eman ta zabal zazu

Universidad del País Vasco
Euskal Herriko Unibertsitatea

Caracterización de defectos sensoriales en quesos de leche cruda de oveja

Ardi-esne gordineko gazten akats sentsorialen karakterizazioa

Sensory defect characterization in ewe´s raw milk cheeses

Tesis Doctoral

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Gaztaegileei

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Resumen

La Tesis Doctoral que se presenta a continuación se encuadra dentro de la línea de investigación*: caracterización de defectos en quesos madurados* del grupo de investigación multidisciplinar Lactiker, que trabaja en el campo de la calidad y seguridad de alimentos de origen animal de la Universidad del País Vasco (UPV/EHU).

El objetivo de la investigación realizada fue aportar un mayor conocimiento científico sobre los defectos sensoriales que se producen en quesos comerciales de leche cruda de oveja. Para ello se tomó como modelo una variedad comercial de queso semi-duro elaborado de forma artesanal a partir de leche cruda de oveja en pequeñas y medianas queserías del centro norte de la Península Ibérica.

En primer lugar, se caracterizó la frecuencia de aparición de defectos sensoriales en los quesos elaborados durante cinco campañas, y se estudiaron las relaciones entre los defectos, el efecto del tiempo de maduración y de la época del año de elaboración sobre la aparición de defectos. El análisis sensorial de los quesos fue realizado por el Laboratorio de Análisis Sensorial de la UPV/EHU (LASEHU) utilizando un método acreditado con un panel formado por siete evaluadores entrenados con una escala discontinua de siete puntos.

Los defectos sensoriales observados con mayor frecuencia fueron los relacionados con la presencia de ojos, donde destacan cavernas y grietas en la pasta. Estos defectos se suelen asociar al crecimiento de microorganismos en la leche o el queso o a un orígen mecánico durante su elaboración. Aunque la frecuencia de aparición de los defectos de flavor en los quesos comerciales fue menor en comparación con la de los otros defectos sensoriales, este

tipo de defectos generan gran preocupación en el sector elaborador y son considerados defectos graves en el queso. La excesiva acidez, los aromas animales (sucios o fecales), sabor excesivamente amargo y rancio fueron los defectos de flavor más frecuentes.

El tiempo de maduración influyó en la presencia de defectos que presentaron los quesos. Los quesos con menor maduración presentaron una pasta demasiado blanca, textura blanda y pastosa, y un exceso de acidez. Los de mayor maduración presentaron un exceso de cerco con un color de la pasta demasiado oscuro, flavor animal y rancio, y marcas en la corteza en comparación con los quesos de corta maduración. Por el contrario, el tiempo de maduración no influyó en la frecuencia de aparición de defectos de ojos en la pasta del queso. Los quesos se analizaron con una maduración mínima de dos meses, por lo que puede deducir que estos defectos aparecen en los quesos en los dos primeros meses de maduración.

En cuanto a la época de elaboración del queso, se observó que el abombamiento, la presencia de cavernas y ojos mal distribuidos en la pasta fueron más frecuentes en las elaboraciones de invierno y primavera que en las de verano. En invierno, en cambio, la frecuencia de aparición de quesos defectuosos, con sabor excesivamente ácido y textura blanda y pastosa, fue mayor que en los quesos elaborados en primavera y verano.

El análisis estadístico multivariante (análisis de correspondencias múltiples) realizado para conocer las relaciones entre los defectos sensoriales, además de poner de manifiesto las agrupaciones de defectos debidas al efecto del tiempo de maduración y a la época de elaboración, mostró cómo los diferentes tipos de aperturas en la pasta (cavernas, grietas, ojos numerosos, redondos y mal distribuidos) se relacionaban con diferentes defectos, sugiriendo que se originan por causas diversas. También reveló relaciones

entre defectos de diferente naturaleza. Estas relaciones podrían ser de interés para predecir defectos de flavor evaluando otros parámetros como la textura y la apariencia.

En segundo lugar, una vez identificados los defectos de flavor de mayor interés (excesivamente ácido, animal y rancio), se seleccionaron muestras de queso comerciales que presentaban estos defectos para su posterior análisis. Asimismo, se seleccionaron muestras de quesos que no presentaron ningún defecto sensorial como muestras control.

Por un lado, los compuestos volátiles de los quesos fueron extraídos mediante la técnica de evaporación del aroma asistida por disolvente (SAFE). Los extractos fueron analizados por cromatografía de gases (GC) con detección simultánea de olfatometría e ionización de llama (FID) y con detección de espectrometría de masas (MS) con objeto de identificar, describir las propiedades de olor y cuantificar los compuestos volátiles de las muestras. De esta forma, se determinaron los compuestos odorantes responsables de los principales defectos de flavor en los quesos comerciales. Por otro lado, se caracterizaron los perfiles de compuestos volátiles relacionados con la presencia de defectos de flavor en queso mediante la técnica de microextracción en fase sólida (SPME) y análisis por GC con detección de MS.

Al analizar los quesos comerciales sin defectos se observó que los compuestos volátiles más abundantes fueron los ácidos grasos libres (AGL) de cadena corta lineal como los ácidos, n-butanoico, n-hexanoico y n-octanoico. En el análisis olfatométrico, los evaluadores sensoriales describieron el olor de estos compuestos como rancio, sudor y quemado. Las cetonas fueron la segunda familia química más abundante en el perfil volátil de los quesos comerciales sin defectos, donde las metil-cetonas fueron las predominantes. Los jueces sensoriales describieron su olor como

floral, metálico y herbal. Entre los ésteres, los principales compuestos fueron el hexanoato y butanoato de etilo, descritos por los jueces con notas de olor floral y afrutado.

Una vez descrito el perfil volátil característico de los quesos comerciales sin defectos, se analizaron los quesos con defectos de flavor ácido, animal y rancio, y se compararon los resultados analíticos con los de los quesos sin defectos. El análisis de regresión por mínimos cuadrados parciales (PLSR) fue utilizado para identificar aquellos compuestos volátiles asociados con los defectos de flavor. El defecto de flavor ácido se relacionó principalmente con una concentración alta de ácido acético y de algunas cetonas como 3-hidroxi-2-butanona y 2-hidroxi-3-pentanona, compuestos originados por la glicolisis de lactosa y citrato en las primeras etapas de maduración del queso. El defecto de flavor animal se asoció con la presencia de compuestos fenólicos y alcoholes como 3-metilbutanol, 2-feniletanol y 4-metilfenol que suelen formarse como resultado de una intensa proteolisis, mientras que el defecto de flavor rancio se relacionó con un exceso de AGL de cadena corta lineal, y con la presencia de ésteres de etilo ambos originados por una actividad lipolítica intensa durante la maduración del queso.

El flavor del queso es el resultado de la interacción y el equilibrio entre los diferentes compuestos químicos que lo componen, por lo que la mera presencia de un único compuesto odorante podría no estar directamente asociada a la aparición de un defecto de flavor. Por esta razón, y con objeto de caracterizar de una manera más precisa el perfil de volátiles de los quesos comerciales con defectos, se buscaron relaciones de abundancia entre los compuestos volátiles previamente relacionados y el resto de compuestos volátiles identificados, con la presencia de defectos en los quesos. Estas relaciones fueron definidas como índice de acidez, índice de

glucólisis, índice de degradación de aminoácidos, relación de alcoholes, índice de lipólisis e índice de AGL. El índice de acidez que refleja la concentración de ácido acético y el índice de glucólisis, que refleja la abundancia de 3-hidroxi-2-butanona y 2,3-butanodiona en relación con el resto de compuestos volátiles, fueron característicos de los quesos con excesivo flavor ácido. Tanto la relación de alcoholes (proporción de todos los alcoholes con respecto al resto de compuestos volátiles de la muestra) como el índice de degradación de aminoácidos basado en la abundancia de algunos alcoholes concretos como el 3-metil-1-butanol, 2-feniletanol y 4-metilfenol, caracterizaba los quesos con flavor animal. Por último, los quesos con flavor rancio se asociaron en general con el índice AGL y con el índice lipólisis que relacionaba la abundancia de los ácidos n-butanóico and n-hexanóico con respecto a los demás volátiles.

Esta Tesis Doctoral aporta nuevos datos de interés sobre los defectos sensoriales en quesos comerciales semi-duros de leche cruda de oveja. Una de las principales contribuciones de este trabajo es poder ofrecer recomendaciones concretas a los productores de queso para mejorar la calidad sensorial final de sus productos. Por otro lado, las relaciones de abundancia de compuestos volátiles propuestas para la caracterización del flavor rancio, animal y excesivamente ácido puede facilitar la detección precoz de defectos sensoriales en queso, siendo ésta una herramienta de control de calidad de gran utilidad para la industria quesera.

Laburpena

Ondoren aurkezten den Doktorego Tesia, Lactiker ikerketa taldearen *heldutako gazten akatsen karakterizazioa* ikerketa ildoan barneratzen da. Lactiker disziplina anitzeko ikerketa taldeak, Euskal Herriko Unibertsitatean (UPV/EHU) animalia-jatorriko elikagaien kalitatea eta segurtasunaren arloan egiten du lan.

Ikerketaren helburu nagusia, ardi-esne gordinarekin egiten diren gaztetan agertzen diren akats sentsorialei buruz ezagutza zientifikoa sakontzea izan zen. Horretarako, modelu gisa Iberiar penintsula ipar-erdialdean gaztategi txiki eta ertainetan ardi-esnea erabiliz eta era artisanalean egiten den gazta erabili zen.

Lehenik eta behin, bost produkzio urteetan zehar gazten akats sentsorialen agerraldiaren maiztasuna aztertu zen. Akatsengan heltze denborak eta elaborazio garaiak zuen eragina, eta akatsen arteko erlazioak aztertu ziren ere. Gazten analisi sentsoriala Euskal Herriko Unibertsitateko (UPV/EHU) LASEHU Laborategi Sentsorialak egin zuen zazpi trebatutako ebaluatzalieen bitartez eta akreditatutako metodo sentsoriala erabiliz.

Gehien agertu ziren akats sentsorialak gaztaren barnealdean begien (zuloen) presentziarekin lotuta zeuden; leizeak, arrailak eta pitzadura txikiak zehatz meatz. Akats hauei esne edo gaztan mikroorganismoen hazkuntzara edota jatorri mekaniko bat atxikitzen zaie. Zaporearekin eta aromarekin erlazionatutako akatsen agerraldiaren maiztasuna beste akats sentsorialekin konparatuta txikiagoa izan zen arren, akats horiek kalitateari eragiten dioten kaltea haundia denez, eta gaztaegileen kezka handienetarikoa denez, akats larriak dira eta beraz, kontutan hartzerakoak. Gehien agertu ziren akatsak zapore garratza, animali tankerako usaia (zikin edota fekala), zapore mingotsa eta zaharmindua izan ziren.

Gazten heltzealdiak akatsetan zuen eragina ere aztertu zen. Heltzealdi motza zuten gaztek, kolore zuriagoa, ehundura bigunagoa eta oretsuagoa eta gehiegizko garratztasuna izan zuten. Heltzealdi luzeko gaztek ordea, colore iluna, animali tankerako usainak eta zapore zaharmindua izaten zuten. Aitzitik, heltze denborak ez zuen eraginik izan gazten begi-akatsen agerraldi maiztasunean. Aztertutako gaztek bi hilabetetako gutxieneko heltze denbora zuten, beraz, akats horiek lehenengo bi hilabeteetan agertzen direla ondorioztatu dezakegu.

Gazta landu zen garaia (negua, udaberria edota uda) kontutan hartzerakoan, neguan eta udaberrian egindako gaztek udan egindako gaztek baino maiztasun handiagoarekin konkorrak, leize erako begiak eta gaizki banandutako zulotxoak agertzen zituztela ikusi zen. Neguan, ordea, zapore garratzegia eta ehundura biguna eta oretsua zuten gazten proportzioa udaberrian edota udan egindako gaztena baino altuagoa izan zen.

Aldagai anitzeko analisi estatistikoa (korrespondentzia anitzeko azterketa) erabili zen akats sentsorialen arteko harremanak aztertu ahal izateko. Akatsen multzokatzea aztertzerakoan, heltze denborarekin eta gazta landutako garaiarekin erlazionatzen zirenez gain, gaztaren barnekaldean agertzen ziren begiak (leitzeak, pitzadura ugari eta borobilduak eta baizki banatuak) akats desberdinekin taldekatzen zirela ikusi zen. Honek, begi mota desberdinak arrazoi desberniengatik agertzen direla adierazi dezake. Analisi estatistiko honek agerian utzi zituen, halaber, izaera desberdinetako akatsen arteko harremanak. Akatsen arteko erlazio horiek itxura aztertuz, ehundura edota aroma akatsak iragartzeko erabilgarriak izan daitezke.

Bigarrenik, behin aztergai izateko zaporearekin lotutako akats interesgarrienak identifikatuta (zapore garratza, animali tankeratua eta zaharmindua) akats hauek agertzen zituzten gazta komertzialak

hautatu ziren beranduago aztertu ahal izateko. Akats sentsorialik agertzen ez zuten gaztak ere hautatu ziren kontrol gisa erabiltzeko.

Alde batetik, gazten konposatu lurrunkorrak disolbatzaileaz lagundutako usain lurrunketa (SAFE) teknikaren bitartez erausi ziren. Erauzkinak gas kromatografia (GC) erabiliz banandu ziren eta aldi berean detektore desberdinak erabiliz; olfatometria eta garraren bidezko ionizazio detektorea (FID) eta masa espektrometria (MS) detektorea aztertu ziren. Honen helburua, konposatu lurrunkorrak identifikatzea, usain ezaugarriak deskribatzea eta kuantifikatzea izan zen. Era honetan, zaporearen akatsa zuten gazta komertzialen aromaren konposatu usainkorrak zehaztu ziren. Beste alde batetik, zapore akatsduna zuten gazten konposatu lurrunkorrark fase solidoko mikroerauzketa (SPME) bidez erauzi ziren eta GC-a eta MS erabiliz gazten konposatu lurrunkorren profilak ezaugarritu ziren.

Akatsik gabeko gazta komertzialen analisia egiterakoan, konposatu lurrunkor ugarienak kate laburreko gantz azido askeak (GAA), batez ere azido n-butanoikoa, n-hexanoikoa eta n-oktanoikoa, zirela ikusi zen. Azterketa olfatometrikoa egiterakoan konposatu hauen usaia izerdia, errea eta zaharkitua bezala deskribatu zen. Zetonak izan ziren bigarren konposatu talde ugariena akatsik gabeko gazta komertzialen konposatu lurrunkorren profilan, non metil zetonak izan ziren ugarienak. Olfatometriaren epaileek familia kimiko honetako konposatuen usaina lore, belar eta metal bezala deskribatu zuten. Esteren artean, konposatu ugarienak etil butanoatoa eta hexanoatoa izan ziren lore eta fruta usainarekin.

Behin akatsik gabeko gazta komertzialen konposatu lurrunkoren profil ezaugarria deskribatuta, zapore garratza, animali tankerako usaina eta zapore zaharmindua zuten gaztak aztertu ziren, eta azterketaren emaitzak akatsik gabeko gazten emaitzekin alderatu ziren. Horretarako, karratu minimo partzialen erregresioa (PLSR)

erabili zen zaporearekin erlazionatutako akatsekin lotutako konposatu lurrunkorrak identifikatzeko. Zapore garratza azido azetikoaren kontzentrazio handieki eta zetona batzuekin, esate baterako 3-hidroxi-2-butanonarekin eta 2-hidroxi-3-pentanonarekin, erlazionatu zen nagusiki. Konposatu hauek laktosa eta zitratoaren glikolisiaren ondorioz agertzen dira gaztaren heltze lehen faseetan. Animali tankerako usaina konposatu fenolikoen eta, besteak beste 3-methylbutanol, 2-feniletanol eta 4-metilfenol bezalako alkoholen presentziarekin, proteolisi bizi baten ondorios eratutakoak, lotu zen. Azkenik, zaharmindu zaporea aktibitate lipolitiko bizi baten eraginez sortutako gehiegizko GAA eta etil esterren kontzentrazioarekin lotu zen.

Gaztaren zaporea konposatu kimiko ezberdinen elkarreraginaren eta orekaren ondorioz eratzen da. Usaindun konposatu bakar baten presentzia soilak ez da zuzenean egoten zaporearen akatsaren agerraldiarekin lotuta. Hori dela eta, aldez aurretik gazten akats sentsorialekin lotutako konposatu lurrunkorrak kontutan hartuz, gazta komertzialen konposatu lurrunkorren profila ezaugarritzen zuten konposatuen arteko ugaritasuna ratioak bilatu ziren. Ratio horiek garratztasun indizea, glikolisi indize, aminoazido degradazio indize, alkohol ratio, lipolisi indize eta GAA indize gisa definitu ziren. Garratztasun indizeak azido azetikoaren kontzentrazioa isladatzen du eta glikolisi indizeak 3-hidroxi-2-butanona eta 2,3-butanediona konposatuen ugaritasuna beste konposatu lurrunkorrekin alderatuta islatzen du. Biak zapore garratza zuten gazten ezaugarria izan ziren. Bai alkohol ratioa (alcohol guztien erlazioa gainerako konposatu lurrunkekiko) bai aminoazido degradazio indizea alkohol zehatz batzuen, hala nola 3-metil-1-butanol, 2-feniletanol eta 4 metilfenol, eta bestelako konposatu lurrunkorren ugaritasuna aldekatuz kalkulatua, animali tankerako zaporea zuten gazten ezaugarri izan ziren. Azkenik, zapore

zaharmindua zuten gaztak GAA indizearekin (laginaren GAA guztiak orokorrean hartuta) eta lipolisi ratioarekin (azido n-butanoikoaren eta n-hexanoikoaen ugaritasuna beste konposatu lurrunkorren aldean) lotu ziren.

Aurkezten den Doktoreko Tesi honek ardi-esne gordinarekin egindako gazta komertzialen akats sentsorialen inguruan datu berriak eskaintzen ditu. Ikerketaren ekarpen nagusietako bat gazten kalitate sentsoriala hobetzeko gazta ekoizleei eskeintzen diren gomendio zehatzak dira. Bestalde, proposatzen diren konposatu lurrunkorren ugaritasun ratioak gaztetan akats sentsorialen presentzia goiz detektatzea erraztu dezakete, hau kalitate kontrol tresna erabilgarria izan daiteke gazta industriarentzat.

Summary

The following Ph.D. Thesis belongs to the research line characterization of defects in ripened cheeses conducted within the Lactiker Research Group at the University of the Basque Country (UPV/EHU). Lactiker is dedicated to perform multidisciplinary research on the field of quality and safety of foods from animal origin.

The overall aim of the present research was to provide scientific knowledge about sensory defects that appear in commercial cheeses produced with ewe´s raw milk, as well as to identify the key odour compounds that may reduce the sensory quality of these type of cheeses. A commercial ewe´s raw milk semi-hard cheese variety produced in small and medium size dairies from the northern center of the Iberian peninsula was used as a model for this study.

Firstly, the appearance frequency of sensory defects in cheeses produced during five yearly cheese productions was characterized. The relationships among sensory defects, cheese ripening and season of cheese production were studied. The sensory analysis was carried out by the Sensory Analysis Laboratory of the UPV/EHU (LASEHU) using an accredited method with a trained panel of seven assessors.

Sensory defects related to the presence of eyes were the most frequent in the commercial cheeses, standing out caverns and cracks inside the cheese. These defects are usually associated with microbial growth in milk or cheese or with a mechanical origin during cheese processing. Among flavour defects, excessive acidity, animal off-flavour (dirty or faecal), bitter taste and rancid off-flavour (mainly present in cheeses with long ripening time) were the most frequent. The appearance frequency of off-flavours was smaller than the other sensory defects in the commercial cheeses, but due

to the great concern about them among the producers, they were considered highly important.

Cheese ripening time had an influence on the appearance of defects in the commercial cheeses. Cheeses with short ripening time showed too white colour, soft and melty texture, and excessive acidity. However, long ripened cheeses presented more frequently an excess of rind halo with too dark colour, rancid and animal off-flavour, and external rind marks in comparison with short ripened cheeses. In contrast, ripening time did not influence the occurrence frequency of eye-related defects. Since cheeses were analyzed with more than two months of ripening, we can deduce that these defects appear in the first two ripening months.

The season of production showed that swollen appearance, presence of caverns and badly distributed eyes were more frequently observed in cheeses produced in winter and spring than the produced in summer. On the other hand, the number of cheeses with acid off-flavour and soft and melty texture was higher in winter productions than in those manufactured in summer and spring.

A multivariate statistical analysis (Multiple Correspondence Analysis) was conducted to find out relationships between sensory defects, in addition to reveal clusters of defects due to ripening time and season of production. The results showed different types of openings/eyes (caverns, cracks, numerous, round and badly distributed eyes) grouped with different defects, thus suggesting different origins. The statistical analysis also revealed relationships between defects of different nature. These relationships may help to predict the presence of off-flavour by evaluating texture and appearance defects.

Once the most interesting off-flavours (excessively acid, animal and rancid) were identified, commercial cheese samples that showed these off-flavours were selected for later analysis. Non-defective commercial cheese samples were also selected as control samples.

On the one hand, the volatile compounds of cheeses were extracted by Solvent-Assisted Flavour Evaporation (SAFE) methodology. The volatiles were analyzed by gas chromatography (GC) with simultaneous olfactometry and Flame Ionization Detection (FID) and Mass Spectrometry Detection (MS) in order to identify, describe odour properties and quantify the volatile compounds in the samples. In this way, the odourant compounds responsible for main off-flavours of the commercial cheeses were determined. On the other hand, volatile compound profiles related to the off-flavours were characterized by solid phase microextraction (SPME) and GC analysis with MS detection.

When analyzing commercial non-defective cheeses, it was observed that the most abundant volatile compounds were the linear short chain free fatty acids (FFA) such as n-butanoic, n-hexanoic and n-octanoic acids. Olfactometric analysis carried out by sensory assessors described the odour of these compounds as sweat, burnt and rancid. Ketones were the second most abundant chemical compounds in the volatile profile of commercial non-defective cheeses, being methyl ketones the predominant ones. Sensory assessors described their odour as floral, metallic and herbal. Among esters, ethyl hexanoate and butanoate were main compounds, described by assessors with floral and fruity odour notes.

Once the characteristic volatile profile of non-defective commercial cheeses was described, cheeses with off-flavours (acid, animal and rancid flavour) were analyzed and compared with non-defective cheeses. Partial least squares regression analysis (PLSR) was used

to identify those volatile compounds associated with off-flavours. Excessive acid flavour was mainly related to a high acetic acid concentration and with some ketones such as 3-hydroxy-2-butanone and 2-hydroxy-3-pentanone. These compounds are originated by lactose and citrate glycolysis in early ripening stages. The animal off-flavour was associated with the presence of some phenolic compounds and alcohols such as 3-methylbutanol, 2-phenylethanol and 4-methylphenol, which are usually formed as a result of an intense proteolysis. Finally, rancid off-flavour was related to an excessive abundance of linear short chain free fatty acids, and to high abundance of ethyl esters, both originated from intense lipolytic activity during cheese ripening.

Cheese flavour is the result of the interaction and balance between the different chemical compounds that compose it, so the presence of a single odourant compound could not be directly associated with an off-flavour. For this reason, in order to characterize more precisely volatile profile of commercial defective cheeses, volatile compound abundance ratios between the volatiles compounds previously related with the presence of off-flavours and the other identified volatile compounds were sought. These relationships were defined as acidity index, glycolysis index, amino acid degradation index, alcohol ratio, lipolysis index and FFA index. The acidity index reproducing acetic acid concentration and glycolysis index, reflecting the abundance of 3-hydroxybutan-2-one and 2,3-butanedione in relation to the other volatile compounds were characteristic of cheeses with excessive acid flavour. Both alcohol ratio (ratio of all alcohols to all other volatile compounds in the sample) and amino acid degradation index focused on some particular alcohols such as 3-methylbutan-1-ol, 2-phenylethanol and 4-methylphenol, characterized animal off-flavoured cheeses. Finally, rancid off-flavour cheeses were associated with the FFA

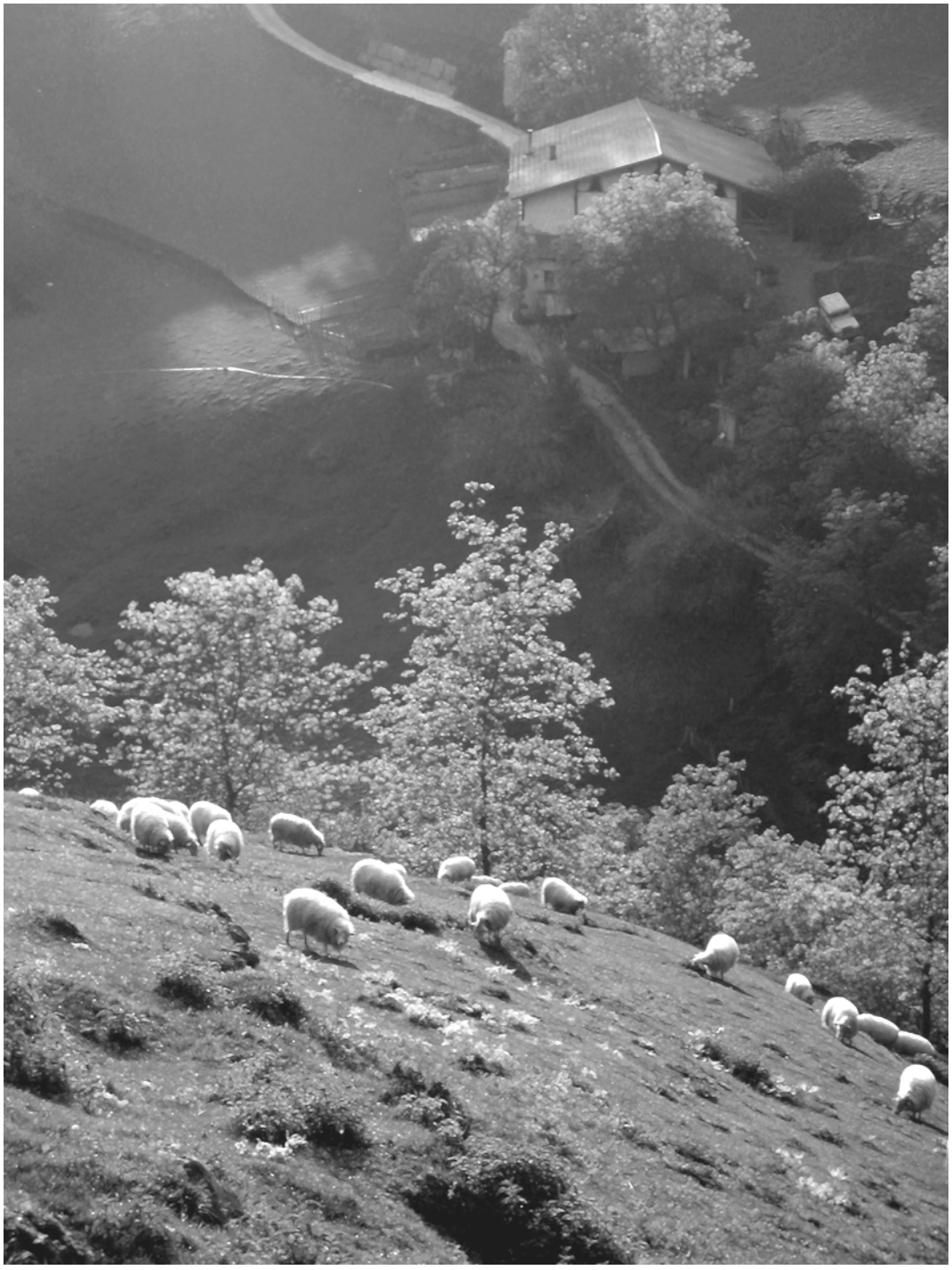
index and with the lipolysis index that was related to the abundance of n-butanoic and n-hexanoic acids with respect to the other volatile compounds.

This study brings new insights of sensory defects in semi-hard commercial ewe´s raw milk cheeses. One of the main contributions of this work is to offer specific recommendations to cheese producers to improve the final sensoryl quality of their products. In addition, abundance ratios of volatile compounds proposed for the characterization of excessively acid, animal an rancid off-flavours can allow an early detection of the presence of a sensory defects, which is a quality control tool of great utility for the cheese industry.

Abreviaturas

AGL	Free fatty acid - Ácido graso libre
ANOVA	Analysis of variance - Análisis de la varianza
DOP	Denominación de Origen Protegida – Protected Denomination of Origin
ETG	Guaranteed Traditional Speciality - Especialidad Tradicional Garantizada
FID	Flame ionization detector - Detector de ionización de llama
GC	Gas chromatography - Cromatografía de gases
IGP	Protected Geographical Indication - Indicación Geográfica Protegida
LASEHU	Sensory Laboratory of the Basque Country - Laboratorio de Análisis Sensorial de la Universidad del País Vasco UPV/EHU
LOD	Limit of detection - Límite de detección
LOQ	Limit of quantification - Límite de cuantificación
LRI	Linear retention index - Índice de retención lineal
MCA	Multiple correspondence analysis - Análisis de correspondencias múltiples
MS	Mass spectrometry - Espectrometría de masas
OZ	Odour zone - Zona de olor
PLSR	Partial least square regression - Regresión por mínimos cuadrados parciales
SAFE	Solvent assisted flavour evaporation - Evaporación de aroma asistida por disolvente
SPME	Solid phase microextraction - Micro-extracción en fase sólida
UPV/EHU	Universidad del País Vasco/Euskal Herriko Unibertsitatea

Índice



1. Thesis structure

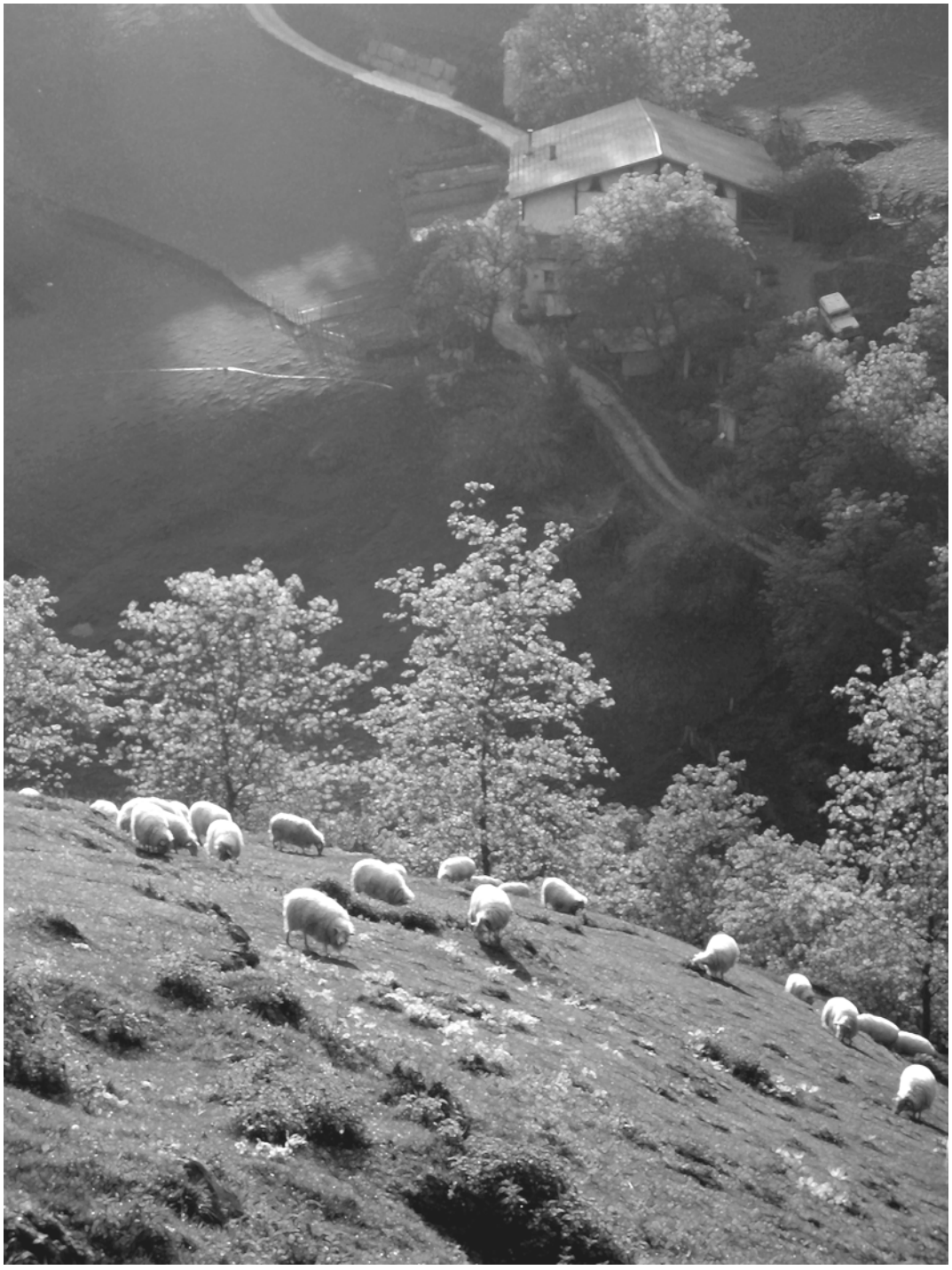
The Ph.D. Thesis presented below begins with a general introduction about artisanal ewe´s raw milk cheeses describing the main factors affecting their quality, as well as, the sensory analysis as an important tool to guarantee product quality. After that, the hypothesis and objectives of the Ph.D. Thesis are established in the section of materials and methods were techniques, equipments and methods used for the analysis of commercial cheeses are described in detail. The results are presented and discussed in depth throughout three scientific publications that constitute the three chapters in which the section of results and discussion is divided:

Chapter I: *Occurrence of sensory defects in semi-hard ewe´s raw milk cheeses*. Laura Zabaleta, Marta Albisu, Mónica Ojeda, Pilar Fernández Gil, Iñaki Etaio, Francisco José Perez-Elortondo, Mertxe de Renobales and Luis Javier R. Barron. Dairy Science and Technology (2016) 96:53–65.

Chapter II: *Identification of odour-active compounds in ewes´ raw milk commercial cheeses with sensory defects*. Laura Zabaleta, Karine Gourrat, Luis Javier R. Barron, Marta Albisu and Elisabeth Guichard. International Dairy Journal (2016) 58:23-30.

Chapter III: *Volatile compounds associated with sensory attributes and off-flavour generation in ewe´s raw milk commercial cheeses*. Laura Zabaleta, Marta Albisu and Luis Javier R. Barron. European Food Research and Technology (2017). DOI 10.1007/s00217-017-2851-0.

The most relevant results of the Ph.D. Thesis are presented in the general discussion section, and lastly, the conclusions of the work and the bibliography used are indicated.



2. Introducción

2.1. El queso de leche cruda de oveja: producción artesanal, relación con el territorio y calidad diferenciada

El origen del queso no se conoce con exactitud pero se estima que surgió hace aproximadamente 10.000 años, como forma de conservar la leche, en la misma época en la que se domestica la oveja. Se cree que los viajeros provenientes de Asia introdujeron el arte de la elaboración del queso en los países de la cuenca mediterránea, en los que hoy en día existen una amplia gama de quesos. Posteriormente, durante el Imperio Romano, la producción de queso se expandió por el resto de Europa (International Dairy Foods Association, 2017; Ramírez, 2005).

Los tipos de quesos pueden clasificarse atendiendo a diferentes criterios como son el animal del que procede la leche con la que se elabora (vaca, cabra, oveja, búfala, camella, otros animales, o mezcla de la leche procedente de ellos); el pre-procesado de la leche (cruda o pasteurizada); el método de coagulación (por acción enzimática del cuajo, por acidificación microbiana o combinada); la textura (pasta blanda, semidura o dura); el tiempo de maduración (frescos o con diferentes tiempos de curación); el contenido de grasa sobre extracto seco (extragraso, semigraso o magro), y el tipo de microorganismos empleados en su elaboración (quesos azules, de moho blanco, con desarrollo bacteriano en la corteza, o madurados por adición de cultivos bacterianos lácticos) (Lay, Kolpin, Sommer, & Rankin, 2007). Debido a la gran variabilidad en la materia prima y en los métodos de elaboración y maduración, los quesos pueden presentar grandes diferencias en su composición, dando lugar a un gran número de tipos y variedades (Walstra, Geurts, Noomen, Jellema, & vanBoekel, 2001).

En el caso de los quesos artesanales, esta diversidad se ve especialmente aumentada al incluir las fuertes variaciones estacionales en la producción de leche, los diferentes tipos de alimentación del ganado, y el factor humano en la elaboración del queso (Caridi, Micari, Caparra, Cufari, & Sarullo, 2003; Nájera et al., 2009) que, en muchos casos, supone una riqueza de aromas y tipos de texturas que deben ser conservadas pero que, en otros casos, desembocan en alteraciones y defectos sensoriales del producto final (Bárcenas, Pérez Elortondo, Salmerón, & Albisu, 1998; Bárcenas, Pérez-Elortondo, Salmerón, & Albisu, 2001; Issanchou, Schlich, & Lesschaeve, 1997).

La estacionalidad es una fuente importante de variación en el caso de los elaboradores de queso de pequeñas explotaciones donde se practica el pastoreo y la producción de leche es estacional (Barron et al., 2001; Farruggia et al., 2014). En esos casos, los efectos de los cambios ocasionados por el tipo de alimentación (concentrados y forraje en invierno y pastoreo a tiempo parcial en primavera/verano), y los debidos a la época de lactación de los animales están asociados y son difíciles de diferenciar (Kindstedt, 2005). Así mismo, el proceso de maduración es un factor esencial determinante de la calidad final y posterior venta (Boyazoglu & Morand-Fehr, 2001) ya que influye de forma decisiva en el desarrollo de las propiedades sensoriales de los quesos. Los cambios debidos a la pérdida de humedad y el desarrollo de actividades enzimáticas como la glucólisis, lipólisis y proteolisis determinarán la textura, sabor y aroma del queso final (Virto et al., 2003). Como resultado de estas fuentes de variabilidad en la elaboración del queso se pueden esperar importantes cambios en las características sensoriales de los quesos, y también en las frecuencias de aparición de defectos sensoriales.

La mayor parte de la producción de leche de oveja se destina a la elaboración de queso (Boyazoglu & Morand-Fehr, 2001) y más del 60 % del queso de leche de oveja del mundo se elabora en países mediterráneos (Figura 1) (FAO, 2017) donde se producen variedades de queso que, en muchos casos, son el elemento económico vertebrador de las zonas rurales, formando parte del sistema socio-cultural (Boyazoglu & Morand-Fehr, 2001; Caridi et al., 2003).

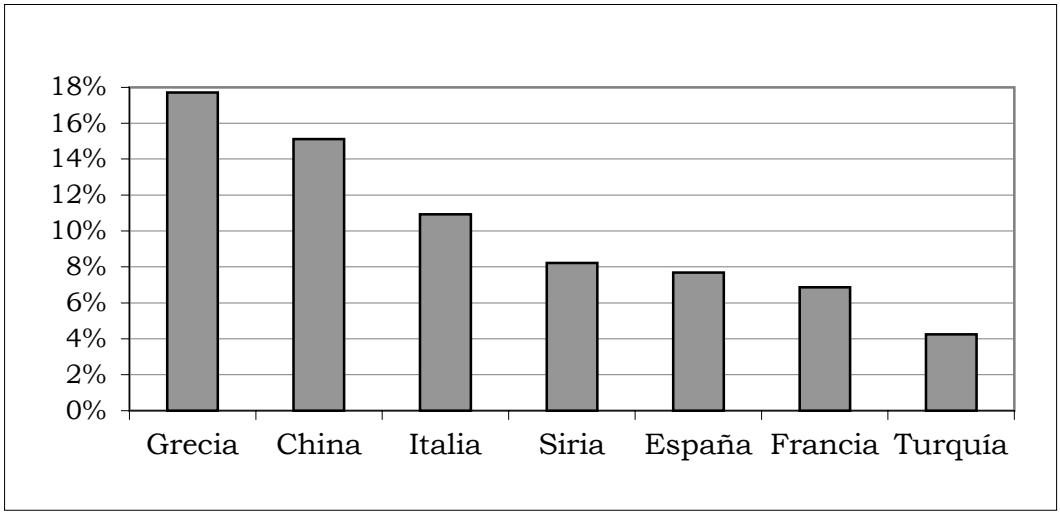


Figura 1: Porcentaje medio de la producción de queso de leche de oveja entre entre 2000 y 2014 respecto a la producción mundial de queso (FAO, 2015).

La producción de quesos artesanos da lugar a alimentos diferenciados cuyas características y tipicidad están estrechamente asociadas con su singularidad histórica y cultural, lo cual es patente a través de su cadena de producción, marketing y consumo. Son productos estrechamente unidos al territorio, parte del patrimonio cultural y gastronómico, en muchos casos protegidos por etiquetas de calidad, que ayudan a la recuperación de razas autóctonas, al mantenimiento de prácticas respetuosas con el medio ambiente y protegen formas de elaboración específicas (Batalla, Pinto, Marijuan, & del Hierro, 2013; Beaufoy & Poux, 2014; Boyazoglu & Morand-Fehr, 2001). Los quesos con etiquetas

de calidad diferenciada están protegidos por una normativa de la Unión Europea que garantiza el cumplimiento de unos requisitos superiores a los exigidos para el resto de los quesos similares, y garantizan que los quesos tengan las características previamente definidas para ellos (Barron, Aldai, Virto, & de Renobales, 2017; MAPAMA, 2017). En la actualidad existen en la Unión Europea 244 etiquetas de calidad diferentes. Ejemplos de estas etiquetas de calidad son las Indicaciones Geográficas Protegidas (IGP), las Denominaciones de Origen Protegidas (DOP) y las Especialidades Tradicionales Garantizadas (ETG). Las IGP protegen los quesos elaborados tradicionalmente en una determinada región, las DOP protegen no sólo que todo el proceso de producción se lleve a cabo en una región concreta, sino que la forma de elaboración e incluso los animales de los que se obtiene la leche pertenezcan a una o varias razas autóctonas concretas. Las ETG, en cambio, protegen alimentos que se distinguen de otros productos similares por utilizar en su elaboración materias primas tradicionales, o por su característica forma de elaboración. Las entidades de control de estas etiquetas de calidad utilizan sistemas de trazabilidad muy exhaustivos y llevan a cabo controles analíticos tanto físico-químicos como sensoriales para garantizar al consumidor que se respete el origen y forma de elaboración de los quesos, y que éstos cumplan con las características físico-químicas y organolépticas exigidas (Barron et al., 2017; MAPAMA, 2017).

Según los últimos datos de la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), se prevé un aumento del 17 % de producción de queso mundial en los próximos diez años relacionado con un mayor consumo de este producto en los países asiáticos. No ocurre lo mismo con el precio del queso, el cual ha sufrido un descenso del 18 % en el año 2015, tendencia que se prevé que continúe a la baja en los próximos años (FAO, 2017).

Esta tendencia descendente ha venido marcada en Europa principalmente por la eliminación de la cuota láctea en marzo del 2015, lo que ha provocado una fuerte crisis en el sector productivo y transformador (European Commission, 2015). Esta alta competitividad del sector lácteo junto con la migración de las nuevas generaciones del entorno rural a las ciudades hacen que la producción de variedades de queso artesanales corra un grave riesgo de desaparecer (Caridi et al., 2003).

Por otra parte, para poder competir con la creciente producción industrial del queso es primordial que los productores artesanales elaboren quesos típicos de alta calidad sensorial y claramente diferenciados de otras variedades de queso. Esto juega un papel muy positivo de cara a su elección por el consumidor (Bárcenas et al., 2001; Boyazoglu & Morand-Fehr, 2001). Debido a que la calidad sensorial del queso está fuertemente determinada por su apariencia, textura y sobre todo por su flavor (Boyazoglu & Morand-Fehr, 2001; Fox & Wallace, 1997), la elaboración de productos que tengan unas características sensoriales tipificadas, y que a su vez no presenten defectos sensoriales, es una de las principales preocupaciones de los productores de queso artesano (Ayad, Awad, El Attar, de Jong, & El-Soda, 2004; Barron et al., 2005). Estas circunstancias ponen de manifiesto la importancia de que el sector de los quesos artesanales cuente con un buen asesoramiento técnico y científico de los procesos de elaboración con objeto de contribuir a la mejora del producto final.

2.2. Control de la calidad del queso: el análisis sensorial

Para garantizar que los quesos cumplen con su definición sensorial, en particular en aquellos quesos protegidos con etiquetas de

calidad, se lleva a cabo el control de calidad mediante análisis sensorial. La evaluación sensorial está constituida por una serie de técnicas cuyo objetivo es obtener medidas precisas de las respuestas humanas a las sensaciones que producen los alimentos minimizando la potencial imparcialidad debida al individuo (Lawless & Heymann, 2010).

El ser humano analiza lo que está a su alrededor, y también los alimentos, a través de sus sentidos. Los sentidos del gusto y el olfato, además de ayudar al consumidor a seleccionar alimentos por la mayor o menor presencia de azúcar, grasa u otros nutrientes, en ocasiones pueden indicar que la ingesta de un alimento podría resultar nociva. El amargor, por ejemplo, es una señal que puede advertir sobre la posible presencia de algunas toxinas, y la sensación de rancidez de la probable contaminación o degradación de un alimento (Clark, Costello, Drake, & Bodyfet, 2009; Delahunty & Drake, 2007). La vista, el olfato y el tacto (para percibir la textura) trabajan conjuntamente con el sentido del gusto para ampliar esta información. La Asociación Española de Normalización y Certificación (AEN, 2016) define el análisis sensorial como "la metodología que permite realizar el examen de las propiedades organolépticas, mediante los sentidos, de los productos de consumo, tales como los alimentos, bebidas, tabaco, productos cosméticos y productos farmacéuticos, incluyendo el empleo de técnicas adecuadas para objetivar los resultados con ayuda de métodos estadísticos apropiados". El proceso normalizado de evaluar la calidad de un producto a través de los sentidos fue el precursor del análisis sensorial (Clark et al., 2009; Lawless & Heymann, 2010). Los primeros evaluadores sensoriales de alimentos comenzaron a tomar importancia a principios del siglo pasado, con la producción industrial de alimentos y bebidas, debido a que empezó a cobrar cada vez más importancia el que la

calidad de cada alimento individual producido representara la calidad de toda la producción. Pronto se comenzó a utilizar esta técnica para la evaluación de la calidad de productos lácteos y se organizaron los primeros concursos de evaluación de estos alimentos. El análisis sensorial no comenzó a utilizarse de forma generalizada en la industria alimentaria hasta los años 70 del siglo pasado, cuando se puso de manifiesto que este tipo de análisis no sólo proporcionaba información sobre la calidad final del producto, sino que también se podía obtener información útil sobre las preferencias de los consumidores, y que podía ser utilizado para ajustar parámetros de elaboración o tomar decisiones durante el proceso. El análisis sensorial se fue definiendo progresivamente, siendo cada vez menos subjetivo, haciendo hincapié en el entrenamiento de los evaluadores y apoyándose en técnicas estadísticas para el diseño de los análisis y el tratamiento de los datos. Hoy en día el análisis sensorial es una herramienta muy utilizada tanto en control de calidad en la industria alimentaria y en investigación, como en marketing (Clark et al., 2009).

Tal y como anteriormente se ha comentado, la presencia de defectos en los quesos puede ocasionar pérdidas económicas importantes para el sector productivo. La alta calidad sensorial del queso artesano es, sin duda, la mejor herramienta de venta del productor artesanal, por lo que reducir la presencia de defectos sensoriales en los quesos debe ser una prioridad. Por lo tanto, es primordial conocer en profundidad los defectos en el queso, y las causas que los producen, para poder orientar a los productores sobre las medidas correctoras que deben tomar durante el proceso de elaboración del queso con objeto de disminuir la presencia de defectos.

2.3. Presencia de defectos en los quesos

Debido a la diversidad y complejidad de la tecnología quesera, los fabricantes, y en particular los artesanos, están expuestos a factores no controlados, contaminaciones o accidentes durante el proceso de elaboración del queso que pueden generar defectos en el producto final. Las características microbiológicas y fisicoquímicas iniciales de la leche cruda tales como la carga y el tipo de microbiota, pH, potencial de óxido-reducción, contenido en caseína y grasa, dimensión de las micelas, polimorfismo genético y contenido en calcio, así como los tratamientos tecnológicos previos de la leche, tienen gran influencia sobre las diferentes fases de la elaboración del queso y, por tanto, sobre su calidad final (Mahaut, Jeanet, & Brulé, 2003). Cambios en las características de las materias primas y/o un procesamiento incorrecto, en particular en los quesos de leche cruda, pueden dar lugar tanto a la pérdida de atributos organolépticos deseables como a la presencia de defectos (Pirisi et al., 2007).

El origen de los defectos sensoriales en el queso puede ser muy diverso, pudiendo estar relacionados tanto con las características de la leche como con las distintas etapas de fabricación (Fatma, Mona, El-Gawad, & Enab, 2013).

Los defectos pueden estar:

- Ligados a sustancias y/o microorganismos que son originados en la leche y que a su vez son transferidas al queso (Feligini et al., 2014; Gómez-Torres, Garde, Peirotén, & Ávila, 2015).
- Vinculados al cuajo utilizado (Pirisi et al., 2007).
- Ligados a las operaciones de corte de la cuajada y desuerado (Kindstedt, 2005; McSweeney, 2007).
- Originados en las etapas de moldeo, prensado y salado de los quesos (Ortiz, 2005).

- Asociados a la acción de determinados microorganismos durante la elaboración y/o maduración (Broadbent et al., 2002; Engel, Nicklaus, Septier, Salles, & Le Quéré, 2001).
- Relacionados con las condiciones de humedad y temperatura en las que se haya producido la maduración y conservación del queso (Kindstedt, 2005).

El defecto más estudiado en la literatura científica en quesos duros y semiduros es el hinchamiento tardío. Este defecto está principalmente causado por la contaminación de la leche y posterior crecimiento de bacterias del género *Clostridium,* que producen gran cantidad de gas hidrógeno y dióxido de carbono, y de ácidos grasos volátiles como butírico y propiónico (Gómez-Torres et al., 2015). Como consecuencia, los quesos con este defecto, además de presentar una apariencia hinchada, tienen defectos en el interior de la pasta con presencia de ojos anormales (grandes cavernas y grietas en la pasta, así como en la corteza en casos severos), y defectos de flavor (rancidez) (Feligini et al., 2014; Garde, Ávila, Gaya, Arias, & Nuñez, 2012; Gómez-Torres et al., 2015; Le Bourhis et al., 2007). Feligini et al. (2014) analizaron muestras de leche y cuajada utilizadas para la elaboración de queso Grana Padano y observaron la presencia de diferentes especies de clostridios (*C. beijerinckii, C. tertium, C. butyricum* y *C. sporogenes*) en la mayoría de las muestras. Estos autores sugirieron que el defecto de hinchamiento tardío ocurría por la acción sinérgica de diferentes especies de clostridios que se reproducen por esporas y contaminan la leche. Por este motivo, la calidad del ensilado, la higiene del animal y del entorno, así como unas prácticas higiénicas adecuadas durante el ordeño, son factores clave para evitar el defecto de hinchamiento tardío del queso madurado. Garde et al. (2012) analizaron la influencia de la presencia de esporas del genero *Clostridium* en leche y queso Manchego en el hinchamiento

tardío. Establecieron una relación entre la presencia de este microorganismo con el abombamiento, las grietas y ojos anormales, y con una concentración mayor a la normal de ácido butírico. También en queso Manchego se estudió el efecto de la adición de esporas de *C. tyrobutyricum, C. beijerinckii*, *C. sporogenes* y *C. butyricum* en la leche de oveja, sobre las características del hinchamiento del queso, presencia de ojos irregulares, grietas y cavernas, y olor desagradable y rancio (Gómez-Torres et al., 2015). Los quesos contaminados con *C. tyrobutyricum* fueron los primeros en presentar el defecto, así como los que mayor hinchamiento, grietas y olor a butírico presentaron tras dos meses de maduración. La contaminación de *C. tyrobutyricum* se asoció con altos niveles de ácido propiónico, butírico y pentanoico y bajos de ácido acético. Otros autores (Le Bourhis et al., 2007) adicionaron esporas de *C beijerinckii*, *C. tyrobutyricum*, *C. sporogenes*, y mezclas de éstas en diferentes proporciones, a leche de vaca en la elaboración de quesos Emmental. Todos los quesos contaminados con esporas de clostridios presentaron el defecto de hinchamiento tardío pero la forma y tamaño de los ojos, y la cantidad de ácido butírico y propiónico, fue diferente en función de la especie de clostridios con la que se contaminó la leche. Estos autores concluyeron que la presencia de *C. tyrobutyricum* era prerrequisito para la aparición del defecto de hinchamiento tardío, y que la presencia de otros clostridios tenía un efecto potenciador en el desarrollo del defecto.

Dado que la apariencia del queso es un parámetro importante que determina su aceptación por parte del consumidor, un color de la pasta inadecuado (por ser demasiado oscuro o claro, o por presentar coloraciones irregulares) es un defecto que preocupa al sector quesero. McSweeney (2007) indicó que un corte inadecuado de la cuajada, dejando gránulos de cuajada de tamaño no homogéneo, puede dar lugar a una coloración irregular del queso.

También señaló que una excesiva humedad de la pasta del queso, ocasionada por un insuficiente prensado, puede dar lugar a pastas demasiado blancas junto a otros defectos como una textura blanda y un flavor excesivamente ácido.

También los defectos de flavor como el amargor y la presencia de olores fecales y animales han sido ampliamente estudiados en la literatura científica, ya que el flavor del queso juega un papel primordial en su calidad (Eknæs, Havrevoll, Volden, & Hovea, 2009; Smit, Smit, & Engels, 2005). La presencia de flavores anormales en el queso limita la aceptación del consumidor y, como consecuencia puede producir pérdidas económicas importantes para el sector (Broadbent et al., 2002; Engel et al., 2001). Eknaes et al. (2009) estudiaron cómo la suplementación del pienso de las cabras con lípidos de diferente origen producía la presencia de defectos de rancidez y amargor en la leche que se trasladaban también al queso. El amargor ha sido objeto de estudio por parte de otros autores (Engel et al., 2001) que investigaron su presencia en queso Camembert utilizando diferentes cepas de *Penicillium camemberti*. El defecto de amargor era producido por una excesiva proteólisis y por un desequilibro del sabor amargo con respecto a otros sabores como el ácido y el salado. En queso Cheddar se encontró que el amargor era producido por la acción de *Lactococcus lactis* durante la maduración del queso (Broadbent et al., 2002). Se ha descrito que los compuestos responsables del amargor en los quesos pueden ser aminoácidos y péptidos de pequeño tamaño producidos por una intensa actividad de enzimas proteolíticos, principalmente provenientes de microorganismos (Smit et al., 2005). En queso Montasio, asociaron la presencia de ácidos grasos libres de corta cadena como el ácido n-hexanoico al flavor animal, también denominado fecal (Innocente, Munari, & Biasutti, 2013). Otros autores (Ayad et al., 2004) asociaron el flavor animal y la presencia

de ojos en la pasta con la acción de coliformes durante la maduración del queso Ras, elaborado con leche cruda de vaca o mezcla de vaca y búfala. Se ha descrito que algunos alcoholes, como el metanotiol, son compuestos odorantes clave en la formación de flavor animal (Smit et al., 2005).

El flavor del queso está determinado por la percepción sensorial de una combinación de una gran variedad de compuestos químicos, tanto volátiles como no volátiles (Innocente et al., 2013; Le Quéré, 2004; Mulder, 1952). La influencia de los microorganismos, y de los compuestos químicos presentes en la leche de origen, en el perfil volátil final del queso es particularmente importante en quesos de leche cruda. El uso de leche cruda confiere al queso un flavor típico y diferenciado de los quesos elaborados a partir de leche pasteurizada aunque puede conllevar un mayor riesgo desde el punto de vista higiénico-sanitario (Ayad et al., 2004; Fatma et al., 2013). La pasteurización de la leche influye sobre la composición volátil del queso debido a que el tratamiento térmico inactiva los enzimas endógenos y reduce la presencia de algunos compuestos volátiles y microorganismos propios de la leche. Por ello, el flavor tiende a ser más intenso y específico en quesos de leche cruda que en los de leche pasteurizada (Fatma et al., 2013). Los compuestos responsables del flavor se forman principalmente durante la maduración del queso como resultado del metabolismo microbiano de la lactosa, lactato y citrato, la formación de AGL, la degradación parcial de la red de caseína en péptidos de pequeño tamaño y aminoácidos libres, y de las subsecuentes reacciones entre los compuestos formados (Fox & Wallace, 1997; Thomsen, Gourrat, Thomas-Danguin, & Guichard, 2014). Aunque un gran número de compuestos volátiles han sido identificados en el queso, sólo un reducido número de ellos han sido asociados con su flavor específico, o identificados como compuestos impacto aromático en

la definición del flavor del queso (Curioni & Bosset, 2002). En muchos casos, los compuestos volátiles más abundantes pueden no ser odorantes, o tener muy poco impacto en el olor, mientras que otros compuestos presentes en concentraciones muy bajas contribuyen en mayor medida al aroma de queso. Por lo tanto, es muy importante identificar cuáles de estos compuestos son compuestos impacto (odorantes clave), ya que determinarán el olor característico del queso (Smit et al., 2005). La búsqueda de los compuestos volátiles responsables del aroma de los quesos es un reto complejo porque el flavor característico suele ser debido a un equilibrio entre diversos compuestos. En este sentido, el estudio de los compuestos responsables de defectos de flavor es de gran interés debido a que ciertos flavores indeseables suelen estar frecuentemente relacionados con un pequeño número de compuestos que tienen un origen específico (Fox, McSweeney, Cogan, & Guinee, 2004; Thomsen et al., 2012).

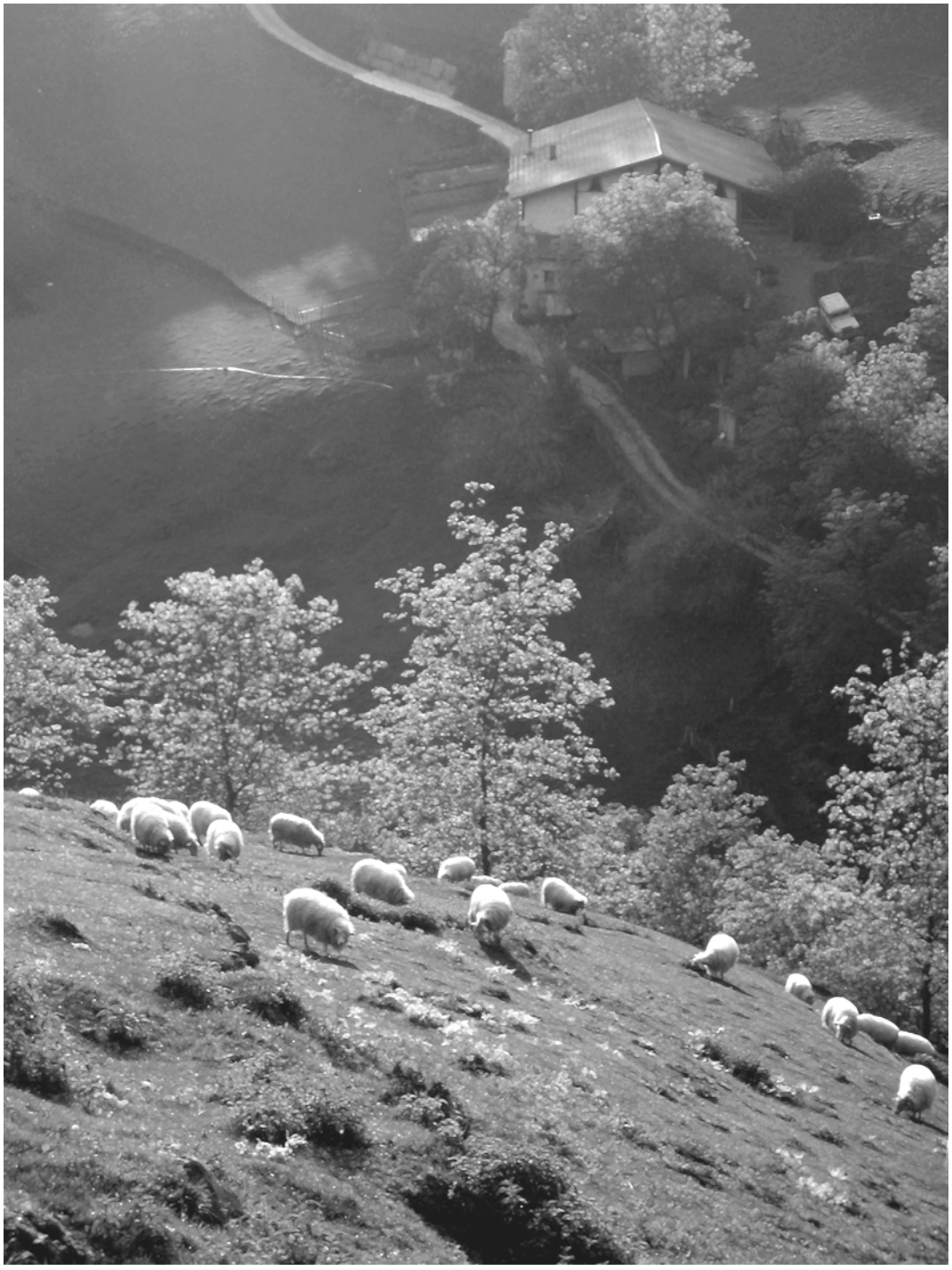
Desde un punto de vista analítico, en general, la detección de sustancias odorantes en los alimentos es todavía hoy en día una tarea difícil debido a que la sensibilidad de los instrumentos de análisis sigue siendo inferior a la de la percepción de la nariz humana (Chamber & Koppel, 2013). No obstante, la técnica de GC con detector de olfatometría ha contribuído en gran medida a la identificación de compuestos odorantes. Esta metodología incorpora la nariz humana como detector de compuestos volátiles separados en una columna cromatográfica, permitiendo identificar zonas de olor (OZ) y descriptores olfativos durante el análisis cromatográfico. La técnica de detección olfatométrica se utiliza en combinación con otros detectores, en la mayoría de los casos de ionización de llama (FID) o de espectrometría de masas (MS), con objeto de identificar y caracterizar compuestos odorantes en la muestra analizada. Esta técnica ha sido ampliamente utilizada para evaluar el aroma

característico en quesos de leche de vaca como el Cheddar (Avsar et al., 2004; Cadwallader, Drake, Carunchia-Whetstine, & Singh, 2006; Frank, Owen, & Patterson, 2004; O'Riordan & Delahunty, 2003), Arzúa-Ulloa (Rodríguez-Alonso, Centeno, & Garabal, 2009), Gouda (Thomsen et al., 2014), quesos de leche de oveja (Bergamini, Wolf, Perotti, & Zalazar, 2010) y queso azul (Frank et al., 2004). Chamber y Koppel (2013) realizaron una revisión sobre la relación entre los resultados de medidas instrumentales y sensoriales de los compuestos químicos para predecir el flavor de alimentos. Estos autores señalaron que la técnica de olfatometría es especialmente eficaz para encontrar compuestos responsables de malos olores en quesos (Chamber & Koppel, 2013). Sin embargo, debe tenerse en cuenta que la técnica de olfatometría analiza y describe el olor de compuestos volátiles de forma individual, y que las posibles interacciones entre compuestos que puedan provocar alteraciones del olor no pueden ser detectadas. En consecuencia, los resultados obtenidos a partir de la olfatometría deben ser complementados con el análisis sensorial del queso (Chamber & Koppel, 2013; Clark et al., 2009; Thomsen et al., 2012).

En la literatura científica apenas se han encontrado estudios que investigan relaciones entre diferentes defectos sensoriales en quesos, ya que en la mayor parte de los estudios los defectos se estudiaron de forma independiente (Engel et al., 2001; Feligini et al., 2014; Garde et al., 2012). Por otra parte, en general, los estudios sobre defectos se han llevado a cabo con quesos experimentales elaborados principalmente a partir de leche de vaca, y en los que los defectos eran inducidos a escala de laboratorio (Gómez-Torres et al., 2015; Le Bourhis et al., 2007).

En relación con la interpretación de los resultados, el estudio del flavor de forma univariante es a menudo insuficiente y no informa sobre los efectos combinados entre compuestos odorantes. El uso

de métodos estadísticos multivariantes como el análisis de correspondencias múltiples (MCA) y la regresión de mínimos cuadrados parciales (PLRS), permiten al investigador descubrir relaciones entre compuestos volátiles, variables procedentes del análisis sensorial, y otras variables de diferente naturaleza (Greenacre, 2008; Ordoñez, Ibañez, Torre, Barcina, & Pérez-Elortondo, 1998; Sourial et al., 2010).



3. Hypothesis and Objectives

This Ph.D. Thesis has been conducted within Lactiker Research Group at the University of the Basque Country (UPV/EHU), which is dedicated to perform multidisciplinary research on the field of Quality and Safety of Foods from Animal Origin. Specifically, this Thesis is part of the research line focused on *Cheese Quality and Safety - Characterization of Defects.*

The research is based on the hypothesis that the identification of cheese´s main sensory defects and the characterization of the volatile profile of defective cheeses can help in elucidating their causes and, thus, could contribute to the improvement of the quality and profitability of commercial cheeses.

The overall aim of the present Ph.D. Thesis is to contribute to the scientific knowledge related to sensory defects commonly present in commercial cheeses produced with ewe´s raw milk, as well as to identify the key odour compounds that may reduce the sensory quality of these types of cheeses.

In order to achieve the overall aim, the following specific objectives were set:

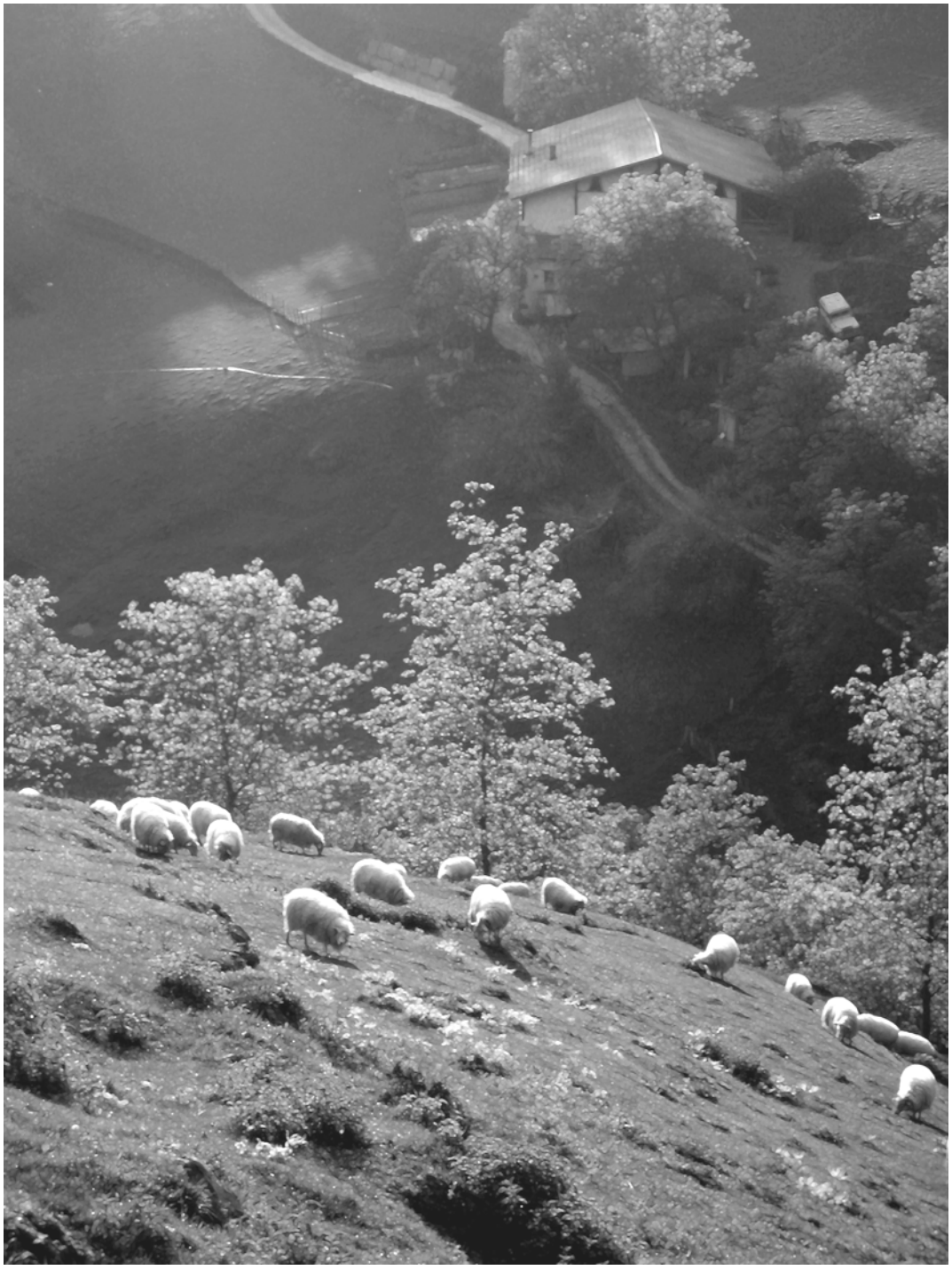
1. To assess the occurrence of sensory defects in commercial cheeses from different yearly cheese productions.

2. To investigate the effect of ripening time and production season on the occurrence of sensory defects in commercial cheeses.

3. To establish relationships among different sensory defects found in commercial cheeses.

4. To characterize key odour compounds responsible for the main off-flavours in ripened cheeses made with ewe´s raw milk.

5. To identify characteristic volatile compound profiles associated to different off-flavours.



4. Materiales y Métodos

4.1. Quesos comerciales

Para la investigación realizada en la presente Tesis Doctoral, se tomó como modelo una variedad comercial de queso semiduro elaborado de forma artesanal a partir de leche cruda de oveja en pequeñas y medianas queserías del norte de la península Ibérica.

Debido a una cláusula de confidencialidad con los elaboradores y fuentes de datos, no se indica la variedad de queso comercial sobre la que se llevó a cabo este estudio, ni se citarán los documentos asociados a sus protocolos de elaboración.

El proceso de elaboración del queso objeto de estudio, similar al de otros quesos de oveja elaborados en la península Ibérica fue el siguiente:

- Leche cruda de oveja acidificada por adición de cultivos iniciadores comerciales.
- Calentamiento a 28-32 ºC.
- Coagulación de la leche por adición de cuajo comercial o pasta de cuajo de cordero artesanal, o por la mezcla de ambos.
- Corte de la cuajada hasta que los gránulos de cuajada alcanzan un tamaño aproximado de 5 milímetros de diámetro.
- Recalentamiento de los gránulos hasta alcanzar aproximadamente 35-37 ºC.
- Desuerado por acción de pre-prensado en la propia cuba.
- Moldeado de la pasta pre-prensada en moldes cilíndricos.
- Prensado de la pasta en los moldes durante 6-10 horas en prensas neumáticas.
- Inmersión de los quesos en salmuera.
- Maduración de los quesos en cámaras entre 8-12 ºC por un período mínimo de 2 meses.

Una vez elaborado el queso, éste debe presentar las siguientes características básicas para su comercialización:

- Porcentaje mínimo de proteína y grasa sobre extracto seco de 25 % y 45 %, respectivamente.
- Características sensoriales:
  - Forma cilíndrica, proporcionada, con las caras planas y talones ligeramente convexos.
  - Corteza desarrollada de color amarillo pálido o gris blanquecino y sin marcas de bandeja, pero con señales de paño.
  - Color interno homogéneo de marfil a amarillo pajizo, con cerco estrecho y ligeramente oscuro.
  - Presencia de ojos no numerosos, repartidos al azar, de forma irregular y pequeños.
  - Ojos escasos, repartidos al azar, irregulares y pequeños.
  - Olor intenso, penetrante, ligeramente ácido, a leche evolucionada de oveja y ausencia de olores extraños.
  - Textura ligeramente elástica, firme y granulosa.
  - Flavor a leche de oveja madurada con notas de cuajo y torrefacto, ligeramente ácido, salado y picante. No debe presentar amargor.
  - Persistencia con las mismas características del flavor.

Figura 2. Imagen de la corteza y pasta del tipo de queso comercial utilizado en el estudio. Autor: L. Zabaleta.

4.2. Muestreo de quesos

Las muestras de queso comerciales de leche cruda de oveja fueron recogidas al azar de 126 pequeñas queserías para su control de calidad sensorial rutinario.

En el *Chapter I* del apartado *Results and discussion* de la Tesis Doctoral se analizan los datos del control de calidad sensorial de 1437 quesos procedentes de queserías artesanales durante cinco años consecutivos (2006-2010). En los *Chapter II y III* se analizan los datos del control de calidad sensorial de 752 quesos procedentes de queserías artesanales de las campañas 2011 y 2013 (ambas incluidas). Del total de quesos analizados en estas tres campañas se muestrearon en diversas queserías artesanales, un total de 34 quesos que presentaron al menos un defecto sensorial de interés. Estas muestras de queso fueron posteriormente sometidas a análisis físico-químicos generales y de compuestos volátiles. Además de estos quesos con defectos, se muestrearon en las queserías artesanales un total de 7 quesos comerciales que no presentaron ningún defecto sensorial, los cuales fueron considerados quesos control. Todos los quesos muestreados fueron troceados en cuñas de aproximadamente 250 gramos, envasados al

vacío en bolsas de plástico, y se mantuvieron congelados a –35 ºC hasta su posterior análisis.

4.3. Datos técnicos de elaboración

Con el fin de recabar información sobre las queserías y sobre los diferentes parámetros utilizados para la elaboración de los quesos, se diseñó una encuesta para los elaboradores (Anexo I). Se visitaron 43 queserías artesanales en las que se realizaron dichas encuestas y en las que se observó la elaboración del queso y se entrevistó a los elaboradores para conocer la opinión del sector sobre la problemática de los defectos sensoriales. Los resultados de la información técnica recabada en las encuestas sirvieron para agrupar las muestras de queso según diferentes parámetros como fueron el tiempo de maduración y la época del año en la que se elaboraron.

Los quesos se clasificaron en tres grupos: quesos de maduración corta (de 60 a 90 días) con un total de 1079 muestras, quesos de maduración media (de 91 a 119 días) con 260 muestras, y quesos de larga maduración (de 119 días en adelante) con 98 muestras. El número de muestras en el grupo de quesos de maduración corta fue muy superior al del grupo de maduración media, y éste a su vez superior al de larga maduración. Estas diferencias en el número de muestras recogidas por cada grupo de tiempo de maduración fueron debidas a que los productores de queso disponían en sus cámaras de maduración de un mayor número de quesos de maduración corta y a que todos los quesos debían ser sometidos al control de calidad sensorial antes de ser comercializados debiendo tener una maduración mínima de 60 días. Por este motivo, la mayor parte de los quesos muestreados tenían entre 60 y 90 días de maduración.

Al ser el queso un producto estacional se consideró la influencia de la época de elaboración en la aparición de defectos sensoriales ya que las campañas anuales de elaboración de queso se llevan a cabo entre los meses de diciembre y agosto. La composición de la leche varía a lo largo del año, por un lado, debido al estado de lactación de los animales, y por otro, a las diferencias en el manejo del rebaño. Em el caso de los quesos comerciales objeto de estudio, este manejo cambia durante la época de producción de leche; en invierno los animales se encuentran estabulados y son alimentados con forrajes y concentrados, mientras que en primavera los animales se encuentran en un manejo de pastoreo a tiempo parcial, el cual va incrementando las horas de pastoreo hacia la época de verano. Las muestras se clasificaron en tres grupos de según la fecha de fabricación de queso: invierno (entre el 1 de diciembre y el 31 de marzo) con un total de 800 muestras, primavera (entre el 1 de abril y el 20 de junio) con 539 muestras, y verano (entre el 21 de Junio y el 31 de agosto) con 98 muestras. El tamaño de muestra de cada grupo fue diferente porque la mayor producción de queso coincide con el máximo del pico de lactación que se produce durante el mes de marzo (Lana, Lasarte, & Lazkanotegui, 2011).

4.4. Análisis sensorial

Las muestras de queso fueron analizadas en el Laboratorio de Análisis Sensorial de la UPV/EHU (LASEHU) por un panel entrenado. El método de control de la calidad sensorial fue descrito previamente por Pérez-Elortondo et al. (2007) y Ojeda et al. (2015), y está incluido en el alcance de la acreditación de LASEHU según la norma ISO 17025 (2005). En cada sesión de análisis sensorial fueron analizadas un máximo de ocho muestras distintas de queso por un panel de siete evaluadores expertos, ubicados en cabinas acondicionadas. Se analizaron ocho parámetros en cada muestra:

forma, corteza, color de la pasta, ojos, olor, textura, sabor/aroma y persistencia. La intensidad de cada parámetro fue evaluada mediante una escala discontinua de siete puntos (siendo uno la puntuación mínima y siete la máxima). En el caso de que un juez otorgara para un parámetro una puntuación inferior a cuatro, éste debía señalar la presencia de al menos un defecto sensorial relacionado con dicho parámetro. Se consideró la presencia de un defecto específico en la muestra de queso cuando al menos cinco de los siete evaluadores la señalaron. El método de control de la calidad sensorial incluye además un catálogo que describe 44 posibles defectos específicos para esta variedad de queso. Para la presente Tesis Doctoral se tomaron en cuenta aquellos defectos que presentaron una frecuencia media mínima de aparición de 2,5 % en los quesos comerciales analizados en los años estudiados.

4.5. Análisis generales

El extracto seco de las muestras de queso se determinó según método normalizado ISO 5534/IDF 4:2004 (IDF, 2004). El contenido total de proteína se midió mediante el método Kjeldahl tal como se describe en la norma ISO 8968-1/IDF 20-1:2014 (IDF, 2014). El contenido de grasa se determinó por extracción sólido-líquido con n-pentano (98 % PRS, Panreac, Barcelona, España) mediante método Soxhlet. La acidez valorable, expresada en milimoles de hidróxido de sodio por 100 g, se analizó de acuerdo a la norma ISO 11869/IDF 150:2012 (IDF, 2012). El pH se midió con un electrodo de penetración (Crison micropH 2000, Barcelona, España) en una mezcla de 10 g de queso rallado con 10 ml de agua MilliQ.

Todos los análisis de composición general de las muestras de queso se realizaron por duplicado.

4.6. Extracción de compuestos volátiles

Se utilizaron dos técnicas diferentes para la extracción de compuestos volátiles de las muestras de queso que fueron previamente descongeladas durante 24 h en refrigeración, y posteriormente mantenidas durante 1 h a temperatura ambiente, en dos experimentos diferentes. En el primer experimento (experimento 1), que se describe en el *Chapter II* de la sección Results and discussion, los compuestos volátiles fueron extraídos mediante la técnica de evaporación de aroma asistida por disolvente (SAFE), y en el segundo experimento (experimento II), descrito en el *Chapter III,* los compuestos volátiles fueron extraídos mediante micro-extracción en fase sólida (SPME).

4.6.1. Método SAFE

Los compuestos volátiles del queso se extrajeron mediante el método descrito por Thomsen (2014). Después de retirar 1 cm de corteza de la muestra, se trituraron 20 ± 0,01 g de queso y se introdujeron en un matraz redondo de vidrio junto con 100 mL de agua MilliQ. El matraz se colocó en el equipo SAFE y se mantuvo a 37 ºC con agitación contínua de la muestra y en condiciones de vacío durante todo el proceso de extracción (2 h). Los compuestos volátiles arrastrados junto con el vapor de agua eran rápidamente condensados en un matraz de vidrio sumergido en un baño de nitrógeno líquido.

Los compuestos volátiles presentes en el destilado acuoso del matraz de recogida se extrajeron por decantación con 40 mL de una solución de 125 ng/mL de n-eicosano (patrón interno; > 99 % de pureza, Sigma-Aldrich, Saint-Quentin Fallavier, Francia) en diclorometano (99,9 % de pureza, Carlo Erba, Val de Reuil, Francia). Esta solución se introdujo en una columna de concentración de volátiles Kuderna Danish en baño termostático a

70 ºC durante 2-3 minutos. El volumen final extraído se ajustó a un volumen total de 400 µL en un vial ámbar (4 mL) con diclorometano, y se conservó a –20 ºC para su posterior análisis.

4.6.2. Método SPME

Los compuestos volátiles del queso se extrajeron de acuerdo al método descrito por Amores et al. (2013). Después de retirar 1 cm de corteza de la muestra, se trituraron 15 g de queso junto con 20 g de sulfato de sodio anhidro (99,5 %, Panreac, Barcelona, España) en un molino de muestra durante 1 min. A continuación se añadieron 200 µL de una solución acuosa (0,5 g/L) de ciclohexanona (patrón interno, 99,8 % de pureza, Sigma-Aldrich, Alcobendas, España), volviéndose a homogeneizar en el molino durante 30 segundos. Se pesaron en una balanza analítica 1 ± 0,005 g de la mezcla resultante y se depositaron en un vial ámbar (4 mL), el cual se cerró herméticamente con un tapón con septum de PTFE/silicona (Supelco, Bellefonte, EEUU).

El vial que contenía la muestra fue introducido en un baño de agua termostático a 45 ºC durante 15 minutos como tiempo de pre-equilibrio de los compuestos volátiles en el espacio en cabeza del vial. A continuación, se introdujo en el vial una fibra DVB/Carboxen/PDMS (50/30 µm, Supelco) de 1 cm de longitud utilizando un soporte manual (Supelco). La extracción y atrapamiento de los compuestos volátiles en la fibra se llevó a cabo a una temperatura de 45 ºC durante 30 min.

4.7. Análisis de compuestos volátiles

Los compuestos volátiles extraídos se analizaron por GC utilizando dos métodos analíticos diferentes. En el experimento I, los volátiles se detectaron por MS y mediante olfatometría combinada con

detección FID, mientras que en el experimento II se utilizó únicamente detección por MS.

4.7.1. Experimento I

El análisis de los compuestos volátiles extraídos mediante el método SAFE se realizó mediante inyección de 2 µL de extracto en modo splitless en un equipo de GC Agilent 6890A (Santa Clara, EEUU) acoplado a un detector MS Agilent 5973. Los compuestos volátiles se separaron en una columna capilar de sílice fundida DBWAX (30 m de longitud; 0,32 mm de diámetro interno; 0,5 µm de espesor de película; Agilent J & W, Santa Clara, EEUU) utilizando helio de alta pureza como gas portador a un flujo de 1,5 mL/min y el siguiente gradiente de temperatura del horno cromatográfico: 40 ºC de temperatura inicial y rampa de calentamiento hasta 240 ºC a una velocidad de 4 ºC/min, e isoterma final a 240 ºC durante 10 min. La línea de transferencia entre el equipo de GC y el detector de MS se mantuvo a 230-250 ºC. El detector de MS trabajó en modo de impacto electrónico (70 eV) y en un rango de masas de 29 a 350 m/z.

La identificación de compuestos volátiles se realizó de forma tentativa por comparación de los índices de retención lineal (LRI) y espectros de masas experimentales con la información correspondiente a cuatro bases de datos comerciales: NIST 2.0 (National Institute of Standards and Technology, Nueva York, EEUU), biblioteca Wiley 138 (Wiley & Sons Inc., Nueva York, EEUU), INRAMass (INRA, Dijon, Francia), y Volatile Compounds in Foods 15.2 (Zeist, Países Bajos), y de forma positiva por comparación de los LRI y espectros de masas de sustancias puras analizadas. Para la comparación de espectros se consideró un factor de coincidencia superior a 800. Los límites de detección (LOD) y cuantificación (LOQ) se calcularon como dos y cuatro veces,

respectivamente, el promedio del ruido de fondo (expresado en unidades arbitrarias de área). Se utilizaron diez blancos experimentales mediante análisis de diclorometano para calcular el valor promedio del ruido de fondo en tres zonas diferentes del cromatograma. La cuantificación de los compuestos volátiles en las muestras de queso se expresó como abundancia relativa frente al patrón interno (expresado en unidades arbitrarias de área) a partir de la expresión:

[1] Abundancia relativa = (área del pico/área patrón interno) x 100

Los extractos obtenidos por el método SAFE fueron asimismo analizados por GC utilizando un equipo similar al descrito anteriormente con detección combinada de FID y olfatometría trabajando con una relación de división de flujo 1:1 para los dos puertos de detección. A la salida del detector de olfatometría se colocó el molde de vidrio que mejor se ajustaba a la nariz de cada evaluador con objeto de que los compuestos volátiles separados por el GC llegaran perfectamente a la nariz con la mínima dispersión en el ambiente.

En la detección olfatométrica participaron 8 evaluadores sensoriales, cada uno de los cuales analizó cada extracto de volátiles de queso por duplicado (16 evaluaciones por muestra). Durante los 40 minutos de duración del análisis olfatométrico los evaluadores sensoriales señalaron con un pulsador la presencia de OZs, a la vez que asignaban un descriptor al olor percibido utilizando su propio vocabulario. Tanto los tiempos de retención cromatográfico correspondientes a las OZs como la descripción de los olores se registraron utilizando el software AcquiSniff® (UR QuaPa / T2A; Saint-Genès-Champanelle, Francia). Sólo se tuvieron en cuenta aquellas OZs que fueron detectadas en al menos 5 de las 16 evaluaciones de cada muestra de queso.

4.7.2. Experimento II

Los compuestos extraídos por SPME se analizaron mediante un equipo de GC 7820A acoplado a un detector de MS 5975 (Agilent, Santa Clara, EEUU) equipado con una columna de sílice SUPELCOWAX (Supelco) (60 m de longitud; 0,25 mm de diámetro interno; 0,25 μm de espesor de fase) utilizando el siguiente programa de temperatura del horno cromatográfico: 40 ºC de temperatura inicial y rampa de calentamiento hasta 110 ºC a una velocidad de 5 ºC/min y, a continuación, una rampa de calentamiento hasta 240 ºC a una velocidad de 10 ºC/min. Helio de alta pureza fue utilizado como gas portador con un flujo de 1 mL/min. La línea de transferencia entre el equipo de GC y el detector de MS se mantuvo a 200 ºC. El detector de MS trabajó en modo de impacto electrónico (70 eV) y en un rango de masas de 33 a 250 m/z.

Los compuestos volátiles fueron identificados de forma tentativa por comparación de sus espectros de masas (factor de coincidencia >800) con los de las librerías comerciales de espectros NIST 8.0 y WileyILEY 4275. La identificación positiva de los compuestos volátiles de las muestras de queso se realizó por comparación de sus LRI y espectros de masas con los de sustancias puras comerciales de alta pureza. El LOD y el LOQ se calcularon de forma análoga al experimento I. Las abundancias relativas de los compuestos volátiles en el espacio de cabeza de las muestras de queso se calcularon de acuerdo a la expresión [1] utilizando en este caso como patrón interno ciclohexanona.

4.8. Análisis estadístico

Los análisis estadísticos se realizaron utilizando el programa estadístico XLSTAT (versión 2011.2, Addinsoft, Francia).

En el *Chapter I* de la sección *Results and discussion*, para la interpretación de la matriz de datos dicotómicos sobre presencia o ausencia de defectos sensoriales en las muestras de queso, se calculó en primer lugar la frecuencia de aparición de los defectos sensoriales en relación con el número total de muestras de queso que presentaron al menos un defecto sensorial. Asimismo, se utilizó el test de Chi cuadrado de Pearson para comparar la frecuencia media de aparición de defectos en los grupos de quesos clasificados en función de su maduración (corta, media o larga) y época de elaboración (invierno, primavera o verano).

Se aplicó un análisis MCA para conocer las relaciones entre los defectos sensoriales presentes en las muestras de quesos. En el modelo de MCA se incluyeron únicamente aquellas variables que presentaron en la solución factorial pesos mayores, en valor absoluto, a 0,5.

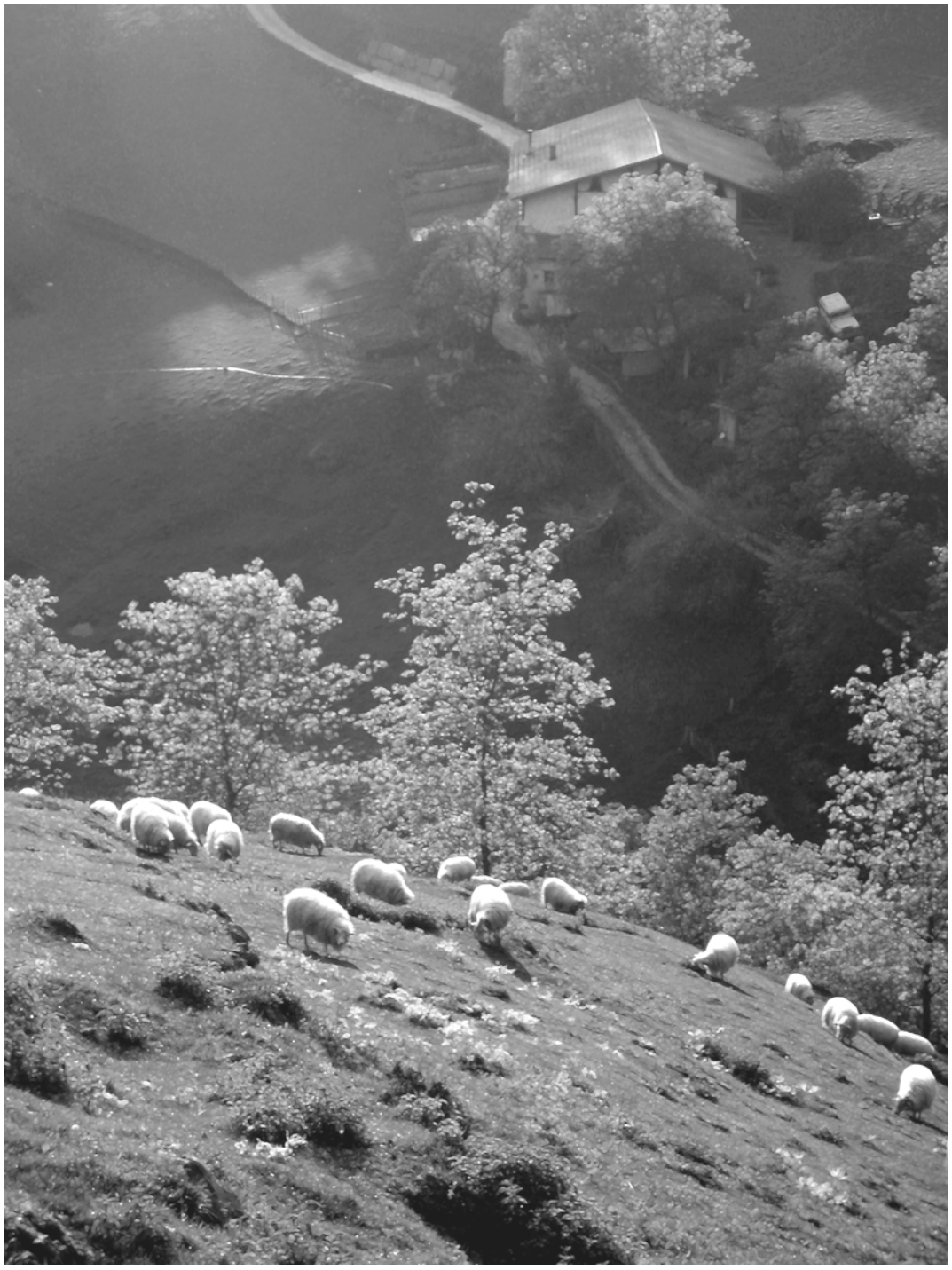
En el *Chapter II* se aplicó el análisis PLSR para estudiar las relaciones entre las abundancias relativas de los compuestos odorantes y los defectos sensoriales presentes en las muestras de queso. Se incluyeron en el modelo lineal aquellas variables que presentaron valores de importancia en la proyección mayores a uno, así como puntuaciones en el modelo mayores, en valor absoluto, a 0,5 en los dos primeros factores. De esta manera se identificaron aquellos compuestos odorantes que presentaban mayor relación con los defectos de flavor.

Se utilizó el modelo lineal general de análisis de la varianza (ANOVA) para examinar las diferencias en la abundancia relativa de las diferentes familias químicas de volátiles y los compuestos odorantes clave entre las muestras de queso con diferentes tipos de defectos, incluyendo también muestras de queso sin defectos. El

test de Tukey fue utilizado para las comparaciones múltiples post-hoc.

En el *Chapter III* se aplicó el análisis PLSR a la abundancia relativa de todos los compuestos volátiles identificados en las muestras de queso que presentaron defectos de flavor para seleccionar compuestos de interés relacionados con la aparición de los defectos ácido, animal y rancio. Para la selección de los compuestos volátiles a incluir en el modelo se utilizaron los mismos criterios aplicados en el análisis PLSR del *Chapter II*. Además, este análisis se aplicó para diferenciar las muestras de queso con defectos de flavor utilizando relaciones de abundancia entre compuestos volátiles. Asimismo, se aplicó el ANOVA y el test de Tukey de comparaciones múltiples post-hoc para comparar las relaciones de abundancia de compuestos volátiles entre quesos con defectos y los quesos sin defectos.

El nivel crítico de significación en todos los análisis estadísticos fue de $\alpha = 0,05$.



5. Results and Discussion

5.1. Chapter I: Occurence of sensory defects in semi-hard ewe´s raw milk cheeses

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ORIGINAL PAPER

Occurrence of sensory defects in semi-hard ewe's raw milk cheeses

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Abstract Sensory quality is one of the most important attributes of cheeses, as consumers demand homogeneous and unaltered products. The presence of sensory defects in the final product causes financial losses and consequently has a great economic impact on cheese makers. Therefore, a study was conducted to find out main defects that affect sensory quality: eyes, paste, rind, flavour, texture and shape of commercial semi-hard ewe's raw milk cheeses. The frequency of occurrence of relevant sensory defects in cheeses with different ripening times and those manufactured in different seasons was also investigated. Samples were collected along 5 years during the annual sensory quality control from different cheese makers. Although flavour is a major determinant of consumer acceptance, most common defects were those related to the internal appearance, especially cracks, which were more frequent in summer cheeses, and caverns, which were more frequent in spring and winter cheeses. White paste, soft texture and acid flavour defects were more frequent in cheeses with short-ripening period. Medium- and long-ripened cheeses presented a higher percentage of an excess of rind halo with a darker paste colour, animal flavour and marks in the rind. Due to compositional milk changes throughout cheesemaking seasons, cheese makers should adapt their manufacturing practises to those changes and try to achieve uniform cheeses. The results found in this study may be useful for the quality improvement of semi-hard ewe's raw milk cheeses.

Keywords Sensory quality · Defect · Ewe's raw milk cheese · Seasonality · Ripening

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1 Introduction

Around 60% of global sheep milk cheese is manufactured in Europe, mainly in Mediterranean countries such as Greece, Italy and Spain (FAO 2014). Spanish sheep milk cheese production increased by 28% between 2002 and 2012 (FAO 2014). Ewes are mainly milked for the manufacture of typical dairy products, in particular cheeses, which often have a regional or local connotation of origin and quality (Boyazoglu and Morand-Fehr 2001). Local cheese production is an important economic activity, especially in Mediterranean countries (Caridi et al. 2003). In some cases, these cheese varieties have a strong brand image that connects the product to characteristic sensory attributes, to a particular animal management conditions and to traditional cheesemaking methods. That plays a positive role in the choice of the product by consumers (Boyazoglu and Morand-Fehr 2001). Sensory quality is one of the most important attributes of a food product, determined by its flavour, texture and appearance. The relative importance of these three quality attributes is interrelated but unfortunately these relationships have not been studied in depth. Initial microbiological and physicochemical characteristics of raw milk, as well as technological treatments, affect the different phases of the production and hence the final quality of the product (Mahaut et al. 2003). Any change in any of these milk characteristics and/or an incorrect processing, particularly in raw milk cheeses, may result in a lack of some desirable organoleptic attributes or in the presence of an undesirable defect (Pirisi et al. 2007). The presence of defects in the final product causes financial losses and consequently has a great economic impact on the dairy industry (Engel et al. 2001).

The most studied defect is late blowing, as it is one of the major causes of spoilage in semi-hard and hard cheeses. It is predominantly caused by the growth of *Clostridium tyrobutyricum* that produces both texture and flavour defects (Feligini et al. 2014; Garde et al. 2012; Le Bourhis et al. 2007). Amongst cheese flavour characteristics, bitterness and unclean flavour (animal) have been particularly studied because of the consequences of economic loss, as these defects limit consumer acceptance (Engel et al. 2001). Studies reported in the literature concerning defects were mainly conducted with non-commercial or experimental cow's milk cheeses with induced defects at laboratory scale. Defects were studied separately and independently rather than investigating interactive effects that could arise amongst them.

Ripening is a major factor in determining the quality of small ruminant dairy products (Boyazoglu and Morand-Fehr 2001). It influences development of sensory characteristics of cheeses due to its effect on the chemical composition such as moisture loss, enzymatic activities including degradation of residual lactose, the lipolysis of fat increasing the total amount of free fatty acids and the proteolysis of casein leading to the production of amino acids and flavour precursors (Virto et al. 2003).

In the same way, seasonality is a major source of variation especially for farmhouse cheese makers that practise pasture feeding and seasonal milk production (Barron et al. 2001; Farruggia et al. 2014). In those cases, the effects of both changes in feed sources and farm practises are interactive and cannot be separated (Kindstedt 2005). As a result of those sources of variability, a change in the frequencies of occurrence of sensory defects or differences in cheese's defect profile could be expected.

The aim of this study was to describe the main sensory defects that appear in semi-hard sheep raw milk cheeses, as well as interactions or relationships that may arise

amongst them. The frequency of occurrence of relevant sensory defects in cheeses with different ripening times and those manufactured in different seasons was also investigated.

2 Materials and methods

2.1 Sample collection

Samples were collected during the annual sensory quality control of a semi-hard sheep's raw milk cheese variety over five consecutive years (2006–2010). Commercial cheeses were manufactured in 126 different dairies in a similar manner. Milk was acidified by starter cultures and coagulated by the addition of rennet (commercial or traditional lamb rennet paste) at 25–35 °C. The resulting curd was cut into rice-sized grains and heated to 35–37 °C. Then, the curd was introduced into cylindrical 1–2 kg moulds, pressed and salted with saturated sodium chloride brine at 10–12 °C for 16–24 h. Cheeses were ripened at 8–10 °C for at least 2 months. Cheese samples with at least one sensory defect were collected (1437 cheese samples).

In order to determine the possible influence of the ripening time on the presence of defects, cheeses were classified into three groups: short-ripened cheeses (from 60 to 90 days) with 1079 samples, medium-ripened cheeses (from 91 to 119 days) with 260 samples and long-ripened cheeses (from 119 days onwards) with 98 samples. Cheeses had to pass the sensory quality control before being sold. Since minimum cheese ripening was 2 months, the number of short-ripened cheese samples submitted for the quality control was larger than medium- and long-ripened cheeses.

Cheeses were manufactured from December to August. Milk composition changes in this period because lactation spans from winter (early lactation) to summer (late lactation), and animal management and feeding are different in winter (animals are fed indoors with forages and concentrate), spring (part-time grazing) and summer (grazing and extensive management). In order to determine if there were differences in the presence of defects amongst cheeses made in different seasons, samples were classified into three different groups according to manufacturing date: winter cheeses (December 1st to March 31st) with 800 samples, 539 samples belonged to spring cheeses (April 1st to June 20th) and 98 samples manufactured in summer (June 21st to August 31st). Sample size of each group was different because cheese production decreased from the beginning (winter) to the end (summer) of lactation period.

2.2 Sensory analysis

Cheese samples were analysed in the Sensory Laboratory of the University of the Basque Country (LASEHU) by a trained sensory panel. Sensory quality control method was previously described (Pérez-Elortondo et al. 2007), and it was included in the accreditation scope of the LASEHU according to ISO 17025 (2005). A maximum of eight samples were analysed in each session by seven trained expert assessors. Six

parameters were analysed: shape, rind, paste, eyes, texture and flavour, on a seven-point category scale (1 being the minimum and 7 the maximum score). The method included a catalogue describing 44 possible specific defects for this cheese variety. The presence of a specific defect in the cheese sample was recorded when at least five of the seven assessors marked it. Defects with a minimum mean frequency of occurrence of 2.5% were selected for this study. Descriptions of these defects and photographs of some of them are shown in Table 1 and Fig. 1, respectively. A database was created with the results of the sensory analyses.

2.3 Statistical analysis

The percentage of occurrence of each sensory quality parameter and defect in relation to the total number of defective cheese samples (samples with at least one defect) was calculated. Relative frequency of occurrence of defects in the cheese groups classified according to ripening time and season were compared using the Pearson's chi-square test ($P \leq 0.05$). Multiple correspondence analysis (MCA) was applied to explore relationships between cheese defects. Those variables that showed weight values higher than $|0.5|$ in the factorial solution were included in the MCA. XLSTAT software (version 2011.2, Addinsoft, France) was used for the statistical analysis of the data.

Table 1 Sensory description of the selected defects

Parameter	Specific defect	Description
Eyes	Caverns	Irregular large holes in paste
	Cracks	Lineal openings in paste
	Numerous	Too many holes in paste
	Round	Round holes in paste
	Badly distributed	Irregular distribution of holes in paste
Paste colour	Excess of rind halo	Too dark or wide paste border
	Irregular	Irregular colorations in paste
	Dark	Too dark paste colour
	White	Too white paste colour
Rind	Black spots	Black spots in rind
	Colourings	Irregular colorations in rind
	Marks	Marks from the trays in the cheese rind
Flavour	Acid	Flavour sensation of white wine vinegar or lactic acid
	Animal	Flavour sensation reminiscent of cowshed or faecal
	Bitter	Elementary taste produced by substances such as quinine
Texture	Melty	Forms a paste with saliva and continuously melts
	Soft	Little resistance to deformation in mouth
Shape	Swollen	Markedly convex faces
	Convex	Markedly convex sides
	Inclined	Sides with different heights

Adapted from Pérez-Elortondo et al. 2007

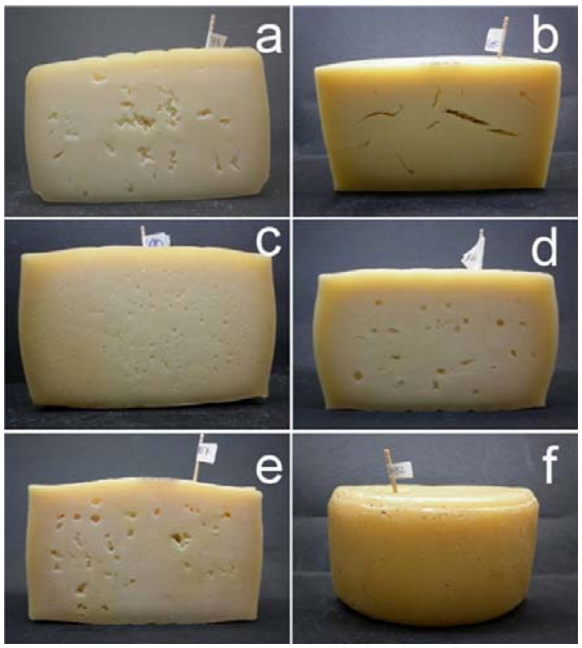

Fig. 1 Cross section of six defective cheeses. Caverns, marks, inclined and white paste (**a**); cracks, dark paste and excess of rind halo (**b**); numerous holes, irregular paste colour and white paste (**c**); round holes and excess of rind halo (**d**); swollen shape, round holes, caverns and paste irregular colour (**e**); and black spots (**f**)

3 Results and discussion

3.1 Frequency of occurrence of relevant sensory defects

Table 1 shows the specific defects found in each of the sensory quality parameters (eyes, paste colour, rind, flavour, texture and shape) during the studied period. This table includes those defects which were found at least with mean frequency of occurrence of 2.5% in the cheese samples. The data from the 5 years studied showed slight variations in the percentages of the different defects, with no major changes in the trend of their appearance. Therefore, the average results of this period were considered.

The most frequently observed defects were those related to the eyes (Table 2). Caverns and cracks in the paste were frequent defects in the cheese samples. Defective openings have been described in the literature in different types of cheese. It is difficult to find a specific cause for this problem because many agents could cause it, such as microbial growth, abnormal mineral composition, inadequate draining and ripening conditions (Mahaut et al. 2003). Eyes are mainly caused by hydrogen gas and carbon dioxide produced by the metabolism of *Clostridium* spp. (Le Bourhis et al. 2007) or other microorganisms such as coliforms, yeasts and heterofermentative lactic acid bacteria that can also be involved in gas production during cheese ripening (Mahaut et al. 2003). The formation of different kinds of holes in ripened cheeses is influenced by the type and amount of gas produced, cheese texture and effect of temperature on gas solubility within the cheese (Sheehan 2011). Openings could also be mechanically originated by an insufficient or fast pressing (Ortiz 2005). Other authors suggested that an excessively intense curd draining could give a friable consistency to the cheese paste originating cracks in paste and rind (Tornadijo et al. 1998).

Table 2 Percentage of defective cheese samples showing at least one specific defect during the period 2006–2010

Defect	Year					Total	
	2006	2007	2008	2009	2010		
	Percentage of cheeses					Mean	Parameter[a]
Caverns	40	55	35	45	39	43	74 Eyes
Cracks	26	21	29	25	29	26	
Numerous	13	9	8	10	6	10	
Round	14	8	7	9	9	9	
Badly distributed	11	8	6	6	9	8	
Excess of rind halo	32	29	24	23	23	27	35 Paste colour
Irregular	7	7	4	4	5	6	
Dark	8	5	6	4	2	5	
White	3	4	4	2	5	3	
Black spots	19	14	12	15	10	14	30 Rind
Colourings	10	13	9	11	14	11	
Marks	9	8	11	11	9	10	
Acid	15	13	11	13	12	13	20 Flavour
Animal	8	3	7	4	3	5	
Bitter	8	3	3	3	1	4	
Melty	25	16	10	8	7	14	16 Texture
Soft	9	4	3	4	2	5	
Swollen	8	4	6	11	11	8	15 Shape
Convex	8	5	3	4	2	4	
Inclined	2	3	4	3	3	3	

[a] Percentage of samples with at least one defect related with the parameter. The same sample could have more than one defect

Main paste colour defects were the presence of an excessive rind halo, an irregular colour and a too dark or too white paste colour (Table 2). As ripening progresses, cheeses lose moisture and the colour of the paste becomes darker. An excess amount of salt or an inadequate ripening, with high temperatures or excessive air velocity, can contribute to an excessive loss of moisture and thus be the cause of the dark paste colour of cheeses. The irregular paste colour is mainly due to a non-homogeneous particle size of the grain during curd cutting, resulting in a paste with non-homogeneous moisture giving defective fermentations (Mahaut et al. 2003; McSweeney 2007).

Black spots, unwanted yellow and orange colourations and tray marks were the predominant rind defects (Table 2). Black spots were described in the paste of Cheddar cheese as a consequence of residual levels of a commercial intramammary teat sealant containing bismuth subnitrate. In this case, the black spots were solid amorphous structures of bismuth salts in the cheese (Lay et al. 2007). The problem of abnormal colouring in the rind may be due to colonisation by yeasts, moulds or bacterial

populations influenced by environmental factors, especially in the airing area and ripening chambers (Amato et al. 2012; Mahaut et al. 2003; McSweeney 2007). This contamination does not usually produce a severe flavour problem (Ortiz 2005). Tray marks are usually caused by an insufficient turning of the cheeses during ripening.

Excessive acidity, animal (dirty) aromas and bitter flavour were the most frequent flavour defects (Table 2). Numerous studies pointed out that the type and amount of starter culture, pH of the milk and curd, brining, ripening conditions, and variations in cheese composition can exert an important influence on cheese flavour (Margrete et al. 2009). However, the origin of a specific flavour defect could be unknown due to the complexity of the cheese matrix and the possible interactions between its components during cheese ripening (Engel et al. 2001; Smit et al. 2005). An excessive acidity could be originated by a large amount of starter culture added to the vat milk, an irregular curd cutting, an insufficient amount of salt, high moisture content of cheese or high temperature in the ripening chamber (Ortiz 2005). In relation with other flavour defects, it has been found that the bacterial metabolism had a significant impact in the development of off-flavours such as bitter, meaty, brothy, putrid, faecal and unclean flavour in Cheddar cheese. These off-flavours can originate from the catabolism of aromatic amino acids during cheese ripening. Catabolism of L-tryptophan, tyrosine and phenylalanine leads to the formation of indole, skatole, tryptamine, *m*-cresol, *p*-cresol, phenol, benzoic acid and phenethanol which are responsible for unclean flavours in some cheese varieties (Ummadi and Weimer 2001). Bitterness has been considered as the major cheese flavour defect for some matured cheese varieties, and in some cases, as for Cheddar cheese, it can become a serious economic concern (Engel et al. 2001). Some authors reported that amino acids and peptides originated by hydrolysis of caseins are responsible for bitter flavour in ripened cheeses (Broadbent et al. 2002; Engel et al. 2001; McSweeney 2007). However, in this study only 4% of the cheese samples showed bitter flavour (Table 2).

Main texture problems were melty (adhesiveness) and excessively soft cheeses. It has been described that it may be due to insufficient curd draining caused by slow acidification originated by too low room temperature when pressing the paste. Consequently, the cheese paste shows high water activity value, which favours enzyme and microbial activities, and therefore an excessive proteolysis (McSweeney 2007). Other authors linked these texture defects with the type and amount of rennet used for cheesemaking (Pirisi et al. 2007).

Shape defects such as swollen and convex cheeses (Table 2) were primarily due to technologically related problems. The swollen defect occurs frequently in many cheese varieties and is usually due to the formation of gas by different types of microorganisms. It could be caused by the starter culture formulation with an excess of lactic acid heterofermentative bacteria, by a lack of hygiene in milking and cheesemaking or by contamination of *Clostridium* spp. that often are associated with animal silage feeding (Garde et al. 2012; McSweeney. 2007). A very low-level contamination (0.01 cfu/mL) of butyric acid bacteria can cause late blowing defect in Gouda-type cheeses (Vissers et al. 2006). As a result of the bacterial metabolism, this contamination often comes with an increase in acidic compounds, mainly butyric acid, and gas production. The resulting cheeses present large caverns and off-flavours. Late blowing is considered one of the major causes of spoilage in semi-hard and hard cheeses (Garde et al. 2012; Le Bourhis et al. 2007).

3.2 Cheeses of different ripening times

Table 3 shows the defects which were significantly ($P \leq 0.05$) different between cheese samples of different ripening times (short-, medium- and long-ripened cheeses). The frequency of cheeses with a white paste and a soft texture was significantly higher in short-ripened cheeses than in medium- and long-ripened cheeses. It was also found that excessive acidity and melty texture affected short-ripened cheeses more than those with a longer ripening period (Table 3). Those defects, which were more frequent in short- and medium-ripened cheeses, could be caused by an insufficient draining of the cheese. Excessive whey retention may be due to a low temperature in the press, an insufficient activity of lactic acid bacteria or a weak pressing of the cheese curd (Kindstedt 2005). Furthermore, cheeses with high moisture have high concentrations of whey and lactose, and therefore the lactic acid bacteria can produce a large amount of lactic acid, and consequently, the acidity of the cheese will be high (Kindstedt 2005). Medium- and

Table 3 Percentage of cheese samples with specific defects according to different ripening times and seasons

Parameter	Defect	Ripening time[1]			Season[2]		
		Short	Medium	Long	Winter	Spring	Summer
Eyes	Caverns	44	41	37	45^{a}	41^{a}	30^{b}
	Cracks	26	25	28	23^{b}	$27^{a,b}$	35^{a}
	Numerous	9	13	7	8^{b}	12^{a}	$9^{a,b}$
	Round	9	8	12	8^{b}	12^{a}	$11^{a,b}$
	Badly distributed	8	7	13	$8^{a,b}$	10^{a}	3^{b}
Paste colour	Excess of rind halo	23^{c}	33^{b}	53^{a}	27^{a}	30^{a}	10^{b}
	Irregular	5	6	9	5^{b}	5^{b}	14^{a}
	Dark	4^{b}	8^{a}	15^{a}	5	6	2
	White	4^{a}	1^{b}	0^{b}	4	3	4
Rind	Black spots	14	13	17	15	13	13
	Colourings	10	15	13	10^{b}	12^{b}	19^{a}
	Marks	8^{b}	13^{a}	15^{a}	9	10	11
Flavour	Acid	14^{a}	$10^{a,b}$	4^{b}	16^{a}	9^{b}	$10^{a,b}$
	Animal	4^{b}	10^{a}	11^{a}	4^{b}	8^{a}	3^{b}
	Bitter	4	4	1	4	4	2
Texture	Melty	15^{a}	12^{a}	5^{b}	16^{a}	11^{b}	$14^{a,b}$
	Soft	6^{a}	1^{b}	0^{b}	7^{a}	2^{b}	1^{b}
Shape	Swollen	9	7	2	10^{a}	5^{b}	5^{b}
	Convex	5	4	5	5	6	7
	Inclined	3	2	5	3	3	3

[a,b,c] Different letters in the same row within the same comparing group indicate significant differences ($P \leq 0.05$)

[1] Short-ripened from 60 to 90 days, medium-ripened from 91 to 119 days and long-ripened from 119 days onwards

[2] Winter from December 1st to March 31st, spring from April 1st to June 20th and summer from June 21st to August 31st

long-ripened cheeses presented a higher percentage of an excess of rind halo with a darker paste colour, stronger animal flavour and more marks in the rind than short-ripened cheeses (Table 3). The compositional changes caused by the loss of moisture and biochemical processes during cheese ripening could affect texture, colour and flavour making them harder, with a darker colour and stronger flavour than the less ripened cheeses. It has been reported that high humidity and temperature in the ripening chambers can cause colour defects (Ortiz 2005).

3.3 Cheeses manufactured in different seasons

Table 3 shows defects with significantly ($P \leq 0.05$) different frequencies of occurrence between cheeses made in winter, spring and summer. Cheeses with swollen shape, caverns and badly distributed eyes were more frequent in winter and spring cheeses than in summer cheeses. Cheese blowing and the presence of abnormally shaped or excessively big eyes have been related to late blowing defect, caused by contamination with spores of *Clostridium* spp. that metabolise lactate producing acetic and butyric acids, and gas (carbon dioxide and hydrogen) (Le Bourhis et al. 2007; Sheehan 2011). Silage feeding, common practise during winter, has been reported as a possible source of this kind of contamination. In contrast, some authors have found higher *Clostridium* spp. spore counts in summer milk than in milk from other seasons (Garde et al. 2011). In addition, frequency of occurrence of cheeses with acid flavour and soft and melty texture was higher in cheeses made in winter than in cheeses manufactured in spring (Table 3). Those defects could be caused by a high moisture content of the cheese paste. As discussed above, low temperatures in the press could affect the draining of the cheese. The low calcium content in winter milk could also (Nájera et al. 2009) hinder curd draining, resulting in a moister paste. For this reason, winter cheeses could be softer and with more lactose available to be transformed into lactic acid than spring and summer cheeses (McSweeney 2007).

Spring cheeses showed higher proportion of numerous and round holes in the paste and stronger animal flavour than winter cheeses. In spring, grazing begins, with high air humidity and a higher likelihood of contamination of the udders. These conditions and the higher temperatures stimulate the growth of microorganisms such as coliforms in milk which can cause these defects (Walstra et al. 2001).

Cracks, irregular paste and rind colourings were frequent in summer cheeses (Table 3). Milk production decreases in summer, so more milkings are often combined to have sufficient milk volume for cheesemaking (Endrizzi et al. 2012). Consequently, milk is cold stored for long periods (up to 48 h) favouring the proliferation of psychrotrophs in milk. Proteolytic activity of psychrotrophs could negatively affect curd formation and consistency (Fonseca et al. 2012; Malacarne et al. 2013), resulting in a paste that cracks easily after pressing. Furthermore, high summer temperatures could increase dairy environmental proliferation of microorganisms, such as *Clostridium* spp., responsible for gas production which could also originate cracks in the paste. Garde et al. (2011) reported that the highest incidence of late blowing defect for Manchego cheeses produced in summer correlated with high clostridial spore counts in summer milk. In addition, milk fat content was higher in summer (at the end of lactation period) than in spring and winter. Summer cheese fat was also more unsaturated due to the extensive grazing management of flocks during this season. As it

is well-known, fresh grass is a source of polyunsaturated fatty acids, and when it is ingested by ruminants, the content of these fatty acids in milk and therefore in cheese increases. Although scientific data are very scarce, particularly for sheep milk, changes in fat content can affect fat globule structure and size (Walstra et al. 2006; Lopez et al. 2007), and the mechanical strength of cheese paste could be reduced resulting in the appearance of cracks during cheese ripening (McSweeney 2007). The increase of fat content could also impede a homogeneous whey draining (Kindstedt 2005), and in consequence, an improper draining could result in an irregular colouring of the cheese paste. The appearance of colourations in the rind may be due to an environmental contamination during ripening by moulds and yeasts, particularly when the temperature is excessively high in the ripening chamber (Mahaut et al. 2003).

3.4 Relationships between defects

Figure 2 shows the factorial map corresponding to the first two factors resulting from the MCA that explained the 66.13% of the total inertia. Factor F1, which explained 45.38% of total inertia grouped (weight values higher than $\left|0.5\right|$) white, soft, bitter, melty, acid, marks, dark, swollen and excess of rind halo defects. In this factor, defects with positive values were those frequently found in short-ripened cheeses, whereas those with negative values were more frequent in long-ripened cheeses. When cheeses present high moisture content, they also retain less salt, favouring microorganism growth, and consequently, a higher proteolysis is produced during cheese ripening. Therefore, the cheese will present a weaker texture and a bitter and acid taste. Other authors also found a soft and sticky texture in cheeses with high moisture (Tornadijo et al. 1998). Bitter flavour and texture defects have also been described when proteolytic enzymes were added to vat milk to accelerate cheese ripening (Izco et al. 2000).

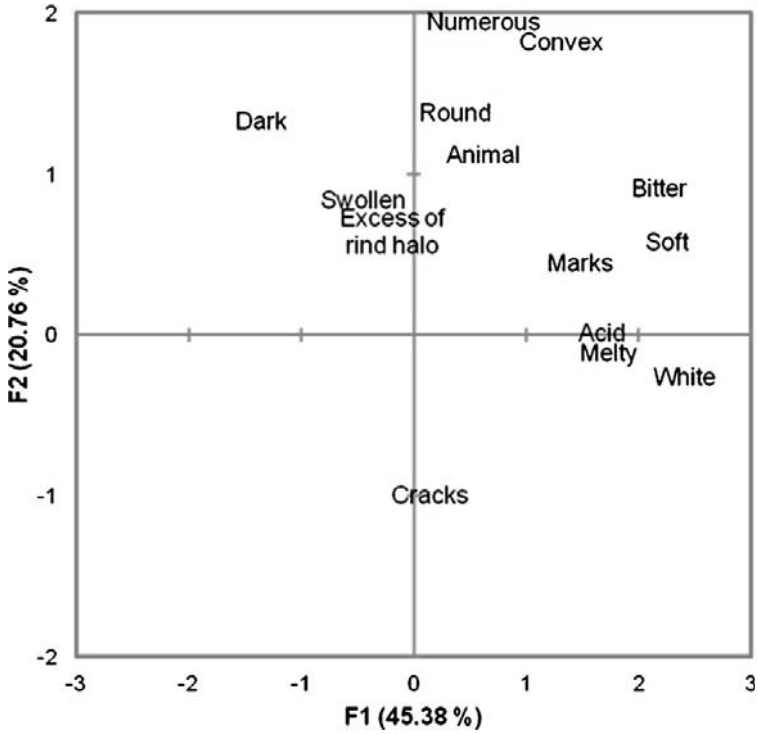

Fig. 2 Multiple correspondence analysis of the defects in cheese samples. Only defects with a minimum weight of $\left|0.5\right|$ in at least one factor are shown

Cheeses with soft texture may exhibit frequent rind marks because they may have not been turned as often as needed.

Defects with negative weight values in factor F1 were swollen, excess of rind halo and dark paste colour. Those defects had been discussed when the cheese samples of different ripening times were compared. As mentioned before, the swollen defect could be the result of the proliferation of *Clostridium* spp.

Factor F2, which accounts for 20.76% of total inertia grouped convex cheeses, numerous and round eyes, and animal flavour all with positive weight values, and cracks with negative weight values. As discussed above, coliforms can be the major cause for the presence of numerous small round eyes, which are often related with dirty and putrid flavour (Ortiz 2005). The origin of cracks, observed more often in summer, may be caused by gas production from the growth of microorganisms as a result of the storage of milk for several days or the metabolism of clostridial bacteria.

4 Conclusion

Flavour is one of the main sensory parameters to assess cheese quality. In this study, it was one of the least affected sensory quality parameters along with texture and shape. The most frequent flavour and texture problems were excessive acidity and melty texture, primary found in short- and medium-ripened cheeses. For this reason, cheeses could be best commercialised after 4 months of ripening. In contrast, the eyes were the most frequently affected sensory quality parameter. Cracks and caverns in the cheese paste were not associated with flavour defects as evidenced by the MCA. The caverns were more frequent in spring and winter cheeses whereas the cracks were more frequent in summer cheeses. Because flock management and cheesemaking conditions differ in winter and in summer, the appearance of different types of openings at these times suggests different origins. However, in the literature, authors often describe different types of openings in an undifferentiated manner as eyes, thus making the interpretation of their results difficult. On the other hand, the ripening period did not affect the frequency of the defects related with eyes and shape, suggesting that these problems may appear within the first 2 months of ripening. Early quality control could avoid the costs associated with the ripening cheeses with defective openings. Due to compositional milk changes and environmental changes throughout cheesemaking seasons, cheese makers should modify their manufacturing practises according to seasons. For example, cheeses made in winter should be turned more often than those made in summer. In order to prevent fermentation by unwanted microorganisms, special care should be taken when environmental temperature is high. After a hygienic milking, milk should be rapidly cooled and stored at low temperature for the shortest time possible prior to cheese manufacture. Hygienic practises should be maintained throughout the whole cheesemaking process, and an active starter culture that rapidly metabolises lactose and acidifies milk should be used. Cutting of curd should be standardised in order to obtain a homogeneous grain size and thus an uniform draining. The results found in this study may be useful for the quality improvement of semi-hard ewe's raw milk cheeses.

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Conflict of interest L. Zabaleta, M. Albisu, M. Ojeda, P. F. Gil, I. Etaio, F.J. Perez-Elortondo, M. de Renobales and L.J.R. Barron declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects performed by any of the authors.

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5.2. Chapter II: Identification of odour active compounds in ewe´s raw milk commercial cheeses with sensory defects

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Identification of odour-active compounds in ewes' raw milk commercial cheeses with sensory defects

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ABSTRACT

The aim of this work was to identify key odorant compounds associated with main off-flavours (acid, rancid and faecal) and one defect related to the internal appearance (big irregular eyes) in ewes' raw milk commercial cheeses. Cheese samples were submitted to solvent assisted flavour evaporation (SAFE) and odorant compounds were detected by gas chromatography–olfactometry (GC–O). Odour-active compounds detected by GC–O were identified and quantified by gas chromatography–mass spectrometry (GC–MS). Partial least square regression was performed to determine relationships between relative abundances of the odour-active compounds and sensory defects of commercial cheeses. An imbalance in the concentration of short-chain free fatty acids, predominant compounds in all cheese samples, was associated with acid and rancid off-flavour, whereas faecal off-flavour was related to minor compounds such as 4-methylphenol and 3-methyl-1-butanol. No volatile compound could be related to the defect of big irregular eyes.

1. Introduction

Flavour plays a key role in the overall quality of cheese and, therefore, the presence of sensory defects, and/or the lack of typical flavour, significantly decreases its quality causing financial losses for the dairy industry (Engel, Nicklaus, Septier, Salles, & Le Quéré, 2001). Cheese flavour is determined by the perception of a combination of large variety of volatile and non-volatile compounds in a particular balance (Le Quéré, 2004).

Volatile compounds are formed mainly during the ripening process. They are the result of microbial metabolism of residual lactose, lactate and citrate, formation of free fatty acids (FFAs), and casein degradation into a range of peptides and free amino acids (FAAs). The degradation of these compounds and the subsequent reactions between them also influences in the flavour composition (McSweeney & Sousa, 2000). Milk pasteurisation influences cheese volatile composition due to heat treatment inactivates enzymes, and reduces the amount of volatile compounds (some of them directly derived from feed) and native microorganisms of raw milk. Therefore, flavour tends to be stronger and more specific in raw milk cheeses than in pasteurised ones (Barron et al., 2007).

Although hundreds of volatile compounds have been identified in cheese, only few of them have been associated with its specific flavour (Curioni & Bosset, 2002). In many cases, the most abundant volatiles may have little odour impact and volatile compounds that are present at very low concentration contribute the most to cheese aroma. It is highly important to understand which compounds are key odorants because they can strongly determine the characteristic odour of the cheese (Smit, Smit, & Engels, 2005). Even though finding the volatile compounds responsible for aroma in dairy products is challenging, the study of compounds responsible for off-flavours could be appealing because each off-flavour often has a specific cause (Fox, McSweeney, Cogan, & Guinee, 2004; Thomsen et al., 2012).

Detection of key odorants is quite difficult because the sensibility of analytical instruments is lower than that of human nose perception (Curioni & Bosset, 2002). Gas chromatography–olfactometry (GC–O) has greatly improved the identification of odour-active compounds, in particular of those compounds responsible for off-flavours (Chamber & Koppel, 2013). Nevertheless, it should be emphasised that volatile compounds are analysed separately by GC–O and possible interactions between them might affect their

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perception. Consequently, this technique should be complemented with the sensory analysis of the food product (Chamber & Koppel, 2013; Thomsen et al., 2012).

Results from earlier studies (Zabaleta et al., 2016) indicated that sensory defects related with different kinds of openings in the cheese were the most frequently observed defects in ewes' raw milk commercial cheeses, and that some eye-related defects were associated with off-flavours. The objective of this study was to identify key odorant compounds associated with acid, rancid and faecal off-flavours, and one defect related to the internal appearance, big irregular eyes, in ewes' raw milk commercial cheeses.

2. Materials and methods

2.1. Commercial cheeses

Ewes's raw milk Idiazabal PDO cheeses were randomly collected from different producers for routine sensory quality control over three consecutive years. Cheeses were manufactured by cheesemakers according to the specifications of the Regulatory Council (Ministerio de Agricultura, Pesca y Alimentación, 1993). Briefly, raw milk was acidified with starter cultures and coagulated by addition of lamb rennet paste (or commercial rennet in some cases) at 28–32 °C. The coagulated milk was cut into rice-sized grains, stirred and heated to 35–37 °C for around 10 min. The curd was manually pressed in the vat and the whey removed. Then, the curd was distributed into cylindrical moulds, pressed about 6–8 h at approximately 20 °C, and placed in saturated sodium chloride brine at 10–12 °C for 16–24 h. Cheeses were ripened at 8–10 °C and around 85–90% relative air humidity for at least 2 months. The final cheese weighed approximately 1–2 kg.

A total of 752 Idiazabal cheeses were submitted to sensory quality control using the sensory analysis method described by Ojeda et al. (2015). Briefly, eight sensory parameters, cheese shape, rind, internal colour, eyes, odour, texture, flavour and persistence were scored by seven expert assessors on a 7-point discontinuous scale. In addition to positive sensory characteristics, sensory defects within each sensory parameter were assessed. Nine defects were selected due to their frequency of occurrence and/or their interest to the cheesemaking sector (acid, rancid, faecal, soft, adherent, pale, short cracks, big irregular eyes, and small rounded eyes; Zabaleta et al., 2016). Also, among all commercial cheeses submitted to sensory quality control, eight cheeses (two different cheeses for each sensory defect) were selected for gas-chromatography analysis because they showed only one of the four defects, namely, acid, rancid and faecal off-flavour, and big irregular eyes. The presence of a defect in the cheese was considered when at least six of the seven assessors indicated it in each session. In addition to these selected defective cheeses, two non-defective commercial cheeses were also selected as control cheeses. Cheeses were cut into 250 g triangular portions, vacuum packed in plastic bags, and frozen at −35 °C until physico-chemical analysis.

Ages of selected defective and non-defective cheeses ranged between 2 and 5 months. Average gross composition of these cheeses (mean ± standard deviation) was the following: dry matter (DM) percentage 65.36 ± 3.46, total fat and protein percentage on DM of 45.94 ± 6.70 and 35.77 ± 2.01, respectively, titratable acidity of 22.97 ± 3.17 mmol of sodium hydroxide per 100 g, and pH of 5.25 ± 0.15.

2.2. Extraction of volatile compounds

Selected defective and non-defective cheeses were submitted to solvent assisted flavour evaporation (SAFE) as previously described (Thomsen, Gourrat, Thomas-Danguin, & Guichard, 2014). The volatile compounds present in the resulting aqueous distillate were extracted with 40 mL of a 125 ng mL^{-1} solution of n-eicosane (internal standard; >99% purity, Sigma–Aldrich, Saint-Quentin Fallavier, France) in dichloromethane (99.9% purity, Carlo Erba Reagents, Val de Reuil, France). After drying, filtering and concentrating the organic phase, the extracted volume was adjusted to 400 μL with dichloromethane and stored at −20 °C for further analysis. Three replicate extracts containing volatile compounds were obtained from each cheese.

2.3. Gas chromatography–mass spectrometry

Volatile compounds present in each of the three replicate extracts obtained from each cheese were analysed by GC–MS by injecting (2 μL) of extract in splitless mode. Separation was performed using an Agilent 6890A gas chromatograph (Hewlett–Packard, Palo Alto, CA) coupled to a 5973 quadrupole mass spectrometer (Agilent, Palo Alto, CA). Volatile compounds were separated in a DBWAX fused silica capillary column (30 m length × 0.32 mm i.d. × 0.5 μm film thickness; Agilent J&W, Santa Clara, USA), using the following temperature gradient: oven temperature was held at 40 °C, then increased at a rate of 4 °C min^{-1} up to 240 °C and held for 10 min. Helium was used as carrier gas at 1.5 mL min^{-1}. Identification of volatile compounds was done by comparison of their experimental linear retention index (LRI), mass spectra with data from four databases: NIST 2.0 (National Institute of Standard and Technology, New York, USA), WILEY 138 library (Wiley & Sons Inc., New York, USA), INRAMass (Internal database achieved using standard compounds, INRA, Dijon, France) and Volatile Compounds in Food 15.2 (Zeist, The Netherlands). Limits of detection (LOD) and quantification (LOQ) were calculated as twice and four times the average noise (arbitrary units) respectively. Ten blanks (analysis of dichloromethane) were used to calculate the average noise in three different parts of the chromatogram. Volatile compound relative abundance (arbitrary units) in each cheese sample was calculated from the total ion current (TIC) multiplying by 100 the division between their peak area and that of internal standard (n-eicosane).

2.4. Gas chromatography–olfactometry

One cheese for each defect (acid, faecal and rancid off-flavour, and big irregular eyes appearance), and one non-defective cheese were selected for GC–O analysis. Three replicate extracts of each cheese were mixed and the resulting solution was analysed by GC–O by a panel of eight assessors, and each cheese extract was analysed in duplicate for each assessor. A total of 16 olfactometric determinations were done for each cheese extract. Odour zones (OZs) were detected using a HP 6890A GC (Agilent Technologies, Santa Clara, USA) coupled to two detection ports (split ratio 1:1); a flame ionisation detector (FID), and an olfactometric detection port (ODP). Chromatographic conditions were the same as for GC–MS analysis. The duration of olfactometric session was 40 min per cheese sample analysis. Each time the assessors detected an OZ, they pointed out one descriptor for the perceived odour using their own vocabulary. Odour descriptions and number of positive determinations for OZs were recorded using AcquiSniff® software (UR QuaPa/T2A; Saint Genès Champanelle, France). Only OZs with 5 or more positive determinations by the assessors for at least one cheese were considered.

2.5. Data treatment and statistical analysis

Three significant figures were used to express (mean and standard deviation) the relative abundance of volatile compounds.

XLSTAT statistical software (version 2011.2, Addinsoft, France) was used for data treatment. One-way analysis of variance (ANOVA) was performed to determine significant differences ($P \leq 0.05$) in the relative abundances of individual volatile compounds, and in the sum of relative abundances of all compounds of the same chemical family, between commercial cheeses with different sensory defects, including also non-defective commercial cheeses. Tukey's test was used for multiple comparisons between different cheeses. Partial least square regression (PLSR) was performed to determine relationships between relative abundances of the odour-active compounds and sensory defects of the cheeses. Only those odour-active compounds which showed scores for Variable Importance in the Projection (VIP) higher than 1, and loadings higher than 0.5 in the first two factors were included.

3. Results and discussion

3.1. Sensory defects in selected commercial cheeses

Fig. 1 shows the number of positive determinations for sensory defects in the selected commercial cheeses with acid, rancid and faecal off-flavour, big irregular eyes, and also in non-defective cheeses. This sensory analysis was made by the seven expert assessors panel on two different cheeses for each defect, and also for the non-defective samples. Acid off-flavour cheeses showed clearly other sensory defects such as pale colour and soft and adherent texture (positive determinations higher than 10). The other off-flavour cheeses did not significantly show other appearance and texture defects although short cracks and small rounded eyes were positively pointed out eight times in rancid and faecal off-flavour cheeses, respectively. Cheeses with big irregular eyes showed adherent texture, whereas the number of positive determinations for selected sensory defects in the non-defective cheeses was lower than four.

3.2. Odour zones

The assessors detected 72 different OZs by GC–O in the five tested cheeses. Most frequently cited odour descriptors and volatile compounds identified in each OZ are given in Table 1. One single volatile compound was identified by GC–MS in 55 OZs. These volatile compounds belonged to different chemical families, comprising acids, ketones, alcohols, esters, furans, lactones, aldehydes, phenolic compounds, pyrazines, sulphur compounds and terpenes.

Each of the five cheeses was described by GC–O with 52–63 different OZs but only 38 OZs were common for all the cheeses, suggesting that the corresponding volatile compounds were the main contributors to the overall cheese odour. Among these common OZs, strong odour notes like vinegar, cheesy, sweat, foot and dust were associated with n-butanoic and n-hexanoic acids and (E)-non-2-enal. It is well known that these compounds in the correct balance are major contributors to the characteristic odour of different types of cheeses (Bosset & Gauch, 1993; Fox et al., 2004; Hernández et al., 2009). Acids were the compounds with the highest relative abundance in the five tested cheeses (Table 2). Those predominant compounds contribute strongly to the aroma of Idiazabal PDO and other different cheese varieties such as, Manchego and Cheddar (Barron et al., 2005b; Frank, Owen, & Patterson, 2004). According to the high content of FFAs in all the cheeses, including non-defective ones (Table 2), lipolysis is a major pathway for flavour generation in this cheese variety. It has been reported that the use of lamb rennet paste which contains a potent pregastric lipase that liberates mainly short-chain fatty acids, gives a characteristic strong and piquant flavour (Amores et al., 2013). Short-chain FFAs contribute to cheese flavour more than long-chain ones since they have a lower flavour threshold (Fox & Wallace, 1997; McSweeney & Sousa, 2000; Ziino, Condurso, Romeo, Giuffrida, & Verzera, 2005).

In addition, γ-hexalactone, γ-nonalactone, γ-decalactone and δ-decalactone, formed by intramolecular esterification of C4 or C5 hydroxyacids (Fox & Wallace, 1997; Smit et al., 2005), and esters such as ethyl butanoate, ethyl hexanoate and ethyl octanoate provided sweet odour notes (floral, fruity and coconut) to cheese aroma, as was also described in other cheese varieties (Frank et al., 2004; Moio, Dekimpe, Etiévant, & Addeo, 1993).

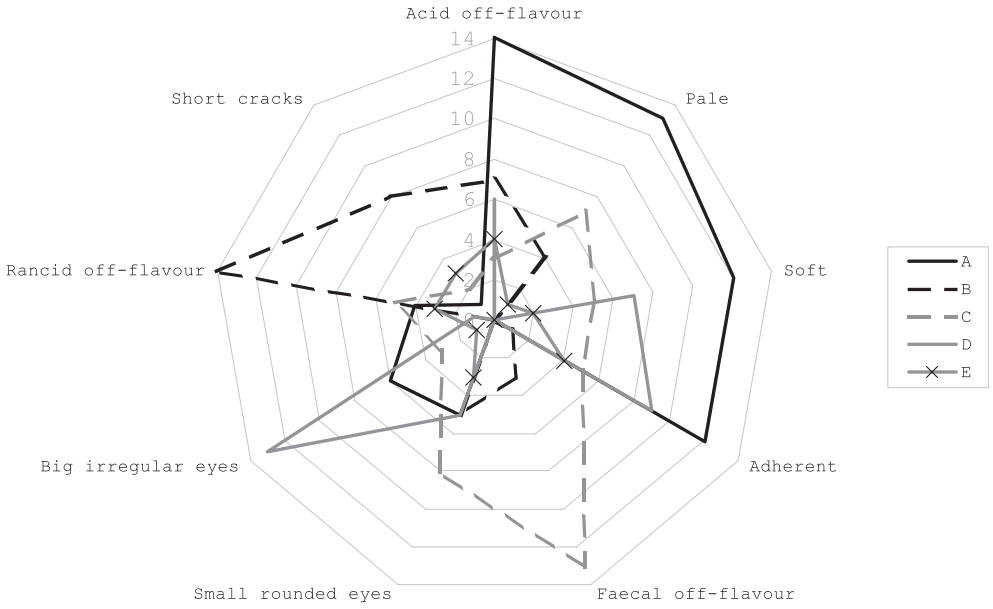

Fig. 1. Number of positive determinations for sensory defects pointed out by the seven expert assessors panel from the sensory quality control of selected commercial cheeses. Each assessor evaluated two cheeses in different sessions for each type of cheese A, B, C, D and E. A (solid black line), acid off-flavour cheeses; B (dashed black line), rancid off-flavour cheeses; C (dashed grey line), faecal off-flavour cheeses; D (solid grey line), cheeses with big irregular eyes; E (grey line marked with ×), non-defective cheeses.

Table 1
Odour zones, main odour descriptors, number of positive determinations by GC–O (8 assessors × duplicate analyses), and volatile compounds identified by GC–MS in tested defective and non-defective commercial cheeses.[a]

OZ	Odour descriptors	Number of positive determinations					$LRI_{(Exp)}$	$LRI_{(Lit)}$	DB	Volatile compounds
		A	B	C	D	E				
										Acids
1	Acid, bitter almond	11	8	15	0	0	1577	1576	1	2-methylpropanoic acid
2	Cheesy, fruity	13	13	16	6	13	1678	1677	3	3-methylbutanoic acid
3	Herbal, cheesy	3	9	2	3	2	1822	1820	3	4-methylpentanoic acid
4	Vinegar, acid	16	14	15	15	13	1457	1455	3	acetic acid
5	Cheesy, sweat	13	15	15	16	15	1628	1627	3	n-butanoic acid
6	Cheesy, burned	6	11	3	3	6	1752	1749	3	n-pentanoic acid
7	Cheesy, foot, faecal	15	15	16	16	13	1850	1853	3	n-hexanoic acid
8	Faecal, rancid	9	10	6	11	1	1968	1965	3	n-heptanoic acid
9	Faecal, dust, cleaner	15	13	15	15	13	2067	2070	1	n-octanoic acid
10	Faecal, burned, fruit	7	8	10	8	7	2184	2173	3	n-nonanoic acid
11	Dust, wax, burned	13	5	11	13	15	2282	2284	3	n-decanoic acid
12	Herbal, flower	0	6	0	1	0	2344	2344	1	n-dec-9-enoic acid
13	Chemical, cowshed	8	4	11	8	9	1948			unknown acid
										Ketones
14	Blue Cheesy, flower	1	2	1	0	13	1185	1184	4	heptan-2-one
15	Butter, flower	7	8	9	11	15	1291	1290	3	3-hydroxybutan-2-one
16	Flower, metal, herbal	15	10	15	14	16	1380	1395	3	1-hydroxybutan-2-one
17	Bread, vegetable	13	0	13	7	10	1817	1815	4	tridecan-2-one
18	Baked, celery	13	0	13	7	10	1447			unknown ketone
										Alcohols
19	Burned, herbal	15	10	15	14	16	1217	1229	3	3-methyl-1-butanol
20	Fruit, herbal	9	0	0	7	12	1330	1332	3	heptan-2-ol
21	Grilled, bread	13	0	13	7	10	1346	1340	3	2-methylpentan-3-ol
22	Fruity	0	0	0	0	11	1592	1566	4	butane-2,3-diol
23	Cookie, flower	1	2	1	0	13	1880	1869	4	phenylmethanol
24	Rose, violet, wine	7	8	9	11	15	1921	1905	4	2-phenylethanol
										Esters
25	Fruit, flower	0	5	0	0	4	1141	1165	3	butan-2-yl butanoate
26	Cabbage, garlic	0	3	3	0	6	1391	1389	3	3-hydroxy-2-butanone, acetate
27	Herbal, humidity	0	13	1	0	2	1603	1604	3	methyl decanoate
28	Fruit, olive	4	1	14	1	6	1071	1070	3	ethyl 3-methylbutanoate
29	Fruit, flower	15	15	14	13	13	1040	1037	3	ethyl butanoate
30	Flower, fruity	13	15	9	12	5	1240	1234	3	ethyl hexanoate
31	Burned, earth, flower	16	14	9	0	0	1444	1446	3	ethyl octanoate
32	Cheesy, fruity	0	7	0	0	6	1562	1553	1	ethyl nonanoate
33	Flower, humidity	4	4	6	0	8	1652	1652	4	ethyl decanoate
34	Flower, vanilla	2	9	5	15	9	1896	1870	4	ethyl dodecanoate
35	Peanut, grilled	0	0	0	0	2	1679	1671	3	ethyl benzoate
36	Flower, fruity	2	11	10	1	3	1429	1421	3	butyl hexanoate
										Furans
37	Chlorine, cleaner	2	0	3	1	6	1620	1616	3	5-methyloxolan-2-one
38	Roasted, fruity	6	6	3	0	6	1887		2	2,5-dimethoxy-2,5-dihydrofuran
										Lactones
39	Baked, vegetable	8	6	13	8	12	1709	1696	3	γ-hexalactone
40	Coconut, fruit, spice	7	9	11	0	13	1980	1977	3	δ-octalactone
41	Cotton candy, coconut, caramel	14	6	6	14	13	2035	2024	3	γ-nonalactone
42	Fruit, apricot, flower	6	5	10	8	5	2154	2173	3	γ-decalactone
43	Peach, celery, flower	11	13	8	14	13	2205	2209	3	δ-decalactone
44	Green, fruity	2	0	3	0	8	2382	2384	3	γ-dodecalactone
45	White flowers, orange	4	5	13	9	7	2233			unknown lactone
										Aldehydes
46	Herbal	4	1	9	3	7	1089	1091	3	n-hexanal
47	Peas, plastic	0	1	3	0	9	1412	1409	3	n-nonanal
48	Chemical, faecal	8	1	1	5	5	2134	2146	3	n-hexadecanal
49	Vegetable, grass	0	6	15	3	0	1438	1434	4	(E)-oct-2-enal
50	Carboard, dust	10	8	15	13	13	1542	1543	4	(E)-non-2-enal
51	Grilled, sweat	0	1	8	4	4	1772	1777	4	(2E,4E)-deca-2,4-dienal
										Phenolic compounds
52	Metal, smoke	4	3	5	6	9	2012	2003	4	phenol
53	Faecal, cowshed	13	4	12	11	13	2088	2068	3	4-methylphenol
54	Sheep, grilled	8	0	10	10	5	2097	2090	3	3-methylphenol
										Pyrazines
55	Bitter almond, roasted potato	6	0	0	0	0	1414	1391	4	2,3,5-trimethylpyrazine
										Sulphur compounds
56	Potato	11	12	0	15	3	1473	1453	4	methional
57	Anis, chlorophylle, flower	13	6	14	12	11	1725	1714	4	methionol
										Terpenes
58	Grass	3	3	2	6	5	1206	1210	3	D-limonene
										Unknown compounds
59	Penetrant, vegetable	2	6	1	0	0	1198			
60	Grilled, vegetable	8	0	0	0	10	1247			

Table 1 (*continued*)

OZ	Odour descriptors	Number of positive determinations					$LRI_{(Exp)}$	$LRI_{(Lit)}$	DB	Volatile compounds
		A	B	C	D	E				
61	Mushroom	15	13	15	15	15	1308			
62	Flower, herbal	15	11	16	14	15	1592			
63	Toasted, vegetable, flower	2	0	0	6	0	1759			
64	Flower, herbal	9	1	4	2	1	1779			
65	Candy, flower	6	5	7	10	8	1869			
66	Purin, solvent	0	2	0	0	9	2122			
67	Spice, cumin	2	6	2	1	1	2268			
68	Fruit, coconut	8	9	12	9	0	2390			
69	Herbal, chemical	7	0	13	10	11	1513			
70	Coconut, candy, sweet	12	8	7	13	10	1927			
71	Fruity, flower	0	7	8	0	5	2110			
72	Faecal, chemical	3	11	11	7	0	2276			

[a] 1 cheese of each type of cheese A, B, C, D and E was analysed: A, acid off-flavour cheese; B, rancid off-flavour cheese; C, faecal off-flavour cheese; D, cheese with big irregular eyes; E, non-defective cheese. Abbreviations are: OZ, odour zone number; $LRI_{(exp)}$, experimental linear retention index; $LRI_{(lit)}$, linear retention index reported in the literature on DB-WAX column. DB, database: 1, NIST 2.0; 2, WILEY 138 library; 3, INRAMass; 4, Volatile Compounds in Food 16.1.

Table 2
Relative abundance of odour-active compounds grouped by chemical families in tested defective and non-defective commercial cheeses.[a]

	Cheeses				
	A	B	C	D	E
Acids	16,500 ± 1900^{b}	35,000 ± 10,200^{a}	9010 ± 2330^{b}	10,400 ± 2420^{b}	10,300 ± 5830^{b}
Ketones	361 ± 77.4^{b}	991 ± 1040^{ab}	300 ± 68.0^{b}	954 ± 568^{ab}	1530 ± 397^{a}
Alcohols	154 ± 91.5^{b}	782 ± 791^{ab}	1090 ± 103^{a}	717 ± 465^{ab}	265 ± 14.6^{b}
Esters	795 ± 97.1^{a}	605 ± 285^{a}	248 ± 195^{a}	589 ± 676^{a}	362 ± 76.6^{a}
Furans	4.44 ± 4.24^{a}	13.9 ± 9.63^{a}	2.68 ± 0.813^{a}	5.72 ± 2.97^{a}	324 ± 893^{a}
Lactones	203 ± 44.0^{a}	215 ± 87.8^{a}	211 ± 34.1^{a}	1720 ± 2140^{a}	575 ± 39.6^{a}
Aldehydes	71.4 ± 53.7^{ab}	59.5 ± 42.1^{ab}	191 ± 171^{a}	22.8 ± 3.45^{b}	87.9 ± 17.5^{b}
Phenolic compounds	18.4 ± 14.9^{b}	18.0 ± 5.92^{b}	88.1 ± 16.1^{a}	15.7 ± 1.59^{b}	9.12 ± 0.456^{b}
Pyrazines	1.69 ± 0.949^{b}	ND	6.66 ± 6.96^{b}	62.8 ± 51.3^{a}	18.5 ± 9.59^{b}
Sulphur compounds	2.73 ± 0.656^{b}	5.90 ± 0.833^{ab}	8.02 ± 4.19^{a}	4.97 ± 1.15^{ab}	7.97 ± 1.59^{a}
Terpenes	1.13 ± 0.607^{ab}	1.02 ± 0.256^{b}	0.710 ± 0.199^{b}	1.10 ± 0.488^{ab}	1.81 ± 0.553^{a}

[a] Values (in arbitrary units; mean ± standard deviation) are data from 2 cheeses × 3 replicate GC–MS analyses for each type of cheese A, B, C, D and E: A, acid off-flavour cheeses; B, rancid off-flavour cheeses; C, faecal off-flavour cheeses; D, cheeses with big irregular eyes; E, non-defective cheeses. Means followed by a different superscript letter in the same row are significantly different ($P \leq 0.05$); ND, peak below limit of detection.

3.3. Relationships between odour-active compounds and sensory defects

PLSR analysis (Fig. 2) showed that the detected odour-active compounds were associated with most of the sensory defects reported in Fig. 1. The first two components (t1 and t2) explained 69.3% of the variability of odour-active compounds. Rancid off-flavour cheeses were separated from the others on the positive values of the first component, while the second component (t2) separated acid from faecal off-flavour cheeses. Cheeses with big irregular eyes and non-defective cheeses were not differentiated by PLSR analysis and could not be associated with any odorant compound. This seems logical because non-defective cheeses did not present any sensory defects and cheeses with big irregular eyes did not show any flavour-related defect (Fig. 1). In this sense, as discussed below for short-cracks and small rounded eyes, it seems that undesirable microbial growth would not be the cause for big irregular eyes in cheese, and that mechanical reasons could be associated to this appearance defect.

3.3.1. Key odorants related to acid off-flavour

According to PLSR results, acid off-flavour, pale, adherent, and soft defects were related to acetic acid and tridecan-2-one (Fig. 2). These results confirmed the above mentioned sensory characterisation of acid off-flavour cheeses (Fig. 1). Acids were the most abundant volatile compounds in acid off-flavour cheeses although the content of which was not significantly different from the other cheeses, even lower than in rancid off-flavour cheeses (Table 2). Acid off-flavour of cheeses has been correlated with high concentration of lactic acid or low pH in cheese (Engel, Nicklaus, Salles, & Le Quéré, 2002). However, no significant differences were found in titratable acidity and pH between cheeses with acid off-flavour and the other tested commercial cheeses. In this sense, other authors attributed this off-flavour to an excessive concentration of short-chain FFAs (McSweeney & Sousa, 2000) or to an excessive or unbalanced proteolysis during cheese ripening (Fox & Wallace, 1997).

Acetic acid, even though being low in relative abundance in comparison with other longer chain acids in tested commercial cheeses, was positively and highly correlated with acid off-flavour, and its relative abundance in acid off-flavour cheeses was significantly higher than in faecal off-flavour cheeses and non-defective cheeses (Table 3). Acetic acid was involved in the typical sharp-vinegar and acid odour notes and it has been considered an important flavour compound in Idiazabal PDO cheeses and other cheese varieties as Cheddar, Gruyére, Emmental, and Manchego (Barron et al., 2005b; Curioni & Bosset, 2002; Frank et al., 2004). In contrast to other acids, its origin is not lipolytic because it is a result of citrate and lactic acid metabolism by lactic acid bacteria, or catabolism of Ala and Ser by starters and other bacteria (McSweeney & Sousa, 2000; Smit et al. 2005; Ziino et al., 2005).

Tridecan-2-one was also positively associated with acid off-flavour (Fig. 2). This medium-chain methyl ketone, which contributes with herb and spice odour notes (Volatile Compounds in Foods 16.1, 2015), has been found in very low concentrations in some blue cheese varieties (Gallois & Langlois, 1990; González de Llano, Ramos, Polo, Sanz, & Martínez-Castro, 1990). Some authors

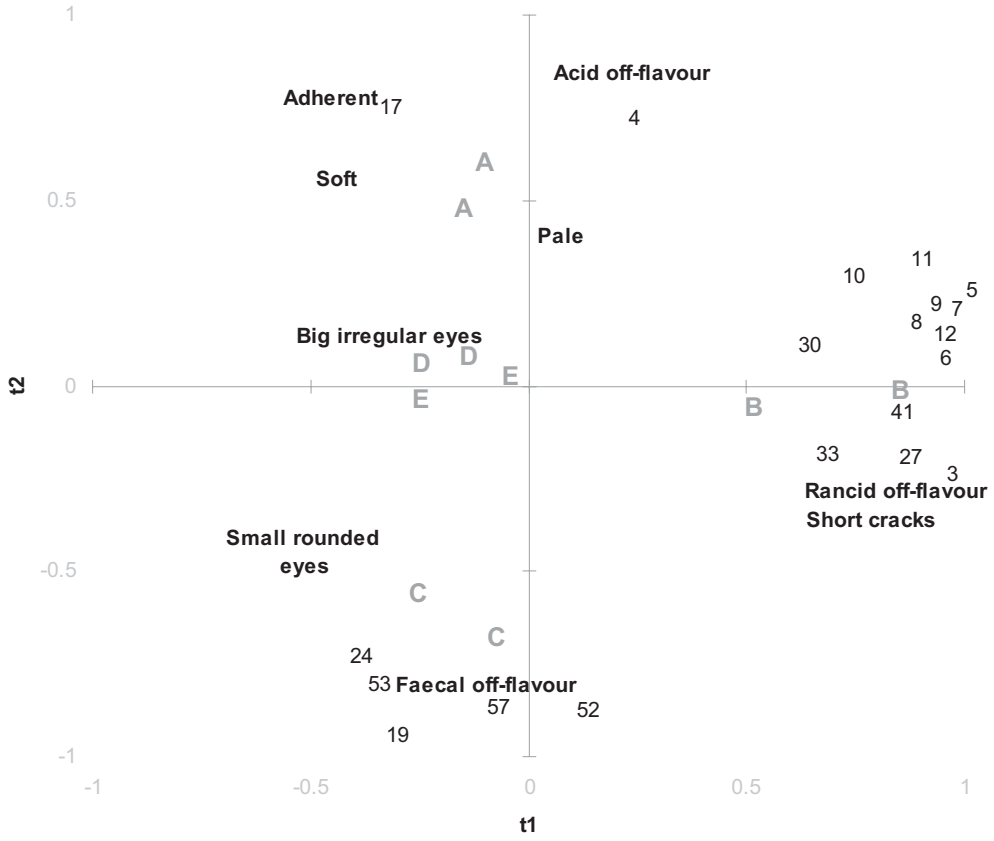

Fig. 2. Partial least square regression (PLSR) plot for relative abundances of odour-active compounds and sensory defects from the sensory quality control of selected commercial cheeses. Explained variance was 69.3% for odour-active compounds and 48.7% for sensory defects. Sensory defects are bold faced, odour-active compounds are numbered by OZ number as in Table 1, and cheese samples are marked in grey. A, acid off-flavour cheeses; B, rancid off-flavour cheeses; C, faecal off-flavour cheeses; D, cheeses with big irregular eyes; E, non-defective cheeses.

Table 3
Relative abundance of key odorant compounds associated with acid, rancid and animal off-flavour in tested defective and non-defective commercial cheeses.[a]

Key odorant compounds	Relative abundance				
	A	B	C	D	E
Acid off-flavour					
n-Acetic acid	260 ± 65.4^{a}	261 ± 71.2^{a}	39.1 ± 33.0^{b}	170 ± 89.5^{ab}	49.3 ± 24.4^{b}
Tridecan-2-one	51.9 ± 31.2^{a}	ND	3.62 ± 4.29^{a}	7.53 ± 3.41^{a}	6.10 ± 6.71^{a}
Rancid off-flavour					
4-Methylpentanoic acid	1.28 ± 1.74^{b}	66.3 ± 21.0^{a}	10.2 ± 6.57^{b}	0.762 ± 0.866^{b}	4.76 ± 2.57^{b}
n-Butanoic acid	2130 ± 241^{b}	7620 ± 2130^{a}	725 ± 249^{b}	1820 ± 323^{b}	1720 ± 959^{b}
n-Pentanoic acid	55.9 ± 23.0^{a}	751 ± 468^{a}	24.4 ± 8.82^{a}	55.3 ± 16.5^{a}	101 ± 88.6^{a}
n-Hexanoic acid	4070 ± 646^{ab}	10,900 ± 3270^{a}	1940 ± 678^{b}	2690 ± 545^{ab}	3060 ± 1930^{ab}
n-Heptanoic acid	251 ± 65.7^{a}	745 ± 385^{a}	116 ± 45.0^{a}	126 ± 67.7^{a}	138 ± 90.3^{a}
n-Octanoic acid	4110 ± 805^{a}	7620 ± 2280^{a}	2590 ± 1030^{a}	2170 ± 437^{a}	2560 ± 1480^{a}
n-Nonanoic acid	264 ± 128^{a}	233 ± 101^{a}	91.9 ± 35.4^{a}	53.8 ± 6.61^{a}	74.0 ± 47.4^{a}
n-Decanoic acid	4190 ± 546^{a}	5670 ± 1760^{a}	2440 ± 552^{a}	2380 ± 297^{a}	2230 ± 1050^{a}
n-Dec-9-enoic acid	306 ± 157^{ab}	738 ± 181^{a}	161 ± 112^{b}	64.7 ± 13.2^{b}	198 ± 172^{b}
Ethyl hexanoate	85.4 ± 46.2^{a}	261 ± 153^{a}	42.4 ± 23.2^{a}	33.6 ± 3.70^{a}	25.9 ± 8.08^{a}
Ethyl decanoate	104 ± 88.1^{a}	203 ± 71.0^{a}	106 ± 81.7^{a}	38.8 ± 27.3^{a}	31.8 ± 16.7^{a}
Methyl decanoate	0.492 ± 0.113^{a}	7.98 ± 7.54^{a}	1.66 ± 1.03^{a}	0.309 ± 0.351^{a}	0.610 ± 0.518^{a}
γ-Nonalactone	11.2 ± 3.62^{a}	79.1 ± 83.1^{a}	9.55 ± 3.23^{a}	8.74 ± 1.63^{a}	9.04 ± 1.38^{a}
Faecal off-flavour					
2-Phenylethanol	67.9 ± 44.6^{b}	44.7 ± 38.4^{b}	486 ± 69.1^{a}	42.0 ± 33.3^{b}	5.14 ± 5.93^{b}
3-Methyl-1-butanol	19.5 ± 5.37^{b}	14.1 ± 10.7^{b}	523 ± 122^{a}	17.0 ± 8.08^{b}	8.84 ± 3.89^{b}
Phenol	ND	2.81 ± 0.535^{a}	4.83 ± 3.27^{a}	2.23 ± 1.22^{a}	0.888 ± 0.990^{a}
4-Methylphenol	11.9 ± 8.48^{b}	5.87 ± 2.34^{b}	63.1 ± 28.0^{a}	8.61 ± 1.49^{b}	4.57 ± 1.21^{b}
Methionol	0.453 ± 0.110^{a}	1.39 ± 0.658^{a}	5.89 ± 3.82^{a}	0.807 ± 0.322^{a}	0.248 ± 0.0404^{a}

[a] Values (in arbitrary units; mean ± standard deviation) are data from 2 cheeses × 3 replicate GC–MS analyses for each type of cheese A, B, C, D and E: A, acid off-flavour cheeses; B, rancid off-flavour cheeses; C, faecal off-flavour cheeses; D, cheeses with big irregular eyes; E, non-defective cheeses. Means followed by a different superscript letter in the same row are significantly different ($P \leq 0.05$); ND, peak below limit of detection. Odour zone number and linear retention index are as given in Table 1.

have reported that this type of methyl ketones could be formed by moulds from ketoacids naturally present in milk fat or by oxidation of monounsaturated fatty acids (Collins, Mc Sweeney, & Wilkinson, 2003).

3.3.2. Key odorants related to rancid off-flavour

Rancid off-flavour was associated with 13 volatile compounds and short cracks defect according to PLSR results (Fig. 2). Short cracks defect was found in rancid off-flavour cheeses according to above mentioned sensory analysis (Fig. 1). Volatile compounds were seven linear saturated fatty acids, from n-butanoic acid to n-decanoic acid, which impart cheesy, sweat, and rancid odours, 4-methylpentanoic acid, described as herbal and cheesy, and n-dec-9-enoic acid, with herbal and flower aroma, three esters, ethyl hexanoate, ethyl decanoate and methyl decanoate, and γ-nonalactone, with flower, fruity, herbal, and coconut odour notes. Relative abundance of acids was significantly higher than in the other tested commercial cheeses (Table 2) mostly due to the high content of n-butanoic acid in rancid off-flavour cheeses (Table 3).

Rancidity in cheese has been attributed to an excessive or unbalanced lipolysis, which leads to an excess of FFAs producing off-flavours (Fox & Wallace, 1997; Fox et al., 2004; McSweeney & Sousa, 2000). n-Butanoic acid, present in significantly higher relative abundance in rancid off-flavour cheeses than in the other tested cheeses (Table 3), has often been described as a key odorant with cheesy or putrid odours (Barron et al., 2005b; Thomsen et al., 2012). High concentration of n-pentanoic acid has also been found in ewes' raw milk cheeses with intense lipolysis (Barron et al., 2005a). The high levels of n-butanoic and n-pentanoic acids found in rancid off-flavour cheeses (Table 3) together with the presence of short cracks defect could be associated to the late blowing defect of cheese (Gómez-Torres, Garde, Peirotén, & Ávila, 2015). FFAs are also precursors of other potent odorant compounds such as methylketones, lactones and esters (Fox & Wallace, 1997; McSweeney & Sousa, 2000; Smit et al., 2005).

3.3.3. Key odorants related to faecal off-flavour

Faecal off-flavour was related to small rounded eyes on the PLSR plot (Fig. 2), as observed in a previous study (Zabaleta et al., 2016). Faecal off-flavour cheeses showed significantly higher abundance of phenolic compounds than the other tested cheeses, and also significantly higher abundance of alcohols and aldehydes than non-defective cheeses (Table 2). PLSR analysis highlighted an association between faecal off-flavour and 2-phenylethanol, 4-methylphenol, 3-methyl-1-butanol, phenol and methionol (Fig. 2).

3-Methyl-1-butanol is generally considered as a result of Strecker degradations of Leu, forming 3-methyl-1-butanal that is rapidly reduced to 3-methyl-1-butanol by the activity of lactic acid bacteria dehydrogenases (Chamber & Koppel, 2013; Curioni & Bosset, 2002; Fox & Wallace, 1997). Panel assessors gave descriptors such as burned and herbal to 3-methyl-1-butanol. Its odour has been described with descriptors such as dark chocolate, green, burned, faecal and sheep (Chamber & Koppel, 2013; Garde, Ávila, Medina, & Nuñez, 2005; McSweeney & Sousa, 2000; Morales, Fernández-García, Gaya, & Nuñez, 2003). Its relationship with unclean flavour and gassy openings was reported in Egyptian Ras cheese (Ayad, Awad, El Attar, de Jong, & El-Soda, 2004).

High concentration of phenolic compounds can exert a negative effect to cheese flavour with unpleasant odour notes (Curioni & Bosset, 2002). 4-Methylphenol (*p*-cresol) was present in significantly higher relative abundance in faecal off-flavour cheeses than in the other cheeses (Table 3). This compound is formed from Strecker degradation of Tyr, being a potent odorant that causes in cheese strong unclean flavours as putrid and strong medicinal aromas, and cowy odour notes (Curioni & Bosset, 2002; Frank et al., 2004; McSweeney & Sousa, 2000).

3.3.4. Key odorants related to non-defective cheeses

As mentioned above, PLSR analysis did not differ between cheeses with big irregular eyes and non-defective cheeses (Fig. 2). Acids were major volatile compounds in both types of cheese, followed by lactones and ketones (Table 2). The last two groups of compounds, even in low concentrations, contribute to the overall cheese aroma with flowery, apricot and coconut odour notes because they have very low perception thresholds (Curioni & Bosset, 2002).

4. Conclusions

Acids, esters, ketones and lactones were predominant volatile compounds in the ten tested commercial defective and non-defective cheeses. Those compounds, in appropriate concentrations, were involved in the basic cheese aroma of these ewes' raw milk commercial cheeses, but an imbalance in the concentration of them caused an unpleasant cheese flavour. This was observed for acids that, in high proportions, led to acid and/or rancid off-flavour, and for some minor odorant compounds such as 4-methylphenol and 3-methyl-1-butanol for faecal off-flavour. The results showed that both the quantification of odour-active compounds and their odour description are necessary to detect off-flavours in cheese. This study contributes to a better understanding of both the control of the sensory quality in ewes' raw milk commercial cheeses and the identification of key odorant compounds associated with off-flavours. Further studies including a higher number of cheeses are required to validate our results.

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5.3. Chapter III: Volatile compounds associated to desirable flavour and off-flavour generation in ewe´s raw milk commercial cheeses

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ORIGINAL PAPER

Volatile compounds associated with desirable flavour and off-flavour generation in ewe´s raw milk commercial cheeses

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Abstract This study investigated relationships between the volatile profile and balanced flavour of high sensory quality ewe's raw milk commercial cheeses, and those relations between certain volatile compounds and off-flavours in defective cheeses. Commercial cheeses were selected in the routine sensory quality control from 27 farmhouses. High sensory quality cheeses showed a particular desirable balanced flavour and volatile profile. However, an imbalanced composition of volatile compounds was found in off-flavour cheeses. Rancid off-flavour was mainly related to an excessive concentration of *n*-butanoic and *n*-hexanoic acids, and their corresponding ethyl esters, generated by strong lipolysis during ripening. Animal off-flavour was associated with the generation of 3-methylbutan-1-ol, 2-phenylethanol and 4-methylphenol originated by amino acid catabolism after intense proteolysis. Finally, acid off-flavour was related to an excessive abundance of acetic acid generated mainly by strong glycolysis at early stages of ripening.

Keywords Off-flavour · Volatile profile · Farmhouse commercial cheeses · Sheep raw milk

Introduction

Sheep and goat dairy products manufacture, especially from Mediterranean countries, has often been linked to particular regions, natural feeding resources (like pasture grazing), native breeds, and traditional production methods. Farmhouse production has preserved the typical quality of these dairy products until nowadays [1]. However, rural dairies are suffering a progressive abandonment in many European areas due to the migration of young people to cities, low economic profitability of farms, and high competitiveness within the dairy sector. Production of standardised traditional high-quality dairy products is one of the last remaining ways to compete with the growing industrial dairy mass production [1, 2].

Consumer's preference of farmhouse dairy products depends largely on their sensory properties, being flavour one of the most important attributes [1, 3]. Thus, the production of a high-quality cheese, and without off-flavour, is a major worry of farmhouse cheese makers [4, 5]. Cheese flavour is the result of the simultaneous complex perception of different sensory signals [6]. The balance among volatile and non-volatile compounds originated by catabolism of lipids, proteins and carbohydrates during cheese ripening (or new compounds formed by reactions between them) determines cheese flavour [7, 8]. Off-flavour in cheese could result from compounds originated in milk and transferred to cheese, by the action of enzymes from raw milk, starter and rennet microorganisms during cheese ripening, and also by the influence of cheesemaking conditions [6, 9, 10]. Even though volatile compounds responsible for cheese flavour have been extensively reported in the scientific literature [11], most studies were on cheeses from cow's milk [12, 13]. Studies on volatile compounds associated with desirable flavour or off-flavour of cheeses from small ruminants' milk are scarce in the scientific literature [14–16]. The study of defective commercial cheeses is of major interest for farmhouse cheese makers. However, there have been few studies conducted on commercial defective

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cheeses [15, 16]. The objective of the present study was to investigate relationships between the volatile profile and balanced flavour of high sensory quality ewe's raw milk commercial cheeses, and those relations between certain volatile compounds and off-flavours in defective commercial cheeses. In a previous study [16], key odorants associated with off-flavours were identified in a small number of ewe's raw cheese samples. To validate and extrapolate these results to commercial farmhouse cheese productions, the present study has been conducted on a greater number of ewe's raw milk commercial cheeses made in three consecutive annual productions.

Materials and methods

Sampling of commercial cheeses

Idiazabal PDO ewe's raw milk cheeses were manufactured in small farmhouses following the specifications of its Regulatory Council [17]. Cheese makers used milk from their own flocks following a feeding strategy as previously described [18]. Cheeses were manufactured in a similar way. Briefly, raw milk was acidified by the addition of mostly homofermentative starter cultures. Then, milk was heated to 28–32 °C and coagulated using commercial rennet, lamb rennet paste or the mixture of both. The curd was then cut into rice-sized grains and heated to 35–37 °C for around 10 min under stirring. After removing the whey, curd grains were placed into cylindrical moulds, pressed for 6–10 h, and salted by immersion in saturated sodium chloride brine at 10–12 °C for 16–24 h. Cheeses (1–2 kg) were ripened for at least 2 months at 8–12 °C and 85–90% relative humidity before sampling. Defective and non-defective commercial cheeses were sampled taking into account the previous assessment carried out by the annual sensory quality control of Idiazabal PDO Regulatory Council, as explained below. A total of 41 commercial cheeses were sampled (34 defective cheeses and 7 non-defective cheeses) from 27 different farmhouses between 2011 and 2013.

Sensory analysis

Sensory quality of Idiazabal PDO cheeses was systematically controlled by the Sensory Laboratory of the University of the Basque Country (LASEHU) following an accredited sensory method according to ISO 1725 [19] by a trained sensory expert assessor panel according to the guidelines of ISO 8586 [20]. The sensory method has been deeply described by Pérez-Elortondo et al. [21] and an improvement of the method has been recently published by Ojeda et al. [22]. Briefly, a panel of 7 sensory expert assessors analysed cheeses separately, scoring eight parameters: shape, rind, paste colour, eyes, odour, texture, flavour and persistence in a discountinous seven point scale. A sensory parameter was considered defective in a cheese sample when the sensory expert assessors scored it with a value lower than four. In this case, each assessor should describe which was the defect (or defects) present in the cheese sample. Based on these records, it was considered the presence of a sensoy defect in a cheese sample when at least five of the seven sensory expert assessors marked it. Desirable attributes and sensory defects registered by the assessors for each parameter have been previously described by Elortondo el al. [21], Ojeda et al. [22] and Zabaleta et al. [23]. From all desirable attributes and sensory defects, five desirable flavours (ewe's milk, slightly acid, natural rennet, medium salty, and slightly pungent) and three main undesirable off-flavours (rancid, animal and very high acid off-flavour) were considered for the present study (Table 1). According to this sensory quality assessment, cheese samples were classified as rancid off-flavour

Table 1 Sensory description of desirable attributes of Idiazabal PDO cheese and off-flavours considered for the present study

Attributes	Sensory description[a]
Desirable attributes	
Ewe's milk	Flavour sensation frequently associated with fermented sheep's milk
Slightly acid	Elemental taste perceived in the mouth with watery solutions of diverse organic substances such as citric or lactic acid
Natural rennet	Combination of typical aromas of traditional rennet paste
Medium salty	Elemental taste caused by diluted watery solutions of diverse solutions such as sodium chloride
Slightly pungent	Mouth feel perceived as an irritation, burning or stinging
Off-flavours	
Rancid	Flavour sensation frequently associated with butyric acid
Animal	Flavour sensation reminiscent of cowshed or faecal (ewe's rear wool)
Very high acid	Flavour sensation of white wine vinegar or high lactic acid intensity

[a]Adapted from Perez-Elortondo et al. [21], Ojeda et al. [22] and Zabaleta et al. [16]

cheeses (RA; 13 samples), animal off-flavour cheeses (AN; 8 samples), very high acid off-flavour cheeses (AC; 13 samples), and non-defective cheeses (ND; 7 samples). Ripening time of cheese samples ranged from 2 to 5 months being AC cheeses (average ripening time of 78 ± 5 days) less mature than the other cheese samples (average ripening time of 98 ± 19 days). Immediately after the sensory quality control, cheese samples were cut into 250 g triangular portions, vacuum packed in plastic bags and stored at −35 °C until physico-chemical analysis.

Cheese gross composition

Dry matter (DM) content was determined according to IDF standard no. 4 [24]. Kjeldahl method was used to analyse crude protein content as described in IDF standard no. 20-1 [25], and fat content was assessed by solid–liquid extraction using *n*-pentane with Soxhlet method. Cheese pH was measured with a penetration electrode (MicropH2000, Crison, Barcelona, Spain) in a mixture of 10 g grated cheese with 10 mL of MiliQ water. Cheese titratable acidity, (mmol of sodium hydroxide per 100 g) was analysed according to the IDF standard no. 150 [26]. All analyses were performed in replicate samples.

Volatile composition

Volatile compounds of cheeses were analysed by solid-phase microextraction–gas chromatography–mass spectrometry (SPME–GC–MS) essentially as previously described [27]. Briefly, volatiles were extracted at 45 °C for 30 min on a 50/30 μm DVB/Carboxen/PDMS fiber (Supelco, Bellefonte, USA) from a mixture of grated cheese and anhydrous sodium sulphate. An aqueous solution (0.5 g L^{-1}) of cyclohexanone (99.8% purity, Sigma–Aldrich, Madrid, Spain) was used as internal standard. Volatiles were separated using a 7820 A gas chromatograph coupled to a 5975 mass spectrometer detector (Agilent Technologies, Santa Clara, USA) on a Supelcowax (Supelco, Bellefonte, USA) column (60 m × 0.250 mm i.d., 0.25 μm film thickness) subjected to temperature gradient from 40 to 240 °C. Volatile compounds were tentatively identified by comparing their mass spectra with those of NIST 8.0 (National Institute of Standard and Technology, New York, USA) and Wiley 4275 (Wiley & Sons Inc., New York, USA) libraries. Positive identification was done by comparison of linear retention index (LRI) and mass spectra of sample peaks with those of high purity standards (Sigma–Aldrich). The limit of detection (LOD) of a compound was established in terms of peak area as twice the average noise value (in arbitrary units) of the chromatogram. The limit of quantification (LOQ) was established as four times the average noise value. The content of volatile compounds in the headspace of the cheese samples was calculated as relative abundance (peak area in arbitrary units relative to that of internal standard) using the Eq. (1):

$$\text{Relative abundance} = (\text{peak area}/\text{internal standard area}) \times 100 \quad (1)$$

Statistical analysis

Statistical analyses were performed using XLSTAT statistical software (Excel 97, vers. 2011.2, Addinsoft, France). Partial least square regression (PLSR) was applied to the relative abundances of volatile compounds found in off-flavour cheese samples to select which compounds were specifically associated with RA, AN and AC off-flavour cheeses. Two criteria were followed to select volatile compounds: (1) those compounds with Variable Importance in the Projection (VIP) scores higher than 1.0, and (2) those compounds with loadings higher than 0.5 in the first two PLSR factors. Furthermore, PLRS analysis was used to differentiate RA, AN and AC off-flavour cheeses using volatile abundance ratios. The presence of absence of significant differences in gross composition ($P \leq 0.05$) and volatile abundance ($P \leq 0.1$) among ND, RA, AN, and AC cheeses was determined by analysis of variance (ANOVA), and Tukey's test was used for pairwise comparisons. Critical level of significance of $P \leq 0.1$ was used due to the high variability expected for the abundance of the volatile compounds in the commercial cheese sample groups.

Results and discussion

Gross composition

Non-significant differences ($P > 0.05$) were found in the gross composition between ND and off-flavour cheeses (RA, AN, and AC cheeses). Average dry matter (DM) percentage of all commercial cheese samples was 65.12 ± 5.28, mean percentage of protein on DM was 36.21 ± 2.89 and that of fat was 46.53 ± 6.76. Average titratable acidity was 22.9 ± 2.87 mmol 100 g^{-1}, and pH 5.28 ± 0.14. Gross composition of cheese samples was quite similar to that of ripened cheeses made from ewe's milk in other Mediterranean areas [10, 28].

Sensory profile

Figure 1 shows the percentage of assessors that marked the presence of desirable flavours and off-flavours in the selected commercial cheeses. Desirable flavours as slightly acid and pungent, medium salty, natural rennet and ewe's milk were marked by more than 78% of the assessors in ND cheeses, whereas off-flavours were hardly detected in these

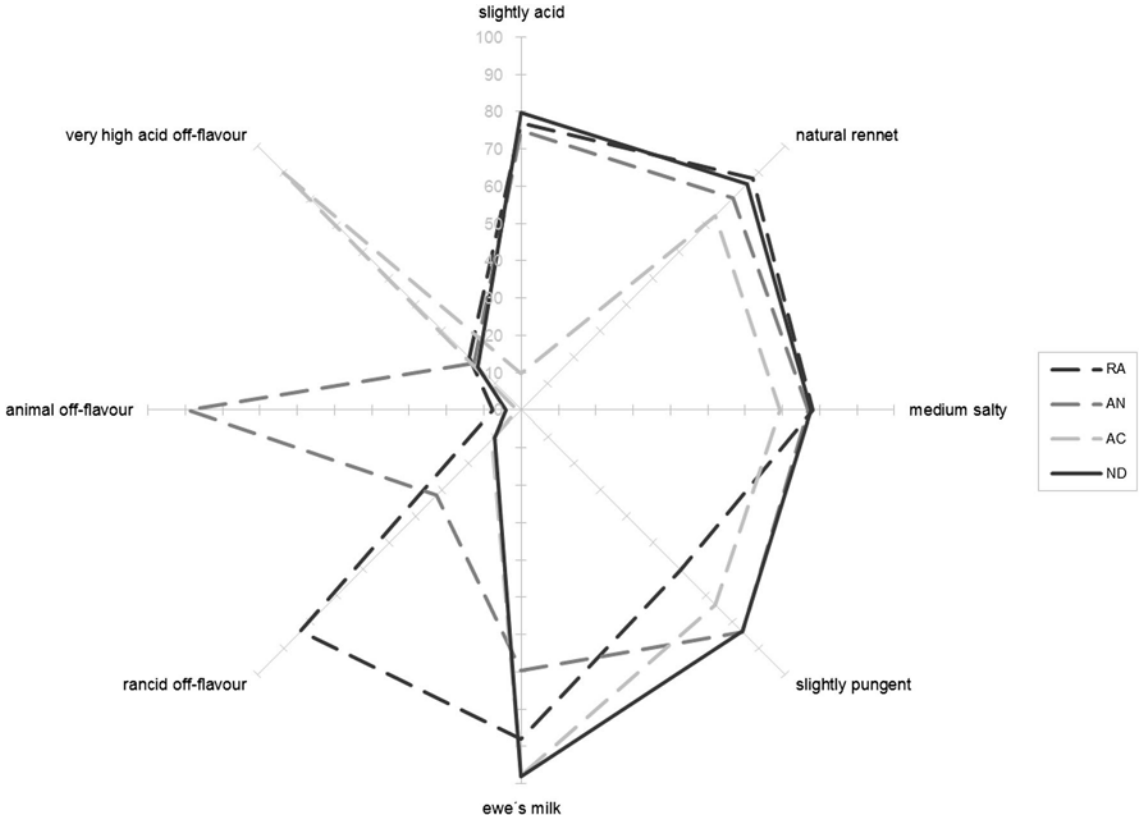

Fig. 1 Graph depicting the percentage of assessors (7) of the quality control panel that marked the presence of desirable flavours and off-flavours in commercial cheeses. *RA* rancid off-flavour cheeses ($n=13$); *AN* animal off-flavour cheeses ($n=8$); *AC* acid off-flavour cheeses ($n=13$); *ND* non-defective cheeses ($n=7$)

samples (<17% of the assessors). In contrast, RA, AN, and AC cheeses were marked with each of their respective off-flavours (rancid, animal and very high acid) by more than 84% of the assessors. With the expected exception of slightly acid flavour in AC cheeses, desirable flavours in off-flavour cheeses were marked at least by the 70% of the assessors. However, slightly pungent flavour was less frequently detected in RA cheeses than in AC and AN cheeses, and ewe's milk flavour was more frequently marked in AC and RA than in AN cheeses. It must be emphasized that even though slightly pungent flavour was marked by 60% of the assessors in RA cheeses, the remaining 40% described these cheeses as very pungent (data not shown). Medium salty and natural rennet flavours were less detected in AC cheeses.

Volatile profile of non-defective cheeses

The headspace of cheeses with desirable and balanced flavours (ND cheeses) showed 77 different volatile compounds belonging to ten chemical families: acids, ketones, alcohols, terpenes, lactones, esters, hydrocarbons, ethers, aldehydes and sulphur compounds (Table 2). ND cheeses (7 samples) were produced on different farmhouses, seasons and were collected with different ripening time (from 82 to 120 days). This fact was probably the main cause of the large variability found in the relative abundance of volatile compounds in these cheese samples.

The volatile profile of ND cheeses, along with their above mentioned sensory characteristics, was concordant with results previously reported on Idiazabal cheese variety [5, 14]. Acids were the most abundant chemical family in ND cheeses mainly due to the content of straight short-chain free fatty acids (FFA), in particular *n*-butanoic, *n*-hexanoic and *n*-octanoic (Table 2). These straights FFA are mainly formed by lipolysis of triglycerides during cheese ripening, but can be also formed from lactose and lactic acid fermentation or even by deamination reaction of amino acids [11, 12]. The contribution of FFA to cheese flavour is especially noteworthy in raw milk cheeses because milk lipoprotein lipase and other enzymes from microorganisms are not inactivated by any heat treatment, so its action can be extended during milk storage, cheesemaking process and ripening time [29]. Some studies have associated these straight volatile acids with desirable and characteristic acid, pungent and rennet flavours of particular

Table 2 Relative abundance in area arbitrary units of volatile compounds found in the headspace of non-defective commercial cheeses ($n=7$)

LRI	Compound	ID	Mean ± SD
	Acids		539 ± 524
1547	Acetic acid	P	4.31 ± 4.34
1614	2-Methylpropanoic acid	P	3.56 ± 2.47
1663	*n*-butanoic acid	P	244 ± 283
1736	3-Methylbutanoic acid	P	3.87 ± 10.2
1849	*n*-pentanoic acid	P	4.64 ± 6.32
1896	*n*-hexanoic acid	P	210 ± 318
2073	*n*-heptanoic acid	P	1.76 ± 2.56
2101	Hex-3-enoic acid	T	0.0734 ± 0.194
2154	*n*-octanoic acid	P	49.7 ± 49.6
2283	*n*-nonanoic acid	P	0.432 ± 0.501
2378	*n*-decanoic acid	P	15.5 ± 14.6
2471	Dec-9-enoic acid	T	0.235 ± 0.46
2638	*n*-dodecanoic acid	T	0.547 ± 0.523
	Ketones		113 ± 62.4
812	Propan-2-one	T	1.03 ± 0.786
895	Butan-2-one	P	42.2 ± 46.4
971	Pentan-2-one	P	11.6 ± 7.45
974	Butane-2,3-dione	P	8.04 ± 10.2
1011	3,7-Dimethyloct-2-ene	T	0.3 ± 0.583
1013	3-Methylpentan-2-one	T	0.308 ± 0.815
1031	3,7-Dimethylocta-1,6-diene	T	0.335 ± 0.347
1113	Hexan-2-one	P	0.439 ± 0.618
1186	Heptan-2-one	P	14.2 ± 14.1
1304	Octan-2-one	P	0.193 ± 0.23
1311	3-Hydroxybutan-2-one	T	24.4 ± 30.4
1383	Heptane-2,4-dione	T	0.14 ± 0.142
1398	Nonan-2-one	P	9.04 ± 13.6
1457	Non-8-en-2-one	T	0.441 ± 0.751
1468	Cyclohex-2-en-1-one	T	0.227 ± 0.173
	Alcohols		30.9 ± 19.2
926	Ethanol	P	8.58 ± 3.03
1021	Butan-2-ol	P	6.43 ± 16.3
1072	2-Ethyl-4-methylpentan-1-ol	T	0.214 ± 0.267
1100	2-Methylpropan-1-ol	T	0.0972 ± 0.228
1122	Prop-2-en-1-ol	T	0.108 ± 0.145
1125	Pentan-2-ol	P	3.71 ± 6.43
1150	Butan-1-ol	P	0.922 ± 1.32
1208	3-Methylbutan-1-ol	P	1.8 ± 2.55
1255	Pentan-1-ol	P	0.126 ± 0.334
1258	3-Methylbut-3-en-1-ol	T	0.592 ± 0.507
1330	Heptan-2-ol	P	0.833 ± 1.08
1335	2-Methylbut-2-en-1-ol	T	0.62 ± 0.818
1362	Hexan-1-ol	P	0.813 ± 1.11
1364	2-Methylpentan-3-ol	T	0.935 ± 1.22
1408	Hex-2-en-1-ol	T	0.0734 ± 0.126
1409	Cyclohexanol	T	0.175 ± 0.31
1464	Heptan-1-ol	P	0.123 ± 0.129
1520	Nonan-2-ol	P	0.165 ± 0.174
1906	Phenylmethanol	T	0.363 ± 0.725
1947	2-Phenylethanol	T	4.2 ± 11.1
	Hydrocarbons		17.9 ± 40.6
794	*n*-octane	P	0.33 ± 0.252
830	Oct-3-ene	T	0.549 ± 0.379
866	2,4-Dimethylhept-1-ene	T	0.156 ± 0.374
992	*n*-decane	P	0.697 ± 0.876
1040	toluene	P	15.1 ± 40.0
1792	dec-1-ene	T	1.02 ± 1.96
	Lactones		11.9 ± 10.5
1755	γ-Caprolactone	T	11.4 ± 10.6
1870	δ-Caprolactone	P	0.334 ± 0.483
2244	δ-Nonalactone	P	0.0819 ± 0.0373
	Esters		11.6 ± 7.96
878	Ethyl acetate	T	0.265 ± 0.584
1036	Ethyl butanoate	P	3.96 ± 3.367
1133	Butan-2-yl butanoate	T	0.178 ± 0.471
1137	Ethyl pentanoate	P	0.0719 ± 0.131
1242	Ethyl hexanoate	P	5.48 ± 6.687
1336	2-Methylpropyl hexanoate	T	0.301 ± 0.797
1377	2-Propan-2-yloxypropane	T	0.52 ± 0.427
1391	3-Oxobutan-2-yl acetate	T	0.247 ± 0.259
1445	Ethyl octanoate	P	0.446 ± 0.358
1653	Ethyl decanoate	P	0.135 ± 0.332
1890	Hexyl 2-methylpropanoate	T	0.344 ± 0.678
	Terpenes		1.75 ± 2.41
1013	α-pinene	P	0.107 ± 0.135
1141	*p*-xylene	P	1.41 ± 2.23
1195	D-limonene	P	0.229 ± 0.400
	Ethers		1.72 ± 1.87
1377	2-Propan-2-yloxypropane	T	1.72 ± 1.87
	Aldehydes		1.20 ± 0.688
911	3-Methylbutanal	P	0.115 ± 0.227
1085	*n*-Hexanal	P	0.423 ± 0.401
1402	*n*-Nonanal	P	0.295 ± 0.411
1562	Benzaldehyde	P	0.27 ± 0.354
	Sulphur compounds		0.0389 ± 0.0126
1481	3-Methylsulfanylpropanal	T	0.0389 ± 0.0126

LRI linear retention index, *ID* identification mode, *T* tentative identification, *P* positive identification, *SD* standard deviation

cheese varieties [5, 12, 30–33]. So, the highest relative abundance of straight acids mostly contributed to the desirable flavours slightly acid, pungent and natural rennet in ND cheeses. Branched-chain short-chain FFAs (2-methylpropanoic and 3-methylbutanoic acid) were detected in low abundance in ND cheeses. These compounds have very low perception thresholds and they impart lactic odour

notes to sheep and goat cheeses [11] and they could contribute, together with other compounds, to the ewe's milk flavour. Ketones were the second mayor chemical family in the headspace of ND cheeses being methyl ketones major compounds (Table 2). Butan-2-one and 3-hydroxybutan-2-one are generated from butane-2,3-one in the metabolism of citrate and lactose mainly by lactic acid bacteria [8, 10]. These ketones, with milky and sweet odour notes, could be key odourants associated with the ewe's milky desirable flavour of ND cheeses, as other authors reported for different cheese varieties [10–12, 33, 34]. Heptan-2-one and nonan-2-one showed considerable relative abundance in ND cheeses in comparison to other minor ketones. Their flavour has been described as musty and pungent [29, 35] and, therefore, they could contribute in part to the desirable slightly pungent flavour of ND cheeses.

A high number of alcohols, most of them in very small abundances, were found in ND cheeses (Table 2). Ethanol, 2-butanol and 2-pentanol were some of the most abundant alcohols but their contribution to the flavour of ND cheeses may be very low due to their high odour thresholds [32, 36]. 2-Phenylethanol found in ND cheese headspace is among the most odorous aromatic alcohol, but its floral rose-like odour note [11] was not described as typical desirable attribute for this cheese variety [36].

Lactones such as γ-caprolactone could be associated with the desirable milky flavour of these cheeses because they have very low odour threshold and contribute with buttery odour notes to cheese aroma [29, 37].

Among esters, ethyl hexanoate and butanoate were the most abundant compounds in ND cheeses as a result of the esterification of ethanol with n-butanoic and n-hexanoic acids, respectively. In particular, these fruity compounds have been associated with the typical and desirable rennet flavour of Idiazabal cheese [36].

Finally, terpenes, aldehydes, sulphur compounds, ethers, and other hydrocarbons were minor volatile compounds in the headspace of ND cheeses. Main aldehydes were n-hexanal and n-nonanal, which flavour was described as sharp and penetrating fruity [11, 38] and, therefore, they could also contribute to the slightly pungent flavour of ND cheeses.

Volatile compounds associated with off-flavour

PLSR analysis was performed on all volatile compounds found in off-flavour cheese samples to select those volatiles, specifically associated with AC, AN, and RA off-flavour cheeses. From the 170 different volatile compounds found in off-flavour cheeses, 13 compounds were specifically associated with RA cheeses, 7 compounds to AN cheeses and 5 compounds to AC cheeses (Table 3).

In general, very high abundance of short-chain FFA and ethyl butanoate was found in RA cheeses in comparison to ND, AN, and AC cheeses ($P \leq 0.1$). As other authors reported, an excessive concentration of carboxylic acids, mainly short and medium chain FFA, can lead to a rancid and lipolyzed flavour of cheese [3, 8]. As mentioned above, FFA are liberated from cheese fat triglycerides by lipases from different origin during ripening, so FFA concentration increases with time [10, 35]. However, RA cheeses were not more ripened than ND cheeses, so excessive lipolysis due to other causes should cause this off-flavour during ripening. Balanced concentrations of ethyl esters of *n*-butanoic and *n*-hexanoic acids have positive contribution to cheese flavour but in high concentration, as in RA cheeses, can contribute to rancid off-flavour [10, 32]. Furthermore, Alewijn et al. [29] found a relation between high levels of esters and short chain FFA, suggesting that the same esterase could be responsible for the release of short chain FFA and the formation of ethyl esters. Some authors found high levels of ethyl esters in cheeses contaminated with specific *Clostridium* strains [10].

3-Methylbutan-1-ol was the volatile compound most strongly associated with animal off-flavour. The abundance of this branched-chain alcohol was much greater in AN cheeses than in ND, RA and AC off-flavour cheeses ($P \leq 0.1$) (Table 3). Its odour has been described as unclean, animal and stable [8, 40] and its presence has been related to undesirable openings in some cheese varieties [4, 16]. This alcohol originates from the reduction of 3-methyl-1-butanal, produced in the Strecker degradation of Leu by lactic acid bacteria during cheese ripening [3, 11, 40]. Other aromatic alcohols were in high concentration in the headspace of AN cheeses in comparison to ND cheeses, and RA and AC off-flavour cheeses ($P \leq 0.1$) (Table 3). 2-Phenylethanol was related to off-flavour when generated in high concentration from catabolism of Phe by yeast and certain strains of lactic streptococci in Cheddar and Gruyere cheese [6, 35]. 4-Methylphenol is a strong odourant with animal, faecal and putrid odour notes which is formed during cheese ripening from catabolism of Tyr by Strecker degradation [8, 11, 34]. It has been found in Cheddar cheese contaminated by salt-resistant lactobacilli from inadequately filtered rennet [6]. The origin of these branched-chain and aromatic alcohols is mainly due to proteolytic spoilage of cheese [43]. Some microorganisms which may be present in raw milk such as *Pseudomona* and *Enterobacteriaceae* can grow at refrigeration temperatures (4 °C) and originate this type of off-flavour in milk and cheese [6, 44].

Volatile compounds mainly related to AC cheeses were acetic acid, 3-hydroxybutan-2-one and 2-hydroxypentan-3-one, the abundance of which was considerably higher in AC than in ND cheeses ($P \leq 0.05$). The flavour of acetic

Table 3 Relative abundance in area arbitrary units (mean ± standard deviation) of volatile compounds associated with off-flavour in selected rancid (RA, $n = 13$), animal (AN, $n = 8$), and very high acid (AC, $n = 13$) off-flavour cheeses

Compound[1]	Associated with off-flavour cheeses	RA cheeses	AN cheeses	AC cheeses	Comparison to ND cheeses			References[9]
					RA	AN	AC	
n-hexanoic acid	RA	1780 ± 2580^{a}	243 ± 306^{b}	282 ± 308^{b}	*	NS	NS	[16, 30, 39]
n-butanoic acid	RA	938 ± 542^{a}	289 ± 324^{b}	339 ± 257^{b}	**	NS	NS	[15, 16, 30, 39]
n-octanoic acid	RA	339 ± 458^{a}	38.6 ± 45.0^{b}	53.5 ± 46.8^{b}	*	NS	NS	[16, 30]
n-decanoic acid	RA	121 ± 174^{a}	11.1 ± 12.6^{b}	17.0 ± 14.0^{b}	*	NS	NS	[16, 30]
Ethyl hexanoate	RA	82.3 ± 143^{a}	4.73 ± 4.66^{b}	5.06 ± 5.95^{b}	NS	NS	NS	[10, 29, 30, 32]
Ethyl butanoate	RA	46.7 ± 61.2^{a}	19.2 ± 15.0^{ab}	7.38 ± 9.76^{b}	*	*	NS	[10, 29, 30, 32]
n-heptanoic acid	RA	11.0 ± 12.8^{a}	1.43 ± 1.83^{b}	1.55 ± 1.64^{b}	*	NS	NS	[16, 30]
Dec-9-enoic acid	RA	3.53 ± 4.16^{a}	0.222 ± 0.411^{b}	0.337 ± 0.646^{b}	**	NS	NS	[16]
n-nonanoic acid	RA	3.22 ± 4.01^{a}	0.358 ± 0.397^{b}	0.420 ± 0.428^{b}	*	NS	NS	[30]
Butyl butanoate[2]	RA	2.69 ± 4.76^{a}	0.0378 ± 0.107^{b}	0.0388 ± 0.109^{b}	†	†	†	[10, 29, 32]
Propyl hexanoate[3]	RA	2.60 ± 4.76	0.131 ± 0.370	Not detected	†	†	†	[10, 29, 32]
Butyl hexanoate[4]	RA	1.33 ± 2.42	Not detected	Not detected	†	†	†	[10, 16, 29, 30, 32]
Ethyl pentanoate	RA	1.14 ± 1.49^{a}	0.100 ± 0.202^{b}	0.0554 ± 0.111^{b}	*	NS	NS	[10, 16, 29, 32]
3-Methylbutan-1-ol	AN	2.97 ± 2.11^{b}	36.9 ± 46.6^{a}	3.30 ± 3.13^{b}	NS	*	NS	[4, 8, 16, 30, 40]
2-Phenylethanol	AN	Not detected	13.5 ± 14.5^{a}	2.01 ± 7.26^{b}	‡	*	NS	[6, 16, 30, 35]
2-Methylpropan-1-ol	AN	0.0563 ± 0.138^{b}	3.28 ± 4.21^{a}	0.198 ± 0.236^{b}	NS	*	NS	[41]
2-Methylpropyl 2-methylbutanoate	AN	0.0329 ± 0.119^{b}	1.28 ± 1.86^{a}	Not detected	NS	NS	NS	[5] [10]
4-Methylphenol[5]	AN	0.0918 ± 0.194^{ab}	0.161 ± 0.141^{a}	0.0153 ± 0.0553^{b}	†	†	†	[6, 8, 11, 16, 34]
Propyl 2-methylbutanoate[6]	AN	0.0865 ± 0.184^{b}	0.156 ± 0.291^{a}	Not detected	†	†	†	[5] [10]
Heptane-2,3-dione[7]	AN	Not detected	0.226 ± 0.00824	0.00824 ± 0.0297^{b}	†	†	†	[42] [10]
3-Hydroxybutan-2-one	AC	28.8 ± 41.3^{ab}	12.3 ± 12.6^{b}	59.7 ± 35.7^{a}	NS	NS	**	[14 ,16, 30]
Acetic acid	AC	19.6 ± 17.9^{b}	18.2 ± 19.2^{b}	52.3 ± 48.5^{a}	**	NS	**	[14, 15, 30]
Butane-2,3-dione	AC	2.72 ± 4.98^{b}	2.97 ± 5.38^{b}	16.8 ± 12.8^{a}	*	NS	*	[11, 34]
2-Methylpentan-3-ol	AC	0.0399 ± 0.144^{b}	0.312 ± 0.579^{b}	2.26 ± 2.21^{a}	**	NS	NS	[11, 34]
2-Hydroxypentan-3-one[8]	AC	0.0565 ± 0.138^{b}	0.115 ± 0.180^{b}	0.886 ± 0.857^{a}	†	†	†	[11, 34]

NS not significant

** $P \leq 0.05$; * $P \leq 0.1$

†Not detected in non-defective cheeses (ND, $n = 7$)

‡Detected in ND cheeses but not in RA cheeses

[a,b]Means of RA, AN and AC cheeses followed by a different superscript letter in the same row are significantly ($P \leq 0.1$) different

[1]LRI values and ID (T or P) for volatile compounds are shown in Table 1. [2]LRI = 1239 and ID = T; [3]LRI=1333 and ID = T; [4]LRI = 1420 and ID = T; [5]LRI = 2107 and ID = T; [6]LRI = 1193 and ID = T; [7]LRI = 1157 and ID = T; [8]LRI = 1376 and ID = T; [9]Literature references where volatile compounds were previously identified in cheese; [10]Position isomers or different chain length of branched-chain esters were identified

acid has been described as acid, vinegar, sour and sharp, while ketones as above mentioned provide milky and sweet odour notes to cheese [11, 34, 38]. Therefore, the very high acid off-flavour of AC cheeses should most likely due to the high abundance of acetic acid. The increase in abundance of ketones and acetic acid in AC cheeses compared to ND and RA and AN cheeses appeared to be related to an strong citrate and lactate metabolism by lactic acid bacteria. Excessive whey retention in cheese caused by weak press and short or slow ripening leads to a high lactose concentration available for lactic acid bacteria, and may cause an excessive glycolysis of citrate and lactate [8, 11, 44]. In fact, as above mentioned, AC cheeses were less ripened than ND cheeses, RA and AN off-flavour cheeses.

Volatile ratios

The mere presence of an individual volatile compound might not be directly associated with the appearance of off-flavour because cheese flavour results from the interaction and balance between different compounds. For this reason, a new approach based on volatile abundance ratios

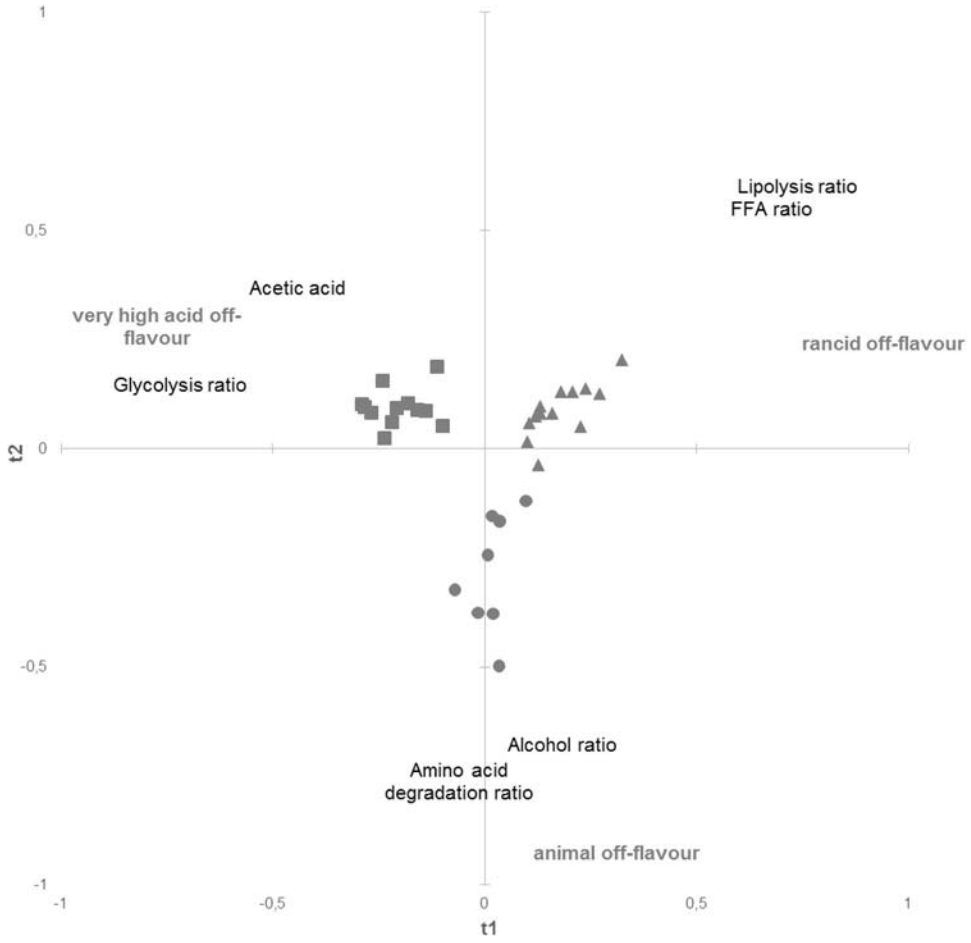

Fig. 2 Plot for the two first components corresponding to the partial least square regression (PLSR) analysis on sensory scores and volatile abundance ratios of off-flavour commercial cheeses. *Filled triangle* rancid off-flavour cheeses; *filled circle* animal off-flavour cheeses; *filled square* acid off-flavour cheeses

was used to explain RA, AN and AC off-flavour generation in cheese. The following ratios were calculated using previously selected volatile compounds (Table 3): glycolysis ratio was the sum of abundance of 3-hydroxybutan-2-one and butane-2,3-dione divided by total volatile abundance; alcohol ratio was the total alcohol abundance divided by total abundance of volatiles excluding alcohols and acids; amino acid degradation ratio was the sum of abundance of 3-methylbutan-1-ol, 2-phenylethanol and 4-methylphenol divided by total volatile abundance; FFA ratio was the total FFA abundance divided by total abundance of volatiles other than acids; and lipolysis ratio was the sum of abundance of *n*-butanoic and *n*-hexanoic acids divided by total abundance of volatiles other than acids. A PLSR analysis was performed on RA, AN and AC cheeses using volatile ratios, acetic acid abundance, and off-flavour sensory scores. Off-flavour cheeses were clearly differentiated in the PLSR plot of the first two components which explained 70.4% of variance for off-flavour sensory scores, and 67.8% for volatile ratios. Along the positive values of the first component axis (t1) rancid off-flavour was associated with FFA and lipolysis ratios, whereas on the negative values of t1 very high acid off-flavour was associated with glycolysis ratio and acetic acid abundance. Animal off-flavour was associated with alcohol and amino acid degradation ratios on the negative values of the second component axis (t2). These results confirmed those above-mentioned potential relationships between the presence of individual volatiles and the generation of off-flavours in the commercial cheeses. Furthermore, t1 component mainly differentiated AC cheeses, with scores placed close to the loadings of glycolysis ratio and acetic acid abundance, from RA cheeses with scores near loadings of lipolysis and FFA ratios. The t2 component differentiated between AC and RA cheese from AN cheeses with scores close to the loadings of amino acid degradation and alcohol ratios (Fig. 2).

Conclusions

The results obtained in this study mainly contribute to the identification of volatile compounds involved in off-flavour generation in cheese. PLSR was a useful tool to select those volatile compounds, and specific volatile abundance ratios, mainly associated with rancid, animal and acid off-flavours in cheese.

Most volatiles associated with off-flavours were found in the headspace of desirable flavour commercial cheeses and the excessive abundance, or imbalanced ratio, of some of them caused off-flavour. Rancid off-flavour was caused by excessive abundance of short-chain fatty acids, in particular *n*-butanoic and *n*-hexanoic acids, generated by a strong lipolytic activity during cheese ripening. Animal off-flavour was associated with an unbalanced proportion of 3-methylbutan-1-ol, 2-phenylethanol and 4-methylphenol which were produced by catabolism of free amino acids generated after intense proteolysis during cheese ripening. Acid off-flavour was strongly related to high proportions of acetic acid and ketones formed mainly by glycolysis at early stages of cheese ripening. The strong sourness of acetic acid was most likely responsible for acid off-flavour in cheese. The identification of specific volatile compounds and abundance volatile ratios associated with off-flavour in cheese could be of interest for dairy sector because it could help in the development of automated techniques to detect off-flavours, and to minimise the risk of off-flavour generation during ripening.

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Compliance with ethical standards

Conflict of interest Laura Zabaleta, Marta Albisu and Luis Javier R. Barron declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

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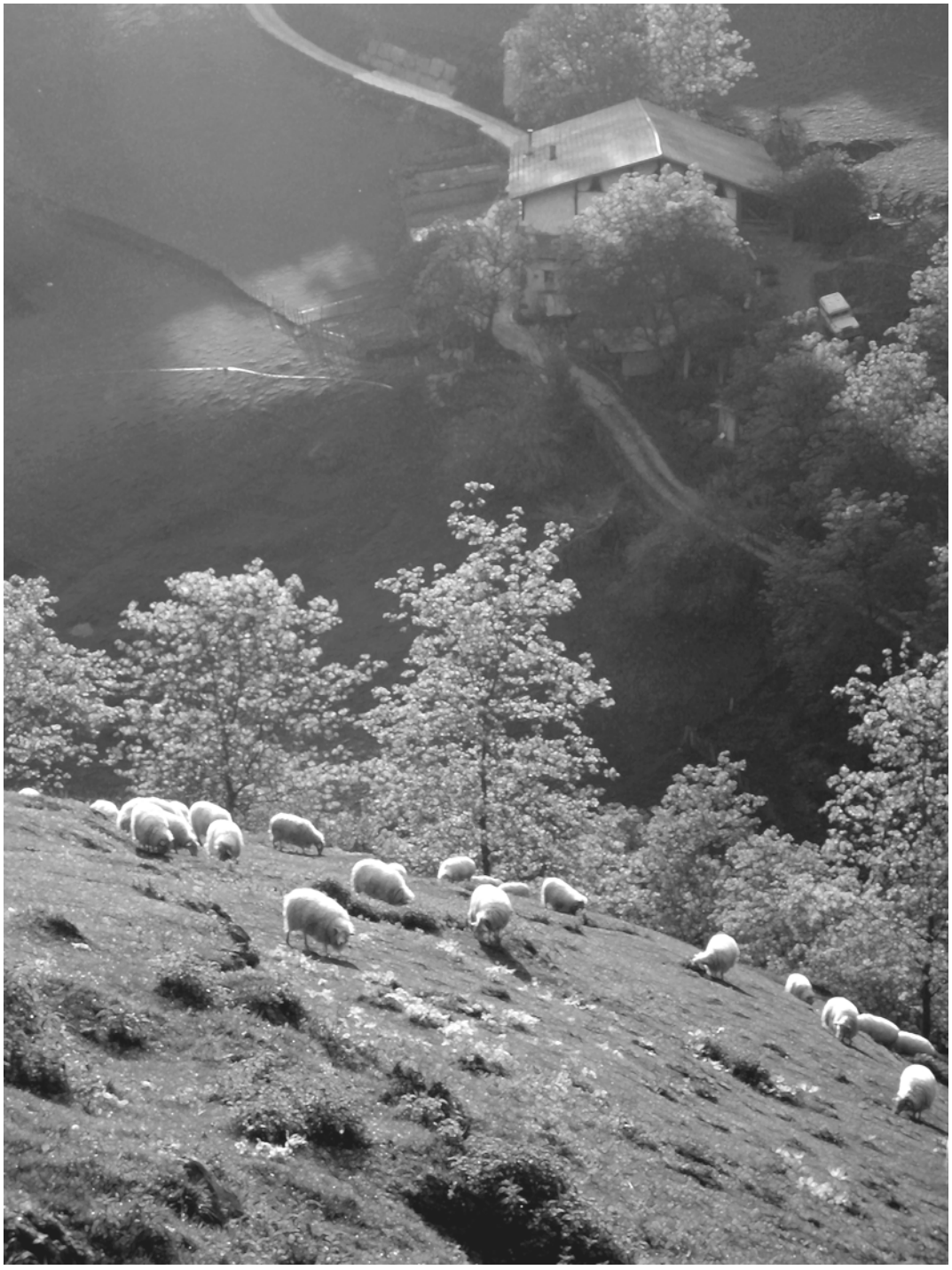


6. Discusión general

En este apartado se discuten los principales resultados de esta Tesis Doctoral. El *Chapter I* de la sección *Results and discussion* se centra en la investigación sobre la frecuencia de aparición de defectos sensoriales en quesos comerciales elaborados a partir de leche cruda de oveja a lo largo de cinco campañas anuales de producción. Los resultados de este primer estudio mostraron que los parámetros de producción que mayor relación presentaban con la aparición de defectos sensoriales en los quesos comerciales fueron el tiempo de maduración y la época de elaboración del queso.

Los *Chapter II y III* recogen las investigaciones dirigidas a analizar la relación entre los compuestos volátiles del queso y la presencia de defectos sensoriales. Se identificaron tres defectos de flavor relevantes para el sector productor quesero: flavor excesivamente ácido, animal y rancio. Mientras el *Chapter II* se centra en la identificación de compuestos odorantes responsables de dichos defectos de flavor, el *Chapter III* compara el perfil de compuestos volátiles de quesos con defectos de flavor ácido, animal y rancio con el de quesos comerciales sin defectos sensoriales.

A continuación se presenta el resumen y la discusión de los resultados más relevantes.

6.1. Frecuencia de aparición de defectos sensoriales en quesos comerciales

En las investigación realizada se compararon las frecuencias de aparición de defectos sensoriales en quesos comerciales de leche cruda de oveja elaborados en cinco campañas de producción consecutivas. El análisis de los datos mostró que las variaciones de las frecuencias de aparición de los defectos fueron pequeñas entre

campañas y que, por lo tanto, los defectos presentaban una tendencia similar en su aparición.

Los defectos sensoriales observados con mayor frecuencia fueron los relacionados con la presencia de ojos, principalmente cavernas y grietas en la pasta. Encontrar una causa específica como origen de estos defectos es difícil ya que los ojos pueden estar provocados por diversos factores que pueden actuar de forma individual o combinada. Algunas posibles causas de este defecto son un crecimiento microbiano no deseado, una composición mineral anormal, un drenaje incompleto del lactosuero, o condiciones inadecuadas de humedad y/o temperatura en las cámaras de maduración (Mahaut et al., 2003).

La aparición de ojos en los quesos puede originarse por acumulación de gases como hidrógeno y dióxido de carbono producidos por el metabolismo de *Clostridium spp*. (Le Bourhis et al., 2007) o de otros microorganismos como coliformes, levaduras y bacterias ácido lácticas heterofermentativas (Mahaut et al., 2003). El tamaño y la forma de los ojos está determinado por factores como la cantidad de gas producido, el efecto de la temperatura sobre la solubilidad del gas, y las características de la textura en la pasta (Sheehan, 2011).

En otras ocasiones, los ojos son originados mecánicamente por un drenaje excesivamente intenso de la cuajada en el corte, que podría conferir una consistencia friable a la pasta, originando grietas tanto en el interior como en la corteza del queso (Tornadijo, Marra, & Carballo, 1998), o por un insuficiente o rápido prensado (Ortiz, 2005).

Los principales defectos de color de la pasta fueron la presencia de cerco excesivamente ancho y/o marcado, color irregular y

demasiado oscuro, o por el contrario, muy blanco. Un exceso de sal en la pasta, temperaturas superiores a 10-12 ºC, y/o excesiva velocidad de aire en las cámaras de maduración pueden contribuir a una pérdida excesiva de humedad y, por lo tanto, ser la causa del color oscuro y de la formación de un cerco demasiado ancho o marcado. Por otra parte, el color irregular se ha relacionado principalmente con un tamaño de granulo no homogéneo durante el corte de la cuajada, dando como resultado una pasta con zonas más húmedas que otras (Mahaut et al., 2003; McSweeney, 2007).

Los defectos predominantes en la corteza fueron coloraciones no deseadas (puntos negros, amarillos y naranjas) así como marcas de bandeja. Las coloraciones anormales en la corteza del queso pueden ser debidas a la actuación de levaduras, mohos o poblaciones bacterianas favorecida por las condiciones ambientales en las cámaras de oreo y de maduración (Amato et al., 2012; Mahaut et al., 2003; McSweeney, 2007). Sin embargo, las marcas de bandeja son causadas generalmente por un volteo insuficiente de los quesos durante el periodo temprano de la maduración.

Aunque la frecuencia de aparición de los defectos de flavor fue menor en comparación con los defectos anteriormente comentados, estos flavores indeseables generan gran preocupación en el sector elaborador y son considerados defectos graves en el queso. La excesiva acidez, los flavores animales (también denominados sucios o fecales) y el sabor excesivamente amargo fueron los defectos de flavor más frecuentes. El flavor rancio, aunque fue poco frecuente, se presentó en mayor proporción en los quesos con una maduración de 3 - 5 meses. Algunos autores han atribuido la rancidez a una lipolisis excesiva o desequilibrada que conduce a una excesiva liberación de AGLs durante la maduración del queso, siendo de especial relevancia la acumulación de AGLs de cadena corta (Fox & Wallace, 1997; Fox et al., 2004; McSweeney & Sousa,

2000). La presencia de una excesiva acidez en el queso podría tener su origen en la adición de una excesiva cantidad de cultivo iniciador, una concentración insuficiente de sal, y/o una concentración excesiva de lactosa en la pasta debido a un prensado demasiado débil junto con bajas temperaturas en la sala de prensado. Las bacterias ácido lácticas metabolizan la lactosa disponible produciendo ácido láctico y contribuyendo al aumento de la acidez del queso (Kindstedt, 2005). En relación con el defecto de flavor animal, el metabolismo bacteriano, principalmente de coliformes, parece ser el principal causante de su desarrollo en diversos tipos de quesos (Ummadi & Weimer, 2001). En algunos tipos de queso como el Cheddar, el amargor se considera el principal defecto de flavor (Engel et al., 2001). Una excesiva concentración de aminoácidos y péptidos de pequeño tamaño, originados por la hidrólisis de las caseínas, podría ser la causa de este defecto en quesos duros y semiduros (Broadbent et al., 2002; McSweeney, 2007). Sin embargo, en los quesos comerciales analizados, sólo el 4 % de las muestras presentó defecto por amargor.

Los principales defectos de textura fueron una excesiva pastosidad (adherencia) y quesos excesivamente blandos. Estos defectos se han descrito en la literatura como consecuencia de un insuficiente drenaje de la pasta (McSweeney, 2007) aunque otros autores los han relacionado con el tipo y cantidad de cuajo utilizado en la fabricación del queso (Pirisi et al., 2007).

El abombamiento y convexidad fueron los defectos de forma más frecuentes en los quesos comerciales. El abombamiento es debido en general a la acumulación de gas producido por diferentes tipos de microorganismos anteriormente comentados, siendo el hinchamiento tardío el defecto más conocido y estudiado. Este

hinchamiento es causado por la actividad de *Clostridium spp.* durante la maduración del queso, microorganismo que metaboliza el lactato produciendo ácido butírico y gases, lo que provoca no solo el abombamiento y grandes grietas en la pasta, sino también defectos de flavor (Garde et al., 2012; Le Bourhis et al., 2007; Sheehan, 2011).

6.2. Efecto de la maduración y la época de elaboración en la presencia de defectos sensoriales

La frecuencia de aparición de quesos comerciales con pasta blanca, textura blanda y pastosa, y exceso de acidez fue significativamente mayor en aquellos con maduración corta. Los quesos de maduración media y larga presentaron con mayor frecuencia un exceso de cerco, con un color de la pasta demasiado oscuro, flavor animal y marcas excesivas de bandeja en la corteza. Los cambios en la composición química causados por la pérdida de humedad y los procesos bioquímicos que se producen durante la maduración pueden afectar a la textura, color y sabor originando quesos más duros, con un color más oscuro y un sabor más intenso. En este sentido, una temperatura demasiado alta en las cámaras de maduración puede acelerar el proceso de maduración y contribuir a la aparición de estos defectos sensoriales (Ortiz, 2005).

En cuanto a la época de elaboración del queso, defectos como abombamiento, presencia de cavernas y ojos mal distribuidos en la pasta fueron más frecuentes en las elaboraciones de invierno y primavera. Estos defectos pueden ser provocados por hinchamiento tardío por contaminación con *Clostridium spp.* que pueden estar asociadas al uso de forrajes ensilados para la alimentación de los animales cuando están estabulados (Garde et al., 2012; McSweeney, 2007). En los quesos elaborados en invierno

aparecieron con más frequencia el sabor excesivamente ácido y la textura blanda y pastosa. En general, la sala de prensa de las queserías muestreadas no se encontraba climatizada y las bajas temperaturas en el invierno podrían haber ocasionado un drenaje insuficiente de la pasta, por lo que estos quesos presentaron una textura más blanda y con más lactosa disponible para ser transformada en ácido láctico (McSweeney, 2007). Los quesos de primavera presentaron con mayor frecuencia de ojos numerosos y redondos y flavor animal. En la primavera comienza el pastoreo de los rebaños, la alta humedad y la templada temperatura ambiental aumenta la probabilidad de contaminación microbiana de las ubres de los animales.y la aparición de estos defectos (Walstra et al., 2001). Grietas en la pasta y presencia de coloraciones irregulares, tanto en pasta como en corteza, fueron más frecuentes en los quesos de verano. Debido a la menor producción de leche de las ovejas en esta época del año (final del periodo de lactación) se combinan ordeños de varios días para tener un volumen suficiente de leche para elaborar los quesos (Endrizzi et al., 2012). En consecuencia, la leche cruda es almacenada en frío (entre 2-6 ºC) durante largos períodos (hasta 48-72 h) lo cual favorece la proliferación de microorganismos psicrótrofos en la leche; cuya actividad proteolítica altera la consistencia del queso, dando como resultado una pasta que se agrieta con más facilidad después del prensado (Fonseca, Bordin, Neff, & Oliveira, 2012; Malacarne et al., 2013).

El estudio de las relaciones entre defectos sensoriales en los quesos se llevó a cabo mediante el análisis MCA. Los resultados mostraron una relación entre los defectos color de pasta blanca, textura blanda y pastosa, flavor ácido y amargo, y marcas en la corteza. Estos defectos están relacionados con una maduración insuficiente

o con un alto contenido de humedad del queso, que puede favorecer el crecimiento de microorganismos y, en consecuencia, ocasionar excesiva proteólisis durante la maduración. (Izco, Irigoyen, Torre, & Barcina, 2000). En el mismo factor de correspondencia, pero con valores negativos, se agruparon: forma abombada, el color de pasta oscuro y el exceso de cerco, defectos presentes especialmente en los quesos comerciales de media y larga maduración.

Un segundo factor agrupó los quesos convexos, con ojos numerosos y redondos, y el flavor animal. Tal y como se ha indicado anteriormente, el crecimiento de coliformes puede ser la principal causa de la presencia de numerosos ojos pequeños y redondos en la pasta del queso, así como del flavor animal (Ayad et al., 2004; Ortiz, 2005).

6.3. Defectos de flavor en quesos comerciales: compuestos odorantes y perfil característico de quesos con defectos

Con objeto de identificar los compuestos odorantes y perfiles de compuestos volátiles relacionados con los defectos de excesiva acidez, rancidez y flavor animal, se estudió en profundidad la composición de compuestos volátiles de quesos comerciales que presentaban de forma independiente estos defectos de flavor, y se comparó con la de quesos comerciales sin defectos.

Los quesos sin defectos se caracterizaron por presentar una alta abundancia de AGLs de cadena corta lineal como los ácidos n-butanoico, n-hexanoico y n-octanoico. Estos compuestos se originan principalmente por hidrólisis de triglicéridos durante la maduración del queso, pero también a partir de la fermentación del ácido láctico o por reacción de desaminación de aminoácidos (Curioni & Bosset, 2002). La lipólisis es especialmente notable en

esta variedad de queso ya que, por un lado, se elabora con leche cruda en la que se mantiene la actividad de la lipoproteína-lipasa y otras lipasas procedentes de microorganismos endógenos de la leche (Alewijn, Sliwinski, & Wouters, 2005). Además, el uso de la pasta de cuajo de cordero artesanal es muy habitual en este tipo de queso. Esta pasta de cuajo contiene una lipasa pregástrica que cataliza una intensa hidrólisis de la posición sn-3 del triglicérido, donde generalmente se encuentran esterificados los ácidos grasos de cadena corta (Amores et al., 2013). Actualmente, se conoce que estos compuestos, en un adecuado equilibrio con el resto de compuestos volátiles contribuyen en gran medida al olor característico a cuajo natural y con flavor ligeramente picante de diferentes variedades de queso semiduro tales como Idiazabal, Ossau-Iraty, Manchego, Reggianito Argentino y Cheddar (Amores et al., 2013; Barron et al., 2005; Bosset & Gauch, 1993; Innocente et al., 2013; Izco et al., 2000; Ortigosa, Torre, & Izco, 2001; Wolf, Perotti, Bernal, & Zalazar, 2010).

Las cetonas como 2-butanona y 3-hidroxi-2-butanona generadas a partir del metabolismos del citrato y la lactosa por las bacterias acido lácticas fueron la segunda familia química más abundante en los quesos sin defectos. Estas metil-cetonas son consideradas compuestos odorantes clave en muchos tipos de queso aportando olores lácteos y dulces (Curioni & Bosset, 2002; Gómez-Torres et al., 2015).

Se detectó un gran número de alcoholes en los quesos sin defectos, la mayoría de ellos presentes en muy baja abundancia. Etanol, 2-butanol y 2-pentanol fueron algunos de los alcoholes más abundantes aunque su olor no fue detectado por los evaluadores sensoriales debido a sus umbrales de olor altos (Barron et al., 2005; Wolf et al., 2010). Entre los ésteres, el hexanoato y butanoato

de etilo fueron los más abundantes y fueron descritos por los evaluadores sensoriales como florales y afrutados, y en estudios previos han sido relacionados también con el flavor a cuajo (Barron et al., 2005). Otros compuestos odorantes como las lactonas con muy bajo umbral de olor, contribuyeron al flavor del queso sin defectos con notas de olor descritas como floral, afrutado, coco, y mantequilla (Alewijn et al., 2005; Fox et al., 2004).

Una vez descrito el perfil volátil de los quesos sin defectos, se analizaron los quesos con defectos de flavor excesivamente ácido, rancio y animal. El análisis PLSR fue utilizado para identificar aquellos compuestos volátiles asociados, y/o responsables, de los defectos de flavor.

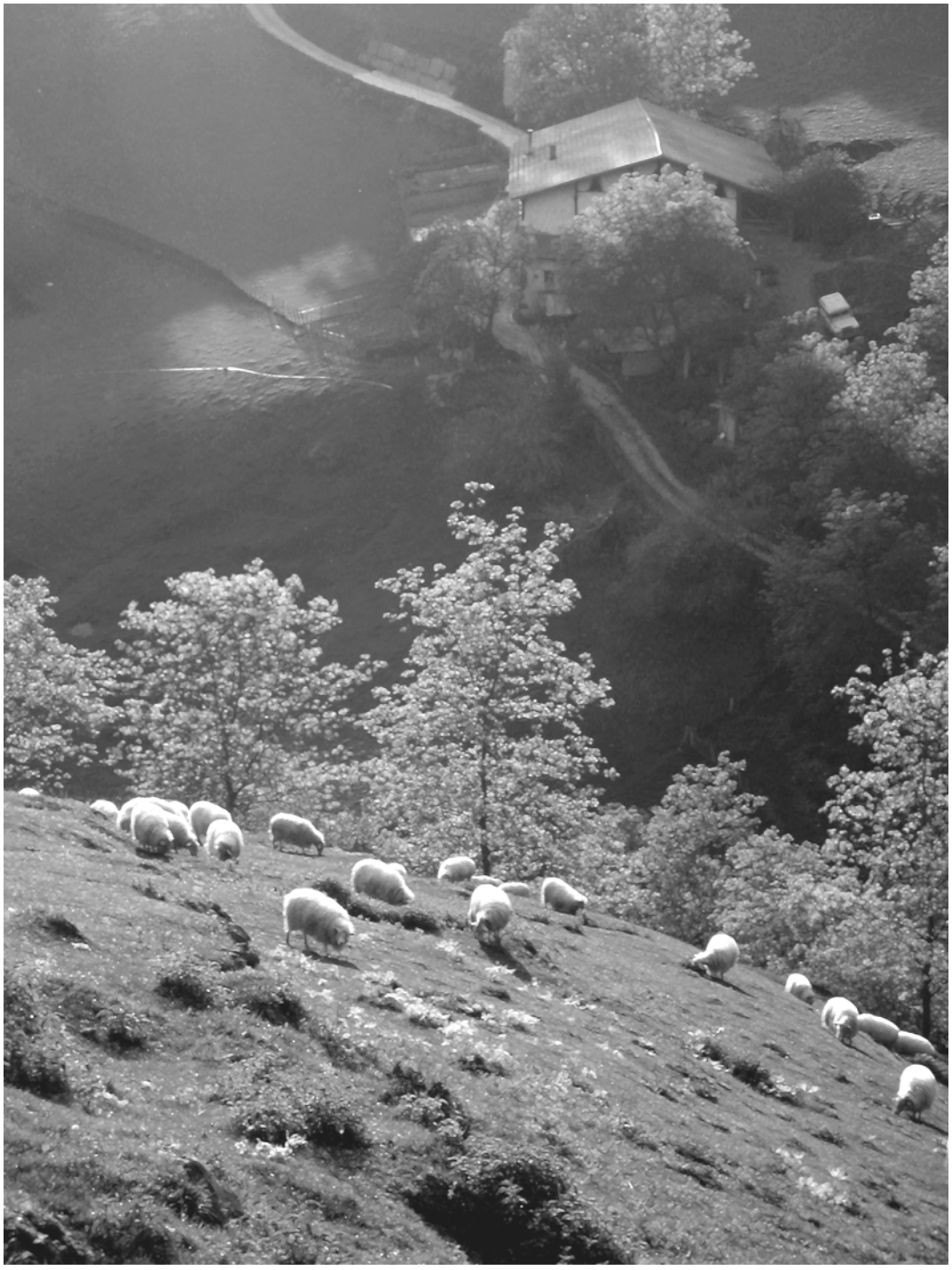
El flavor excesivamente ácido se relacionó principalmente con una alta concentración de ácido acético en el queso. El análisis olfatométrico del ácido acético fue descrito como ácido y a vinagre. A diferencia de otros ácidos volátiles, su origen no es lipolítico sino que se origina como resultado del metabolismo del lactato y citrato por las bacterias ácido lácticas, o bien por catabolismo de los aminoácidos Ala y Ser (McSweeney & Sousa, 2000; Ziino, Condurso, Romeo, Giuffrida, & Verzera, 2005). En este sentido, el análisis PLSR asoció también la abundancia de algunas cetonas como 3-hidroxi-2-butanona y 2-hidroxi-3-pentanona, generadas también a partir de la glucólisis, con los quesos con defecto de acidez. El tiempo medio de maduración de los quesos con defecto ácido fue de 78 ± 5 días; inferior al tiempo medio de maduración del resto de los quesos comerciales analizados (98 ± 19 días). Este puede ser uno de los motivos por los que la concentración de suero sea mayor en la pasta de quesos con defecto de flavor ácido, defecto que puede ir desapareciendo progresivamente con el tiempo de maduración.

El defecto de flavor animal se asoció con la presencia de algunos compuestos fenólicos y alcoholes. El compuesto volátil más fuertemente asociado con el defecto de flavor animal fue 3-metilbutanol, el cual fue descrito por los evaluadores sensoriales como quemado y herbal, aunque en la literatura se ha descrito también como animal y establo (Garde, Ávila, Medina, & Nuñez, 2005; McSweeney & Sousa, 2000; Morales, Fernández-García, Gaya, & Nuñez, 2003). El origen de este compuesto proviene de la reducción de 3-metil-1-butanal, producido por catabolismo del aminoácido Leu (Curioni & Bosset, 2002). El 4-metilfenol (*p-cresol*), originado por el catabolismo de la Tyr, también fue asociado con el flavor animal. Este compuesto se caracteriza por un bajo umbral de reconocimiento y su olor fue descrito como animal, a heces y a pútrido por los evaluadores.

Los quesos con flavor rancio se asociaron con una abundancia excesiva de AGLs de cadena corta lineal (del ácido n-butanoico a n-decanoico), cuyo olor fue descrito como sudor, queso y rancio, y de esteres de etilo como el hexanoato de etilo. La aparición del flavor rancio se produjo de igual manera en quesos de corta, media o larga maduración, por lo que una lipólisis excesiva para su tiempo de maduración podría ser la causa de este defecto. Los AGLs son también precursores de otros compuestos odorantes debido a su oxidación a metil-cetonas, y posterior reducción a alcoholes secundarios, o también a la formación de ésteres con alcoholes primarios (Fox & Wallace, 1997; McSweeney & Sousa, 2000; Smit et al., 2005).

Como se ha indicado anteriormente, el flavor del queso es el resultado de la interacción y el equilibrio entre los diferentes compuestos químicos que lo componen, por lo que la mera presencia de un único compuesto odorante podría no estar

directamente asociada a la aparición de un defecto de flavor. Por esta razón se planteó un nuevo enfoque basado enla identificación de relaciones de abundancia entre compuestos volátiles presentes en los quesos con defectos de flavor. Las relaciones entre estos compuestos, detalladas en el manuscrito correspondiente al *Chapter III*, hacen referencia a compuestos odorantes y rutas bioquímicas responsables de la generación de flavor en el queso. El grado de asociación entre relaciones de compuestos volátiles y los defectos de flavor se estudió mediante el análisis PLSR. Los resultados mostraron asociaciones diferentes para los quesos en función del defecto que presentaron: excesiva acidez, rancidez y flavor animal. Los quesos excesivamente ácidos se asociaron fuertemente con el *índice de acidez y glucólisis*, los quesos con flavor rancio con el *índice de lipólisis* y la *relación de AGLs*, y el *índice de degradación de aminoácidos* y la *relación de alcoholes* con los quesos con flavor animal. Por lo tanto, estos resultados indicaron que la determinación de estos índices y/o relaciones entre compuestos volátiles puede ser útil para la identificación y prevención de flavores no deseados en el queso.



7. Conclusions

From this research conducted on the characterization of sensory defects in commercial cheeses, the following conclusions were drawn:

1. The most frequent sensory defects found over five consecutive yearly cheese productions were undesired openings inside the cheeses that could be associated to the presence of microorganisms in raw milk or to mechanical origin. Undesired openings appeared during the first two months of ripening while the ripening time had no influence on their frequency. An early detection of the openings could improve the profitability of the production.

2. The presence of off-flavour was less frequent compared to texture and appearance defects in commercial cheeses. However, their study is of great importance since their presence can negatively influence the final quality of cheeses. Excessive acidity, bitternes, animal an rancid off-flavours were the most abundant ones.

3. The relationships found among sensory defects may help to predict the presence of off-flavour by evaluating texture and appearance defects.

4. Different types of openings have been associated with different off-flavour and specific seasons suggesting different origins. Therefore, openings should be described in a differentiated manner.

5. Cheeses ripened over short and medium periods of time (2 to 4 months) showed high presence of flavour and texture defects mainly related to excessive acidity and melty texture.

Therefore, it is recommended to ripen the cheeses over four months in order to avoid the display of these defects.

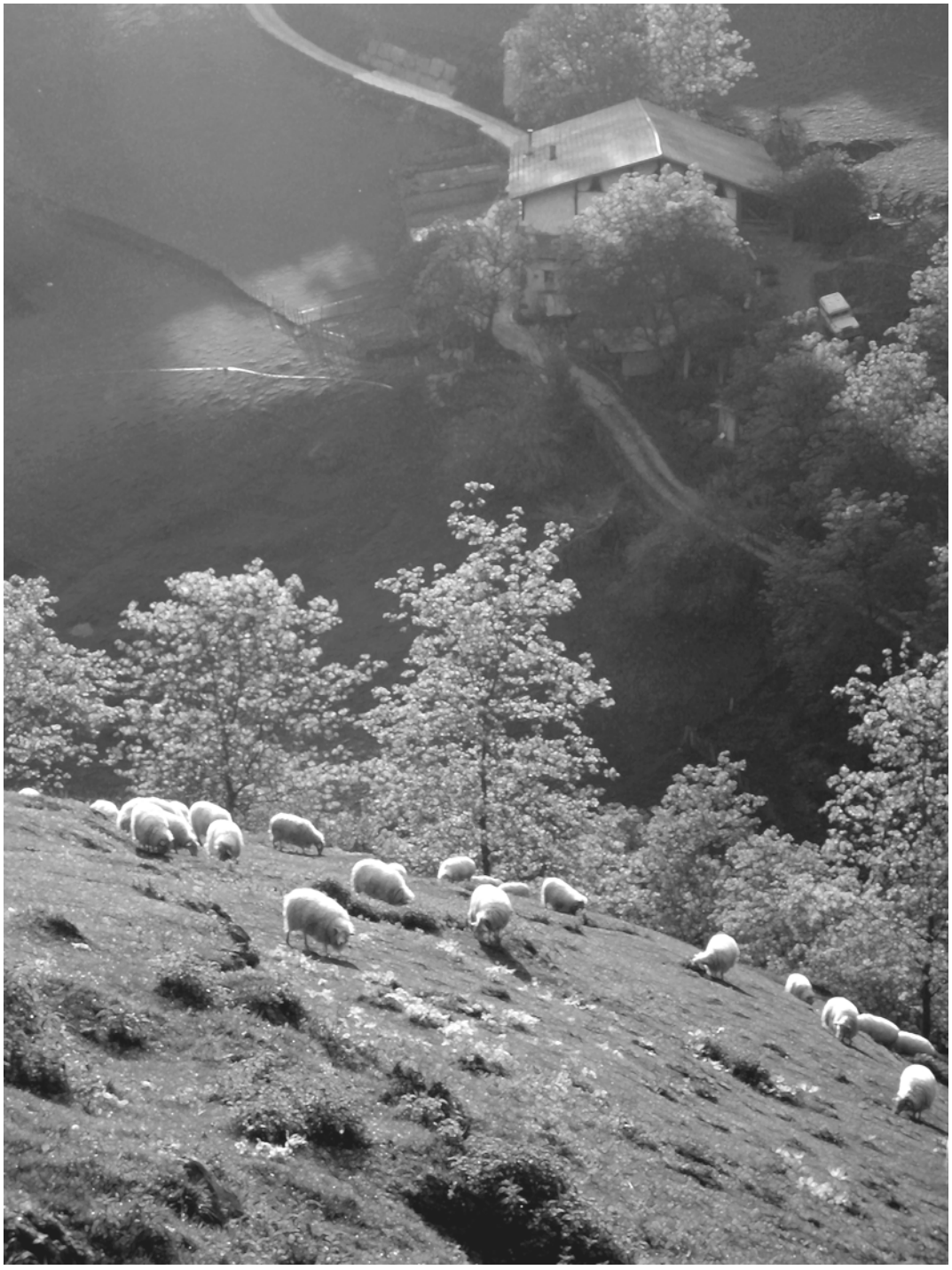
6. Cheesemaking season had an influence in the occurrence of sensory defects. In winter, soft texture was common and, therefore, cheeses should be turned more frequently to avoid rind marks. In spring and summer, off-flavour and openings was the major problem, probably caused by the growth of undesirable microorganisms. Milking conditions, milk refrigeration time, and hygiene and sanitary conditions of dairy facilities should be closely monitored especially in the warm seasons.

7. Cheeses with excessive acidity showed by acetic acid and ketones, originated from the glycolysis of lactose and citrate in early stages of cheese ripening.

8. Cheeses with animal off-flavour showed considerable abundance of odourant compounds such as 3-methylbutanol and 4-methylphenol commonly generated after an intense proteolysis during cheese ripening.

9. Cheeses with rancid off-flavour provided an excessive proportion of short-chain free fatty acids usually generated by a strong lipolytic activity during ripening.

10. The identification of specific odourants as well as specific profiles based on abundance ratios of volatile compounds can assist in the development of methods for an effective early detection of undesirable off-flavour happening in commercial cheeses produced with raw milk from ewes.



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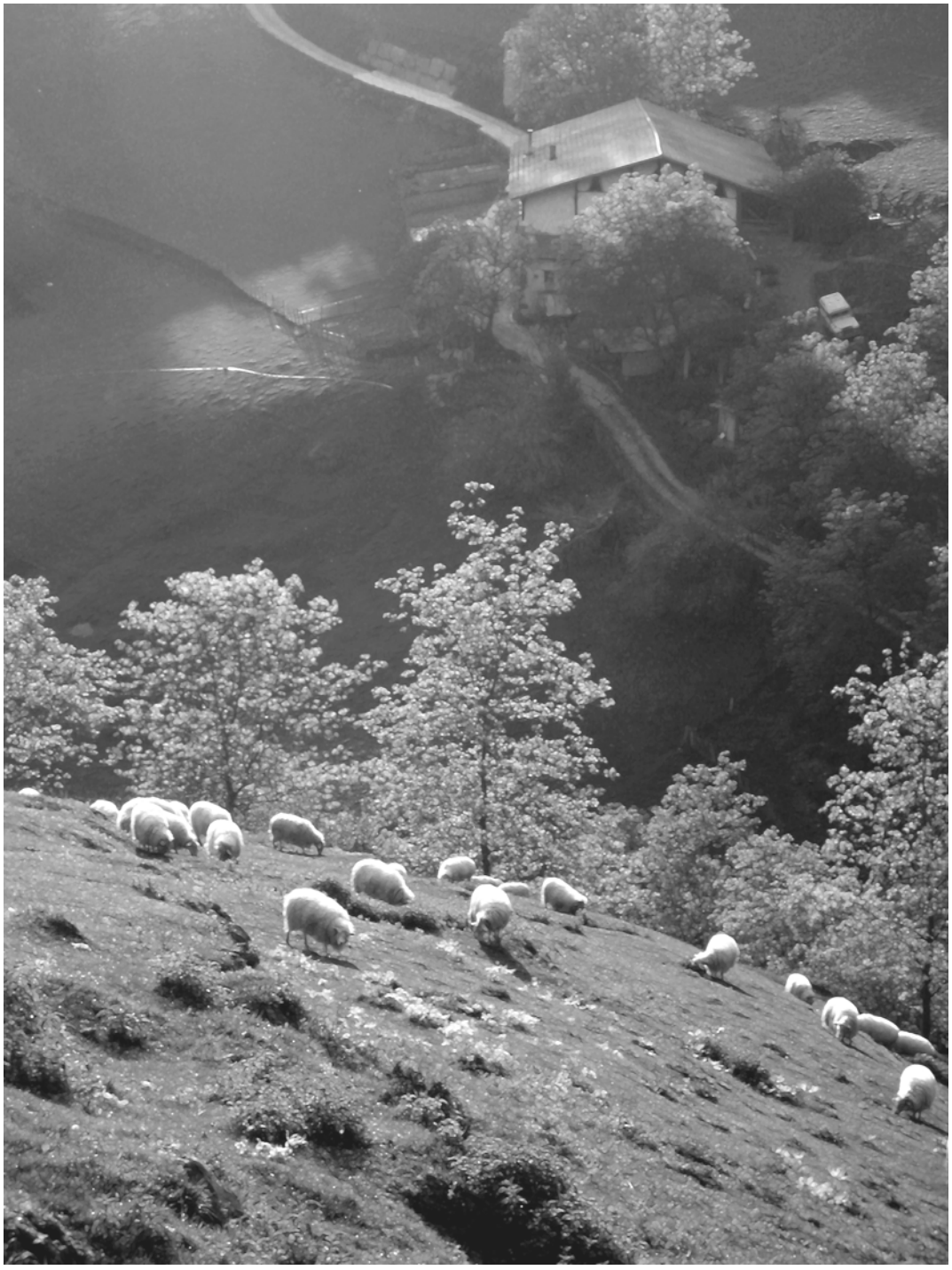

9. Anexo

Anexo I. Plantilla de encuesta sobre la quesería.

QUESERÍA (Q):					
PROVINCIA:		Tel:			
Fecha:					
REBAÑO					
Propio: SI/NO	Compran leche: SI/NO		Comentarios:		
Número de cabezas:					
Esquilado:					
Alimentación:					
Fecha de partos:					
Comentarios:					
LECHE					
Tª en tanque:					
Volumen:					
Ubicación:					
Transporte:	Tª:		Tiempo:		Distancia:
Nº ordeños:					
Comentarios:					
FERMENTO					
Fermento	Composición:			Parada durante fermentación SI/NO	
Forma de añadir:		Cantidad:			
Comentarios:					
CUAJO					
Cuajo	**Líquido**	Marca:	mL:	**Natural**	gr:
Forma de añadir:					
Recalentamiento:					
Comentarios:					
Lisozima	SI/NO	mL:			
CÁMARAS (oreo/maduración)					
Localización:					
Humedad:					
Temperatura:					
Espacio:					
Forma y lugar de realización:					
Comentarios:					
PRENSA					
Forma:					
Control Tª, pH:					
Comentarios:					
SALMUERA					
Tª:					
pH:					
Controles:			Cambio:	Aditivos:	
Sal:		Marca:			

PINTURA	
SI/NO	Marca:

QUESOS			
Ahumados:SI/NO	Forma de ahumar:		
Tamaño:	Pequeño (%):	Mediano(%):	Grande(%):

OBSERVACIONES

Grupo de Investigación

Lactiker

Ikerketa Taldea