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Hot topic: Changes in angiotensin-converting enzyme inhibition and concentrations of the tripeptides Val-Pro-Pro and Ile-Pro-Pro during ripening of different Swiss cheese varieties

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ABSTRACT

The angiotensin-converting enzyme (ACE) inhibitory activity and the concentration of the 2 ACE-inhibiting tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) were studied during cheese ripening in 7 Swiss cheese varieties. The semi-hard cheeses Tilsiter, Appenzeller 1/4 fat, Tête de Moine, and Vacherin fribourgeois and the extra-hard and hard cheeses Berner Hobelkäse, Le Gruyère, and Emmentaler were investigated. Three loaves of each variety manufactured in different cheese factories were purchased at the beginning of commercial ripeness and investigated at constant intervals until the end of the usual sale period. Good agreement was found between ACE-inhibitory activity and the total concentration of VPP and IPP at advanced ripening stages. In most of the investigated varieties ACE-inhibitory activity and the concentration of the 2 tripeptides initially increased during the study period. A decline in the concentration of VPP and IPP was obtained toward the end of the investigated period for Tilsiter and Gruyère. The ratio of VPP/IPP decreased during ripening in all varieties with the exception of Emmentaler. However, large variations were observed among the cheese varieties as well as the individual loaves of the same variety. Chemical characterization of the investigated cheeses revealed that qualitative differences in the proteolysis pattern, not quantitative differences in the degree of proteolysis, are responsible for the observed variations in the concentrations of VPP and IPP. The presence of *Lactobacillus helveticus* in the starter culture was associated with elevated concentrations of VPP and IPP. The results of the present study show that concentrations of VPP and IPP above 100 mg/kg are attainable in semi-hard cheese varieties after ripening periods of about 4 to 7 mo and that stable concentrations of the 2 antihypertensive tripeptides can be expected over several weeks of cheese ripening.

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INTRODUCTION

Cheese ripening is a complex process that involves a large number of biochemical reactions. Detailed studies on cheese ripening have been carried out for various Swiss cheese varieties such as Appenzeller (Steffen et al., 1993b), Tilsiter (Steffen et al., 1993a), Raclette (Schär et al., 1992), Emmentaler (Bachmann et al., 1999), Gruyère (Steffen et al., 1992), and Sbrinz (Sollberger et al., 1991). During secondary proteolysis a large number of peptides varying in chain length are released from caseins through the action of proteolytic enzymes from milk, raw milk flora, milk-clotting enzymes, and microbial proteases from lactic starter cultures and adjunct cultures. In a study of Emmentaler, 91 peptides were found (Bütikofer et al., 1998) and in another report more than 100 peptides were detected in the water-soluble extracts (Gagnaire et al., 2001). A total of 107 peptides were reported in different fractions of artisanal or industrial Manchego cheese (Gómez-Ruiz et al., 2007).

Milk protein is a rich source of biologically active peptides that may be released through the action of proteolytic enzymes during cheese ripening. Some of these peptides have been shown to inhibit angiotensin-converting enzyme (**ACE**) and are therefore associated with antihypertensive properties (Bachmann et al., 2003). The study by Okamoto et al. (1995) was one of the first to describe ACE inhibition in cheese. Large differences in ACE inhibition were shown in vitro in water-soluble extracts (Meisel et al., 1997; Smacchi and Gobbetti, 1998; Haileselassie et al., 1999; Saito et al., 2000; Stepaniak et al., 2001; Ong et al., 2007; Ong and Shah, 2008a, b) as well as in the ethanol-soluble fraction (Pripp et al., 2006) of various cheese varieties.

In recent years a large number of ACE-inhibiting peptides from various cheese varieties have been isolated and identified. For instance, 22 peptides could be identified in Manchego aged 8 mo (Gómez-Ruiz et al., 2002) and a total of 41 peptides were found in 6

other Spanish cheeses (Gómez-Ruiz et al., 2006). In a new type of low-fat ripened cheese, 3 peptides with high ACE-inhibiting activity were found (Ryhänen et al., 2001) and in ovine and caprine cheese-like systems manufactured with proteases from *Cynara cardunculus*, 4 such peptides (Silva et al., 2006) were identified.

Among the various ACE-inhibiting peptides, the lactotripeptides Val-Pro-Pro (**VPP**) and Ile-Pro-Pro (**IPP**), first isolated from *Lactobacillus helveticus*-fermented milk (Nakamura et al., 1995a), have been the subject of various in vivo studies. The antihypertensive activity of these 2 peptides was demonstrated several times in spontaneously hypertensive rats (Nakamura et al., 1995b; Masuda et al., 1996; Sipola et al., 2001) as well as in patients with mild hypertension (Hata et al., 1996; Seppo et al., 2002, 2003; Tuomilehto et al., 2004; Jauhiainen et al., 2005). Two recent studies showed that the 2 tripeptides may be naturally present in several cheese varieties of Swiss origin at concentrations similar to those found in fermented milk products with blood-pressure-lowering properties (Bütikofer et al., 2007, 2008). In an initial study with 44 individual samples of various cheese varieties, the concentrations of VPP and IPP varied between 0 mg/kg in Mozzarella and 320 mg/kg in Berner Hobelkäse (Bütikofer et al., 2007). Although Bernard et al. (2005) previously reported a total concentration of 566.88 mg/kg of VPP and IPP in blue cheese, only moderate concentrations were found in the investigated soft cheeses probably because of poor proteolysis (Bütikofer et al., 2007). Therefore, in a second study, our investigations focused on semi-hard, hard, and extra-hard cheeses made from raw or thermized milk. In the group of semi-hard cheeses (n = 28), the total concentration of VPP and IPP was on average at 89 mg/kg (range: 7 to 317 mg/kg) and in 73 samples of extra-hard and hard cheeses, a mean concentration of 96 mg/kg (range: 2 to 425 mg/kg) of VPP and IPP was obtained (Bütikofer et al., 2008).

The results of several studies suggest that ACE-inhibitory activity and the concentration of the involved peptides increased with cheese ripening, but later declined when proteolysis exceeded a certain degree (Haque and Chand, 2008). For example, in 3 samples of Emmentaler manufactured in a factory using a traditional whey culture the total concentrations of VPP and IPP were 139.3, 184.6, and 165.6 mg/kg at 4, 9, and 12 mo of aging, respectively, indicating a maximum value in medium-aged cheese (Bütikofer et al., 2008). Meisel et al. (1997) measured the ACE inhibition of young, medium, and old Gouda cheeses and also obtained an optimum in medium-aged cheese. Saito et al. (2000) obtained a significantly greater reduction of blood pressure in spontaneously hypertensive rats with Gouda aged for 8 mo compared with Gouda aged for 24 mo. In Festivo, a newly developed functional cheese, ACE inhibition increased to over 50% at wk 13 and then decreased in cheese aged 20 wk (Ryhänen et al., 2001). However, the monitoring of 5 ACE-inhibiting peptides in 4 batches of Manchego during a 12-mo ripening period did not show an age-dependent trend (Gómez-Ruiz et al., 2004). In Cheddar cheeses produced with probiotic adjunct cultures increased ACE inhibition was obtained after 24 wk of ripening at 4 and 8°C compared with control cheese (Ong and Shah, 2008a). In Cheddar cheeses made with starter lactococci (control), *Lactobacillus acidophilus* L10 and starter lactococci, or *Lactobacillus acidophilus* L10, *Lactobacillus helveticus* H100, and starter lactococci, the percentage of ACE inhibition increased by increasing the temperatures from 4 to 8 to 12°C, but the ACE-inhibitory activity among these cheeses did not differ significantly (Ong and Shah, 2008b). In our previous studies wide variations were observed in the concentrations of VPP and IPP within samples of the same age from the same variety (Bütikofer et al., 2007, 2008) underlining the fact that other factors besides ripening may have a more pronounced effect on the concentrations of ACE-inhibiting peptides. The results of recent studies show that a better understanding of the factors affecting the concentrations of ACE-inhibitory peptides in cheese is still needed to develop functional cheeses with antihypertensive potential. The aim of the present study was therefore to monitor the ACE-inhibitory activity and concentrations of the 2 lactotripeptides, VPP and IPP, in individual loaves of Appenzeller 1/4 fat, Tilsiter, Tête de Moine, Vacherin fribourgeois, Emmentaler, Gruyère, and Berner Hobelkäse from the beginning of commercial ripeness until full ripeness and to investigate differences among the cheeses manufactured by several producers of the same variety.

MATERIALS AND METHODS

Selection of Cheeses and Ripening Conditions

Three loaves each of Appenzeller 1/4 fat, Tilsiter, Tête de Moine, Vacherin fribourgeois, Emmentaler, Gruyère, and Berner Hobelkäse manufactured in different cheese factories were purchased before the usual sale date and further ripened and cured under typical conditions in the ripening cellars of the research station Agroscope (Liebefeld, Switzerland) until the end of the usual sale period. The cheeses were manufactured according to the directions of the individual cheese associations as described previously (Bütikofer et al., 2008).

Two of the semi-hard cheeses, Appenzeller 1/4 fat and Tilsiter, were investigated from 3 to 10 mo of aging, and the other two, Tête de Moine and Vacherin