Meat Science 97 (2014) 459–467

Contents lists available at ScienceDirect

Meat Science

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

Antioxidant and antimicrobial activity of *Kitaibelia vitifolia* extract as alternative to the added nitrite in fermented dry sausage

Vladimir S. Kurćubić [a,*], Pavle Z. Mašković [a,1], Jelena M. Vujić [b,1], Danijela V. Vranić [c,2], Slavica M. Vesković-Moračanin [c,2], Đorđe G. Okanović [d,3], Slobodan V. Lilić [c,2]

[a] Department of Food Technology, Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Republic of Serbia
[b] Department of Chemistry and Chemical Engineering, Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Republic of Serbia
[c] Institute for Meat Hygiene and Technology, Kaćanskog 13, 11000 Belgrade, Republic of Serbia
[d] Institute of Food Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Republic of Serbia

ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form 9 March 2014
Accepted 19 March 2014
Available online 26 March 2014

Keywords:
Kitaibelia vitifolia
Ethanol extract
Fermented dry sausage
Antioxidant activity
Antimicrobial activity
Proximate composition

ABSTRACT

Fermented dry sausages (FDS) without nitrite added, fortified with bioactive phenol and flavonoid compounds originating from the ethanol extract of *Kitaibelia vitifolia* were food matrix for investigation of its antioxidant and antimicrobial potency. These activities were researched in order to improve the sausages' shelf-life, safety, and provide health benefits to consumers as well. The oxidative stability of the FDS, containing two different levels of natural preservative, was evaluated using five different contemporary methods for antioxidative activity. The activity was tested on the 20th day of the refrigerated storage. Minimum inhibitory concentrations of the sausage extract were determined against six microorganisms, using a micro dilution method. Determined optimal effective concentration of dissolved *K. vitifolia* extract (12.5 g/kg of meat dough) revealed strong antioxidant activity, and moderate antimicrobial activity against *Escherichia coli* (minimum inhibitory concentrations = 15.625 μg/mL). The modified sausages had typical chemical–physical characteristics of FDS, controlled on 0, 13, 26 d of ripening and 20, 40 and 60 d of storage.

1. Introduction

Fermented sausages are meat products made without heat treatment during the production process, which enables the biological values of nutritionally essential elements (proteins, vitamins, minerals) to stay unchanged. Despite the widespread production, Europe is still a major producer and consumer of fermented dry sausages (FDS), with the highest figures in Germany, Italy, Spain, and France (Lücke, 1998; FICT, 2002; Di Cagno et al., 2008).

Modification of the conventional composition of fermented sausages in order to make them healthier is technologically possible thanks to the addition of plant extracts, fibers and vegetables, elimination/partial replacement of fats, and reduction of different additives (Mendoza, Garcia, Casa, and Slgas, 2001; Muguerza, Gimeno, Ansorena, and Astiasaran, 2004; Fernández-Ginés, Fernández-López, Sayas-Barberá, and Pérez-Alvarez, 2005; Müller, 2006; Vasilev et al., 2010). Nitrite reactions result in change in the color of cured meat, microbial inhibition, antioxidant effects and flavor (Schrader, 2010). Reduction in the use of nitrites has become a one of the most important aims for the meat processors. Nitrite is recognized as a potentially toxic compound of cured meat, including chemical toxicity, formation of carcinogens (reactions with some biogenic amines and formation of N-nitrosamines) in food or after ingestion, and reproductive and developmental toxicity (Coughlin, 2006). Some experiments on nitrites reduction were successfully applied (Sebranek and Bacus, 2007; Yilmaz and Zorba, 2010; Şükrü & Ömer, 2011; Hospital, Hierro, and Fernández, 2012). Schrader (2010) reports that the two types of "uncured, no-nitrate-or-nitrite-added" meat products are available on the marketplace, as natural or organic, with a higher level of safety than the traditional products. The first type is a truly uncured product, with no replacement for nitrate or nitrite, whose typical properties showed to be more variable than that observed in conventionally cured meats. The second type is produced using alternative methods which utilize naturally occurring nitrates and nitrites found in vegetables and sea salts and demonstrate traditional color and flavor characteristics.

The above mentioned results suggest that active principles of plant species *Kitaibelia vitifolia* have potential for being used in preservation of meat products, without available literature data on its traditional use.

* Corresponding author. Tel.: +381 32 30 34 00, +381 64 125 94 52 (mobile); fax: +381 32 30 34 01.
E-mail addresses: vkurcubic@kg.ac.rs, vkurcubic@open.telekom.rs (V.S. Kurćubić), pavlem@kg.ac.rs (P.Z. Mašković), jelenav@kg.ac.rs (J.M. Vujić), daniv@inmesbgd.com (D.V. Vranić), slavica@inmesbgd.com (S.M. Vesković-Moračanin), djordje.okanovic@fins.uns.ac.rs (Đ.G. Okanović), slobo@inmesbgd.com (S.V. Lilić).
[1] Tel.: +381 32 30 34 00; fax: +381 32 30 34 01.
[2] Tel.: +381 11 265 06 55; fax: +381 11 265 18 25.
[3] Tel.: +381 21 48 53 707; fax: +381 21 45 07 30.

http://dx.doi.org/10.1016/j.meatsci.2014.03.012
0309-1740/

The purpose of the present study was to develop no-nitrite-added fermented dry sausages, modified by using ethanol extract from the overground part (stems, leaves and flowers) of *K. vitifolia* as a functional ingredient. The primary aim of study was to select the most suitable effective concentration of extract to be added, on the basis of the oxidative and microbiological stability of FDS during storage under different conditions (aerobic or anaerobic packs). The impact of the nitrites replacement on the quality aspects of FDS was yet another aim.

2. Material and methods

2.1. Plant material

K. vitifolia is a member of the *Malvaceae* family, an imposing and undemanding Mallow from ex-Yugoslavia. The above-ground part of the test plant was collected in Central Serbia (latitude 40.1965, longitude 20.28352, and 322 m above sea) in May 2009, at its flowering stage. This robust, perennial, shade-loving plant has bold, maple-like leaves (silvery when young) and a stalk-full of small white-to-pink flowers. Plant hardiness of *K. vitifolia* is in the zone 6 (−23.33 °C to −17.78 °C). The species was identified and the voucher specimen was deposited at the Department of Botany, Faculty of Biology, University of Belgrade (16350 BEOU, Lakušić Dmitar).

2.2. Preparation of herb extract

Samples prepared from the over ground part of the plant *K. vitifolia* (10.0 g) were extracted by 96% ethanol (100.0 mL) as a solvent. The extraction process was carried out using an ultrasonic bath (Brason and Smith-Kline Company, B-220) at room temperature for 1 h. The goal was the highest extraction yield of phenol acids. After filtration, 5 mL of the liquid extract was used for extraction yield determination. The solvent was removed by a rotary evaporator (Devarot, Elektromedicina, Ljubljana) under vacuum, and was dried at 60 °C to constant weight. The dried extracts were stored in glass bottles at 4 °C to prevent oxidative damage until analysis. Extract of the *K. vitifolia* was dissolved in sterile distilled water at concentrations of 3% (w/v) and 10% (w/v), before adding in fortified production batches PB II and PB III of FDS. Value of pH of the prepared extract was 4.5. Spectrophotometric measurements were performed using a UV–VIS spectrophotometer MA9523-SPEKOL 211 (ISKRA, Horjul, Slovenia). The chemical composition of ethanol herb extract of *K. vitifolia* used in the present study was previously determined by Mašković et al. (2011).

2.3. Sausage formulation and processing

Three production batches (PB) of fermented dry sausages (FDS), about 20 kg each, were prepared according to the procedure described below. Three formulations of FDS were made with frozen pork shoulder (40%), beef meat II category (loin, back, shoulder — 30%) and frozen pork back fat (30%). The following additives were added in gram per kilogram quantities to the meat mixture of PB I: spice for Kulen (Lay Gewürze) 11 g/kg, Paprika extract — Oleorezin 30.000 FE (Lay Gewürze) 1 g/kg, nitrite salt 27 g/kg, TARI® S77 [GdL (E575), sugars, salt, and sodium-isoascorbate (E 316)] 9 g/kg. This original mixture was used as control sample. To assess the influence of the various concentrations of herb extract, nitrite was replaced by dissolved herb extracts of the *K. vitifolia* in effective concentration of 30.0 g/kg of meat dough in PB II and 12.5 g/kg of meat dough in PB III. To avoid the influence of its own antioxidant potential of hot pepper and garlic to the accuracy of the results of determining antioxidant effects, these traditional spices were not added to the experimental sausages.

The FDS were prepared on the same day and in an identical manner in a small-scale plant "Kotlenik-promet" Ltd (Lađevci, Central Serbia), in accordance with industrial processing. Partially defrosted meat and pork fat were first cut into small pieces using a guillotine (Sind Šabac, Serbia). The meat was minced using a meat grinder (REX Technologie GmbH & Co. KG) down to the size of about 8 mm, and then transferred to the cutter. All of the other ingredients were added and mixed with minced meat in a cutter for 4 min at the temperature of −1 to −3 °C. Herb extract of *K. vitifolia* or nitrite salt was added to the prepared meat dough in accordance with the following recipe: PB I was prepared as a control group with nitrites; PB II was manufactured with *K. vitifolia* extract prepared at concentrations of 3% (w/v), and added to meat dough in quantity of 30.0 g/kg. PB III was produced with *K. vitifolia* extract prepared at concentrations of 10% (w/v), and added to meat dough in quantity of 12.5 g/kg. As a following process, each stuffing was filled into natural pork casings of 36–38 mm diameter (country of origin: Spain), using a filling machine (VEMAG, model ROBBY-2, 1998) at 2 °C. Filled sausages were hand-paired, hung on metal rods, set on the cart and transported to an air-conditioned chamber for ripening, smoking and drying.

Temperature and relative humidity (RH) in the air-conditioned chamber during the process of ripening, smoking and drying were changed: 22 °C/92% on 1st day, 20 °C/88% on 2nd day, 19 °C/86% on 3rd day, 18 °C/82% for 4th day, 17 °C/78% for 5th day and 15 °C/72% from the 6th to 26th day. Sausages were smoked 5 h daily (from the 3rd to the 5th day) with filtrated smoke from beech wood, smoked temperature 18 °C. At the end of the ripening process, which lasted for 26 d, sausages were stored at 4 °C. Each PB of sausages was divided in two groups: first was aerobically packed and the second one was vacuum packed (Inauen Machinen, AG VC 999) using polyethylene (PE) bag thickness 0.07 mm (Blik-produkt Kikinda, Serbia). Chemical–physical analysis was carried out during processing (ripening: 0th, 13th and 26th day) and storage at 4 °C (after 20, 40 and 60 d). Antioxidant experiments were conducted on 20th day of storage (46 d from the 0 d).

2.4. Preparation of sausage extract

Wrappers were removed from FDS samples, stuffed, chopped, and then homogenized in the blender. Ten grams of homogenized sausages sample was taken and dissolved in 10 mL of distilled water to obtain a concentration of 1 mg per 1 mL of distilled water. The aqueous extract of FDS was prepared for antioxidant and antimicrobial activity testing.

2.5. Determination of total phenol content in FDS

Total phenols in experimental FDS were estimated according to Singleton, Orthofer, and Lamuela-Raventos (1999). The aqueous extract of sausages was diluted to the concentration of 1 mg/mL, and aliquots of 0.5 mL were mixed with 2.5 mL of Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) and 2 mL of $NaHCO_3$ (7.5%). After 15 min at 45 °C, the absorbance was measured at 765 nm using a spectrophotometer against a blank sample. Total phenols were determined as gallic acid equivalents (mg GA/g extract), and the values are presented as mean of three determinations.

2.6. Determination of flavonoid content in FDS

Total flavonoids in experimental FDS were determined according to Brighente, Dias, Verdi, and Pizzolatti (2007). A total of 0.5 mL of 2% aluminum chloride ($AlCl_3$) in methanol was mixed with the same volume of methanol solution of plant extract. After 1 h at room temperature, the absorbance was measured at 415 nm in a spectrophotometer, compared to a blank sample. Total flavonoids were determined as rutin equivalents (mg RU/g dry extract), and the values are presented as mean of three determinations.

2.7. Determination of total antioxidant (AOX) capacity

The total antioxidant activity was evaluated by the phosphormolybdenum method (Prieto, Pineda, and Aguilar, 1999). The assay is