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Oxidation phenomena and color properties of grape pomace on nitrite-reduced meat emulsion systems

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ABSTRACT

The present study focuses on the effect of different levels of red grape pomace (1 and 2%, w/w) on the color changes, lipid oxidation (TBARS), antioxidant activity, microbial counts, total phenol content and sensory attributes of the sausages formulated with various levels of sodium nitrite (30, 60 and 120 mg/kg). It was found that the addition of grape pomace (1%, w/w) in combination of reduced nitrite levels to the beef sausage samples reduced TBARS content and the degree of lipid oxidation. Antioxidant activity and total phenol contents were further evaluated based on DPPH scavenging activity method. A significant reduction in lightness (L^*) and yellowness (b^*) of systems containing grape pomace was observed, following by an increase in the oxidative stability and the radical scavenging activity. Acceptability of beef sausages was not significantly ($P > 0.05$) affected by the addition of grape pomace and had relatively greater scores from a sensory point of view.

1. Introduction

Natural and organic processed meats have recently showed a remarkable demand growth in the world consumption. Cooked sausages are one of the most important and widespread foodstuffs throughout the meat products. Traditionally, nitrite/nitrate are added to processed meat products for curing purposes. It basically contributes to the development of a characteristic pink color and lipid oxidation inhibition by reacting with myoglobin. In addition, it hinders the growth of pathogenic and spoilage microorganisms, particularly, *Clostridium botulinum* (Cammack et al., 1999). However, despite its important technological value, the presence of free nitrite in meat products can be associated with potential health risks (Deda, Bloukas, & Fista, 2007). Several studies have reported mechanism and effects of nitrite reduction during processing and curing of meat products (Hammes, 2012; Hernández-Hernández, Ponce-Alquicira, Jaramillo-Flores, & Legarreta, 2009).

In response to the consumer's demand and industry growing concern for the production of healthier and safer products, many researches have been conducted with the substitution of herbal extracts, including natural preservatives such as phenolic compounds. The main compounds responsible for the high antioxidant activity are phenol diterpenes, such as carnosic acid, carnosol and rosmarinic acid in conjunction with the presence of flavonoid species (Rhee, Ziprin, & Calhoun, 2001). Natural antioxidants sourced from herbs and spices have been practically used in ground beef, aiming to reducing mutagen formation effects at high-temperatures. It is postulated that addition of antioxidants could retard the formation of highly reactive substances in lipid oxidation process, leading to a color alteration (Fernández-López et al., 2004; Hernández-Hernández et al., 2009).

Grape pomace as an agro-by-product in grape juice production, contains abundantly levels of polysaccharides and phenolic compounds, extracting from peels, seeds and stems (Montealegre, Peces, Vozmediano, Gascueña, & Romero, 2006). Anthocyanins, catechins, procyanidins, flavonol glycosides, phenolic acids and stilbenes are the major phenolic compounds extensively found in red grape pomace (Yu & Ahmedna, 2013). It has been previously reported that the addition of grape antioxidant dietary fiber could considerably improve the oxidative stability and the radical scavenging activity in raw and cooked chicken hamburgers (Sáyago-Ayerdi, Brenes, & Goñi, 2009). Sánchez-Alonso and Borderías (2008) showed that addition of grape antioxidant dietary fiber to mince fish muscle inhibits the lipid oxidation during frozen storage. Moreover, grape seeds extracts showed anti-microbial effect on *Listeria innocua*, *Salmonella enteritidis* and *E. coli* (Su Cha, Choi, Chinnan, & Park, 2002). In other study by Sáyago-Ayerdi, Brenes, Viveros, and Goñi (2009) also stated that dietary grape pomace concentrate could be effective in inhibiting lipid oxidation of chilled and long-term frozen stored chicken patties. Brannan (2009) investigated the effect of grape seed extract on descriptive sensory analysis of ground chicken during refrigerated storage, reporting a significant darker sensory scores and redder (a^*) and less yellow (b^*) patties. Furthermore,

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grape seed extract has been shown to be effective in reducing the amount of primary and secondary lipid oxidation products in beef and chicken raw meats (Banon, Díaz, Rodríguez, Garrido, & Price, 2007; Lau & King, 2003). Therefore, there is increasing evidence demonstrating the ability of grape by-products to retard lipid oxidation in meat during storage. In the sense of these characteristics, it appears that grape pomace might be a noble option for improving the organoleptic and technological quality aspects of sausage formulations. Most previous aforementioned studies have focused on relatively textural, nutritional antioxidant activity characteristics of meat/patties containing grape seeds. However, there are no reports in the literature on the antioxidant activity and color, sensory attributes of cooked sausage emulsions containing red grape pomace extracted from various parts of grape. Therefore, the present work aims to investigate the effect of grape pomace powder on the antioxidant capacity and microbial activity and residual phenol content of samples during 30 days storage time at 4 °C. Moreover, the effects of grape pomace on color and other properties of beef cooked sausages in conjunction with lessened nitrite levels were evaluated.

2. Material and methods

2.1. Dry red grape pomace (DRGP) preparation

Fresh grape pomace (*Vitis vinifera* L. var. Siahe sardasht) was provided by the Azarkam (Azarkam Juicing Co., Urmia, Iran) as a by-product of the process of the grape juice production. The grape pomace mainly consisted of stems, skins and seeds (with no separation of components). Preparation of grape pomace was carried out according to the method described by Larrauri, Rupérez, and Saura-Calixto (1997). Grape pomace was dried in an industrial counter-current drier (WPS 700, UK) at 50–55 °C for 12 h to a moisture content of 11.15% (wet-weight basis). Dried red grape pomace (DRGP) was milled using an industrial mill and was sieved through a no. 60 mesh (250 μm) to obtain merely fine particles. Then, dried grape pomace was irradiated with the dosage of 25 kGy (dose-rate ~2.74 kGy/s) for 150 min at ambient temperature in the atomic energy organization (Tehran, Iran) to eliminate microbial contaminations and stored in the dark in high-density polyethylene bags at 4 °C for further experiments.

2.2. Physicochemical analysis

The fat content of grape pomace was determined using analytical standards of AOAC (AOAC, 1995). Lipids were extracted through the Soxhlet method using hexane as solvent under reflux for 6 h. The protein content was determined by the micro-Kjeldahl method (AOAC, 1995) using a conversion factor of 6.25 to covert the nitrogen content into the protein content. Total dietary fiber (TDF) was analyzed according to the enzymatic-gravimetric method of Prosky et al. (1984), using the addition of the soluble and insoluble fractions. The pH was measured by direct reading on the potentiometer, (MS Tecnopon, model mPA210) calibrated in buffer solutions of pH 4.0 and 7.0. The moisture and ash content of the grape pomace were also determined using AOAC methods. The difference method was used to measure the total carbohydrate content (Sousa et al., 2014):

$$100-(\text{weight in grams [moisture} + \text{ash} + \text{protein} + \text{total fat} + \text{total dietary fiber in 100 g of food]} \tag{1}$$

All measurements were performed in triplicate.

2.3. Beef sausage emulsion preparation

The sausages were prepared according to the formulation provided by a major sausage manufacture, including 90 cl topside beef leg (55%), ice/water (16%), sunflower oil (14%), wheat flour (2%), wheat starch (3%), gluten (2.3%), soy protein isolate (3%), mixed spices and ascorbic acid (1.25%), sodium chloride (NaCl) (1.5%), sodium polyphosphate (0.3%), sugar (0.7%) and frozen garlic (1%). The sausages were manufactured in a pilot plant according to industrial procedures. The initial mixture was produced without nitrite as the blank sample and for analyzing the influence of grape pomace on beef sausage, two concentrations of DRGP (1 and 2 kg/100 kg emulsion) were added to each formulation in combination of different nitrite levels (30 and 60 mg/kg) (Table 1). Control sample was then manufactured to compare the outcomes of this investigation with practical sausages. To achieve the correct measure of variability to compare treatments, the entire sausage emulsion processing procedure (including base mix and T_1, T_2 T_3 T_4 and control treatments) was made in two batches (duplicate) on two different days.

Blank sausage emulsion (without nitrite) was manufactured by adding ground beef (3 mm) to the cutter followed by sodium phosphate, sodium chloride, ice slurry, Soy protein isolate, vegetable oil, mixed spices, flour and wheat starch. After homogenization for 5 min, various proportions of nitrite and DRGP were added to the mixture in separate batches. Then, sausages were stuffed into an polyamide casing (previously soaked in tepid water) called Arta (Arta Co., Iran) with an 50 mm. Polyamide casings were used in this study to reduce the possibility of case breaking and minimize the cooking and moisture losses during processing time (manufacturing, cooking and chilling). The sausages were kept in the cooking room until the temperature at the center of each sample, reached 72 °C. The temperature of the product was monitored using a thermocouple probe (Omega Engineering, Inc., Stamford, CT) positioned in the geometric center of the sausages. The sausages were promptly chilled using cold water and then, samples were transferred to the laboratory for further experiments. Three samples (approx. 500 g) of each sausage replication were vacuum packed and stored at 4 °C until analyzed. Further analyses on color changes, lipid oxidation, total phenol and antioxidant activity were carried out at 0, 10, 20 and 30 days.

2.4. Color determination of cooked sausages

Color measurements were performed using a chamber (with the dimensions of 30 ∗ 30 ∗ 30 cm and a white background) to establish stable conditions. Two 30 W xenon lamps were used for lighting. The angle between the light incidence axis and the camera lens axis was 45° and the light intensity was uniformed with an opaque sheet. Image acquisition was performed in triplicate using a digital camera (Panasonic, Lumix DMC-TZ5, Japan, 3456 ∗ 2592 pixels, 2× digital zoom). The color parameters of Hunterlab system (L^*, a^* and b^*) were achieved by Photoshop software (Adobe systems, version 8:0) after transferring the JPEG images to a computer. This model includes L^* (lightness), a^* (redness-greenness) and b^* (yellowness-blueness) color coordinates and were determined according to the ISO/CIE standard color space system proposed by Commission Internationale de l'Eclairage (Joint ISO/CIE Standard, 2008). The three parameters of this model denote the lightness of color (L^*) ranging from 0 to 100 (black to white), its position between red and green (a^*, values in the range of −120 and +120) and its

Table 1
Beef sausage formulations based on nitrite (mg/kg) and DRGP (%) contents.

Samples	Formulation	
	Sodium nitrite (mg/kg)	Grape pomace (%)
Blank	–	–
Control	120.00	–
T_1	60.00	1.00
T_2	30.00	1.00
T_3	60.00	2.00
T_4	30.00	2.00