VOLATILE COMPOUNDS IN SUPERCRITICAL CARBON DIOXIDE EXTRACTS FROM MUSCLE AND SUBCUTANEOUS FAT OF IBERIAN HAM

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BACKGROUND

To date, studies of food volatile compounds have employed different techniques, obtaining satisfactory results in fruits, cheeses, wines, meat and meat products (Berdagué et al.,1993; Ang and Liu, 1996). In recent years, the extraction of volatile compounds with supercritical carbon dioxide has increased, initially in vegetable products and more recently in meats and meat products (Merkle and Larick, 1994; Taylor and Larick, 1995). Researchers have reported that flavor compound extracted using this thecnique were of higher quality and more true to the source than those from other methods. Also, it permits the treatment of thermally unstable samples and the obtention of different classes of compounds, through slight variations in fluid density (Majors, 1991).

OBJETIVES

The purpose of this study was to identify the volatile compounds obtained from muscle and subcutaneous fat of Iberian ham using supercritical fluid extraction.

METHODS

Samples were taken from *Biceps femoris* muscle and subcutaneous fat from five high quality dry cured hams. All samples were ground and 3.5g of muscle samples and 2g of fat samples were transferred into a thimble.

The extractor is a Hewlett-Packard 7680A. The analytes were trapped on a solid sorbent (tenax). 7ml thimbles were used. Extractions were performed at 40°C of temperature and 0.5g/ml of density (91 atm of pressure). After complete extraction, the trap, containing the analytes, was rinsed with acetone.

Volatile compounds were analyzed by capillary GC-FID. Separation was realized in a 50m x 0.32mm i.d. HP (Crosslinked 5% Ph Me silicone) column with a 0.52µm film thickness. The column inlet pressure was 10 psi. Helium was used as the carrier gas. Injector and detector temperatures were of 230 and 240°C repectively. The GC was temperatured programmed from 35°C at 10°C/min to 200°C, from 200°C at 20°C/min to a final temperature of 230°C and held for 50min.

We weighed 28g of muscle and the same amount of fat when we realized volatile identification. Compounds were identified using capillary GC-MS. The mass detector temperature was of 180°C and the EMVolts of 1756. The column, the temperature program and other relevant GC conditions were the same as described for capiliary GC-FID. Volatile compounds were tentatively identified using the following procedures: comparison of their GC retention times and Kovats indices to those of standard compounds and comparison of their mass spectra to those of standard compounds in the Wiley and NIST/EPA/NIH library.

1µl of supercritical carbon dioxide extracts was injected directly onto the column in both kind of analysis.

RESULTS AND DISCUSSIONS

We used the total thimble capacity to make the extraction of samples. In this way, 3.5g of muscle were transferred into the thimbles but only 2g were weighed in the fat extractions. It was necessary to decrease the amount of fat since larger amounts cause persistent plugging in the lines on the extractor.

After analyzing the extracts by GC-FID, we saw that larger number and higher concentration of compounds were obtained in fat extracts (chromatograms in figure 1), in spite of using lower sample amount in this case. The best results obtained in subcutaneous fat confirm that the process is easier in fat than muscle. Probably, this is due to the fact that muscle matrix is more complex than fat one. So, the extraction is hindered by the interaction between volatile compounds and components of muscle.These last ones retain the first compounds and may hamper carbon dioxide penetration.

We increased muscle amount to identify volatiles by GC-MS. Thus, we realized eight extractions of each muscle sample, then we used 28g of sample. Volatile identification is easier since we increased the area of peaks.

78 and 58 compounds were identified in muscle and subcutaneous fat extracts respectively. They were grouped into 8 classes in the first extracts and into 9 in the second ones (table 1). It is remarkable the fact that the nonpresent compounds in fat extracts correspond to the ones with a low molecular weight in all classes.

Most of aldehydes identified in muscle and subcutaneous fat extracts have already been studied in Iberian ham using different techniques (García et al., 1991; López et al., 1992).

On the contrary, most of ketones (excepting 2propanone-1hidroxy, 2butanone-3hidroxy, 2heptanone and cyclohexanone) have not been reported in ham. This may be due to the particular solvent selectivity of the supercritical carbon dioxide (Taylor and Larick, 1995). This ketones have been extracted from muscle and only three have been obtained from fat. This could be due to the intense oxidative reactions which take place in subcutaneous fat during ripening of Iberian ham due to a direct exposing to oxygen, light and temperature. Thus, this ketones might become another compounds.

Most of alcohols, aliphatic hydrocarbons, carboxylic acids and esters have already been described in Iberian ham. We saw that these compounds were different depending on the studied extract (muscle or fat). This fact can be explained by the greater intensity of oxidative reactions in subcutaneous fat. Also, this may be the reason why furans and nitrogenous compounds were only extracted in fat samples. However, some of this compounds have already been identified in Iberian ham by López et al. (1992).

Occurrence of a elevated aromatic hydrocarbons number confirms the observation of Merkle and Larick (1994) that supercritical carbon dioxide has high selectivity for extracting this compounds.

Only one lactone was present in muscle extracts, butyrolactone, which was previously reported by García et al. (1991) and López et al. (1992) studying Iberian ham.

CONCLUSIONS

SFE combined with GC-MS has been shown to be succesful in the analysis of Iberian ham flavor. Several new compounds not previously reported by other authors have been tentatively identified in this study. Extraction of compounds from subcutaneous fat is

easier than muscle since this first one needs lower sample amount. The differences between volatiles in muscle and fat extracts may be due to the more intense oxidative reactions in subcutaneous fat than muscle.

PERTINENT LITERATURE

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FIGURE 1.- GC-FID chromatograms of volatile compounds obtained from (a) musle extract and (b) subcutaneous fat extract

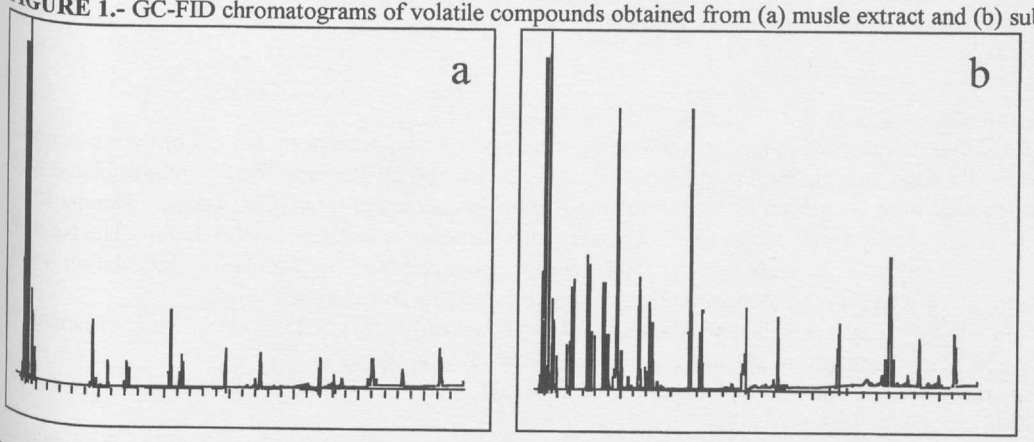

Table 1.- Compounds identified by GC-MS in muscle and subcutaneous fat extracts.

COMPOUNDS	IK	Muscle Extract	Fat Extract
Aldehydes			
butanal	607	+	
butanal3methyl	662	+	
pentanal	683	+	
hexanal	794	+	+
heptanal	905	+	+
benzaldehyde	961	+	
octanal	1008	+	+
2octenal	1067		+
benzeneacetaldehyde	1074	+	
nonanal	1110	+	+
2nonenal	1169		+
decanal	1208	+	+
2,4nonadienal	1218	+	+
2decenal	1270		+
2,4decadienal	1317	+	+
2undecenal	1370		+
dodecanal	1399	+	+
Ketones			
2propanone1hidroxy	674	+	
2butanone3hydroxy	716	+	
3penten2one	750	+	
3hexen2one	804	+	
4pentanone4hydroxy4methy	845	+	
4heptanone	851	+	+
2heptanone	877	+	
cyclohexanone	894	+	
2heptanone4methyl	913	+	
3hexanone5hydroxy3methyl	943	+	
3nonen2one	1016	+	
3decen2one	1146	+	
cicloheptanone2methyl	1233	+	
2pentadecanone	1249		+
Alcohols	1704		+
1,3butanediol			
2,3butanediol	784	+	
2pentanol4methyl	784	+	
1hexanol	821	+	
1heptanol	871	+	
1octen3ol	975		+
4heptanol3methyl	985		+
	997	+	

COMPOUNDS	IK	Muscle Extract	Fat Extract
1hexanol2ethyl	1033	+	
benzenemethanol	1051	+	
1octanol	1076		+
benzeneethanol	1139	+	
1nonanol	1194		+
1decanol	1281		+
endobornylacetate	1318		+
bicyclo(5,1,0)octan4ol	1457		+
dodecanol	1579	+	
Aliphatic Hydrocarbons			
1,3pentadiene4methyl	645		+
heptane	700		+
cyclohexanemethyl	737		+
octane	800		+
nonane	900	+	
decane	1000		+
decane2methyl	1024	+	
limonene	1047	+	
cyclohexane2propenyl	1054		+
decane5methyl	1057	+	
1undecene4methyl	1085	+	
undecano	1100	+	+
1dodecene	1192	+	
dodecane	1200		+
2dodecene	1203	+	
3dodecene	1211	+	
dodecane5methyl	1257	+	
tridecane	1300		+
1tetradecene	1389	+	
tetradecane	1400		+
2tetradecene	1403	+	
1dodecene2ethyl	1407	+	
3tetradecene	1416	+	
cyclododecane	1486	+	+
pentadecano	1500	+	
1hexadecene	1593	+	
3hexadecene	1604	+	
1octadecene	1795	+	
Aromatic Hydrocarbons			
toluene	778	+	+
benzene propil	971	+	
benzene4ethyl1,2dimethyl	1095	+	

COMPOUNDS	IK	Muscle Extract	Fat Extract
1ethyl3,5dimethylbenzene	1106	+	
benzene1,3,4,5tetramethyl	1146	+	
naftalene	1222	+	
naftalene1methyl	1346		+
bencene1buthylheptyl	1645		+
bencene1propyloctyl	1657		+
bencene1methyldecyl	1720		+
Carboxylic acids			
acetic acid	622	+	
propanoic acid	702	+	
butanoic acid	792	+	+
pentanoic acid	881	+	+
hexanoic acid	981	+	+
heptanoic acid	1069	+	+
octanoic acid	1172	+	+
benzoic acid	1180	+	
nonanoic acid	1243	+	+
decanoic acid	1349	+	+
undecanoic acid	1421		+
dodecanoic acid	1558	+	+
tetradecanoic acid	1767	+	+
Esters			
propanoic acid 2methyl	767	+	
butanoic acid2methyl	839	+	
butanoic acid 3 methyl	861	+	+
tricaproin	1072		+
tetradecanoic acid ethyl ester	1796		+
Furans			
tetrahydrofurane	640		+
furan2pentyl	1000		+
furanonedihydro5pentyl	1384		+
Nitrogenous Compounds			
2propanamine1methylethyl	664		+
hexanenitrile	811		+
piperidine2methyl	1366		+
Lactones			
butyrolactone	927	+	
Others			
cloroformo	629	+	+
benzenedicarboxylacidiethylether	1617	+	

\+ The compound is present in the extract