

Quantification of ACE-inhibiting tripeptides Val-Pro-Pro and Ile-Pro-Pro in traditional cheese varieties with LC-MS³ and determination of the IC₅₀ values

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Introduction

During cheese ripening caseins are degraded by proteinases to large peptides and in a second step by peptidases to smaller peptides and free amino acids. Some of the liberated peptides show biological activity in the human organism such as antihypertensive, immunomodulating, opioid and antimicrobial effects. Among the various bioactive peptides, the ACE-inhibiting peptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) are of special interest, because they can influence blood pressure.

A new quantitative HPLC method with MS³ detection was developed for the quantitative determination of VPP and IPP in cheese. Additionally, the inhibition of the angiotensin converting enzyme (ACE) *in vitro* was investigated by determination of IC₅₀ values of the filtered water-soluble extracts

Materials and Methods

A total of 43 cheese samples mainly originated from Switzerland were purchased.

Determination of ACE-inhibiting peptides VPP and IPP

The HPLC separation was performed on a PLRP-S column 1 x 150 mm (300 Å, 3 µm). LC-MS experiments were carried out on a Finnigan LTQ linear ion trap mass spectrometer coupled with a CTC HTS Autosampler. Pro-Pro-Pro-Pro (PPPP) has been used as internal standard. A five point calibration was used for calibration of VPP.

Determination of IC₅₀

The *in vitro* investigation of ACE activities were performed according to the method of Cushman and Cheung (1), modified by Parrot et al. (2). The IC₅₀ values were determined in filtered water-soluble extracts (cut off 3 kD).

Results

The correlation coefficients between the IC₅₀ values (expressed as weight of cheese) and the sum of the two peptides VPP and IPP of soft, semi-hard, hard and extra-hard cheeses were at $r = -0.357$, $r = -0.534$, $r = -0.721$ and $r = -0.878$, respectively, indicating that in hard and extra-hard cheeses there is a good correlation between ACE-inhibiting activity and the presence of these two peptides (Table 1). In fresh and soft cheese ($n = 11$) only low concentrations (0 – 11 mg/kg) of VPP and IPP could be found. In semi-hard cheese ($n = 17$) the concentration range was from 1 to 120 mg/kg for VPP and 0 to 47 mg/kg for IPP. The highest concentrations in semi-hard cheese were found in non-pasteurized Tilsit and Vacherin fribourgeois (Table 2).

Table 1 Concentration of VPP+IPP and IC₅₀ values

Cheese type	n	VPP+IPP [mg/kg]		IC ₅₀ mg cheese / ml		correlation VPP+IPP & IC ₅₀
		Mean	SD	Mean	SD	r
Soft cheese	11	3	± 6	17	± 6	-0.36
Semi-hard cheese	17	36	± 40	13	± 7	-0.53
Hard cheese	11	93	± 53	11	± 7	-0.72
Extra-hard cheese	5	127	± 128	13	± 11	-0.88

In hard and extra-hard cheeses ($n = 16$) the concentration range was from 2 to 224 mg/kg for VPP and 1 to 95 mg/kg for IPP. The highest concentration of both peptides were found in a long ripened raw milk extra-hard cheese from the Bernese Oberland (Hobelkäse) which contained 224 mg/kg VPP and 95 mg/kg IPP.

Table 2 Cheese samples with high VPP and IPP content

	Cheese variety	Age	Origin	VPP+IPP [mg/kg]	Ratio VPP/IPP	IC ₅₀ mg cheese / ml
1	Hobelkäse Bernese Oberland	> 18 m	CH	319.5	2.5	2.6
2	Emmental, organic	> 4 m	CH	189.5	4.5	7.5
3	Gouda old	?	NL	187.8	1.1	2.0
4	Appenzeller, 1/4 fat	7-10 m	CH	159.3	1.5	4.2
5	Tilsit, raw milk	> 90 d	CH	150.3	4.2	4.4
6	Winzerkäse	> 4 m	CH	124.7	3.8	9.9
7	Emmental, cave ripened	> 12 m	CH	123.6	4.5	7.1
8	Tête de moine	> 75 d	CH	113.5	6.6	7.1
9	Vacherin fribourgeois	> 60 d	CH	108.8	1.4	5.0
10	Gruyère, salted	> 10 m	CH	89.1	3.5	14.2

Conclusions

The results indicate that various traditional cheese varieties, mainly extra-hard, hard and semi-soft cheeses contain considerably amounts of VPP and IPP.

Based on results of clinical studies in human with daily consumption of fermented milks containing a total of 5 mg VPP and IPP, it can be speculated that regular consumption of traditional cheese varieties rich in these two bioactive peptides is in favour for lowering blood pressure in mildly hypertensive patients.

To cover a daily intake of 5 mg VPP and IPP, it would be necessary to consume in average 1500 g soft cheese, 140 g semi hard cheese, 50 g hard cheese or 40 g extra-hard cheese. Further studies will show the variation and development of VPP and IPP within individual cheese varieties.



¹ Cushman, D. W., & Cheung, H. S. (1970). *Biochemical Pharmacology* 20, 1637-1648

² Parrot, S., Degraeve, P., Curia, C., & Martial-Gros, A. (2003). *Nahrung* 47, 87-94.