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High-pressure processing decelerates lipolysis and formation of volatile compounds in ovine milk blue-veined cheese

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ABSTRACT

Enzyme-rich cheeses are prone to over-ripening during refrigerated storage. Blue-veined cheeses fall within this category because of the profuse growth of *Penicillium roqueforti* in their interior, which results in the production of highly active proteinases, lipases, and other enzymes responsible for the formation of a great number of flavor compounds. To control the excessive formation of free fatty acids (FFA) and volatile compounds, blue-veined cheeses were submitted to high-pressure processing (HPP) at 400 or 600 MPa on d 21, 42, or 63 after manufacture. Cheeses were ripened for 30 d at 10°C and 93% relative humidity, followed by 60 d at 5°C, and then held at 3°C until d 360. High-pressure processing influenced the concentrations of acetic acid and short-chain, medium-chain, and long-chain FFA. The effect was dependent on treatment conditions (pressure level and cheese age at the time of treatment). The lowest concentrations of acetic acid and FFA were recorded for cheeses treated at 600 MPa on d 21; these cheeses showed the lowest esterase activity values. Acetic acid and all FFA groups increased during ripening and refrigerated storage. The 102 volatile compounds detected in cheese belonged to 10 chemical groups (5 aldehydes, 12 ketones, 17 alcohols, 12 acids, 35 esters, 9 hydrocarbons, 5 aromatic compounds, 3 nitrogen compounds, 3 terpenes, and 1 sulfur compound). High-pressure processing influenced the levels of 97 individual compounds, whereas 68 individual compounds varied during refrigerated storage. Total concentrations of all groups of volatile compounds were influenced by HPP, but only ketones, acids, esters, and sulfur compounds varied during refrigerated storage. The lowest total concentrations for most groups of volatile compounds were recorded for the cheese pressurized at 600 MPa on d 21. A principal component analysis combining total concentrations of groups of FFA and volatile compounds discriminated cheeses by age and by the pressure level applied to HPP cheeses.

Key words: high-pressure processing, lipolysis, volatile compound, blue-veined cheese

INTRODUCTION

Flavor, rheological properties, and visual appearance determine cheese quality (Fox and Wallace, 1997). Cheese flavor, probably the main trait influencing its quality, is caused by the interaction of many compounds responsible for taste and aroma. These compounds are produced during manufacture and ripening through the metabolism of lactose, lactate, and citrate, the liberation of FFA, and the degradation of caseins to peptides and free amino acids (McSweeney and Sousa, 2000; Collins et al., 2003). Primary degradation is followed by the secondary catabolism of the resulting products to compounds that, in many cases, have higher flavor impact than their respective precursors. More than 600 volatile compounds have been identified in cheese, most of which have been associated with particular odor and aroma notes (Molimard and Spinnler, 1996; Curioni and Bosset, 2002).

Coagulant enzymes, together with lactic starter cultures and their enzymes, are responsible for the biochemical changes occurring during the manufacture and ripening of semihard and hard cheeses made from pasteurized milk, because most of the microorganisms and enzymes present in raw milk have been inactivated by the thermal treatment. In the case of blue-veined cheeses, *Penicillium roqueforti* is an additional major ripening agent responsible for their unique flavor. *Penicillium roqueforti* consumes lactic acid, causing an increase in cheese pH value favorable for many chemical reactions, produces extracellular proteinases and lipases (Gripon et al., 1977; Lamberet and Menassa, 1983), and has the ability to form methyl ketones through the β-oxidation of FFA followed by a decarboxylation reaction (Kinsella and Hwang, 1976).

The activity of enzymes and microorganisms persists during the refrigerated storage of ripe cheese at distribution and retail, which can cause over-ripening if levels of flavor compounds above the desired balanced concentrations for a particular cheese variety are attained. Thus, the cheese purchased by the con-

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sumer may have a stronger or different flavor than the manufacturer intended (Wick et al., 2004). Blue-veined cheeses, because of their richness in enzymatic activities, seem particularly prone to over-ripening defects during the refrigerated storage of ripe cheese. An approach to prevent over-ripening and prolong the shelf life of ripe cheese is frozen storage. Although cheese flavor remains unchanged at thawing, both texture and visual appearance are negatively affected by freezing (Tejada et al., 2000; Van Hekken et al., 2005).

High-pressure processing (**HPP**), with a negligible effect on flavor characteristics, meets the increasing consumer demand for fresh-tasting, minimally processed foods. It has been successfully applied to milk and cheese for the inactivation of pathogenic and spoilage microorganisms (O'Reilly et al., 2000; Arqués et al., 2006). In addition, HPP may be a useful tool for the inactivation of enzymes present in cheese such as proteinases (García-Risco et al., 2003; Huppertz et al., 2004), peptidases (Malone et al., 2003; Juan et al., 2007), and esterases (Ávila et al., 2007). The formation of volatile compounds in cheese is also influenced by HPP, at a variable degree that depends on the pressure level applied and the age of cheese at the time of treatment (Ávila et al., 2006; Arqués et al., 2007). Consequently, HPP seems a feasible procedure to prevent over-ripening during the refrigerated storage of blue-veined cheese.

In a previous study, we reported the effect of HPP on the proteolysis and formation of biogenic amines in blue-veined cheese made from ovine milk (Calzada et al., 2013). However, the effects of HPP on the lipolysis and formation of volatile compounds in blue-veined cheese are not well known. In the only work published on the subject (Voigt et al., 2010), the authors did not find significant differences in the concentrations of FFA and methyl ketones when comparing pressurized and control blue-veined cheeses, a result that could be ascribed to the short refrigerated storage period of cheeses after HPP (only 28 d). In the present work, we investigated the influence of HPP applied to ovine milk blue-veined cheese at 400 or 600 MPa on d 21, 42, or 63 after manufacture on the lipolysis and formation of volatile compounds during a 90-d ripening period and a further 270-d refrigerated storage period.

MATERIALS AND METHODS

Cheese Manufacture and HPP

The manufacturing procedure of blue-veined cheese from pasteurized ovine milk was described in a previous work (Calzada et al., 2013). Two batches of blue-veined cheese were made on consecutive days, each from 1,200 L of milk inoculated with lactic cultures and *P. roqueforti*. Cheeses, 18 cm in diameter and 10 cm high, were ripened at 10°C and 93% relative humidity until d 30 and then at 5°C from d 30 to d 90. After 90 d, they were held at 3°C until d 360.

Cheeses were pressurized at 400 or 600 MPa for 5 min, after 21, 42, or 63 d of ripening, as described by Calzada et al. (2013). Treatments were coded as **400W3**, **600W3**, **400W6**, **600W6**, **400W9**, and **600W9** according to the pressure level applied (400 or 600 MPa) and the age of cheese (3, 6, or 9 wk) at pressurization. Cheeses were unpackaged after HPP, and ripening and storage proceeded under the same conditions as for control cheese.

A different cheese per treatment (1 control and 6 HPP) was sampled at each of the times of analysis. Two 100-g pieces per cheese were wrapped in aluminum foil, vacuum-packaged, and frozen at −40°C for chemical analyses.

FFA Determination

Acetic acid, propionic acid, and FFA from butyric ($C_{4:0}$) to linolenic acid ($C_{18:3}$) in cheese were determined by gas chromatography with flame-ionization detection, as described by Fernández-García et al. (2006), with elution in 8 mL of diethyl ether containing 2% formic acid. Frozen cheese pieces were thawed overnight at 4°C before analysis. At all sampling times, acids were extracted from cheeses using a solid-phase extraction technique, with pentanoic, nonanoic, and heptadecanoic acids added as internal standards. A Hewlett-Packard 6890 gas chromatograph (Agilent Technologies, Las Rozas, Spain) equipped with an automatic sampler (HP 7683), a split/splitless injector, a FFAP column (Agilent Technologies, 30 m × 0.32 mm i.d. × 0.25 μm film thickness) and a flame-ionization detector was used for the analysis. Injection (1 μL of sample) was performed in split mode at 1:20 split ratio, at 260°C. Helium was the carrier gas, with the flow set for maintaining a constant pressure of 0.80 kg/cm^2. For chromatographic separation, the temperature was increased from 65 to 240°C at a rate of 10°C/min, and held at 240°C for 12.5 min. Fifteen standard solutions of FA were used for the calculation of calibration curves. Individual FFA were separated, identified, and quantified, and their concentrations expressed in milligrams per gram of cheese DM.

Determination of Esterase Activity

Esterase activity was determined in duplicate on cheese extracts according to the method described by Ávila et al. (2007) with some modifications. Ten grams