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Bitter Fractions in Soft Cheese

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Introduction

Consumers accept bitterness as a desired flavour characteristic only in few foodstuffs, such as grapefruit, specific vegetables like broccoli, cocoa, beer, tea or coffee. In general, bitterness of foods is considered a flavour defect. Because of its considerable economic importance bitterness in cheese has been investigated by several authors [1,2]. Soft cheeses and fat reduced cheeses are known to be more susceptible to develop bitter tastes than hard cheeses. Bitterness of cheeses is mainly due to bitter tasting hydrophobic peptides present at concentrations above their taste thresholds [3]. Cultures employed for cheesemaking contribute considerably to the proteolysis and to the formation of bitter peptides by the specificity and activity of their proteolytic enzymes. Many of the known bitter peptides derive from α_{s1}- and ß-casein [1-3].
In the present study a soft cheese manufactured with addition of a *Lactobacillus casei* strain was assessed sensorially as more bitter compared to the control cheese produced without adjunct culture. The aqueous extracts of the cheeses were ultrafiltered and fractionated by gel permeation chromatography prior to peptide analysis by HPLC-MS^n.

Materials and methods

Samples: Two Camembert type soft cheeses, EH 12 and EH 15, were produced from pasteurised milk. EH 12 was manufactured using a standard culture and EH 15 contained a mesophilic culture mix and additionally a *Lactobacillus casei* strain (FAM 18121). Both cheeses were ripened for 30 days.

Cheese extract: Each cheese was suspended in Milli Q water and extracted at ambient temperature. The suspension was adjusted to pH 4.6 and further extracted for 1h.

Fractionation: Ultrafiltration (M_r < 10 kDa) and gel permeation chromatography (Sephadex G 15) allowed to isolate low molecular weight peptides.

Peptide analysis: The bitter fractions were analysed by RP-HPLC-MS^n (XTerra MS C18 column). Mass spectra were obtained by electrospray ionisation mass spectrometry using a linear ion trap mass spectrometer. Peptide identification was performed using BioWorks 3.1.

Sensory evaluation: The lyophilised fractions dissolved in water were evaluated for their bitter taste intensities by seven trained panellists.

Results

* The gel permeation chromatograms of the two cheese extracts depicted in *Figure 1* show more intense UV signals of the fractions F2, F3 and F4 of EH 15 compared to the control cheese EH 12. The sensory evaluation of the fractions by a trained sensory panel revealed the fractions F1 and F2 of both cheeses as bitter. Particularly fraction F2 of EH 15 was assessed as strongly bitter, whereas F1 was less bitter compared to F1 of the control cheese. Further investigation was focused on the peptides in fraction F2.

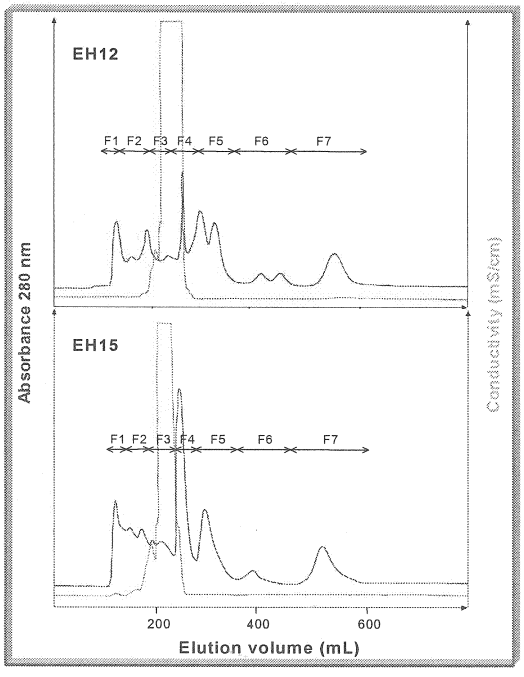

Figure 1. Gel permeation chromatography of ultrafiltered cheese extracts

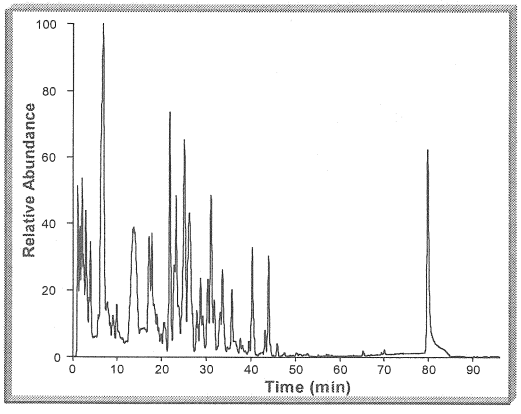

Figure 2. HPLC-MS chromatogram of the bitter fraction F2 of EH 15

* Reversed-phase HPLC in combination with mass spectrometry of the two cheese fractions allowed to compare the peptide profiles of the two fractions. *Figure 2* displays the base peak LC-MS chromatogram of fraction F2 of EH 15. EH 15 showed more signals in the molecular weight fraction $M_r \leq 1500$ compared to EH 12. The preliminary peptide identification revealed 62 peptides in EH 15, of which 26 derived from α_{s1}- casein and 36 peptides from ß-casein C.

* *Table 1* lists a selection of the peptides detected in fraction 2 of EH 15 which were different from the ones found in EH 12. The peptides are of low molecular weight and stem mainly from the C-terminus of ß-casein C or from the N-terminal region of α_{s1}-casein. These two casein fractions comprise around 80 % of the casein composition of cheese. Probably for this reason most of the known bitter peptides in cheeses were identified in these two casein fractions [1,3].

* Visser et al [3] identified the bitter peptide sequence f(193-209) from ß-casein in Gouda cheese. Many of the peptides detected in this study are fragments of this ß-casein fragment. Other peptides stem from α_{s1}-casein which has been described as source of bitter tasting peptides in cheeses, too [1]. Especially the sequence f(21-23) and f(23-34) exhibited bitter tastes in Cheddar cheese. Manifold factors may explain the presence of the bitter peptides detected in the present study.

Table 1. Selected putatively bitter peptides detected in fraction 2 of EH 15

RT [min]	Peptide sequence	Casein fraction	MW $[M+H]^+$
1.37	GPVRG	β f(199-203)	485.6
2.80	VLGPVRG	β f(197-203)	697.9
5.60	EPVLGPVRG	β f(195-203)	924.1
5.61	PVLGPVRG	β f(196-203)	795.0
5.74	EPVLGPVR	β f(195-202)	867.0
5.76	PVLGPVR	β f(196-202)	737.9
7.64	PVLGPVRG	β f(196-203)	795.0
10.43	EPVLGPVRGP	β f(195-204)	1021.2
16.68	FPEVFGKE	α_{s1} f(28-35)	953.1
16.94	PEVFGKE	α_{s1} f(29-35)	805.9
17.31	FPEVFGKEK	α_{s1} f(28-36)	1081.3
20.61	VPSERYLGYL	α_{s1} f(86-95)	1197.4
22.50	GPVRGPFPI	β f(199-207)	940.1
24.53	PEVFGKE	α_{s1} f(29-35)	805.9
24.59	VLGPVRGPF	β f(197-205)	942.1
30.02	GPFPIIV	β f(203-209)	742.9
33.14	SLVYPFPGPIPN	β f(57-68)	1301.5

* All of the peptides listed in *Table 1* contain leucine and/or valine and also at least one proline residue, some other peptides also one or more phenylalanine residues. According to Ney [5] and Matoba and Hata [6] the bitterness of peptides is positively correlated to their mean hydrophobicity. Aromatic amino acids as well as leucine, isoleucine, and proline residues (single letter codes F, Y, W, L, I, P, respectively) contribute most to the bitter taste of peptides. Bitter peptides are present in bitter cheeses but also in taste-wise faultless cheeses. The concentration of the peptides is crucial for the development of bitter taste.

Further investigation of the fractions to elucidate which ones of the identified peptides contribute most to the bitter taste is currently ongoing.

References

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