (COP) extraction efficiency, totaling 19 experiments. The independent variables were sample weight, KOH concentration, saponification time, volume of water used to transfer the saponified sample to a separatory funnel, number of extractions with hexane and number of washes for cleanup of the unsaponifiable matter. After that, a central composite design 2^2 with four axial points and four central points was developed using the statistically significant variables ($p < 0.10$). The cholesterol content (mg/100 g) and the recovery of 7-keto (%) were set as the response variables.

The method was validated for linearity, recovery, repeatability and detection and quantification limits. Linearity was observed through the correlation coefficients (R^2) of analytical curves constructed with six points of standard solutions, with concentrations ranging from 0.05 to 4 mg/mL for cholesterol, and from 0.5 to 100 μg/mL for the cholesterol oxides. The limits of detection (LOD) and quantification (LOQ) were calculated using the analytical curves (Ribani, Bottoli, Collins, Jardim, & Melo, 2004). The recovery tests were carried out in two levels with 10 replicates each (cholesterol: 20 and 40 mg/g; 20α-OH, 22R-OH, 22S-OH, 25-OH, 24-OH, 5,6α-EP, 5,6β-EP, 7-keto, 7β-OH and 7α-OH, 20 and 40 μg/g). Repeatability was evaluated using the relative standard deviations (RSD).

A liquid chromatograph (HPLC) equipped with UV (SPD-10 AVVP) and RI (RID-10 A) detectors (Shimadzu, Kyoto, Japan) was used to determine cholesterol and cholesterol oxides, as previously described by Saldanha, Sawaya, Eberlin, and Bragagnolo (2006) and Mariutti et al. (2008). Cholesterol and the epimeric 5,6-epoxides were quantified using the RI detector and the other compounds, using the UV detector at

Table 1
Levels of the independent variables of the factorial screening design 2^{6-2}.

Variables	−1	0	+1
Sample weight (g)	0.5	1.0	1.5
KOH concentration (%)	10	20	30
Saponification time (h)	20	22	24
Volume of water used to transfer the saponified sample to a separatory funnel (mL)	10	15	20
Number of extractions with hexane	3	4	5
Number of washes for cleanup of the unsaponifiable matter	2	3	4