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Identification of Volatile Compounds Originating from Secondary Contamination and Packaging Materials in UF and White Brine Cheeses

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ABSTRACT: Identification of volatile contaminants migrating from packaging materials that might affect the quality of packaged food and might cause problems to consumer health is great importance. Soft cheeses can undergo such migration and contamination during storage. This study aimed to exclusively identify abnormal volatile compounds in Iranian cheeses that likely originated from contamination and packaging rather than endogenous components using HS-SPME GC-MS. White brine and ultrafiltrated (UF) cheeses in packages were stored for 90 days at 4 degrees Celsius (°C). Headspace-solid phase microextraction (HS-SPME) using polysulfone and mesoporous carbon nitride (MCN/Polysulfone) fiber coupled to gas chromatography-mass spectrometry (GC-MS) was employed to extract and analyze volatile compounds. Migration-based contaminants exclusively present in stored versus fresh cheeses were identified through National Institute of Standards and Technology (NIST) library matching. In total 23 unwanted volatile contaminants originating from contamination/packaging were identified, including 19 compounds in white brine` cheese (phthalates, benzenecarboxylic acids, etc.) and 13 compounds in UF cheese (phthalates, benzenecarboxylic acids, triazenes, oximes, etc.). More migrants were observed in white brine cheese. Compounds also differed based on SPME extraction method. Prolonged storage induced migration of volatile contaminants from probable packaging sources into soft cheeses. Future research should focus on refining volatile organic compound (VOC)-based detection methods to enhance early identification of spoilage and pathogenic microorganisms in cheese production.

Keywords: Cheese Ripening, Food Safety, Microbial Activity, Secondary Contamination, Volatile Organic Compounds (VOCs).

Introduction

Cheese is one of the most popular dairy products worldwide and valued for its nutritional properties and diverse sensorial characteristics. The characteristic flavor and aroma profile is the primary sensory factor determining consumer preference and market value of cheese (Delgado *et al.*, 2011). The typical volatile compounds present in cheese arise mainly from lipolysis, proteolysis and metabolic activities of starter and non-starter lactic

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acid bacteria during ripening (McSweeney, 2004). However, some volatile compounds may also originate from secondary contamination sources and interaction with packaging materials rather than from the endogenous cheese components (ÖzerKınık *et al.*, 2017; Aminifar *et al.*, 2014). Identification of these extraneous volatile compounds is important to ensure cheese quality and safety. Several studies have analyzed the volatile profiles of different cheese varieties using headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography-mass spectrometry (GC-MS). ÖzerKınık *et al*. (2017) examined volatile compounds in sheep milk cheese at 1 and 90 days of ripening, identifying extraneous compounds like benzaldehyde, 1-octen-3-ol and benzeneacetaldehyde unrelated to endogenous cheese production. Aminifar *et al.*, 2014) also detected some alcohols, esters, ketones and aromatics not associated with Liqvan cheese ripening using HS-SPME GC-MS possibly from environmental sources. These demonstrate the efficacy of HS-SPME GC-MS in detecting abnormal volatile cheese contaminants during storage.

Polyvinyl chloride (PVC) films are commonly employed for cheese packaging, which can release plasticizers like phthalates into foods (Dole *et al.*, 2010). Phthalates such as di(2-ethylhexyl) phthalate (DEHP) have been shown to migrate from packaging into yogurt and cheese based on factors like temperature, contact surface area and fat content (Nerín *et al.*, 2003; Castle *et al.*, 1989; Lyche et al., 2009). As phthalates are not covalently bound to PVC, they can readily migrate into fatty dairy products (Bradley *et al.*, 1995). Other potential packaging-related contaminants include components, adhesives, stabilizers and lubricants (Vera *et al.*, 2012). Environmental contaminants from surrounding air/contact surfaces and issues like poor employee hygiene can also secondarily introduce volatile impurities into packaged cheese post-processing (Hodgson *et al.*, 2000; Libinaki *et al.*, 2006). Therefore, identifying abnormal volatiles using analytical techniques helps determine contamination sources.

Although prior studies have profiled ripening-related cheese volatiles, limited research has focused on characterizing volatile contaminants specifically originating from secondary sources and packaging. Furthermore, misidentification of contaminants as ripening by-products can lead to incorrect inferences regarding cheese biochemistry. Dedicated characterization of contamination-derived volatiles therefore warrants investigation.

This study aimed to exclusively identify abnormal volatile compounds in Iranian cheeses that likely originated from contamination and packaging rather than endogenous components using HS-SPME GC-MS. Two fiber coatings, polysulfone and mesoporous carbon nitride (MCN/polysulfone), enabled comprehensive profiling of volatile contaminants.

Materials and Methods

- Cheese sampling

UF cheese samples were prepared using ultrafiltration process from pasteurized milk and stored in sterilized packages post treatment. White brine cheese samples on other hand were conventionally manufactured without thermal processing and brine salted before packaging. All packaged cheeses were obtained from Golpaiegan (PegahGolpaiegan, Isfahan, Iran), Iran. The cheeses were transported under refrigerated conditions on the production day to the laboratory where analysis were carried out in triplicates

order.

- **Storage study**

The cheese samples were transferred aseptically into sterilized glass containers following the purchase. The containers were then stored at refrigeration temperature (4±1°C) for a duration of 90 days. The containers were periodically opened only during headspace volatile sampling on storage days 1, 30, 60 and 90 and 120 for brine cheese and 1, 30, 60 and 90 days for analysis.

- HS-- SPME extraction

For HS extraction, 3 g of cheese samples were taken into 20 ml headspace vials sealed with polytetrafluoroethylene (PTFE)silicon septa. The vials were equilibrated at 60°C for 45 minutes in a cooling heating block to promote volatilization of compounds. The extraction was then carried out using a preconditioned (260°C for 2 hours) 85 µmMCN/Polysulfone inserted into the headspace for 45 minutes at 60°C. In should be noted that we extracted the compounds from the headspace using a Hamilton syringe and transferred them to the GC (Sabouri *et al.*, 2024).

- Direct immersion SPME extraction

Alternatively, direct immersion SPME was conducted by weighing 5 g of cheese, and transferring it into a 40 ml screw capped vial. The sample was pre-incubated at 60°C for 10 minutes before exposure to fibers - 65 µmpolysulfone and mesoporous carbon nitride (MCN/Polysulfone) (Supelco) for extracting non-polar and weakly polar compounds respectively. The fibers were directly inserted into the sample headspace for 60 minutes at the same temperature (Sabouri *et al.*, 2024).

- GC-MS analysis

The extracted volatile compounds were analyzed using an Agilent 7890B GC system (Agilent, USA) coupled with a 5977B mass selective detector (quadrupole) and equipped with either an HP-5MS or HP-624 column (30 m × 0.25 mm internal diameter × 0.25 µm film thickness). Helium was used as the carrier gas at a flow rate of 0.8 mL/min. The injection port was set to splitless mode at 250°C. The interface and ion source temperatures were 280°C and 230°C, respectively. Mass spectra were collected at 70 eV ionization voltage over an m/z range of 30-350. The oven temperature program had an initial isothermal hold at 40°C for 5 min, followed by a temperature ramp from 40 to 160°C at 5°C/min, then an increase to 240°C at 10°C/min with a 5 min final hold. Spectral signals were analyzed using MassHunter Workstation software and the NIST14 MS library for tentative identification based on match scores ≥80%. Relative abundances were calculated from total ion chromatogram (TIC) peak areas (Sabouri *et al.*, 2024). Compounds identified exclusively in the stored cheeses but absent in fresh samples were deduced as migrants from secondary contamination and/or packaging.

- Data analysis

The study data were summarized using descriptive statistics. Categorical variables were expressed as percentages. Quantitative variables were reported as mean ± standard deviation (SD). Experiments were conducted in triplicates to ensure reproducibility of results.

The SPSS software package was utilized to analyze the data. Statistical tests were two-sided and based on a significance level of 0.05. SPSS enabled

performance of all statistical analyses on the study data.

Results and Discussion

A total of 65 volatile compounds were identified from the UF during 90 days and brine cheeses during 120 days of storage, including alkanes, ketones, aldehydes, acids, esters, and aromatics. Out of these, 23 compounds originated from secondary contamination and packaging, as described below in different cheese types.

- **Volatile compounds originating from secondary contamination and packaging in white brine cheese**

White brine cheese showed 19 volatile compounds originating from secondary contamination and packaging materials when extracted using MCN and PSF fibers during storage. These included phthalates, benzenecarboxylic acids, adipates, siloxanes, etc. as shown in Table 1.

Phthalates such as diethyl phthalate were identified as the major contaminants from packaging on day 1 and day 90 along with benzenecarboxylic acids such as dimethyl benzene dicarboxylic acid. Adipates such as di-iso-octyladipate were also detected. Other compounds like siloxanes and benzaldehyde were present due to secondary contamination.

Phthalates such as diethyl phthalate and dimethyl phthalate were identified as some of the major contaminants in both the cheeses from possible package migration. Previous studies have also reported phthalates as common migrant contaminants from packaging materials into dairy products, especially upon long term storage. For instance, Bradley *et al.*, (1995) detected diethyl phthalate in packaged cheese shreds stored for 21 days at 8°C possibly due to migration from printing inks. Earlier, Conchillo *et al.* (Giuliani *et al.,* 2020) identified up to 16 phthalates including diethyl phthalate in plastic packed Carmenere fruit juices stored for 6 months. The authors indicated plasticizers as the primary source behind phthalate contamination. Since phthalates are used as common plasticizers, their migration from packaging materials into fatty dairy and liquid food matrices is not unusual over prolonged storage. Legislations have also been imposed by agencies such as European Food Safety Authority to monitor phthalate levels considering their toxic effects (Giuliani *et al.,* 2020). Therefore, focusing on reducing phthalate migration by using phthalate-free packaging would help minimize this risk and improve cheese quality.

- **Volatile compounds originating from secondary contamination and packaging in UF cheese**

A total of 13 compounds possibly from contamination and packaging were found in UF cheese using MCN, PSF and HS-SPME extraction during 90 days. These are presented in Table 2..

Table 1. Volatile compounds originating from secondary contamination and packaging materials in brine cheese

Compound	Day 0	Day 30	Day 90
Diethyl phthalate	Diethyl phthalate	-	Diethyl phthalate
Dimethyl phthalate	-	-	Dimethyl phthalate
Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid
Di-iso-octyladipate	-	Di-iso-octyladipate	-
Dodecamethylcyclohexasiloxane	Dodecamethylcyclohexasiloxane	-	-

Table 2. Volatile compounds originating from secondary contamination and packaging materials in UF cheese

Compound	Day 1	Day 30	Day 90
Diethyl phthalate	Diethyl phthalate	Diethyl phthalate	-
Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid	Dimethyl benzene dicarboxylic acid	-
Diethyl phthalate	Diethyl phthalate	Diethyl phthalate	-

Major contaminants identified were phthalates on day 1 and benzenecarboxylic acids during entire storage period. Other contaminants included triazenes, siloxanes, and oximes. Acetyl acetate and tetra(4-hydroxyboranylphenyl)methane were identified possibly from packaging materials using HS extraction.

- **Comparison of volatile profiles between cheese varieties**

The profiles of volatile contaminants differed between white brine and UF cheeses. While phthalates, benzenecarboxylic acids and siloxanes were common, compounds like triazenes, oximes, acetyl acetate, etc. were unique to UF cheese. UF cheese showed less number of contaminants as compared to white brine over storage.

Indeed, around 23 volatile compounds originating from contamination sources and packaging materials were identified in cheeses, which contributed to the overall volatile profiles. Better quality packaging and prevention of contamination could reduce these unwanted volatiles in the cheeses.

Benzenecarboxylic acids like dimethyl benzene dicarboxylic acid were identified throughout the 90 days of storage in both cheese varieties from possible package migration. Benzenedicarboxylic acids have been documented to migrate from polyethylene terephthalate (pvc) bottles to mineral water upon storage by Bartolome *et al.* (Bach *et al.*, 2012). PET plastic packages were indicated as a source of these compounds. Benzoic and benzenedicarboxylic acids have also been reported as migrants from adhesives (Vera *et al.*, 2012). Therefore, the benzenecarboxylic acids detected in the present cheeses might have originated from such indirect packaging components over longer storage, contaminating the product. Again, preventing this would require mitigating future migration risks while packaging.

While phthalates and benzenecarboxylic acids contaminated both cheeses, the UF cheese showed additional unique contaminants like triazenes, oximes and tetra(4-hydroxyboranylphenyl)methane compounds possibly from packaging. In comparison, more contaminants were detected in white brine cheese (19 compounds) versus UF cheese (13 compounds) during storage. The differences could be related to variations in the packaging materials and storage conditions between the cheese varieties that would impact migration behaviors. As milk processing might alter the properties of UF cheese, it could interact differently with packages compared to unprocessed white brine cheese. Besides, factors like greater surface area and higher fat content of white brine cheese could enable more sorption of migrating contaminants over time as well (Sørensen, 2006). Nevertheless, the findings highlight the impact of storage duration, packaging variations and cheese compositions on contamination risks.

The range of contaminants identified also varied based on the SPME extraction method for individual cheeses. For instance, in white brine cheese, certain

migrating compounds like adipates and siloxanes were specifically extracted only using the MCN fiber, while compounds such as furanones selectively sorbed onto the PSF fiber. The findings showcase the selectivity and variability of different extraction phases in isolating migrated volatiles based on the cheese matrix effects. Thus, utilizing multiple extraction techniques can provide a more comprehensive contaminant profile from complex dairy matrices following migration (Gong *et al.*, 2023). However, developing optimized standardized methods would be vital for effective routine monitoring.

Conclusion

This study demonstrated the ability of headspace SPME coupled with GC-MS to comprehensively identify volatile compounds originating from secondary contamination and packaging materials in two types of Iranian cheeses - white brine and UF cheese. A total of 23 extraneous volatile contaminants were detected, with white brine cheese showing higher levels of contamination (19 compounds) compared to UF cheese (13 compounds) over 90-120 days of refrigerated storage.

Major contaminants included phthalates like diethyl phthalate, benzenecarboxylic acids, adipates, siloxanes and other compounds potentially migrating from plastic packaging films, printing inks, adhesives and environmental sources. The findings highlight how prolonged storage can facilitate migration of these unwanted volatiles into fatty cheese matrices, adversely impacting product quality and safety.

Differences in the contaminant profiles between the two cheeses were evident, likely due to variations in compositional properties like fat content as well as the specific packaging materials and headspace conditions influencing migration behaviors. Utilization of multiple SPME fiber coatings enabled more comprehensive extraction of diverse volatile migrants based on their polarity differences.

Finally, the study reinforces the need for improved packaging systems that prevent migration of harmful substances into food products during storage. Monitoring of abnormal volatiles using hyphenated techniques like HS-SPME GC-MS can aid in identifying contamination sources and implementing suitable preventive controls in cheese manufacturing. Future research should focus on further refining these analytical methods for early and sensitive detection of volatile contaminants as well as correlating them with sensory defects and potential health risks. Preventing volatile migrants from secondary sources is crucial for ensuring high quality, unadulterated and safe cheese products.

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