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The formation mechanism of lactones in Gouda cheese

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Abstract

Lactones are fat-derived aroma compounds, but the formation mechanism of these compounds during ripening of Gouda cheese is unknown. Both enzymatic and chemical formation pathways were investigated in this study. Lactone formation from milk triglycerides or free fatty acids by lactic acid bacteria enzymatic activity was not observed. Instead, the mechanism of the lactone formation in cheese was a one-step, non-enzymatic reaction, where a hydroxy fatty acid, esterified in a triglyceride undergoes *trans*-esterification to release the lactone directly. The lactone reaction potential was determined for all major γ- and δ-lactones by controlled heating of the fat. The chemical formation of lactones was temperature-dependent and the Arrhenius parameters of the formation reaction were estimated for each of the lactones. The kinetics of the reaction were first-order, and could be used to explain the formation of lactones in ripening Gouda cheese up to about 40 weeks.

Keywords: Lactones; Cheese; Milk fat; Chemical formation; Reaction mechanism

1. Introduction

Lactones are potent fat-derived flavour compounds that play a role in the overall flavour of cheese (Curioni & Bosset, 2002; Dufosse, Latrasse, & Spinnler, 1994; Moio, Etievant, Langlois, de Kimpe, & Addeo, 1994; Singh, Drake, & Cadwallader, 2003; Urbach, 1997). Lactones are found in Cheddar (Chin, Bernhard, & Rosenberg, 1996; Rehman et al., 2000; Wong, Ellis, & LaCroix, 1975), Gouda (Alewijn, Sliwinski, & Wouters, 2003, 2005; Dirinck & de Winne, 1999), Gruyère (Bosset & Liardon, 1985), ewes' milk cheeses (Larrayoz, Addis, Gauch, & Bosset, 2001), Parmesan (Barbieri et al., 1994) and Blue-type cheeses (Gallois & Langlois, 1990). The sensory properties of lactones in dairy products were recognized long ago (Keeney & Patton, 1956), and the aroma thresholds of the individual lactones have been established (Sick, Albin, Sather, & Lindsay, 1971), which can be as low as 1 ppm in oil systems, and even lower in water.

It has been known for several decades that heating of milk fat results in the formation of lactones (Boldingh & Taylor, 1962; Keeney & Patton, 1956). Urbach and Stark (1978) defined the lactone potential of milk as the amount of lactones released when the fat fraction is heated at 180 °C in the presence of water and in the absence of air. Hydroxy fatty acids, esterified in glycerides are thought to be the precursors of the lactones formed after heating (Jurriens & Oele, 1965; Mattick, Patton, & Keeney, 1959; Wyatt, Pereira, & Day, 1967). These precursors are produced in the cow, and their production depends upon the feeding regime and stage of lactation (Mattick et al., 1959; Parliment, Nawar, & Fagerson, 1966; Urbach, 1982; Urbach & Stark, 1978; Walker, Patton, & Dimick, 1968). When milk fat is heated, esterified hydroxy acids are hydrolysed from the glyceride to form a free hydroxy acid, which may then spontaneously lactonize, as described by Mattick et al. (1959). Although this mechanism is accepted for the formation of lactones under quite extreme temperatures (180 °C), the formation mechanism of lactones during cheese ripening has not been studied in detail. It is generally assumed that during cheese ripening, lactones are formed by a mechanism starting from

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esterified hydroxy acids with free hydroxy acids as intermediates (Collins, McSweeney, & Wilkinson, 2003; Marilley & Casey, 2004; McSweeney & Sousa, 2000; Molimard & Spinnler, 1996), although an alternative mechanism was proposed, where lactic acid bacteria transform unsaturated free fatty acids to free hydroxy acids and lactones (Wanikawa, Hosoi, & Kato, 2000; Wanikawa, Shoji, Hosoi, & Nakagawa, 2002). It was recently shown that the release of aliphatic free fatty acids and formation of lactones followed a different time pattern during the ripening of Gouda cheese (Alewijn et al., 2005). This study showed that the formation rate of lactones was considerably higher than that of free fatty acids during cheese ripening. If lactones would have to be formed from free hydroxy acids, or from unsaturated acids produced by lipolysis, a very specific release of these acids from the triglycerides would then be required, which is not very likely. Therefore, it is presumed that the formation mechanism of lactones is somewhat different than suggested to date.

The major aim of this research was to clarify the mechanism of lactone formation during the normal (13 °C) ripening of Gouda cheese, and to determine whether this formation is an enzymatic process or resembles the mechanism that occurs during heating. Since the method of determining lactone potentials has not been comprehensively reported (Urbach & Stark, 1978), and the published lactone potentials do not include all individual major lactones (Stark, Urbach, Cook, & Ashes, 1978), potentials of individual lactones in different milk fats were established. The proposed mechanism and the validity of its kinetic properties was verified by comparing the mechanistically predicted values of lactone concentration with the real data in a ripening (Gouda) cheese.

2. Materials and methods

2.1. Samples

Six commercially available milk samples were obtained from local supermarkets, and an additional six milk samples, ready for Gouda cheese production, were collected from several cheese factories. All milk was fresh (pasteurized), full fat, obtained between February 2001 and October 2002, and stored at −25 °C prior to analysis. Full-fat Gouda-type cheese was prepared and ripened as described previously (Alewijn et al., 2005). The dry matter content was calculated from the weight difference of a sample mixed with clean dry sand, before and after 2 h of drying in a 103 °C hot air oven.

2.2. Microbial formation of lactones

Three strains of *Lactococcus lactis* subsp. *cremoris*, B697, B1492 and B1157, were obtained from the NIZO culture collection (NIZO Food Research, Ede, The Netherlands), and a common Gouda cheese starter mixture (BK2) was obtained from CSK Food Enrichment (Ede, The Netherlands). The strains were cultured at 30 °C in sterilized (15 min at 121 °C) M17 broth (Difco, Detroit, MI, USA) with lactose. Cells in their late exponential growth phase or in the late stationary phase were incubated with 10-undecenoic acid (C10:1), *cis*-5-dodecenoic acid (C12:1), *cis*-9-tetradecenoic acid (C14:1), *cis*-10-heptanoic acid (C17:1), oleic acid (C18: 1Δ9), linoleic acid (C18: 2Δ9, 12) or ricinoleic acid (12-hydroxy-C18: 1Δ9), all obtained from Sigma-Aldrich (St. Louis, MO, USA), and minimally 97% pure, or with water-washed milk fat. To the bacterial culture (10 mL), a 10% (w/w) solution of fatty material in 70% ethanol (50 μL) was added. The same incubations were also carried out with cell-free extracts of the cultures, obtained after cell disruption using 0.1 mm zirconia/silica beads (Biospec, Bartlesville, OK, USA). Incubations were carried out at 13 or 30 °C for 12–96 h, and were shaken or left at rest in the presence or absence of oxygen (GasPack, BBL Microbiology, Kansas City, MO, USA). The aqueous broth was extracted three times with ether/heptane, and an aliquot was analysed for lactones on the GC/MS system as described below.

2.3. Fat collection and lactone determination

Fresh milk (100 mL) was mixed 1:1 (v/v) with 96% ethanol, and extracted with 10 mL of ether/heptane (1:1, v/v) at 40 °C. After centrifugation at 700 × *g* for 5 min, the top layer was collected, and the extraction was repeated twice to obtain a large part of the milk triglycerides. The ether/heptane from the combined organic layers was evaporated at low temperature (< 20 °C). Cheese fat was isolated in a similar fashion.

Independent duplicates of milk fat (0.4 g) and water (20 μL) were kept frozen (−20 °C) until analysis. Lactones were extracted with acetonitrile (1 mL) at 30 °C. The acetonitrile-layer was analysed directly by GC/MS, consisting of a 8000^{top} GC (CE Instruments, Milan, Italy) equipped with a FFAP GC column (J&W Scientific, Palo Alto, CA, USA) and a Finnigan Automass II (Finnigan, Bremen, Germany). Myristylbromide (18 μg mL^{-1}) was used as an internal standard. To correct for extraction losses that occurred with the acetonitrile extraction of the fat, the distribution coefficients of all individual lactones in this extraction were determined by re-extracting the residue of the first extraction with another 1 mL of acetonitrile. Using the distribution coefficient, the lactone concentration in the fat was calculated, as described earlier (Alewijn et al., 2003).

2.4. Lactone potentials and temperature dependency

To determine the lactone potentials (LP), samples of fat were kept in closed tubes at 160 °C for 5–220 min and analysed for lactones. All data points (five to nine independent duplicates per milk fat) were used and fitted to Eq. (3), minimizing the sum of squares. A 95%

confidence interval $LP_{95\%CI}$ was calculated assuming normally distributed errors using the following:

$$LP_{95\%CI} = (LP \pm 1.96 \cdot \sigma) \quad (1)$$

with a standard deviation of,

$$\sigma = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(lac_i - lac_{fit,i})^2},$$

where n is the number of observations, and lac_i and $lac_{fit,i}$ the respective observed and fitted lactone concentrations. To determine the temperature dependency of lactone formation, three different milk fat samples, in duplicate, were similarly kept at 20, 40, 60 and 140 °C. Heating times (5 min–174 h) were chosen to obtain well-distributed data points over the particular formation curve at each temperature, and the free lactone concentrations were determined for each fat sample. The resulting 42 data points seemed to be described by the Arrhenius equation (4). The Arrhenius parameters and 95% confidence intervals were estimated after linear transformation of Eq. (5), minimizing the sum of squares, using the Microsoft Excel Analysis ToolPak add-in (Microsoft, Redmond, WA, USA).

3. Results and discussion

3.1. Microbial formation of lactones

Lactones can be produced from unsaturated free fatty acids by lactic acid bacteria (Wanikawa et al., 2002). In the case of a ripening Gouda cheese, the starter lactic acid bacteria could be candidates to perform such a conversion. To check the likelihood of this conversion, three strains of *Lc. lactis* subsp. *cremoris*, representative of the starter for Gouda cheese, the mixed strain starter culture itself, and cell-free extracts from these strains were tested for lactone formation with a number of free unsaturated fatty acids, as described above. However, under the in vitro conditions tested, no significant amounts of lactones from the various fatty acids were produced. It should be noted that the in vitro circumstances were certainly different from those in cheese, and that the number of strains and conditions tested were not exhaustive. Nevertheless, the experiments showed that the conversion of unsaturated fatty acids into lactones by lactic acid bacteria is a reaction that does not occur readily.

3.2. Lactone potentials

Lactone potentials were determined by heating the fat at 160 °C, rather than at 180 °C as suggested by Stark et al. (1978), to be able to follow the formation of lactones during heating more closely. The concentrations of δ-lactones during heating time are shown in Fig. 1. Lactone formation was fast in the beginning, but then slowed down quickly and lead to an almost constant concentration after 45 min of heating at 160 °C. It is presumed that the precursors of the lactones became exhausted. Obviously, under these circumstances the reaction responsible for the conversion of precursor molecules to free lactones is a chemical reaction, not catalysed by enzymes.

The formation of lactones showed kinetics that resembled a first-order reaction mechanism. Generally, a first-order reaction can be described by

$$\ln\left(\frac{[C]}{[C_0]}\right) = -k \times t, \quad (2)$$

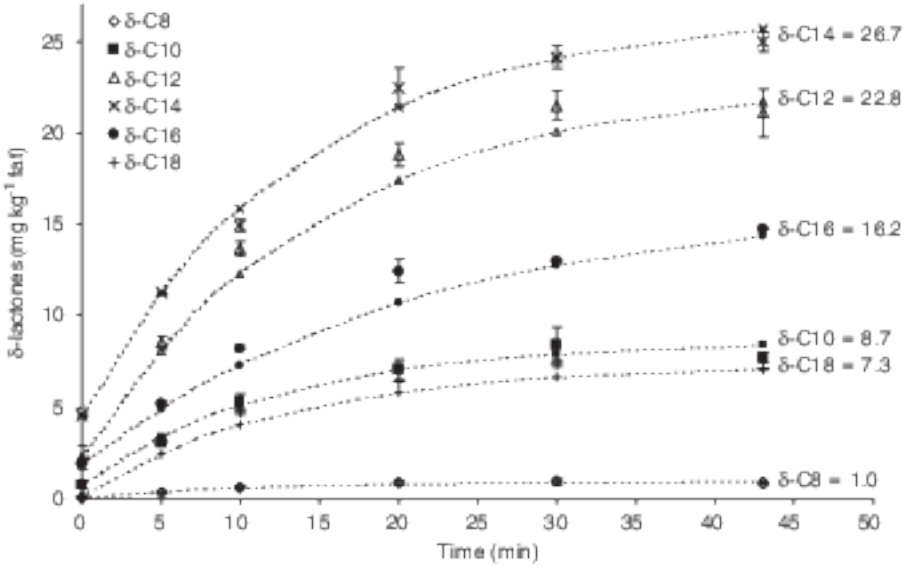

Fig. 1. Formation of δ-lactones (mg kg^{-1}) in milk fat B, heated at 160 °C. Symbols are experimentally obtained values, dotted lines are calculated trends, as explained in the text. The values on the right-hand side are the predicted lactone potentials in mg kg^{-1} fat. Error bars represent standard deviation of duplicates.

where C_0 is the initial concentration, C the concentration at time (t), and k is the rate constant. In the current experiment, $[C_0]$ is the lactone potential (LP), and $[C]$ is the remaining concentration of lactone precursor at a given time. The products of the reaction, free lactones (FL), were measured over time, and with

$$LP = [C] + [FL]$$

the current reaction could be described by

$$\ln\left(\frac{LP - [FL]}{LP}\right) = -k \times t. \quad (3)$$

The formation of all δ-lactones (C8, C10, C12, C14, C16) in heated milk fat can clearly be explained by a first order reaction (Fig. 1), which was also true for all major γ-lactones (C12, C12:1, C14, C16) (data not shown).

To determine the lactone potential, the maximum lactone concentration after a particular heating time has been used (Stark et al., 1978). However, since the concentration of free lactones approached the lactone potential in an asymptotic way, it was difficult to determine when the heating time was sufficient to estimate the lactone potential. Therefore, a mathematical approach was used. All time and free lactone concentration combinations, five to nine independent duplicates for each sample of milk fat, were used to fit LP and k in Eq. (3), minimizing the sum of squared differences between observed and predicted data. The obtained lactone potentials of individual lactones in different milk fats are given in Table 1, showing considerable differences between different milk fats. The exact origin of these milk samples was not always known, and these differences might have been due to seasonal variations and different feeding regimes of the cows (Stark et al., 1978; Urbach, 1982). Despite the differences in concentrations, the ratios of individual lactones varied only slightly in the samples investigated. The different lactone potentials can influence the final aroma of cheeses prepared from these milk sources, or the flavour (defect) when this milk is extensively heated.

3.3. Short chain γ-lactones

Short chain γ-lactones (γ-C6, γ-C8 and γ-C10) clearly showed a different formation pattern than the δ-lactones described above. When the milk fat was heated, their formation rate was slow in the beginning, and increased gradually for 2 h (Fig. 2). Also, in contrast to the other γ- and δ-lactones, the formation of short-chain γ-lactones was stimulated by water amounts up to 20% (v/v) (data not shown). The short-chain γ-lactones also behaved differently from the other lactones in cheese ripening, as described below. In the current experiment, no lactone potentials could be established for the short-chain γ-lactones, and a formation mechanism different from that of the longer-chain γ-lactones and the δ-lactones must therefore be considered. This remains to be investigated.

Table 1
Lactone potentials (in mg kg^{-1}) of milk fats from twelve different full fat milk samples

	A[a]	B	C	D	E	F	G	H	I	J	K	L	Average
γ-C12	7.4 (±0.94)[b]	8.8 (±0.37)	8.0 (±1.56)	7.7 (±0.80)	10.0 (±0.78)	7.8 (±1.11)	8.7 (±1.06)	5.8 (±0.50)	10.5 (±0.51)	9.5 (±1.47)	11.0 (±1.30)	9.9 (±0.87)	8.8
γ-C12:1	2.2 (±0.42)	2.3 (±0.09)	1.6 (±0.32)	2.1 (±0.11)	2.4 (±0.21)	1.9 (±0.23)	1.8 (±0.16)	1.4 (±0.17)	2.3 (±0.18)	2.3 (±0.41)	2.6 (±0.37)	2.4 (±0.23)	2.1
γ-C14	4.4 (±0.23)	4.8 (±0.34)	2.6 (±0.28)	n.q.[c]	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	3.9
γ-C16	8.5 (±2.51)	1.8 (±0.15)	4.5 (±0.70)	n.q.	4.1 (±0.41)	5.6 (±0.69)	6.3 (±0.69)	6.2 (±0.97)	6.9 (±0.67)	7.5 (±0.92)	6.7 (±1.00)	4.9 (±0.68)	5.7
Sum γ	22	18	17	10	16	15	17	13	20	19	20	17	17
δ-C8	1.9 (±0.17)	1.0 (±0.14)	1.1 (±0.19)	0.9 (±0.09)	1.3 (±0.16)	1.6 (±0.14)	1.4 (±0.14)	1.8 (±0.21)	1.8 (±0.14)	1.8 (±0.20)	1.7 (±0.13)	1.6 (±0.18)	1.5
δ-C10	20 (±3.96)	9 (±0.84)	8 (±1.74)	10 (±1.04)	12 (±1.81)	13 (±1.38)	13 (±1.27)	15 (±1.43)	14 (±1.42)	13 (±1.43)	15 (±2.17)	14 (±1.79)	13.1
δ-C12	50 (±9.05)	23 (±1.16)	21 (±4.49)	24 (±2.96)	28 (±2.40)	31 (±3.06)	31 (±2.12)	35 (±3.04)	33 (±2.87)	29 (±2.48)	42 (±7.04)	34 (±4.01)	31.7
δ-C14	66 (±10.3)	27 (±1.38)	26 (±6.61)	30 (±2.92)	40 (±3.87)	48 (±5.57)	47 (±6.69)	57 (±6.43)	51 (±5.79)	45 (±5.73)	58 (±6.53)	53 (±7.88)	45.7
δ-C16	31 (±7.12)	16 (±0.97)	35 (±7.16)	16 (±2.34)	26 (±3.96)	26 (±4.35)	27 (±3.49)	31 (±3.42)	31 (±3.26)	29 (±3.19)	24 (±3.01)	37 (±3.31)	27.5
δ-C18	14.1 (±1.70)	7.3 (±0.37)	5.8 (±0.87)	n.q.	10 (±1.17)	10 (±1.55)	12 (±1.62)	13 (±1.57)	12 (±0.90)	10 (±1.51)	12 (±1.20)	11 (±0.90)	10.7
Sum δ	170	75	92	82	107	120	120	139	131	117	141	140	119
γ/δ ratio (%)	13	23	18	12	15	13	14	10	15	16	14	12	14
Total lactone[d]	193	93	108	92	123	135	136	152	151	137	162	157	137

[a] A through L represent the different milk samples studied.
[b] The values in parentheses represent a 95% confidence interval of the lactone potential.
[c] n.q. = not quantified. Reliable quantification was not possible due to co-elution with a fatty acid.
[d] Excludes γ-C6, C7, C8, and C10 lactones, for which a lactone potential could not be determined, as described in the text.

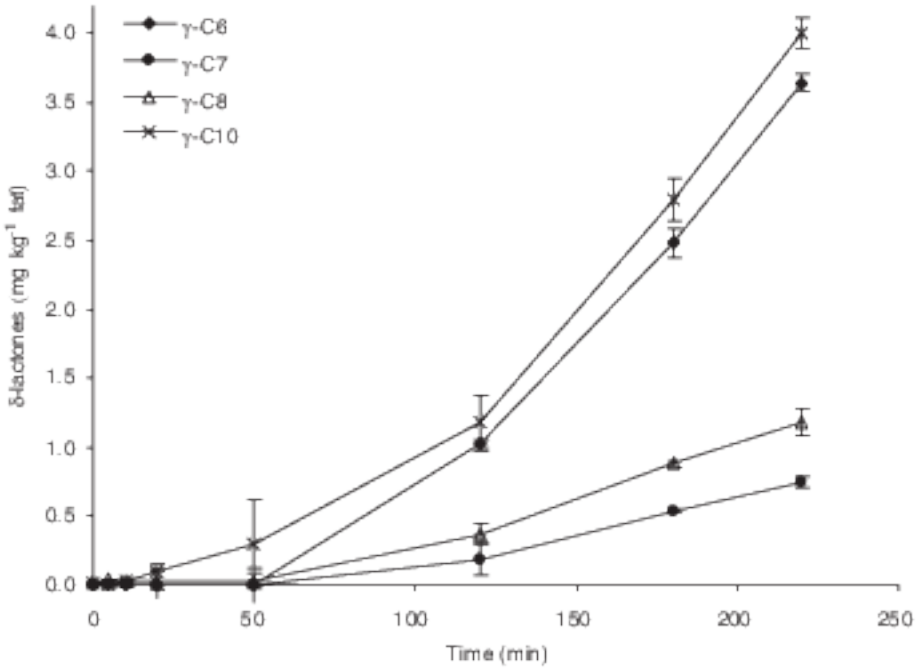

Fig. 2. Formation of short chain δ-lactones (mg kg^{-1}) in milk fat B, during heating at 160 °C. Error bars represent standard deviations of duplicates.

3.4. Lactone formation mechanism

Lactones are thought to originate from hydroxy fatty acids (Jurriens & Oele, 1965; McSweeney & Sousa, 2000; Wyatt et al., 1967). These are present in milk fat, incorporated in the milk fat triglycerides, but are not present in their free form. Free hydroxy acids might be produced by hydrolysis of the hydroxy acid from the triglyceride, as originally suggested by Mattick et al. (1959). However, whereas milk fat consists of about 99% of triglycerides esterified with aliphatic fatty acids (Walstra, Geurts, Noomen, Jellema, & van Boekel, 1999), less than 1% of the available aliphatic fatty acids were hydrolysed from the triglycerides after heating milk fat at 140 °C for 220 min. In contrast, the amount of lactones produced reached almost the maximal potential of the fat at that time. It is therefore unlikely that the formation mechanism of lactones includes a hydrolysis of the hydroxy fatty acid from the triglyceride. Addition of diluted acid (HCl) and diluted NaOH (both up to 300 μmol g^{-1} fat) had no substantial effect on the formation of the major lactones, whereas the hydrolysis of aliphatic free fatty acids was greatly enhanced (results not shown). This also indicated that the formation of lactones does not include a hydrolysis step followed by lactonisation, but is a direct lactonisation instead. Based on these observations, a reaction mechanism for δ- and long-chain γ-lactones is proposed (Fig. 3). When the starting molecule (I in Fig. 3) is in the correct conformation, the reaction starts by a nucleophilic attack of the hydroxy group on the carboxyl C-atom. A negative charge is temporary stored on the neighbouring oxygen atom. The reaction proceeds slowly without water addition prior to heating. Addition of 5% (v/v) water enhances the

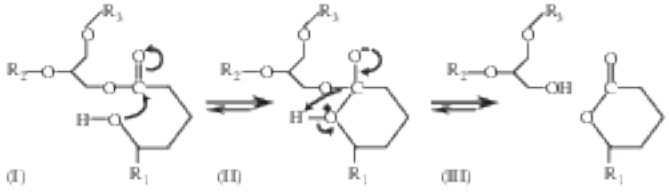

Fig. 3. Proposed formation mechanism of δ-lactones from esterified hydroxy fatty acids in milk fat. Long-chain γ-lactones form 5-membered rings analogously, with the hydroxy group on C4 rather than the depicted C5 for δ-lactones. $R_1 = (CH_2)_6-CH_3$; R_2 and $R_3 = CO-(CH_2)_6-CH_3$.

formation rate substantially, whereas adding more water had no additional effect (data not shown). This indicates that water plays a catalytic role in the reaction, probably by shielding the charge of the intermediate structure (II) from its non-polar environment. In the next step, the reaction terminates by splitting off the lactone, leaving a glycerolate anion, which will readily become a (di)glyceride after addition of the proton from the former hydroxy group. The unimolecular nature of the proposed reaction agrees well with the observation that first-order reaction kinetics can be used to describe the reaction.

3.5. Temperature dependency

Temperature dependency of chemical reaction rates can be described by the Arrhenius equation,

$$k = A \cdot e^{(-E_a/R \cdot T)}. \quad (4)$$

The formation of individual lactones in pure milk fat was determined at 20, 40, 60 and 140 °C, and used to calculate the relevant Arrhenius parameters. It was reported earlier

Table 2
Kinetic parameters[a] for the formation of the major lactones in milk fat

	E_a (kJ mol^{-1})	A ($\times 10^6$ h^{-1})	k at 13 °C ($\times 10^{-3}$ h^{-1})	k at 160 °C ($\times 10^{-3}$ h^{-1})
γ-C12	62.1 (±4.0)	192 (±8.4)	0.87	34.7
γ-C12:1	62.6 (±4.1)	187 (±3.7)	0.69	28.4
γ-C14	63.4 (±8.8)	115 (±1.2)	0.31	13.3
γ-C16	61.2 (±4.5)	104 (±2.6)	0.69	26.0
δ-C8	56.1 (±3.0)	32.6 (±2.5)	1.82	51.0
δ-C10	56.8 (±2.9)	34.4 (±2.8)	1.43	41.7
δ-C12	56.9 (±3.0)	29.4 (±2.6)	1.18	34.7
δ-C14	56.8 (±2.9)	30.5 (±2.6)	1.29	37.6
δ-C16	54.1 (±3.0)	9.6 (±1.0)	1.27	31.5
δ-C18	51.8 (±7.2)	8.4 (±0.9)	2.86	62.0

[a] E_a (energy of activation) and A (the pre-exponential factor) are the terms calculated for the Arrhenius equation (4), with $R = 8.31$ J K^{-1} mol^{-1} and T = absolute temperature (K). The values in parentheses represent the 95% confidence intervals for E_a and A. The reaction rate constant, k, is calculated as an example for 13 and 160 °C.

that the Arrhenius parameters E_a and A are strongly correlated when using a relatively narrow temperature range, so the following reparameterization was used (Brands, 2002):

$$k = -\frac{1}{t}\left(\frac{\mathrm{LP} - [\mathrm{FL}]}{\mathrm{LP}}\right) = X \cdot e^{(-Y \cdot E_a)} \quad (5)$$

with $X = k_0 \cdot e^{(-E_a/R \cdot T_{av})}$,

$$Y = \frac{1}{R}\left(\frac{1}{T} - \frac{1}{T_{av}}\right),$$

$$T_{av} = \frac{\sum T}{n}.$$

In these equations, E_a is the activation energy (kJ mol^{-1}), R the gas constant (8.31 J mol^{-1} K^{-1}), k_0 a dimensionless pre-exponential factor, and the rate constant k was substituted as shown in Eq. (3).

The LP was calculated (Section 3.2) and corrected for the initial lactone concentration. All the data (42 measurements) for milk fat heated at 20, 40, 60 and 140 °C at the different time points were used simultaneously to fit X and Y in Eq. (5), minimizing the sum of squares. This yielded the activation energies and pre-exponential factors for the major lactones in cheese (Table 2).

3.6. Fit with experimental cheese data

To check the model data for the formation of lactones, a typical Gouda cheese was prepared and the formation of lactones was followed during ripening. The ripening of this cheese was previously described in more detail (Alewijn et al., 2005). The potentials of the individual lactones are listed (Table 1), with milk fat 'K' being a sample from the milk used to prepare this cheese. Together with the kinetic data on the formation of lactones (Table 2) and the fat content in dry cheese matter (data not shown), the formation of individual lactones during ripening of this cheese at 13 °C was predicted. The formation of the individual δ-lactones in the actual cheese environment could be accurately predicted for up to 40 weeks of ripening time (Fig. 4).

The formation of the major γ-lactones (C12, C12:1 and C14) could be predicted in a similar way (data not shown). Only δ-octalactone (δ-C8) showed a clear (and unpredicted) decrease after 8 weeks of ripening time, which was observed in other cheeses as well. For this deviant behaviour no clear explanation could be found. Moreover, the short-chain γ-lactones consistently showed a formation pattern in ripening cheese resembling the curves obtained at 140 °C (Fig. 2), although the formation rate was much lower at the ripening temperature. This reaction cannot be described by a first-order reaction as for the formation of the other γ- and δ-lactones. The mechanism of the short-chain γ-lactones is not yet understood and the formation in cheese ripening could therefore not be predicted.

Similar to the cheese described above, the formation of long-chain γ-lactones and δ-lactones could also be predicted for several other cheeses that were followed during ripening (Alewijn et al., 2005). This suggests that the mechanism presented above is the predominant mechanism in most Gouda cheeses, and probably in most other cheese types as well. Since the formation of lactones is a non-enzymatic process, only limited control of lactone formation is possible in ripening cheese with a given lactone potential, as the key influencing factors, temperature and water activity have rather strict practical limits.

4. Conclusions

The presented method describes a relatively fast and easy way to determine the lactone potential of milk fat. With this lactone potential, which varies for different batches of milk, the calculated kinetic parameters can be used to predict the concentration of the major individual lactones for Gouda cheese ripened at 13 °C for up to 40 weeks. This enables an accurate estimation of the formation of individual lactones in all cheeses where no additional microbial activity contributes to the lactone formation. Such a microbial activity was never convincingly observed

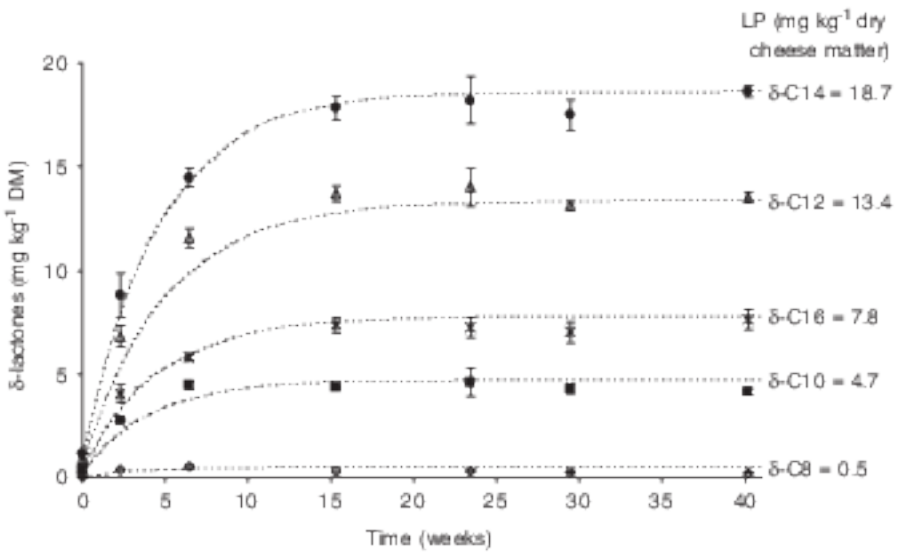

Fig. 4. Concentrations of major δ-lactones (mg kg^{-1} dry cheese matter) during the ripening of a typical Gouda cheese. Symbols represent lactone concentrations (independent duplicates) determined in cheese samples as described in the text, with error bars representing standard deviations. Dotted lines represent calculated lactone concentration trends based on the E_a and A values in Table 2, and the lactone potential (LP) of this particular cheese is expressed in mg kg^{-1} cheese dry matter (DM).

with the Gouda-related strains that were used in this study. However, the mechanism of small chain γ-lactones proved to be different from that of the other lactones, and their formation could not be predicted with the data presented.

The results presented here indicate that the originally proposed two-step formation mechanism (hydrolysis of the hydroxy fatty acid followed by ring-closure) of the δ-lactones and major γ-lactones at 140 °C is not very likely, and that the formation of lactones is a one-step intramolecular *trans*-esterification reaction. It was also shown that the same (chemical) reaction occurs under cheese ripening conditions.

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