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COMPARISON OF DIFFERENT SOLID PHASE MICROEXTRACTION FIBERS IN EXTRACTION OF VOLATILE COMPOUNDS FROM ADIPOSE TISSUE

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ABSTRACT
The volatile compounds (VCs) in headspace of adipose tissues (perirenal, tail, caul) were extracted by solid-phase microextraction (SPME) fiber coated with carboxen/ polydimethylsiloxane (CAR/PDMS) or divinylbenzene/ carboxen/ polydimethylsloxane (DVB/CAR/PDMS). In extraction with CAR/PDMS coating compared to DVB/CAR/PDMS, more VCs were obtained in perirenal (53 versus 41), tail (51 vs 47) and caul (35 vs 33) fats. Increased retention index for aldehydes increased the adsorption on DVB/CAR/PDMS. This fiber also provided a good yield for carboxylic acids. CAR/PDMS fiber could be recommended for VCs from adipose tissues if the goal is to identify aldehydes, especially fatty acid oxidation products and low molecular weight hydrocarbons, as well as greater number of VCs. However, in case of targeting carboxylic acids, esters, and also high molecular weight aldehydes, DVB/CAR/PDMS could be utilized due to its high affinity for mentioned compounds. Furthermore, the optimum extraction time should be studied to determine the time for VCs to reach equilibrium
Keywords: Tail fat, aldehydes, polydimethylsiloxane, linoleic acid

YAĞ DOKUSUNDAN UÇUCU BİLEŞENLERİN EKSTRAKSİYONUNDA FARKLI KATI FAZ MİKROEKSTRAKSİYON FİBERLERİNİN KARŞILAŞTIRILMASI

ÖZ
Adipoz dokuların (böbrek, kuyruk ve gömlek yağ) tepe boşluğundan uçucu bileşenlerin ekstraksiyonu, farklı adsorbant karboksen/ polidimetilsiloksan, (CAR/PDMS) ya da divinilbenzen/ karboksen/ polidimetilsiloksan (DVB/CAR/PDMS) kaplamalı katı faz mikroekstraksiyon fiberleri ile gerçekleştirilmiştir. DVB/CAR/PDMS ile karşılaştırıldığında CAR/PDMS kaplamalı fiber ile ekstraksiyonda böbrek (53'e 41), kuyruk (51'e 47) ve gömlek (35'e 33) yağlarda daha fazla sayıda uçucu bileşen belirlenmiştir. Aldehitlerin alıkonma indeksinin artması, DVB/CAR/PDMS'e adsorpsiyonu artırmıştır. Bu fiber karboksilik asitler için de etkili bir affinite göstermiştir. Adipoz dokularda, özellikle yağ asidi oksidasyon ürünü aldehitlerin, düşük molekül ağırlıklı hidrokarbonların ve ayrıca daha fazla uçucu bileşenin tanımlanması için CAR/PDMS fiberi önerilebilir. Ayrıca, karboksilli asitler, esterler ve yüksek molekül ağırlıklı aldehitlerin belirlenmesinde ise söz edilen bileşenlere

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affinitesinin yüksek olması nedeniyle DVB/CAR/PDMS kullanılabilir. Bununla birlikte uçucu bileşenlerin dengeye ulaşma zamanını belirlemek için optimum ekstraksiyon sürelerinin araştırılması gerekli olabilmektedir.
Anahtar kelimeler: Kuyruk yağı, aldehitler, polidimetilsiloksan, linoleik asit

INTRODUCTION

The development of gas chromatography-mass spectrometry (GC-MS) has allowed a detailed analysis of the volatile compounds in foods. The methods used in isolating volatile compounds from foods include headspace, distillation, solvent extraction, solid-phase extraction and direct injection (Alonso et al., 2009; Güler 2007; 2014; Güler et al., 2009). Among them, headspace is one of the simplest methods used. Headspace sampling involves static and dynamic headspace techniques. The first is by direct injection of the headspace vapors above a food via "syringe" or "loop headspace", the latter involves an active cycling of the headspace vapor (Razote et al., 2002; Balasubramanian and Panigrahi, 2010). Nowadays, static headspace (HS)-solid phase microextraction (SPME) is well known and widely used since it is a simple, fast, and solvent-free technique, and also the most satisfactory for determination of VCs (Serrano et al., 2009; Güler et al., 2016; 2019). Because in SPME heating of the tissue sample is minimal and the recovery of VCs is closer to their real content than the values obtained by heating techniques such as distillation or solvent extraction (Alonso et al., 2009). Besides, it does not require an additional sample preparation step that could result in possible loss of analyte and sample contamination (Balasubramanian and Panigrahi, 2010).

HS-SPME is also defined as a sampling technique based on the adsorption of VCs on a polymeric material, namely stationary phase, covering silica fiber, and an equilibrium is reached between the food matrix and the stationary phase (Lorenzo, 2014). The use of single-coated SPME fibers (polydimethylsiloxane vs polyacrylate) with dynamic headspace sampling was not recommended for trace analysis (Elmore et al., 1997). There are 7 types of stationary phase including non-polar polydimethylsiloxane (PDMS), polar (polyacrylate, divinylbenzene (DVB), carbowax-polyethylene glycol (PEG)) and mixed polarity (PDMS/DVB, CAR/PDMS and DVB/CAR/PDMS). In particular, combined coated has been commonly used for the analysis of VCs with higher recoveries (Lorenzo, 2014). The choice of the fiber coating is an important factor, since the VCs have a wide variation in polarity. Compared to CAR and DVB, which are polar phases, PDMS is not sensitive to polar VCs because it is non-polar (Gianelli et al., 2002). Therefore, the type of stationary phase affecting SPME fiber performance affects the analysis results (Elmore et al., 2001; Marco et al., 2004; Lorenzo, 2014).

HS-SPME technique is also well-suited in the investigation of biodegradation pathways of foods (Balasubramanian and Panigrahi, 2010) such as volatiles from lipid degradation pathways by lipase and lipoxygenase. As lipids are solvents for most VCs, it is important to evaluate their flavor profile (Calkins and Hodgen, 2007). However, the food matrix and target VCs dictate the complexity level of sample extraction. Page and Lacroix (1993) stated that as the lipid content of foods increased, the release of VCs into headspace decreased. So much so that the extraction of VCs may differ from food to food. For vegetables (Jelen et al., 2000) and olive oil (Cavalli et al., 2004), VC extraction is also applied at room temperature (20-25°C), but for animal fats or adipose tissue, the extraction temperature can be up to 100°C (Watkins et al., 2012). Since animal products (meat and/or dairy) release a wide variety of VC, a mid-polar affinity-coated SPME fiber such as CAR/PDMS or DVB/CAR/PDMS could demonstrated as an ideal choice to extract a majority of the volatiles (Balasubramanian and Panigrahi, 2010). It has been reported that CAR/PDMS coated fiber is ideal for VCs with low molecular weight while the DVB/CAR/PDMS coated fiber is for VCs with large molecular weight, namely found in dry-cured ham (Garcia-Esteban et al., 2004). A higher affinity of DVB/CAR/PDMS for aldehydes and CAR/PDMS for ester compounds was reported by Marco et al. (2004) in fermented sausages.

Dynamic headspace techniques have been widely applied to determine VCs of adipose tissues (Priolo et al., 2004; Sivadier et al., 2008; Young et al., 1997; 2003). But, Marsilli (1999) has shown that the cheaper static HS-SPME technique is superior to dynamic headspace analysis in terms of reproducibility and proper baseline with less noise. Moreover, static HS-SPME was used for the analysis of VCs from beef and sheep fats (Watkins et al., 2012) and lamb fat (Vasta et al., 2012). As far as we know, there is no study on which SPME fiber can be recommended for adipose tissues. In addition to volatile compounds (VCs), fatty acids (FAs) composition is an important parameter in terms of fat or adipose tissue quality (Bas et al., 2007). Because FAs can not only directly contribute to flavor but also are precursors for most flavor compounds (Tekin and Güler, 2021).

We aimed to compare extraction performance of CAR/PDMS and DVB/CAR/PDMS coating fibers in headspaces of different adipose tissues (perirenal, tail and caul fats) and to determine whether volatile compound profile and abundance can change depending on fatty acids of adipose tissues.

MATERIAL AND METHODS

The adipose tissues were obtained from Karagül (Karakul) sheep, three randomly selected depending on same age (24-months) and living weight (50-55 kg), from breed's flock (approximately 100 heads) reared in Muş, province of Türkiye. The adipose tissues (perirenal, tail and caul) were collected within 30-45 min of slaughter and transferred to the laboratory in ice-boxes and then they were vacuum packaged and frozen at -18°C until analysis.

Gas chromatography analyzes

Fatty acid composition of adipose tissues was determined as fatty acid methyl ester derivatives using GC-MS (Agilent 6890 chromatography and 5973N mass spectrophotometry; Agilent, Palo Alto, USA) coupled with a capillary column (Supelco SP-2830, 60 m x 0.25 mm id x 0.20 μm film thickness) according to the procedure described by Rule (1997). GC-MS operating conditions were applied with minor modifications in procedure reported by Peng et al. (2010).

The HS-SPME technique was used to analyze the VCs of adipose tissues according to the method described by Watkins et al. (2012) with minor modifications. Firstly, adipose tissues (10 g) were rendered using a bench-top heater (IKA HS7, Deutschland, Germany) at 100°C. Seven g of molten fat were transferred to 20 mL headspace vials and sealed with polytetrafluoroethylene (PTFE)/silicone septa and steel caps. The vials with sample were pre-heated at 65°C for 5 min prior to insertion of the SPME fiber. Two different coating SPME fibers were used: CAR/PDMS (50/30 μm) and DVB/CAR/PDMS (75 μm). Before the analysis, the fibers were preconditioned in the injection port of GC as indicated by the manufacturer. SPME fiber was injected into the headspace where it was held for 45 min. The VCs were chromatographed on a capillary column (HP-INNOWAX; 60 m × 0,25 mm id × 0.25 μm film thickness). As reported by Dursun and Güler (2019), GC-MS conditions and VCs identification were done.

Data Analysis

The relative amounts of both VCs and FAs were estimated by the area normalization method. The peak area of each identified compound was integrated, and total peak area was calculated from the equation reported by Güler et al. (2022). Statistical differences between the mean values of adipose tissues were determined one-way ANOVA, and between the mean values of VCs determined with SPME fibers (CAR/PDMS and DVB/CAR/PDMS) were determined t-test by using SPSS program (Version 22, IBM, USA). Duncan's multiple comparison test was used to compare significance being determined at $P < 0.05$. Principal component analysis (PCA), based on the correlation matrix, was conducted to determine components which account for most of the total variation in VCs and FAs. The principal components scores presented in a bi-plot and VCs profile presented in cell-plot were

carried out by using JMP Version 13 software (SAS Institute, USA).

RESULTS AND DISCUSSION

Fatty acid profile of adipose tissues

A total of 22 fatty acids were determined in adipose tissues, including 11 saturated fatty acids (SFA), 6 monounsaturated fatty acids (MUFA), and 5 polyunsaturated fatty acids (PUFA) as shown in Table 1. Stearic acid (C18:0), saturated fatty acid, was found to be in perirenal fat, as the main FA, and oleic acid (*cis9*-C18:1), mono-unsaturated fatty acid, in caul and tail tissues. Similar results were previously reported in perirenal (Bas et al., 2007) and tail (Mahachi et al., 2020) fats. Palmitic acid (C16:0) and margaric acid (C17:0) were the second and third most abundant SFA, respectively, and did not differ between the adipose tissues. Myristic acid (C14:0), was also substantially determined in adipose tissues, which was highest in tail fat. The remaining SFAs were detected in trace. Oleic acid, the second most abundant FA in perirenal fat, was less than the other ones (P <0.001). The tail and caul fats with high *cis9*-C18:1 and also *cis9*-C16:1 had low stearic acid. This finding was confirmed with the results of Shingfield and Wallace (2014), who stated an inverse relationship between ruminal oleic acid metabolism and its bio-hydrogenation intermediates including *trans11*-C18:1 and C18:0. Furthermore, the externally located adipose tissue may have much more Δ9- desaturase activity (Jiang et al., 2018) than internal ones, thereby replacing stearic acid with oleic acid. Like oleic acid, palmitoleic (*cis9*-C16:1) and elaidic (*trans9*-C18:1) acid were also lowest in perirenal fat (Table 1). Among the PUFA, linoleic, conjugated linoleic (CLA) and α-linolenic acids, routinely determined in all adipose tissues, were highest in tail fats (P <0.01).

Table 1. Fatty acid methyl esters of adipose tissues

Fatty acids	Adipose tissues				P
	Perirenal	Tail	Caul	Mean	
C10:0	0.08±0.01b	0.15±0.01a	0.08±0.00b	0.11±0.04	***
C12:0	0.12±0.00b	0.23±0.03a	0.05±0.00c	0.14±0.08	***
C13:0	0.03±0.01	0.03±0.00	0.03±0.00	0.03±0.01	ns
C14:0	3.55±0.05b	4.90±0.48a	2.68±0.02c	3.84±0.99	***
C15:0	1.07±0.02a	1.03±0.09a	0.55±0.01b	0.92±0.24	***
C16:0	20.6±0.25	22.2±0.97	21.9±0.67	21.5±0.99	ns
C17:0	4.50±0.09	4.61±0.48	4.22±0.11	4.47±0.31	ns
C18:0	37.8±0.76a	23.0±1.51c	27.1±0.38b	29.6±7.12	***
C19:0	0.91±0.03a	0.65±0.06b	0.72±0.00b	0.76±0.13	***
C20:0	0.66±0.02a	0.24±0.03b	0.16±0.01c	0.38±0.24	***
C22:0	0.12±0.02	-	-	0.04±0.06	-
cis9-C14:1	-	0.14±0.03	-	0.05±0.07	-
cis9-C16:1	0.68±0.03c	1.73±0.06b	1.93±0.04a	1.39±0.59	***
cis9-C18:1	22.0±0.68b	32.3±2.12a	32.1±1.03a	28.4±5.44	***
trans11-C18:1	3.69±0.06	2.94±0.08	2.96±1.45	3.23±0.67	ns
trans9-C18:1	0.58±0.02b	0.87±0.03b	1.21±0.29a	0.85±0.28	**
cis11-C20:1	0.12±0.00	0.12±0.02	0.13±0.01	0.12±0.01	ns
cis9,12-C18:2	2.30±0.02c	3.27±0.08a	3.07±0.07b	2.86±0.47	***
cis9,trans11-C18:2 (CLA)	0.50±0.01c	0.78±0.05a	0.64±0.00b	0.64±0.13	***
cis9,12,15-C18:3	0.69±0.01a	0.75±0.09a	0.48±0.01b	0.66±0.13	**
cis8,11,14-C20:3	-	0.03±0.01	-	0.01±0.02	-
cis5,8,11,14-C20:4	-	0.06±0.02	0.08±0.01	0.04±0.04	-

Data were expressed as mean±standard deviation. The mean values followed by different letters in the same row indicate the significance differences between adipose tissues at **P <0.01 and ***P <0.001 levels; ns: not significant (P >0.05); -: not detected.

Volatile compounds of adipose tissues

As shown in Fig. 1, a total of 58 VCs were identified in headspace of adipose tissues using CAR/PDMS or DVB/CAR/PDMS for extraction. The volatiles were classified into eight chemical groups: aldehydes (21 compounds), hydrocarbons (9), acids (9), ketones (7), phenyl and phenols (4), heterocyclic compounds (3), alcohols (3) and esters (2). The number of VCs identified was similar to that (55) reported by Vasta et al. (2012) in lamb fat, but lower than the finding of Watkins et al. (2012) in beef and sheep fat. This could be attributed to several factors such as breed, grazing, age, etc. affecting also the adipose tissue fatty acid composition.

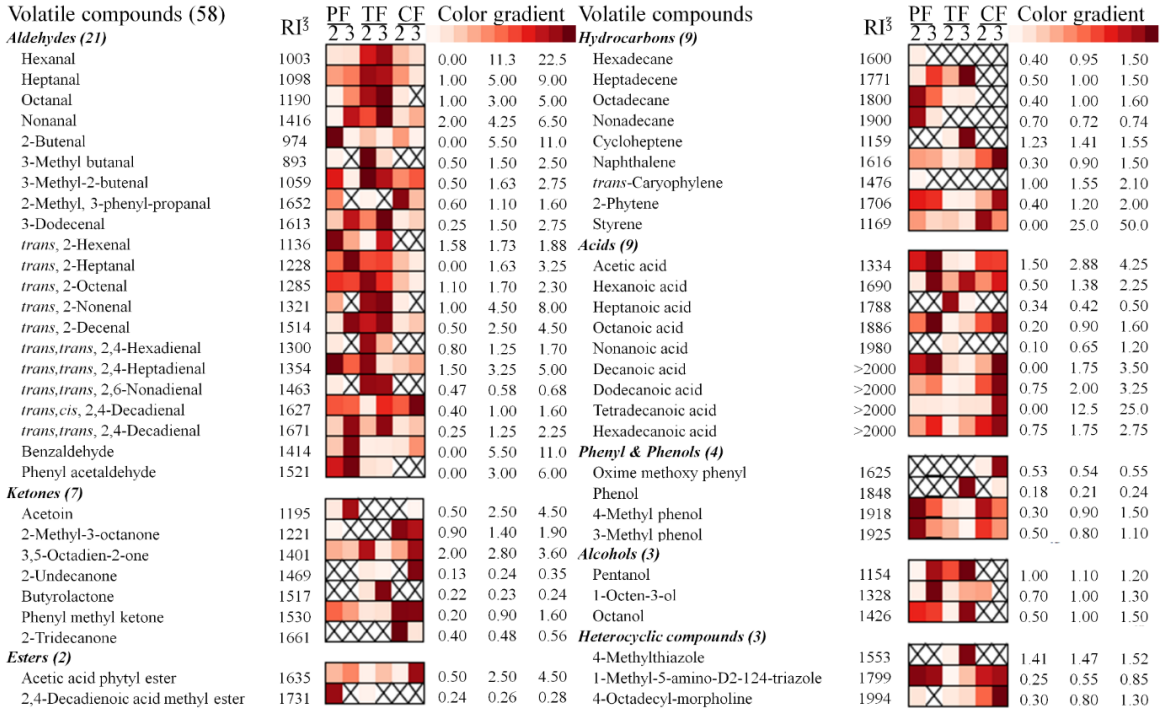

Fig. 1. Cell-plot for the volatile compounds identified in perirenal fat (PF), tail fat (TF) and caul fat (CF) by SPME fiber coating with CAR/PDMS (2) and DVB/CAR/PDMS (3). RI: Retention index based on identified compound retention times, calculated from the linear equation between each pair of straight alkane series (C8-C20). X: not detected.

Only 28 out of 58 VCs were common to all the adipose tissues, which were extracted by both the SPME fibers. The CAR/PDMS fiber extracted a greater number of VCs from perirenal (53 in number), tail (51) and caul (35) fats than DVB/CAR/PDMS (41, 47 and 33, respectively) one.

A remarkable difference in VCs number between SPME fibers was observed for perirenal tissue. Although DVB/CAR/PDMS coating extracted 41 VCs, CAR/PDMS extracted 12 additional VCs, including 3-methyl butenal, *trans*,*trans*,2,4-hexadienal, *trans*, 2-nonenal,*trans*,*trans*,2,6, nonadienal, 2-methyl-3-phenyl-propanal, 2,4-decadienoic acid methyl ester, 4-octadecyl-morpholine, hexadecane, *trans*-caryophylene, 2-methyl-3-octanone, 2-undecanone, and phenol. Marco et al. (2004) also identified 24 additional VCs in fermented sausage in the case of using CAR/PDMS coating compared to DVB/CAR/PDMS. In contrast, Gianelli et al. (2002) found that DVB/CAR/PDMS extracted VCs in greater number than CAR/PDMS (60 vs 41 VCs, respectively).

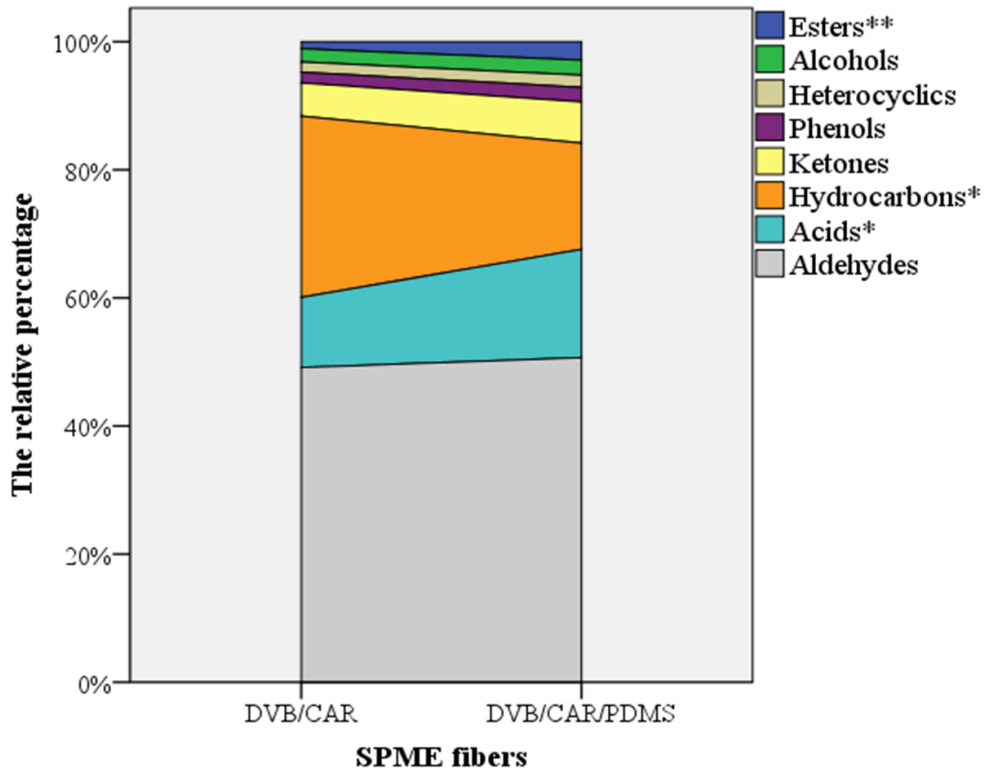

Fig. 2. The mean percentage composition of the chemical groups of VCs in adipose tissues. The mean values were significant different at *P <0.05 and **P <0.01 levels.

Aldehydes were the principal chemical group in headspace of adipose tissues, except for caul fat, in terms of their number and their percentage composition (Fig. 1-2). It was not surprising as the high temperature (100°C) used for rendering fat could result in an increased oxidation or degradation products of adipose tissues such as aldehydes and hydrocarbons. Regardless of tissues, a significantly higher percentage of nonanal, benzaldehyde, *trans*, 2-decenal, 3-dodecanal, *trans*,*cis*, 2,4-decadienal and *trans*,*trans*, 2,4-decadienal was extracted by DVB/CAR/PDMS fiber. It was observed that as the retention index of aldehydes increased, there were more adsorbed on DVB/CAR/PDMS coating than on CAR/PDMS one. Similar findings were reported previously (Garcia-Esteban et al., 2004; Yu et al., 2008; Lorenzo et al., 2014). Among aldehydes hexanal was determined in tail fat as the principal aldehyde and volatile compound by both SPME fibers. This finding was confirmed by high PUFA level in tail fat since hexanal is one of the main oxidation products of *cis9,12*-C18:2 fatty acid. Similarly, as stated by Pan et al. (2005) and Elmore et al. (2005), aldehydes 3-methyl-2-butenal, heptanal, octanal, nonanal, and *trans* isomers of hexenal, heptenal, octenal, nonenal and decenal, derived from oxidation of unsaturated fatty acids, were also determined in highest in tail fat. Besides, their abundance did not a significant between SPME fibers, irrespectively of tissues (Table 2). On the other hand, the relative percentages of 2-butenal in all the adipose tissues and *trans*,*trans*-2,4-heptadienal in tail and perirenal fats significantly increased in extraction by CAR/PDMS coating. 2-Butenal was identified as the second most abundant volatile compound following styrene in perirenal tissue. In contrast, the relative percentage of benzaldehyde, derived mainly from phenylalanine amino acid, showed a tendency to increase in case of using DVB/CAR/PDMS fiber. This was in consistent with the finding of Marco et al. (2004), but was in disagreement with

that of Garcia-Esteban et al. (2004). These conflicts reports may be related to the applied extraction time since benzaldehyde requires more time to reach equilibrium with CAR/PDMS than with DVB/CAR/PDMS, as stated by Lorenzo (2014). Regardless of tissues, a high extraction proportion by CAR/PDMS coating for 2-butenal may closely be associated with its molecular weight since it is aldehyde with the lowest molecular weight among aldehydes identified. This finding is confirmed that CAR/PDMS fiber is suitable for volatile compounds with small molecular weight, irrespectively of the functional group of chemical compound (Garcia-Esteban et al., 2004). Nevertheless, the increased yield of *trans*,*trans*, 2,4-heptadienal in extraction by CAR/PDMS coating fiber is probably due to its competitions with *trans*, 2-heptanal at the same extraction. Because the latter aldehyde showed a good yield in extraction by DVB/CAR/PDMS fiber for perirenal and tail fats.

Generally, a relatively high proportion of aldehydes in extraction by DVB/CAR/PDMS coating fiber (Fig.1) could be attributed to its high affinity for aldehydes, especially for molecular weights above 100, as stated by Lorenzo et al. (2014) and Marco et al. (2004).

Regarding to perirenal fat, the most abundant VCs analyzed by CAR/PDMS and DVB/CAR/PDMS were styrene and benzaldehyde, respectively (Fig. 1; Table 2). The first has been reported previously in caudal subcutaneous adipose tissue (Sivadier et al., 2008) and the second in perirenal fat of lambs (Vasta et al., 2012). Possible precursors of styrene are carotenoids, long-chain fatty acid esters, aldehydes, phenylalanine amino acid (Pagot et al., 2007). High styrene presence in perirenal fat may support carotenoid precursor depending on animals' grazing on highland pasture. In addition, oxidation of styrene may produce benzaldehyde. The present study shows that perirenal and caul fats are a good source for styrene and benzaldehyde, irrespectively of fiber coating used. Styrene and 2-phytene abundance in caul fat may utilize as a fingerprint of the feeding system.

Hydrocarbons were the second most abundant chemical group identified in adipose tissues except for caul fat. The CAR/PDMS fiber extracted a significantly (P <0.001) high percentage of hydrocarbons compared to the DVB/CAR/PDMS one (Fig. 1). Besides, 2 additional hydrocarbons (hexadecane and *trans*-caryophylene) were extracted by the CAR/PDMS coating. Among hydrocarbons, styrene was the principal hydrocarbon, and also VC in perirenal and caul fats. This hydrocarbon was decisive in separating the perirenal and caul fats extracted by CAR/PDMS coating from the others, according to PCA applied on data obtained from volatile compounds (Fig. 3A). This may be related to both the coating fiber and high styrene level in above-mentioned tissues. As shown in Table 2, another principal hydrocarbon, 2-phytene, was obtained at a higher relative percentage in caul fat in extraction with DVB/CAR/PDMS coating compared to CAR/PDMS. But, 2-phytene was both considerably less than styrene and had a molecular weight of about 3 times higher. 2-Phytene, derived from the degradation of chlorophyll by rumen microorganisms (Body, 1977), showed no significant difference in percent composition between the extraction coatings in either perirenal fat or tail fat.

Carboxylic acids were the third most abundant chemical group identified, in terms of their percentage composition. As shown in Fig. 2, acid chemical group was found to be lower by CAR/PDMS fiber than the other one, irrespectively of tissues. As reported by Lorenzo et al. (2014), CAR/PDMS coating showed a lower affinity to acids. Among carboxylic acids, acetic acid was the most abundant acid identified in perirenal fat and had a similar the percentage value in all adipose tissues extracted by both SPME fibers (Table 2). But, tetradecanoic acid was determined in tail and caul fats as the major acid. DVB/CAR/PDMS fiber extraction gave rise to higher proportion of tetradecanoic acid. Similarly, DVB/CAR/PDMS coating extraction increased the proportions of hexanoic, decanoic, dodecanoic, and hexadecanoic acids in perirenal and caul fats.

Table 2. Major volatile compounds of adipose tissues

Major volatile compounds[þ]	Perirenal fat		*P*	Tail fat		*P*	Caul fat		*P*
	S (2)	S (3)		S (2)	S (3)		S (2)	S (3)	
Acetic acid	3.50±1.11	4.18±0.31X	*ns*	1.70±0.55	1.56±0.10Y	*ns*	3.23±0.59	3.92±0.20X	*ns*
Hexanoic acid	0.62±0.19y	2.19±0.11	***	1.01±0.26x	2.00±0.17	**	1.18±0.10x	2.16±0.23	**
Decanoic acid	2.77±0.13x	3.38±0.24X	*	0.49±0.05z	0.14±0.00Y	***	1.00±0.37y	4.25±1.09X	**
Dodecanoic acid	1.51±0.14y	1.86±0.05Y	*	0.81±0.18z	0.91±0.03Z	*ns*	2.17±0.25x	3.84±0.23X	***
Tetradecanoic acid	1.93±0.20xy	1.78±0.26Y	*ns*	1.07±0.50y	2.56±0.71Y	*	2.73±0.87x	5.15±0.67X	*
Hexadecanoic acid	1.39±0.06y	2.11±0.18Y	**	0.84±0.27z	1.37±0.21Z	*ns*	2.15±0.08x	3.18±0.34X	**
2-Butenal	10.9±1.45x	0.28±0.05	***	2.46±0.45y	0.40±0.03	***	3.85±1.81y	0.30±0.10	*
Hexanal	2.24±0.49y	2.83±0.48Y	*ns*	16.4±2.51x	20.3±1.90X	*ns*	5.37±2.15y	3.62±0.62Y	*ns*
3-Methyl-2-butenal	2.09±0.29xy	0.60±0.11Z	***	2.75±0.65x	2.37±0.06X	*ns*	1.40±0.21y	2.13±0.03Y	**
Heptanal	3.72±0.36y	4.56±0.23Y	*	8.17±1.34x	7.77±0.31X	*ns*	3.92±0.71y	2.15±0.03Z	*
trans, 2-Hexenal	1.86±0.35x	1.67±0.24X	*ns*	1.58±0.26x	1.80±0.10X	*ns*	-	-	
Octanal	1.11±0.24y	2.61±0.33Y	**	4.49±0.33x	4.95±0.43X	*ns*	1.31±0.02y	-	***
trans, 2-Heptanal	1.60±0.14x	3.17±0.36X	**	1.88±0.43x	1.99±0.17Y	*ns*	0.75±0.22y	0.24±0.03Z	*
Nonanal	2.08±0.32y	5.58±0.38Y	***	4.63±1.03x	6.45±0.64X	*ns*	2.54±0.37y	4.06±0.08Z	**
trans, 2-Octenal	1.87±0.31xy	1.83±0.20X	*ns*	2.22±0.38x	1.89±0.10X	*ns*	1.37±0.15y	1.41±0.12Y	*ns*
trans,*trans*, 2,4-Heptadienal	4.95±0.36x	3.34±0.07X	**	4.77±0.59x	1.88±0.04Z	***	2.17±0.37y	3.12±0.07Y	*
Benzaldehyde	2.30±0.32x	10.4±0.35X	***	0.55±0.11z	0.63±0.04Z	*ns*	0.99±0.17y	5.10±0.23Y	***
trans, 2-Nonenal	3.13±0.41y	-	***	7.37±0.21x	7.68±0.64	*ns*	1.37±0.07z	-	***
trans, 2-Decenal	0.86±0.12y	4.25±0.77X	**	3.59±0.60x	4.25±0.12X	*ns*	0.89±0.11y	1.64±0.11Y	***
Phenyl acetaldehyde	4.43±0.86x	5.84±0.80X	*ns*	0.69±0.04y	0.46±0.03Y	***	-	-	
3-Dodecenal	0.77±0.10y	2.24±0.10Y	***	1.31±0.17x	2.72±0.07X	***	0.42±0.07z	0.86±0.02Z	***
trans,*cis*, 2,4-Decadienal	1.06±0.15x	1.03±0.19Y	*ns*	0.47±0.07y	1.20±0.03Y	***	1.05±0.08x	1.92±0.22X	***
trans,*trans*, 2,4-Decadienal	0.62±0.13	2.08±0.28X	***	0.75±0.14	2.10±0.08X	***	0.46±0.13	0.85±0.11Y	*
Acetic acid phytyl ester	1.53±0.46x	2.16±0.55Y	*ns*	0.62±0.12y	1.42±0.11Y	***	0.64±0.15y	5.02±0.50X	***
Styrene	17.7±1.89y	8.03±0.42Y	***	9.91±0.92z	3.75±0.26Z	***	45.6±3.06x	24.5±1.57X	***
2-Phytene	1.51±0.28x	1.44±0.22Y	*ns*	0.44±0.02z	0.47±0.04Z	*ns*	0.85±0.09y	2.24±0.10X	***
3,5-Octadien-2-one	2.47±0.42	2.35±0.15Y	*ns*	3.34±0.71	2.10±0.20Y	*	2.50±0.42	4.20±0.15X	**

[þ] The compounds with the mean relative percent value above 1% were taken as major volatile compounds. Data were expressed as mean±standard deviation. The mean values followed by different letters in the same row indicate significant differences between perirenal, tail and caul fats adsorbed on DVB/CAR (S2) fiber [x,y,z] and on DVB/CAR/PDMS (S3) fiber [X,Y,Z] (P <0.05). -: not detected; ns: not significant (P >0.05); *P <0.005, **P <0.01, ***P <0.001

We detected 7 ketone compounds. Among them 3,5-octanedien-2-one and phenyl methyl ketone were found only in all adipose tissues. The first was also the most abundant ketone as reported by Sivadier et al. (2008) who determined the 3,5-octanedien-2-one as a major compound in adipose tissues. The extraction with different coatings did not differ in ketone number. The highest proportion of 3,5-octanedien-2-one was found to be in caul fat in extraction by DVB/CAR/PDMS, followed by tail fat by CAR/PDMS coating (Table 2). It might have taken longer to reach equilibrium with DVB/CAR/PDMS at the same extraction time due to a greater number of volatile compounds in tail fat as consequently different affinities of volatiles for the fiber coating. Nevertheless, fiber difference was not affected proportion of 3,5-octanedien-2-one, irrespectively of adipose tissues (Table 2).

Phenols, alcohols, and esters were in less proportion and number in adipose tissues compared to other compounds. 3- and 4- Methyl

phenols were routinely determined. The type of fiber coating did not affect the proportion of phenol in tail fat, but DVB/CAR/PDMS coating had an enhancing effect on these in perirenal and caul fats (Fig. 1). As shown in Fig. 1, alcohols were detected in adipose tissues except for caul fat by DVB/CAR/PDMS. In perirenal and tail fat high alcohol proportions were determined by DVB/CAR/PDMS, like esters. This trend was in disagreement with the findings of Marco et al. (2004) who reported that CAR/PDMS fiber had a higher affinity for lower molecular weight compounds. These differences could be attributed to extraction time since a 15-min extraction with CAR/PDMS coating fiber provided a good yield for esters in headspace of foal dry-cured loin, but extended extraction time up to 45 min with DVB/CAR/PDMS coating as in the present study increased the proportion of ester and decreased the proportion of alcohol, which was highest in sample extracted by DVB/CAR/PDMS fiber for 15 min (Lorenzo, 2014). In addition, identifying a greater number of volatile components by CAR/PDMS coating may result in a reduction in their relative percent composition.

Principal component analysis

In order to discriminate of SPME fibers, principal component analysis (PCA) was conducted on the data obtained from major VCs identified in adipose tissues extracted by the both fibers (Fig. 3A). PC1 and PC2 explained 67% of total variance in VCs in headspace of adipose tissues. Also, PCA analysis was applied on data from FAs together with VCs. As can be seen in Fig. 3B, the variation between the adipose tissues can all be explained by two components. Tail fat that has a higher aldehyde (Fig. 3A) and PUFA (Fig. 3B) proportion separated from the other ones and positioned on the positive side of PC1. A differentiation between the SPME fibers was proved along PC2 (Fig. 3A), those analyzed with CAR/PDMS and DVB/CAR/PDMS were in the negative and positive sides of PC2, respectively. As can be seen in Fig. 3A, a marked variation between the SPME fibers was also observed for perirenal fat. This may be a consequence of the fact that VCs in more number (12) were detected by DVB/CAR than by DVB/CAR/PDMS. Most of the major VCs were also located on the above the bi-plot chart. In particular styrene and 2-butenal, which have a higher negative eigenvector of PC2 component, resulted in a higher relative percentage in the analysis of adipose tissues by CAR/PDMS. Carboxylic acids, 2-phytene and acetic acid phytyl ester determined in perirenal and caul fats and aldehydes (*trans*,*trans*,2,4-decadienal, nonanal, 3-dodecenal, *trans*-2 isomers of hexenal, heptenal, octenal, and decenal) found in tail fat extracted by DVB/CAR/PDMS in a high relative percentage were lined along PC2 with high positive eigenvectors (Fig. 3A). But, aldehydes derived from oxidation of fatty acids were clearly separated tail fats from caul and perirenal fats. These aldehydes which have higher levels in tail fat coincide with the fact that tail fat has more oleic, linoleic, CLA and linolenic acids (Fig. 3B). The difference in aldehyde proportions according to the fibers used also clearly distinguished the tail fats from each other.

CONCLUSION

SPME is an appropriate tool for qualitative analysis of VCs in the headspace of adipose tissues. Both CAR/PDMS and DVB/CAR/PDMS fiber coatings are feasible for studying volatiles from adipose tissue. However, the number of VCs detected in headspace of adipose tissues varied from the tissue to tissue. It was observed that VCs in greater number were extracted by DVB/CAR coating than DVB/CAR/PDMS. Probably due to the detection of VCs in less number, an increase in the relative percentage of VCs extracted by DVB/CAR/PDMS was observed. Tail fat with high oleic, linoleic and linolenic acids was clearly separated from other tissues based on aldehydes, which are fatty acid oxidation products, mainly hexanal. Aldehydes, namely octanal, nonanal, and decanal produced from C18:1; hexanal, *trans*-2-heptenal, *trans*-2-octenal, *trans,trans*-2,4-decadienal, and benzaldehyde from C18:2, and also *trans,trans*-2,4-decadienal, decanal, benzaldehyde from C18:3 can give an information about fatty acid profile of adipose tissue.

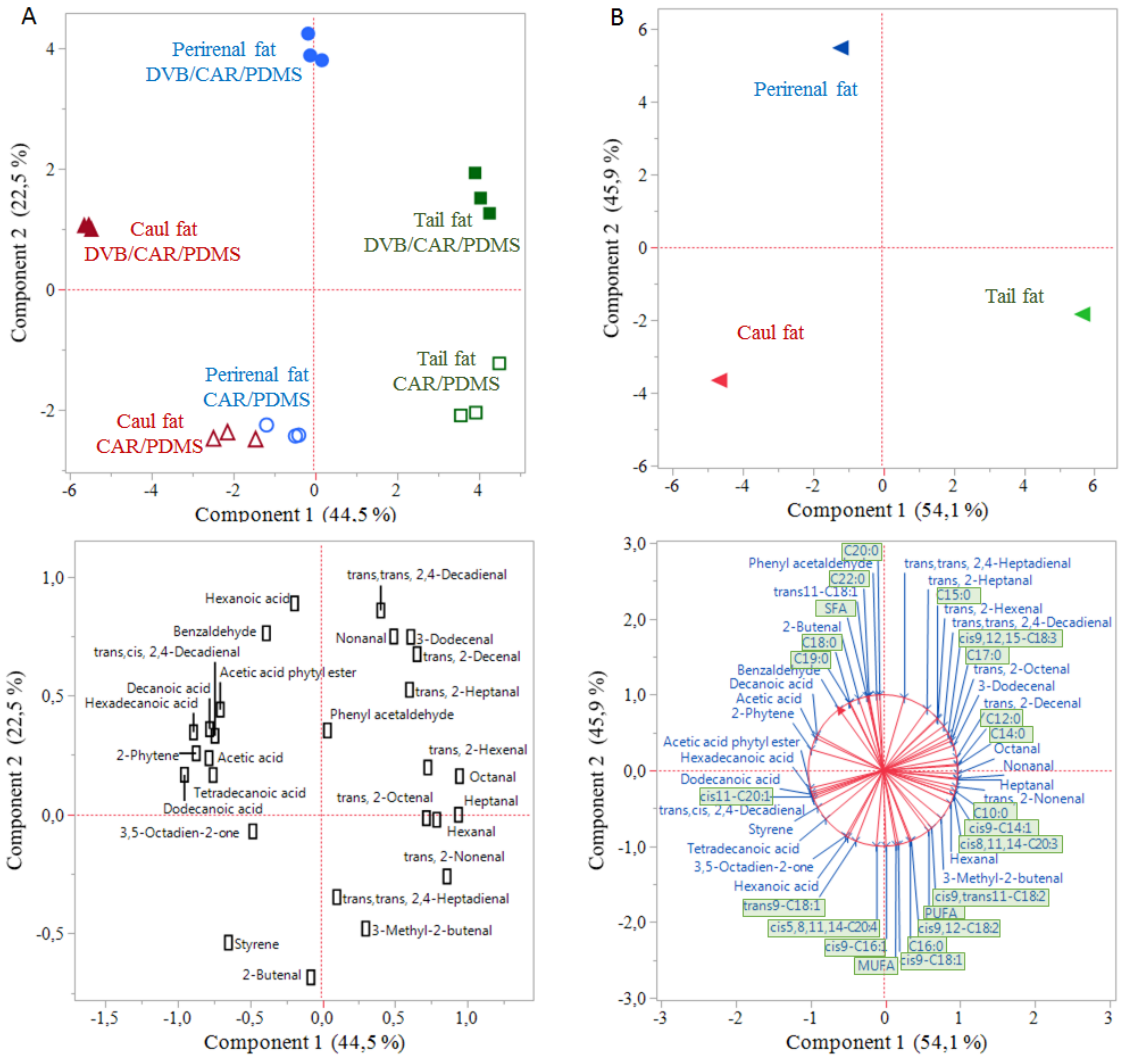

Fig. 3. Results of principal component analysis of VCs (A) and FAs together with VCs (B) identified in perirenal (○), tail (□) and caul (△) fats by CAR/PDMS (empty symbols) and DVB/CAR/PDMS (filled symbols).

Extraction by DVB/CAR/PDMS coating provided a good yield for carboxylic acids and benzaldehyde. If the goal is to identify aldehydes, especially fatty acid oxidation products and hydrocarbons with low molecular weights, as well as a greater number of volatile compounds, CAR/PDMS fiber seems to be recommended for volatile compounds from adipose tissues. But if the target is carboxylic acids, esters, and also aldehydes with high molecular weight, DVB/CAR/PDMS coating may be utilized due to its high affinity for mentioned compounds. Even so, the optimum extraction time should be examined to determine the time to reach equilibrium avoiding the presence of lipid oxidation products such as aldehydes favored by prolonged exposure.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

AUTHOR CONTRIBUTIONS

Zehra Güler: Project administration, methodology, writing-review & editing. Ahmet

Dursun: Formal analysis, statistical analysis, visualization.

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