J. Dairy Sci. 99:4220–4232
http://dx.doi.org/10.3168/jds.2015-9907

Effect of high-pressure treatment on hard cheese proteolysis

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ABSTRACT

The application of high hydrostatic pressure (HHP) treatment has been proposed to reduce the ripening time of cheese via modifications in the enzymatic activities or the substrate reactivity. Investigations on the effect of HHP on cheese proteolysis have been undertaken with either encouraging results or little effect according to the treatment conditions and the type of cheese, but information concerning the effect of HHP on the ripening of hard cooked cheese is still lacking. In this report, we describe the effect of HHP treatment on Reggianito cheese proteolysis. For that purpose, 1-d-old miniature cheeses (5.5-cm diameter and 6-cm height) were treated at 100 or 400 MPa and 20°C for 5 or 10 min, and control cheeses in the trial were not pressurized. All cheeses were ripened at 12°C during 90 d. The HHP did not affect gross composition of the cheeses, but microbial load changed, especially because the starter culture count was significantly lower at the beginning of the ripening of the cheeses treated at 400 MPa than in controls and cheeses treated at 100 MPa. Cheeses treated at 400 MPa for 10 min had significantly higher plasmin activity than did the others; the residual coagulant activity was not affected by HHP. Proteolysis assessment showed that most severe treatments (400 MPa) also resulted in cheeses with increased breakdown of α_{S1}- and β-CN. In addition, nitrogen content in soluble fractions was significantly higher in cheeses treated at 400 MPa, as well as soluble peptides and free AA production. Peptide profiles and individual and total content of free AA in 60-d-old treated cheese were as high as in fully ripened control cheeses (90 d). Holding time had an effect only on pH-4.6-soluble nitrogen fraction and plasmin activity; cheese treated for 10 min showed higher values than those treated for 5 min, at both levels of pressure assayed. We concluded that HHP treatments at 400 MPa applied 1 d after cheesemaking increased the rate of proteolysis, leading to an acceleration of the ripening process in Reggianito Argentino cheese, whereas 100-MPa treatments did not lead to significant changes.
Key words: high hydrostatic pressure, Reggianito cheese, ripening acceleration, proteolysis

INTRODUCTION

High hydrostatic pressure (**HHP**) treatment, a nonthermal method of food preservation used for a wide range of products, has similar benefits to thermal treatment in food safety while causing minimal changes in food quality (Torres and Velazquez, 2005). Research into the application of HHP processing dates back to the end of the 19th century when Hite (1899) demonstrated that the shelf life of milk could be extended by high-pressure treatment. However, the unavailability of suitable equipment hampered further research for the first half of the 20th century. The first HHP-treated products appeared on the market in Japan in 1991 (Cheftel, 1995). In milk and dairy products, the current state of knowledge on the subject is primarily derived from research done since 1990. In recent years, interest in the effect of HHP treatment on cheese has increased. The main objective is to accelerate cheese ripening and reduce the ripening time, during which the biochemical transformations of proteins, fats, and carbohydrates that will result in the texture, flavor, and functionality of different cheeses will proceed (Trujillo et al., 2000; O'Reilly et al., 2001).

The potential use of HHP treatment for the acceleration of cheese ripening was first studied by Yokoyama et al. (1992). These researchers claimed that cheese exposed to HHP treatment at 50 MPa for 3 d at 25°C had characteristics typical of a 6-mo-old commercial Cheddar cheese. However, further studies in Cheddar

Received June 3, 2015.
Accepted February 14, 2016.
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(O'Reilly et al., 2000, 2001), Gouda (Messens et al., 1999), and goat cheese (Saldo et al., 2001) generally concluded that HHP treatments similar to those used by Yokoyama et al. (1992) had only minor effects on cheese ripening. These contradictory results were observed because the levels of starter bacteria added to the cheese milk by Yokoyama et al. (1992) were at least 10-fold higher than those used in the other studies, which probably influenced the acceleration of cheese ripening.

O'Reilly and colleagues studied the effect of different pressure ranges (50 to 400 MPa) and times both long (of the order of 3.5 and 81.5 h) and short (under 20 min) in Cheddar cheese (O'Reilly et al., 2000, 2002, 2003). Overall, these researchers determined that whereas cheese treated at low pressures and long holding times (~50–200 MPa during up to 82 h) had increased levels of primary proteolysis, HHP treatment at higher pressures for relatively shorter processing times (200–400 MPa for ~20 min) produced changes primarily in the protein structure, which improved the functional properties. These authors also found that the time during ripening in which the HHP treatment was applied also influenced the results, exhibiting stronger effects when the HHP treatment was applied to 1-d-old cheeses than to 15-d-old cheeses. Also for Cheddar cheese, studies performed by Rynne et al. (2008) using a pressure level of 400 MPa for 10 min had little effect on primary proteolysis compared with control cheeses. Ozturk et al. (2013a) studied the effect of pressure levels between 50 and 400 MPa and holding times of 5 and 20 min in reduced-fat Cheddar cheese. These researchers concluded that HHP treatment did not change the rate of proteolysis but improved the texture of the cheese. No change in the indexes of proteolysis was encountered when a treatment of 405 MPa for 3 min was applied on regular and reduced, low, and no salt added Cheddar cheeses, but certain microbiological, textural, and rheological properties were affected by HHP (Ozturk et al., 2013b). Similar results in proteolysis were observed in Edam cheese, where a pressure level between 200 and 500 MPa for a holding time of 30 min did not alter the rate of proteolysis, but treated cheese had an improvement of the consistency compared with control cheeses (Iwaczak and Winiewska, 2005). In a hard uncooked cheese, in which pressure levels between 200 and 500 MPa were applied for 10 min, an increase in the rate of proteolysis at 300 MPa was observed (Yang et al., 2014). In Mozzarella cheese, HHP treatment at 400 MPa for 5 min had no effect on the composition and functional properties (Sheehan et al., 2005). Studies in hard goat cheese treated at 400 MPa showed an increase in the rate of proteolysis and changes in the composition of the HHP-treated cheese with respect to controls, although no change in plasmin activity and a decrease in the activity of the residual coagulant were observed (Saldo et al., 2002). In sheep cheeses, HHP treatment at 300 MPa for 10 min 1 d after manufacture resulted in an increase in the rate of proteolysis, higher levels of free AA (**FAA**), and an improvement in the texture of the cheese compared with controls (Juan et al., 2008). Brie cheese and blue-veined cheese, treated at 400 and 600 MPa for 5 and 10 min, respectively, exhibited a decrease in microbial counts compared with the control cheese (Voigtet et al., 2010; Calzada et al., 2014). In addition, these researchers observed decelerated proteolysis of HHP-treated cheeses.

Different combinations of pressure level and holding time have been applied in several types of cheeses. Regarding hard cheeses, most studies have investigated Cheddar cheese and hard sheep cheeses. However, hard cooked cheeses, such as Parmigiano Reggiano, Grana Padano, Sbrinz, or Reggianito, that are ripened for periods ranging from 6 mo to 2 yr have not been subjected to HHP to date. Technological approaches to accelerate the ripening of hard cooked cheeses is a topic of interest in dairy technology, as keeping large volumes of cheese for such a long period is both expensive and time consuming.

Reggianito is a hard cooked cheese derived from Italian Grana and Parmesan cheeses. This cheese has existed in Argentina since the late 19th century. Italian immigrants first produced Reggianito cheese, which has since diverged from the original varieties but is also different from Parmesan-like American or generic products (Zannoni et al., 1994). Reggianito has a higher moisture content (between 31.3 and 34.9%), smaller size (between 5 and 10 kg of weight), and shorter ripening period (minimum 6 mo) than Italian hard cheeses, who have a moisture between 29.9 and 31.7%, weight of 37 kg on average, and minimum ripening time of 12 mo (Zannoni et al., 1994; Sihufe et al., 2012). Reggianito cheese is the most important hard cheese variety manufactured in Argentina, and it is exported primarily to Brazil, Russia, the United States, Chile, and Venezuela.

In this work, we studied the effect of pressure level (100 and 400 MPa) and holding time (5 and 10 min) on Reggianito cheese proteolysis.

MATERIALS AND METHODS

Experimental Design

We assessed the influence of pressure and holding time on Reggianito cheese proteolysis. Both factors were studied at 2 levels (pressure: 100 and 400 MPa; holding time: 5 and 10 min), which accounted for 4 treatments: **T1** (100 MPa, 5 min), **T2** (100 MPa, 10 min), **T3**