Comparative analysis of volatile compounds in leather using solvent-assisted flavor evaporation and solid-phase microextraction

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Abstract

The volatile component of leather is a mixture of different compounds. Gas chromatography-mass spectrometry (GC/MS) is used to analyze volatile compounds. Pretreatment is required before the sample is introduced into the GC. Solid-phase microextraction (SPME) is a solvent-free sample preparation method that uses coated fibers to extract analytes from samples prior to GC. SPME is a simple method, but the adsorbed compounds depend on the type of fiber. Quantitative analysis is difficult because the gas phase is injected into the GC. To understand the totality of volatiles in leather, we need to look at solvent extraction. Solvent Assisted Flavor Evaporation (SAFE) distillation is a well-known in the food sector. In combination with a high vacuum pump (5×10^{-3} Pa), high boiling compounds can be removed by high-vacuum distillation. In this study, volatile compounds in leather were extracted using two extractions, SAFE and SPME. The eluate was concentrated to 1 mL using a Kuderna-Danish concentrator. Volatile compounds were identified by GC/MS. 35 volatile compounds were identified by SPME and 31 by SAFE. SAFE showed a good extraction effect on alcohols, and SPME showed a good extraction effect on aldehydes, linear alkanes, and ethers.

Keywords: solvent-assisted flavour evaporation, solid-phase microextraction, gas chromatography-mass spectrometry

1. Introduction

Volatile compounds are generated by the chemicals used in the various stages of the leather manufacturing process. Leather manufacturing inevitably involves the use of chemicals, including organic solvents and dyes. These additives can remain in the finished product as volatile compounds. Gas chromatography-mass spectrometry (GC/MS) is used to analyze volatile compounds. GC/MS is suitable for the measurement of gaseous and liquid samples and is characterized by the ability to determine multiple components rapidly and simultaneously, both qualitatively and quantitatively, and by excellent reproducibility of analytical values. Automation of injection with autosamplers is an advantage. There are variations of injection systems such as headspace (HS), solid phase microextraction (SPME) and liquid. SPME injection is simple, has a concentration effect and is widely used in leather analysis [1-4]. The SPME method can be used with gas chromatography-olfactometry (GC-O) to identify an odor-active compound in leather [5]. Adsorption compounds depend on the type of SPME fiber, and quantitative analysis is difficult as the gas phase is injected into the GC. In mass spectrometry for trace analysis, selectivity by gas chromatography–tandem mass spectrometry (GC-MS/MS) is essential to detect target compounds with high sensitivity. The sample should be highly concentrated before GC-MS/MS analysis. While the above injection systems are also effective, solvent assisted flavor evaporation (SAFE), in connection with a high vacuum pump, allows the isolation of volatiles from either solvent extracts, aqueous foods such as milk or beer, aqueous food suspensions such as fruit pulp,

or even matrices with high oil content [6]. SAFE extraction is used primarily in the food industry and can distill high boiling compounds at low temperatures. The extract is further concentrated without heating and subjected to analysis. Leather is heated to denature the collagen fiber and contains fat or oil such as fatliquoring agents. Using SAFE, the volatile compounds in leather can be extracted at low temperatures, and a fatliquoring agent can be separated.
In this study, volatile compounds in leather were extracted using two different methods, solid-phase microextraction (SPME) and solvent-assisted flavor evaporation (SAFE) extraction. The extracts were further concentrated and subjected to GC/MS analysis to compare the extracted compounds from each extraction method for trace analysis.

2. Material and Methods

2.1 Material

Finished leather with a urethane coating was used. Prior to analysis, the sample was kept at a temperature of 20 °C and a relative humidity of 65% for 2 days or more days. After conditioning, the sample was finely cut into 2 mm squares.

2.2 Extraction of volatile compounds by solid phase micro extraction (SPME)

Leather sample (0.5 g) was added to a 20 mL headspace vial. Extraction was performed at 80 °C, and the vial was preheated for 5 min. For the sampling of volatile compounds in leather, a 10 mm long carbon wide range/PDMS fiber (95 µm, Restek corporation, Switzerland) was preconditioned at 280 °C in the conditioning port. It was inserted into the headspace vial to extract the volatiles for 10 min, and then the fiber was inserted into the injection port of the GC-MS system.

2.3 Extraction of volatile compounds by solvent-assisted flavour evaporation (SAFE)

10 g of the leather was weighed and transferred to a 500 mL Erlenmeyer flask to which 200 mL of dichloromethane was added, and the contents of the flask were shaken overnight at room temperature. After this time, the solution was filtered and subjected to volatile extraction in a Solvent-Assisted Flavor Evaporation (SAFE) apparatus AB25-I-2 (Kiriyama Glass Corp., Tokyo, Japan) as described by Engel et al [4]. During the procedure, the temperature of the sample was maintained at 30°C and vacuum was applied using a two-stage vacuum system DS-A212Z (Diavac Limited, Chiba, Japan). The vacuum level was confirmed using a hot cathode ionization meter TRI-10-N25Y (Diavac Limited, Chiba, Japan). When the pressure of the system decreased to 5×10^{-3} Pa, the sample was poured into the dropping funnel. Then, the dropping funnel was opened, and the liquid nitrogen was added to the cooling trap. After the sample was added, the extraction continued for 30 minutes. Then, the extract in the receiving flask was dried over anhydrous sodium sulfate and concentrated to approximately 1.0 mL in a Kuderna-Danish concentrator (Kiriyama Glass Corp., Tokyo, Japan).

2.4 Gas chromatography–mass spectrometry (GC-MS) analysis

The GC conditions of SPME and SAFE were the same except for the injection mode. The content of volatile compounds was determined by the gas chromatography method. GC-MS analysis was performed using a gas chromatograph-mass spectrometer GCMS TQ-8040 (Shimadzu Corp., Kyoto, Japan) with an AOC-6000 multifunctional autosampler (Shimadzu Corp., Kyoto, Japan). The separation of compounds was performed on a fused silica capillary column InertCap-WAX (30 m x 0.25 mm, 0.25 µm film thickness, GL Sciences Inc., Tokyo, Japan).
The flow of carrier gas (helium) was controlled by constant linear velocity and set at 50.0 cm/sec, and the temperature of the injection port was 240 °C. SPME extracts were injected in splitless mode, the sample desorption time was 1 min, while SAFE extracts were injected in split mode (split ratio 10: 1) at 1 µL. The heating program was as follows: The initial temperature was 40 °C and was maintained for 5 min. The temperature was increased to 240 °C at a rate of 10 °C/min and held for 10 min.
The mass spectrometer was operated in electron ionization (EI) mode at 70 eV with a scan range of m/z 28-400. The ion source temperature was 200 °C and the interface temperature was 250 °C. Volatile compounds were characterized using the National Institute of Standards and Technology (NIST) library search program.

3. Results and Discussion

3.1 SAFE extraction

Figure 1 shows the SAFE extraction: the pressure of the system was reduced to 5×10^{-3} Pa, the sample was poured into the dropping funnel. The dropping funnel was then opened, and a sample of the solution was immediately flash distilled at the sample inlet. Most of the solvent and low-boiling compounds evaporated and condensed into a sample collection vessel (300 mL) via a thermostatic distillation head. The high-boiling compounds hit the separator in the head and returned to the distillation vessel (100 mL). Frost formed on the inside of the inlet due to the latent heat of evaporation. This was removed by heating with a heat gun. Low boiling compounds that have an affinity for solvents evaporate predominantly. Dichloromethane, which has strong elution tendency, was used. The collection vessel at the end of the SAFE distillation had a characteristic odor of dichloromethane. The eluate was then concentrated to 1 mL using a Kuderna-Danish concentrator, the odor of the concentrate was different from that of dichloromethane. The odor compounds in the extract were concentrated and above the threshold and the odor of dichloromethane was no longer detectable to the human nose.

Fig.1 SAFE extraction

3.2 Evaluation of SAFE and SPME Extracts

The SPME and SAFE extraction methods were compared to evaluate their suitability for the determination of volatile compounds in leather. Initially, the SAFE concentrate was analyzed in splitless mode as for SPME extraction, but the concentration of the extract was too high and the peaks in the total ion chromatogram (TIC) were too large and insufficiently separated, so it was analyzed in split mode (split ratio 10: 1). 35 volatile compounds were identified by SPME and 31 by SAFE. These compounds are listed in Table 1. A total of 25 volatile compounds were identified by SPME and SAFE together, including butyl acetate, p-xylene, 1-butanol, acetic acid, 2-(2-ethoxyethoxy)-ethanol, 1-nonanol. The SPME method extracted more volatile compounds than SAFE. Aldehydes such as hexanal and octanal, linear alkanes such as hexadecane and heptadecane, and ethers such as hexadecyl nonyl ether and dodecyl nonyl ether were detected only by SPME extraction. Alcohols such as 3-penten-2-ol and 3-methoxy-3-methylbutanol were detected only by SAFE extraction. N, N-dimethylformamide (DMF) was also detected only by SAFE extraction. The number and concentration of volatile compounds extracted by SPME and SAFE were different. SAFE showed a good extraction effect on alcohols, and SPME showed a good extraction effect on aldehydes, linear alkanes, and ethers.

Table 1. Volatile compounds identified using gas chromatography-mass spectrometry (GC-MS) in finished leather after extraction using two methods: solid-phase microextraction (SPME), and solvent-assisted flavor evaporation (SAFE).

No.	Volatile compounds	SPME	SAFE
1	butyl acetate	+	+
2	hexanal	+	-
3	p-xylene	+	+
4	1-butanol	+	+
5	3-penten-2-ol	-	+
6	o-xylene	+	+
7	cyclohexanone	+	+
8	octanal	+	-
9	2-(2-methoxy-1-methylethoxy)-1-propanol	+	+
10	N,N-dimethyl-formamide	-	+
11	nonanal	+	-
12	3-methoxy-3-methylbutanol	-	+
13	acetic acid	+	+
14	2-butoxyethyl acetate	+	+
15	1-(2-methoxy-1-methylethoxy)- 2-propanol	+	+
16	2-ethyl- 1-hexanol	+	+
17	hexadecane	+	-
18	(E)-2-nonenal	+	+
19	2-ethyl-2-Hexen-1-ol	+	+
20	2-(2-ethoxyethoxy)-ethanol	+	+
21	6-methyl-1-octanol	+	+
22	1-nonanol	+	+
23	heptadecane	+	-
24	2-(2-butoxyethoxy)-ethanol	+	+
25	hexadecyl nonyl ether	+	-
26	nonyl tetradecyl ether	+	-
27	1,1'-oxybis- 2-propanol	-	+
28	dodecyl nonyl ether	+	-
29	nonyl octacosyl ether	+	-
30	2-(2-hydroxypropoxy)- 1-propanol	-	+
31	isoquinoline	-	+
32	benzothiazole	+	+
33	1-dodecanol	+	+
34	octanoic acid	+	+
35	p-cresol	+	+
36	m-cresol	+	+
37	2,4,7,9-tetramethyl-5-decyn-4,7-diol	+	+
38	1-methyl-2-(4-methylphenoxy)- benzene	+	+
39	nonanoic acid	+	+
40	1-tetradecanol	+	+
41	diethyl phthalate	+	-

SPME is a headspace method and is characterized by being solvent-free. The SPME fiber layer has different adsorption capacity for compounds. The fiber used in this study was carbon wide range/PDMS for low molecular weight and low boiling point compounds. SAFE has solvent loss in the extraction process, so the volatile compounds extracted by the two methods are not consistent. Therefore, a combination of the two methods can give good results. The total ion chromatogram (TIC) of the finished leather is shown in Figure 2.

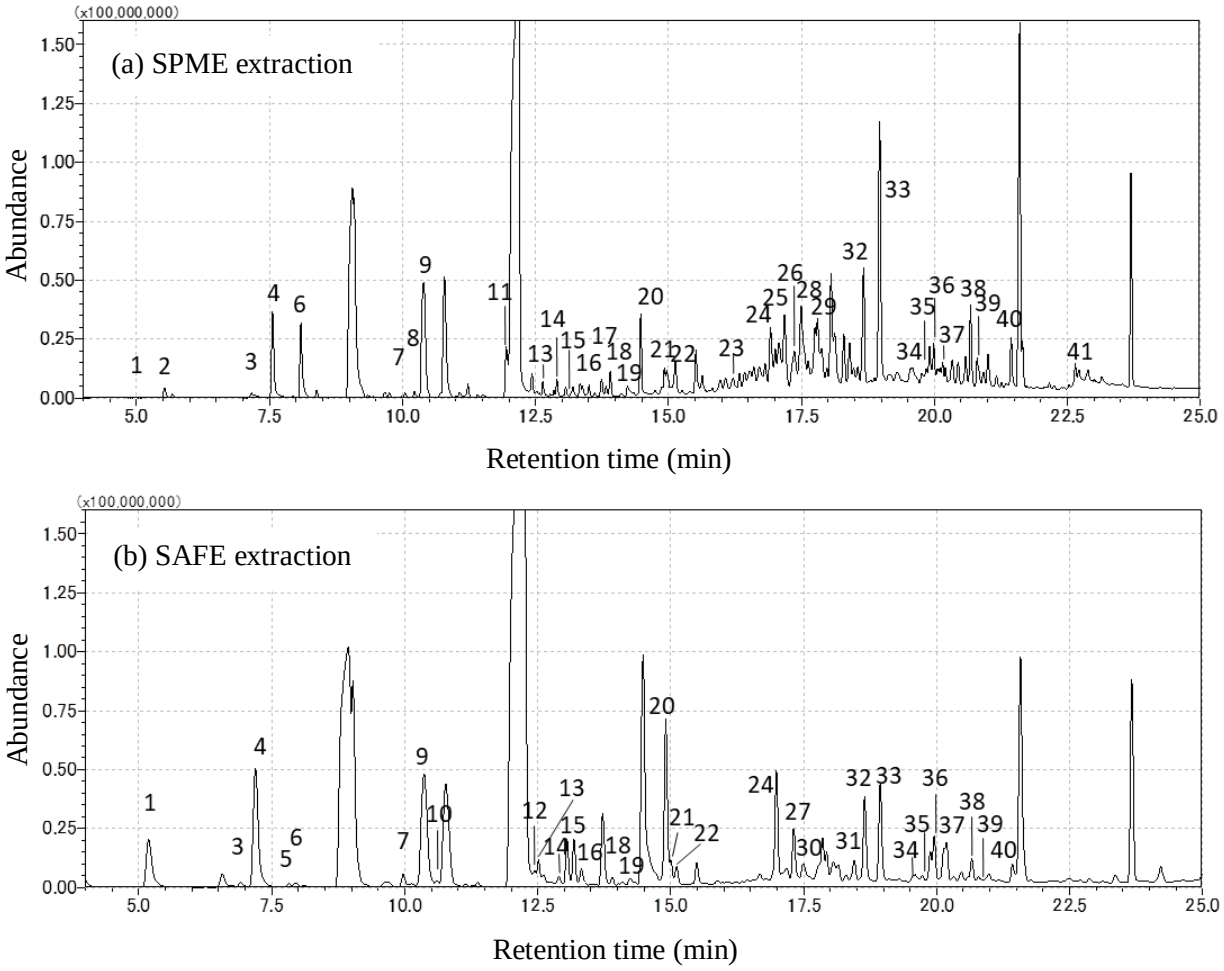

Fig.2 Total ion chromatogram (TIC) of finished leather: (a) SPME extraction (b) SAFE extraction

As mentioned above, differences in the identified compounds were observed between the two extraction methods. Comparing the shape of the TIC of the SPME and SAFE extracts, an aggregation of peaks can be seen in the retention time after 15 min. Peak 25 and peak 26 represent hexadecyl nonyl ether and nonyl tetradecyl ether. These ethers were identified more frequently by SPME extraction than by SAFE extraction. As a result of the removal of high boiling compounds from the leather by SAFE distillation, the TIC of SAFE extraction did not show peak aggregates as SPME.

4. Conclusion

Volatile compounds in leather were extracted using two different methods, SPME and SAFE extraction. The extracts were concentrated in a Kuderna-Danish concentrator and subjected to GC/MS analysis to compare the extracted compounds from each extraction method. 35 volatile compounds were identified by SPME and 31 by SAFE. SAFE showed a good extraction effect on alcohols, and SPME showed a good extraction effect on aldehydes, linear alkanes, and ethers. As a result of the removal of high boiling compounds from the leather by SAFE distillation, the TIC of SAFE extraction did not show peak aggregates as SPME.

5. References

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