Meat Science 145 (2018) 389–398

Contents lists available at ScienceDirect

Meat Science

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

Effect of natural antioxidants from grape seed and chestnut in combination with hydroxytyrosol, as sodium nitrite substitutes in Cinta Senese dry-fermented sausages

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ARTICLE INFO

Keywords:
Volatile compounds
Meat quality
GC-olfactometry
Local pig breed
Lipid oxidation
Pork products

ABSTRACT

Dry-fermented pork sausages, from Cinta Senese local breed, were manufactured replacing sodium nitrite (NIT) with two mixtures of natural antioxidants consisting of: i) grape seed extract and olive pomace hydroxytyrosol (GSE); ii) chestnut extract and olive pomace hydroxytyrosol (CHE). The effects on physical-chemical, aromatic and sensory traits, as well as the microbiological safety, were tested. Nitrite replacement lowered the pH in GSE and CHE samples and resulted in several differences in physical traits between CHE and NIT samples. *Listeria monocytogenes, Salmonella* and *Clostridium botulinum* were not found in any samples. GSE and CHE mixtures showed a slightly lower antioxidant activity. Volatile profile showed a similar aromatic profile among the three treatments with differences mainly to abundance of the single compounds, indicating that replacement of nitrite by natural antioxidants did not affect the overall aroma profile, as outlined by olfactometry results. In addition, the replacement did not affect the overall acceptability, except for color-related traits, underscored in GSE and CHE products.

1. Introduction

Dry cured meat products are typical of the Mediterranean area and they represent a high-value production in European countries, considering that curing process allows extension of meat shelf-life (Marco, Navarro, & Flores, 2006) and leads to typical pork products with specific eating quality and regional identity (Pugliese & Sirtori, 2012). In Southern Europe, salami and dry-fermented sausages, are generally characterized by slowly air-drying and mold-ripening (Flores, 1997). This curing process leads to peculiar characteristics and flavors that are widely appreciated by consumers; but is also related to longer curing times that may cause higher lipid oxidation levels. Moreover, natural fermentation, avoiding the addition of lactic acid-producing starter cultures, is more susceptible to the growth of harmful bacteria, such as *Listeria monocytogenes* or *Clostridium botulinum* (Lücke, 2000). Thus, to avoid a severe deterioration of nutritive and organoleptic attributes, as well as to ensure food safety, several synthetic food preservatives are commonly included. Among them, the most used are nitrites and nitrates (Hammes, 2012). Nitrite positively affects color, inhibits the growth of pathogenic bacteria, contributes to the development of typical cured meat flavor and delays oxidative rancidity (Marco et al., 2006). Despite their effectiveness as curing agents, the nitrite/nitrate intake represents a risk to human health, i.e. the formation of carcinogenic nitrosamines is one of the most current concerns (De Mey, De Maere, Paelinck, & Fraeye, 2017). Several studies have focused on nitrate/nitrite reduction or substitution (Özvural & Vural, 2014; Pateiro, Bermúdez, Lorenzo, & Franco, 2015; Purriños, García Fontán, Carballo, & Lorenzo, 2013), but the main issue remains finding an alternative able to address the multiple activities they perform. Up until now, most of the alternatives proposed are plant extracts, largely obtained from agricultural by-products. These compounds are very rich in polyphenols, flavonoids and terpenoids and are able to perform a double antioxidant-antimicrobial functions (Falowo, Fayemi, & Muchenje, 2014; Hygreeva, Pandey, & Radhakrishna, 2014; Shah, Bosco, & Mir, 2014). These compounds might also constitute a great opportunity to exploit agricultural by-products, which otherwise would be wasted. The aim of this study was to assess the feasibility of producing dry-fermented sausages by replacing sodium nitrite with natural antioxidants

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https://doi.org/10.1016/j.meatsci.2018.07.019
Received 26 April 2018; Received in revised form 13 July 2018; Accepted 13 July 2018
Available online 17 July 2018
0309-1740/

Table 1
Phenolic profile of olive pomace and defatted grape seed and chestnut extracts.

Olive pomace (g/L)		Grape seed		Chestnut	
(hydroxytyrosol)		(mg/g)		(mg/g)	
Hydroxytyrosol	11.65	Gallic acid	0.01	Vescalin	9.34
Tyrosol & hydroxytyrosol derived compounds	15.13	Catechin B3 (dimers)	2.22	Castalin	8.99
Verbascosid	5.84	Catechin	11.07	Pedunculagin I	3.88
		Catechin (trimers)	3.21	Monogalloil glucose I	3.58
		Catechin B6 (dimers)	2.61	Gallic acid	18.50
		Catechin B2 (dimers)	5.37	Monogalloil glucose II	2.73
		Epicatechin	13.62	Roburin D	10.51
		Catechina trimer	3.71	Vescalagin	32.15
		Epicatechin gallate (PM 730)	6.65	*C*-glucoside tergallic dehydrate	2.73
		Epicatechin gallate (PM 442)	6.10	Castalagin	31.03
		Oligomers (tetramers)	54.88	Digalloil glucose I	10.03
		Epicatechin gallate (PM 882)	180.65	Digalloil glucose II	2.09
		Epicatechin gallate oligomers (trimers)	382.97	Hydrolyzable tannin *m/z* 1085	8.05
		Epicatechin gallate oligomers (trimers)	149.66	Trigalloil glucose I	4.61
				Trigalloil glucose II	6.74
				Tetragalloil glucose	2.05
				Ellagic acid	4.08

while trying to maintain quality traits. Grape seed extract, chestnut extract and hydroxytyrosol (extracted from defatted olive pomace), were chosen due to their great availability as by-products of important Tuscan agricultural products. Moreover, among the investigated plant extracts, they have shown an interesting potential both for antioxidant activity and microbial inhibition. This innovation also aimed to valorize Cinta Senese, a local pig breed strongly linked to the Tuscan region.

2. Materials and methods

2.1. Antioxidant mixtures

The natural antioxidants employed in the present studies were provided by Phytolab (Sesto Fiorentino, Florence, Italy). They consisted of grape seed and chestnut extracts, tocopherol and hydroxytyrosol (extracted by defatted olive pomace). The manufacturer provided the phenolic profile (Table 1), total phenolic content and antiradical scavenging activity (EC_{50}) (Table 2) of each extract. The grape seed and chestnut extracts were combined with the same amount of hydroxytyrosol and tocopherol to form two different mixtures; grape seed (GSE) and chestnut (CHE) mixtures.

2.2. Sausages manufacturing

In an industrial plant (Azienda Agricola Savigni, Pistoia, Italy), 24 kg of pork lean and 6 kg of subcutaneous backfat from Cinta Senese pig breed were minced and equally divided in three batches. Salt (23 g/kg), sucrose (35 g/kg) and black pepper (0.2 g/kg) were added to each batch following the recipe traditionally used by the manufacturer. Thirty ppm of sodium nitrite (E250) were added to the first batch to constitute the control (NIT). In second batch, 10 g/kg of GSE mixture were used to replace sodium nitrite, while 10 g/kg of CHE were added to the third batch. Sausages were weighed, dried at 28 °C and RH 85% for 4 days and then ripened 21 days (T 13 °C, RH 70%). Once ripened, six samples of each batch were collected, pH, color, and processing loss were immediately measured. Samples were vacuum packed and stored at −80 °C for physical, chemical and aromatic analysis. Another 3 samples of each batch were stored at 4 °C to be employed for sensory analysis the following day. This design was replicated to have two totally independent batches for each treatment.

Table 2
Total phenolic content and radical scavenging activity of natural antioxidant constituting the mixtures.

	Total phenolic content	Antiradical scavenging activity (EC_{50})
Grape seed extract	822.709 (mg/g)	0.147
Chestnut extract	161.091 (mg/g)	0.085
Olive pomace (hydroxytyrosol)	32.62 (g/l)	0.196
α-tocopherol	–	0.184

2.3. Physical, chemical and microbiological parameters

At the end of ripening, physical parameters were assessed on 12 samples of each batch (6 for each replication). Sausage pH was measured at room temperature (20 °C) using a pH meter Crison GLP21 (Barcelona, Spain), the instrument was introduced in a sausage portion. Color (L*, a* and b*) was determined by a Minolta Chromameter CR-200 (Tokyo, Japan) immediately after slicing. a_w was measured following the method ISO 21807:2004. Two 10 mm-thick and 10 mm-width slices of each sample, were cut and immediately analyzed at room temperature (22 °C), using a Zwick Roell Z2.5 apparatus (Ulm, Germany) with a loading cell of 1 kN at the crosshead speed of 1 mm/s. Texture profile analysis (TPA) was performed assessing the following parameters: hardness, cohesiveness, gumminess, springiness and chewiness. Moisture was determined by lyophilizing to constant weight 40 g of sample, according to AOAC methods (1990). Weight loss was measured as the difference between weight at time zero and end of ripening (after 24 days). Total protein, fat and ash contents were determined following AOAC (1990) methods. Lipid oxidation was determined according Vyncke (1970), using a PerkinElmer Lambda EZ150 spectrophotometer (Waltham, MA, USA). Results were expressed as mg of malondialdehyde (MDA)/kg of samples. Fatty acids were determined using a Varian GC-430 apparatus equipped with a flame ionization detector (FID) (Palo Alto, CA, USA) as reported by Sirtori et al. (2015). The individual methyl esters were identified by their retention time using an analytical standard (F.A.M.E. Mix, C8-C22 Supelco 18,920-1AMP). Response factors based on the internal standard (C19:0) were used for quantification and results were expressed as mg/100 g of sample. The fatty acid content was reported as saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Microbiological analyses were carried out in an external accredited laboratory to determine the products' safety. The following bacteria were investigated: *Escherichia coli* (ISO 16649–2:2001), *Listeria monocytogenes* (UNI EN ISO 11290–1:2005), coagulase positive *Staphylococcus* spp. (UNI EN ISO 6888–1:2004), *Clostridium botulinum* (ISO 15213:2003) and *Salmonella* spp. (UNI EN ISO 6579:2008).