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Effect of high-pressure treatments on proteolysis, volatile compounds, texture, colour, and sensory characteristics of semi-hard raw ewe milk cheese

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ABSTRACT

High pressure (HP) offers potential industrial applications in cheese preservation, but it is essential to provide knowledge concerning their effects on the ripening process and sensory characteristics. In this study, we investigated the effect of different HP treatments (200–500 MPa at 14 °C for 10 min on day 7) on proteolysis, texture, colour, volatile compounds and sensory characteristics of semi-hard raw ewe milk cheese. HP treatments did not affect pH or dry matter values of 60-day-old cheeses. Treatments at pressure levels up to 400 MPa led to significant ($P < 0.01$) increases in the total free amino acids (FAA) content at 60 days, compared to control cheese, although the cell-free aminopeptidase activity was lower. HP retarded the formation of some volatile compounds in cheeses, the number of compounds affected by HP being higher as the pressure level increased. Cheeses pressurized at 300–500 MPa had lower levels of 2-butanone, 2-butanol, 2-propen-1-ol, 1-butanol and acetic acid than control cheese, cheeses pressurized at 400–500 MPa lower levels of 1-propanol, 2-pentanol, and butyric and hexanoic acids, and cheeses pressurized at 500 MPa lower levels of ethanol, 3-methyl-1-butanol and 3-methyl-2-buten-1-ol. All HP-treated cheeses showed higher fracturability values, and higher Hue angle and lower a^* values than control cheese. Despite the differences detected by instrumental analyses between HP-cheeses and control cheese, few significant differences were found between the sensory characteristics of HP-cheeses and control cheese. Only the pressurization of cheese at 500 MPa affected significantly ($P < 0.01$) some of the sensory characteristics, with a negative effect on taste intensity but a positive effect on aroma quality.

In summary, HP treatments at 200 and 300 MPa showed the mildest effects on the characteristics of semi-hard raw ewe milk cheese. HP treatment of this cheese variety at 300, 400 and 500 MPa prevented late blowing defect caused by *Clostridium tyrobutyricum* (Ávila et al., 2016, Food Microbiol. 60, 165–173). Thus, it may be concluded that HP treatment at 300 MPa is the most adequate procedure, able to prevent late blowing with minimum changes in cheese characteristics.

1. Introduction

High-pressure (HP) treatment is a non-thermal method to eliminate undesirable microorganisms in foods, ensuring their microbiological safety and extending their shelf life, with low impact on its nutritional and sensory characteristics. Most HP applications for cheese are related to the inactivation or reduction of pathogenic and spoilage microorganisms (Martínez-Rodríguez et al., 2012). In addition, several works have studied the application of HP treatments to accelerate cheese ripening (Martínez-Rodríguez et al., 2012), as well as to prevent overripening (Calzada, del Olmo, Picón, Gaya, & Nuñez, 2013a,b, 2014a,b). However, to date most HP-related cheese studies involved the use of cow milk, existing very limited information for ewe milk cheeses.

Regarding cheeses made from pasteurized ewe milk, HP treatment of a semi-hard cheese at 300 MPa on day 1 accelerated cheese proteolysis, but the treatment at 500 MPa decelerated it (Juan, Ferragut, Buffa, Guamis, & Trujillo, 2007). In the same type of cheese, the application of HP limited the formation of some volatile compounds, including acids, alcohols, ketones, aldehydes, and sulfur compounds (Juan, Barron, Ferragut, & Trujillo, 2007). In addition, treatments at 200 and 300 MPa enhanced the firmness of cheeses but pressurization at 500 MPa produced a weakening effect in the casein matrix (Juan, Trujillo, Guamis, Buffa, & Ferragut, 2007). In contrast, pressurization of ovine brined cheese at 200 MPa on day 15 did not affect cheese

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composition or microbiota (Moschopoulou, Anisa, Katsaros, Taoukis, & Moatsou, 2010). However, HP treatment at 500 MPa reduced levels of total aerobic mesophilic bacteria, thermophilic lactococci, thermophilic lactobacilli and non-starter lactic acid bacteria without altering cheese composition. In the same cheese variety, HP treatment at 200 MPa on day 7 accelerated secondary proteolysis and cheese ripening (Giannoglou et al., 2016). Furthermore, pressurization did not avoid over-ripening of blue-veined cheese (Calzada et al., 2013a).

The effect of HP on cheeses made from ewe raw milk has been investigated in semi-soft to soft varieties La Serena, Casar and Serra da Estrela, which use an extract of *Cynara cardunculus* L. (cardoon) flowers as milk coagulant. Pressurization of La Serena cheese on day 50 of ripening at 400 MPa was the most adequate treatment, among those tested (300 or 400 MPa on day 2 or 50), to improve its microbiological safety while maintaining its sensory characteristics (Arqués, Garde, Gaya, Fernandez-García, & Nuñez, 2007; Arqués, Garde, Gaya, Medina, & Nuñez, 2006; Garde, Arqués, Gaya, Medina, & Nuñez, 2007). In Casar cheese, pressurization at 600 MPa also prevented over-ripening (Calzada et al., 2014b). HP treatment (400, 500 or 600 MPa) of Serra da Estrela cheese reduced bacteria counts and prevented lipid oxidation, thereby increasing cheese shelf life during refrigerated storage (Inácio, Fidalgo, Santos, Queirós, & Saraiva, 2014). In a previous study performed by our research group on industrial sized semi-hard raw ewe milk cheeses concerning the effect of HP (200–500 MPa at 14 °C for 10 min on day 7) on spoilage by *Clostridium tyrobutyricum*, we observed that HP-treatments at or above 300 MPa were effective in preventing the late blowing defect (Ávila, Gómez-Torres, Delgado, Gaya, & Garde, 2016). However, the effects of HP on the ripening process itself and on cheese sensory characteristics were not determined, which are essential when aiming to industrial implementation.

In this work our aim was to study the effect of HP treatments (200–500 MPa at 14 °C for 10 min on day 7) on proteolysis, texture, colour, volatile compounds and sensory characteristics of industrial sized semi-hard raw ewe milk cheeses, in order to fully validate the HP process. This is the first time to our knowledge that the effect of HP on the characteristics of a semi-hard raw ewe milk cheese has been evaluated.

2. Materials and methods

2.1. Cheese manufacture

Industrial size semi-hard cheeses (Castellano type) were manufactured from raw ewe milk in duplicate cheese trials carried out on consecutive days at the pilot plant of the Estación Tecnológica de la Leche of ITACyL (Palencia, Spain). Direct vat-set starter cultures containing *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris* blend (Choozit™ MA 11, Danisco, Laboratorios Arroyo, Santander, Spain) and *Streptococcus thermophilus* (Choozit™ TA 54, Danisco) were used following manufacturer's instructions. Each cheese trial consisted of one vat containing 50 L of milk and calcium chloride (0.01%), warmed at 32 °C and inoculated with starters MA 11 and TA 54. Following a 25 min incubation, calf rennet (18 mL/50 L milk, 1:15,000 strength, Laboratorios Arroyo) was added. After 25 min coagulation, the curd was cut into small grains, gently stirred and scalded up in the vat in its own whey to 37–38 °C for 40–50 min. Then the whey was drained off, the curd was distributed into cylindrical moulds (15 cm diameter, 10 cm height) and then were pressed at 1.5–2 kg cm^{-2} until curd pH was 5.5, and salted for 4.5 h at 12 °C in brine (190 g of NaCl/L). Six cheeses (1.5 kg average weight out of the press) were obtained from the vat and were ripened for a total period of 60 days at 12 °C and 82% relative humidity. Cheese rinds were coated on day 9 with two layers of an antifungal formulation containing natamycin and potassium sorbate (Fungirol DP 10 TLS, Laboratorios Arroyo).

2.2. Sampling and high-pressure processing of cheeses

One of the six cheeses obtained from each vat was analysed on day 1, four of them were pressurized at 200, 300, 400 and 500 MPa on day 7, and the sixth one remained unpressurized and served as control cheese. The last five cheeses were analysed at the end of the ripening period (60 days). Cheeses were aseptically sampled after discarding the cheese rind. Cheese pH, dry matter (DM), aminopeptidase activity, overall proteolysis, texture, colour and sensory analyses were determined on fresh samples. For analysis of amino acids and volatile compounds, cheese pieces were wrapped in aluminium foil, vacuum packed and frozen at − 40 °C until analysis.

For high-pressure processing, cheeses were vacuum-packed in CN300 bags (Cryovac Grace S. A., Barcelona, Spain) on day 7 post-manufacture and HP-treated for 10 min at 200, 300, 400 or 500 MPa by means of a 135-L capacity discontinuous isostatic press (Wave 6000/135 Hyperbaric S.A., Burgos, Spain). Temperature of the water used as pressure-transmitting fluid at the beginning of the process was between 13.9 and 14.4 °C. Times to reach 200, 300, 400 or 500 MPa were 143, 170, 212 and 273 s, respectively, with a depressurization time of 2 s. After treatments, HP-treated cheeses were unpackaged and continued ripening at 12 °C.

2.3. Cheese pH and dry matter

The pH was measured with a Crison penetration electrode (model 52-3.2) connected to a Crison pH meter GPL 22 (Crison Instruments, Barcelona, Spain). DM content was determined in duplicate after drying to constant weight in a vacuum oven at 100 °C (IDF, 1982).

2.4. Aminopeptidase activity

Aminopeptidase activity was determined by a method adapted from Garde et al. (2007). Briefly, 2 g of cheese were homogenized with 4 mL of 10 mM sodium phosphate buffer, pH 7, at 20 °C for 1 min in an Ultraturrax T18 (IKA Werke GmbH, Staufen, Germany), followed by centrifugation (10,000 × *g*, 15 min, 4 °C) and filtering through Whatman No. 2 paper to finally obtain a cheese extract. Aminopeptidase activity in duplicate cheese extracts was determined with lysine *p*-nitroanilide (Lys-*p*-NA) and leucine *p*-nitroanilide (Leu-*p*-NA) as substrates, by measuring the *p*-nitroaniline released by action of aminopeptidases. Release of *p*-nitroaniline was measured at 410 nm by means of a Beckman DU 650 spectrophotometer (Beckman Instruments España SA). The assay mixture contained 200 μL cheese extract, 50 μL substrate (25 mM in methanol) and 500 μL 100 mM phosphate buffer, pH 7.0. One unit of aminopeptidase activity was defined as the amount of enzyme producing 1 nmol *p*-nitroaniline per min per g of cheese.

2.5. Assessment of proteolysis in cheeses

Overall proteolysis of the cheeses was determined in duplicate samples by the *o*-phthaldialdehyde (OPA) test, based on the reaction of released α-amino groups with this compound and with β-mercaptoethanol to form an adduct that absorbs strongly at 340 nm (Church, Swaisgood, Porter, & Catignani, 1983). Prior to the OPA test, 5 g of cheese were homogenized with 25 mL of sodium citrate (2% w/v) in an Ultraturrax T18. To a 5-mL homogenate was added 1 mL of ultrapure water and 10 mL of 20% trichloroacetic acid while vortexing. After 10 min, the suspension was filtered through Whatman No. 2 paper. The mixture of the OPA test containing 150 μL of filtered cheese extract and 3 mL of OPA reagent (25 mL of 100 mM sodium tetraborate, 2.5 mL of 20% (w/w) SDS, 40 mg of OPA (dissolved in 1 mL of methanol), 100 μL of β-mercaptoethanol and 21.4 mL of ultrapure water) was incubated at room temperature for 2 min. Absorbance was measured by means of a Beckman DU 650 spectrophotometer and proteolysis was expressed as absorbance at 340 nm.