Effect of plant extracts combined with vitamin E in polyunsaturated fatty acidsrich diets given to cull cows on lipid oxydation of meats a 9 month frozen storage

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Plant extracts combined with vitamin E in PUFA-rich diets given to cull cows protect a 9 month frozen storage meats towards lipid peroxidation.

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Abstract— **We investigated the effectiveness of vitamin E (vit E) added with a mixture of plant extracts rich in polyphenols (P) in the diet of cull cows to protect their meats against lipid oxidation after a 9 month frozen storage.**

Normand cows (n=5/group) were given for a 100d finishing period a concentrate/straw based diet (70/30) supplemented with extruded linseeds (40 g oil/kg DM; C group), or the same diet plus vit E (155 IU/kg DM; E group) or plus vit E and a plant extracts mixture (0.7 g/kg DM; EP group). *Semintendinosus* (ST) and *Longissimus thoracis* (LT) muscles were aged at 4°C for 12d in carcass or in pieces under vacuum and then stored under vacuum and froze at -20 °C for 9 months.

Malondialdehyde (MDA), a marker of lipid peroxidation, was lower in frozen beef aged under vacuum (-36%; P=0.03) than in beef aged in carcass. Vacuum ageing would better protect frozen beef from lipid peroxidation than carcass ageing.

Moreover, MDA values highly decreased in frozen beef from the EP group (-40%; P=0.003) compared to that in the C group. In frozen beef from LT muscles aged in carcass, MDA strongly decreased in the EP group (-51%; P=0.01) compared to that in the C group. Addition of plant extracts with vit E in diets would exert a better antioxidant action than vit E provided alone.

Our study shows the possibility to decrease lipid peroxidation of frozen beef by an original dietary antioxidant strategy during the finishing period of cull cows.

Keywords— **Lipid peroxidation, plant extracts, antioxidants, ageing, freezing, beef, cull cows.**

I. INTRODUCTION

Addition of extruded linseeds rich in polyunsaturated fatty acids (PUFA) in diets of ruminants can reduce the atherogenic fatty acids (FA) content of meats and favour their content in beneficial FA such as n-3 PUFA. However, n-3 PUFA were known to favour the oxidative process in meat FA of which final products are considered to be responsible for developing rancidity in stored meats [1].

Vitamin E plays a role of chain-breaking antioxidant, limiting propagation of the lipid peroxidation reaction by captation of radical electrons. But, a high concentration of vitamin E in the diet would not be efficient, because the excess of vitamin E would be catabolised or excreted [2] or favoured pro oxidant reaction against lipids.

In this context, new dietary antioxidant molecules provided together with vitamin E could be more effective for preventing lipid peroxidation. Thus, a mixture of vitamin E (as α-tocopherol acetate) associated with plant extracts rich in polyphenols would preserve the animal health against lipid peroxidation as shown in rats [3] and in dairy cows [4] given lipid supplements rich in n-3 PUFA.

The objective of the present study was to investigate in the finishing period of cull cows, the effectiveness of this dietary antioxidant mixture to protect meats against lipid peroxidation during a 9 month frozen storage.

II. MATERIELS AND METHODS

A. Animals and diets

The experiment was performed with 15 Normand cull cows selected for their live weight (642kg), age (48-60 months) and body fat score (3.5) for a 100d finishing period. Animals were assigned at random to three isoenergetic and isonitrogenous rations (n=5 for each diet). All rations were straw (30%) and concentrate (70%) -based supplemented with extruded linseeds (40g oil/kg diet DM). Animals were given the basal diet without any supplement (C group), or supplemented with vitamin E (155 IU/kg diet DM; E group) or with vitamin E and plant extracts (P) rich in

polyphenols from rosemary, grape, citrus and marigold (7g/kg diet DM; EP group).

B. Meat treatments

Longissimus thoracis (LT) and *semitendinosus* (ST) muscles were removed from the right half carcass, vacuum packed and refrigerated at +4°C for 12d (vacuum ageing). The left half carcass was whole-matured at +4°C for 12d (carcass ageing) and LT and ST muscles were subsequently removed.

After ageing, the two muscles were cut into 10-15 mm (LT) and 8-10 mm (ST) thick steaks, as commonly found on the French market. Samples were packed under vacuum and stored at −20°C for 9 months. After the freezing storage, each beef sample was ground immediately after opening the pack into a fine homogenous powder in liquid N_2 and stored at −80°C until analysis.

C. Malondialdehyde (MDA)

As a specific marker of lipid peroxidation intensity, MDA was extracted by hexane from muscle powder separated by HPLC and quantified by fluorescence (excitation at 515 nm, emission at 553 nm) using a tetraethoxipropane calibration curve.

D. Determination of α-tocopherol acetate (Vit E) and retinol (Vit A) content

Beef α-tocopherol acetate and retinol were assayed by HPLC and UV absorption as described by [5]. Before the extraction step, α-tocotrienol acetate was added as internal standard to beef powder samples to determine the extraction efficiency. Beef powder was treated by heating and α-tocopherol was extracted with hexane. After elimination of hexane by evaporation, α-tocopherol and α-tocotrienol acetate were dissolved in tetrahydrofurane and methanol/dichloromethane (65/35, vol/vol). Finally, α-tocopherol, α-tocotrienol and retinol beef levels were determined by UV spectrophotometry at 292 nm.

E. Determination of total antioxidant status (TAS)

Total antioxidant status (TAS) was determined by the method described by [5] for bovine tissues and based on the absorption ABTS+ cation [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)]. Beef powder (250mg) was homogeneized in 3 mL of 0.01 M, pH 7.4 phosphate buffer. The homogenate was centrifuged and the supernatant filtered. Production of ABTS+ was initiated by adding 450 µM H_20_2 in quartz microcells containing 12 µL of filtered solution, 0.5 mM ABTS and 48.8 µM metmyoglobin in 5 mM, pH 7.4 phosphate buffer solution (PBS). Absorbance was measured by double-beam spectrophotometry at 732nm immediately (A_0) and 3min following addition of hydrogen peroxide at 37°C (A_3). Blank absorbance (i.e., containing PBS instead of plasma) was determined to calculate the inhibition percentage of the reaction as follows:

$$\% \text{ inhibition} = \frac{(A_0 - A_3)\text{ blank} - (A_0 - A_3)\text{ plasma} \times 100}{(A_0 - A_3)\text{ blank}}$$

Inhibition percentages were converted into Trolox Equivalent Antioxidant Capacity (TEAC), expressed as µmol TEAC/g fresh tissue, representing the capacity of tissues to resist oxidation reactions.

F. Statistical analysis

All data were subjected to analysis by ANOVA using the general linear model procedure of Statistical Analysis System software (SAS Institute, Cary, USA, 2000). A P-value lower to 0.05 was considered to be significant and a P-value lower to 0.1 was considered as a trend.

"Diet", "muscle", "ageing" effects and their interactions were analysed. When interactions were not significant (P>0.05), they were removed from the model.

III. RESULTS

As regard the "ageing" effect in the both frozen muscles (Table 1), mean MDA in beef from carcass ageing were higher (+36%) than those from vacuum ageing. Mean of vit E tended to be higher (+22%) in vacuum aged than in carcass aged muscles. TAS and vit A were not significantly changed.

Table 1. Ageing effect on mean frozen beef MDA and vit E (µg/g tissue) from the both muscles.

	Carcass ageing	Vacuum ageing	P values
MDA	0.19 [a]	0.14 [b]	0.03
Vit E	3.6	4.4	0.06

[a-b] In each column, superscript letters indicated differences at P<0.05.

As regard the "muscle" effect in frozen beef (Table 2) and considering the both ageing methods, LT muscles were characterized by a higher vit A level (x5.4) and a higher TAS intensity (+11%) than ST muscle.

Table 2. Muscle effect on mean frozen beef vit A (µg/g tissue) and TAS (µmol TEAC/g tissue) from the both ageing methods.

	LT	ST	P values
Vit A	1.74 [a]	0.32 [b]	<0.0001
TAS	5.45 [a]	4.90 [b]	0.02

[a-b] In each column, superscript letters indicated differences at P<0.05.

As regard the "diet" effect, means of MDA and antioxidants values (vit E, vit A and TAS) from aged and frozen stored muscles are reported in Table 3. **Mean beef MDA** highly decreased in frozen beef from the EP group compared to that in the C group (-40%, P=0.003). **Mean Vit E value** increased more than twice in the E and EP groups as compared with the C group (P<0.05). **Mean Vit A and TAS values** did not change within the different diets.

Table 3. Diet effect on mean frozen beef MDA, vit E, vit A (µg/g tissue) and TAS (µmol TEAC/g tissue) from the both muscles.

	C	E	EP	P values
MDA	0.207 [a]	0.166 [ab]	0.124 [b]	0.01
Vit E	2.03 [a]	4.75 [b]	5.29 [b]	<0.0001
Vit A	0.84	0.87	1.37	NS
TAS	5.27	5.21	5.05	NS

[a-b] In each row, superscript letters indicated differences at P<0.05.

Detailed MDA values for LT and ST muscles are given in Table 4. **In the E group** and in all conditions tested, MDA slightly decreased as compared with C group, but not significantly. **In the EP group**, particularly in LT muscle aged in carcass, MDA value was significantly reduced as compared with C group (-51%, P=0.01). In other conditions, MDA values decreased under values obtained with vit E supplementation, but not significantly.

Table 4. MDA concentrations (µg/g tissue) in steaks from LT and ST muscles having a 12d-ageing at 4°C followed by a 9 month-frozen storage at -20°C.

	LT		ST	
	Carcass ageing	Vacuum ageing	Carcass ageing	Vacuum ageing
C	0.265 [a]	0.176 [a]	0.210 [a]	0.174 [a]
E	0.236 [ab]	0.136 [a]	0.162 [a]	0.127 [a]
EP	0.130 [b]	0.124 [a]	0.136 [a]	0.105 [a]

[a-b] In each column, superscript letters indicated differences at P<0.05. Within each diet, "muscle" and "ageing" effects are not significant.

Detailed beef vit E values are given in Table 5. In all tested conditions, vit E values largely increased in the E and EP groups as compared to the C group (P<0.05).

Table 5. Vit E concentrations (µg/g tissue) in steaks from LT and ST muscles having a 12d-ageing at 4°C followed by a 9 month-frozen storage at -20°C.

	LT		ST	
	Carcass ageing	Vacuum ageing	Carcass ageing	Vacuum ageing
C	2.06 [a]	1.82 [a]	1.86 [a]	2.36 [a]
E	4.08 [ab]	6.24 [b]	3.50 [ab]	5.15 [b]
EP	5.25 [b]	6.27 [b]	4.94 [b]	4.69 [b]

[a-b] In each column, superscript letters indicated differences at P<0.05. Within each diet, "muscle" and "ageing" effects are not significant.

IV. DISCUSSION

As regard the two ageing methods (Table 1), the vacuum ageing did not favour largely intensity of lipid peroxidation in frozen beef of C group (as means 0.175 µg/g) compared to that measured at slaughter by (0.160 µg/g) [6]. Whatever the muscle, intensity of lipid peroxidation was higher in frozen stored meats aged for 12d at 4°C in carcass than under vacuum. Carcass ageing would increase muscle's exposure to the O_2 of atmosphere favouring reactions of lipid peroxidation. Carcass ageing tended to decrease vit E content of meats as compared to vacuum ageing (P=0.06). During the carcass ageing, lipid peroxidation could have been intensive and vit E could have been depleted in order to limit these reactions.

Whatever the ageing method, frozen beef from LT muscle exhibited a higher antioxidant capacity (+11%, P<0.02) than ST beef, partly due to its higher vit A content (x5.4, P<0.0001) and probably due to the different origin and metabolic properties of these muscles. We can hypothesize that the higher oxido-glycolytic metabolic activity of LT compared with ST favors the utilization of O_2 and increase the antioxidant capacity of these muscle to resist to radical oxygenated species attacks. This antioxidant capacity of LT is in accordance with our previous observations on beef lipid peroxidation in different packaging systems [7].

Global lipid peroxidation intensity decreased in frozen beef from animals given the plant extracts combined with vit E supplement as compared to control animals (P=0.01; Table 3). In spite of the higher vit E content in frozen meats from E group animals, the vit E dose used in this trial and given alone in diet was not enough effective to limit MDA formation (NS). In the study of [7], high level of dietary vit E supplementation (2000 UI/kg/d) resulted in a two times decrease of lipid oxidation intensity in beef LT samples after a 8 weeks of storage (without ageing) compared to control beef. The vit E, as chain breaking antioxidant, could partly explain the decrease of lipid peroxidation intensity in the EP group of our study. Clearly, the combination of vit E and plant extracts in the diet was more effective than vit E alone in preventing lipid peroxidation reactions. These results on frozen beef are in agreement with our previous observations comparing the effect of antioxidant combinations on the prevention of beef lipid peroxidation in different packaging systems [6].

In the particular case of the most deleterious conditions, ie carcass ageing, lipid peroxidation intensity of the LT muscle was effectively limited by the combination of vit E and plant extracts. As proposed in a study on vit E oxidation in frozen fish muscle [8], authors suggested a synergy of actions of antioxidants, such as a recycling of vit E by phenolic antioxidants, to reduce lipid oxidation. Plant extracts effectiveness could be attributed to a recycling of vit E. However, in our frozen meats, no significant differences of vit E concentrations appeared between the E and EP groups. Antioxidant activity of plant extracts could be expressed by another distinct way, such as the activation of antioxidant enzymes in the muscle as already observed in rat [9].

V. CONCLUSIONS

The under vacuum method of beef ageing could contribute to the prevention of lipid peroxidation in a long term storage of frozen beef.

Association of dietary plant extracts with vit E would exert an effective antioxidant action preserving frozen stored meats towards lipid peroxidation, particularly in beef from the LT muscle aged in carcass.

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