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Effect of the molecular weight and concentration of chitosan in pork model burgers

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ABSTRACT

Chitosan of high and low molecular weights was added at 0%, 0.25%, 0.5% and 1% concentrations to a burger model system. Burgers were evaluated by physicochemical analysis, cooking characteristic and storage stability. The antioxidant activity of chitosan was studied in vitro. The addition of chitosan influenced pH and color properties, in molecular weight and concentration dependent ways. Cooking properties were significantly affected by the chitosan. High molecular weight chitosan improved all cooking characteristics compared with control samples. Low molecular weight chitosan increased the shelf life of burgers, enhanced the red color and reduced total viable counts, compared with control and high molecular weight chitosan samples. The antioxidant activity of chitosan was dependent on molecular weight and concentration. The results indicate that high molecular weight chitosan (HMWC) improves all cooking characteristics and antioxidant activity while low molecular weight chitosan extends the red color and reduces total viable counts.

1. Introduction

Meat is prone to both microbial and oxidative spoilage and therefore it is desirable to use a preservative with both antioxidant and antimicrobial properties. While lipid oxidation contributes to the development of unacceptable organoleptic characteristics, microbial growth may cause both spoilage and disease (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007) both phenomena reduce food safety. In order to protect lipids, avoid deterioration of appearance and microbial growth, meat product manufacturers have used several food additives with antimicrobial and antioxidant properties. Nowadays, there is an increased demand for healthier and organic food products, without chemical preservatives, resulting in a need to avoid the use of synthetic additives. This has favored the use of natural additives or alternative methods to extend shelf life and/or improve safety.

Chitosan is a deacetylated form of chitin and a straight-chain polymer of glucosamine and N-acetylglucosamine (Muzzarelli, Muzarelli, & Terbojerich, 1997; Vinsova & Vavrikova, 2008). Chitin is obtained from the shell of crustaceans, the cuticles of insects and the cell wall of fungi, it is the second most abundant biopolymer in nature (Knorr, 1991; Vinsova & Vavrikova, 2008). Chitosan, a versatile biopolymer, composed of polymeric 1,4-linked 2-amino-2-deoxy-b-D-glucose, possesses many beneficially biological properties such as antimicrobial activity (Wang, 1992; Darmadji & Izumimoto, 1994a, 1994b; Shahidi, Arachchi & Jeon, 1999; Jeon, Shahidi, & Kim, 2000; Kim, Thomas, Lee, & Park, 2003; Zheng & Zhu, 2003; Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001), antioxidant properties (Lin & Chou, 2004; Kim & Thomas, 2007; Yen, Yang, & Mau, 2008a), chelating activity that selectively binds protein and metals (Yen, Yang, & Mau, 2008b), biodegradability, hemostatic activity and wound healing properties (Shepherd, Reader, & Falshaw, 1997; Ravi-Kumar, 2000; Kachanechai, Jantawat, & Pichyangkura, 2008; Kanatt, Chander, & Sharma, 2008; Vinsova & Vavrikova, 2008).

Due to its properties, chitosan and its derivatives have been proposed for applications in biomedical, food, agricultural, biotechnological and pharmaceutical fields (Felse & Panda, 1999; Ravi-Kumar, 2000; Shahidi, Arachchi, & Jeon, 1999). In food products, chitosan offers a range of applications (Devlieghere, Vermeulen, & Debevere, 2004; Jumaa, Furkert, & Müller, 2002; Tsai, Su, Chen, & Pan, 2002; Kanatt et al., 2008) including antimicrobial and antioxidative activities, consequently chitosan has attracted attention as a potential natural food preservative (El-Ghaouth, Aru, Ponnampalam, & Castaige, 1992; Darmadji & Izumimoto, 1994a, 1994b; Chen, Liau, & Tsai, 1998; Kittur, Kumar, & Tharanathan, 1998; Shahidi et al., 1999; Roller & Covill, 1999; Rhoades & Roller, 2000; Tsai, Wu, & Su, 2000). Chitosan exhibits antimicrobial activity in vitro against a wide range of foodborne filamentous fungi, yeasts and bacteria. Chitosan possesses other properties including intestinal lipid binding (Razdan & Pettersson, 1994); and serum cholesterol lowering effects (Maezaki, Tsuji, Nakagawa, Kawai, & Akimoto, 1993; Liao, Shieh, Chang, & Chien, 2007; Xia, Liu, Zhang, & Chen, 2010; Osman, Fayed, Mahmoud, & Romeilah, 2010). However, the application of this polysaccharide in the food industry and medicine is limited because of its high molecular weight (MW) resulting in low solubility in aqueous media (Kim & Thomas, 2007). It has been reported that antioxidant and antimicrobial activity of chitosan is dependent on

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its molecular weight (Xing et al., 2005; Kim & Thomas; Dutta, Tripathi, Mehrotra, & Dutta, 2009).

Burgers are meat products that are consumed regularly, especially by the young. Due to the high fat content and raw nature of burgers (lack of thermal processing), they have low oxidative stability and high susceptibility to microbial growth, resulting in a short self-life. Many efforts have been made to improve the quality and stability of burgers (Aleson-Carbonell, Fernández-López, Pérez-Alvarez, & Kuri, 2005). Nowadays, consumers demand for natural additives that extend food shelf-life together with the need to accomplish this with increasingly restrictive legislation have created a need for alternative preservation systems for meat and meat products. Studies in real meat systems are needed to assess the commercial potential of chitosan. The objective of the present study was to evaluate the potential of chitosan (high and low molecular weights) as a preservative and technological ingredient in pork burgers.

2. Materials and methods

2.1. Chemicals

All chemicals used were obtained from commercial sources and were of analytical grade. 20cp (low molecular weight chitosan, LMWC) and 40cp (high molecular weight chitosan, HMWC) chitosans were obtained from Sigma-Aldrich.

2.2. Antioxidant activity of chitosan

In vitro antioxidant capacity of chitosan was determined by a radical scavenging method (DPPH) and the induction period (IP) by RANCIMAT.

2.2.1. Determination of antioxidant activity using the 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity of L chitosan was measured in terms of hydrogen-donating or radical scavenging ability, using the stable radical DPPH (Brand-Williams, Cuvelie, & Berset, 1995). Fifty μl of a methanol stock solution of LMW and HMW chitosans of five different concentrations (0.2, 0.4, 0.6, 0.8 and 1 w/v) was put into a cuvette, and 2 ml 6×10^{-5} M methanol solution of DPPH was added. Butyl Hydroxy Toluene (BHT) was used as reference. The mixtures were shaken in a vortex (2500 rpm) for 1 min and then placed in a dark room. The decrease in absorbance at 517 nm after 1 h was determined using a HP 8451 spectrophotometer (Hewlett-Packard). Methanol was used as the blank. The DPPH scavenging activity was expressed as the inhibition of free radical DPPH (%).

$$\%\, I = [(A_B - A_S)/A_B]\times 100$$

where A_B is the absorbance of the control and A_S the absorbance of test sample.

2.2.2. Rancimat assay

The induction period (IP) measured volatile products such as aldehydes, acids, and alcohols occurring during the secondary phase of pork lard oxidation treated with chitosan at various concentrations (0.5, 1 and 2%). A Metrohm Rancimat® (743 Rancimat®, Brinkmann Instruments, Inc., Westbury, NY) was used to determine IP. Air flow was 20 ml/min through 2.5 gram samples at 120 ± 0.2 °C. The air was then passed through deionised water and the conductivity of the water was measured. The IP (h) was recorded automatically. The antioxidant activity index (AAI) was calculated from the measured induction times, as: (AAI = IP of lard with chitosan/IP of pure lard) (Viuda-Martos, Ruiz-Navajas, Sánchez-Zapata, Férnandez-López, & Pérez-Alvarez, 2010).

An antioxidant activity index >1 indicates inhibition lipid oxidation; the higher the value, the better the antioxidant activity (Lalas & Dourtoglou, 2003).

2.3. Preparation of a burger model system

Ground fresh pork meat was mixed with 0.7% salt. The salt concentration was selected to enhance meat binding properties with minimal effect on water activity. Pork lean at 4 ± 1 °C, was ground (MAINCA PM-98) with a 5-mm mesh plate. The ground salted lean was divided into 7 equal batches and chitosan was added at different concentrations: control (0% chitosan), 0.25% LMWC, 0.50% LMWC, 1% LMWC, 0.25% HMWC, 0.50% HMWC and 1% HMWC, a total of seven different formulas. All batches were treated identically, under hygienic conditions and were formed in a manual burger mold and covered with shaped plastic films. Burger models were 1 cm thick and weighed about 50 g. Samples were packed under modified atmosphere (30% CO_2 and 70% O_2) and kept at 4 ± 1 °C for 8 days.

Samples were taken at 0, 1, 4 and 8 days for sensory evaluation, pH, color, and microbiological analysis. Moisture, fat and cooking characteristics were determined at day 0 on raw and cooked burgers.

2.4. Analysis of pork model burgers

2.4.1. Chemical analysis

Moisture and fat content were determined by AOAC methods 24.003 and 24.005, respectively (AOAC, 1995). Moisture (g water/100 g sample) was determined by drying to constant weight at 105 °C. Total content of lipid (g fat/100 g sample) was determined gravimetrically by extraction with diethyl ether using a Soxhlet apparatus (Selecta, Barcelona, Spain). Moisture and fat were determined in raw and cooked burgers in duplicate.

2.4.2. Physicochemical analysis

The CIE LAB color space was used Cassens et al. (1995). The following color coordinates were determined: lightness (L*), a* (redness/greenness, ±) and b* (yellowness/blueness, ±). Color determinations were made, at 12 ± 2 °C by means of a Minolta CM-2002 (Minolta Camera Co., Osaka, Japan) spectrophotometer with illuminant D_{65}, 10° observer, SCI mode, 11 mm aperture for illumination and 8 mm for measurement. Spectrally pure glass (CR-A51: Minolta Co.) was put between the sample and the equipment. American Meat Science Association guidelines for color measurements were followed (Hunt et al., 1991). Nine replicate measurements were taken for each sample.

pH was directly measured by using a Crison pH meter (Model 507, Crison, Barcelona, Spain) equipped with a Crison combination electrode (Cat. No. 52, Crison, Barcelona, Spain). Duplicate measurements were taken.

2.4.3. Cooking characteristics

Water, fat, total cooking losses and diameter shrinkage were determined. Three burgers from each formulation were cooked at 150 °C in a forced draught oven (Balay, Spain) to a core temperature of 72 °C. Internal temperature was determined at the geometric center of the burgers by inserting thermocouples. After cooking, they were cooled to 21 °C for 1 h and blotted before weighing. Samples were weighed and measured before and after cooking. To estimate the amount of fat and moisture retained in the samples, the cooking yield and dimensional changes, the following calculations were performed:

$$\%\,\text{Fat retention} = 100\times\frac{\text{cooked weight(g)}\times\%\text{ fat in cooked sample}}{\text{raw weight(g)}\times\%\text{ fat in raw sample}}$$

$$\%\,\text{Moisture retention} = 100\times\frac{\text{cooked weight(g)}\times\%\text{ moisture in cooked sample}}{\text{raw weight(g)}\times\%\text{ moisture in raw sample}}$$