LWT - Food Science and Technology 85 (2017) 89–95

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Effect of phenols extracted from a by-product of the oil mill on the shelf-life of raw and cooked fresh pork sausages in the absence of chemical additives

Stefania Balzan [a], Agnese Taticchi [b], Barbara Cardazzo [a], Stefania Urbani [b], Maurizio Servili [b], Giuseppe Di Lecce [c], Izaskun Berasategi Zabalza [d], Maria Teresa Rodriguez-Estrada [e], Enrico Novelli [a, *], Luca Fasolato [a]

[a] Department of Comparative Biomedicine and Food Science, University of Padova, viale Università 16, I-35020, Legnaro, Italy
[b] Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, I-06121, Perugia, Italy
[c] Department of Agricultural and Food Sciences, Alma Mater Studiorum-Università di Bologna, piazza Goidanich 60, I-47023, Cesena, Italy
[d] Department of Nutrition, Food Science and Physiology, University of Navarra, C/ Irunlarrea 1 E-31008 Pamplona, Spain
[e] Department of Agricultural and Food Sciences, Alma Mater Studiorum-Università di Bologna, Viale Fanin 40, I-40127, Bologna, Italy

ARTICLE INFO

Article history:
Received 27 January 2017
Received in revised form
31 May 2017
Accepted 2 July 2017
Available online 3 July 2017

Keywords:
Olive vegetation water
Phenols
Fresh pork sausage
Lipid oxidation
Shelf-life

ABSTRACT

Replacing chemical additives in meat preparations with natural compounds is a matter of great interest today, both to consumers and to the food sector. The effect of an extract rich in phenols obtained from olive vegetation water (an agricultural by-product) on the pH, weight loss after cooking, diacylglycerols (DAGs), peroxide value (POV), thiobarbituric acid reactive species (TBARS), and cholesterol oxidation products (COPs) of raw and cooked fresh pork sausages prepared without chemical additives was evaluated before and after aerobic storage at 2 ± 2 °C for 14 d. Adding the extract at concentrations of 0.075 and 0.15 g/100 g resulted in a decrease in pH, DAGs, POV, TBARS and COPs; notably, the COPs levels were 4- and 17-fold lower in raw and cooked sausages, respectively. Sensory analysis revealed significant differences between control samples and those enriched with the extract, but the enriched samples were never considered unpleasant by the panellists. Storing the raw sausages for 14 d and subsequently cooking them led to 58% and 49% decreases in phenols, respectively. The purified phenols from olive oil wastewater proved to be an effective antioxidant, thus demonstrating themselves to be a potential ingredient to ensure the quality and safety of meat preparations.

1. Introduction

The consumption of fresh ground meat preparations is widespread due to their pleasant taste and ease of cooking. The lipid fraction of ground meat can rapidly oxidize due to its large surface/mass ratio. Rancidity causes a general deterioration of the sensory quality while generating free radicals and non-radical reactive derivatives (reactive oxygen species (ROS) and reactive nitrogen species (RNS)). After ingestion, ROS and RNS are decomposed or metabolized into free radicals, which are involved in a series of chronic degenerative pathologies other than cancer (World Cancer Research Fund, 2007). Food additives, such as antioxidants, are generally used with the goal of controlling lipid oxidation during food processing and storage. Even if consumers are aware of the benefits of using additives, they have a strong expectation of foods with the fewest or lowest possible level of additives (Brockman & Beeren, 2011). Although the current regulations do not distinguish between natural and synthetic additives, consumers tend to sharply distinguish between the two (Tarnavölgyi, 2003). When an item existing in both natural and artificial versions (but chemically identical) is submitted to consumer evaluation, those who generally prefer natural products continue to prefer the natural one. However, the preference for natural products appears to be mainly an ideological concept rather than an evaluation of objective and

* Corresponding author.
E-mail addresses: stefania.balzan@unipd.it (S. Balzan), agnese.taticchi@unipg.it (A. Taticchi), barbara.cardazzo@unipd.it (B. Cardazzo), stefania.urbani@libero.it (S. Urbani), maurizio.servili@unipg.it (M. Servili), leccegius@hotmail.com (G. Di Lecce), izaskun.nutricion@gmail.com (I.B. Zabalza), maria.rodriguez@unibo.it (M.T. Rodriguez-Estrada), enrico.novelli@unipd.it (E. Novelli), luca.fasolato@unipd.it (L. Fasolato).

http://dx.doi.org/10.1016/j.lwt.2017.07.001
0023-6438/

measurable superior qualities (Rozin et al., 2004). There is a large and public perception that processed meat is unhealthy (Tobin, O'Sullivan, Hamill, & Kerry, 2014). According to a survey on the health benefits of processed meats, half of the respondents believed that processed meat contains large quantities of harmful chemicals (Tobin et al., 2014).

One alternative approach to the use of chemical additives could be the use of natural antioxidants. Free radical or active oxygen scavenging capacity has been detected in several phenolic compounds (Brown & Rice-Evan, 1998). Virgin olive oil (VOO) is a basic component of the Mediterranean diet with a well-established role in contributing to human health, mainly attributed to the antioxidant actions of a composite class of hydrophilic phenols (Covas, 2008). During VOO production by a three-phase centrifugation system, a by-product known as olive vegetation water (OVW) is formed (between 50 and 90 L of OVW/100 kg of olive paste) (Servili et al., 2011a). OVW is an emulsion made of oil, mucilage and pectin, with organic compounds that range between 3 and 16 g/100 mL of which 0.3–1.1 g/100 mL are phenols. Currently, resolving the OVW disposal problem is fundamental from an ecological viewpoint, and the recovery of the phenols provides an added value to OVW that would otherwise represent only a cost for oil mills. The purified phenolic extract (PE) with a 65 g/100 g concentration of phenolic compounds used in the present study was obtained from fresh OVW arising from Moraiolo Cv. Olives, grown in Umbria region (Central Italy), according to Esposto et al. (2015). The PE was stored a −20 °C in the dark, its qualitative and quantitative composition was evaluated at its using time. It largely contained the aglycone of the hydroxylated form of the tyrosol ester of elenolic acid (3,4-DHPEA-EDA), followed by verbascoside (VB), hydroxytyrosol (3,4-DHPEA) and tyrosol (*p*-HPEA). The OVW is the result of a simple mechanical separation, whereas the membrane filtration process at room temperature allows to obtain the PE. Therefore, its phenolic compounds are the same occurring in olive fruits and in VOO, thus guaranteeing its genuineness and naturalness.

The present study had two main goals. The first was to assess the storage stability of fresh sausages intended for eating after cooking. The sausages were made using only ground meat and salt as ingredients according to a simple recipe and were supplemented with two levels of a PE obtained from OVW. The second goal was to monitor the kinetics of the decrease in the concentration of phenols in the raw sausages during refrigerated storage as well as after cooking. To the best of our knowledge, this is the first study to monitor the kinetics of the transformation and degradation of phenolic compounds during the shelf-life of a refrigerated fresh meat preparation.

2. Materials and methods

2.1. Manufacture, storage and cooking of sausages

The entire experiment was performed in three replicates, each one using slightly more than 40 kg of shoulder and belly pork (approximately 50/50, w/w) that was ground with a professional meat mincer equipped with a steel plate with 5 mm diameter holes (Cavalli Meat Processing Machinery Srl, Felino, Italy). The minced meat was mixed with salt (1.5 g/100 g) using an electric mixer (Cavalli Meat Processing Machinery Srl), after which the dough was divided into three batches: i) Control, the minced meat plus salt alone; ii) L1, Control plus PE equivalent to 75 mg of phenols/100 g of dough; and iii) L2, Control plus PE equivalent to 150 mg of phenols/100 g of dough. With the goal of avoiding secondary and non-standardizable antioxidant actions from other ingredients, no spices were added. Each batch was further mixed for 1 min, stuffed into 40 mm diameter bovine casings by an hydraulic piston-type stuffer (Cavalli Meat Processing Machinery Srl). The sausages, nearly 100 g each, were left to drip at 15 ± 1 °C for 6 h and then stored without packaging (to simulate a widespread commercialization mode at retail and butcheries) in a display cabinet under alternating exposure to fluorescent light (12 h dark and 12 h light; Osram Natura De Luxe L36 W/76–1, Munich, Germany) at 2 ± 2 °C for 14 d. At 0, 7 and 14 d, a representative number of sausages from each batch were sampled and frozen in liquid nitrogen before being stored at −80 °C until analysis. At the same sampling times, the same number of sausages from each batch were cooked at 200 °C in a ventilated electrical oven (MPM Instrument Srl, Bernareggio, Italy) to an internal temperature of 85 °C (Testo 700 digital thermometer probe, Testotherm, Postfach 1140, Lenzkirch/Schwartzwald, Germany). After cooking, the sausages were cooled in an ice bath, stored for 72 h at 2–4 °C and then frozen in liquid nitrogen before being stored at −80 °C until analysis.

2.2. Extraction and HPLC evaluation of phenolic compounds in sausages

Ten grams of sausage were mixed with 100 mL of methanol and water (80/20, v/v) containing 20 mg/L of butylated hydroxytoluene (BHT). The system was homogenized using a rod disperser (IKA, T50 Ultra-Turrax, Werke, Staufen, Germany) for 1 min at 7000 rpm, centrifuged at 5000 rpm for 10 min and the supernatant recovered. The operation was repeated twice, and the collected extract was then concentrated by a rotary evaporator (Buchi Rotavapor, R-215, Flawil, Switzerland) until reaching a final volume of 50 mL, which was used for the extraction of phenols by solid-phase extraction (SPE). A C18 SPE cartridge, previously activated with 10 mL of methanol and 10 mL of water, was loaded with 2 mL of aqueous extract. The elution was performed with 50 mL of methanol. After solvent removal under vacuum, the phenolic extract was solubilized in 0.5 mL of methanol and filtered with a 0.2 μm PVDF filter. The extract was submitted to HPLC analysis (Montedoro et al., 1993). Each measurement was done in duplicate.

2.3. Proximate composition and salt

Moisture and crude protein were measured according to AOAC (1990). Crude fat was determined according to Boselli, Velazco, Caboni, and Lercker (2001). The salt content was determined using the Volhard method (AOAC, 1990). Each measurement was done in duplicate.

2.4. pH, cooking loss and diacylglycerols

The pH was determined after blending 10 g of sample with 90 mL of distilled water using a Portamess pH meter (Knick 910, Berlin, Germany) equipped with an INLAB 427 electrode (Mettler Toledo, Urdof, Switzerland). To measure cooking loss, samples were weighed before and after cooking. The cooking loss was calculated as [(fresh weight−cooked weight)/fresh weight] × 100. Diacylglycerols (DAGs) were determined by gas chromatography (Bonoli, Caboni, Rodriguez-Estrada, & Lercker, 2007). Each measurement was done in duplicate.

2.5. Peroxide values and thiobarbituric acid reactive substances

Peroxide values (POV) were determined in 15–40 mg of lipid extract (Shantha & Decker, 1994). Thiobarbituric acid reactive substances (TBARS) were determined in 2 g of sample (Witte, Krause, & Bailey, 1970). Each measurement was performed in duplicate.