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ScienceDirect
International Dairy Journal 18 (2008) 147–157
INTERNATIONAL DAIRY JOURNAL
www.elsevier.com/locate/idairyj

Terpenoids and benzenoids in La Serena cheese made at different seasons of the year with a *Cynara cardunculus* extract as coagulant

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Received 22 January 2007; accepted 21 August 2007

Abstract

The objective was to determine the influence of the season of manufacture and the ripening time on the volatile terpenoids and benzenoids of La Serena cheese made in summer, winter and spring, using a plant coagulant. Ewes were fed with formulated feed in summer, grazing on natural pastures in winter and spring. Most terpenoids and benzenoids present in cheese were observed in the formulated feed (mainly terpenes), and in the *Cynara cardunculus* extract (mainly benzenoids). The enantiomeric α(+)/α(−)−pinene ratio changed during ripening. γ-Curcumene, α-humulene, ethyl benzene and propyl benzene and all xylene isomers decreased, while α-terpineol, verbenone, benzyl alcohol, 2-phenyl-ethanol, benzoic acid methyl ester, and the phenolic compounds increased significantly during ripening. Safranal, geranyl acetone, γ-curcumene and α-curcumene were more abundant in spring than in winter cheeses. Alkyl benzenes and other benzenoids were significantly more abundant in summer than in winter or spring cheeses.

Keywords: La Serena cheese; Terpenoid compounds; Benzenoid compounds; Dynamic headspace; GC-MS

1. Introduction

La Serena is a soft cheese made in Extremadura (Western Spain), from Merino ewes' raw milk, protected by a designation of origin (PDO). No starter cultures are added, and a plant coagulant, obtained from an aqueous maceration of the thistle *Cynara cardunculus* L. (CC) flowers, are used to coagulate the milk. The use of Merino ewes' raw milk and plant coagulant gives the cheese its peculiar slightly bitter taste and a spreadable texture. La Serena cheese is consumed after a minimum ripening period of 60 d. General characteristics of this cheese variety have previously been studied (Fernández del Pozo, Gaya, Medina, Rodríguez-Marín, & Nuñez, 1988a, b). The seasonal variability of the volatile fraction (Carbonell, Nuñez, & Fernández-García, 2002a, b) and the free fatty acid fraction (Fernández-García, Carbonell, Calzada, & Nuñez, 2006) have also been investigated.

Terpenes are secondary plant metabolites, well known for their biological activity in plants and as key aroma compounds of essential oils. More than 20,000 individual terpenoids have been described, (Connolly & Hill, 1991). Some terpenoids supposedly of plant origin, have been found in animal products such as milk and cheese, although terpenoids of microbiological origin have also been reported (Carrau et al., 2005; Imhof, Glättli, & Bosset, 1995). Terpenes have been shown to be useful markers for the origin of dairy products (Buchin et al., 2002; Viallon et al., 2000), particularly useful for the differentiation of highland and lowland cheeses (Bosset, Bütikofer, Gauch, & Sieber, 1994; Bugaud, Buchin, Coulon, Hauwuy, & Dupont, 2001). It has also been reported that feeding concentrates to milking animals causes a decrease of terpene concentration in milk (Claps, Rubino, Fedele, Morone, & Di Trana, 2005). However, the terpene profile of milk and cheeses does not reflect the

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doi:10.1016/j.idairyj.2007.08.007

extremely rich composition observed in the plants on which the milking animals graze (Mariaca et al., 1997; Viallon et al., 1999). Terpenes have been reported to undergo microbial bioconversions (Agrawal & Joseph, 2000; Tan & Day, 1998) and to be metabolised when incubated with rumen fluid (Schlichtherle-Cerny, Imhof, Fernández-García, & Bosset, 2004). All terpenes are probably chiral. For this reason, a chiral column has been used in the current study to monitor their enantiomeric ratios during ripening.

Benzene compounds, found in many cheeses, could come from the metabolism of aromatic amino acids and subsequent reactions (Christensen, Dudley, Pederson, & Steele, 1999; Yvon, Berthelot, & Gripon, 1998), from plants (Buchin et al., 2002) as well as probably from accumulation of environmental pollutants (Bosset, Gubler, Bütikofer, & Gauch, 2000).

The use of thistle flowers in the manufacture of this particular cheese variety may influence the level of chemicals of plant origin. However, feeding milking ewes on pasture or on feed concentrates could also modify the terpenoid and benzenoid composition of the volatile fraction of La Serena cheese. Our objective was to determine the influence of the season of manufacture, the ripening process and the use of a plant coagulant on the presence of volatile compounds of plant origin in La Serena cheeses, focusing on benzenoid and terpenoid compounds, including the enantiomeric ratios.

2. Materials and methods

2.1. Cheeses

La Serena cheese was produced from Merino ewes' raw milk in the traditional manner (Carbonell et al., 2002a) at one selected PDO farm using an extract of CC flowers as coagulant. Thistle flowers were collected manually from natural fields and dried. The coagulant is traditionally prepared by overnight maceration of the flowers in tap water (1:10) at room temperature. The filtrate is added to the cheese milk at a ratio of 1:100. La Serena cheeses used in this study were manufactured in different seasons of the year as part of the normal farm production. Three batches were manufactured on three different days in September (Summer cheeses), representative of a very dry summer, the ewes being fed only with a formulated feed (FF) (COVAP, Pozoblanco, Spain) containing 40% barley, 20% maize, 17% soy flour, 5% wheat and 5% gluten. Three batches were manufactured in January (Winter cheeses), after the autumn rains, when ewes were part time in the fields grazing on natural pastures. The last three batches were made in March (Spring cheeses) when the ewes were grazing on natural pastures most of the time. In winter, as well as in spring, there was a supplementation of the pasture with FF. After 1, 30 and 60 days of ripening cheeses were vacuum packaged and frozen at −40 °C until the analysis for volatile compounds.

2.2. Dynamic headspace extraction and GC-MS analyses

Frozen cheese samples were grated and homogenised with ultra-pure water (1:3) prior to dynamic headspace extraction. Aliquots (15 g) of all sample types, i.e., the milk samples (non-diluted), the CC extracts, the aqueous homogenates of FF and the aqueous homogenates of cheeses, were subjected to dynamic headspace extraction of volatile compounds at 55 °C, using an automatic purge and trap apparatus (LSC 2000, Tekmar, Cincinnati, USA) equipped with a Tenax trap at 36 °C and a cryofocusing unit. The analyses were run in duplicates. The conditions were as follows: equilibration time 5 min, purge gas nitrogen at 40 mL min^{-1} flow; purge time 20 min, dry purge time 12 min, desorption time 5 min, transfer line to GC at 180 °C. The cryofocusing unit was cooled to −150 °C at desorption and then heated up to 225 °C for injection (3 min). GC-MS analysis was performed with a HP 5890 Series II GC equipped with a 5971A mass selective detector (MSD) system (Agilent Technologies, Palo Alto, USA) and a CP-Chirasil-Dex CB capillary column, 25 m × 0.25 mm × 0.25 μm film thickness (Stehlin, Basel, Switzerland). The oven temperature program was 35 °C (0.5 min hold) to 130 °C at 2.5 °C min^{-1} and to 215 °C at 6 °C min^{-1}, using helium as carrier gas at 0.5 bar. The MSD operated in EI ionization mode at 70 eV, with the GC-MS interface at 280 °C, and scanned from mz^{-1} 26 to 350 at 0.8 s^{-1}.

The monoterpenes were identified by comparison of the mass spectra with the bibliographic data from the Wiley 275 library (Wiley & Sons, Inc., Germany), and with the mass spectra and the linear retention indices (LRI) of authentic standards (Sigma-Aldrich, Buchs, Switzerland) calculated by running a paraffin series (from C7 to C17) under the same working conditions. Terpenes other than monoterpenes and benzenoid compounds were tentatively identified by comparison of the mass spectra with the bibliographic data from the Wiley 275 library, the identification quality for a compound with a given LRI being higher than 90% at least in one sample type. For many compounds, the identification quality was higher than 95%. Characteristic single ion areas were used for the semi-quantitative analysis (arbitrary units) by comparing the peak areas of the samples.

2.3. Volatile fraction of formulated feed and C. cardunculus

Two samples per season of the FF and two samples per season of CC were analysed for volatiles. FF was finely grated and homogenised with ultra-pure water (at a ratio of 1:10) prior to dynamic headspace extraction. Dried thistle flowers were finely grated and macerated overnight at room temperature (22 ± 1 °C) in ultra-pure water (1:10).