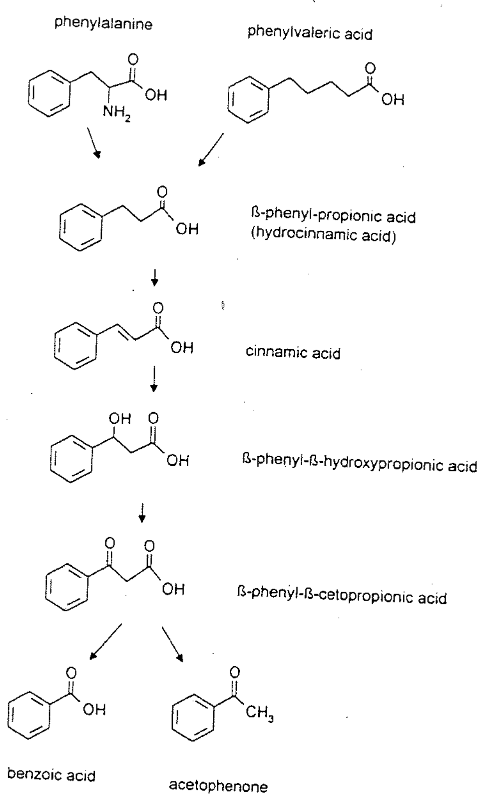
FAM-INFORMATION

May 1995 / 301 P/W

Federal Dairy Research Institute
CH-3097 Liebefeld-Berne

Benzoic Acid as a Natural Compound in Cultured Dairy Products and Cheese



. Breakdown of phenylalanine as an alternative pathway for the biogenesis of benzoic acid.

R. Sieber, J.O. Bosset
and U. Bütikofer

Int. Dairy Journal **5**, 227-246 (1995)

Benzoic Acid as a Natural Compound in Cultured Dairy Products and Cheese

R. Sieber, U. Bütikofer & J.O. Bosset

Federal Dairy Research Institute, CH-3097 Liebefeld-Bern, Switzerland

(Received 24 November 1993; revised version accepted 7 April 1994)

ABSTRACT

This review deals with the 'state of the art' of benzoic acid in cultured dairy products and cheese. During fermentation, benzoic acid is produced from hippuric acid, a component of milk naturally present at concentrations of up to 50 mg/kg. In smear-ripened cheese, however, higher benzoic acid concentrations have often been measured on the surface or even inside such cheeses. A second metabolic pathway has been proposed: during ripening, an additional quantity of benzoic acid originates from phenylalanine degradation, with β-phenyl-propionic (hydrocinnamic) acid and cinnamic acid as intermediate products. Acetophenone is a by-product of this breakdown, which essentially occurs in the rind and the smear. The presence of these intermediate products and their concentration gradient, the concentration of benzoic acid and its formation during cheese ripening, and the simultaneous production of ammonia resulting from deamination support the validity of this second metabolic pathway. A third way could be the auto-oxidation of benzaldehyde, produced by certain strains of lactic acid bacteria. In addition to the transformation of hippuric acid, these two sources (phenylalanine degradation, auto-oxidation of benzaldehyde) supply benzoic acid in cheese.

INTRODUCTION

Benzoic acid is widely used in the food industry as a preservative in acid foods, under code number E210, owing to its antimicrobial activity against various bacteria, yeasts and fungi involved in food poisoning and food spoilage, such as *Escherichia coli*, *Listeria monocytogenes*, *Aspergillus sp.* and

Penicillium sp. (Chipley, 1993). Benzoic acid occurs naturally in different foods, such as fruits, vegetables, spices and nuts (Nagayama *et al.*, 1983, 1986; Heimhuber & Herrmann, 1990) and also in milk, especially dairy products, at low concentration.

The objective of this paper is to collect together some of the results obtained by the authors from previous investigations on benzoic acid in cultured dairy products and cheese. The results have been supplemented by data from recently published literature. A review on the occurrence of benzoic acid in various foods has been published by Sieber *et al.* (1989).

The intake of benzoic acid differs in different countries. In the Netherlands, the mean total daily intake of benzoic acid by 16–18-year-old male adolescents was 34 mg and the maximum was 66 mg (van Dokkum *et al.*, 1982). In Finland, a comparable consumption of 40 mg benzoic acid per person and per day was estimated (Penttilä *et al.*, 1988). The daily intake of Japanese people during the period 1976–81 was calculated to be 10·9 mg benzoic acid and 1·01 mg of esters of *p*-hydroxybenzoic acid (Toyoda *et al.*, 1983). The FAO/WHO evaluated acceptable daily intake (ADI) is in the order of 5 mg benzoic acid/kg body weight.

Benzoic acid is a colourless aromatic compound. Its acidic character ($pK = 4{\cdot}19$ at 25°C) is due to its carboxylic group. Its solubility in water and/or in organic solvents depends primarily on its degree of protonation, i.e. on the pH of the medium. Its main chemical and physical properties are summarized in Table 1.

The first methods for the determination of benzoic acid in processed cheese were described in the 1930s (Hostettler, 1933). For analytical purposes, benzoic acid is usually extracted from foods by hot steam distillation after acidification (stripping) or by liquid/liquid extraction (Brandl & Binder, 1982). A preliminary alkaline pre-concentration may be required. The liquid/liquid extraction technique is more often used because it is more rapid than distillation. Benzoic acid can be quantified in foods by using different analytical methods, such as polarography, (spectro)photometry, isotachophoresis, gas chromatography with different detectors (FID, MSD), or high performance liquid chromatography (HPLC) with UV detection (Table 2). A provisional

TABLE 1

Some Physical and Chemical Properties of Benzoic Acid*

Other names	Phenylformic acid, benzenecarboxylic acid
Formula	C_6H_5COOH
Molecular weight	122·1
In pure form	Colourless, white needles, leaflets
Solubility	1·8, 2·7, 3·4, 22 g/L of water at 4°C, 18°C, room temperature and 75°C, respectively 10–20 g/kg of fatty oils Soluble in anhydrous ethanol
Melting point	122°C

*From Lück (1980) and Chipley (1993).

TABLE 2
Principal Methods for Determination of Preservatives

Product	*Method*	*Recovery (%)*	*Detection limit (mg/kg or L)*	*Compound*	*First-name author*
Milk	HPLC	95·2–98·7		B	Wiench (1984)
Milk, yoghurt	Polarography			B	Vogel (1965*b*)
Milk, sour milk	HPLC	>98	0·1	B, H	Hatanaka (1986)
Milk, dairy products	GC/MS			B	Drawert (1978)
Liquid food	HPLC/UV	95–101·5	5	B, S, E	Matsunaga (1985)
Yoghurt	HPLC/UV	91·2		B, S	Arimoto (1983)
Yoghurt	HPLC/UV	93·3–96·7	0·2	B, S	Ito (1983)
Yoghurt	LC	71–74	20	B, S	Puttemans (1985)
Yoghurt	HPLC/UV		5	B, H, S	Stijve (1984)
Yoghurt, cheese	HPLC/UV	98·5, 96·3		B, S, E	Leuenberger (1979)
Quarg, cheese	LC	95	1	B, S	Küppers (1988)
Cheese	GC			S, B	Stark (1976)
Cheese	GC/MS	96·4		B	Kurisaki (1973)
Cheese	HPLC		1–2	B	Toppino (1987*b*, 1990)
Dairy products	UV	92/75		B, S	Raissouni (1991)
Dairy products	HPLC/UV	98·8–100·8	1	B, S, E	Gieger (1982)
Dairy products	GC/MS	95·2	0·2	B	Richardson (1981)
Dairy products	HPLC/UV		3	B, S	Bütikofer (1988)
Dairy products	HPLC/UV		5	B, S	Anonymous (1988*a*)
Food	GC			B, S	Vogel (1965*a*)
Food	LC	90–105	20	B, S	Bui (1987)
Food (cheese)	GC/MS	95–102	0·1–0·25	B, S	Kakemoto (1992)
Beverage, jam	HPLC	98–104	<1	B, S	Hannisdal (1992)
Fatty foods	HPLC/UV			B, S, E	Aitzetmüller (1984)
Food	SP	96–103		B, S	Zoneveld (1975)
Water	HPLC/UV	>95	0·1	B	Dietz (1993)

(HP)LC(UV) = (high performance) liquid chromatography (with UV-detection), GC(MS) = gas chromatography (with mass spectrometric detection), SP = spectrophotometry.
B = benzoic acid; H = hippuric acid; S = sorbic acid; E = ester of *p*-hydroxybenzoic acid.

IDF standard method is available (Anon, 1987; 1988*a*). A HPLC method has been developed at the Swiss Dairy Research Institute to determine benzoic acid alone or together with other preservatives such as *p*-hydroxybenzoic acid methylester, -ethylester, -propylester, sorbic and hippuric acid and pimaricin (Bütikofer *et al.*, 1988).

OCCURRENCE OF BENZOIC ACID IN FERMENTED DAIRY PRODUCTS

Cultured or fermented dairy products such as yoghurt, fruit yoghurt, sour milk with bifidobacteria and kefir contain up to 50 mg/kg (0·41 mmol/kg) benzoic acid with most mean values around 20 mg/kg (Tables 3 and 4), but milk contains only a few mg/kg (Vogel & Deshusses, 1965*b*; Nishimoto *et al.*, 1968*a*, 1969; Chandan *et al.*, 1977; Drawert & Leupold, 1978; Richardson & Gray, 1981; Sajko *et al.*, 1984; Hatanaka & Kaneda, 1986; Nagayama *et al.*, 1986). According to Nishimoto *et al.* (1968*b*, 1969), lactic acid bacteria convert hippuric acid naturally present in milk (Fleischmann, 1932; Karabinos & Dittiner, 1943; Kieffer *et al.*, 1964; Svensen, 1974; Marsili *et al.*, 1981; Wolfschoon-Pombo & Klostermeyer, 1981; Lavanchy & Steiger, 1984; Hatanaka & Kaneda, 1986) to benzoic acid so that the latter could also be considered as a natural component of milk and milk products (Fig. 1). Wolfschoon-Pombo & Klostermeyer (1981) found a maximum of 62·4 mg/kg (0·35 mmol/kg) of hippuric acid in evening milk, which could

TABLE 3

Benzoic Acid Content of Some Yoghurt and Fruit Yoghurt (mg/kg)

Product	*N*	$\bar{x}$	*s*	Δ	$\tilde{x}$	*First-named Author*
Yoghurt	4	33·9		27–47		Vogel (1965*b*)
Yoghurt plain				16–25		Chandan (1977)
Yoghurt	4			21–25		Gieger (1982)
Yoghurt	11	25		13–39		Van Renterghem (1982)
Yoghurt	2	13·0		12, 14		Hatanaka (1986)
Yoghurt natural	8	18·2		9–25	19·1	Sieber (1990*a*)
Yoghurt fat-free				53–56		Chandan (1977)
Yoghurt skim milk	6			24–30		Gieger (1982)
Yoghurt skim milk	4	25		13–34		Stijve (1984)
Yoghurt skimmed	2	19·3		13, 25		Toppino (1990)
Yoghurt low fat				31–35		Chandan (1977)
Yoghurt 1% fat	32	14·8	3·4			Obentraut (1983)
Yoghurt 3·6% fat	40	14·3	4·0			Obentraut (1983)
Yoghurt cream	3			10–17		Gieger (1982)
Yoghurt full cream	8	26		18–37		Stijve (1984)
Fruit yoghurt				18–26		Chandan (1977)
Fruit yoghurt	10	25		19–33		Van Renterghem (1982)
Fruit yoghurt	92	15·0	4·0			Obentraut (1983)
Fruit yoghurt	18	24·4		15–39	23·0	Stijve (1984)
Fruit yoghurt	105	16·4	4·7	5–32	17·0	Sieber (1990*a*)
Fruit yoghurt	5	12·2		8–16	12·2	Toppino (1990)

N = Number of samples.
$\bar{x}$ = Mean.
s = Standard deviation.
Δ = Range.
$\tilde{x}$ = Median.

TABLE 4
Benzoic Acid Content of Some Other Fermented Dairy Products (mg/kg)

Product	*N*	$\bar{x}$	Δ	$\tilde{x}$	*First-named Author*
Bifidus sour milk	5	12·5	5–23	10·1	Sieber (1990*a*)
Cream, sour			11–18		Chandan (1977)
Cream, sour, 10% fat	4		10–16		Gieger (1982)
Buttermilk			11–16		Chandan (1977)
Buttermilk	4		10–19		Gieger (1982)
Kefir	3		18–23		Gieger (1982)
Kefir	3	14·5	8–19	16·5	Sieber (1990*a*)
Whey	3	12·6	12–13		Toppino (1990)
Whey powder	6	50·0	23-75		Kopp (1992)

N, $\bar{x}$, Δ, $\tilde{x}$: See footnotes to Table 3.

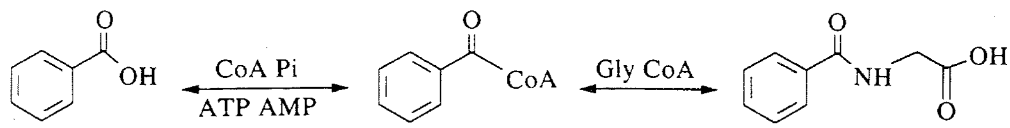


Fig. 1. Stoichiometric (reversible) conversion of hippuric to benzoic acid (Pi = phosphate; gly = glycine).

account for a maximum concentration of 0·35 mmol/kg of benzoic acid according to a stoichiometrical conversion. According to Tschager *et al.* (1993), there was a maximal hippuric acid content of 113 mg/L milk. In addition, *Lactococcus lactis*, *Escherichia coli* and *Pseudomonas fluorescens* can synthetize benzoic acid in milk (Sajko *et al.*, 1984).

OCCURRENCE OF BENZOIC ACID IN CHEESE

Benzoic acid is also present in fresh cheeses, quargs and various cheeses (Tables 5–7). Its concentration is normally below the above-mentioned, tolerated threshold of 50 mg/kg, except for some cheese varieties for which data were reported by Svensen (1974), Van Renterghem and Waes (1987), Müller (1990), Sieber *et al.* (1990*a*), and Kopp (1992). In these publications, the mean and median can be different. According to Toppino *et al.* (1990), higher concentrations of benzoic acid were also detected in Provolone cheese samples, where an additional amount of benzoyl peroxide, equivalent to 60 mg benzoic acid per kg, was permitted (Toppino *et al.*, 1987*b*); the concentration of benzoic acid remained unchanged during ripening (Toppino *et al.*, 1987*a*). Benzoic acid was also found in lactic casein (13·3 mg/kg), in lactic casein whey (15·0 mg/kg), and in lactic casein whey protein concentrate powder (50·4 mg/kg), but not in sulphuric acid and rennet casein (Richardson & Gray, 1981).

Non-smear-ripened cheeses contain relatively low concentrations of benzoic acid, comparable with those in fresh cultured dairy products. However, significantly higher concentrations were found at the centre and especially in the rind of

TABLE 5
Benzoic Acid Content of Fresh Cheeses and Various Quargs (mg/kg)

Cheese	*N*	$\bar{x}$	*s*	Δ	$\tilde{x}$	*First-named Author*
Fresh cheese	3			5–8		Grieger (1982)
Fresh cheese	82	26		– 68		Van Renterghem (1987)
Fresh cheese	5	8		1–17	3·0	Sieber (1990*a*)
Cottage cheese	4			9–18		Chandan (1977)
Cottage cheese	6	9	6	2–18		Takeba (1990)
Quarg, skim	4			17–29		Gieger (1982)
Quarg, skim	18	39·1	2·5			Obentraut (1983)
Quarg, skim	3	24·1		23–25	24·0	Sieber (1990*a*)
Q. 20% fat dry matter	9	23·9	2·2			Obentraut (1983)
Q. 40% fat dry matter	2			11, 13		Gieger (1982)
Q. 40% fat dry matter	12	14·0	2·9			Obentraut (1983)
Quarg, cream	3	9·9		6–13	11·0	Sieber (1990*a*)
Quarg, sour milk	7	39·4		31–48		Anonymous (1988*b*)
Quarg with fruit	11	16·0		10–25	15·3	Sieber (1990*a*)

N, $\bar{x}$, s, Δ, $\tilde{x}$: See footnotes to Table 3.

smear-ripened cheeses such as Bündner mountain cheese, Tilsit from pasteurised milk, Vacherin fribourgeois and Vacherin Mont d'Or (Fig. 2).

By excluding any prohibited adulteration of the cheeses or addition of preservatives to these cheeses, such high concentrations of benzoic acid cannot be explained by a stoichiometric conversion of hippuric acid, which clearly indicates the existence of at least one more metabolic pathway.

FORMATION AND DISTRIBUTION OF BENZOIC ACID DURING MANUFACTURE AND RIPENING OF CHEESE, ESPECIALLY OF SMEAR-RIPENED CHEESE

The formation and distribution of benzoic acid during the manufacture of cheese was investigated by Chandan *et al.* (1977), Sieber *et al.* (1990*b*) and Kopp (1992). During the manufacture of cottage cheese, Chandan *et al.* (1977) observed nearly the same low concentration of benzoic acid in fermented skim milk and in the cheese curd. Sieber *et al.* (1990*b*) and Kopp (1992) studied the formation and distribution of benzoic acid during the manufacture of smear-ripened cheeses and red smear-ripened cheese, respectively. In the study of Sieber *et al.* (1990*b*), milk was stored overnight at 3–7°C without or with supplementation with benzoic acid (variants I and II, respectively) and also at room temperature after inoculation with a mixed starter culture without or with added hippuric acid (variants III and IV, respectively). The cheese was manufactured, and the behaviour and fate of both acids were studied.

TABLE 6
Benzoic Acid Content of Various Types of Cheese (mg/kg)

Cheese	*N*	$\bar{x}$	*s*	Δ	$\tilde{x}$	*First-named Author*
Cheese	24	9·7		2–41	6·1	Kurisaki (1973)
Cheese	11			trace–35		Chandan (1977)
Cheese		– 70				Överström (1972)
Cheese	7	11·3		4–24	11·5	Nagayama (1986)
Cheese	3	17·7		12–22		Anonymous (1988*b*)
Cheese	2			98, 118		Anonymous (1989)
Cheese	71			< 10–200		Müller (1989)
Cheese	75	10·4		0–52	8·8	Toppino (1990)
Provolone[a]	8	96·4		8–341	73	Toppino (1990)
Cheese	36	5·9		0–22		Takeba (1990)
Cheese	59	5·7		1–20		Takeba (1991)
Cheese	7	10·9		< 2–18	10·8	Kopp (1992)
Whey cheese	44	45·1		18–111	41·0	Svensen (1974)
Cheese non-smear-ripened						
centre	16	5·8		0–20	3·5	Sieber (1990*a*)
rind	16	6·3		0–19	3·5	Sieber (1990*a*)
centre	5	16·5		12–25	12·9	Kopp (1992)
rind	4	15·9		5–41	9·1	Kopp (1992)
Cheese smear-ripened						
centre	84	27·7		0–174	15·0	Sieber (1990*a*)
rind	84	61·8		0–622	18·5	Sieber (1990*a*)
centre	4	36·6		9–66	28·9	Kopp (1992)
rind	4	86·2		8–285	56·4	Kopp (1992)

N, $\bar{x}$, s, Δ, $\tilde{x}$: See footnotes to Table 3.
[a]Benzoyl peroxide declared on label.

TABLE 7
Benzoic Acid Content of Various Types of Processed Cheese (mg/kg)

Processed cheese	*N*	$\bar{x}$	*s*	Δ	*First-named Author*
Without ingredients					
Austrian	22	9·4	4·3	4–17	Luf (1986)
Imported	24	9·2	4·4	5–19	Luf (1986)
	19	11·5		0–29	Sieber (1990*a*)
	17	8	3	4–13	Takeba (1990)
	10	6		0–21	Toppino (1990)
With ingredients					
Austrian	11	10·1	4·4	7–22	Luf (1986)
Imported	8	13·1	4·9	8–24	Luf (1986)
	10	11·8		0–20	Sieber (1990*a*)

N, $\bar{x}$, s, Δ: See footnotes to Table 3.

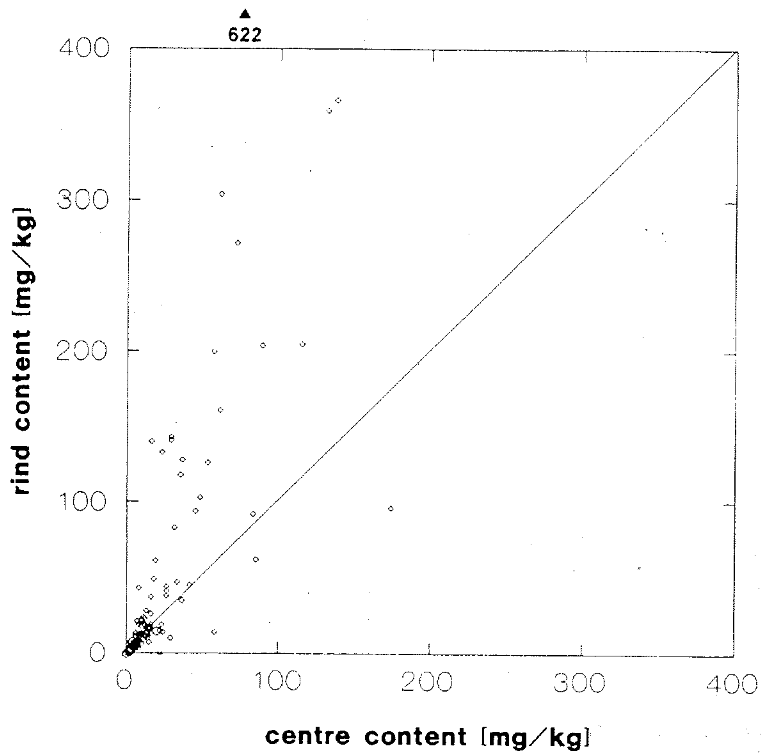


Fig. 2. Occurrence of benzoic acid in various smear-ripened cheeses. (From Sieber, R., Bütikofer, U., Baumann, H. & Bosset, J.O. (1990) Über das Vorkommen der Benzoesäure in Sauermilchprodukten und käse. *Mitt. Geb. Lebensm. Hyg.*, **81**, 484–93. Reprinted by permission.)

No benzoic acid was found before cheese manufacture (Table 8), but hippuric acid remained at a practically constant concentration. The distribution determined during cheese manufacture was as follows: 9% in curd and 91% in whey for benzoic acid and 7% in curd and 93% in whey for hippuric acid. Benzoic acid was transferred from milk into the cheese in the same way as hippuric acid (variant II). Less than 10% of the benzoic acid added to the milk was found in the curd. During cheese ripening, additional benzoic acid also appeared in this variant, lying significantly outside the expected balance (Table 8).

Formation of benzoic acid from hippuric acid during cheese manufacture

Nishimoto *et al.* (1968*b*, 1969) showed that lactic acid bacteria convert the indigenous hippuric acid in milk to benzoic acid. Considering that during the first hours of Swiss cheese manufacture, only the streptococci, and then, a few hours later, the lactobacilli, grow, i.e. during pressing of the cheese loaf (Steffen, 1971), it could be assumed that hippuric acid is converted to benzoic acid by the latter rather than by the former organisms.

The data in Table 8 confirm the stoichiometric conversion of hippuric acid (Sieber *et al.*, 1990*b*). No difference of metabolism could be found between variants I and IV. In both variants, no biogenesis of benzoic acid was observed

TABLE 8

Balance of Hippuric and Benzoic Acid (mmol) under Different Cheese Manufacturing and Ripening Conditions*

Step/Product	*Variant I*†		*Variant II*†		*Variant III*†		*Variant IV*†	
	Benzoic acid	*Hippuric + benzoic acids*	*Benzoic acid*	*Hippuric + benzoic acids*	*Benzoic acid*	*Hippuric + benzoic acids*	*Benzoic acid*	*Hippuric + benzoic acids*
Milk	0	6·7	63·9	70·7	0	7·1	0·3	50·1
Vat milk	0	6·7	64·4	71·3	0	6·7	0·4	50·4
Vat milk + rennet + mixed starter culture	0·6	7·4	62·4	69·0	0	6·7	0	50·4
Curd/whey	0/0·5	7·2	5·1/56·1	67·7	0/0	6·5	0/1·1	49·3
After pressing: cheese/whey	0·4/0	7·2	3·7/0·3	66·3	0·4/0	6·6	2·8/0·1	49·1
Cheese 1-month-old[a]	0·5	7·4	3·5	66·1	0·6	6·7	2·8	49·1
Cheese 3-month-old[a]	0·5	7·4	6·7	69·3	6·7	12·9	9·6	55·9

*From Sieber, R., Bütikofer, U., Baumann, E. & Bosset, J.O. (1990). Über die Benzoesäurebildung und -verteilung während der Herstellung und Reifung von geschmierten Käsen. *Mitt. Geb. Lebensm. Hyg.*, **81**, 722–30. Reprinted by permission.

†Variant I: stored at 3–7°C overnight.
Variant II: as I with a total supplementation of 49·2 mmol sodium benzoate.
Variant III: stored at room temperature overnight, after inoculation with a mixed starter culture.
Variant IV: as III, with a total supplementation of 33·6 mmol sodium hippurate.

[a]The balance also included the portion of whey lost by the preparation and pressing of the curd.

either in milk or in vat (Kessi) milk or even in curd. In variant I, milk with added rennet and mixed starter culture already contained a low concentration of benzoic acid (Table 8). In variant III, benzoic acid was present in cheese only after pressing. Variants I and III contained no more hippuric acid after this manufacturing step.

To determine whether an initially higher concentration of hippuric acid could enhance the formation of benzoic acid by lactic acid bacteria, milk was inoculated with a mixed starter culture, enriched with hippuric acid, and then ripened overnight at room temperature (variant IV). Such a procedure led to the production of only minute amounts of benzoic acid in the stored milk. This experiment showed that most of the hippuric acid drained off and was lost with whey and that less than 10% of the hippuric acid (indigenous or added) present was carried over into the fresh curd.

TABLE 9

Concentration (mg/kg) of Benzoic, Hydrocinnamic, and Cinnamic Acid in 1- and 3-month-old Cheese after Different Storage Conditions of the Vat Milk With and Without Supplementation of Hippuric and Benzoic Acid*

	1-month-old cheese			*3-month-old cheese*		
	Centre	*Rind (flat side)*	*Rind (hoop side)*	*Centre*	*Rind (flat side)*	*Rind (hoop side)*
Variant I†						
Cinnamic acid	nd	7	nd	nd	12	nd
Hydrocinnamic acid	nd	54	45	41	202	53
Benzoic acid	9	39	10	9	126	39
Variant II†						
Cinnamic acid	nd	7	nd	nd	13	nd
Hydrocinnamic acid	nd	122	13	29	226	48
Benzoic acid	62	160	4	119	172	62
Variant III†						
Cinnamic acid	nd	28	nd	nd	20	nd
Hydrocinnamic acid	nd	97	10	41	323	46
Benzoic acid	10	144	18	114	212	55
Variant IV†						
Cinnamic acid	nd	8	nd	nd	19	nd
Hydrocinnamic acid	nd	95	8	69	292	54
Benzoic acid	46	133	44	159	247	99

*From Sieber, R., Bütikofer, U., Baumann, E. & Bosset, J.O. (1990). Über die Benzoesäurebildung und -verteilung während der Herstellung und Reifung von geschmierten Käsen. *Mitt. Geb. Lebensm. Hyg.*, **81**, 722–30. Reprinted by permission.

†For variants I–IV, see footnotes to Table 8.

nd = not detected (below the detection limit).

Formation of benzoic acid in smear-ripened cheeses

During cheese ripening (1 and 3 months), the formation of benzoic acid was demonstrated by Sieber *et al.* (1990*b*). In spite of a very low residual concentration of hippuric acid (due to its conversion to benzoic acid) in cheese just after pressing, a significant increase in the concentration of benzoic acid was found in 3-month-old cheese in variant III, showing a non-stoichiometric balance between these acids (Table 8). In variant I, the concentration of benzoic acid remained practically constant in the centre of the loaf but increased particularly in the rind of the flat sides and, to a lesser extent, in the rind on the hoop side (Table 9). In short, a stoichiometric transformation of the total amount of hippuric acid available in milk to benzoic acid cannot account for the high concentration of this substance in the outer zone of these cheese loaves. This observation leads to the hypothesis that one or more natural pathways exist for its formation.

BREAKDOWN OF PHENYLALANINE AS AN ALTERNATIVE PATHWAY FOR THE BIOGENESIS OF BENZOIC ACID IN SMEAR-RIPENED CHEESES

Degradation of phenylalanine as a possible metabolic pathway for the formation of benzoic acid

According to Leuthardt (1977), another metabolic pathway can explain the biogenesis of benzoic acid from phenylalanine, as well as the simultaneous production of acetophenone (Bosset & Liardon, 1984, 1985) and ammonia (Blanc *et al.*, 1983). According to Leuthardt (1977), acetophenone and benzoic acid are formed from phenylvaleric acid with the intermediate product, phenylpropionic acid, which can also be generated through the deamination of phenylalanine (Fig. 3). The degradation of phenylalanine to benzoic acid was also demonstrated in cell-free extracts of *Sporobolomyces roseus* (Moore *et al.*, 1968) and in intact cells of *Rhodotorula rubra* (Uchiyama *et al.*, 1969); cinnamic acid was an intermediate.

Evidence of metabolites of phenylalanine degradation in cheeses

According to Teuber (1987), benzoic acid can be formed at up to 200 mg/kg (1·64 mmol/kg) in Harzer cheese by fermentation of aromatic amino acids. Kurisaki *et al.* (1973) showed that, in yeast-ripened cheese, benzoic acid might be produced from phenylalanine but not from tyrosine. The yield from the conversion of phenylalanine amounted to 0·06%, which was determined by using radioactively labelled phenylalanine in curd and benzoic acid in ripened cheese. Using GC/MS analysis, Kuzdzal-Savoie *et al.* (1971, 1973) identified β-phenylpropionic acid (hydrocinnamic acid), phenylacetic acid and benzoic acid in Munster and Livarot cheese. Simonart and Mayaudon (1956), Jarczynski and Kiermeier (1955) and Kiermeier and Jarczynski (1960) found *p*-hydroxybenzoic acid in cheese. According to Schormüller *et al.* (1954), this substance can be an intermediate product from the degradation of tyrosine during cheese ripening. Tyrosine itself is not converted into benzoic acid (Kurisaki *et al.*, 1973).

The following metabolites (Fig. 3) were identified by GC/MS or even quantified by HPLC by studying the ripening of smear-ripened cheeses (Swiss Gruyère and Vacherin fribourgeois):

phenylalanine by ion exchange chromatography (Lavanchy & Bühlmann, 1983):
β-phenylpropionic acid by HPLC (Bosset *et al.*, 1990; Sieber *et al.*, 1990*b*) and GC/MS (Bosset *et al.*, 1993);
cinnamic acid by HPLC (Bosset *et al.*, 1990; Sieber *et al.*, 1990*b*);
acetophenone by GC/MS (Bosset & Liardon, 1984, 1985); and
benzoic acid by HPLC (Bosset *et al.*, 1990; Sieber *et al.*, 1990*b*) and by GC/MS (Bosset *et al.*, 1993).

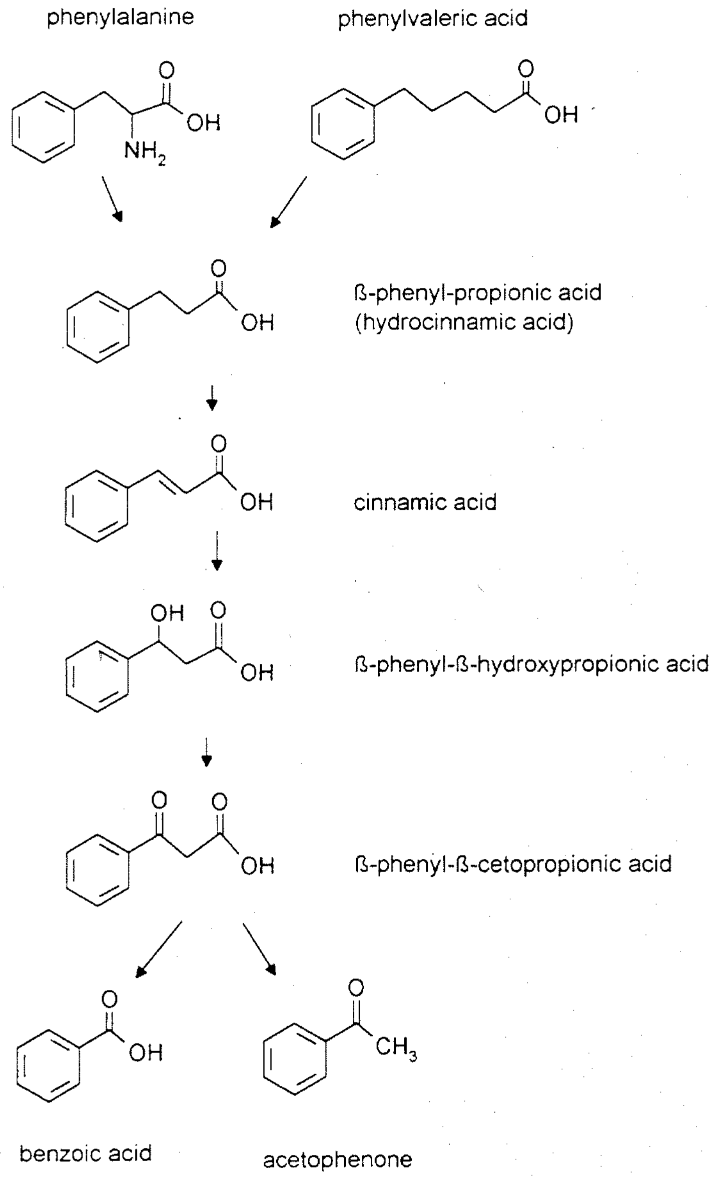
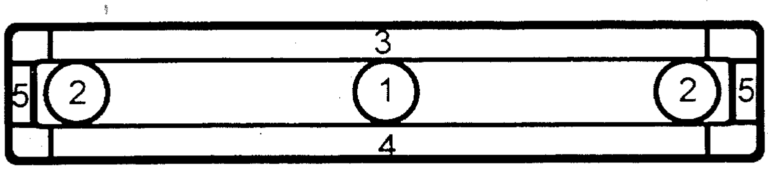


Fig. 3. Breakdown of phenylalanine as an alternative pathway for the biogenesis of benzoic acid.

These metabolites were found almost exclusively in the rind, including smear (Bosset & Liardon, 1984, 1985; Bosset *et al.*, 1993). Their concentrations increased markedly (Bosset & Liardon, 1984; Bosset *et al.*, 1993) during maturation and an intensive emission of ammonia was observed (Blanc *et al.*, 1983). The relatively low concentration of benzoic acid measured in the middle and centre of most cheese loaves could be ascribed to the relatively slow diffusion of this substance from the rind to the centre. Similar concentration gradients were observed for many other smear-produced polar compounds such as pyrazines, aldehydes, and methyl ketones (Bosset & Liardon, 1984, 1985; Liardon *et al.*, 1982).

A further observation could support the validity of this theory: loaves of smear-ripened Swiss Gruyère cheese ripened in a gas-tight container specially designed for the determination of respiratory quotients under a controlled anaerobic atmosphere (Bosset *et al.*, 1982) showed a much lower tendency to become mouldy than loaves of smearless Swiss Emmental cheese stored under similar conditions (Flückiger, 1983, personal communication). This can be interpreted as a consequence of the biogenesis of a sufficient amount of benzoic acid from phenylalanine by yeasts on the smear-ripened cheese. It is known that this acid can inhibit the growth of bacteria, yeasts and moulds (Chipley, 1993).

Free phenylalanine and other amino acids are present at relatively high concentrations in different ripened cheeses (Lavanchy & Bühlmann, 1983; Lavanchy & Sieber, 1993). Assuming that free or even peptide-bound phenylalanine of cheese could be stoichiometrically converted to benzoic acid, concentrations of several g/kg of benzoic acid could be expected. Owing to the more intensive degradation of protein and peptides by the smear, the available concentration of this (free) amino acid should be higher in the rind than in the centre of cheese. It is also known that benzoic acid can be degraded anaerobically (Londry & Fedorak, 1992).



Sample	benzoic acid content [mg/kg cheese]	hydrocinnamic acid content [mg/kg cheese]
1. centre	8	0
2. centre hoop site	9	25
3. rind, upper site	10	24
4. rind lower site	24	33
5. rind, hoop site	81	230

Fig. 4. Distribution of benzoic and hydrocinnamic acid in Vacherin fribourgeois cheeses. (From Bosset, J.O., Bütikofer, U. & Sieber, R. (1990). Phenylalaninabbau—ein weiterer Weg zur natürlichen Bildung der Benzoesäure in geschmierten Käsen. *Schweiz. Milchwirtsch. Forsch.*, **19**, 46–50. Reprinted by permission.)

TABLE 10
Concentration of Benzoic and Hydrocinnamic Acid (mg/kg) in Vacherin Fribourgeois from Different Manufacturers*

Manufacturer	*1st investigation*		*2nd investigation*			
	Centre	*Rind*	*Centre*	*Rind*	*Hoop side*	
	Benzoic acid	*Benzoic acid*	*Benzoic acid*	*Benzoic acid*	*Benzoic acid*	*Hydro-cinnamic acid*
A	61	161	nd	nd	nd	nd
B	89/41[a]	204/45[a]	131	359	229	168
C	22	0	137	366	454	313
D	16	26	5	7	3	nd
E	57	200	13	17	8	nd
F	45	94	12	11	22	nd
G	nd	nd	5	7	15	nd
H	72/115[a]	272/205[a]	35	118	123	114
I	29	143	16	140	140	484
K	61	304	7	21	43	nd

*From Bosset, J.O., Bütikofer, U. & Sieber, R. (1990). Phenylalaninabbau—ein weiterer Weg zur natürlichen Bildung der Benzoesäure in geschmierten Käsen. *Schweiz. Milchwirtsch. Forsch.*, **19**, 46–50. Reprinted by permission.
nd = not detected.
[a]Two samples within 3 weeks.

Model studies with Vacherin fribourgeois

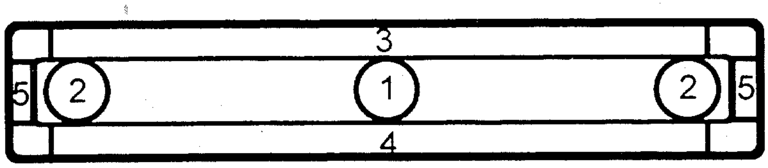
Owing to the high concentrations of benzoic acid in many smear-ripened cheese varieties, Vacherin fribourgeois was chosen as a model for further studies, which should provide more information on the occurrence of various substances formed during the degradation of phenylalanine (Bosset *et al.*, 1990). The different zones investigated showed a variable concentration of benzoic acid (Fig. 4). The highest content was in the rind on the hoop side of cheese, which thus supported an independent observation by Marchon and Deillon (1990, personal communication). Simultaneously, a high concentration of hydrocinnamic acid was measured in the same region. Other samples of Vacherin fribourgeois containing high concentrations of benzoic acid had at the same time a high content of hydrocinnamic acid in the rind of the hoop side (Table 10).

This surprising distribution of benzoic and hydrocinnamic acids can, however, be explained. Only the rind on the hoop side is exposed all the time to aerobic conditions. The formation of benzoic acid needs such conditions. In comparison, the lower concentrations in the bottom and upper flat side are subjected to different ripening and washing conditions: these sides rest (in close contact) alternately on the wood and are therefore exposed to aerobic conditions for only half of the ripening period. Moreover, the surfaces of the cheese loaves are peri-

These metabolites were found almost exclusively in the rind, including smear (Bosset & Liardon, 1984, 1985; Bosset *et al.*, 1993). Their concentrations increased markedly (Bosset & Liardon, 1984; Bosset *et al.*, 1993) during maturation and an intensive emission of ammonia was observed (Blanc *et al.*, 1983). The relatively low concentration of benzoic acid measured in the middle and centre of most cheese loaves could be ascribed to the relatively slow diffusion of this substance from the rind to the centre. Similar concentration gradients were observed for many other smear-produced polar compounds such as pyrazines, aldehydes, and methyl ketones (Bosset & Liardon, 1984, 1985; Liardon *et al.*, 1982).

A further observation could support the validity of this theory: loaves of smear-ripened Swiss Gruyère cheese ripened in a gas-tight container specially designed for the determination of respiratory quotients under a controlled anaerobic atmosphere (Bosset *et al.*, 1982) showed a much lower tendency to become mouldy than loaves of smearless Swiss Emmental cheese stored under similar conditions (Flückiger, 1983, personal communication). This can be interpreted as a consequence of the biogenesis of a sufficient amount of benzoic acid from phenylalanine by yeasts on the smear-ripened cheese. It is known that this acid can inhibit the growth of bacteria, yeasts and moulds (Chipley, 1993).

Free phenylalanine and other amino acids are present at relatively high concentrations in different ripened cheeses (Lavanchy & Bühlmann, 1983; Lavanchy & Sieber, 1993). Assuming that free or even peptide-bound phenylalanine of cheese could be stoichiometrically converted to benzoic acid, concentrations of several g/kg of benzoic acid could be expected. Owing to the more intensive degradation of protein and peptides by the smear, the available concentration of this (free) amino acid should be higher in the rind than in the centre of cheese. It is also known that benzoic acid can be degraded anaerobically (Londry & Fedorak, 1992).



Sample	benzoic acid content [mg/kg cheese]	hydrocinnamic acid content [mg/kg cheese]
1. centre	8	0
2. centre hoop site	9	25
3. rind, upper site	10	24
4. rind lower site	24	33
5. rind, hoop site	81	230

Fig. 4. Distribution of benzoic and hydrocinnamic acid in Vacherin fribourgeois cheeses. (From Bosset, J.O., Bütikofer, U. & Sieber, R. (1990). Phenylalaninabbau—ein weiterer Weg zur natürlichen Bildung der Benzoesäure in geschmierten Käsen. *Schweiz. Milchwirtsch. Forsch.*, **19**, 46–50. Reprinted by permission.)

TABLE 10
Concentration of Benzoic and Hydrocinnamic Acid (mg/kg) in Vacherin Fribourgeois from Different Manufacturers*

Manufacturer	*1st investigation*		*2nd investigation*			
	Centre	*Rind*	*Centre*	*Rind*	*Hoop side*	
	Benzoic acid	*Benzoic acid*	*Benzoic acid*	*Benzoic acid*	*Benzoic acid*	*Hydro-cinnamic acid*
A	61	161	nd	nd	nd	nd
B	89/41[a]	204/45[a]	131	359	229	168
C	22	0	137	366	454	313
D	16	26	5	7	3	nd
E	57	200	13	17	8	nd
F	45	94	12	11	22	nd
G	nd	nd	5	7	15	nd
H	72/115[a]	272/205[a]	35	118	123	114
I	29	143	16	140	140	484
K	61	304	7	21	43	nd

*From Bosset, J.O., Bütikofer, U. & Sieber, R. (1990). Phenylalaninabbau—ein weiterer Weg zur natürlichen Bildung der Benzoesäure in geschmierten Käsen. *Schweiz. Milchwirtsch. Forsch.*, **19**, 46–50. Reprinted by permission.
nd = not detected.
[a]Two samples within 3 weeks.

Model studies with Vacherin fribourgeois

Owing to the high concentrations of benzoic acid in many smear-ripened cheese varieties, Vacherin fribourgeois was chosen as a model for further studies, which should provide more information on the occurrence of various substances formed during the degradation of phenylalanine (Bosset *et al.*, 1990). The different zones investigated showed a variable concentration of benzoic acid (Fig. 4). The highest content was in the rind on the hoop side of cheese, which thus supported an independent observation by Marchon and Deillon (1990, personal communication). Simultaneously, a high concentration of hydrocinnamic acid was measured in the same region. Other samples of Vacherin fribourgeois containing high concentrations of benzoic acid had at the same time a high content of hydrocinnamic acid in the rind of the hoop side (Table 10).

This surprising distribution of benzoic and hydrocinnamic acids can, however, be explained. Only the rind on the hoop side is exposed all the time to aerobic conditions. The formation of benzoic acid needs such conditions. In comparison, the lower concentrations in the bottom and upper flat side are subjected to different ripening and washing conditions: these sides rest (in close contact) alternately on the wood and are therefore exposed to aerobic conditions for only half of the ripening period. Moreover, the surfaces of the cheese loaves are periodically washed and therefore subjected to mechanical rubbing, which is certainly more intensive on the flat surfaces than on the curved hoop side.

Both hydrocinnamic and cinnamic acids are metabolites of phenylalanine degradation (Bosset *et al.*, 1990). In all variants (I–IV) discussed above, the concentration of hydrocinnamic acid in the rind of the flat surfaces as well as in the hoop side of the 1- and 3-month-ripe cheese loaves was comparable with that of benzoic acid. Cinnamic acid was found at lower concentrations practically only in the rind of the flat surfaces (Table 9).

FURTHER POSSIBLE SOURCES OF BENZOIC ACID IN SMEAR-RIPENED CHEESES

A study in progress on the origin and content of volatile flavour components in cultured dairy products revealed the occurrence of relatively high concentrations of benzaldehyde in many of them (Imhof *et al.*, 1994, 1995). This substance seems to be produced by certain strains of lactic acid bacteria. Benzaldehyde has been characterized as a product of phenylacetic acid oxidation by *Penicillium chrysogenum* (Hockenhull *et al.*, 1952). Benzaldehyde is known to be subjected to auto-oxidation in the presence of air (Bosset *et al.*, 1982), a condition that is fulfilled in the smear-ripened rind. This reaction therefore explains the presence of benzoic acid in the outer zone of cheese loaves.

Another possible mechanism for the formation of benzoic acid could be the anaerobic metabolism of phenol (Londry & Fedorak, 1992), which was found at low concentrations and almost exclusively in the outer zone of Gruyère, but also in other cheeses (Bosset *et al.*, 1993).

CONCLUSION

Benzoic acid is a natural constituent of fermented dairy products, particularly cheese. It is produced from hippuric acid, another naturally occurring compound in dairy products, according to a stoichiometric reaction (1:1). The high concentrations of benzoic acid found in some smear-ripened cheeses can be explained by the breakdown of phenylalanine during ripening as well as, probably, to a certain extent by the auto-oxidation of benzaldehyde, a constituent frequently identified in cultured milk products produced with lactic acid bacteria.

The variable concentration of benzoic acid in the bottom and upper sides, as well as in the hoop side, of smear-ripened cheese loaves, depends on many factors, such as the composition of the microbiological flora, the frequency and kind of cheese curing and ripening conditions in the cellar (temperature, humidity, duration, etc.). The species of microorganisms able to break down phenylalanine to benzoic acid have not yet been identified: lactobacilli, rather than streptococci, seem to be involved. In addition to its role by generating cheese flavour compounds, the smear seems to act as a natural preservative against yeast and mould contamination on the surface of smear-ripened cheese loaves. This natural protection could be due to the age-old practical knowledge (empiricism!) of cheesemakers, whose critical observation on traditional cheese manufacture could have anticipated in this field the newest developments and knowledge of modern food science and technology!

REFERENCES

Aitzetmüller, K. & Arzberger, E. (1984). Analyse von Konservierungsstoffen in fetthaltigen Lebensmitteln mittels HPLC. *Z. Lebensm. Unters. Forsch.*, **178**, 279–83.

Anon. (1987). Milk, dried milk, yoghurt and other fermented milks: determination of benzoic and sorbic acid content. Standard 139, International Dairy Federation, Brussels.

Anon. (1988*a*). Milch, Milchpulver, Joghurt und andere gesäuerte Milch — Bestimmung des Gehalts an Benzoe- und Sorbinsäure. *Milchwissenschaft*, **43**, 366–8.

Anon. (1988*b*). Jahresbericht 1988. Milchwirtschaftliche Untersuchungs- und Versuchsanstalt, Kempten, pp. 17, 59.

Anon. (1989). Jahresbericht 1989. Milchwirtschaftliche Untersuchungs- und Versuchsanstalt, Kempten, p. 17.

Arimoto, M., Tsujisawa, H. & Yokoyama, T. (1983). Rapid determination of benzoic acid and sorbic acid in yoghurt by HPLC. *Ann. Rep. Wakayama Prefectural Res. Center Environm, Public Health*, 193–7 (29), cited in *Dairy Sci. Abstr.*, **49**, 613 (1987).

Blanc, B., Bosset, J.O., Martin, B. & Jimeno, J. (1983). Echanges gazeux à la surface du fromage de gruyère en cours de maturation. *Schweiz. Milchwirtsch. Forsch.*, **12**, 30–4.

Bosset, J.O. & Liardon, R. (1984). The aroma composition of Swiss Gruyère cheese. II: the neutral volatile components. *Lebensm.-Wiss. u.-Technol.*, **17**, 359–62.

Bosset, J.O. & Liardon, R. (1985). The aroma composition of Swiss Gruyère cheese. III: relative changes in the content of alkaline and neutral components during ripening. *Lebensm.-Wiss. u.-Technol.*, **18**, 178–85.

Bosset, J.O., Graber, B., Martin, B. & Knecht, P. (1982). Nouveau dispositif pour déterminer la consommation d'oxygène: application au mesurage de diverses oxydations et de quotients respiratoires. *Trav. Chim. Aliment. Hyg.*, **73**, 420–37.

Bosset, J.O., Bütikofer, U. & Sieber, R. (1990). Phenylalaninabbau-ein weiterer Weg zur natürlichen Bildung der Benzoesäure in geschmierten Käsen. *Schweiz. Milchwirtsch. Forsch.*, **19**, 46–50.

Bosset, J.O., Collomb, M. & Sieber, R. (1993). The aroma composition of Swiss Gruyère cheese. IV: the acidic volatile components and their changes in content during ripening. *Lebensm.-Wiss. u.-Technol.*, **26**, 581–92.

Brandl, E. & Binder, E. (1982). Prüfung von Nachweismethoden für Zustazstoffe unter besonderer Berücksichtigung des 'Carry over effects' bei Benzoesäure in Milch und Milchprodukten. *Beiträge Umweltschutz Lebensm. Veterinärverw.*, (1) 217–75.

Bui, L.V. & Cooper, C. (1987). Reverse-phase liquid chromatographic determination of benzoic and sorbic acids in foods. *J. Assoc. Off. Anal. Chem.*, **70**, 892–6.

Bütikofer, U., Baumann, E. & Bosset, J.O. (1988). Eine verbesserte HPLC-Methode zur Bestimmung der Sorbinsäure in Milchprodukten unter spezieller Berücksichtigung von Artefakten. *Mitt. Geb. Lebensm. Hyg.*, **79**, 392–405.

Chandan, R.C., Gordon, J.F. & Morrison, A. (1977). Natural benzoate content of dairy products. *Milchwissenschaft*, **32**, 534–7.

Chipley, J.R. (1993). Sodium benzoate and benzoic acid. In *Antimicrobials in Foods*, 2nd edition, ed. P.M. Davidson & A.L. Branen. Marcel Dekker, New York, NY, USA, pp. 11–48.

Dietz, E.A., Cortellucci, N.J. & Singley, K.F. (1993). Determination of benzoic acid, chlorobenzoic acids and chlorendic acid in water. *J. Liq. Chromatogr.*, **16**, 3331–47.

Drawert, F. & Leupold, G. (1978). Über das Vorkommen von Benzoesäure in Milch und Milcherzeugnissen. *Lebensmittelchem. gerichtl. Chem.*, **32**, 76–7.

Fleischmann, W. (1932). *Lehrbuch der Milchwirtschaft.* 7. Auflage. P. Parey, Berlin, Germany, p. 84.

Gieger, U. (1982). Benzoesäure in Sauermilcherzeugnissen und Frischkäse. HPLC-Analyse von Benzoesäure, Sorbinsäure und PHB-Estern in Lebensmitteln. *Lebensmittelchem. gerichtl. Chem.*, **36**, 109–10.

Hannisdal, A. (1992). Analysis of acesulfame-K, saccharin and preservatives in beverages and jams by HPLC. *Z. Lebensm. Unters. Forsch.*, **194**, 517–9.

Hatanaka, H. & Kaneda, Y. (1986). Rapid and simultaneous analysis of hippuric and benzoic acids in fermented milk or raw milk by high performance liquid chromatography. *J. Food Hyg. Soc. Japan*, **27**, 81–6.

Heimhuber, B. & Herrmann, K. (1990). Benzoe-, Phenylessig-, 3-Phenylpropan- und Zimtsäure sowie Benzoylglucosen in einigen Obst- und Fruchtgemüsearten. *Dtsch. Lebensm. Rundsch.*, **86**, 205–9.

Hockenhull, D.J.D., Walker, A.D., Wilkin, A.D. & Winder, F.G. (1952). Oxidation of phenylacetic acid by *Penicillium chrysogenum. Biochem. J.*, **50**, 605–9.

Hostettler, H. (1933). Ueber die Bestimmung von Salicylsäure, *p*-Oxybenzoesäureestern, Benzoesäure und *p*-Chlorbenzoesäure in Schmelzkäse. *Mitt. Geb. Lebensm. Unters. Hyg.*, **24**, 1–12.

Ito, Y., Tonogai, Y., Ogawa, S., Yokoyama, T., Honda, T. & Iwaida, M. (1983). Rapid simultaneous determination of benzoate and sorbate in yoghurt by use of high-performance liquid chromatography. *Jap. J. Dairy Food Sci.*, **32**, A89–A94.

Imhof, R., Glättli, H. & Bosset, J.O. (1994). Volatile organic aroma compounds product by thermophilic and mesophilic mixed strain dairy starter cultures. *Lebensm. - Wiss. u. - Technol.*, **27**, 442–9.

Imhof, R., Glättli, H. & Bosset, J.O. (1995). Volatile organic aroma compounds product by thermophilic and mesophilic single strain dairy starter cultures. *Lebensm. - Wiss. u. - Technol.*, in press.

Jarczynski, R. & Kiermeier, F. (1955). Ueber das Vorkommen von Paraoxy-Benzoesäure als natürliches Reifungsprodukt in Käse. *Z. Lebensm. Unters. Forsch.*, **100**, 195–200.

Kakemoto, M. (1992). Simultaneous determination of sorbic acid, dehydroacetic acid and benzoic acid by gas chromatography–mass spectrometry. *J. Chromatogr.*, **594**, 253–7.

Karabinos, J.V. & Dittiner, K. (1943). Presence of hippuric acid in milk. *Proc. Soc. Exp. Biol. Med.*, **53**, 111–2.

Kieffer, F., Solms, J. & Egli, R.H. (1964). Vorkommen von Nucleotiden und verwandten Verbindungen in Milch und Milchprodukten. *Z. Lebensm. Unters. Forsch.*, **125**, 346–50.

Kiermeier, F. & Jarczynski, R. (1960). Ueber die quantitative Bestimmung von *p*-Hydroxybenzoesäure in Käse. *Z. Lebensm. Unters. Forsch.*, **113**, 370–4.

Kisza, J., Sajko, W., Sadowska, D. & Tyszkiewicz, K. (1984). Benzoic acid in dairy starters and cultured milk beverages. *Zesz. Nauk. Akad. Roln. Techn. Olsztynie Technol. Zywnosci*, (19) 69–77.

Kopp, G. (1992). Benzoesäure und Sorbinsäure in Milchprodukten. Bericht, Milchwirtschaftliche Untersuchungs- und Versuchanstalt Kempten, pp. 1–34.

Küppers, F.J.E.M. & Jans, J.A. (1988). Reverse-phase liquid chromatographic determination of benzoic acid and sorbic acids in fresh cheese. *J. Assoc. Off. Anal. Chem.*, **71**, 1068–71.

Kurisaki, J., Sasago, K., Tsugo, T. & Yamauchi, K. (1973). Formation of benzoic acid in cheese. *J. Food Hyg. Soc. Japan*, **14**, 25–30.

Kuzdzal-Savoie, S., Losi, G., Kuzdzal, W. & Goto, K. (1971). Mise en évidence des acides benzoique, phenylacétique (ou alpha-toluique) et phenylpropionique (ou hydrocinnamique) dans les acides gras libres du Munster et du Livarot. *Techn. Lait.*, (724) 15–21.

Kuzdzal Savoie, S., Kuzdzal, W., Losi, G. & Goto, K. (1973). Riconoscimento degli acidi benzoico, fenilacetica (α-toluico) e fenil-propionico (idrocinnamico) presenti negli acidi grassi liberi del Munster e del Livarot. *Sci. Tecnol. Aliment.*, **3**, 229–33.

Lavanchy, P. & Bühlman, C. (1983). Valeurs normales de certains paramètres importants du métabolisme pour des fromages frabriqués en Suisse. *Schweiz. Milchwirtsch. Forsch.*, **12**, 3–12.

Lavanchy, P. & Sieber, R. (1993). Proteolyse in Hart- und Halbhartkäsen: 1. Freie Aminosäuren. *Schweiz. milchw. Forsch.*, **22**, 59–64.

Lavanchy, P. & Steiger, G. (1984). Determination of orotic acid and of other carboxylic acids in milk and dairy products by HPLC. In *Challenges to Contemporary Dairy Analytical Techniques* (Special Publication No. 49), Royal Society of Chemistry, London, pp. 317–8.

Leuenberger, U., Gauch, P. & Baumgartner, E. (1979). Determination of food preservatives and saccharin by high-performance liquid chromatography. *J. Chromatogr.*, **173**, 343–8.

Leuthardt, F. (1977). *Intermediärstoffwechsel.* W. de Gruyter, Berlin, Germany, pp. 505, 657.

Liardon, R., Bosset, J.O. & Blanc, B. (1982). The aroma composition of Swiss Gruyère cheese. 1: the alkaline volatile components. *Lebensm.-Wiss. u.-Technol.*, **15**, 143–7.

Londry, K.L. & Fedorak, P.M. (1992). Benzoic acid intermediates in the anaerobic biodegradations of phenols. *Can. J. Microbiol.*, **38**, 1–11.

Lück, E. (1980). *Antimicrobial Food Additives: Characteristics, Uses, Effects.* Springer Verlag, Berlin, Germany.

Luf, W. & Brandl, E. (1986). Der originäre Gehalt an Benzoesäure in Schmelzkäse. *Oest. Milchwirtsch.*, **41**, 43–6 (Wiss.Beilage 4).

Marsili, R.T., Ostapenko, H., Simmons, R.E. & Green, D.E. (1981). High performance liquid chromatographic determination of organic acids in dairy products. *J. Food Sci.*, **46**, 52–7.

Matsunaga, A., Yasmamoto, A. & Makimo, M. (1985). Simultaneous determination of saccharin, sorbic acid, benzoic acid and five esters of *p*-hydroxybenzoic acid in liquid foods by isocratic high-performance liquid chromatography. *J. Hyg. Chem.*, **31**, 269–73; cited in *Food Sci. Technol. Abstr.*, **19**, 187 (1987).

Moore, K., Subba Rao, P.V. & Towers, G.H.N. (1968). Degradation of phenylalanine and tyrosine by *Sporobolomyces roseus. Biochem. J.*, **106**, 507–14.

Müller, U. (1989). Jahresbericht 1988. Kantonales Laboratorium, Bern, Switzerland, pp. 34-7.

Müller, U. (1990). Jahresbericht 1989. Kantonales Laboratorium, Bern, Switzerland, p. 37.

Nagayama, T., Nishijima, M., Yasuda, K., Saito, K., Kamimura, H., Ibe, A., Ushiyama, H., Nagayama, M. & Naoi, Y. (1983). Benzoic acid in fruits and fruit products. *J. Food Hyg. Soc. Japan*, **24**, 416–22.

Nagayama, T., Nishijima, M., Yasuda, K., Saito, K., Kamimura, H., Ibe, A., Ushiyama, H., Naoi, Y. & Nishima, T. (1986). Benzoic acid in agricultural food products and processed foods. *J. Food Hyg. Soc. Japan*, **27**, 316–25.

Nishimoto, T., Uyeta, M. & Taue, S. (1968*a*). On benzoic acid in fermented dairy products. *J. Food Hyg. Soc. Japan*, **9**, 60–2.

Nishimoto, T., Uyeta, M., Taue, S. & Takebayashi, I. (1968*b*). Bacterial formation of benzoic acid in milk. *J. Food Hyg. Soc. Japan*, **9**, 58–9.

Nishimoto, T. Uyeta, M. & Taue, S., (1969). Precursor of benzoic acid in fermented milk. *J. Food Hyg. Soc. Japan*, **10**, 410–3.

Obentraut, S., Binder, E. & Brandl, E. (1983). Benzoesäure in fermentierten Milchprodukten. *Wien. tierärztl. Mschr.*, **70**, 276–82.

Överström, H., Reigo, J. & Borgström, S. (1972). Preliminary study concerning formation of benzoic acid in cheese. *Milchwissenschaft*, **27**, 705–7.

Patton, S. (1953). The presence of hippuric acid in milk. *J. Dairy Sci.*, **36**, 943–7.

Penttilä, P.-L., Salminen, S. & Niemi, E. (1988). Estimation of the intake of food additives in Finland. *Z. Lebensm. Unters. Forsch.*, **186**, 11–5.

Puttemans, M.L., Branders, C., Dryon, L. & Massart, D.L. (1985). Extraction of organic acids by ion-pair formation with tri-n-octylamine. VI: determination of sorbic acid, benzoic acid, and saccharin in yoghurt. *J. Assoc. Off. Anal. Chem.*, **68**, 80–2.

Raissouni, B., Ouedrhiri, M. & Bourgriane, J. (1991). Etude de deux additifs par spectrophotométrie UV: l'acide benzoique (E 210) et l'acide sorbique (E 200) dans les boissons gazeuses et quelques produits laitiers commercialisés au Maroc. *Act. Inst. Agric. Vet.*, **11**, 27–33, cited in *Dairy Sci. Abstr.*, **55**, 305 (1993).

Richardson, R.K. & Gray, I.K. (1981). The levels of natural benzoic acid in casein and whey by-products. *N.Z. J. Dairy Sci. Technol.*, **16**, 179–86.

Sajko, W., Kisza, J. & Niedziecki, J. (1984). Benzoic acid in raw cow milk. *Zesz. Nauk. Akad. Roln.-Techn. Olsztynie, Technol. Zywnosci*, (19) 61–8.

Schormüller, J., Birn, K.J. & Winter, H. (1954). Beiträge zur Biochemie der Käsereifung. 4. Das Verhalten des Tyrosins im Verlauf der Reifung. *Z. Lebensm. Unters. Forsch.*, **98**, 411–24.

Sieber, R., Bütikofer, U., Bosset, J.O. & Rüegg, M. (1989). Benzoesäure als natürlicher Bestandteil von Lebensmitteln – eine Übersicht. *Mitt. Geb. Lebensm. Hyg.*, **80**, 345–62.

Sieber, R., Bütikofer, U., Baumann, H. & Bosset, J.O. (1990*a*). Über das Vorkommen der Benzoesäure in Sauermilchprodukten und Käse. *Mitt. Geb. Lebensm. Hyg.*, **81**, 484–93.

Sieber, R., Bütikofer, U., Baumann, E. & Bosset, J.O. (1990*b*). Über die Benzoesäurebildung und -verteilung während der Herstellung und Reifung von geschmierten Käsen. *Mitt. Geb. Lebensm. Hyg.*, **81**, 722–30.

Simonart, P. & Mayaudon, J. (1956). Etude chromatographique des fromages. III: Acides aromatiques. *Neth. Milk Dairy J.*, **10**, 261–7.

Stark, W.,Urbach, G., Crow, J.W. & Young, B. (1976). Estimation of sorbic acid in cheese at the 1 ppm level. *Aust. J. Dairy Technol.*, **31**, 88–91.

Steffen, C. (1971). Konzentration und Konfiguration der Milchsäure im reifenden Emmentalerkäse. Dissertation Nr. 4630, Eidg. Techn. Hochschule Zürich, Switzerland.

Stijve, T. & Hischenhuber, C. (1984). High performance liquid chromatographic determination of low levels of benzoic acid and sorbic acid in yoghurts. *Dtsch. Lebensm. Rundsch.*, **80**, 81–4.

Svensen, A. (1974). Hippursyre og benzoesyre i melk og forskjellige meieriprodukter. *Meieriposten*, **63**, 704–7, 731–4, 761–7, 789–92.

Takeba, K., Umeki, F., Murakami, F., Nakama, A., Igusa, K. Maruyama, T. & Matsumoto, M. (1990). Estimation of chemical and microbiological quality of domestic cheese. *Ann. Rep. Tokyo Metrop. Res. Lab. Public Health*, **41**, 95–100.

Takeba, K., Umeki, F., Murakami, F., Nakama, A., Igusa, K., Maruyama, T. & Matsumoto, M. (1991). Estimation of chemical and microbiological quality of imported cheese. *Ann. Rep. Tokyo Metropol. Res. Lab. Publ. Health*, **42**, 102–11.

Teuber, M. (1987). Grundriss der praktischen Microbiologie für das Molkereifach. *Molkereitechnik*, **59/60**, 124.

Toppino, P.M., Contarini, G. & Degano, L. (1987*a*). Valutazione quantitiva di formalina, acido benzoico e lisozima nel Provolone. *Sci. Tecnol. Latt. Cas.*, **38**, 538–46.

Toppino, P.M., Contarini, G., Degano, L., Battistotti, B. & Brambilla, E. (1987*b*). Studio delle metodiche di determinazione dell'acido benzoico nei prodotti lattiero-caseari. *Sci. Technol. Latt. Cas.*, **38**, 397–407.

Toppino, P.M., Volpato, R., Amelotti, G. & Contarini, G. (1990). Determinazione quantitativa HPLC di acido sorbico e acido benzoico nei prodotti lattiero-caseari. *Sci. Tecnol. Latt. Cas.*, **41**, 137–52.

Toyoda, M., Ito, Y., Isshiki, K., Onishi, K., Kato, T., Kamikura, M., Shiroishi, Y., Harada, Y., Fukasawa, Y., Yokoyama, T., Yoneda, M., Hirayama, Y. & Iwaida, M. (1983). Daily intake of preservatives, benzoic acid, dehydroacetic acid, propionic acid and their salts, and esters of *p*-hydroxybenzoic acid in Japan. *J. Jap. Soc. Nutr. Food Sci.*, **36**, 467–80.

Tschager, E., Lang, E.C. & Kneifel, W. (1993). Einfluss von Fütterung und Haltung auf Mikronährstoffe, Vitamine und Sensorik der Milch. *Milchwirtsch. Berichte* (116/117), 164.

Uchiyama, K., Kawaguchi, K., Tochikura, T. & Ogata, K. (1969). Metabolism of aromatic acids in microorganisms. Part III: metabolism of cinnamic acid in *Rhodotorula*. *Agric. Biol. Chem.*, **33**, 755–63.

Van Dokkum, W., Vos, R.H. de, Cloughley, F.A., Hulshof, K.F.A.M., Dukel, F. & Wijsman, J.A. (1982). Food additives and food components in total diets in the Netherlands. *Br. J. Nutr.*, **48**, 223–31.

Van Renterghem, R. (1982). Het naturlijke benzoezuurgehalte van yoghurt. *Belg. Arch. Soc. Geneesk. Hyg. Arbeidsgen. Ger. Geneesk.*, **40**, 530–8.

Van Renterghem, R. & Waes, G. (1987). Recherche relative à la teneuen en acide sorbique et en acide benzoique et à la présence de moisissures et de levures dans le fromage frais. *Rev. Agric.*, **40**, 141–7.

Vogel, J. & Deshusses, J. (1965*a*). Identification des acides déhydroacétique, sorbique, benzoique, ortho-chlorobenzoique, para-chlorobenzoique, salicylique, des esters méthylique, éthylique, propylique et butylique de l'acide para-hydroxybenzoique par chromatographie en phase gazeuse. *Trav. Chim. Aliment. Hyg.*, **56**, 35–7.

Vogel, J. & Deshusses, J. (1965*b*). Dosage polarographique de faibles quantités d'acide benzoique; application de la méthode au dosage de l'acide benzoique dans le lait normal et les yoghurts. *Trav. Chim. Aliment. Hyg.*, **56**, 63–7.

Wiench, L., Bierska, J., Lysakowski, K. & Smoczynski, S. (1984). HPLC method for determining benzoic acid in milk. *Rocz. Inst. Przem. Mlecz.*, **26**, 5–10.

Wolfschoon-Pombo, A. & Klostermeyer, H. (1981). Vorkommen und Bestimmung von Hippursäure in der NPN-Fraktion der Milch. *Z. Lebensm. Unters. Forsch.*, **172**, 269–71.

Zonneveld, H. (1975). Spectrophotometric estimation of benzoic and sorbic acid. *J. Sci. Food Agric.*, **26**, 879–85.