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Effect of nutmeg (*Myristica fragrans*) essential oil on the oxidative and microbial stability of cooked sausage during refrigerated storage

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1,1,3,3-Tetraethoxypropane (PubChem CID: 67147)

ABSTRACT

This paper examines the oxidative and microbial stability of cooked sausages, produced with the addition of 10 ppm (NO1) and 20 ppm (NO2) nutmeg (*Myristica fragrans*) essential oil. Instrumental parameters of color (CIE L^*, CIE a^* and CIE b^*), Thiobarbituric acid-reactive substance (TBARS) values, microbial profile and sensory properties of aroma have been determined on the 1st, 30th, 45th, and 60th day of storage. Addition of the nutmeg essential oil had no effect on the color of cooked sausages. At the end of the storage, NO2 sausages had the best oxidative and microbial stability. TBARS values of NO1 and NO2 sausages were 1.21 mg MDA/kg and 0.95 mg MDA/kg, respectively, and were significantly lower ($P < 0.05$) compared to control (1.53 mg MDA/kg). Total number of aerobic mesophilic bacteria was lowest in NO2 sausages (78.3 cfu/g) and highest in control (185 cfu/g). After 45 and 60 days of storage, sensory properties of aroma of NO1 (4.21; 3.92) and NO2 sausages (4.39, 4.28) were better compared to those in control (4.07, 3.25). Hence, the addition of nutmeg essential oil in amount of 20 ppm can be successfully applied in order to extend the shelf life of cooked sausages.

1. Introduction

Production of cooked sausages makes up about 50% of the total industrial production of meat in Serbia (Šojić et al., 2011). According to Serbian legislation (Serbian Regulations, 2012), cooked sausages are made from minced meat, mechanically deboned meat (MDM), fat tissue, water, soy proteins, starch, salt, spices and additives (nitrites, polyphosphates, antioxidants etc.). After the filling of the casings, they are treated with heat at a temperature of pasteurization (in steam or hot water – internal temperature of 71 °C), cooking (in boiled water – 100 °C to $F_0 = 0.3$) or sterilization (in steam – t > 100 °C, $F_0 = 3$). Due to the variability of the used meat, spices and other ingredients, effects of high temperatures in thermal treatment and different storage conditions, cooked sausages are subjected to chemical (Hayes, Stepanyan, Allen, O'Grady, & Kerry, 2011; Kulkarni, De Santos, Kattamuri, Rossi, & Brewer, 2011), microbiological (Sachindra, Sakhare, Yashoda, & Rao, 2005) and sensory degradation (Hayes et al., 2011). The most common cause of chemical degradation is the lipid oxidation (Jayawardana et al., 2011). Lipid oxidation leads to rancidness, discoloration and accumulation of potentially toxic compounds that are harmful to human health (Hayes et al., 2011; Qi & Zhou, 2013). One way to slow down the oxidation of lipids is the use of antioxidants. Due to the fact that synthetic antioxidants such as butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) have toxic properties and present a potential health hazard to consumers, the attention of many researchers is focused on the study of natural antioxidants

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isolated from plants (Huang et al.; 2011; Jayawardana et al., 2011; Kulkarni et al., 2011; Shah, Bosco, & Mir, 2014).

Nutmeg (*Myristica fragrans*) is a widely used spice and aromatic component in the food industry, because it contains compounds such as flavonoids, carotenoids, vitamins, phenols. The main chemical components of nutmeg oil are borneol, geraniol, linalool, terpineol, eugenol, myristicin, safrole, camphene, dipentene and pinene (Shekarforoush, Nazer, Firouzi, & Rostami, 2007). Nutmeg has been reported to have excellent activities in scavenging radicals, reducing metal ions and inhibiting lipid oxidation (Gupta, Bansal, Babu, & Maithil, 2013), with possible anti-inflammatory and antimicrobial activities (Chatterje et al., 2007; Firouzi, Shekarforoush, Nazer, Borumand, & Jooyandeh, 2007; Gupta et al., 2013; Murcia et al., 2004; Verluyten, Leroy, Vuyst, & 2004). Nutmeg essential oil was able to suppress lipid oxidation in chicken tissue homogenates and egg yolk fat (Dorman, Deans, Noble, & Surai, 1995).

The aim of this paper was to evaluate the effect of nutmeg essential oil on oxidative and microbial stability of cooked sausages over 60 days of refrigerated (4 °C) storage. PH, color and sensory attributes of aroma were also determined.

2. Material and methods

2.1. Material

Fresh chicken breast, pork shoulder (90% visibly lean) and pork back fat were purchased from a local meat supplier. Mechanically deboned chicken meat (MDCM) was produced from breasts after removal of most meat. Yield of MDCM was 67%. Spice mix and commercially nutmeg essential oil (37.83 w/w) were supplied by Milex Co., Ltd, Novi Sad, Serbia.

2.2. Preparation of cooked sausage

Cooked sausages were produced in local industrial plant. The main mixture consisted of meat from chicken breast (22.5 kg), pork shoulder (15.0 kg), MDCM (15.0 kg), pork back fat (15 kg), ice (15 kg), maize starch (3 kg), textured soy protein (2 kg), nitrite salt (1.6 kg), dextrose (0.1 kg), polyphosphate (0.3 kg), and spice mix (0.5 kg). The minced meat was mixed with all other ingredients in a bowl chopper (Taifun 200, Nowicki, Poland) to obtain sausage batter.

Nutmeg essential oil was added to the sausage batters at concentration of 10 ppm (NO1) and 20 ppm (NO2). The sausage batter without nutmeg essential oil addition was used as control. All batches were stuffed into artificial cellulose casings (diameter of 65 mm) and pasteurized (in steam at 75 °C) until an internal temperature of 71 °C was reached. Immediately after the heating process sausages were cooled with combination of water/air cooling for 45 min. (till reduced internal sausage temperature to 25 °C), followed by air cooling in the chamber (to 4 °C). The sausages were stored at 4 °C until analysis. Processing was repeated three times for each batch (control, NO1, NO2).

2.3. Physical analyses

The pH was measured using the portable pH meter (Consort T651, Turnhout, Belgium) equipped with an insertion glass combination electrode (Mettler Toledo Greifensee, Switzerland). PH was measured on three samples from each batch in duplicate.

Color of each sample of the cooked sausage was measured immediately after slicing. The CIE L^* (lightness), CIE a^* (redness) and CIE b^* (yellowness) color coordinates (CIE, 1976) were determined using MINOLTA Chroma Meter CR-400 (Minolta Co., Ltd., Osaka, Japan) using D-65 lighting, a 2° standard observer angle and an 8-mm aperture in the measuring head (Honikel, 1998; Tomović et al., 2013). The Chroma Meter was calibrated using a Minolta calibration plate (No. 11333090; Y = 92.9, x = 0.3159; y = 0.3322). Fifteen replicate measures of surface color were performed on three samples from each batch.

2.4. TBARS determination

TBARS (2-thiobarbituric acid reactive substances) test was performed according to the method of Bostoglou et al. (1994), with modifications. Total volume of trichloroacetic acid (TCA) was added to the sample and extraction was performed in ultrasonic bath XUB 12 (Grant Instruments, Cambridge, UK) (Mandić, 2007). Spectrophotometer Jenway 6300 (Jenway, Felsted, United Kingdom) was used. TBARS values were expressed as milligrams of malondialdehyde per kilogram of sample. TBARS test was performed on three samples from each batch in duplicate.

2.5. Microbiological analysis

Microbiological analyses were performed on three samples from each batch in duplicate. Twenty grams of samples were homogenized for 10 min at 200 rpm (Unimax 1010, Heidolph, Germany) in 180 mL 1 g/L buffered peptone water (Merk, Darmstadt) and then serial of decimal dilution were prepared (up to 10−3). One milliliter of each dilution was placed in a sterile Petri plate and poured with appropriate media depending on the type of tested microorganism.

Total aerobic mesophilic bacteria count was enumerated in Plate Count Agar (PCA) (Merk, Darmstadt, Germany) and incubated at 30 °C for 72 h; total yeasts and molds count was enumerated in Dichloran Rose Bengal Chloramphenicol agar (DRBC) (Merk, Darmstadt, Germany) and incubated at 25 °C for 5 days; *Escherichia coli* was determined on Tryptone Bile Glucuronic Agar (TBX agar) (Merk, Darmstadt) after an incubation at 37 °C for 24–48 h; *Clostridium* spp. was determined on Tryptone Sulfite Cycloserine (TSC) Agar (Merk, Darmstadt) after an incubation at 37 °C for 24–48 h under anaerobic conditions; total *Enterobacteriaceae* count was enumerated in Violet Red Bile Glucose Agar (VRBGA) (Merk, Darmstadt, Germany) at 37 °C for 24–48 h.

After incubation, typical and atypical grown colonies have been identified by microscopic observation of cell morphology and also with biochemical tests. Results were expressed as a number of colony forming units per gram (cfu/g).

2.6. Sensory evaluation of aroma

A panel consisting of seven trained members of different ages performed sensory evaluation of aroma (ISO 8586-1, 1993; ISO 5492, 2008; ISO 8586-2, 2008). The casing was removed; the sausages were cut into slices of approximately 4 mm thickness and served at room temperature on white plastic dishes. Three slices were served from each batch. Water and unsalted toasts were provided to cleanse the palate between samples. Evaluation was performed according to quantitative descriptive analysis (QDA), using a scale from 0 to 5, with a sensitivity threshold of 0.25 points (ISO 4121, 2003; Stone & Sidel, 2004). Each mark means distinctive quality level, described as follows: 5 – extraordinary, typical, optimal quality; 4 – observable deviations or insignificant quality defects; 3 – drawbacks and defects of quality; 2 – distinct to very distinct drawbacks and defects of quality; 1 – fully changed, atypical properties, product unacceptable; 0 – visible mechanical or microbiological contamination, atypical product.