# EFFECT OF THAWING BY MICROWAVE HEATING ON THE EXTRACTION OF TOCOPHEROLS IN FAT FROM IBERIAN PIGS

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Abstract – The quantification of gamma and alpha-tocopherol in Iberian pig tissues is a feasible method for distinguishing Iberian pig feeding systems. The aim of this research was to study the effect of previous microwave heating on gamma and alpha-tocopherol extraction in fat samples from Iberian pigs; and to establish the most adequate microwave parameters to optimise this process. Fat samples of approximately 39.3  $g \pm 6.2$ were taken. Some were melted using different microwave heating treatments (700 W, 595 W and 462 W at 1, 1.5 and 2 min.) while other samples were not heated. Saponification of a small amount (0.05 g) of either melted fat or unmelted fat samples was used for tocopherol extraction. The second trial evaluated sample size (12.6g  $\pm$  1.04 vs.  $38.5g \pm 9.5$ ) (700 W) and heating time (1-2 min). Microwave heating (at the evaluated parameters) did not modify the alpha and gamma-tocopherol concentration of fat samples when compared to those unmelted samples. Heating times below or equal to 2 min. at maximum power did not modify the tocopherol content in 40g samples. However, in 10g samples heating (700 W) for 1-2 min. improved tocopherol extraction compared to the 40g samples.

Key Words – extraction method, tocopherols, pigs.

### I. INTRODUCTION

Some of the most expensive meat products in the world are those obtained from Iberian pigs extensive conditions fed under and. consequently, quality control measures have been widely proposed. Tocopherols are present in tissue membranes and their concentrations have been found to be directly related to the feeding regime [1]. Pigs fed under free-range conditions have a higher proportion of both forms of tocopherol (gamma- and alpha-) than those fed with grass, which mainly has a high proportion of alpha-tocopherol, or those fed with diets in intensive situations. Consequently, the quantification of gamma and alpha-tocopherol in Iberian pig tissues can be used as a reliable method for distinguishing Iberian pig feeding systems and in 89% of the cases it correctly classifies the meat into one of the three qualities defined by the Spanish Quality Policy for the Iberian [2]. Moreover, tocopherol pig concentration has been found to be one of the most accurate methods recently tested [3]. The analytical methods for tocopherol quantifications require the previous extraction of these compounds directly or by saponification from fresh samples. Recent studies in vegetables indicate that heating previous to extraction may increase tocopherol content in that matrix [4]. However, there is not to our knowledge any information on the effect of microwave heating on the  $\alpha$  and  $\gamma$ -tocopherol concentrations in fat samples or the microwave parameters required to minimize its destruction. The objective of this research was to study how thawing fat using microwave heating affects tocopherol concentration and to study the most adequate microwave parameters for tocopherol extraction.

# II. MATERIALS AND METHODS

In a first trial, twenty seven fat samples from Iberian pigs were taken at the height of the tail insertion and kept frozen at  $-20^{\circ}$ C until analysis. Ten total treatment were considered, one group of samples was not heated and the nine others were heated by microwave heating at 3 powers (700, 595 and 462) x 3 times (1, 1.5 and 2 min.) (Fig. 1). Hence, tocopherols (alpha and gamma forms) were extracted using the method described by Rey et al. [1] from fat samples without any previous treatment (n=27). At the same time a piece of fat of approximately 39.3 g  $\pm$  6.2 was subject to different microwave heating treatments using a Teka microwave oven MWE 23 G. The microwave parameters used were 3 heating powers (700 W, 595 W and 462 W) and 3 different heating times (1, 1.5 and 2 min.) (n=3)samples per treatment). The tocopherol extraction procedure for all treatments consisted of the saponification of 0.05 g of fat in the presence of KCl (1.15%) and KOH (50%) [1]. Tocopherols were quantified by reverse phase HPLC (HP 1100, equipped with a diode array fluorescence detector) and (Agilent Technologies, Waldbronn, Germany). The fluorescence detector was operated at excitation and emission wavelengths of 295 nm and 330 nm respectively.

In a second trial, experimental design followed a factorial arrangement (2 X 3): fat samples of two different sizes (12.6g  $\pm$  1.04 and 38.5g  $\pm$  9.5) were weighed and melted in a microwave oven (700 W) for 3 times (1, 1.5 and 2 minutes) previous to the tocopherol extraction analysis.

Data were analysed using the general linear model procedure in SAS 9 [5] to study the effects of the treatment, sample size, microwave power and heating time. Means were analysed using the Duncan test.

Figure 1. Effect of thawing by microwave heating on gamma- and alpha-tocopherol concentrations in fat samples from Iberian pigs.

Treatments exp. 1 (n=27)									
Without heating	Microwave heating (n=27)								
(n=27)	700 W			595 W			462 W		
	1 m	1,5 m	2 m	1 m	1,5 m	2 m	1 m	1,5 m	2 m

## III. RESULTS AND DISCUSSION

Microwave heating did not modify the alpha and gamma-tocopherol concentration of fat samples when compared to the extraction without thawing (Fig. 2). When the effect of microwave power and heating time were evaluated in samples of approximately 40 g, it was found that the effect of the microwave power on tocopherol concentration was not statistically different. However, samples melted at 700 W had numerically higher concentrations of gamma and alpha-tocopherols (Fig. 3) when compared to those melted at medium or low power. Also, the 1.5 and 2 min. thawing times produced numerically higher tocopherol concentrations in 40g fat samples than a heating time of 1 min., although the differences were not statistically significant (Fig. 4).













Kreps et al. [6] reported the rate of tocopherol degradation in heated oils; however these authors used longer heating times and a different sample type. Conversely, Carballo et al. [7] used microwave radiation on the high-performance liquid chromatography separation of tocopherols to obtain a more efficient separation. Borges et al. [8] studied the effect of microwave heating on baru oil using crude soybean oil for comparison. These authors found that for times up to 3 min. (1000 W) no significant adverse changes were observed in either oil; however, longer exposition times produce adverse effects and tocopherols dropped abruptly. The results of our study are consistent with those presented by Borges, since heating times were lower than 2 min.

In order to look at the effect of the sample size on tocopherol extraction in samples melted by microwave heating, two different sizes were heated for three different lengths of time at maximum power (experiment 2). The sample size affected the gamma- and alpha-tocopherol concentration (P=0.0001) (fig. 5). The smaller fat samples that had been previously melted in a microwave oven had higher gamma- and alphatocopherol concentrations. The heating time in this second trial did not significantly affect the tocopherol concentration of the fat samples.





 $\square \gamma$ -tocopherol  $\square \alpha$ -tocopherol

To our knowledge no previous information is available on the effect of sample size, since most of the studies were carried out using oil. The results of the present study show that microwave heating improves tocopherol extraction in 10g samples at 700 W up to 2 min.

#### CONCLUSION

Sample size is an important characteristic that affected tocopherol extraction in fat melted by microwave heating. Samples weighing approximately 10 g resulted in higher tocopherol extraction than 40g samples at 700 W for short heating times.

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