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Occurrence of the Angiotensin-Converting Enzyme–Inhibiting Tripeptides Val-Pro-Pro and Ile-Pro-Pro in Different Cheese Varieties of Swiss Origin

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ABSTRACT

The contents of the 2 antihypertensive peptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) were determined in 101 samples from 10 different Swiss cheese varieties using HPLC with subsequent triple mass spectrometry. In the category of extra hard and hard cheeses, the Protected Denomination of Origin cheeses Berner Alpkäse and Berner Hobelkäse, L'Etivaz à rebibes, Le Gruyère, Sbrinz, Emmentaler (organic and conventional) and in the category of semihard cheeses, the varieties Tilsiter, Appenzeller ¼ fat and full fat, Tête de Moine, and Vacherin fribourgeois were screened in the study. The average concentration of the sum of VPP and IPP in the screened cheese varieties varied to a large extent, and substantial variations were obtained for individual samples within the cheese varieties. The lowest average concentration of the 2 tripetides was found in L'Etivaz à rebibes (n = 3) at 19.1 mg/kg, whereas Appenzeller ¼ fat (n = 4) contained the greatest concentration at 182.2 mg/kg. In individual samples, the total concentration of VPP and IPP varied between 1.6 and 424.5 mg/kg. With the exception of a 10-yr-old cheese, VPP was always present at greater concentrations than IPP. Milk pretreatment, cultures, scalding conditions, and ripening time were identified as the key factors influencing the concentration of these 2 naturally occurring bioactive peptides in cheese. The results of the present study show that various traditional cheese varieties contain, on average, similar concentrations of the 2 antihypertensive peptides to the recently developed fermented milk products with blood pressure–lowering property. This may serve as a basis for the development of a functional cheese with blood pressure–lowering property.
Key words: angiotensin-converting enzyme-inhibiting peptide, Val-Pro-Pro, Ile-Pro-Pro, Swiss cheese variety

INTRODUCTION

Angiotensin-converting enzyme (**ACE**)-inhibiting peptide sequences are found in the AA sequence of different proteins, and the peptides can be formed during the fermentation of foods or by digestion of food proteins (Bachmann et al., 2003). These bioactive peptides are of special interest because of their blood pressure–lowering activities. The conversion of angiotensin I to angiotensin II, which causes the contraction of blood vessels and increases blood pressure, is inhibited and the degradation of bradykinin. This, in turn, leads to a dilation of the blood vessels and to a decrease in blood pressure to inactive fragments, is prevented (Li et al., 2004).

Different foods have been shown to contain these ACE-inhibiting peptides and also ACE-inhibiting activity (Li et al., 2004). Water-soluble extracts of several cheese varieties such as Norvegia, Jarlsberg, Cheddar, Blue (Stepaniak et al., 2001), Gouda, Emmentaler, Camembert, Edam, and Havarti (Saito et al., 2000) as well as several Italian cheeses with short and medium ripening times (Smacchi and Gobbetti, 1998) showed large differences in ACE-inhibitory activity in vitro. In addition, ACE-inhibitory potential was measured in the ethanol-soluble fraction of different cheeses such as Gamalost, Pultost, Norvegia, Castello, French Brie, Port Salut, and Kesam (Pripp et al., 2006). Furthermore, a large number of individual peptides with ACE-inhibitory activity could be identified in various cheese varieties. In water-soluble extracts of an 8-mo-old Manchego manufactured from sheep milk, 22 (Gómez-Ruiz et al., 2002) or 75 (Gómez-Ruiz et al., 2004) peptides with ACE-inhibitory activity were identified. These peptides varied in size from dipeptides to decapeptides. Recently, the same research group found 41 ACE-inhibitory peptides (<1,000 Da) in the permeate of different Spanish cheeses analyzed by HPLC-MS/MS and off-line MS/MS, in which all peptides showed moderate or low ACE-inhibitory activity (Gómez-Ruiz et al., 2006). In an 8-mo-old Gouda 2 peptides, α_{s1}-casein f(1–9) and β-CN f(60–68), showed efficient ACE-inhibiting activity (Saito et al., 2000). In cheese, the

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formation of antihypertensive peptides can be influenced by milk pretreatment, processing, and ripening time (Bütikofer et al., 2007). Cheese made from raw milk showed greater ACE-inhibition than that from pasteurized milk. The ACE-inhibiting activity found in Harz, Camembert, Tilsiter, Edam, Leerdam, Roquefort, Emmentaler, Cheddar, and Parmesan was strongly dependent on the degree of proteolysis and the age of the cheese (Meisel et al., 1997). However, the blood pressure of spontaneously hypertensive rats was significantly reduced to a greater extent after feeding of Gouda cheese aged for 8 mo compared with 24-mo-old Gouda. The younger cheese contained 2 nonapeptides, an undecapeptide, and a tridecapeptide (Saito et al., 2000).

In vivo, antihypertensive effects of bioactive peptides were demonstrated in spontaneously hypertensive rats (Nakamura et al., 1995a; Muguerza et al., 2006; Quirós et al., 2007) and in hypertensive human subjects (Hata et al., 1996; Seppo et al., 2002, 2003; Tuomilehto et al., 2004; Mizuno et al., 2005) after administration of *Lactobacillus helveticus*-fermented milk (Nakamura et al., 1995a; Hata et al., 1996; Seppo et al., 2002, 2003; Tuomilehto et al., 2004; Jauhiainen et al., 2005), *Enterococcus faecalis*-fermented milk (Muguerza et al., 2006; Quirós et al., 2007), or a casein hydrolysate prepared using an *Aspergillus oryzae* protease (Mizuno et al., 2005; Sano et al., 2005). The tripeptides valyl-prolyl-proline (Val-Pro-Pro; **VPP**) and isoleucyl-prolyl-proline (Ile-Pro-Pro; **IPP**) from *L. helveticus*-fermented milk were identified as the most potent antihypertensive peptides (Nakamura et al., 1995b). The products given to mildly hypertensive or hypertensive patients contained between 2 and 5 mg of the 2 tripeptides (Hata et al., 1996; Seppo et al., 2002, 2003; Tuomilehto et al., 2004; Mizuno et al., 2005). However, 10-fold-greater doses of 50 mg/d have also been used in clinical studies (Jauhiainen et al., 2005). In a double-blind, placebo-controlled crossover study with 6 healthy subjects, plasma concentrations of IPP were increased after consumption of a lactotripeptide-enriched milk beverage (Foltz et al., 2007).

In a recent study, we quantified the VPP and IPP tripeptides in individual samples of various extra hard, hard, semihard, and soft cheeses with a new method using HPLC coupled with triple MS detection (**HPLC-MS^3**; Bütikofer et al., 2007). In the 44 samples we identified 9 different cheese varieties (Berner Hobelkäse, Emmentaler, Gouda, Appenzeller full fat and ¼ fat, Tilsiter, Winzerkäse, Tête de Moine, and Vacherin fribourgeois) that contained VPP and IPP at concentrations above 100 mg/kg. Because these 2 ACE-inhibiting peptides in recently developed fermented milk products have been shown to be effective against hypertension at a dose of about 5 mg/d, the daily consumption of a 50-g portion of cheese containing similar amounts of these peptides could be of interest as a dietary recommendation for individuals with mild hypertension. However, because of the large number of small-scale cheese factories in Switzerland and due to differences in cultures, processing, and ripening conditions from factory to factory, it is difficult to specify the concentration of VPP and IPP for any individual cheese variety. Nevertheless, a better knowledge of the naturally occurring concentrations of VPP and IPP in traditional cheese varieties would be helpful for the development of a reproducible cheese-making process yielding these antihypertensive peptides at high concentrations. The aim of the present work was therefore to measure the concentration of these 2 ACE-inhibitory peptides in a series of traditional Swiss cheese varieties.

MATERIALS AND METHODS

Cheese Samples

A total of 101 commercial cheese samples from the following Swiss cheese varieties were analyzed: Berner Alpkäse (n = 11) and Berner Hobelkäse (n = 23), L'Etivaz à rebibes (n = 3), Sbrinz (n = 7), Le Gruyère (n = 9), Emmentaler of conventional (n = 12) and organic (n = 8) production, Tilsiter (n = 6), Appenzeller full-fat (n = 6), Appenzeller ¼ fat (n = 4), Tête de Moine (n = 6), and Vacherin fribourgeois (n = 6). With the exception of Appenzeller and Tilsiter, the included cheese varieties are registered as products with Protected Designation of Origin (**PDO**) with well-defined manufacturing procedures. The most important technological characteristics of the cheese varieties are summarized in Table 1. Cheese samples were purchased randomly from local cheese factories or obtained directly from cheese associations. The production date and the approval number of the manufacturer were registered to obtain further information and to determine the exact age of the cheese samples. The cheese samples were stored at –20°C until analysis. All samples were analyzed for moisture, protein, TCA-soluble nitrogen (**TCA-SN**) and fat using standard methods as previously described (Bütikofer et al., 2007). Furthermore, fat in DM and moisture on a fat-free basis were calculated.

HPLC-MS^3 Determination of ACE-Inhibiting Peptides VPP and IPP

Analytical grade formic acid and HPLC-grade acetonitrile were purchased from Sigma-Aldrich (Buchs, Switzerland). Deionized water was prepared on a