ANTIOXIDANT EFFECT OF POMEGRANATE PEEL AND ACORN CUPULE EXTRACTS DURING *IN VITRO* DIGESTION OF UNCURED DRY SAUSAGES

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I. INTRODUCTION

Lipids and proteins in meat and meat product can be altered due to oxidative damage during digestion [1]. Fruit and vegetable extracts have been shown to exert a strong antioxidant effect on meat and meat products during *in vitro* digestion. Pomegranate juice and extracts effectively prevent oxidation phenomena under simulated digestion [2]. In contrast, no information is available on the antioxidant activity of acorn cupule and the formation of lipid and protein oxidation-derived compounds. This study aimed to investigate the gastrointestinal behaviour under simulated conditions of a pomegranate peel and an acorn cupule extract at two levels of phenolic compounds as antioxidants against lipid and protein oxidation of an uncured dry sausage.

II. MATERIALS AND METHODS

Dried and ground pomegranate peel and acorn cupule (1:9 w/v) were used to obtain the water: acetone (3:7 v/v) extracts (Pomegranate peel extract: PPE and acorn cupule extract: ACE). Acetone was evaporated and the obtained aqueous extract was freeze-dried. Lyophilized extracts were analyzed according to Medina *et al.* [3] to determine the content of total phenolic compounds (TPC). Working extracts were prepared at 25.0 and 2.5 mg TPC/mL, and 500 μL of these extracts were used.
Uncured dry sausages, produced under commercial conditions, were used for *in vitro* digestion trials. Six *in vitro* digestion conditions were assayed using different extracts and levels of addition: 1. Control (CON); 2. Quercetin (0.005 mg quercetin/digest) (QUER); 3. PPE containing 12.5 mg TPC/digest (PPE_12.5); 4. PPE containing 1.25 mg TPC/digest (PPE_1.25); 5: ACE containing 12.5 mg TPC/digest (ACE_12.5), and 6. ACE containing 1.25 mg TPC/digest (ACE_1.25).
In vitro digestions were performed following the standardized static COST INFO-GEST protocol [4]. The 4-HNE and hexanal contents were determined by HPLC-FD [1]. The protein carbonyl content was determined by the method of Soglia *et al*. [5]. The thiol content was assayed according to Martínez *et al.* [6]. Data were analyzed using ANOVA and Tukey's test.

III. RESULTS AND DISCUSSION

The amounts of 4-HNE and hexanal increased throughout the simulated digestion phases, the intensity of which was affected by the presence/absence of compounds with antioxidant activity. In CON digests, the formation of 4-HNE and hexanal was boosted during *in vitro* digestion, but quercetin addition satisfactorily inhibited their formation. Similar effects were produced by PPE and ACE, being their concentrations in digests significantly lower ($P < .05$) than in the Control group. The inhibition of the formation of both compounds in PPE and ACE digest was dose-dependent, being lower the amounts of 4-HNE and hexanal, the higher the level of TPC added. In any case, PPE or ACE-added digests showed lower ($P < .05$) hexanal concentrations than Control digests.
In CON and PPE_1.25 digests, protein carbonylation increased throughout simulated digestion; meanwhile, carbonyl contents remained unchanged in QUER, PPE_12.5, and both ACE digests. All digest-containing antioxidants (QUER, PPE, or ACE, irrespective of the digestion step) significantly ($P < .05$) reduced their amounts of carbonyls in comparison with CON digests. QUER digests contained the lowest amounts of carbonyls in any of the three stages of the simulated digestion. PPE or ACE

added efficiently controlled proteins from oxidative deterioration during *in vitro* digestion. In the digests, thiol contents showed the opposite behaviour to that described for carbonyls, with a steady decrease in their contents as *in vitro* digestion progressed. The presence of phenolic compounds restrained the loss of -SH groups. The amounts of thiols in the PPE and ACE digests in the intestinal phase showed an undisputed dose effect, with lower -SH contents in digests added with 12.5 than those with 1.25 mg TPC, regardless of the added extract. PPE at 12.5 mg/digest exacerbated the loss of thiols in the digests, with significantly (P <.05) lower values than their counterparts in the three gastrointestinal phases of the *in vitro* digestion.

Table 1 Lipid and protein oxidation markers in digest throughout *in vitro* digestion

			Pomegranate peel extract		Acorn cupule extract	
	Control	Quercetin	PPE_12.5	PPE_1.25	ACE_12.5	ACE_1.25
			4-Hidroxynonenal (pmol /mL digest)			
Oral	917 b z	416 c y	633 c y	1063 ab y	551 c z	1303 a y
Gastric	2136 a y	469 d y	1415 bc y	1578 b y	953 cd y	1389 bc y
Intestinal	6268 a x	983 c x	1874 c x	3838 b x	1858 c x	3137 b x
			Hexanal (pmol/mL digest)			
Oral	5808 a z	841 c z	1395 c y	5510 a z	2410 b y	6033 a y
Gastric	10746 a y	1388 e y	4373 cd x	6854 b y	3176 de xy	6234 bc y
Intestinal	16567 a x	2333 d x	5282 c x	10120 b x	3456 d x	9324 b x
			Carbonyls (nmol/mg protein)			
Oral	25.5 a y	18.8 b	21.7 ab	20.7 b y	20.6 b	21.6 ab
Gastric	24.6 a y	16.9 c	21.0 ab	21.7 ab y	19.7 bc	22.5 ab
Intestinal	33.7 a x	16.4 c	19.7 bc	29.1 a x	20.3 bc	22.8 b
			Thiols (mg Cys equiv/mg protein)			
Oral	37.6 a x	29.0 b x	6.2 d x	29.0 b x	20.9 c x	31.5 b x
Gastric	26.6 ab y	28.9 a x	3.2 d y	23.7 b y	16.6 c y	23.7 b y
Intestinal	9.1 c z	18.2 a y	2.3 d y	13.4 b z	10.2 c z	13.7 b z

a,b,c,d: Means within the same time of sampling with different letter are significantly different (Tukey's test, $P < .05$).
x,y,z: Means within the same experimental group with different letter are significantly different (Tukey's test, $P < .05$).

IV. CONCLUSION

Digestion of uncured sausages induced the generation and accumulation of 4-HNE, hexanal and carbonyls. PPE is an effective strategy to control the intensity of lipid peroxidation and protein carbonylation during *in vitro* digestion. ACE provides antioxidant effects equivalent to those produced by the pomegranate extract, presenting itself as a promising new natural source of phenolic compounds for the control of oxidative deterioration during digestion.

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