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Olive mill wastewater phenols as natural antioxidant against protein oxidative deterioration in beef hamburger
(#480)

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Introduction

The preparation and sale of minced meat and some of its fresh preparations, such as hamburgers, is a widespread commercial practice both at the retail level and at the restaurant one. The mincing of meat, which usually is made mixing lean and fat primal meat cuts, increase the surface area of lipid and protein directly exposed to oxygen. At the same time the cellular compartmentalization become less efficient as it is for the antioxidative defense system of the muscular cell, the various pro-oxidants factors come in direct contact with lipids and proteins that, in the presence of molecular oxygen, become more susceptible to the attack by ROS (Soladoye *et al*., 2015). At the same time there is a growing interest in the use of natural antioxidants, especialy for those coming from the agriculture's by-products. Purpose of the present study was to test the antioxidant effect, versus protein oxidation, of a concentrate rich in phenols obtained from Olive Mill Wastewater (OMW) added to fresh minced meat prepared as *hamburger*.

Methods

Minced meat was prepared using beef (shoulder and belly cuts). Salt was added to the minced meat (0.8%) followed by a short kneading (1 min). The mixture was divided into 3 batches: C (Control), L1 (control + phenols 87.5 mg/kg), L2 (control + phenols 175 mg/kg). The phenols concentrate was in the form of a powder (spray dry technology) in which the phenols were suspended in a vehicle made of maltodextrin. Therefore, 0.35% of maltodextrin was added to the control batch (Maltodextrin Glucidex 19 - Roquette, France) whereas phenols were added to L1 and L2 batches that were kneaded for another 1 min each one. The forming of the *hamburgers* (80 g) was carried out using a manual device. Subsequently, the hamburgers were packaged on a rigid tray heat-sealed in protective atmosphere (2 pieces per tray) using a gas mixture containing 50% nitrogen, 20% oxygen, 30% carbon dioxide. The trays were randomly divided and placed in two different refrigerated cabinets at 4 ± 2 °C, submitted to a fluorescent light (Osram Natura De Luxe L36 W/76-1, Munich, Germany) from 9:00 am to 8:00 pm to simulate the vending conditions in a retail shop. The refrigerated cabinets used were the following: Costan 32020 (Costan, Limana-BL, Italy) and Majolo Plus 100 (Majolo, Cadoneghe-PD, Italy). *Hamburgers* were sampled for analytical measures in the same day of manufacturing (day 0) and after 5 and 8 days of storage, half *hamburger* was analysed raw (three replicates for each one) the other half was packaged under vacuum and cooked for 20 min at 90 °C in a waterbath. At the end of cooking the samples were chilled under tap water and analysed according three replicates for each sample (it was analysed a total of 36 *hamburgers*). The determination of the content of carbonyl groups (nmol DNPH/mg protein) was carried out according to the method of Oliver et *al.* (1986) as modified by Zakrys *et al.* (2008). The determination of the content of sulfhydryl groups (nmol SH/mg protein) was carried out according to the method of Eymard *et al*. (2009) as updated by Winther and Thorpe (2014). To test the effect of *i*) phenols addition (and the level of addition), *ii*) days of storage and *iii*) cooking treatment, the data were submitted to the ANOVA test by means of IBM SPSS Statistics package (version 25).

Results

In Table 1 the analytical results of protein oxidation of *hamburgers* have been summarized. Carbonyls, were reduced by the phenols added at the lower level, but in the L2 batch they were even higher than control one. Sulfhydrils, evidenced an oxidative action of phenols according to a dose-dependent manner. The length of storage at 4 °C showed a progressive and significant protein oxidation according to free sulfhydryls reduction (from the beginning onwards to day 8), whereas carbonyls seemed not affected. Surprisingly, at day 8 the hamburgers of the batch L2 showed an inversion of the pro-oxidant trend, with a reduction of the carbonyls concentration and a rise of the sulfhydryls compared to the data observed at day 5. Cooking had a pro-oxidant effect on protein as can be seen by the significant rise of the carbonyls and decrease of the sulfhydryls, respectively from 7.5 to 9.5 and from 34.9 to 21.2. A significant interaction showed that carbonyls growing more in the cooked hamburgers of the L2 batch than in those of the L1 batch.

Conclusion

The results observed showed that phenols of olive mill wastewater can act as antioxidant and as pro-oxidant toward proteins, according to their chemical state in hydroxyl or quinone form respectively. The balance between the two-redox forms of the phenols added to meat depends by the meat species itself, by the oxidation state of transition metals, the contextual presence of other antioxidant molecules (e.g. tocopherols) or by the availability of hydrogen peroxide to activate myoglobin. Several analytical investigation will be necessary to fully understand the chemistry of OMW phenols added to meaty foods.

Notes

	Phenols (P)			Days (D)			Treatment (T)			*P*					
	C	L1	L2	0	5	8	Raw	Cooked	Sem	P	D	T	PxD	PxT	PxDxT
Carbonyls	8.9	5.1	11.5	8.2	8.3	9.0	7.5	9.5	0.27	***	ns	***	***	***	***
Sulfhydryls	34.0	26.6	23.6	35.1	26.8	22.3	34.9	21.2	0.84	***	***	***	***	ns	***

Table 1.
Carbonyls (nmol DNPH/mg protein) and sulfhydrils (nmol SH/mg protein) in beef hamburgers

Notes