

## Vitamin E homologues in wild boar meat from *Montado*

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### Abstract

The wild boar meat from *Montado* (savannah-like pastures where cork and holm oaks are the predominant trees) has gathered enthusiastic consumers among the Portuguese population. However, little information is available regarding its nutritional quality. The objective of this study was to evaluate the contents of vitamin E homologues in wild boar meat. Twenty-five wild boars were used in this study. After sanitary inspection, *psaos major* muscles were collected and transported under refrigeration to the laboratory, trimmed and frozen (-20 °C) until analysis, which was performed by normal-phase HPLC, using fluorescence detection. The most important vitamin E homologues in wild boar meat were  $\alpha$ -tocopherol (17.4±3.3 µg/g meat),  $\gamma$ -tocopherol (2.6±1.3 µg/g meat) and  $\gamma$ -tocotrienol (0.8±1.1 µg/g meat). Minor isomers were detected just in some animals, as  $\delta$ -tocopherol (n=16; 0.1±0.08 µg/g meat),  $\delta$ -tocotrienol (n=13; 0.04±0.04 µg/g meat),  $\beta$ -tocopherol (n=12; 0.06±0.02 µg/g meat) and  $\alpha$ -tocotrienol (n=6; 0.06±0.02 µg/g meat). These results suggest that  $\alpha$ -tocopherol content in wild boar meat is above the values found in domestic pigs fed with  $\alpha$ -tocopherol supplemented diets and above the values that are needed to prevent lipid oxidation. Six other vitamin E homologues with health promoting effects were also found in wild boar meat.

### Introduction

*Montado* is a traditional agro-silvo-pastoral system characteristic of Alentejo, a southern province of Portugal. The Portuguese *Montado* closely resembles the Spanish *Dehesa* and both constitute examples of land-use systems well adapted to the environmental restrictions of the Mediterranean region, characterized by low edaphic and climatic potential. The predominant trees of *Montado*, cork and holm oaks (*Quercus suber* and *Quercus rotundifolia*, respectively) contribute with their fruits to both domestic mammals and wild life feeding, representing an important nutritional supplement during the autumn and winter seasons, while the predominant shrubs are the strawberry tree (*Arbutus unedo*), *Cistus ladanifer*, and *Rosmarinus officinalis*.

Nowadays, this traditional agro-silvo-pastoral system is changing, and big game hunting reserves are replacing the traditional agro-pastoral activities. Among the most appreciated animals for big game hunting is the wild boar, and *Montado* provides more than 60% of all the wild boars slaughter in Portugal.

Game hunting is an economic activity on its own, but the commerce of big game meat, a surplus of the hunting activity is of economical importance and has gathered enthusiastic consumers among the Portuguese population.

*Montado* wild boar meat should reflect the omnivorous feeding behaviour and the richness of *Montado*. Nevertheless, very little information is available regarding wild boar meat nutritional quality. Therefore it was this study objective to evaluate the *Montado*'s wild boar meat contents in vitamin E, since vitamin E, major lipid soluble antioxidant in meat, is of particular importance to the preservation of meat nutritional quality, by the inhibition of lipid oxidation.

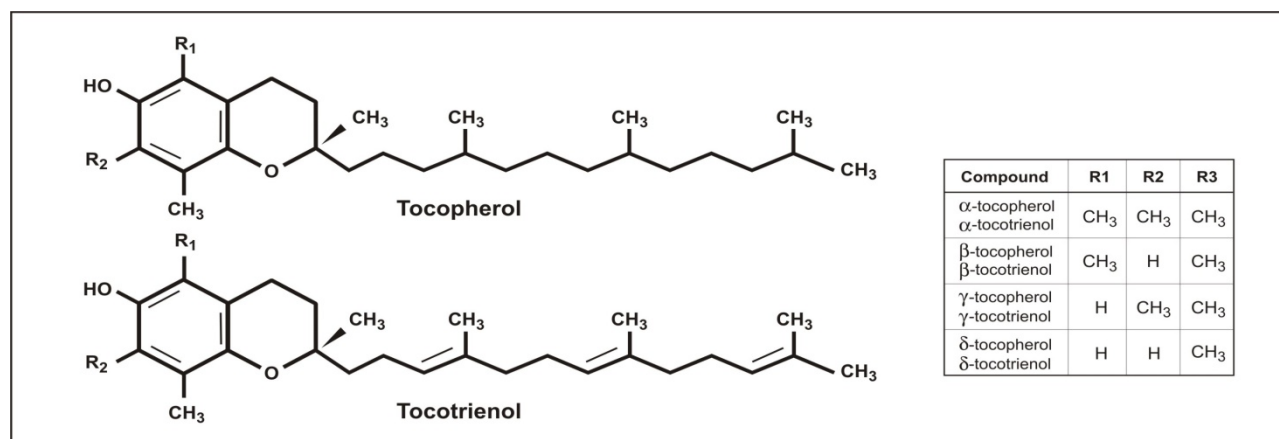
### Material and methods

Twenty-five wild boars of both genders and different maturity stages were gathered after being shot during battues, performed in a big game hunting reserve. Sampling was performed during the afternoon, immediately after the sanitary inspection, by the collection of *psaos major* muscles, which were transported under refrigeration (+5° C.), stored under refrigeration and processed on the day after. All meat samples were trimmed of connective, adipose tissue and blood coagulum before blending in a food processor, afterwards, meat samples were vacuum-packed and stored under freezing (-20° C.) until analysis, which was performed within a month.

The quantification of vitamin E isomers was performed by a HPLC system (Agilent 1100 Series, Agilent Technologies Inc.). The analysis of tocopherols and tocotrienols in meat was performed using a normal-phase silica column (Zorbax RX-Sil) with fluorescence detection (excitation wavelength of 295 nm and emission wavelength of 325 nm). The injection volumes used varied between 10 and 100  $\mu\text{l}$  in order to get values inside the linearity range of the standard curves, as was previously described by Mestre Prates *et al.* [1].

## Results and discussion

Vitamin E is, unlike other vitamins with a single well-defined chemical structure, a collective term used to describe all the natural tocopherols and tocotrienols (Figure 1). Both tocopherols and tocotrienols have four natural homologues, designated as  $\alpha$ -,  $\beta$ -,  $\gamma$ - and  $\delta$ - that differ in the number and position of methyl groups attached to the chromanol ring, sharing in common the antioxidant activity of the  $\alpha$ -tocopherol.



**Figure 1.** The vitamin E family, with the tocopherol and tocotrienol families and their homologues.

Mammals are unable to synthesize vitamin E homologues, consequently their contents in meat reflect the diet contents in vitamin E.  $\alpha$ -Tocopherol is the prime vitamin E homologue in beef and meat (pig, lamb and poultry), while  $\gamma$ -tocopherol appears as a secondary tocopherol in meat, but no other tocopherols and tocotrienols have been described in both beef and meat, with the exception of poultry [2]. The  $\alpha$ -tocopherol predominance over other homologues results from the conjugation of two important factors: 1) preference of the  $\alpha$ -TTP for the  $\alpha$ -tocopherol (the  $\alpha$ -TTP is a hepatic enzyme that is responsible for vitamin E metabolism in mammals) and 2) high availability of  $\alpha$ -tocopherol in animal feeds, as grass and cereal.

Vitamin E has been considered of particular importance in meat industry thanks to its antioxidant function, since it influences the rate of lipid peroxidation, meat colour stability, water-holding-capacity and cholesterol oxidation [3, 4]. Thereby, vitamin E is a key factor in the preservation of meat nutritional and nutraceutical properties.

Vitamin E homologues present in wild boar *psaos major* are presented in Table 1.  $\alpha$ -Tocopherol, major vitamin E isomer in muscle ( $17.4 \pm 3.33 \mu\text{g/g}$  of meat), accounts for 77% of all the vitamin E homologues in wild boar *psaos major* muscle, while  $\gamma$ -tocopherol, the second major isomer, accounts for 11.5% of all vitamin E ( $2.59 \pm 1.32 \mu\text{g/g}$  of meat). Together, these two isomers ( $\alpha$ - and  $\gamma$ -tocopherol) accounts for 89% of all vitamin E isomers in wild boar *psaos major* muscle. Minor isomers were detected in all animals  $\gamma$ -tocotrienol ( $0.81 \pm 1.11 \mu\text{g/g}$  of meat), or just in some animals, as  $\delta$ -tocopherol ( $n=16$ ;  $0.1 \pm 0.08 \mu\text{g/g}$  meat),  $\delta$ -tocotrienol ( $n=13$ ;  $0.04 \pm 0.04 \mu\text{g/g}$  meat),  $\beta$ -tocopherol ( $n=12$ ;  $0.06 \pm 0.02 \mu\text{g/g}$  meat) and  $\alpha$ -tocotrienol ( $n=6$ ;  $1.53 \pm 0.82 \mu\text{g/g}$  meat).

**Table 1.** Vitamin E homologues contents in wild boar *psaos major* ( $\mu\text{g/g}$  meat)

	TF	$\alpha$ -	TT	$\alpha$ -	TF	$\beta$ -	TF	$\gamma$ -	TT	$\gamma$ -	TF	$\delta$ -	TT	$\delta$ -
Average	42	17.	3	1.5	6	0.0	9	2.5	1	0.8	0	0.1	4	0.0
Standard Deviation	3	3.3	2	0.8	2	0.0	2	1.3	1	1.1	8	0.0	4	0.0
n		25		6		12		25		25		16		13

\*TF stands for tocopherol, while TT stands for tocotrienol

The wild boar vitamin E contents in wild boar meat can be characterized as unique, due to its high contents in  $\alpha$ -tocopherol and to the presence of other six vitamin E homologues. Together, these vitamin E homologues protect wild boar meat from lipid oxidation, a very important role in meat particularly in game meat.

The  $\alpha$ -tocopherol content in wild boar *psaos major* is considerably above the contents found in pigs supplemented with high levels of  $\alpha$ -tocopherol. The supplementation of pigs with 100, 200 and 700 mg of  $\alpha$ -tocopherol per kg of feed provides *psaos major* muscle with an  $\alpha$ -tocopherol contents of 9.8-10.0, 11.4-12.0 and 15.1-16.3  $\mu\text{g/g}$  of meat, respectively [5], values that are below the  $\alpha$ -tocopherol values found in wild boars. Such situation is difficult to explain, nevertheless, we have thought in some possible explanations to this event: 1) the wild boar has an increased efficiency in the process of gastrointestinal absorption of vitamin E, which is considered quite inefficient in other species, or has an improved muscle uptake of vitamin E; 2) Montado acorns proportionate a high bioavailability of vitamin E, considering that Iberian pig daily consumption of acorns has been estimate between 7 and 10 kg [6], therefore, the daily intake of  $\alpha$ - and  $\gamma$ -tocopherol would fluctuate depending on acorn species between 107.4–153.1 mg/day of  $\alpha$ -tocopherol and 638.5-910.8 mg/day of  $\gamma$ -tocopherol (*Quercus rotundifolia*), and 214.7–306.3 mg/day of  $\alpha$ -tocopherol and 361.6-515.8 mg/day of  $\gamma$ -tocopherol (*Quercus suber*). Assuming that wild boar and Iberian pig have similar daily intake of acorns, we estimate that they will ingest 160-456 IU of  $\alpha$ -tocopherol ( $\alpha$ -tocopherol 1.49 IU/mg) and 361.6-515.8 mg/day of  $\gamma$ -tocopherol, an amount that is still quite below the values associated with vitamin E supplementation.

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