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Effect of high pressure treatment applied on starter culture or on semi-ripened cheese in the quality and ripening of cheese in brine

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ABSTRACT

Cheese ripening acceleration is of continuous interest for the industry. High-pressure (HP) treatment of starter cultures used in cheese-manufacturing offers the potential to accelerate ripening by increasing the activity of their intracellular peptidases that contribute in the development of desired cheese organoleptic characteristics. The objective of the present research was the investigation of the effect of HP treatment (200 MPa-20 °C - 20 min) directly on white brined cheese or on the starter culture used for its manufacture (*Str. thermophilus:L. lactis:L. bugaricus 2:1:1*). For this purpose, the microbial, textural, physicochemical and organoleptic characteristics and proteolysis were assessed during the 2nd stage of ripening in cold stores. Control cheese without any treatment was also studied.

Cheeses made with HP-treated starters had increased secondary proteolysis. Organoleptic scoring of these cheeses was higher during the whole storage period compared to control and HP-treated cheese. Their superiority was evident even at the early stages of ripening in cold stores, since no bitterness was detected. On the contrary, although HP treated cheeses showed the highest increase in aminopeptidases activities, this was not correlated with the studied ripening indices or the organoleptic characteristics.

According to the results, HP-treated starter culture can accelerate proteolysis and potentially the ripening of cheese-in-brine.

Industrial relevance: The data obtained from this work suggest that application of HP treatment under optimized conditions on cheeses in brine starter cultures or on whole cheeses can be effectively used for the production of products with reduced ripening time. This is of great importance for the cheese industries, since the storage period for ripening is long (higher than two months), while applying HP treatment as suggested in this study, this time may be reduced to less than one month, producing cheeses of superior quality.

1. Introduction

White brined cheeses (WBCs) are cheeses ripened and preserved in brine made from curds that are not subjected to any heat treatment, like Feta and similar cheese varieties. Their flavour is slightly acidic and salty that sometimes becomes rancid and piquant. Their colour is pure white, when they are made from ewe's and goat's milk. The cheese mass has no rind, no gas holes or other openings, except for small mechanical openings and its texture is smooth and rather soft but always sliceable. Feta and other white brined cheeses ripen in two distinct periods. Firstly they are kept at 16–18 °C to be acidified and drained properly (pH ≤ 4.6), for 7 to 15 days. This period is the 1st stage of ripening. Then, cheeses packaged with brine are transferred to cold stores for the continuation of ripening, up to at least two months. This cold storage period is the second stage of ripening (Moatsou & Govaris, 2011).

Cheese maturation is a complex process involving many biochemical changes such as glycolysis, lipolysis and mainly proteolysis (Kalit, Havranek, Kaps, Perko, & Cubric, 2005; Mc Sweeney & Sousa, 2000). Proteolysis in cheeses takes place in two stages. In the primary proteolysis, residual rennet enzymes along with endogenous milk proteases, hydrolyse casein resulting in the production of rather large peptides or medium-sized peptides. In the secondary proteolysis, proteins and large peptides are gradually hydrolysed to smaller peptides and amino acids due to the action of intracellular and extracellular enzymes of starter cultures or other cheese microorganisms (Addeo et al., 1992; Gagnaire, Mollé, Herrouin, & Léonil, 2001; Ur-Rehman, Fox, & Mc

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Sweeney, 2000). Therefore, part of proteinase and peptidase activities during cheese ripening comes from the starter cultures. According to several research findings, an increase of the activity of these peptidases may accelerate the ripening step of cheese production (Christensen, Dudley, Pedersen, & Steele, 1999; Katsaros, Giannoglou, & Taoukis, 2009; Mierau et al., 1996), which can lead to economic and technological advantages for the producers by reducing the cost of storage of cheeses (O'Reilly et al., 2003; Saldo, Sendra, & Guamis, 2000).

High Pressure (HP) treatment induces conformational changes of proteins, affecting directly the enzyme modulation sites or active sites (Rovere, 1995). These changes can lead to enzyme activation or inactivation (Saldo et al., 2000). The effect of HP on ripening indices of cheeses in relation to potential ripening acceleration is under investigation. Studies have been carried out on the application of HP in the starter cultures used in cheese manufacturing or directly to the cheese itself, in order to investigate the effect of HP on cheese proteolysis. In the international literature there is a number of publications on the effect of HP on aminopeptidases of starter cultures and on the effect of HP on the ripening stage of different types of cheeses.

As regards the effect of HP on aminopeptidases, Miyakawa, Anjitsu, Ishibashi, and Shimamura (1994) studied the effect of pressure (400 MPa at 30 °C for 10 min) on the activity of *Lb. helveticus* peptidases. HP treatment increased the activities of aminopeptidase (AP) and X-prolyl dipeptidyl aminopeptidase (PepX), which are important for the acceleration of cheese ripening. Malone, Wick, Shellhammer, and Courtney (2003) studied the activity of aminopeptidases of *L. lactis,* used in Cheddar cheese manufacturing, after treatment at various HP levels, at 25 °C for 5 min and various HP conditions. They reported activation, inactivation or no effect of proteolytic and glycolytic enzymes depending on the HP conditions and enzyme. Katsaros et al. (2009), carried out a kinetic study on the effect of HP on the aminopeptidases PepN, PepX, PepA, PepC and PepY from *Lb. bulgaricus* ssp. *delbrueckii*. Mathematical models were developed to describe the effect of HP on aminopeptidases' activity. They reported increased activities for all the studied HP-treated aminopeptidases which were maximized after treatment at 200 MPa for 20 min at 20 °C. Giannoglou, Katsaros, and Taoukis (2016) observed similar effect of HP and temperature on the activity of the abovementioned aminopeptidases of the starter cultures, *Str. thermophilus* and *L. lactis*.

Some indicative studies concerning the effect of HP on cheese ripening acceleration, are presented below. Yokoyama, Chiba, and Yoshikawa (1992) studied the acceleration of cheese ripening using HP and reported that Cheddar cheese could ripen in 3 days by treatment at 50 MPa, in contrast to a 6 month ripening period for conventional Cheddar cheese. O'Reilly, Kelly, Murphy, and Beresford (2000) treated Cheddar cheese at various stage of ripening at 50 MPa for 3 days at 25 °C and concluded that the levels of proteolysis in HP-treated cheeses were higher compared to control cheeses. Although, the increase was not in accordance to those reported by Yokoyama et al. (1992), who applied similar treatments. Capellas, Mor-Mur, Sendra, and Guamis (2001) studied the effect of HP at 500 MPa for 5, 15 and 30 min at 10 °C or 25 °C on Mató cheese and reported that the total nitrogen content of the HP-treated samples, which was higher in comparison to control samples. Maniou et al. (2013) investigated the effect of a DVI starter mixture HP-treated at 200 MPa, 20 °C and 15 min on ripening indices of Feta cheese and reported that the use of HP-treated starter increased secondary proteolysis in cheese.

The objective of the present research was the investigation of the effect of HP treatment directly on white brined cheese manufactured similarly to Feta cheese or on the starter culture used for its manufacture. In order to investigate the potential of these treatments to accelerate ripening, the microbial, textural, physicochemical and organoleptic characteristics and proteolysis were assessed during the 2nd stage of cheese ripening in cold stores.

2. Materials and methods

2.1. Starter culture preparation

The starter cultures used in cheesemaking were a mixture of the bacterial strains *Lactobacillus delbrueckii* subsp. *bulgaricus* ACA-DC 0105, *Streptococcus thermophilus* ACA-DC 0022 and *Lactococcus lactis* ACA-DC 0049 (kindly provided from Collection ACA-DC of the Agricultural University of Athens, Greece). *Lactobacillus bulgaricus* was grown anaerobically at 45 °C and *Streptococcus thermophilus* and *Lactococcus lactis* were grown aerobically at 35 °C, in reconstituted skimmed milk (10% TS). The bacteria cultures were used in cheesemakings after reaching the late exponential phase, i.e. approximately 10^8 CFU/g. At this stage, the cultured milk precipitate resuspended in appropriate culture medium (MRS broth for lactobacilli and M-17 broth for cocci) showed an absorbance of $A_{600nm} = 4.2$.

2.2. Cheese manufacture

Four cheeses were manufactured using ovine milk (5.8% fat content), according to Feta cheese-making and ripening conditions as described by Moschopoulou, Anisa, Katsaros, Taoukis, and Moatsou (2010). Two replicate experiments were carried out within two consecutive weeks, i.e. two cheesevat replicates were studied for each experimental cheese.

Cheeses coded as Control samples were manufactured using 2% v/v of untreated starter culture containing 50% *Lactococcus lactis* and 50% *Streptococcus thermophilus*/*Lactobacillus delbrueckii* subsp. *bulgaricus* 1:1. After the pre-maturation stage, half of the Control cheese quantity produced, was packed in laminated pouches and treated with HP. This cheese was coded as HPC.

A mixture of untreated and HP-treated starter culture (40:60 ratio) was used for the manufacture of HPA cheeses. An amount of 2.5% v/v of the pre-mentioned starter culture was used.

Only HP treated starter mixture was used for the manufacture of HPB samples. An amount of 3% v/v of the pre-mentioned starter culture was used for HPB.

The mixture ratio in the cases of HPA and HPB experimental cheese (2.5 and 3% v/v, respectively), was defined based on unpublished data on the effect of HP treatment on the ability of starter cultures to produce lactic acid. In all cases, the produced lactic acid per liter of milk was the same (i.e., more starter culture was used in the case of HP treated mixture compared to untreated culture).

After the 1st stage of ripening (approximately 1 week) all the produced cheeses were packed with brine (7% w/w NaCl) in plastic containers with a capacity of 500 mL each (two cheese blocks in brine in each container) and were stored at 4 °C for the 2nd stage of ripening. Cheese samples were analysed in triplicate after 1, 7, 14, 21, 30, 45, 60 and 90 days of their placement in cold stores at 4 °C.

2.3. High pressure processing

Predetermined volumes of each bacterial strain for HPA and HPB samples, were vacuum packed separately in sterile laminated pouches. In the case of HPC samples, Feta cheese was cut in pieces after the 1st stage of ripening and the samples were also vacuum packed in sterile laminated pouches. The treatment of the starter cultures and HPC cheese, was carried out in a laboratory-scale HP system with a maximum operating pressure of 1000 MPa (Food Pressure Unit FPU 1.01, Resato International BV, Roden, Holland), as described by Katsaros et al. (2009). The bacterial strains and the HPC cheese were treated under 200 MPa, at 20 °C for 20 min. The applied HP conditions were selected according to the findings of Katsaros et al. (2009) and our own unpublished data that show that the subjection of the starter culture under these HP conditions, leads to the maximum activity of the starter culture aminopeptidases. The pressure transmitting fluid was polyglycol ISO viscosity class VG 15 (Resato International BV, Roden,