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Original article

Characterization of cheese ripening by free amino acids and biogenic amines and influence of bactofugation and heat-treatment of milk

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Summary — An efficient method for the simultaneous determination of free amino acids (FAA) and biogenic amines (BA) was applied to the analysis of presumed raw milk cheeses of the following types: Emmental ($n = 18$), Bergkäse ($n = 28$), semi-hard cheeses ($n = 18$) and soft cheeses ($n = 41$). The absolute amounts of FAA and BA largely varied, but the relative amounts could be classified into four groups independently of cheese type and ripening: i) glutamic acid, lysine, leucine (10–20% of total FAA); ii) proline, phenylalanine, valine (6–10%); iii) methionine, alanine, glutamine, isoleucine (3–6%); iv) BA and residual FAA (< 3%). As the formation of BA is strictly dependent on the decarboxylase activity of microorganisms – raw milk flora, starter, ripening and contaminating bacteria – a large variation within cheese types and even individual cheeses occurred. The use of bactofugated milk for the production of Emmental cheese resulted in a decrease of putrescine and cadaverine, but did not significantly influence the formation of histamine and tyramine. When comparing cheese samples of the same type but from different heat treatments of cheese milk or curd, FAA and BA metabolites showed various tendencies; in particular, BA were not generally reduced upon higher heat treatment. Furthermore, hard cheese samples with strong sensory defects showed considerable amounts of cadaverine (up to 1800 mg/kg) as well as δ-amino valeric acid (up to 2200 mg/kg), which in regular cheese samples of the same type and age was found to be remarkably lower (100–200 mg/kg). The combined data for FAA and BA provide useful information on cheese ripening and quality control.

free amino acid / biogenic amine / cheese ripening

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Résumé — Caractérisation de la maturation du fromage par ses acides aminés libres et ses amines biogènes, et influence de la bactofugation et du traitement thermique du lait. Une méthode performante a été utilisée pour l'analyse simultanée des acides aminés libres (AAL) et des amines biogènes (AB) de fromages supposés être fabriqués au lait cru, de types emmental ($n = 18$), de montagne ($n = 28$), à pâtes mi-dure ($n = 18$) et molle ($n = 41$). La teneur absolue totale en AAL et en AB variait beaucoup, mais a pu néanmoins être distribuée en quatre classes distinctes, indépendantes du type de fromage considéré et du degré d'affinage, soit : i) l'acide glutamique, la lysine et la leucine (10–20 % du total) ; ii) la proline, la phénylalanine et la valine (6–10 %) ; iii) la méthionine, l'alanine, la glutamine et l'isoleucine (3–6 %) ; iv) les AB et les autres acides aminés (< 3 %). Comme la formation des AB ne dépend que de l'activité de la décarboxylase des micro-organismes, de la flore originelle du lait, des levains, des bactéries d'affinage et des contaminants, on a observé une grande variation de concentration de ces constituants, tant entre les différentes sortes qu'à l'intérieur d'un même type de fromage. L'emploi de laits bactofugés pour la fabrication d'emmental se traduit par une diminution des teneurs en putrescine et en cadavérine, mais n'influence pas de façon significative celles en histamine et en tyramine. Si l'on compare des échantillons de fromage «normaux» de même type, mais provenant de laits ou de caillés ayant subi des traitements thermiques différents, on constate que les métabolites suivis présentaient des tendances très variables, les AB n'étant pas significativement réduits en général par un traitement thermique plus élevé. Les échantillons de fromage à pâte dure avec des défauts marqués de flaveur contenaient tous des quantités considérables de cadavérine (jusqu'à 1 800 mg/kg) et d'acide δ-amino-valérique (jusqu'à 2 200 mg/kg), alors que des fromages de même type et de même âge contenaient des teneurs beaucoup plus faibles (100–200 mg/kg) de ces mêmes composants. Les résultats des analyses de AAL et de AB donnent donc de précieuses informations quant à l'affinage et au contrôle de la qualité des fromages.

acide aminé libre / amine biogène / affinage / fromage

INTRODUCTION

Many attempts have been made to monitor proteolysis in cheese ripening by analyzing the different nitrogen fractions (McSweeney and Fox, 1993), but little effort has been made to identify the entity of individual compounds of such relatively complex fractions. Peptide analysis reveals specific information on proteolytic pathways, provided that the primary products of proteolysis have been identified by sequencing (Polo et al, 1985; Addeo et al, 1992; Belitz and Kaiser, 1993).

The liberation of amino acids in cheese ripening was recognized very early (Ritter et al, 1966; Schormüller, 1968), and their contribution to cheese flavour has been discussed (Fox et al, 1993). It is well established that most microorganisms participating in the different stages of cheese ripening require certain free amino acids (FAA) as growth factors (Cogan and Hill, 1993). Furthermore, FAA composition has been evaluated to serve as typicality and quality index of several cheese varieties (Resmini et al, 1985, 1993; Resmini and Pellegrino, 1986). Although the absolute amounts of FAA vary to a large extent according to cheese type and age, relative amounts (percentage of individual FAA in total FAA) reveal significant differences in several cheese varieties or different ripening technologies applied to one cheese variety (Bütikofer and Fuchs, 1997). Several non-proteinogenic FAA (eg, α- and γ-amino butyric acid, ε-amino caproic acid) formed during amino acid metabolism may serve as early indicators of quality defects as a consequence of undesired fermentation or infection, as they may inhibit further proteolysis.

Aside from the toxicological potential of histamine, tyramine, 2-phenylethylamine (Taylor et al, 1982; Joosten, 1988) and the undesired flavour of putrescine, cadaverine, spermine, spermidine (Askar and Treptow, 1986), biogenic amines (BA) formed by decarboxylation of amino acid precursors provide additional information on cheese ripening and quality. In combination with FAA values, an extended set of data is available for the individual characterization of the ripening processes.

We have recently developed a method for the simultaneous determination of FAA and BA in various food and biological matrices (Krause et al, 1995) using pre-column derivatization via dabsyl chloride. The present paper describes the application of this method to the analysis of presumed raw milk cheeses.

MATERIALS AND METHODS

Cheese samples were obtained from the market or directly from several producers. Alkaline phosphatase activity was determined according to Rocco (1990) to ascertain whether raw milk had really been used for cheese production. Extraction of FAA and BA, deproteination of the extracts by micro-scale ultrafiltration, derivatization with dabsyl chloride and chromatography were performed as described elsewhere (Krause et al, 1995; Bockhardt et al, 1996).

RESULTS AND DISCUSSION

Procedures

For the analysis of cheese samples, extracts prepared by homogenization with 0.1 mol/L hydrochloric acid were simply but effectively deproteinized by micro-scale ultrafiltration. Without further pre-treatment, the resulting filtrates containing FAA and BA were directly analyzed using an automated pre-column derivatizer/autosampler. The elution profiles of an amino acid and biogenic amine standard mixture (A) and a hard cheese sample with abnormal flavour (B) are given in figure 1, illustrating the high separation efficiency of the chromatographic system. More than 40 amino acids and their metabolites could be separated simultaneously. Peak areas and concentrations were found to be linearly related from 1.25 to 1250 pmol and the detection limits ranged between 0.12 and 0.52 pmol. The average repeatabilities ranged between 1.3 and 3.1% and the recovery values were between 98 and 104% (Krause et al, 1995).

FAA and BA of raw milk cheese

Analysis of 103 cheese samples, presumed to be from raw milk, revealed that absolute amounts of FAA and BA varied to a large extent according to cheese age, type and origin, as has been reported by several authors (eg, Schormüller, 1968; Sieber et al, 1988; Lavanchy and Sieber, 1993; McSweeney and Fox, 1993; Bütikofer and Fuchs, 1997). Glutamic acid dominated in all cheese samples analyzed, the absolute amount reaching values up to 8 000 mg/kg, where its flavour-enhancing properties should be taken into consideration (Preininger et al, 1996). Total amounts of FAA and BA were highest in hard cheeses, ranging from 10–40 g/kg, whereas for semi-hard cheese amounts usually lower than 10 g/kg were observed. In fully ripened soft cheese samples amounts were below 15 g/kg, and pre-mature soft cheeses ranged within 5 and 7 g/kg. Independently of cheese type, age and origin, the relative proportions of FAA and BA could be divided into four classes with different levels. In the first group (i), the principal FAA were glutamic acid, leucine and lysine (10–20% of total FAA), followed by proline, valine and phenylalanine (6–10% of total FAA) in the second group (ii). The third group (iii) consisted of alanine, methionine, glutamine and isoleucine (3–6% of total FAA). The last group (iv) contained residual FAA and BA ranging between 0 and 3%. Similar results for different types of cheese have been obtained by other authors (summarized by McSweeney and Fox, 1993).

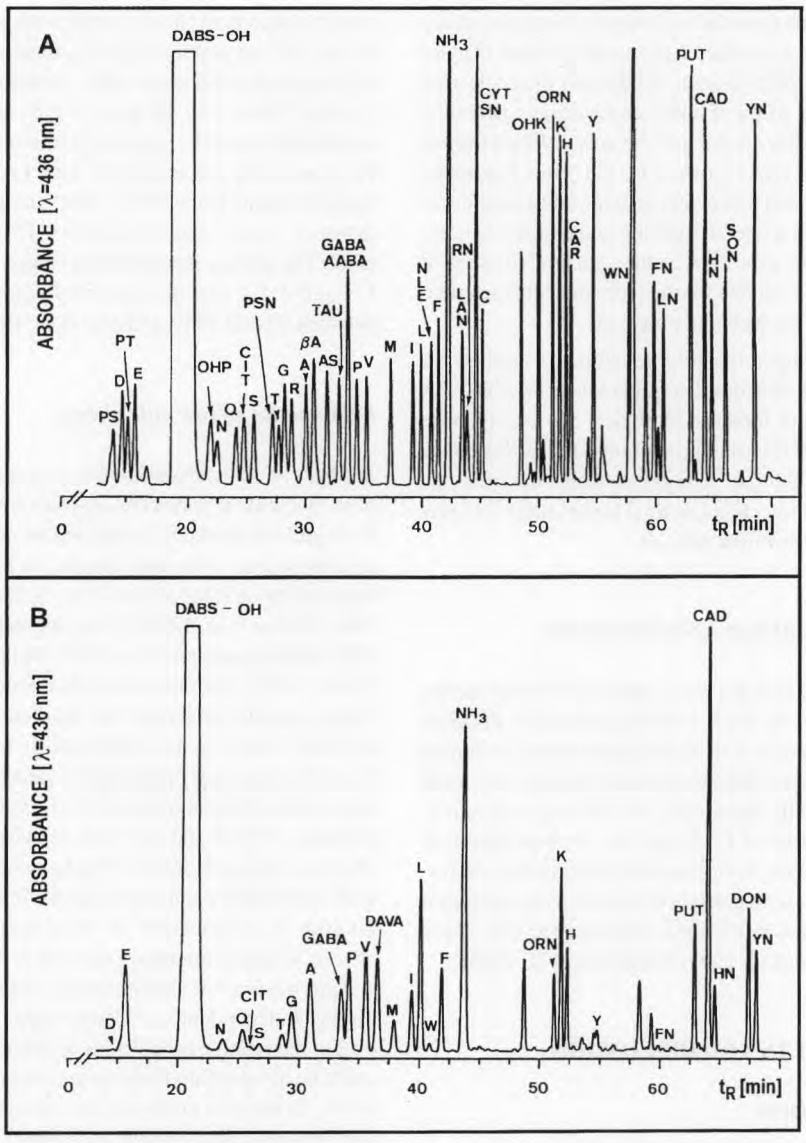

Fig 1. RP–HPLC separation of dabsyl derivatives from FAA and BA standard mixture (**A**) and Emmental cheese (**B**). Abbreviations for amino acids in one-letter code. AABA: α-amino butyric acid, AS: anserine, βA: β-alanine, CAD: cadaverine, CAR: carnosine, CIT: citrulline, CYT: cystathionine, DABS-OH: dabsonate, DAVA: δ-amino valeric acid, DON: 1,8 diamino octane (int standard), FN: 2-phenyl ethylamine, GABA: γ-amino butyric acid, HN: histamine, LAN: lanthionine, LN: 3-methyl butylamine, NLE: norleucine, OHK: hydroxylysine, OHP: hydroxyproline, ORN: ornithine, PS: *o*-phosphoserine, PSN: *o*-phosphoethanolamine, PT: *o*-phosphothreonine, PUT: putrescine, RN: agmatine, SN: ethanolamine, SON: serotonin, TAU: taurine, WN: tryptamine, YN: tyramine.

*Séparation par RP–HPLC de dérivés dabsyl de AAL et de AB dans un mélange standard (**A**) et dans un fromage d'emmental (**B**).*

In our investigations, special attention has been paid to selected FAA and their secondary metabolites (table I). In several hard and semi-hard cheeses, significant levels of histamine and tyramine were reached (up to 1000 mg/kg), whereas the maximum level of tyramine, cadaverine and putrescine was close to 2000 mg/kg. If high levels of BA were present in cheese samples, their amino acid precursors decreased correspondingly.

We have also compared data on FAA and BA of selected raw milk cheeses (Emmental, Bergkäse, semi-hard and soft cheeses) with phosphatase activity above 200 mU/g to those of the same cheese type but with low residual phosphatase activity (table II). In these samples, it was concluded that either the cheese milk has been low-temperature pasteurized or elevated cooking temperatures had been applied. Depending on the cheese type, the levels of selected FAA and their corresponding metabolites showed distinguishable tendencies. Histidine content was insensitive to heating, whereas histamine levels decreased in Emmental and Bergkäse with low phosphatase activity, but not in semi-hard and soft cheese. Tyrosine and lysine levels decreased in Emmental and Bergkäse with low phosphatase activity, but increased in semi-hard and soft cheese, whereas tyramine level decreased remarkably only in semi-hard and soft cheese. Cadaverine level was obviously increased in Emmental and Bergkäse with low phosphatase activity, but decreased in semi-hard and soft cheese. Within the group of arginine metabolites (table I) different tendencies occurred for individual cheese types. With the exception of soft cheeses, glutamic acid was generally lower upon heating, whereas γ-amino butyric acid increased, especially in Bergkäse.

In a further study, the influence of partial bactofugation (10 and 90%) of milk used for the production of Emmental cheese was investigated. Use of 90% bactofugated milk led to an almost complete decrease in putrescine and cadaverine (fig 2), but did not significantly influence the formation of histamine, tyramine and 3-methylbutylamine. Although the significance of these results has not yet been proved, the data clearly demonstrate the efficiency of the method, being capable of simultaneously determining

Table I. Amino acid precursors and selected metabolites.
Précurseurs des acides aminés et métabolites.

Amino acid [a]		*Metabolite(s)* [b]
Arg (R)	→	Citrulline (CIT) → Ornithine (ORN) → Putrescine (PUT) / δ-Amino valeric acid (DAVA)
Asp (D)	→	β-Alanine (βA)
Glu (E)	→	α-/γ-Amino butyric acid (AABA/GABA)
His (H)	→	Histamine (HN)
Leu (L)	→	3-Methyl butylamine (LN)
Lys (K)	→	Cadaverine (CAD)
Phe (F)	→	2-Phenyl ethylamine (FN)
Tyr (Y)	→	Tyramine (YN)

[a] Amino acid abbreviation: 3-letter code (1 letter code). [b] Abbreviation for metabolites: mutual.
[a] *Abréviation pour des acides aminés : code de trois lettres (code d'une lettre).* [b] *Abréviation pour des métabolites.*

Table II. Free amino acids and related metabolites (average in mg/kg) in cheese with different heat treatments.
Acides aminés libres et métabolites correspondants (moyennes en mg/kg) dans des fromages après divers traitements thermiques.

Type	*Emmental*		*Bergkäse*		*Semi-hard cheese*		*Soft cheese*	
Heating [a]	–	+	–	+	–	+	–	+
ALP (mU/g)	*200–500*	*< 50*	*200–1000*	*< 100*	*500–1500*	*< 200*	*500–2000*	*< 200*
n	*13*	*5*	*20*	*6*	*8*	*10*	*14*	*8*
H	480	432	546	521	177	151	315	358
HN	239	91	297	75	65	41	55	28
Y	835	652	628	433	179	259	266	484
YN	150	106	131	199	325	67	315	187
K	2211	1577	3524	1636	534	695	871	1164
CAD	23	463	65	402	682	61	467	220
R	176	243	153	114	48	189	98	534
CIT	378	441	522	355	99	109	225	271
ORN	795	398	838	427	223	247	228	225
PUT	35	136	87	131	207	39	132	56
DAVA	277	61	475	136	362	39	201	244
E	2790	1901	3582	2165	900	669	1366	1439
GABA	240	448	184	711	165	218	153	170

[a] –: raw milk cheese; +: low-temperature pasteurized cheese milk or elevated cooking temperatures as judged by ALP activity; for abbreviations, see table I; ALP: alkaline phosphatase.

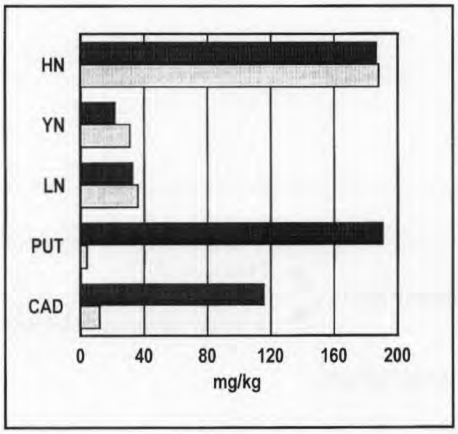

Fig 2. Effect of partial replacement of raw milk by bactofugated milk (■: 10%, ■: 90%) on the formation of BA in Emmental cheese ($n = 6$). For abbreviations: see figure 1.
Effet d'un remplacement partiel de lait cru par du lait bactofugé (■ : 10 %, ■: 90 %) sur la formation de AB dans un fromage d'emmental (n = *6).*

closely-related amino acid metabolites and thus providing data on the influence of technological processes on the quality of cheese as judged by their FAA and BA content.

In figure 3, the FAA and BA profile of a regular Emmental cheese is compared to that of the same cheese type with a pronounced sensory defect; a chromatogram of the latter is shown in figure 1B.

In addition to a high level of cadaverine which possibly contributes to the abnormal flavour, high amounts of δ-amino-valeric acid (up to 2200 mg/kg) were observed throughout in all cheese samples showing the same flavour defect ($n = 12$). This arginine metabolite (table I), which in regular cheese samples of the same type and age was found to be remarkably lower (100–200 mg/kg), might serve as an indicator of anaerobic contaminating microorganisms (Bockhardt et al, 1997).

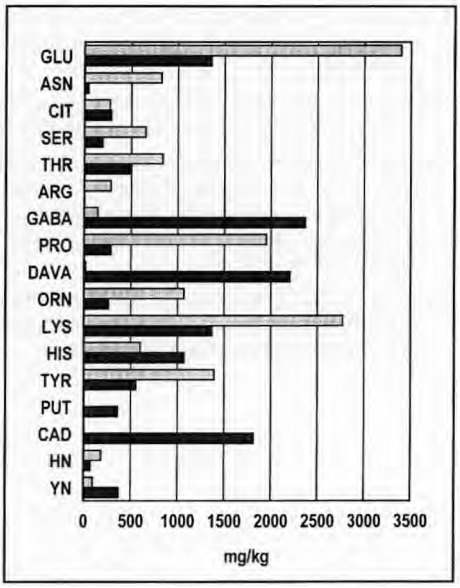

Fig 3. Profiles of selected FAA and BA in one Emmental cheese with regular flavour ( ) and off-flavour ( ). For abbreviations, see figure 1.
Profils de quelques AAL et AB d'un fromage d'emmental présentant une flaveur normale ( ) et un défaut de flaveur ( ).

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