PROCYANIDINS COULD PREVENT OXIDATION OF CHICKEN MEAT PROTEIN AND LIPID

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Abstract – Procyanidins (condensed tannins) have biological activities such as antioxidant activity and could be added to meat products to prevent oxidative processes. The aim was to evaluate the antioxidant properties of a procyanidin fraction on meat oxidation with 2 models representing the structure (ground meat) and its absence (meat protein solution). Colour (L*a*b*), TBARS, carbonyls content and granulometry were measured for the ground meat model. The meat protein solution was heated in presence or not of oxidant (0.05mM ascorbic acid; FeCl3 0.1mM) and procyanidins. TBARS, carbonyls and granulometry were measured. We clearly demonstrated that the incorporation of natural antioxidant such as procyanidins to prevent oxidation processes was successful but only in the case of the meat protein solution model. In the meat protein solution model, addition of procyanidins clearly protected against lipid and protein oxidation and a dose related effect was evidenced. In the ground meat model, the accessibility for the procyanidins to their substrates to exert their biological effect has been possibly hinder by the structure of muscle cell.

Key Words – Oxidation, Procyanidin, Meat, Protein, model

I. INTRODUCTION

Procyanidins (condensed tannins) are a class of flavan-3-ol oligomers and polymers found in many plants, foods, and beverages. They have been shown to have biological activities such as a powerful free radical scavenging activity, antioxidant activity, and anti-tumor-promoting effect (Ariga et al 1998; Landrautet al 2001; Zhao et al. 1999). Perry pears and cider apples used in the beverage industry are particularly rich in procyanidins, a part of which being recovered in the pomace often considered as a waste product. From the meat industry there is a growing attention for preserving meat from oxidation processes. In fact, during storage and more generally during processing proteins and lipids are oxidized leading to an overall deterioration of the sensory properties and a decrease of the nutritional value of meat. The possible association of different natural antioxidants could counteract the oxidation. However in the case of meat or meat products, the ability of scavenging free radicals will depend on the accessibility of the antioxidant to its substrate. In other words, the accessibility will be linked to the structure of the product. The aim of this study was to evaluate the antioxidant properties of a procyanidin fraction purified from pear on meat protein and lipid oxidation with 2 models representing the structure (ground meat) and its absence (meat protein solution).

II. MATERIALS AND METHODS

The *Pectoralis major* muscles from 3 certified chicken were collected in a commercial slaughterplant at 24h *post mortem*.

Procyanidins were purified from a perry pear juice (obtained from the Fausset variety) by reversed phase preparative HPLC according to Es-Safi *et al*., (2006). After concentration and freeze-drying, the fraction was analyzed by reversed phase analytical HPLC coupled to the thiolysis reaction (Guyot et al., 2001). The fraction was kept at -20°C under argon atmosphere until used). This procyanidin fraction contained circa 90 % of polymerized procyanidin with an average degree of polymerization close to 28 and a few percentage of cafeoylquinic acid.

Model of ground meat: The meat was ground in mincers using blades with 8 mm holes. Two solutions of procyanidin were used: 2mg/ml and 10 mg/ml. Five ml of procyanidin were added and mixed to 100 g ground meat and divided in 10 replicates. The samples were plastic wrapped and placed at 4°C for 7 days. Colour (L*a*b*),

TBARS, carbonyls content and granulometry were measured at 24h *post mortem*, day 4 and day7 (Lynch and Frei, 1993; Oliver et al., 1987; Promeyrat et al., 2010).

Model of meat protein solution: 5g of meat was homogenised in 50ml buffer (50mM Tris-HCl, 100mM KCl pH 7.4) after centrifugation at 3000g during 15 min at 4°C, supernatant was collected and proteins were quantified using Bradford method. The protein solution was heated in presence or not of oxidant (0.05mM ascorbic acid; FeCl3 0.1mM) and procyanidins. After incubation the solutions were placed in ice for one hour before the assessment of TBARS, carbonyls and granulometry. The experimental design is shown in table 1.

Table 1 experimental design for the model of meat protein solution

	Control	**T°**	**OH°**	**Procyan. 2mg/ml**	**Procyan. 10mg/ml**
supernatant	5ml	5ml	5ml	5ml	5ml
H2O	200 µl	200 µl	-	-	-
Oxidant solution	-	-	200 µl	200 µl	200 µl
Incubation at 37°C during 4h	-	+	+	+	+
Procyanidins	-	-	-	500 µl	500 µl
	N=2	N=3	N=3	N=3	N=3

III. RESULTS AND DISCUSSION

Ground meat model

Similar pattern was shown for colour coordinates whatever procyanidins were added or not. Luminosity L* values remained comprised between 46 and 50 and redness a* did not change according to storage duration or procyanidins addition. Only yellowness b* showed an increase with duration of storage. Compared to control, the basal yellowness of ground meat mixed with procyanidin tended to be slightly higher (Fig 1). However after 7 days, no difference was found. The procyandin may contribute to the yellow coloration but again the differences were too subtle to conclude.

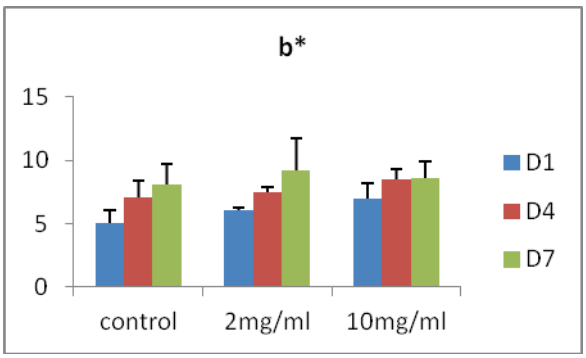

Figure 1. Colour coordinate b*. Yellowness evolution in ground meat added with procyanidins solution (control without procyanidin, 2g/ml, and 10 mg/ml)

The difference in reflectance between 630nm and 580nm reflects the oxidation of myoglobin. After 4 and 7 days of storage, ΔR630-R580, 30% reduction was observed in control and procyanidin samples. Oxidation of basic amino acids measured by carbonyls did not change whatever the storage duration or the samples, neither the TBARS values. The possible antioxidant effect of procyanidins was not observed despite their demonstrable potential to scavenge free radicals (Ariga et Ammano, 1990). This activity is mainly explained by a strong protein-binding capacity, which seemed to be enhanced of highly polymerized procyanidins (Saito et al., 1998). In the case of ground meat, this absence could be explained by a hindered accessibility and therefore an impossibility of exerting their antioxidant ability.

Granulometry measurements showed that the circularity of the particles for the control was stable over time of storage. On contrary ground meat added with procyanidins exhibited a decrease in circularity inversely proportional to the concentration of procyanidins (Fig 2). Recently, Bax et al (2012) reported that circularity increased in cooked meat compared to raw meat. These authors emphasized the occurrence of heating-induced particles aggregation and compaction effect. In our study the decrease could mean an interaction between meat particles and procyanidin. This ability to form complex with protein is intrinsically linked to procyanidins. Hamauzu et al. (2007) demonstrated that procyanidins extracted from pear bound strongly with the proteins and exerted a protective effect against free radicals. In their study, the proteins were in the gastric mucosa and procyanidins displayed a local protective

effect against free radicals produced by activated leukocytes.

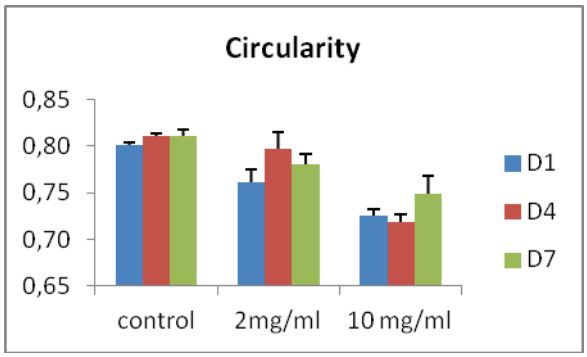

Figure 2. Granulometry measurement. Circularity of during storage in ground meat added with procyanidins solution (control without procyanidins, 2g/ml, and 10 mg/ml)

Meat protein solution

The effect of heating, of the addition of oxidant generating a large family of oxygenated free radical including OH° and the inclusion of antioxidant such as procyanidin was evaluated on colour coordinates of meat protein solution. The addition of procyanidin 10mg/ml provided a couldy appearance of the solution which was not noticed in the other conditions. This may be due to an increase of opacity with higher procyanidin concentrations. Berké & de Freitas (2005) indicated an establishment of a special hydrogen-bonded network between water and procyanidins, which may result in procyanidin conformation modifying the water network in favour of a more or less important interaction with other component. Moreover, temperature was shown to increase redness of dehydrated apple stored at 40°C (Lavelli & Vantaggi, 2009).

Oxidation of basic amino acids increased dramatically after heating and oxidant addition. Interestingly, adding procyanidins with 2mg/ml in the meat protein solution reduced the carbonyl content to the level of the control. Indeed The reduction reached 30% compared to control with a concentration of 10 mg/ml procyanidins. Here we clearly evidenced a dose-related effect on protection from protein oxidation (Fig. 3). Lipid oxidation presented a similar pattern, but the decrease due to procyanidins adding was even more remarkable (Fig 4).

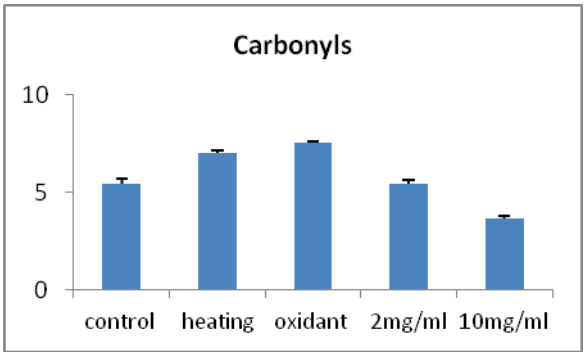

Figure 3. Carbonyls content (nmol DNPH/mg protein) in meat protein solution (control), after heating at 37° C during 4h (heating), after heating + addition of FeCl3, ascorbic acid (oxidant) and after heating + oxidant + procyanidin (2mg/ml or 10mg/ml).

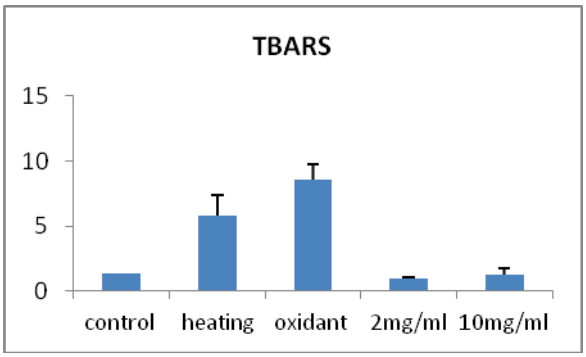

Figure 4. TBARS content (mg MDA/g meat) in meat protein solution (control), after heating at 37° C during 4h (heating), after heating + addition of FeCl3, ascorbic acid (oxidant) and after heating + oxidant + procyanidin (2mg/ml or 10mg/ml).

The granulometry measurements indicated an increase in the circularity and the Ferret ratio in the presence of procyanidins. This observation is often associated with a folding of fibrous-shape particles mainly through hydrophobic interaction. In this study, protein hydrophobicity was not measured because the experimental conditions were designed to promote oxidation processes through the generation of OH°. The analysis of the images revealed changes which were not taken into account by the granulometer system such as an increase in opacity and an aggregation of fibrils (Fig 5). In the presence of procyanidins, myofibrils had a shape of an irregular star, rarely seen in previous studies, and possibly due to the bond of the tannins.

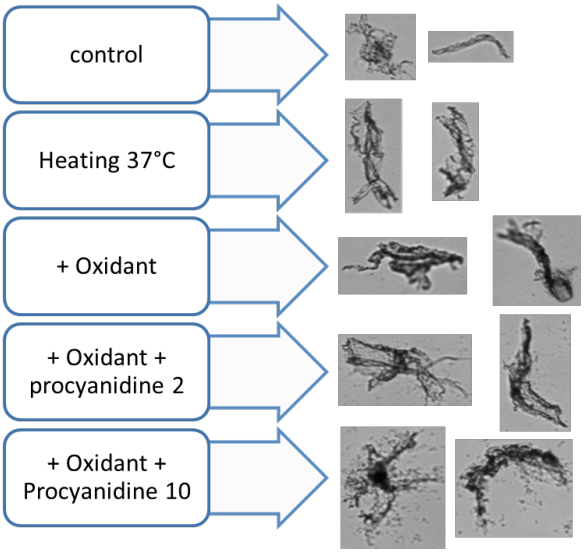

Figure 5. Summary of particles analysis by granulometry.

IV. CONCLUSION

We clearly demonstrated that the incorporation of natural antioxidant such as procyanidins to prevent oxidation processes was successful but only in the case of the meat protein solution model. In the meat protein solution model, addition of procyanidins clearly protected against lipid and protein oxidation and a dose related effect was evidenced. In the ground meat model, the accessibility for the procyanidins to their substrates to exert their biological effect has been possibly hinder by the structure of muscle cell. However, besides the accessibility in other words the structural properties of the meat, another explanation has to be stressed by mentioning the water activity which differed widely in the two models, which may have affected the stability of antioxidant properties of procyanidins.

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