

# Quantitative Determination of the Bioactive Peptides Val-Pro-Pro and Ile-Pro-Pro in Cheese with LC/MS<sup>3</sup>

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## Introduction

Proteolysis, glycolysis and lipolysis are the main factors for the development of texture and flavour in cheese. During cheese ripening, caseins are degraded to polypeptides and oligopeptides and finally to free amino acids. A large number of these peptides have biological activities; such as antihypertensive, immunomodulating, opioid and antimicrobial effects.

The antihypertensive activity of the two  $\beta$ -casein derived peptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) has been demonstrated in various human studies at a total intake of 5 mg/day.

The quantitative determination of VPP and IPP in casein hydrolysate with HPLC-MS was reported by Matsuura et al. (1). They used single stage MS detection of VPP at m/z 312.1 and IPP at m/z 326.1.

In cheese samples, much more low molecular peptides are present which might interfere in the quantification of VPP and IPP. Therefore a suitable technique with MS<sup>3</sup> detection was developed for the quantification of VPP and IPP in cheese.

## Materials and Methods

In total, 44 commercial cheese samples were purchased. The water soluble extract from the cheese samples were prepared according to Gomez et al. (2). The water soluble extracts were ultrafiltered using a 3 kDa cut-off membrane.

The HPLC separation was performed on a PLRP-S column 1 x 150 mm (300 A, 3  $\mu$ m). LC-MS Experiments were carried out on a Finnigan LTQ linear ion trap mass spectrometer coupled with a CTC HTS Autosampler (see fig. 1).



Figure 1: HPLC MS system

### HPLC parameters

The following solvent system was used for the analysis. A: 0.5 % (v/v) formic acid in water and B: 0.5 % (v/v) formic acid in acetonitrile. The flow was 70  $\mu$ L/min. The gradient was programmed in the following manner: 0–30 min, 0–40 %B. The column was kept at 25°C. The retention time of VPP, IPP and PPPP (internal standard) were 8, 11 and 11.6 min, respectively (see figure 2).

### MS parameters

Separate MS experiments were set up for VPP, IPP and PPPP:

Peptide	[M+H] <sup>+</sup>	Precursor ion
VPP	312.2	213.1
IPP	326.2	213.1
PPPP	407.3	310.2

## Results

### Validation

	VPP	IPP
Mean $\pm$ standard dev. (N=6)	71.4 $\pm$ 3.2 mg/kg	6.7 $\pm$ 1.0 mg/kg
Limit of detection	0.07 mg/kg	0.01 mg/kg
Limit of determination	0.22 mg/kg	0.03 mg/kg
Recovery (N=6)	96.6 % (87-103 %)	99.5 % (98-101 %)

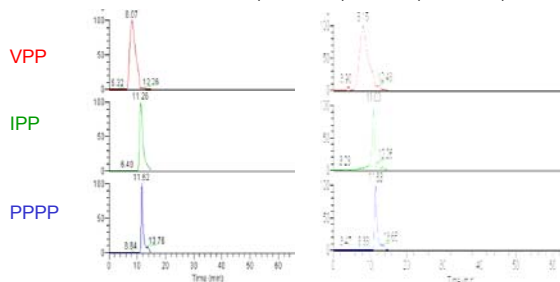


Figure 2: MS<sup>3</sup> signals of VPP, IPP and PPPP in standard solution (left) and in a cheese sample (right)

### VPP and IPP in cheese

From the 44 cheeses, 9 samples showed a total concentration (VPP + IPP) over 100 mg/kg (see table 1). 14 cheese samples were in the range from 30–90 mg/kg and 21 samples had a concentration below 30 mg/kg.

Table 1: Cheese samples with high VPP and IPP content

Cheese variety	Age [months]	VPP [mg/kg]	IPP [mg/kg]	VPP+IPP [mg/kg]
Hobelkäse Bernese Oberland	> 18	224	95	320
Emmentaler, organic	> 4	154	36	189
Gouda, old	unknown	98	90	188
Appenzeller, 1/4 fat	> 7	94	65	159
Tilsiter from raw milk	> 3	120	30	150
Winzerkäse	> 4	98	27	125
Emmentaler, cave ripened	> 12	100	23	124
Tête de Moine	> 2.5	98	16	114
Vacherin Fribourgeois	> 2	62	47	109

## Conclusions

A new HPLC method with MS<sup>3</sup> detection was developed for the quantitative determination of VPP and IPP in cheese with PPPP as internal standard.

In fresh and soft cheeses (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 1–120 mg/kg for VPP and 0.1–47 mg/kg for IPP. The highest concentrations in semi hard cheeses were observed in a non-pasteurized Tilsiter and in a Vacherin fribourgeois. In hard and extra-hard cheeses (N = 16) the concentration range was from 2–224 mg/kg for VPP and 1–95 mg/kg for IPP. The highest concentration of both peptides were detected in a long ripened raw milk cheese from the Bernese Oberland (Hobelkäse).

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.

## References

- Matsuura, K., Mizuno, S., Nishimura, S., Gotou, T., & Yamamoto, N. (2005). *Milchwissenschaft-Milk Science International*, 60(1), 24-27.
- Gomez-Ruiz, J. A., Ramos, M., & Recio, I. (2002). *International Dairy Journal*, 12(8), 697-706.