Z Lebensm Unters Forsch (1994) 198:267–276

Zeitschrift für
Lebensmittel-Untersuchung und -Forschung

Review

Relationships between micro-organisms and formation of aroma compounds in fermented dairy products

René Imhof, Jacques O. Bosset *

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

Received October 11, 1993

Beziehungen zwischen Mikroorganismen und der Bildung von Aromakomponenten in fermentierten Milchprodukten

Zusammenfassung. Der vorliegende Artikel enthält eine Zusammenstellung von Publikationen über Analyse und Bildung von Aromakomponenten, die durch milchwirtschaftlich genutzte Mikroorganismen produziert werden. Zwischen 1970 und 1993 erschienene Artikel werden besprochen, während die vor 1970 erschienene Literatur durch eine Bibliographie von Übersichtsartikeln abgedeckt wird. Schwerpunkt ist der Beitrag von Lactobacillen, Lactococcen, Streptococcen und Bifidobakterien zur Aromabildung bei fermentierten Milchprodukten. Tabellen mit vergleichbaren quantitativen Daten und den zugehörigen Literaturzitaten gewährleisten mit Hilfe von Schlüsselbegriffen einen schnellen Zugang zu einem Interessengebiet.

Abstract. A compilation of publications on the analysis and formation of aroma compounds produced by micro-organisms used in dairy production is presented. Literature published between 1970 and 1993 is reviewed whereas literature published before 1970 is covered by a bibliography of review articles. Special attention was given to the contribution of lactobacilli, streptococci, lactococci, and bifidobacteria on the formation of volatile aroma compounds in dairy products. Comparative quantitative data including complete citation of the references are listed in tables thus providing a quick access to an area of special interest with the help of key words.

Introduction

There exists a widespread spectrum of fermented dairy products, from fresh products such as yoghurt or cottage cheese via soft and semi hard cheese to hard cheese with a maturation time of several months. The experimental knowledge on the production of fermented milk products is enormous. Yet the scientific knowledge of the detailed mechanisms and pathways followed during formation of the characteristic aroma of the respective product, despite all the scientific research done over the past 50 years, is only just beginning.

In fresh products the main stage of aroma formation occurs during the first hours of production and is dominated by the starter cultures used. During this time the microorganisms principally metabolise lactate from lactose. Thus the resulting aroma is limited to the main product and the by-products of glycolysis. The formation of the characteristic aroma of semi hard and hard cheeses on the other hand is essentially determined by proteolytic and lipolytic processes as well as by the development of a secondary microbial flora. For these products it is therefore extremely difficult to appraise the direct influence of the starter cultures on the resulting aroma.

The aim of this study is to give an overview of the scientific work that has been done in the field of analysis and formation of aroma compounds by micro-organisms used in dairy production. Special attention is given to the literature published between 1970 and 1993. Literature published before 1970 is covered by a bibliography of review articles. The main focus lies in the contribution of micro-organisms to the formation of aroma. Consequently, aroma compounds produced by starter cultures are listed in tables giving comparative quantitative data and complete citation of the references.

Bibliography of review articles

The literature concerning scientific aroma research published before 1970 is considered only in form of a listing of review articles published between 1970 and 1993 [1–59]. The contents of the articles listed in Table 1 are described through the key words of such categories, such as products, micro-organisms, maturation, aroma, and some further key words.

* *Correspondence to:* J. O. Bosset

Table 1. Review articles concerning aroma production in fermented dairy products

Ref.	Products			Micro-organisms					Maturation			Aroma		Key words	
	Cheese	Cultured milks	Dairy products	Lactobacilli	Streptococci	Lactococci	Leuconostoc	Bifidobacteria	Enzymes	Proteolysis	Lipolysis	Volatiles	Sensor		Number of References cited in the article
[1]	×								×	×		×			80
[2]		×		×	×	×			×			×			84
[3]	×			×	×	×	×		×	×	×			Mould ripening	258
[4]	×	×		×	×	×	×			×	×	×		Dairy products	111
[5]	×	×	×	×	×	×	×					×		Dairy products	143
[6]	×		×						×	×	×	×		Metabolic pathways	58
[7]	×	×		×	×	×	×		×	×	×	×		Metabolic pathways	90
[8]	×											×		Metabolic pathways	102
[9]	×					×	×		×	×		×		Metabolic pathways	73
[10]	×								×	×	×	×		Baterial metabolism, pH. redox potential	85
[11]	×											×		Redox reactions	11
[12]	×			×	×	×	×		×	×	×				107
[13]	×								×			×		Metabolic pathways, off-flavour	26
[14]	×											×		Metabolic pathways	63
[15]	×											×		Off-flavours	70
[16]	×			×	×	×			×	×	×	×	×	Free amino acids, analytical methods	55
[17]	×									×	×	×	×		45
[18]	×			×	×	×	×			×	×	×		Flavour defects	111
[19]		×		×	×	×	×					×		Metabolic pathways	138
[20]	×			×	×	×	×		×	×	×	×			35
[21]			×			×	×					×		Metabolic pathways	22
[22]	×								×	×	×			Pediococci, micrococci	87
[23]	×											×		Mould-ripened blue cheeses	55
[24]				×	×	×	×		×	×					97
[25]	×			×					×	×				Non starter lactic acid bacteria	123
[26]	×			×	×	×			×	×	×			Synergisms	114
[27]	×	×		×	×	×	×					×		Genetics	17
[28]	×			×	×	×				×	×			Cheese defects	230
[29]				×	×	×	×		×	×				Metabolic pathways	149
[30]	×			×		×			×	×	×			Accelerated ripening	58
[31]	×											×		Free fatty acids, free amino acids	107
[32]	×			×	×	×								Milk quality	
[33]	×			×	×	×								Starters, propionibacteria	
[34]	×			×	×	×			×	×	×	×		Free amino acids	
[35]	×			×	×							×		Cheese defects	470
[36]	×											×	×	Swiss cheese flavour	66
[37]	×			×	×							×		Manufacture	146
[38]		×		×	×					×	×	×		Manufacture	
[39]	×	×							×			×		Redox potential, accelerated ripening	68
[40]			×	×	×	×	×					×		Metabolic pathways	66
[41]	×	×	×		×	×	×		×			×			43
[42]	×	×	×	×	×	×	×		×			×		Flavour defects	86
[43]	×	×	×	×		×	×		×	×	×	×	×	Accelerated ripening	62
[44]	×	×	×									×	×	Lactones, thresholds	159
[45]				×	×	×	×		×			×		Metabolic pathways	18
[46]			×	×	×	×	×		×			×		Metabolic pathways	135
[47]		×	×	×	×	×	×		×			×		Metabolic pathways	76
[48]	×											×		Flavour formation	39
[49]	×			×					×	×	×	×		Cheese defects	159
[50]	×					×			×	×	×	×			181
[51]		×		×	×									Synergetic effects	21
[52]		×		×	×							×			51
[53]		×		×	×				×	×		×		Biochemistry, technology	538
[54]		×		×	×							×		Flavour variation	43
[55]	×	×	×	×	×	×	×		×			×		Metabolism of starters	95
[56]		×		×	×	×	×		×			×		Fermentation types	135
[57]		×		×	×									Manufacture, storage	132
[58]	×				×	×			×			×		Flavour development, non starter bacteria	39
[59]			×	×	×	×	×	×		×		×	×	Quarg, selection criteria	17

The products are grouped into three subgroups concerning the main subject of the articles: cheese, cultured milks, and articles that deal with dairy products in general. The micro-organisms considered in this study are lactobacilli, streptococci (namely *Streptococcus salivarius subsp. thermophilus*), lactococci, leuconostoc, and bifidobacteria. Key words in the category maturation give information as to whether the article deals with enzymes in general or with proteolysis or lipolysis in particular. The category aroma gives hints about instrumental analyses of (mostly volatile) organic compounds or about sensory evaluation of food aromas. Supplementary key words as to the contents describe the review articles even further. Finally the number of reference citations found in the respective article is noted. The aim of Table 1 is to provide rapid access to a special field of interest with the help of key words.

There are different approaches possible to the complex mechanisms and processes occurring during aroma formation. As a consequence of the rapid technical development of the early 1960s, the instrumental aroma analysis was then intensified in the field of glycolysis and proteolysis [1]. Some authors, e.g. Forss [1], Adda [4], Adda et al. [10, 15], Behnke [8] or Collins [21] preferred a purely chemical and analytical approach. First the product itself and its different aroma compounds were analysed. In a second step the evaluated data were used to postulate and identify metabolic pathways for each of the compounds analysed. Other workers like Law [3, 7, 9, 18], Law et al. [24, 28, 30, 58], Marshall [19], Marshall et al. 29, 55] or Sandine et al. [41, 42] characterised the micro-organisms in connection with analyses of the end product. Finally a third group of authors, e.g. Kandler [45] or Keenan and Bills [40] have characterised the micro-organisms of starter cultures. This approach reveals the potential of aroma-producing capacities by breeding the micro-organisms under ideal conditions in artificial growth media.

Bibliography of research articles concerning micro-organisms

In this section references are cited that contain data on micro-organisms and their ability to produce volatile aroma compounds [60–85]. Information on the compound classes produced by lactic acid bacteria are shown in Table 2 (lactobacilli and bifidobacteria) and Table 3 (S. salivarius subsp. thermophilus and lactococci).

The compounds analysed belonged to the following compound classes: alcohols, aldehydes (mostly as acetaldehyde), diacetyl, acetoin, ketones, volatile organic

Table 2. References providing data on the production of volatile aroma compounds by *lactobacillus subsp.* and *bifidobacteria*

Volatile aroma compounds	*L. delbrückii*		*L. helveticus*	*L. acidophilus*	*L. casei*	*Bifidobacterium*		
	subsp. bulgaricus	*subsp. lactis*			*subsp. casei*	*longum*	*bifidum*	*breve*
Alcohols	[60–64]	[64]	[63]		[64, 65]	[60]	[60]	[60]
Aldehydes	[60–62, 66–70]			[66]	[65]	[60]	[60]	[60]
Diacetyl	[60, 62, 67, 68, 71]			[72–75]	[76]	[60]	[60]	[60]
Acetoin	[63, 64]	[64]	[63]	[72, 74, 75]	[64, 65, 76]			
Ketones	[60, 62, 63, 67]		[63]			[60]	[60]	[60]
Esters					[65]			
Free fatty acids	[60]				[65]	[60]	[60]	[60]
Organic acids	[60, 64]	[64]			[64, 65]	[60]	[60]	[60]
Sulfur c. c.	[60]				[65]	[60]	[60]	[60]
Pyrazines			[77]					
Amines			[77]					
Phenols			[77]					

c. c. = Containing compounds

Table 3. References providing data on the production of volatile aroma compounds by *Streptococcus salivarius subsp. thermophilus* and *Lactococcus lactis subsp.*

Volatile aroma compounds	*Streptococcus salivarius*	*Lactococcus lactis*		
	subsp. thermophilus	*subsp. lactis*	*subsp. diacetylactis*	*subsp. cremoris*
Alcohols	[60, 62, 64]	[61, 62, 64, 81]	[61, 62]	[61, 64]
Aldehydes	[60, 62, 67, 70, 82, 83]	[61, 62, 81, 83]	[61, 62, 83]	[61, 83]
Diacetyl	[60, 62, 67, 71, 83]	[62, 81, 83, 84]	[62, 71, 83]	[76, 83]
Acetoin	[64]	[64, 81, 84]		[64, 76]
Ketones	[60, 62, 67]	[62]	[62]	
Free fatty acids	[60]			
Organic acids	[60, 64, 85]	[64]		[64]
Sulfur c. c.	[60]			

c. c. = containing compounds

acids and compounds containing sulphur or nitrogen. There was only one reference reporting nitrogen-containing compounds such as pyrazines or amines, which would indicate a substantial proteolytic activity.

References providing quantative data on the production of aroma compounds are discussed below. The listing includes only articles containing results of single-strain investigations either in fermented milks or in model systems. If the fermentation medium is not explicitly specified, all experiments were carried out with milk. The best defined species in this field are *Lactobacillus delbrückii subsp. bulgaricus* and *S. salivarius subsp. thermophilus,* the classical yoghurt starters.

Lb. delbrückii subsp. bulgaricus

Of all secondary metabolites of *L. delbrückii subsp. bulgaricus* listed in Table 4, acetaldehyde is of primary interest because of its key role in yoghurt aroma.

Lees and Jago [61] investigated the role of acetaldehyde as an intermediate in the formation of ethanol from glucose by lactic acid bacteria. They found a weak enzyme activity of aldehyde dehydrogenase but none of alcohol dehydrogenase. These findings are confirmed by the research of Marshall and Cole [66], who also substantiated threonine metabolism as a pathway leading to acetaldehyde production. The lack of alcohol dehydrogenase is essential to account for the relatively high acetaldehyde concentration in single strain cultures of *L. delbrückii subsp. bulgaricus.* There exist at least two pathways leading to acetaldehyde as intermediate but there is no way of reducing it to ethanol. The result is an accumulation of acetaldehyde in milks fermented with this organism.

Hamadan et al. [70] reported that single strains of *L. delbrückii subsp. bulgaricus* and *S. salivarius subsp. thermophilus* produced significantly less acetaldehyde than combined mixtures of it, a fact that could not be confirmed by the work of Yuguchi et al. [60] and Zouari et al. [69]. Scolari et al. [62] and Zouari et al. [69] characterised several strains for their ability to produce volatile aroma compounds and their respective amount of titrable acidity with respect to the preparation of new starter cultures. Principal component analysis, including further parameters such as viscosity, maximum acidification rate and corresponding time and pH value, permitted additional definition of some groups of strains showing similar behaviour [69]. Acetaldehyde production was considerably reduced after a storage for 21 days at 4° C [69].

Dutta et al. [71] reported the effects of different heat treatments of milk on changes in acid and flavour production by single-strain cultures of *L. delbrückii subsp. bulgaricus.* The organisms did not produce diacetyl in steam-sterilised milk, whereas equal amounts were found in milk exposed to different pasteurisation treatments. Thornhill and Cogan [64] presented a new gas chromatographic method for the analysis of volatile metabolites of lactic acid bacteria. All the strains tested were grown in MRS medium at 30° C (mesophilic species) and 37° C (thermophilic species), respectively. Besides the quantified compounds ethanol, acetoin and acetic acid, further compounds such as propanone, isobutanol, n-butanol, 2,3-butyleneglycol and propionic and butyric acid, were traced qualitatively.

L. acidophilus

References providing quantitative data on the ability of *L. acidophilus* to produce volatile aroma compounds are listed in Table 5. This organism is used in the manufacture of acidophilus milk, non-fermented acidophilus milk, as a complement to normal yoghurt cultures or in cultured bifidus-acidophilus milk. It is of interest for its capacity to produce acetaldehyde and diacetyl.

Marshall and Cole [66] examined threonine metabolism as a pathway for producing acetaldehyde. Addition of threonine to milk fortified with whey proteins caused L. acidophilus to produce a well flavoured product with a taste and aroma similar to that of yoghurt. This is not the case in milk without threonine addition. Enzyme assays of threonine aldolase and alcohol dehydrogenase re-

Table 5. References providing quantitative data on *L. acidophilus*

Volatile aroma compounds	References				
	[66] (ppm)	[72] (mg/l)	[73] (mg/l)	[74] (μg/mg cell suspension)	[75] (mg/l)
Acetaldehyde	3–41				
Diacetyl			14–62	1.0–1.5	1–18
Acetoin				10–20	4–794
Diacetyl + Acetoin		100–7150			

Table 4. References providing quantitative data on *Lactobacillus delbrückii subsp. bulgaricus*

Volatile aroma compounds	References						
	[60] (μg/l)	[62] (ppm)	[64] (mg/l)	[66] (ppm)	[69] (ppm)	[70] (ppm)	[71] (ppm)
Ethanol	13.05	0–38	5.8				
Acetaldehyde	44.28	0.5–30		42–45	6.5–15	10	
Diacetyl	0.84	0–1					12–13
Propanone	12.58	0.2–1.5					
2-Butanone	0.53						
Acetic acid			1.2				
Dimethyl sulphide	22.31						

vealed modest activities of both enzymes in the organism. In fermentations without excess threonine the acetaldehyde is continuously reduced to ethanol due to the alcohol dehydrogenase activity. Compared to acetaldehyde, ethanol has a very high flavour threshold and its production will reduce the flavour quality and intensity of the product. This may explain the lack of flavour in milks fermented with *L. acidophilus*. As the activity of the alcohol dehydrogenase is rather low, a slight surplus of threonine easily saturates the enzyme with substrate, and acetaldehyde may be expected to accumulate.

The second metabolite of interest in *L. acidophilus* is diacetyl. Benito de Cardenas et al. [72–75] tested production of acetoin and diacetyl by lactic acid bacteria including the species *L. acidophilus*. All the tests were carried out in complex or synthetic media. L. acidophilus produces only small amounts of acetoin + diacetyl (about 100 mg/l medium) grown in basal medium containing glucose (BMG). The addition of 1.1% sodium pyruvate to BMG (=BMGP) induced the diacetyl + acetoin forming system and the production increased up to 7150 mg/l medium. Additional increments of lactic acid to the BMGP medium slightly decreased the production. A pH value below 4.25 due to the addition of more lactic acid, inhibited production of aroma compounds and growth [72].

L. acidophilus grown in complex or synthetic media produced significantly more diacetyl and acetoin in the presence of pyruvate, although pyruvate caused distinct reduction in growth [73, 74], and also produced significantly more diacetyl and acetoin at elevated temperatures, reaching an optimum at 45° C [75].

Further lactobacilli and bifidobacteria

References containing quantative data for *L. casei ssp casei* and *L. delbrückii ssp lactis* are listed in Table 6.

Hegazi and Abo-Elnaga [76] investigated the effects due to changes in pyruvate concentration, incubation period and associative growth on the production of acetoin and diacetyl by various lactic acid bacteria including *L. casei subsp. casei*. The higher the concentrations of pyruvate added to the medium, the higher the amounts of diacetyl and acetoin produced. The time to reach the maximum concentration of both aroma compounds was dependent on the added pyruvate concentration. There was no diacetyl reductase activity detected for *L. casei subsp. casei*. The amounts of diacetyl produced in media with high concentrations of pyruvate either remained constant or increased slightly at the end of the observation period.

Table 6. References (Ref) providing quantitative data about *L. casei subsp. casei* and *L. delbrückii subsp. lactis*

Volatile aroma compounds	*L. casei subsp. casei*		*L. delbrückii subsp. lactis*
	Ref [64] (mg/l)	Ref [76] (mg/l)	Ref [64] (mg/l)
Ethanol	4.2–8.4		13.9
Acetoin	0–43.1		
Diacetyl		34–57	
Diacetyl + Acetoin		127–2884	
Acetic acid	28.8–540		81.6

Table 7. References providing quantitative data on *Bifidobacterium subsp.*

Volatile aroma compounds	*B. longum* Ref [60] (μg/l)	*B. bifidum* Ref [60] (μg/l)	*B. breve* Ref [60] (μg/l)
Ethanol	13.4–71	54,6	62.10
Acetyldehyde	31.0–45.3	12.5	77.5
Diacetyl	0.8–2.1	0.6	0.7
Propanone	11.0–11.4	12.2	11.6
2-Butanone	0.5–0.6	0.6	0.6
Dimethyl sulphide	19.5–27.6	23.6	27.4

Thornhill and Cogan [64] presented a gas-chromatographic method for the analysis of volatile metabolites of lactic acid bacteria. All the strains tested were grown in MRS medium at 30° C (mesophilic species) and 37° C (thermophilic species), respectively. The compounds quantified were ethanol, acetoin and acetic acid; further compounds could not be detected.

Quantitative data on the volatile compounds produced by bifidobacteria were found in only one publication and are listed in Table 7.

Yuguchi et al. [60] fermented sterilised milk either with a single culture consisting of one of five strains of bifidobacterium, a strain of *L. delbrückii ssp bulgaricus*, a strain of *S. salivarius subsp. thermophilus* or with mixed cultures consisting of one strain of each species. The highest production of acetaldehyde was detected with *B. breve*. In addition some organic acids were traced: citric, malic, succinic, lactic, and acetic acid were determined by HPLC.

S. salivarius subsp. thermophilus

S. salivarius subsp. thermophilus together with *L. delbrückii subsp. bulgaricus* are the classical yoghurt starter organisms, and therefore the production of acetaldehyde is of primary interest. References providing quantitative data about production of acetaldehyde and other volatile flavour compounds are listed in Table 8.

Comparisons of strains of both species showed that single-strain cultures of *S. salivarius subsp. thermophilus* produce smaller amounts of acetaldehyde than strains of *L. delbrückii subsp. bulgaricus* [53]. Lees and Jago [61] confirmed the existence of aldehyde dehydrogenase and in two of four strains of alcohol dehydrogenase in *S. salivarius subsp. thermophilus,* and established that glucose was metabolised to acetaldehyde and ethanol.

Alternative pathways for the production of acetaldehyde were published by Raya et al. [78] although reports of enzyme activities of aldehyde dehydrogenase and alcohol dehydrogenase in this organism [61] could not be confirmed in their experiments. Of the total amount of acetaldehyde produced, only a small portion resulted from

Table 8. References providing quantitative data on *S. salivarius subsp. thermophilus*

Volatile aroma compounds	References								
	[60] (µg/l)	[62] (ppm)	[64] (mg/l)	[70] (ppm)	[71] (ppm)	[76] (mg/l)	[82] (ppm)	[83] (mg/l)	[85] (mg/l)
Ethanol	6.9	0–4.9	10.1						
Acetaldehyde	25.0	3.5–5.1		3.0			2.5–6.5	1.6–7.5	
Diacetyl	1.8	0–19.6			12–13	39–61		0.1–3.8	
Diacetyl + acetoin						181–1536			
Propanone	9.2	1.2–1.8							
2-Butanone	0.5								
Formic acid									18–32
Acetic acid			54						
Dimethyl sulphide	16.9								

the decarboxylation of pyruvate, since the α-carboxylase activity was rather low. Production via the hexose monophosphate shunt was excluded because phosphoketolase activity was not detected. In contrast to Lees and Jago [61], deoxyribonuclease activity was found, whereas activity of threonine aldolase could not be detected in strains tested [78].

To evaluate acetaldehyde synthesis from threonine, Wilkins et al. [79] worked with a model system using radioactive labelled threonine as a precursor of acetaldehyde. As a result, only 2% of the total amount of produced acetaldehyde showed radioactive incorporation deriving from threonine. Compared to *L. delbrückii subsp. bulgaricus* the strains of *S. salivarius subsp. thermophilus* revealed significantly higher activities of threonine aldolase.

Dutta et al. [71] reported the effects of different heat treatments of milk on acid and flavour production by single-strain cultures of various lactic acid bacteria including *S. salivarius subsp. thermophilus*. The organism produced only half the amount of acetaldehyde in steam-sterilised milk compared to the different pasteurisation treatments evaluated.

Hegazi and Abo-Elnaga [76] investigated effects due to changes in pyruvate concentrations, incubation period and associative growth on the production of acetoin and diacetyl by various lactic acid bacteria including *S. salivarius subsp. bulgaricus*. The higher the concentrations of pyruvate added to the medium, the higher the amounts of diacetyl and acetoin produced. There was no diacetyl reductase activity detected as the amounts of diacetyl and acetoin produced in media with high concentrations of pyruvate either remained constant or increased slightly at the end of the observation period.

Zouari et al. [82] characterised 20 strains of *S. salivarius subsp. thermophilus* isolated from Greek yoghurt. The strains were compared on the basis of titrable acidity, viscosity, amount of acetaldehyde produced, corresponding time to acidification rate and pH value and urease activity. Principal component analysis was used for strain grouping. Acetaldehyde was reduced by about 30% after a storage of 21 days at 4° C, apparently without alcohol dehydrogenase activity, since an increase in ethanol was not reported.

Production of formate was evaluated on three strains of *S. salivarius subsp. thermophilus* in whole milk and complex media by Perez et al. [85]. Formate production was dependent on strain, culture medium and temperature. The maximum formate concentration was detected during the late period of exponential growth. Formate is a key component in the synergisitic effect between *S. salivarius subsp. thermophilus* and *L. delbrückii spp. bulgaricus,* acting as a stimulus for the latter organism.

Lactococci

The production of lactic acid from lactose is the primary function of starter cultures. Besides this function some mesophilic starter organisms show the capacity to form diacetyl and acetoin by metabolising citrate. Many research articles concerned with the metabolism of citrate by lactococcus and leuconostoc sp. have been discussed

Table 9. References providing quantitative data on *Lactococcus lactis subsp. lactis*

Volatile aroma compounds	References		
	[62] (ppm)	[64] (mg/l)	[83] (mg/l)
Ethanol	26.5–40.0	16.4	
Acetaldehyde	1.7–8.4		0.7–6.5
Diacetyl	2.4–4.4		0.05–0.6
Acetoin			
Propanone	1.3–1.6		
Acetic acid		12.0	

Table 10. References providing quantitative data about *L. lactis subsp. cremoris*

Volatile aroma compounds	References		
	[64] (mg/l)	[76] (mg/l)	[83] (mg/l)
Ethanol	16.8		
Acetaldehyde			0.3–8.0
Diacetyl		39.0–42.0	0.05–2.3
Acetoin	16.2		
Diacetyl + acetoin		52–3404	

Table 11. References providing quantitative data on *L. lactis subsp. lactis var. diacetylactis*

Volatile aroma compounds	References				
	[64] (mg/l)	[71] (ppm)	[81] (mg/l)	[83] (mg/l)	[84] (mg/kg)
Ethanol	25.2–183.1		13.1–26.4		
Acetaldehyde			2.5–7.1	2.2–11.4	
Diacetyl		12.0–15.0	1.0–1.9	0.6–55.0	1.5–2.5
Acetoin	0–200	90–100	212–311		250–270
Acetic acid	444–840				

Table 12. References to the compound classes of substances analysed in fermented dairy products

Compound classes	Cheese							Cultured milks	
	Cheddar	Swiss	Gruyère	Semi hard	Soft	Blue	Other	Yogurt	Other
Alcohols	[87–100]	[34, 101]	[102–108]	[105, 108–110]	[105, 111]	[105, 111]	[98, 105, 108, 112–116]	[60, 117–129]	[130, 131]
Aldehydes	[88, 89, 94, 95, 98–100, 132]	[34, 101, 133, 134]	[102, 103, 104, 106–108]	[108, 109, 134]	[134, 135]	[134]	[98, 108, 113, 114, 134, 136]	[38, 60, 67, 117–129]	[130, 131]
Diacetyl	[88, 92, 95, 100, 132]	[34, 101, 133, 137]	[108]	[108]	[111, 135]			[38, 60, 67, 117–129]	[130]
Acetoin	[93, 95, 99, 100, 132]		[102]				[113]	[38, 60, 119–123]	
Ketones	[87–100, 132]	[34, 101, 133, 134]	[102–108]	[105, 108–110, 134]	[105, 111, 134, 135, 138]	[105, 111, 134]	[98, 105, 108, 112–116, 134, 136]	[38, 60, 67, 117–122, 124–129]	[130, 131]
Esters	[88, 91, 93–95, 98, 100]	[34, 101]	[102, 103, 106–108]	[108–110]	[135, 138]		[98, 108, 112–116]	[120, 121, 124–126, 128]	[131]
Lactones	[88, 89, 94, 99, 139]	[34, 101, 134, 133]	[102, 103, 106, 107]	[134]	[134, 135]		[112–115]	[119]	[131]
Free fatty acids	[89, 90, 92–94, 99, 100, 140]	[34, 133, 134, 137, 141, 142]	[143, 144]	[109, 134]	[134, 135]	[134]	[112, 114–116, 134]	[38, 60, 117, 120 121, 126, 145]	[130, 131]
Organic acids	[89, 90, 140, 146]	[34]			[146]	[146]	[112, 114, 147]	[60, 146]	[130, 146]
Sulfur c. c.	[91–94, 96–98, 100]	[34, 101]	[102–104, 106–108]	[108, 109]	[135, 138]		[98, 108, 113, 114]	[60, 119, 125]	
Hydrocarbons	[91, 93, 94, 100]	[34]	[102, 106–108]	[108]			[108, 112–115]	[125]	[131]
Pyrazines	[77]	[77, 133, 148]	[102, 104, 106, 107, 149]				[77, 113, 114, 150]		[131]
Amines	[77, 140]	[77, 142]		[109]			[77, 147]		
Nitrogen c. c.		[34]	[102]	[109]	[138]		[113]		
Phenols	[77, 89, 99]	[77]	[102, 108]	[108]			[77, 108, 113, 114]		

c. c. = containing compounds

by Cogan [80]. References providing quantitative data about the production of volatile aroma compounds by lactococci are listed in Tables 9 to 11.

Bottazzi and Dellaglio [83] evaluated the acetaldehyde and diacetyl production in single-strain cultures of lactococci and *S. salivarius subsp. thermophilus*. They found considerably higher amounts of both compounds in *Lactococcus lactis subsp. lactis var. diacetylactis* than in those of *L. lactis subsp. lactis* and *subsp. cremoris* respectively, either in complex medium or in autoclaved skim milk. The diacetyl: acetaldehyde ratios under the different experimental conditions in most strains were <1, an unfavourable ratio because of the danger to develop "green flavour" in the product with ratios <2.

Dutta et al. [71] reported effects of different heat treatments of milk in acid and flavour production in selected lactococci. Neither in *L. lactis subsp. lactis* nor in *subsp. cremoris* could production of diacetyl and acetoin be detected. The biovariety *diacetylactis* produced considerably smaller amounts of diacetyl in steam-sterilised milk than in pasteurised milks whereas for acetoin higher amounts were produced in milks treated at higher temperatures.

Clementi [84] evaluated the production of a frozen dessert from an ice cream mix with *L. lactis subsp. lactis var. diacetylactis*. Various culture conditions were investigated in order to obtain a product with high diacetyl content together with pleasant acidic taste. Under standard conditions diacetyl accumulated to a maximum level in up to 24 h. After 36 h production then reached a steady state, probably due to an equilibrium between diacetyl synthesis and its reduction to acetoin. Accumulation of diacetyl and acetoin were promoted by pH values below 5.5. A considerable increase was achieved when milk was supplemented with additional citric acid. The temperature optimum was observed at 30° C and growth under stirred conditions resulted in a significant increase of diacetyl and acetoin amounts.

Libudzisz and Galewska [81] investigated the formation of diacetyl, acetoin, 2,3-butylene glycol, acetaldehyde, ethanol and acetic acid during 24 h of cultivation in milk or milk with supplemented citric acid. An increase in the citrate concentration in milk to 0.5% resulted in an increase in the production of diacetyl from 58 to 74% and of acetoin by 2.8 to 3.7 times. Increased concentrations of citrate in milk stimulated the production of diacetyl and acetaldehyde to a similar extent without deteriorating the organoleptic qualities of starters and product.

Bibliography of research articles about analysis of aroma compounds

During this literature search many research articles dealing with analysing aroma compounds or compilations of references on chemical compounds found in milk and dairy products, like that published by van Straten et al. [86], were found. But none of them provided special investigation into a possible contribution of either starter cultures or secondary flora to the aroma. As a result Table 12 lists references that report the analysis of volatile organic compounds. Again the table format provides rapid access to either different classes of cheeses and cultured milks or various chemical compound classes [87–150].

Obviously most of the research done in the field of aroma compounds in fermented milks has been undertaken with cheese. Most of the reports deal with only three types of cheese: Cheddar, Swiss Emmental, and Gruyére. A similar tendency was observed with cultured milks. In this field about nine out of ten publications are concerned with yoghurt, whereas for other products such as quarg or cottage cheese no research reports could be found. On the other hand, when it comes to the chemical compound classes, the research on fermented dairy products was almost complete.

Future outlook

Research work on the influence of secondary metabolites from micro-organisms on the formation and quality of the aroma of fermented dairy products should be consolidated. More knowledge about the synergistic effects between species as well as single- and mixed-strain cultures would be of enormous interest. Gaining more insight into these mechanisms and effects would also provide desirable progress in the field of quality influencing and quality control. Qualitative and quantitative analysis of the volatile aroma compounds can only be the first step towards achieving this goal. In a second step the threshold of perception of every single compound in the matrix used should be known in order to assess its "aromatic influence" on the resulting aroma. Unfortunately, most of the available data about perception thresholds are determined with water or oil and only few with milk as matrix. Much work remains to be done until a more complete understanding of the formation and factors influencing the formation of the aroma of fermented dairy products are reached.

References

1. Forss DA (1979) J Dairy Res 46:691–706
2. Vedamuthu ER (1982) In: Rose AH (ed) Fermented Foods. Academic Press, London, pp 199–223
3. Law BA (1982) In: Rose AH (ed) Fermented Foods. Academic Press, London, pp 148–198
4. Adda J (1986) In: Birch GG, Lindley MG (eds) Development in food flavours. Elsevier Applied Science, London, pp 151–172
5. Badings HT, Neeter R (1980) Neth Milk Dairy J 34:9–30
6. Dumont JP, Adda J (1979) In: Land DG, Nursten HE (eds) Progress in flavour research. Applied Science Publishers, London, pp 245–262
7. Law BA (1981) Dairy Sci Abstr 43:143–154
8. Behnke U (1980) Die Nahrung 24:71–83
9. Law BA (1982) Perfumer & Flavourist 7:9–21
10. Adda J, Grippon JC, Vassal L (1982) Food Chemistry: 115–129
11. Kristoffersen T (1985) Milchwissenschaft 40:197–199
12. Reiter B, Fryer TF, Sharpe M Elisabeth, Lawrence RC (1966) J Appl Bact 29:231–243
13. Moskowitz GJ (1980) In: Charalambous G (ed) The analysis and control of less desirable flavors in foods and beverages. Academic Press, London, pp 53–70
14. Panousse JJ, Masson J-D, Truong Thanh Tong (1972) Industr Alim Agr 89:133–140
15. Adda J (1984) In: Eck A (ed) Le fromage. Diffusion Lavoisier, Paris, pp 330–340
16. Ney KH (1981) In: Charalambous G, Inglett G (eds) The quality of foods and beverages, Chemistry and Technology, vol 1. Academic Press, London, pp 389–435
17. Farkye NY, Fox PF (1990) Trends Food Sci Technol: 37–40
18. Law BA (1984) In: Davies LF, Law BA (eds) Advances in the microbiology and biochemistry of cheese and fermented milk. Elsevier, London, pp 187–208

19. Marshall VME (1984) In: Davies FL, Law BA (eds) Advances in the microbiology and biochemistry of cheese and fermented milk. Elsevier, London, pp 153–186
20. Moskowitz GJ, LaBelle GG (1981) In: Charalambous G, Inglett G (eds) The quality of foods and beverages, Chemistry and Technology, vol 2. Academic Press, London, pp 21–35
21. Collins EB (1971) J Dairy Sci 55:1022–1028
22. Bhowmik T, Marth E (1990) J Dairy Sci 73:859–866
23. Nelson JH, Richardson GH (1967) In: Peppler HJ (ed) Microbial technology. Reinold, New York, pp 82–106
24. Law BA, Kolstad J (1983) Antonie van Leeuwenhoek 49:225–245
25. Peterson SD, Marshall RT (1990) J Dairy Sci 73:1395–1410
26. Olson NF (1990) FEMS Microbiol Rev 87:131–148
27. Mabbit LA, Davies FL, Law BA, Marshall Valerie M (1987) In: Norris JR, Pettipher GL (eds) Essays in agricultural and food microbiology. Wiley, Chichester, pp 135–166
28. Law BA (1984) Prog Ind Microbiol 19:245–283
29. Marshall VME, Law BA (1984) In: Davies FL, Law BA (eds) Advances in the microbiology and biochemistry of cheese and fermented milk. Elsevier, London, pp 76–98
30. Law BA (1984) In: Davies FL, Law BA (eds) Advances in the microbiology and biochemistry of cheese and fermented milk. Elsevier, London, pp 209–228
31. Aston JW, Dulley JR (1982) Aust J Dairy Technol 37:59–64
32. Langsrud TW, Reinbold GW (1973) J Milk Food Technol 36:487–490
33. Langsrud T, Reinbold GW (1973) J Milk Food Technol 36:531–542
34. Langsrud T, Reinbold GW (1973) J Milk Food Technol 36:593–609
35. Langsrud T, Reinbold GW (1974) J Milk Food Technol 37:26–41
36. Biede S (1977) In: a study of the chemical and flavor profiles of Swiss Cheese, Dissertation, Iowa State University, Ames, Iowa, USA
37. Moquot G (1979) J Dairy Res 46:133–160
38. Rasic JL, Kurmann JA (1978) In: Rasic JL, Kurmann JA (eds) Fermented fresh milks. Technical Dairy Publishing House, Copenhagen DK
39. Green Margaret L, Manning DJ (1982) J Dairy Res 49:737–748
40. Keenan TW, Bills DD (1968) J Dairy Sci 51:1561–1567
41. Sandine WE, Elliker PR (1970) J Agric Food Chem 18:557–562
42. Sandine WE, Daly C, Elliker PR, Vedmuthu ER (1972) J Dairy Sci 55:1031–1039
43. Seitz EW (1990) J Dairy Sci 73:3664–3691
44. Maga JA (1976) Crit Rev Food Sci Nutr 8:1–55
45. Kandler O (1961) Milchwissenschaft 16:523–531
46. Lees GJ, Jago GR (1978) J Dairy Sci 61:1204–1215
47. Lees GJ, Jago GR (1978) J Dairy Sci 61:1216–1224
48. Lamparsky D, Klimes I (1981) In: Schreier P (ed) Flavour '81. de Gruyter, Berlin, pp 557–577
49. Khalid NM, Marth E (1990) J Dairy Sci 73:2669–2684
50. Kamaly KM, Marth EH (1989) J Dairy Sci 72:1945–1956
51. Tramer J (1973) J Soc Dairy Technol 26:16–21
52. De Haast J, Lategan PM, Novello JC (1979) S Afr J Dairy Technol 11:11–15
53. Tamime AC, Deeth HC (1980) J Food Prot 43:939–977
54. Casalis J (1975) Industr Alim Agr 92:1253–1262
55. Marshall VM (1987) J Dairy Res 54:559–574
56. Tamime AY, Robinson RK (1988) J Dairy Res 55:281–307
57. Matalon ME, Sandine WE (1986) Cult Dairy Prod J 11:6–12
58. Law BA, Sharpe M Elisabeth (1977) Dairy Ind Int 42:10–14
59. Kurmann JA (1983) Lebensm-Technol 16:9–15
60. Yuguchi H, Hiramatsu A, Doi K (1989) Jpn J Zootech Sci 60:734–741
61. Lees GJ, Jago GR (1976) J Dairy Res 43:63–73
62. Scolari G, Bottazzi V, Brambilla E (1985) Scienza, Tecnica Lattiero-Casearia 36:593–602
63. Bottazzi V, Battistotti B, Bersani C (1978) Scienza, Tecnica Lattiero-Casearia 29:68–80
64. Thornhill PJ, Cogan TM (1984) Appl Environ Microbiol 47:1250–1254
65. Tracey RP, Brytz TJ (1989) Appl Environ Microbiol 55:1617–1623
66. Marshall Valerie M, Cole Wendy M (1983) J Dairy Res 50:375–379
67. Hu JH, Saito M, Lee KH (1985) Korean J Anim Sci 27:42–46
68. Reps A, Hammond EA, Glatz BA (1987) J Dairy Sci 70:559–562
69. Zouari A, Desmazeaud MJ (1991) Lait 71:463–482
70. Hamdan IY, Kunsman JE, Deane DD (1971) J Dairy Sci 54:1080–1082
71. Dutta SM, Kuila RK, Ranganathan B (1973) Milchwissenschaft 28:231–232
72. Benito de Cardenas I, Ledesma OV, Pesce de Ruiz Holgado AA, Oliver G (1985) J Dairy Sci 68:1897–1901
73. Benito de Cardenas IL, Ledesma O, Oliver G (1989) Milchwissenschaft 44:347–350
74. Benito de Cardenas I, Cerutti de Guglielmone G, Lesdema O, Oliver G (1990) Milchwissenschaft 45:775–777
75. Benito de Cardenas IL, Peral de Portillo MC Albizzatti de Rivadeneira MC, Oliver G (1991) Microbiol Aliments Nutr 9:369–374
76. Hegazi FZ, Abo-Elnaga IG (1990) Nahrung 34:791–801
77. Kowalewska J, Zelazowska H, Babuchowski A, Hammond EG, Glatz BA, Ross F (1985) J Dairy Sci 68:2165–2171
78. Raya RR, Manca de Nadra MC, Pesce de Ruiz Holgado A, Oliver G (1986) Milchwissenschaft 41:397–399
79. Wilkins DW, Schmidt RH, Shireman RB, Smith KL, Jezeski JJ (1986) J Dairy Sci 69:1219–1224
80. Cogan TM (1983) Ir J Food Sci Technol 7:1–13
81. Libudzsiz Z, Galewska E (1991) Nahrung 35:611–618
82. Zouari A, Roger S. Chabanet C, Desmazeaud MJ (1991) Lait 71:445–461
83. Bottazzi V, Dellaglio F (1967) J Dairy Res 34:109–113
84. Clementi F (1991) Milchwissenschaft 46:696–700
85. Perez PF, De Antoni GL, Añon MC (1990) J Dairy Sci 74:2850–2854
86. Straten S van, Vrjier FI de, Beauvesser JC de (1977) In: Straten S van, Vrjier FI de, Beauvesser JC de (eds) Volatile compounds in food. Central Institute for Nutrition and Food Research TNO, Zeist NL
87. Keen AR (1974) In: La formation de 2-Butanol dans le Cheddar. Proceedings XIXth Intern. Dairy Congress, pp 320–321
88. Day EA, Libbey LM (1964) J Food Sci 29:583–589
89. Vandeweghe P, Reineccius GA (1990) J Agric Food Chem 38:1549–1552
90. Marsili R (1985) J Dairy Sci 68:3155–3161
91. McGugan WA, Howsam SG, Elliot JA, Emmons DB, Reiter B, Sharpe ME (1968) J Dairy Res 35:237–245
92. Barlow I, Lloyd GT, Ramshaw EH, Miller AJ, McCabe GP, McCabe L (1989) Aust J Dairy Technol 44:7–18
93. Czulak J, Hammond LA, Horwood JF (1974) Aust J Dairy Technol 29:128–131
94. Liebich HM, Douglas DR, Bayer E, Zlatkis A (1970) J Chromatogr Sci 8:355–359
95. Bradley RL, Stine Jr, Stine CM (1968) J Gas Chromatography 6:344–348
96. Manning DJ (1978) J Dairy Res 45:479–490
97. Manning DJ (1979) J Dairy Res 46:523–529
98. Manning DJ, Moore C (1979) J Dairy Res 46:539–545
99. Dunn HC, Lindsay RC (1985) J Dairy Sci 68:2853–2858
100. Horwood JF (1989) Aust J Dairy Technol 44:91–96
101. Langler JE, Libbey LM, Day EA (1967) J Agric Food Chem 15:386–391
102. Dumont JP, Pradel G, Roger S, Adda J (1976) Lait 551:18–38
103. Bosset JO, Liardon R (1984) Lebensm-Wiss-Technol 17:359–362

104. Bosset JO, Liardon R (1985) Lebensm-Wiss-Technol 18:178–185
105. Groux M, Moinas M (1974) Lait 531:44–52
106. Dumont JP, Adda J, Rousseaux P (1981) Lebensm-Wiss-Technol 14:198–202
107. Guichard E, Berdague JL, Grappin R, Fournier N (1987) Lait 67:319–338
108. Bosset JO, Gauch R (1993) Int Dairy J 3:359–377
109. Ney KH (1985) Fette, Seifen, Anstrichm 7:289–294
110. Sloot D, Harkes PD (1975) J Agric Food Chem 23:356–357
111. Moinas M, Groux M, Horman I (1973) Lait 53:601–609
112. Martinez-Castro I, Sanz J, Amigo L, Ramos M, Martin-Alvarez P (1991) J Dairy Res 58:239–246
113. Dumont JP, Adda J (1978) J Agric Food Chem 26:364–367
114. Meinhart E, Schreier P (1986) Milchwissenschaft 41:689–691
115. Frutos M de, Sanz J, Martinez-Castro I (1991) J Agric Food Chem 39:524–530
116. Frutos M de, Sanz J, Martinez-Castro I (1988) Chromatographia 25:861–864
117. Görner F (1980) Nahrung 24:63–69
118. Görner F, Palo V, Bertan M (1968) Milchwissenschaft 23:94–100
119. Viani R, Horman I (1973) Trav Chim Aliment Hyg 64:66–70
120. Dumont JP, Adda J (1973) Lait 53:12–22
121. Gyosheva B (1985) Nahrung 29:185–190
122. Degorce-Dumas R, Goursaud J, Leveau JY (1986) Industr Alim Agr 103:805–808
123. Rysstad G, Abrahamsen RK (1987) J Dairy Res 54:257–266
124. Kondratenko MS, Guiocheva BC (1978) Lait 58:390–396
125. Labropoulos AE, Palmer JK, Tao P (1982) J Dairy Sci 65:191–196
126. Gyosheva H (1982) Milchwissenschaft 37:267–269
127. Ulberth F (1991) J Assoc Off Anal Chem 74:630–634
128. Gyosheva H, Rusev P (1979) Nahrung 23:385–392
129. Hild J (1979) Milchwissenschaft 34:281–283
130. Marsili RT (1981) J Chromatogr Sci 19:451–456
131. Mick S, Mick W, Schreier P (1982) Milchwissenschaft 37:661–665
132. Vedamuthu ER, Sandine WE, Elliker PR (1966) J Dairy Sci 49:151–157
133. Biede SL, Hammond EG (1979) J Dairy Sci 62:227–237
134. Hanspach I (1981) In: Untersuchungen über freie Fettsäuren als Aromasubstanzen verschiedener Käsesorten. Dissertation, Justus-Liebig-Universität, Giessen D, pp 1–147
135. Parliment TH, Kolor MG, Rizzo DJ (1982) J Agric Food Chem 30:1006–1008
136. Golovnya RV, Uralets VP (1972) Nahrung 16:497–506
137. Mitchell GE (1981) Aust J Dairy Technol 36:21–25
138. Moinas M, Groux M, Horman I (1975) Lait: 414–417
139. Wong NP, Ellis R, LaCroix DE (1975) J Dairy Sci 58:1437–1441
140. Ney KH, Wirotoma IPG (1971) Z Lebensm Unters Forsch 146:337–343
141. Kiermeier F, Mayr A, Hanusch J (1968) Z Lebensm Unters Forsch 136:193–203
142. Ney KH, Wirotoma IPG (1972) Z Lebensm Unters Forsch 149:347–349
143. Berdague JL, Jeunet R, Grappin R (1987) Lait 67:246–263
144. Bosset JO, Collomb M, Sieber R (1993) Lebensm-Wiss-Technol 26:581–592
145. Gyosheva H (1982) Nahrung 26:853–856
146. Marsili RT, Ostapenko H, Simmons RE, Green DE (1981) J Food Sci 46:52–57
147. Ney KH, Wirotoma IPG (1978) Fette, Seifen, Anstrichm 80:249–251
148. Sloot D, Hofmar HJ (1975) J Agric Food Chem 23:358
149. Liardon R, Bosset JO, Blanc B (1982) Lebensm-Wiss-Technol 15:143–147
150. Lin SS (1976) J Agric Food Chem 24:1252–1254