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REGULAR ARTICLE

Use of smart nose and GC/MS/O analysis to define volatile fingerprint of a goatskin bag cheese "*Bouhezza*"

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ABSTRACT

Bouhezza is an Algerian ripened cheese manufactured specifically in a goatskin bag with "*Lben*", salt and raw milk for several weeks. The aim of this study was to characterize the unknown aromatic profile of the *Bouhezza* using SMart Nose, gas chromatography/mass spectrometry (GC/MS) and GC/MS/Olfactometry. In a first step, four farmhouse *Bouhezza* cheeses were analyzed by SMart Nose and by GC/MS to investigate volatile profile at 75 and 150 ripening days. In a second step, two *Bouhezza* cheeses produced under controlled conditions according to the traditional process were analyzed during manufacturing-ripening by GC/MS/Olfactometry, to detect odor active compounds changes. Results showed higher variability of volatiles in cheeses at 75 days ripening compared to 150 days. Farmhouse and experimental cheeses showed esters the most abundant volatile compounds detected with GC/MS and GC/MS/Olfactometry. Ten odor active compounds were common between cheeses whatever is the origin or the age. These compounds can be considered as marker of the aromatic profile of *Bouhezza* cheese, not yet established.

Keywords: *Bouhezza* cheese; Goatskin bag; SMart Nose; Olfactometry; Volatile profile

INTRODUCTION

Cheeses are consumed and appreciated worldwide for their nutritional and sensorial characteristics. The pleasant odor and aroma of cheeses depends on several factors and principally on manufacturing and ripening processes. In literature, it is well known that volatile compounds and aroma precursors' generation in cheeses during ripening is mainly due to biochemical mechanisms; glycolysis, proteolysis and lipolysis (McSweeney and Sousa, 2000).

Several studies had well characterized the volatile profile of different cheeses. In general, ripened cheeses were studied using different aroma extraction, detection and identification techniques. To put it differently, many researchers used static headspace SPME (solid phase micro-extraction) technique for the extraction of volatile compounds (VOCs). This technique has many advantages, as reported by Deibler et al. (1999). Compounds from SPME are usually separated and identified by gas chromatography (GC) coupled to mass spectrometry (MS), to flame ionization detection (FID) and/or Olfactometry (Carpino et al., 2004; Mallia et al., 2008; Poveda et al., 2008; Serhan et al., 2010; Delgado et al., 2011).

Chesses manufactured or ripened in animal skin bags have been poorly studied. Those cheeses are known and produced only in some areas in a few countries worldwide. Some authors have studied such ripened cheeses to specify changes in manufacturing process and the aroma profile (Hayaloglu et al., 2007; Kalit et al., 2010; Serhan et al., 2010).

Bouhezza cheese is manufactured and ripened inside an animal skin bag using raw cow's, goat's or ewe's milk. This traditional process has been handed down from generation to generation and still preserved in some localities at the *Chaouia* area, East of Algeria. Aissaoui Zitoun et al. (2011) described that traditional process of using a goatskin bag

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"*Chekoua*" and fermented churned milk called '*Lben*'. Salted *Lben* is regularly added into the goatskin bag during several weeks. Draining and ripening are simultaneous processes with salted *Lben* additions. When the cheese is ripened for at least one month, whole raw milk is added, during one to two weeks, to adjust cheese acidity and salt content. All through the manufacturing process, the *Chekoua* is suspended and cleaned from the outside, on a daily basis, by rinsing with water. Sometimes a mixture of a few quantity of salt and juniper berry is powdered on the outer surface of the *Chekoua*. Once the cheese-making process is over, the *Chekoua* is emptied and the cheese is spiced with red hot pepper, in a form of powder or pasta, for habitual consumption.

A few previous studies have described the cheese-making process and the physicochemical, biochemical and microbiological changes during ten weeks of *Bouhezza* cheese- making (Aissaoui Zitoun et al., 2011; Aissaoui Zitoun et al., 2012). *Bouhezza* is a soft and a mid-fat cheese mainly ripened in the mass, as argued in the above-cited studies.

This experimental study might provide original information on the unknown volatile profile of *Bouhezza* cheese. The present study attempts at completing the global characterization of this authentic cheese through two aims. The first aim was to delineate the volatile profile of farmhouse *Bouhezza* cheese by SMart Nose and Solid-phase micro-extraction–gas chromatography–mass spectrometry (SPME/GC/MS) analysis. The second one was to study the change in aroma profile of experimental cheese samples during the manufacturing and ripening period by SPME/GC/MS/Olfactometry analysis.

MATERIALS AND METHODS

Farmhouse *Bouhezza* cheese sampling

Farmhouse samples of *Bouhezza* cheese, made from cow's milk (F1, F2, F3 and F4) were collected from four different farmhouses located in *Chaouia* area, Eastern Algeria. F1 and F2 cheeses have been manufactured and ripened for 75 days, and the two other cheeses, F3 and F4, for 150 days.

Experimental *Bouhezza* cheese manufacturing and sampling

Two batches of traditional *Bouhezza* cheese, L1 and L2, have been processed in two separated goatskin bags "*Chekoua*" by means of the same *Lben* and raw milk for a period of 70 days following the same process. The two *Chekouates* were prepared identically; the full traditional process of *Chekoua* preparation and cheese-making conditions was previously delineated in Aissaoui Zitoun et al. (2012).

Analyses were performed on samples taken from L1 and L2 at 7, 14, 21, 42, 56 and 70 days. These samples were stored at –20 °C pending analysis. The chemical composition of those samples was given in Aissaoui Zitoun et al. (2012).

SMart nose: Analytical instruments and conditions

A first screening was performed by means of an electronic nose, SMart Nose® (SMart Nose 1.51, LDZ, CH-2074 Marin-Epagnier, Switzerland) system, based on mass spectrometry, which allows the analysis of headspace volatile compounds without chromatographic separation. Extraction of volatile compounds was made using a gas-tight syringe, performing a headspace injection. SMart Nose system incorporates the Combi Pal autosampler CTC Analytics AG (CTC Combi Pal) with the Cycle Composer software; SMart Nose 1.52, statistical software delivered with SMart Nose system, a high-sensitivity quadrupole mass spectrometer (Inficon AG) with an ionic mass detection ranging from 1 to 200 amu and which is equipped with a specific statistical software (SMart Nose 1.51) allowing application of a multivariate analysis on data acquisition. A sample of four grams of *Bouhezza* cheese was filled into 20-mL vials (adapted for the Combi Pal autosampler) then closed with a silicone/PTFE septum and a magnetic cap. The vials with cheese samples were randomly placed in the autosampler trays to avoid any biases attributable to previous sample and/or external factors. For each sample three replicates were measured. Sample incubation temperature and time were 60 °C and 30 min, respectively. The volume of the injection volume was 2.5 mL. The syringe and the injector temperatures were set respectively at 100 °C and at 160 °C. Nitrogen was used as purge gas, with a purge flow of 200 mL min−1. Smart Nose analysis operated in EI ionization mode at 70 eV, a scan speed of 0.5 s mass−1, mass range of 10–160 amu and scanning electron microscope voltage at 1540. The total acquisition time was fixed at 170 s and three cycles for each injection were measured.

Volatile compounds (VOCs) extraction by solid phase micro extraction (SPME) method

Volatile Compounds (VOCs) were extracted from the static headspace by using solid-phase microextraction (SPME) technique. A DVB/CAR/PDMS coating (Supelco, Bellefonte, PA, USA) fiber (50/30 mm) was used to adsorb volatile compounds from headspace samples. The cheese sample was weighed (3 g) and finely dispersed in 30 ml of MilliQ water. Homogenization is done using an Ultraturrax (Heidolph DIAX 900) in 1% strength for 30 seconds.Two mL of mixture were filled into a 22 mL vial and conditioned at 37°C for 30 min into thermostatic bath in order to establish volatile equilibrium between the mixture sample and the headspace. For fiber exposition, 30 min were required to establish the volatile compounds equilibrium between samples' headspace and fiber solid phase.

The fiber was pre-conditioned before initial use by inserting it into the injector port of a gas chromatography/mass (GC/MS) instrument for 1 h at 225°C. The fiber was reconditioned at the same temperature for 5 min, before each analysis.

Gas chromatography/mass spectrometry (GC/MS) analysis

For the GC/MS analysis and the identification of the volatile compounds an Agilent 7890A Series GC system (NY, USA) coupled with an Agilent 5975C Mass Selective Detector (NY, USA) (triple axis) was utilized. In order to separate the volatiles components an HP-5 capillary column (30 m × 0.25 mm ID × 0.25 μm film thickness, Agilent Technologies, USA) was used. The chromatographic operatory conditions were as follows: Splitless injector at 220°C; oven program conditions: 35°C for 3 min, 6°C·min^{-1} to 200°C, 30°C·min^{-1} to 240°C for 3 min. Helium pressure (carrier gas) was fixed at 13.6 psi and the gas flow was 1.0 mL·min−1. The mass selective detector operated in the scan mode, 5.15 scan·s^{-1}, with 70 eV IE. The identification of the obtained peaks was carried out by comparison of mass spectra with the linear retention indices (LRI) of authentic standards (Sigma-Aldrich) calculated by running a paraffin series (from C5 to C20) under the same working conditions and with the bibliographic data from the Wiley 175 library (Wiley & Sons, Inc., Germany).

Gas chromatography/mass spectrometry/olfactometry (GC/MS/O) analysis

An HP 6890 Series GC system gas chromatograph olfactometry coupled with an HP 5973 Mass Selective Detector was used for the separations and the identification of the volatile compounds. The HP-5 capillary column (30 m x 0.25 mm ID x 0.25 μm film thickness) (Agilent Technologies, USA) was used to separate the volatile compounds. Splitless injection was performed at 220°C. The oven temperature program and the spectrometer detector calibration were set at the same conditions as reported in GC/MS analysis.

For GC/MS/O analysis, the trained human nose (sniffer) was used as a final detector simultaneously with a Mass Detector. The eluted compounds were mixed with humidified air (Acree and Barnard, 1994) and the sniffer was continuously exposed to this source for 30 min, as reported by Rapisarda et al. (2013). During the olfactometric analysis, the sniffer described the perceptions and duration of odors (Acree et al., 1976). The volatile odor active compounds recognition was performed using the single sniff method which was first developed by Marin et al. (1988). The sniffer is trained with reference chemicals to perform reproducible odor results and to avoid the influence of his/her potential psychophysical conditions (Mallia et al., 2008). Those reference chemicals consisted of a group of eight compounds used to evaluate olfactory acuity as well as to determine if a "sniffer" has specific anosmia for certain odors. The "sniffer" used in this study had no specific anosmia for these standards.

Statistical analysis of Smart nose data

Results obtained by SMart Nose were treated by a specific statistical software (SMart Nose 1.51) as cited by Rapisarda et al. (2013). Treated data among the different groups of the cheese samples allowed the selection of the most discriminant ions used for the PCA analysis. Even if PCA is not a classification method, the statistical software program of the Smart Nose gives the possibility of making a group assignment by Euclidean distances in the multidimensional space created by the PCA. For each separation pattern, a new set of parameters was chosen so as to calculate scores of the principal components.

RESULTS AND DISSCUSSION

Volatile compounds of farmhouse *Bouhezza* cheese by SMart nose and gas chromatography/mass spectrometry (GC/MS) analysis

For a first screening, farmhouse *Bouhezza* cheeses at 75 and 150 days of ripening were analyzed by SMart Nose, to detect differences in volatile compounds among the samples. A PCA analysis was performed on standardized means value and the obtained results revealed that the first two components, with an eigenvalue higher than 1 (Kaiser criterion: Massart et al., 1988), accounted for 95.3% of data variability. The first component was the most determinant and explained 84.91% of total variability, whereas the second component explained 10.39% of total variability in cheese volatiles composition. The data score plot is shown in Fig. 1, which highlighted some differences among farmhouse samples volatile fingerprints. These dissimilarities are likely due to the difference in the cheese's quality and the making process at the level of farms. The main causes of the aforementioned differences might be the traditional method of *Lben* preparation, the goatskin bag's characteristics and the type of hot red pepper spice added to the ripened cheese before consumption. Taking into account the second PC, cheeses showed a separation depending on their ripening time. *Bouhezza* cheese samples ripened to 150 days were separated from cheeses of 75 days ripening. Also, higher variability was

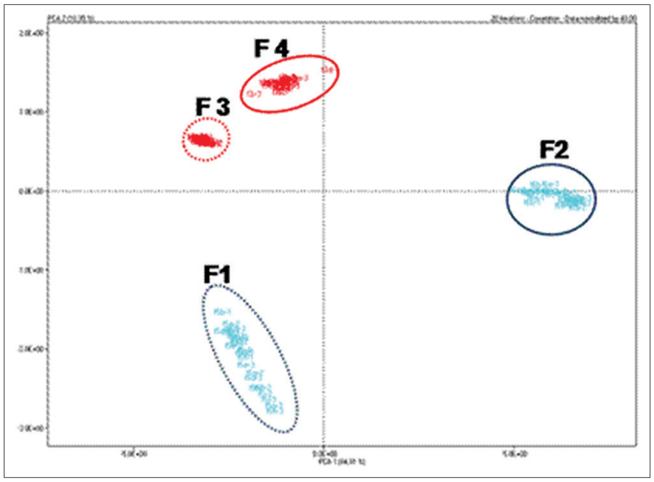

Fig 1. Traditional farmhouse Bouhezza cheese volatile fingerprint profiles by Smart Nose (75 days: F1 and F2; 150 days: F3 and F4)

noted between F1 and F2 samples. These results indicated that marginally (PC2 10.39%), ripening time influenced *Bouhezza* cheese volatile fingerprint. Therefore, PCA results showed that both the cheese-making process and the ripening time could affect *Bouhezza* cheese volatile profile.

Gas Chromatography/Mass Spectometry (GC/MS) qualitative analysis was also performed in farmhouse *Bouhezza* cheese to identify the volatile organic compounds (VOCs) and to determine the compounds responsible for the higher variability of cheeses at 75 days (F1 and F2), as shown by SMart Nose. Using GC/MS system, a total of 50 VOCs were detected and chemically identified in eight classes: Ester (28 VOCs), alcohol (7 VOCs), acid (5 VOCs), aldehyde (2 VOCs), ketone (2 VOCs), terpenes (2 VOCs), and other compounds identified as hydrocarbons (3 VOCs) and ether (1 VOC). For each VOC, an area value was considered to detect the most important differences in volatile profile among the farms. GC/MS results permit the identification of the ester class as the most important chemical class for the number of compounds.

Table 1 showed the area value of esters found in farmhouse cheeses. Within the esters' class, 1-butanol,3-methyl acetate, n-propyl acetate, 2-phenylethyl acetate, propyl propanoate and propyl butanoate were the most representative volatile compounds. Thirteen esters VOCs were detected as common compounds in all farmhouse cheeses (F1, F2, F3 and F4) and among these, 1-butanol,3-methyl acetate showed the highest area value in F1. Two esters, propyl butanoate and 2-phenylethyl propanoate were detected in 75% of samples. In comparison with Turkish Tulum cheese, wich is also ripened in a goatskin bag, nine esters detected in farmhouse *Bouhezza* cheeses, were also found in Tulum (ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, n-propyl acetate, propyl hexanoate, 2-methyl-propyl acetate and 3-methyl-butyl butanoate) (Hayaloglu et al., 2007). Furthermore, ethyl ester of butanoic and hexanoic acids smelled like apple and orange notes, respectively, were also present in ripened cheeses made of both cow's and ewe's milks (Carpino et al., 2004; Horne et al., 2005). Ester chemical class that was responsible for fruity and floral notes in cheeses (Horne et al., 2005). is generated by esterification and alcoholysis enzymatic reactions (Liu et al., 2004).

Farmhouse *Bouhezza* alcohol profile was described in Table 1. Among the alcohol class, 3-methyl, 1-butanol, phenylethyl alcohol and 2-heptanol showed the highest area value. They were also detected in Tulum cheese (Hayaloglu et al., 2007. and were described to have gassy and oily perceptions by Panseri et al. (2008). Besides, 1-octanol and phenylethyl alcohol were detected as common compounds in farmhouse *Bouhezza* cheeses, in Darfiyeh goatskin bag ripened cheese (Serhan et al., 2010) and also in cow's raw milk (Rapisarda et al., 2013). Alcohol profile in *Bouhezza* cheese might be due to the adding of raw milk at the end of the cheese manufacturing process. Raw milk has an effect on the generation of alcohols as a source of lactose and amino acids required by micro-organisms metabolism (Molimard and Spinnler, 1996).

In farmhouse *Bouhezza* cheeses, five free fatty acids were also detected by GC/MS (Table 1). The most discriminant representative acids with the highest area value were, in decreasing order, hexanoic acid, 3-methyl butanoic acid and octanoic acid. The same compounds were detected in Tulum cheese (Hayaloglu et al., 2007) and in Queso Ibores ripened cheese (Delgado et al., 2011). Previous studies on the aroma profile of cow's milk ripened Ragusano, sheep's milk ripened Piacentunu Ennese and goat's milk Spanish ripened cheeses described butanoic and hexanoic acids to have rancid/dirty sock and sweaty perception, in that order (Carpino et al., 2004; Horne et al., 2005; Poveda et al., 2008). Hexanoic and octanoic acids were found as common compounds. Carboxylic acids might be originated from different biochemical reactions, viz. lipolysis, proteolysis, lactose fermentation (Curioni and Bosset, 2002) and aldehyde oxidation by lactic acid bacteria (Smit et al., 2005). Lipolysis contributes directly to the flavor generation producing free fatty acids (McSweeney and Sousa, 2000). Free fatty acids are important contributors to the flavor properties of a wide type of cheeses (Delgado et al., 2011; Poveda et al., 2008).

Table 1: Volatile compounds (Area value x 10^6).[1] Isolated in farmhouse *Bouhezza* cheese by SPME/GC/MS analysis

Compound	RT[ab]	F1	F2	F3	F4
		75 days		150 days	
Acides					
2-Methyl, butanoic acid	7.51	0.00	0.00	1.78	0.00
3-Methyl, butanoic acid	7.30	0.00	0.00	16.82	0.00
Hexanoic acid*	10.82	4.11	17.55	4.32	2.52
Hctanoic acid *	15.89	3.78	15.08	2.08	0.73
n-Decanoic acid	20.35	0.00	0.86	0.00	0.00
Alcohols					
3-Methyl,1-butanol	4.12	64.33	0.00	0.00	55.60
1-Heptanol	10.51	0.00	0.00	0.00	3.57
2-Heptanol	8.53	8.31	0.00	0.00	19.73
1-Octanol	13.29	6.56	4.51	2.56	5.62
1-Nonanol	15.90	3.78	0.00	2.08	3.17
2-Nonanol	14.13	3.01	0.00	1.35	4.48
Phenylethyl alcohol**	14.44	18.25	8.03	43.31	9.77
Aldehyds					
Nonanal	14.19	1.85	2.83	1.83	3.59
Decanal	16.75	1.52	0.00	1.62	2.14
Esters					
Ethyl acetate	3.23	1.56	0.00	0.00	0.00
Ethyl butanoate	5.7	3.13	9.27	1.49	3.70
Ethyl hexanoate*	11.34	10.19	50.93	13.08	14.18
Ethyl heptanoate	13.99	0.00	0.00	0.00	0.75
Ethyl octanoate*	16.52	11.28	36.95	12.87	7.07
Ethyl nonanoate	18.87	0.43	0.83	0.51	0.39
Ethyl 9-decanoate	20.92	0.00	4.11	0.00	0.00
Ethyl decanoate*	21.10	9.43	25.58	7.24	3.08
Ethyl dodecanoate	25.21	1.34	5.36	1.00	0.53
Ethyl tetradecanoate	28.91	0.00	2.19	0.43	0.00
Ethyl hexadecanoate	31.73	0.00	2.09	0.00	0.00
N-propyl acetate**	3.71	1.13	29.94	97.15	14.89
Propyl propanoate**	5.95	0.00	0.00	72.64	0.00
Propyl butanoate*	8.45	0.00	80.89	6.26	19.08
Propyl hexanoate*	13.93	0.49	36.87	6.48	1.58
Propyl octanoate*	18.78	0.65	25.96	3.78	1.73
Propyl decanoate*	23.12	0.78	13.22	1.92	0.73
Isopentyl hexanoate	17.83	0.44	1.12	0.47	0.16
Hexyl acetate	11.70	0.52	0.00	0.40	0.81
2-methyl-propyl acetate	5.03	9.99	0.87	0.00	0.00
2-methyl-3-methyl-butyl propanoate	12.91	1.15	0.00	2.08	0.00
3-methyl-butyl butanoate	12.90	0.00	0.00	2.11	1.22
1-butanol, 3-methyl acetate*	7.87	220.02	62.70	21.73	20.79
1-butanol, 3-methyl propanoate	10.51	0.00	0.00	2.51	0.00
2-phenylethyl acetate*	17.89	75.15	24.95	12.91	9.67
2-phenylethyl propanoate	20.10	0.00	4.91	3.64	1.60
2-6-octadien-1-ol, 3-7-dimethyl acetate	20.71	0.00	1.03	0.00	0.00
Ketons					
2-Nonanone	13.81	0.35	0.00	1.51	0.00
2-Tridecanone	23.21	0.00	0.79	0.00	0.00
Terpens					
α-Pinene	9.40	0.87	1.33	0.00	0.00
d-Limonene*	12.14	2.60	122.05	2.12	1.72
Others					
1,4-Cyclohexadien-1-methyl-4,1-methylethyl**	12.93	0.00	0.00	0.00	10.19
1,6-Octadien-3-ol, 3,7-dimethyl **	14.06	0.00	0.00	2.40	29.34
Trans-cyclohexanone, 2-methyl-5-(1-methylethenyl.**	17.69	0.00	0.00	0.00	92.77
Cyclohexane, 1,2-methyl, 5-methyl ether **	16.56	0.00	0.00	1.20	12.47

[a]RT: Retention time, HP-5 capillary column; [b]Identification: MS (Wiley library). *Compound with the highest area value in cheeses at 75 days; **Compound with the highest area value in cheeses at 150 days

Aldehydes (2 VOCs), ketones (2 VOCs) and terpenes (2 VOCs) were presented in Table 1. Nonanal was detected as common compound, while decanal was found in 75% of the samples. Nonanal was perceived like milk note in Trachanas dairy product (Carpino et al., 2010), in milk and Questo fresco cheese (Tunick et al., 2013) and was also found in extra-hard cheeses varieties (Gobbetti, 2004) and in Parmigiano Reggiano cheese (Qian and Reineccius, 2006). Decanal was smelled as floral/nut odor in milk (Rapisarda et al., 2013). Aldehydes result from the decarboxylation of fatty acids or the deamination of amino acids (Dunn and Lindsay, 1985). The low presence of this chemical class in *Bouhezza* cheese is caused by the rapid transformation of aldehydes in alcohols or related acids, as pointed by Dunn and Lindsay (1985).

The two ketones (Table 1) were represented by 2-nonanone found in F1 and F3 samples and 2-tridecanone found only in F2 sample. 2-nonanone was detected in Cheddar, Tulum, and in goat's milk Spanish cheeses (Urbach, 1993; Hayaloglu et al., 2007; Delgado et al., 2011). The most representative terpene compounds were d-limonene with a high area value in F2 sample and detected as common compounds. Belitz and Grosch (1986) reported that terpenes in cheeses are of a plant origin and not of microbial origin. Terpenes are also transferred to milk and to cheese affecting their flavor properties (Bugaud et al., 2001). Terpenes presence in *Bouhezza* cheese might be due to animal feeding and red hot pepper used for *Bouhezza* consumption. Another interpretation of the terpene results could be in the use of juniper berry during the preparation of a goatskin bag (Aissaoui Zitoun et al., 2011). Essential oil of juniper berry is characterized by α-pinene and limonene terpenes (Mazari et al., 2010).

Table 1 showed other compounds found in farm *Bouhezza* cheeses. Among these compounds, the most representative in area value was trans-cyclohexanone,2-methyl-5-(1-methylethenyl) that was detected as only in F2 sample. 1,6-octadien-3-ol, 3,7-dimethyl was detected in F1 and F2 samples, whereas 1,4-cyclohexadien-1-methyl-4,1-methylethyl was found only in F2 sample. An ether compound, cyclohexane, 1,2-methyl,5-methyl ether was detected in F1 and F2.

Comparing SMart Nose with GC/MS results, the higher variability showed by F1 and F2 samples could be explained by the area value showed by some esters, free fatty acids and terpenes. Furthermore, the low variability showed by F3 and F4 cheeses might be due to the high value of two esters and one alcohol detected in F3, and three hydrocarbons and one ether in F4 cheese (Table 1).

Odor active compounds (OACs) of experimental *Bouhezza* cheese by GC/MS/O analysis

This part of the study was to describe the evolution in odor active compounds profile of *Bouhezza* cheeses during the manufacturing and the ripening processes as well as to give odors active compounds in ripened *Bouhezza* cheese. Two experimental manufacturing, L1 and L2 samples at 7, 14, 21, 42, 56 and 70 days, were qualitatively analyzed by SPME/GC/MS/O. Table 2 shows the OACs perceived and chemically identified in experimental *Bouhezza* cheese. Total odors active compounds during the 70 days of manufacturing-ripening changed from 26 to 18 OACs. *Bouhezza* cheese showed the richest aroma profile at the beginning of the manufacturing. Samples at 7 days of ripening showed 26 OACs whereas samples at 70 days showed a lower aroma profile characterized by 18 OACs (Fig. 2). Considering the "aromagrams" of both cheeses, sample at 7 days (Fig. 2a) showed OACs with higher value of percent duration whereas, ripened samples (Fig. 2b) showed OACs with higher value of intensity of perception. Butyric and spicy notes, with the highest percent duration, were smelled only in a ripened sample. The higher number of OACs after a week of *Bouhezza* cheese manufacturing could be due to the use of '*Lben*' and the treated goatskin bag with juniper berry. Samet-Balli et al. (2010) have noted that aroma profile of Tunisian leben (*Lben*) was characterized by only four volatile compounds: Acetaldehyde, ethanol, diacetyl and acetoin.

In ripened *Bouhezza* cheese, the GC/MS/O has shown the presence of some odorants compounds which are common in dairy products as acetic and butyric acids, methional and 1-octen-3-one. Those OACs gives different odors notes: Pungent, rancid- sour, boiled potato-like, mushroom-like notes (Zellner et al. 2008) and are also detected in Cheddar cheese flavor (Avsar and al. 2004). Sensorial analysis on *Bouhezza* cheese showed that the lactic family of odor and flavor (subfamily Lactic acid bacteria, curdled acidified) was the principal characteristic of the ripened and spiced cheese Aissaoui Zitoun et al. (2011).

In general, GC/MS/O did not show many differences in OACs during the ripening time. *Bouhezza* aroma profile was mainly characterized by esters (12 VOCs), aldehydes (9 VOCs), and ketones (5 VOCs).

Results from GC/MS and GC/MS/O analysis on farmhouse and experimental *Bouhezza* cheese, respectively, shows presence of ten volatile fingerprint compounds in both samples. The common volatile fingerprint compounds are seven esters (ethyl butanoate, propyl butanoate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, 2-phenylethyl acetate, ethyl nonanoate), one acid (octanoic acid), one

Table 2: Odor active compounds presence/absence in experimental *Bouhezza* cheese samples

Compounds	Descriptor	RI[a]	Ident[b]	7 d		14 d		21 d		42 d		56 d		70 d	
				L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	L1	L2
NI	Fried oil	1055	_	x	x		x	x							
Acid															
Acetic acid	Pungent	678	MS										x		x
Butanoic acid	Butyric	860	MS, PI, ST		x				x	x	x	x	x	x	x
Octanoic acid	Rancid	1191	MS	x	x			x	x				x		
Alcohol															
3-methyl,1-butanol	Apple	748	MS, PI	x	x	x		x	x	x	x		x	x	x
Guaiacol	Burnt	1094	PI	x	x		x					x	x	x	
Benzeneethanol	Floral, wine	1110	MS	x	x	x	x	x	x	x	x	x	x	x	x
Aldehyde															
2-hexenal	Orange	850	PI							x					
4-heptenal	Rancid	896	PI, ST	x	x		x	x		x		x	x	x	x
Methional	Potato	903	PI, ST	x	x	x	x	x	x	x	x	x	x	x	x
Octanal	Milk	1006	PI, ST	x	x		x	x							
(E)-2-nonenal	Green	1143	PI, ST	x	x	x	x	x	x	x	x	x	x	x	x
2,6-nonadienal	Cucumber	1149	PI, ST	x	x	x	x	x	x	x	x	x	x	x	x
(Z)-2-nonenal	Hay	1157	PI, ST	x	x	x	x	x	x	x	x	x	x	x	x
p-anisaldehyde	Sweet	1266	PI	x	x		x	x		x		x	x		
Hydrocarbon															
Phenyl cianide	Rancid, spicy	1245	PI	x	x	x	x					x	x	x	
Ester															
Ethyl butanoate	Apple	798	MS,PI,ST	x	x	x	x	x	x	x	x	x	x	x	x
1-butanol,3-methyl Acetate	Sweet	875	MS	x		x		x							
Propyl butanoate	Apple	899	MS						x	x					
Ethyl isohexanoate	Orange	965	PI							x	x	x	x	x	x
Ethyl hexanoate	Orange	999	MS, PI, ST	x	x	x	x	x	x	x	x	x	x	x	x
Ethyl heptanoate	Wine	1096	MS			x			x	x					
2-methyl propyl hexanoate	Orange	1128	MS	x	x	x	x	x	x	x	x	x	x	x	x
Ethyl octanoate	Wine	1194	MS, PI	x	x	x	x	x	x	x	x	x	x	x	x
Ethyl octenoate	Fried oil	1210	PI	x	x	x	x	x	x	x		x	x		x
Hexyl methylbutyrate	Sweet	1241	PI		x		x	x		x			x		
2-phenyl ethyl acetate	Floral, wine, stable	1254	MS	x	x	x	x	x	x	x	x		x	x	x
Ethyl nonanoate	Wine	1294	MS	x											
Ketone															
Diacetyl	Butter	640	PI			x									
1-octen-3-one	Mushroom	976	PI, ST	x	x	x	x	x	x	x	x	x	x	x	x
nonenone	Mushroom, leatherfaeces	1077	PI, ST	x	x	x		x	x	x	x	x	x		
3,5-octadienone	Mushroom	1090	PI			x		x							
2-nonanone	Milk	1098	PI, ST	x	x	x	x	x	x		x	x	x	x	x
Sulphur															
Methylthiazoline	Garlic	960	PI							x	x				
Methildithiofurane	Stable	1168	PI	x		x		x		x		x	x		
Terpene															
Isodihydrocarveol	Sweet	1214	PI		x										
(+)-carvone	Spicy, stable	1258	PI	x			x		x		x		x		x
Safrole	Sweet	1279	PI		x										
TOTAL OACs				26	26	21	21	24	20	24	18	20	26	18	19

[a]RI: Retention Index, HP-5 capillary column; [b]Identification: MS (Wiley library); PI (Internet Data Base: Flavornet); ST (chemical standard); NI: Not identified

alcohol (3-methyl, 1-butanol) and one ketone (2-nonanone). Esters contributed to fruity, wine and fried oil note, the acid to rancid note, the alcohol to apple note and the ketone to milk note to *Bouhezza* cheese.

CONCLUSIONS

This work provided the first characterization of the volatile and aroma profile of the traditional *Bouhezza*

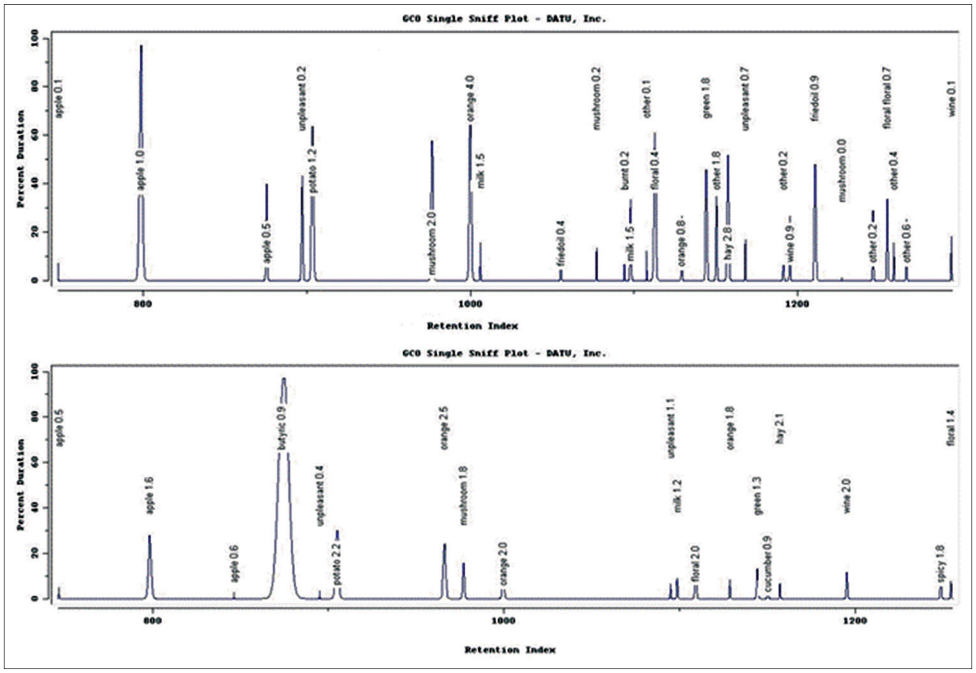

Fig 2. Comparison between "aromagrams" of experimental Bouhezza cheese at 7 days (a) and 70 days (b) of manufacturing-ripening

cheese made with cow's milk. SMart Nose results showed that farmhouse *Bouhezza* volatile profile depended on both the cheese-making process and the ripening time. Gas Chromatography/MS and GC/MS/O analysis made, correspondingly, on farmhouse and experimental samples allowed to define the main key volatile and odor compounds. Ten aromatic compounds were the principal key composites that contributed to the definition of *Bouhezza* volatile profile. The cheese aroma profile was rich and mainly characterized by esters, aldehydes and ketones. Further qualitative and quantitative analyses are required to better investigate the changes in volatile profile of *Bouhezza* cheese starting from the volatile profile of traditional fermented milk *Lben*. Aroma development in *Lben* during the first week of the manufacturing process may contribute to the aromatic precursors' generation of the cheese.

Authors' contributions

O. A. Z: She has manufactured the cheese, provided the research design, participated in the research analysis and has draft and revised the paper.; S. C.: She has participated in the research design and revised carefully the paper.; T. R.: She has participated in the research analysis and has draft and revised the paper.; G. B.: He has participated in the analysis and discussed the results.; G. L.: He has participated in the analysis design.; M. N. E. Z.: He has participated in the cheese manufacturing and provided the research design and revised the paper.

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