The incorporation of a pomegranate peel extract in the formulation of dry sausages without added nitrites protects proteins from oxidation during in vitro digestion

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Introduction: During the digestion of meat products, proteins are susceptible to oxidative processes that generate carbonyls and produce the loss of thiols. Nitrite can minimize these oxidation reactions (Lavado et al., 2021). However, previous studies have shown that the combination of nitrites with certain amines leads to the formation of nitrosamines (Mortensen et al., 2017).

Because of this concern, consumers demand healthier products, and the meat industry searches for the manufacture of meat products without chemical preservatives (Sebranek & Bacus, 2007). It is here where the idea of adding natural extracts arises to try to control the oxidation of the products before and during digestion.

In this work, the effects of sodium nitrite and pomegranate peel extract in the dry sausage formulation on the formation of protein oxidation products during in vitro digestion were evaluated. In addition, the residual antioxidant activity was evaluated during the three phases of in vitro digestion

Materials and methods: Four batches of dry sausages manufactured with different concentrations of sodium nitrite ($NaNO_2$) and pomegranate peel extract (PPE) were used for in vitro digestion assays:

1). 0ppm $NaNO_2$ + 0% PPE (Con-ve); 2). 150 ppm $NaNO_2$ + 0% PPE (Con+ve); 3). 0 ppm $NaNO_2$ + 1% (v/w) PPE (1%PPE) and 4). 0 ppm $NaNO_2$ + 2% PPE (v/w) (2%PPE).

The in vitro gastrointestinal digestions (GID) (n=5/batch/digestion phase) were performed following the COST INFOGEST standardized static protocol (Brodkorb et al., 2019). At the end of the oral (t 2 min), gastric (t 120 min), and intestinal phase (t 240 min) samples were taken and carbonyls (Vossen & De Smet, 2015) and thiols (Ellman, 1959) were analysed as markers of protein oxidation. In digests, residual antioxidant activities ABTS (Re et al., 1999), DPPH (Brand-Williams et al., 1995), and FRAP (Benzie & Strain, 1996) were assessed.

Data were analysed by the one-way Analysis of Variance (ANOVA) procedure of SPSS, version 22.0 (IBM SPSS Statistics, 2013).

Results: In the oral phase digests, carbonyl contents increased in the order Con+ve < 1%PPE = 2%PPE < Con-ve. Throughout GID phases, the carbonyl contents increased in Con-ve ($p<0.001$) and Con+ve ($p<0.01$) digests, meanwhile decreased ($p<0.05$) in the 1%PPE digests and remained unchanged in the 2%PPE digests. The Con+ve and PPE (1%PPE and 2%PPE) digests had lower ($p<0.001$) protein oxidation than in non-added PPE uncured sausages digests (Con-ve) during the oral, gastric, and intestinal phases.

The thiol contents increased in the order 2%PPE < 1%PPE < Con-ve < Con+ve. In all digests, the thiol content decreased ($p<0.001$) as in vitro digestion progressed. In all three digestion stages, thiols contents were higher ($p<0.001$) in Con+ve than in the Con-ve digests, while the digests of sausages formulated without nitrites and 1% or 2% of PPE had the lowest thiol values.

Residual antioxidant activities were detected during the stages of simulated digestion. At the end of the intestinal phase, no significant differences were found in the ABTS activity, while FRAP and DPPH activities were higher ($p<0.001$) in the 2%PPE digests than in the other groups.

Conclusions: The removal of nitrites in the formulation of dry sausages favours the formation of protein oxidation products during simulated gastrointestinal digestion. However, the use of different levels of PPE in the formulation of uncured sausages was an effective strategy to minimize the oxidative deterioration of proteins under in vitro digestion. In addition to the technological effect of PPE in dry sausages, its use makes it possible to obtain a healthier product by reducing the generation of toxic products derived from the oxidation of proteins during digestion.

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