Comparison of antioxidant content in different meat products from the Croatian market

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Abstract

Food additives are defined as any substance not normally consumed as a food itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value. Antioxidants are a class of food additives that can be added to meat products to prevent lipid oxidation, retard development of off-flavours, or improve colour stability. Ascorbic acid (AA) and erythorbic acid (EA) are antioxidants permitted for use in meat preparations and meat products. This study aimed to compare the content of AA and EA in dry and semi-dry sausages collected on the Croatian market during a 4-year period and to determine their conformity with consumer safety regulations. AA and EA were analysed by a validated UHPLC method with a limit of detection and limit of quantification below 3 and 10 mg/L, respectively. The concentration of AA in dry sausages ranged from 13 to 457 mg/kg and in semi-dry sausages from 170 to 545 mg/kg, while the concentration of EA ranged from 198 to 472 mg/kg in dry sausages and from 14 to 400 mg/kg in semi-dry sausages. In total, 12 samples (8.9%) were non-compliant due to an excessive concentration of EA.

Key words: *additives; L-ascorbic acid; erythorbic acid; sausages*

Introduction

Processed food is an important part of the human diet, so the number of chemicals added to the food as additives with varying functions is continuously increasing. Food additives are defined as any substance not normally consumed as a food itself and not normally used as a characteristic ingredient of food, whether or not it has nutritive value. Additives are substances added to food to preserve flavour or enhance its taste, appearance, or any other qualities (Blekas, 2016; Wu et al., 2021).

Based on origin, additives are divided into two groups: natural or synthetic additives. Natural additives are extracted and purified from animal or plant sources while synthetic additives are the result of a chemical or enzymatic reaction (André, 2010; Scotter, 2011). In addition to classification according to origin, additives can be classified according to their function:

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sweeteners, colorants, preservatives, antioxidants, carriers, acids, acidity regulators, anticaking agents, antifoaming agents, bulking agents, emulsifiers, emulsifying salts, firming agents, flavour enhancers, foaming agents, gelling agents, glazing agents, humectants, modified starches, packaging gases, propellants, raising agents, sequestrates, stabilizers, thickeners, and flour treatment agents (Council Regulation (EC) 1333/2008). Certain additives have several functions and can thus be included in different groups. Additives are assigned with unique numbers and the numbering method in European Union gives each additive a unique number called an "E number". This numbering scheme has now been adopted and extended by the Codex Alimentarius Commission (Wu et al., 2021).

Antioxidants are a class of additives, among other food categories, that are added to meat preparations and meat products to prevent lipid oxidation, retard development of off-flavours, and improve colour stability (Kumar et al., 2015). The antioxidants permitted in meat and meat products are ascorbic acid (AA) and citric acid (CA) including their salts. Ascorbic acid is widely used as food additive and the food industry uses chemically synthesised, nature-identical L-ascorbic acid. It is an excellent reducing agent, preventing not only oxidation responsible for discolouring in meat during storage, but also the formation of metmyoglobin. The addition of ascorbic acid also improves meat colour and shelf life (Cenci-Goga, 2020) so it is widely used in fresh meat preparations. Erythorbic acid (EA) is a stereoisomer of ascorbic acid with similar physicochemical properties. It is also widely used as an antioxidant in processed foods including meat products, but also in fishery products, i.e., for preventing melanosis in shrimps (Fidler et al., 2004; Varavara et al., 2016; Toktas and Gokoglu, 2020). The use of AA and EA as food additives in different food categories is regulated by Commission Regulation (EU) No 1129/2011 (European Commission, 2011). While the legislation has not defined a maximum permitted level (MPL) for AA and its usage is defined by *quantum satis* (except in not prepacked meat preparations in which AA is not intended for use), the MPL of EA is defined for several food categories and has been set at 500 mg/kg for meat products. Unlike meat products, EA is not permitted for use in meat preparations such as minced meat and hamburgers.

The present study aimed to compare the content of AA and EA in dry and semi-dry sausages from the Croatian market during a four-year sampling period and to determine conformity of these meat products with safety regulations for human consumption.

Materials and Methods

Collection of samples

The study was conducted on 134 samples of meat products, all declared to contain either AA or EA, collected during a 4-year period in the scope of National Monitoring Plan for food of animal origin. Samples of semi-dry (n=69) and dry sausages (n=65) were sampled from the Croatian market in the amount of 500 g per sample. Samples were delivered to the laboratory, immediately grounded using a Grindomix GM 200 laboratory mill (Retsch, Germany) and stored in the freezer at -20°C pending analysis.

Reagents and standards

Potassium dihydrogen phosphate, potassium hydrogen phosphate trihydrate, sodium dihydrogen phosphate,

phosphoric acid and hydrochloride acid were procured from Sigma (Missouri, United States). Analytical standard L-ascorbic acid (≥ 99.7%) was obtained from the European Pharmacopoeia (EP) Reference Standard and erythorbic acid (≥ 99.7%) from the United States Pharmacopeia (USP) Reference Standard. Ultra-pure water with electrolytic conductivity of ≤ 0.05 S/cm was obtained using the Millipore Direct-Q 3UV system (Merck, Germany).

Instrumental analysis

A HPLC (1290 series, Agilent Technologies, Santa Clara, CA, USA) consisting of a degasser, quaternary pump, autosampler and column compartment was coupled with a UV-Diode Array Detection detector (Agilent Technologies, Santa Clara, CA, USA). The analytical HPLC column with PS/DVB stationary phase (PLRP-S, 100 A, 250 x 4.6 mm, 5 µm) was delivered by Agilent Technologies (Santa Clara, CA, USA). The isocratic elution was employed with mobile phase consisted of 0.2 M sodium dihydrogen phosphate, pH=2.14 and the absorbance signals were detected at 244 nm.

Sample preparation

A 4 g sample was mixed with 40 mL phosphate buffer 0.01 M pH 3.5, obtained by dissolving 1.36 g potassium phosphate monobasic and 1.74 g potassium phosphate dibasic in 1000 mL ultrapure water, and then correcting the pH value to 3.5 by the addition of phosphoric acid. The mixture was shaken on a head-over-head shaker for one minute. After centrifugation for 5 min at 4600 rpm at room temperature, the supernatant (about 1 mL) was filtered through a PTFE syringe filter, 0.2 µm pore size (Phenomenex, Torrance, CA, USA) directly into the vial prior to instrumental analysis.

Method validation

The method was validated according to the Eurachem Guide: The Fitness for Purpose of Analytical Methods (Magnusson and Örnemark, 2014). The parameters evaluated were: linearity, repeatability, limit of detection and quantification (LOD and LOQ), and trueness. For each batch, a 5-point solvent calibration curve was prepared with concentrations as follows: 10, 25, 50, 100 and 200 mg/L. The slope of the calibration curves and signal abundances were then used to calculate the limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ were calculated according to the following equations: LOD= 3.3 SE/b and LOQ= 10 SE/b, where SE is the standard error of the regression and b is the slope of the linear regression. Repeatability was tested by analysing six replicates of the same samples while trueness, due to a lack of certified reference material, was tested through interlaboratory comparison.

Statistical Analysis

Statistical analyses were performed using the SPSS Statistics Software 22.0 (IBM, New York, NY, USA). The results were tested for normality of distribution using the Shapiro-Wilks test. The t-test was used to determine the statistical significance of the differences in AA and EA concentrations among the two sausage types, the One-Way ANOVA with Tamhane's T2 post hoc was used to determine differences among sampling years. Decisions on statistical relevance were made at the significance level of $P \leq 0.05$.

Results and discussion

Method validation resulted in LOD values for AA and EA of 2.7 mg/L and 2.5 mg/L, respectively, while LOQ values were 8.3 mg/L and 7.6 mg/L, respectively.

Linearity was tested in the concentration range from 10 to 250 mg/L and showed a good correlation coefficient (≥ 0.99) for both AA and EA. Method repeatability resulted in satisfactory relative standard deviation (RSD) values that were below 10%; 4.30% for AA and 8.23% for EA, while the determination of trueness resulted in differences below 15% for both AA and EA. The validation results were satisfactory so the method was applied for the analysis of dry and semi-dry sausages from the market.

The concentrations of AA and EA determined in dry and semi-dry sausages from the Croatian market are presented in Table 1. None of the tested samples contained both AA and EA, while in 17.9% of samples, either AA or EA weren't detected although all tested samples were declared to contain either AA or EA. These results, that are below LOQ, are pointing that sometimes AA or EA are added in amounts that are not detectable in the final product. Results revealed that there was no statistically significant difference in the concentration of AA (P=0.247) or EA (P=0.555) according to sausage type. A statistically significant difference was observed for sampling year as follows: 2023 for AA (P=0.001) was statistically different from all other sampling years, and 2020 was statistically different for EA (P=0.004) in comparison to 2022 and 2023.

For both sausage types, and for both additives, large standard deviations were observed. The concentration of AA in dry sausages ranged from 13 to 457 mg/kg and in semi-dry sausages from 170 to 545 mg/kg. In dry sausages, the concentration of EA ranged from 198 to 472 mg/kg and in semi-dry sausages from 14 to 400 mg/kg. The data concerning the usage and amounts of AA and EA in meat products are limited.

The data distribution for AA concentration according to sausage type is presented in Figure 1. High concentrations of AA (> 500 mg/kg) were observed both in group of dry and semi-dry sausages. Although these results are high, all samples were compliant with the regulations since AA in meat products is allowed in *quantum satis* amount.

The data distribution for EA concentration according to sausage type is presented in Figure 2. Unlike AA, the amount of

Table 1. Concentration of AA and EA in dry and semi-dry sausages over a 4-year period

Year	Ascorbic acid (mean of positives mg/kg±SD)		Erythorbic acid (mean of positives mg/kg±SD)	
	Dry sausages	Semi-dry sausages	Dry sausages	Semi-dry sausages
2020	190±132^{ab}	170±129^{a}	207±30^{b}	147±128^{a}
2021	237±193^{ab}	266±370^{ab}	472±193^{a}	293±245^{b}
2022	135±157^{a}	293±292^{ab}	234±122^{b}	399±308^{b}
2023	457±320^{b}	545±482^{b}	198±176^{b}	400±251^{b}
***P*-value**	0.019	0.013	0.046	0.002

^{a,b} values within a column with no common superscript differ significantly ($P< 0.05$); P-value refers to results of analysed parameter per column among the four sampling years

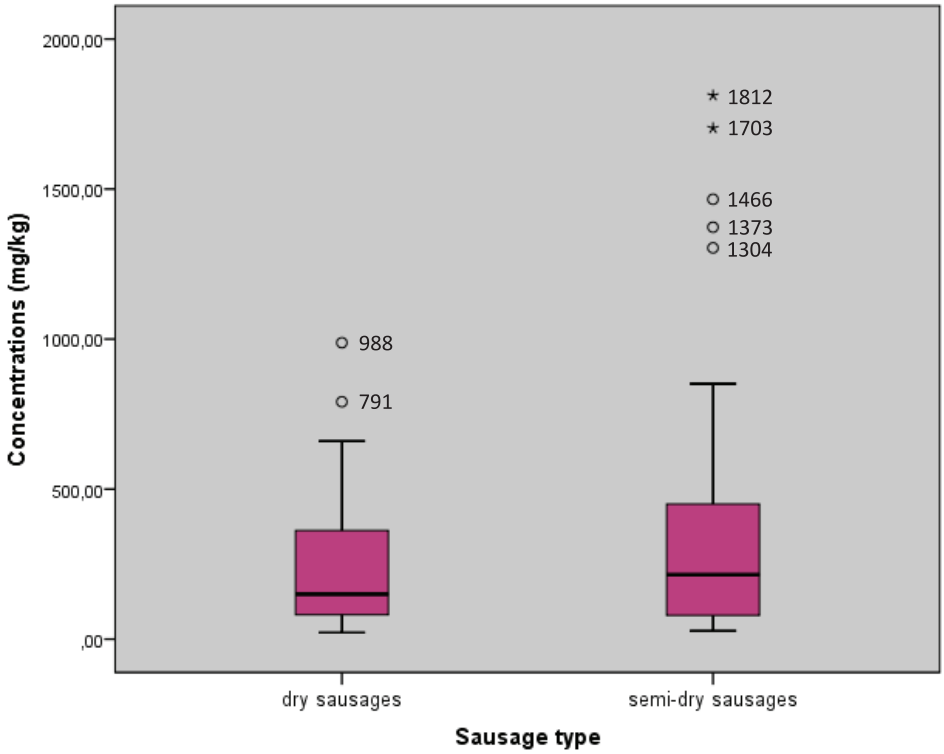

Figure 1. Box plot of ascorbic acid concentrations (mg/kg) according to sausage type (°mild extreme, *high extreme)

EA when added as food additive in meat products is defined by the legislative and the maximum permitted level is 500 mg/kg (European Commission, 2011). During the 4-year sampling period, 12 samples were found to be non-compliant due to the high concentration of EA. In this group of non-compliant samples, 11 of the 12 samples were from the group of semi-dry sausages and one sample was from the group of dry sausages. Few studies have assessed the amounts of added antioxidants in meat products and meat preparations. Iammarino (2012) developed a HPLC method for the determination of AA in not prepacked meat preparations, *i.e.* hamburgers, sausages and other products that are not packed, whereas this additive is not permitted for use. The study included 180 samples of different meat preparations and indicated that although the use of AA is not permitted in not prepacked meat preparations 18.3% of the samples contained AA in amounts above the limit of quantification (20.1 mg/kg). A study on Polish meat products (Czech-Załubska et al., 2023) indicated that 39.8% of tested samples contained erythorbic acid and/or sodium erythorbate. Another study, organised by the European Food Safety Authority, collected analytical results for 4505 samples from Germany and Slovakia. This study revealed that 35 samples of meat preparations were above the MPL value set for authorised use of EA and EA sodium salt as food additives. The results ranged between 503 and 18,256 mg/kg (EFSA, 2016).

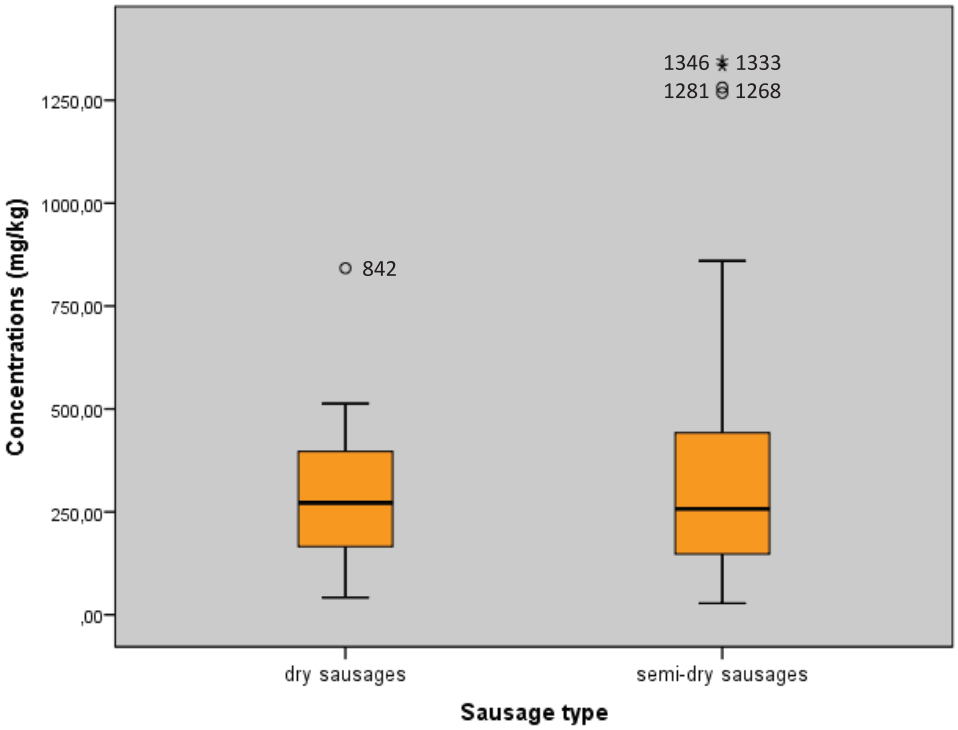

Figure 2. Box plot of erythorbic acid concentrations (mg/kg) according to sausage type (°mild extreme, *high extreme)

Conclusion

Ascorbic acid and erythorbic acid are often used in different categories of meat products as antioxidants, and over 80% of all tested samples contained either AA or EA. Non-compliant results concerning the EA concentration were more frequent in the group of semi-dry sausages than in group of dry sausages. The surveillance of erythorbic acid in meat products is of great interest, since in 8.9% of samples, the EA concentration exceeded the maximum permitted level defined by legislation. It should be considered that this acid can increase iron bioavailability, and ultimately cause negative consequences for individuals with iron deposition disorders.

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Usporedba sadržaja antioksidansa u različitim mesnim proizvodima s hrvatskog tržišta

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Prehrambeni se aditivi definiraju kao svaka tvar koja se sama po sebi ne konzumira kao hrana, niti je prepoznatljiv kao sastojak određene hrane, bez obzira na hranjivu vrijednost. Antioksidansi su klasa prehrambenih aditiva koji se mogu dodavati mesnim proizvodima kako bi se spriječila oksidacija lipida, usporio razvoj neugodnih okusa te poboljšala stabilnost boje. Askorbinska kiselina (AK) i eritorbinska kiselina (EA) su antioksidansi čija je uporaba dopuštena u mesnim pripravcima i mesnim prerađevinama. Cilj je ovoga istraživanja bio usporediti sadržaj AK i EK u trajnim i polutrajnim kobasicama prikupljenim na hrvatskom tržištu tijekom četverogodišnjeg razdoblja i utvrditi njihovu sukladnost u pogledu sigurnosti za konzumaciju. AK i EK analizirane su validiranom UHPLC (tekućinska kromatografija ultra visoke učinkovitosti) metodom s granicom detekcije i granicom kvantifikacije nižom od 3 mg/L, odnosno 10 mg/L. Koncentracija AK u trajnim kobasicama iznosila je od 13 mg/kg do 457 mg/kg, a u polutrajnim kobasicama od 170 mg/kg do 545 mg/kg, dok se koncentracija EK kretala od 198 mg/kg do 472 mg/kg u trajnim kobasicama, a u polutrajnim kobasicama od 14 mg/kg do 400 mg/kg. Zbog visoke koncentracije EK ukupno je nesukladno bilo 12 uzoraka (8,9 %).

Ključne riječi: *aditivi, L-askorbinska kiselina, eritorbinska kiselina, kobasice*