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Sensory-Directed Identification of Creaminess-Enhancing Volatiles and Semivolatiles in Full-Fat Cream

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Aimed at defining the chemical nature of creaminess-related flavor compounds in dairy products on a molecular level, a full-fat cream was analyzed for sensorially active volatiles and semivolatiles by means of activity-guided screening techniques. Application of the aroma extract dilution analysis on an aroma distillate prepared from pasteurized cream (30% fat) revealed δ-decalactone, (*Z*)-6-dodeceno-γ-lactone, γ-dodecalactone, δ-dodecalactone, and 3-methylindole with by far the highest flavor dilution (FD) factors among the 34 odor-active volatiles identified. Using a complementary approach involving silica column chromatography and fractionated high-vacuum distillation combined with sensory experiments enabled the additional identification of δ-tetradecalactone, δ-hexadecalactone, γ-tetradecalactone, γ-hexadecalactone, and δ-octadecalactone as semivolatile flavor components in the cream fat. Although a series of lactones is present in dairy cream, spiking of cream samples with individual lactones revealed that only the δ-tetradecalactone is able to enhance the typical retronasal creamy flavor of the product when added in amounts above its theshold level of 66 μmol/kg. Rather than contributing to the retronasal aroma of cream, first evidence was found that, particularly, γ- and δ-octadecalactones and γ- and δ-eicosalactones are able to influence the melting behavior of cream in the oral cavity.

KEYWORDS: Creaminess; cream; aroma; taste; lactones; fat taste

INTRODUCTION

Human flavor perception is based on the integrative interplay between the olfactory sensation caused by volatiles and the gustatory sensation caused by semi- and nonvolatiles. In addition, oral somatosensations induced either by temperature, tactile stimulation or by the activation of chemosensory receptors on the perigemmal fibers contribute to the overall sensation perceived during eating. Thus, in combination, this array of biosensors provides valuable information on the overall flavor quality of the food we eat. However, in particular, the development of healthier food products, for example, reduced in fat, sugar, or salt, respectively, may sometimes induce nonacceptable flavor defects in the products and has, thus, created unexpected flavor challenges for the food industry. In response to the consumers' demand for healthy but tasty foods, novel ingredient discovery is essential to overcome such flavor challenges associated with the production of, in particular, fat-reduced foods. In particular, the creaminess is associated with indicators of kokumi, richness, and high quality of food products, such as cream, yogurt, cheese, or mayonnaise, and is a well-known key driver of consumers' preference for such fat-containing products. However, one crucial step to make healthy, fat-reduced food taste better is to find ways to impart a desirable creaminess sensation to low-fat food products (*1*, *2*).

Creaminess of dairy products is accepted to be a highly integrated perception encompassing both flavor and texture sensations (*3*, *4*), which are believed to be inexorably linked in the experience of individuals, because these are physically linked in authentic dairy products. Thus, for a better scientific understanding, creaminess perception needs to be deconstructed into its underlying sensory components by means of descriptive training of the sensory panel (*5*). The textural component of creaminess perception of dairy products is believed to be due to tactile oral sensations (*3*), described as a slippery and greasy (*6*) or a creamy mouthfeel (*7*). Moreover, it is agreed that the presence of small, even-sized fat globules coupled with adequate viscosity of the dairy product enhances the oral perception of creaminess (*8*). In contradiction to that, margarine is usually

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not rated with the same scores for creaminess as butter, thus indicating that the desirable creaminess sensation not only might be due to the physical–chemical assembly of the fat matrix but might be influenced by flavor components as well.

Recent studies discussed the concept of flavor in terms of a physiological construct produced by the integration of all of our different senses including taste and smell (*9*). Implicit in the concept is the idea that mixtures of odorants and tastants are sensed as unique flavor entities rather than as the sum of their individual components. A common example is the pairing of strawberry odor with sucrose, which enhances the perceived sweetness of the mixture, whereas the same pairing with peanut butter does not show the same effect (*10*). Similar interactions might also be the clue to understanding creaminess perception on the basis of a suitable flavor enhancing the overall creaminess impression of a fat-containing dairy base. Supplementing dairy products with vanilla (*11*) or dairy flavor (*12*), respectively, was reported to result in a higher fat content perceived and in a significantly enhanced creaminess perception. Thus, flavor might play a more crucial role in the perception of creaminess in foods than some previous studies might suggest.

With the ambition to create an international protocol for the sensory rating of dairy products, the term "creamy" was already used as a flavor descriptor to evaluate the contribution of smell and taste for creaminess (*13*). However, although multiple attempts have been made to identify creamy flavor compounds in foods, just a few volatiles including (*Z*)-4-heptenal (*14*) or a mixture of free fatty acids, tetradecanone, and aldehydes as well as lactones (*15*) have been reported so far to induce some kind of creamy sensation when added to low-fat products. However, it is still unclear whether these volatiles or other previously not reported constituents, including semivolatiles and nonvolatiles, are among the key compounds contributing to the creaminess of dairy products.

Because individuals use the term creaminess interchangeably to describe flavor and textural perceptions in dairy products, most often not distinguishing between them, the sensory evaluation of isolates obtained by, for example, fractionation experiments needs to be done in a creamy matrix such as whipped cream against the same matrix as the control. This opens the possibility of evaluating the impact of these isolates independently from the matrix.

Aimed at defining the chemical nature of creaminess-related flavor compounds in dairy products on a molecular level, the objectives of the present investigation were, therefore, to screen full-fat cream for sensorially active volatile and semi- and nonvolatile compounds by means of sensory-guided screening techniques such as the aroma extract dilution analysis (*16*) or the taste dilution analysis (*17*), to identify the key flavor components, and to evaluate their impact on creaminess perception of cream by sensory experiments.

MATERIALS AND METHODS

Cream Samples. Prepared under controlled conditions from the same batch of cream (30% fat content), fresh nonheated and high-heat-treated cream samples, respectively, were obtained from the dairy industry. Heat treatment was carried out at 95 °C for 6 s by means of a plate heat exchanger, and whipping was done using a kitchen-type mixer.

Chemicals. The following compounds were obtained commercially: 2-acetyl-2-thiazoline, *o*-aminoacetophenone, butanoic acid, (*E*,*E*)-2,4-decadienal, γ-decalactone, δ-decalactone, decanoic acid, dimethyl disulfide, dimethyl sulfide, γ-dodecalactone, δ-dodecalactone, dodecanoic acid, (*Z*)-6-dodeceno-γ-lactone, (*E*)-2-dodecenal, 3-ethylphenol, hexanal, hexanoic acid, (*Z*)-3-hexenal, methanethiol, 2- and 3-methylbutanoic acid, 3-methylindole, 4-methylphenol, 3-(methylthio)propanal, (*E*,*Z*)-2,6-nonadienal, γ-nonalactone, (*E*)-2-nonenal, γ-octalactone, δ-octalactone, octanoic acid, phenylacetic acid, phenylpropanoic acid, δ-tetradecalactone, γ-undecalactone, and 4-hydroxy-3-methoxybenzaldehyde (Sigma-Aldrich, Steinheim, Germany); 1-hexen-3-one and 1-octen-3-one (Lancaster, Mühlheim, Germany); 2-phenylethanol (Fluka, Neu-Ulm, Germany). Sorbitan tristearate (Span 65) was purchased from Sigma-Aldrich and soy lecithin (Emultop) from Degussa (Hamburg, Germany). Solvents were of HPLC grade (Merck), and dichloromethane and diethyl ether were distilled prior to use. Bottled water (Evian) was used for sensory experiments. Acetyl-1-pyrroline (*18*), *trans*-4,5-epoxy-(*E*)-2-decenal (*19*), and (*Z*)-4-nonenal (*20*) were synthesized according to procedures reported in the literature.

Syntheses. *Pentadecanal and Heptadecanal.* According to a procedure reported in the literature (*21*), a solution of 1-pentadecanol or 1-heptadecanol (6 mmol) in dichloromethane (20 mL) was added dropwise within 10 min to a solution of Dess-Martin periodinane (6.6 mmol) in dichloromethane (20 mL) at room temperature with stirring under an atmosphere of argon. After 5 h, diethyl ether (100 mL), followed by a $NaHCO_3$-saturated aqueous solution of sodium thiosulfate (100 mL; 1 mol/L) was added to the cloudy mixture, and the mixture was vigorously stirred for 10 min until the organic phase became clear. The organic layer was sequentially extracted with an aqueous solution of sodium thiosulfate (50 mL; 1 mol/L), a saturated solution of sodium hydrogen carbonate (100 mL), and, finally, distilled water (100 mL). After drying of the organic phase over Na_2SO_4, the solvent was carefully removed by means of a rotary evaporator to afford the title respective compound as a white solid (5.1 mmol; yield: 85%).

Pentadecanal: HRGC-MS-EI, *m*/*z* (%) 57 (100), 82 (95), 43 (80), 96 (65), 68 (58), 109 (25), 124 (18), 182 (12), (11), 138 (8), 152 (5), 226 (1; $[M]^+$); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, $J = 6.57$ Hz, H-C(15)), 1.23–1.36 (m, 11 × 2H, H-C(4–15)), 1.60 (m, 2H, H-C(3)), 2.38 (m, 2H, H-C(2)), 9.76 (t, 1H, $J = 2.02$ Hz, H-C(1)).

Heptadecanal: HRGC-MS-EI, *m*/*z* (%) 57 (100), 82 (90), 43 (80), 96 (65), 68 (58), 109 (25), 124 (18), 210 (12), 138 (8), 152 (5), 226 (1; $[M]^+$); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, $J = 6.57$ Hz, H-C(15)), 1.23–1.36 (m, 11 × 2H, H-C(4–14)), 1.60 (m, 2H, H-C(3)), 2.38 (m, 2H, H-C(2)), 9.76 (t, 1H, $J = 2.02$ Hz, H-C(1)).

γ-Tetradecalactone, γ-Hexadecalactone, γ-Octadecalactone, and γ-Eicosalactone. Following a procedure reported in the literature (*22*) with some modifications, a solution of either undecanal, tridecanal, pentadecanal, or heptadecanal (5 mmol), respectively, and ethyl acrylate (5 mmol) in *tert*-butanol (5 mmol) and tetrahydrofuran (15 mL) was added at 0 °C to a solution (100 mL) of samarium(II) iodide (0.1 mol/L, 10 mmol) in tetrahydrofuran with stirring under an atmosphere of argon. After 5 h, aqueous hydrochloric acid (50 mL, 2 mol/L) was added to the yellowish solution, the mixture was extracted with diethyl ether (3 × 50 mL), and, finally, the combined organic layers were extracted with an aqueous solution of sodium thiosulfate (50 mL, 20% in water), followed by tap water (3 × 50 mL). After drying over Na_2SO_4, the solvent was carefully removed under vacuum, the oily residue was taken up in *n*-pentane (10 mL), and the respective compound was purified by chromatography using a water-cooled glass column (30 × 2 cm i.d.) filled with silica gel (30 g, silica gel 60, Merck, Darmstadt, Germany) conditioned with 5% water. After application of an aliquot (5 mL) of the crude material onto the top of the column conditioned with *n*-pentane, chromatography was performed using *n*-pentane/diethyl ether (95:5, v/v; 100 mL; fraction I), followed by *n*-pentane/diethyl ether (90:10, v/v; 100 mL; fraction II), *n*-pentane/diethyl ether (80:20, v/v; 100 mL; fraction III), *n*- pentane/diethyl ether (60:40, v/v; 100 mL; fraction IV), *n*-pentane/diethyl ether (20:80, v/v; 100 mL; fraction V), and diethyl ether (100 mL; fraction VI). Fraction IV containing γ-tetradecalactone (or fraction V containing γ-hexadecalactone, γ-octadecalactone, and γ-eicosalactone) was collected, freed from solvent under vacuum, taken up in *tert*-butyl methyl ether (1 mL), and, finally, purified using a water-cooled glass column (30 × 2 cm i.d.) filled with alumina oxide (neutral, 30 g; Woelm Pharma) using acetonitrile/*tert*-butyl methyl ether (95:5, v/v; 100 mL) as the solvent. After removal of the solvent under vacuum, γ-tetradecalactone (2.45 mmol; yield = 49%) and γ-hexadecalactone (2.2 mmol; yield = 44%) were obtained as colorless oils and γ-octadecalactone (1.95 mmol; yield = 39%) and γ-eicosalactone (1.75 mmol; yield = 35%) as white solids.

The identities as well as the purities of the lactones were confirmed by HRGC-MS-EI as well as by ^{1}H NMR spectroscopy.

γ-Tetradecalactone: HRGC-MS-EI, *m/z* (%) 85 (100), 41 (25), 55 (25), 69 (19), 97 (12), 128 (10), 111 (9), 208 (3), 226 (1, $[M]^+$); HRGC-MS-CI, *m/z* 227 (100, $[M + 1]^+$), 41 (80), 209 (25), 255 (10), 191 (9), 267 (8); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, J = 6.57 Hz, H-C(14)), 1.22–1.45 (m, 8 × 2H, H-C(6–13)), 1.58 (m, 2H, H-C(5)), 1.81 (m, 2H, H-C(3)), 2.51 (m, 2H, H-C(2)), 4.47 (m, 1H, H-C(4)).

γ-Hexadecalactone: HRGC-MS-EI, *m/z* (%) 85 (100), 41 (25), 55 (25), 69 (19), 97 (12), 128 (10), 111 (9), 208 (3), 226 (1, $[M]^+$); HRGC-MS-CI, *m/z* 255 (100, $[M + 1]^+$), 41 (80), 237 (25), 283 (10), 219 (9), 295 (8); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, J = 6.57 Hz, H-C(16)), 1.19–1.41 (m, 10 × 2H, H-C(6–15)), 1.57 (m, 2H, H-C(5)), 1.83 (m, 2H, H-C(3)), 2.50 (m, 2H, H-C(2)), 4.48- (m, 1H, H-C(4)).

γ-Octadecalactone: HRGC-MS-EI, *m/z* (%) 85 (100), 41 (25), 55 (25), 69 (19), 97 (12), 128 (10), 111 (9), 208 (3), 226 (1, $[M]^+$); HRGC-MS-CI, *m/z* 283 (100, $[M + 1]^+$), 41 (80), 265 (25), 311 (10), 247 (9), 323 (8); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, J = 6.56 Hz, H-C(18)), 1.17–1.48 (m, 12 × 2H, H-C(6–17)), 1.59 (m, 2H, H-C(5)), 1.82 (m, 2H, H-C(3)), 2.52 (m, 2H, H-C(2)), 4.48 (m, 1H, H-C(4)).

γ-Eicosalactone: HRGC-MS-EI, *m/z* (%) 85 (100), 55 (45), 69 (41), 97 (40), 111 (25), 292 (23), 310 (2, $[M]^+$); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, J = 6.85 Hz, H-C(20)), 1.22–1.49 (m, 14 × 2H, H-C(6–19)), 1.58 (m, 2H, H-C(5)), 1.85 (m, 2H, H-C(3)), 2.53 (m, 2H, H-C(2)), 4.45 (m, 1H, H-C(4)).

δ-Hexadecalactone, δ-Octadecalactone, and δ-Eicosalactone. According to a literature procedure with some modifications (*23*), either 1-bromodecane, 1-bromododecane, or 1-bromotetradecane (20 mmol each), respectively, was added to a solution of KOH (1.12 g) and 1,2-cyclohexanedione (20 mmol) in water/dioxane (6:5, by volume; 11 mL). After 20 h of refluxing and cooling to room temperature, an aqueous KOH solution (3% in water; 50 mL) was added, and the mixture was extracted with diethyl ether (3 × 50 mL). The aqueous layer was acidified with hydrochloric acid (10% in water) and extracted with diethyl ether (3 × 100 mL), and the ethereal solution was freed from solvent in vacuum. The residue was taken up in an aqueous solution of Na_2CO_3 (30% in water; 50 mL), heated for 30 h under reflux, and, after cooling to room temperature, acidified again with hydrochloric acid (10% in water) and extracted with diethyl ether (3 × 75 mL). The organic layer was freed from solvent under vacuum, the residue was dissolved in aqueous KOH (5% in water), and then sodium borohydride (500 mg) was added. After the mixture had been maintained for 8 h at 40 °C, the solution was acidified with hydrochloric acid (35% in water), and the crude product was extracted with diethyl ether (3 × 75 mL). After removal of the solvent under vacuum, the title compound was purified using a water-cooled 30 × 2 cm i.d. glass column filled with silica gel (30 g, silica gel 60, Merck, Darmstadt, Germany) conditioned to 5% water. After application of an aliquot (5 mL) of the crude material onto the top of the column conditioned with *n*-pentane, chromatography was performed using *n*-pentane/diethyl ether (95:5, v/v; 100 mL; fraction I), followed by *n*-pentane/diethyl ether (90:10, v/v; 100 mL; fraction II), *n*-pentane/diethyl ether (80:20, v/v; 100 mL; fraction III), *n*-pentane/diethyl ether (60:40, v/v; 100 mL; fraction IV), *n*-pentane/diethyl ether (20:80, v/v; 100 mL; fraction V), and diethyl ether (100 mL; fraction VI). Collection of fraction V and removal of the solvent under vacuum yielded either *δ*-hexadecalactone (4.0 mmol; yield = 20%), *δ*-octadecalactone (3.4 mmol; yield = 17%), or *δ*-eicosalactone (3.2 mmol; yield = 12%), respectively, as colorless solids. The identity as well as the purity of the lactones was confirmed by HRGC-MS-EI as well as by ^{1}H NMR spectroscopy.

δ-Hexadecalactone: HRGC-MS-EI, *m/z* (%) 99 (100), 55 (51), 43 (38), 83 (24), 114 (21), 134 (9), 236 (8), 254 (1, $[M]^+$); HRGC-MS-CI, *m/z* 255 (100, $[M + 1]^+$), 41 (80), 237 (25), 283 (10), 219 (9), 295 (8); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, J = 6.22 Hz, H-C(16)), 1.15–1.34 (m, 9 × 2H, H-C(7–15)), 1.55 (m, 2H, H-C(6)), 1.69–1.99 (m, 2 × 2H, H-C(3), H-C(4)), 2.15–2.65 (m, 2H, H-(C2)), 4.20 (m, 1H, H-C(1)).

δ-Octadecalactone: HRGC-MS-EI, *m/z* (%) 99 (100), 55 (51), 43 (38), 83 (24), 114 (21), 136 (11), 264 (8), 282 (1, $[M]^+$); HRGC-MS-CI, *m/z* 283 (100, $[M + 1]^+$), 41 (80), 265 (25), 311 (10), 247 (9), 323 (8); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, J = 6.82 Hz, H-C(18)), 1.21–1.75 (m, 12 × 2H, H-C(6–17)), 1.80–1.98 [m, 2 × 2H, H-C(3), H-C(4)), 2.51 [m, 2H, H-C(2)), 4.28 [m, 1H, H-C(1)).

δ-Eicosalactone: HRGC-MS-EI, *m/z* (%) 99 (100), 55 (53), 69 (41), 97 (40), 114 (25), 292 (19), 310 (2, $[M]^+$); ^{1}H NMR (400 MHz, $CDCl_3$), δ 0.88 (t, 3H, J = 6.84 Hz, H-C(20)), 1.19–1.45 (m, 14 × 2H, H-C(6–19)), 1.59 (m, 2H, H-C(5)), 1.89 (m, 2H, H-C(3)), 2.55 (m, 2H, H-C(2)), 4.35 (m, 1H, H-C(4)).

Isolation and Fractionation of Aroma-Active Volatiles from Cream. An aliquot (200 g) of the cream was extracted with dichloromethane (100 mL) for 1 h with stirring. The volatiles were separated from the nonvolatiles by high-vacuum transfer using the solvent-assisted flavor evaporation (SAFE) method (*24*). The distillate obtained was extracted with aqueous sodium bicarbonate (0.5 mol/L, 2 × 50 mL), followed by tap water (2 × 30 mL). The organic layer was dried over sodium sulfate to yield the neutral/basic fraction of the volatiles (NBF). The combined aqueous layers were acidified to pH 2 with aqueous hydrochloric acid (2 mol/L) and extracted with dichloromethane (4 × 50 mL), and the combined organic extracts were dried over sodium sulfate to yield the acidic fraction (AF). Both fractions, AF and NBF, were concentrated to about 1 mL using a Vigreux column (60 × 1 cm i.d.).

Isolation of the Fat. Cream (100 g) was mixed with distilled water (100 mL) and extracted with diethyl ether (5 × 250 mL). The combined organic layers were dried over sodium sulfate, concentrated to about 100 mL, and freed from the solvent using the SAFE method (*24*), thus affording the cream fat, which was stored at −20 °C until use.

Isolation of Semivolatiles from Fat. An aliquot (40 g) of the fat was vigorously stirred in a round flask (250 mL) connected to a high-vacuum sublimation device (10^{-6} bar) for 30 min at 50 °C to remove the volatiles without cold trapping. Then, the temperature was increased to 150 °C, and the cream fat was thermally treated for 30 min while the semivolatile compounds released were cryofocused by means of a trap cooled with ice–water. After the trapped compounds had been dissolved in diethyl ether (10 mL), the solvent was removed under high vacuum, thus affording a colorless oil (yield = 0.25%).

Silica Gel Fractionation of the Fat. An aliquot (10 g) of the cream fat dissolved in *n*-pentane (15 mL) was placed onto the top of a water-cooled 48 × 3.6 cm i.d. glass column filled with a slurry of silica gel (150 g, silica gel 60, Merck) in *n*-pentane. Chromatography was performed using *n*-pentane/diethyl ether (95:5, v/v; 750 mL; fraction A), followed by *n*-pentane/diethyl ether (90:10, v/v; 750 mL; fraction B), *n*-pentane/diethyl ether (80:20, v/v; 750 mL; fraction C), *n*-pentane/diethyl ether (60:40, v/v; 750 mL; fraction D), and diethyl ether (750 mL; fraction E). The effluents were collected, dried over Na_2SO_4, filtered, and then freed from solvent under high vacuum to yield the following residues: A, 39.6%; B, 49.9%; C, 4.9%; D, 3.4%; and E, 0.9%. All fractions were stored at −20 °C until use.

Extraction of Fraction D. Following a literature procedure (*25*) with some modifications, an aliquot (1 g) of fraction D was placed into a centrifuge tube with a screw cap (15 mL, Greiner-tube, Roth), then acetonitrile (2 mL) was added, and the mixture was shaken for 10 min at 45 °C. After cooling to 4 °C, the tube was centrifuged at 800 rpm for 4 min at 4 °C and then kept at 4 °C for 15 min. The supernatant was transferred into a tube (2 mL; Save-Lock Tube, Eppendorf), cooled to −20 °C, and then maintained at −20 °C for 30 min. After an additional centrifugation (2500 rpm) for 1 min, the supernatant was freed from solvent under high vacuum and used for sensory experiments as well as for HRGC-MS analysis.

High-Resolution Gas Chromatography–Olfactometry (HRGC-O). For the analysis of odor-active volatiles, HRGC was performed by means of a gas chromatograph type 5300 Mega Series (Carlo Erba Instruments, Hofheim, Germany) using the following thin film capillaries: capillary FFAP (30 m × 0.32 mm fused silica capillary) (J&W Scientific, Folsom, CA), capillary SE-54 (50 m × 0.32 mm fused silica capillary) (Macherey & Nagel, Düren, Germany), and capillary OV-1701 (30 m × 0.25 mm fused silica capillary) (J&W Scientific). The samples were applied with the cool-on-column technique at 40 °C. After 2 min, the temperature was raised at 8 °C/min to 240 °C and then held for 5 min. At the end of the capillary the effluent was split 1:1 into the flame ionization detector (FID) and a sniffing port using deactivated fused silica capillaries and a Y-type effluent splitter. The flow rate of

the carrier gas helium was between 2.0 and 2.2 mL/min. The retention indices (RI) were calculated as described previously (*25*).

For the analysis of the distillate containing the semivolatiles, a HP 6890-type gas chromatograph (Hewlett-Packard) equipped with a split/splitless injector (split 1:10; 310 °C) and an FID was coupled with a capillary column (J&W DB-5MS, 30 m, 0.25 μm, 250 μm). Hydrogen was used as the carrier gas with a flow of 1.5 mL/min. After sample injection, the temperature was kept isothermally at 80 °C for 2 min, then the temperature was increased to 300 °C at a rate of 10 °C/min, and, finally, was held at 300 °C isothermally for 10 min.

Static Headspace Olfactometry (SHO). SHO was performed with a CP-9001 gas chromatograph interfaced to the purge and trap system TCT/PTI 4001 (Chrompack, Frankfurt, Germany). An aliquot of the cream sample (50 g) was equilibrated in a septum-sealed glass vessel (200 mL) at 30 °C for 30 min. Ten milliliters of the headspace gas was withdrawn by means of a gastight syringe and injected onto a cold trap (−110 °C, 400 × 0.53 mm fused silica capillary coated with CP-SIL 8, 5 μm). After 10 min, the trapped volatiles were desorbed onto an RTX-5 column (30 m × 0.53 mm, fused silica capillary, Supelco, Sulzbach, Germany) held at 0 °C. After 10 min, the temperature was raised at 8 °C/min to 230 °C and held for 3 min.

High-Resolution Gas Chromatography–Mass Spectrometry (HRGC-MS). For the identification of odor-active volatiles, mass spectra were acquired using a gas chromatograph 5890 Sseries II (Hewlett Packard, Waldbronn, Germany) coupled to an MAT 95S type sector field mass spectrometer (Finnigan, Bremen, Germany) operating either in the electron impact mode (MS-EI) at 70 eV or in the chemical ionization mode (MS-CI) at 115 eV with isobutane as reagent gas.

For the analysis of the semivolatiles, a HP 6890-type gas chromatograph (Hewlett-Packard) equipped with a 30 m × 0.25 mm i.d., 0.25 μm, capillary column (J&W DB-5MS) was coupled to a HP 5973-type quadrupole mass spectrometer (Hewlett-Packard) operating in the electron impact mode at 70 eV. Using helium (1.5 mL/min) as the carrier gas, the GC was operated with an injector temperature of 310 °C (split 1:10). For HRGC-MS in the chemical ionization mode, chromatography was performed on a capillary column (Agilent HP-5MS, 30 m, 0.25 μm, 0.25 mm), and methane was used as the reactant gas. After injection, the temperature was kept constant for 6 min at 80 °C and then raised to 300 °C with an increase of 8 °C/min, and the final temperature was maintained for 15 min.

High-Performance Liquid Chromatography (HPLC). The HPLC system (Merck Hitachi, Eching, Germany) consisted of an L-6200 pump, an AS-2000 autosampler, a Jetstream 2-type column stove, a DG-1310 degasser (VDV optilab, Degasys), and a Sedex 55 type light scattering detector (ERC). Analytical HPLC was performed on a 250 × 4.6 mm i.d., 5 μm, Microsorb-MV 100 C18 column (Varian) maintained at 30 °C and a flow rate of 0.8 mL/min. Using acetonitrile/*tert*-butyl methyl ether (85:15, v/v) as solvent A and acetonitrile/*tert*-butyl methyl ether (65:35, v/v) as solvent mixture B, chromatography was performed starting with solvent mixture A (100%), then increasing mixture B to 80% within 150 min and, finally, to 100% within 10 min, and then keeping 100% mixture B for another 10 min.

Aroma Extract Dilution Analysis (AEDA). Flavor dilution (FD) factors of the odorants in the SAFE destillate as well as the fractions NBF and AF, respectively, were determined by AEDA (*16*) using the FFAP capillary. Extracts were diluted stepwise with solvent in a 1:1 ratio, and each dilution was analyzed by HRGC-O. The AEDA was performed by three experienced assessors. For a minimum of 1 year, these subjects underwent regular HRGC-O training sessions including sniffing of about 50 reference compounds in amounts adjusted 5-fold above their thresholds in air.

Sensory Analyses. *Panel Training.* Fourteen trained assessors were recruited from the German Research Center for Food Chemistry to recognize and quantify the odor (given in parentheses) of solutions of the following reference odorants dissolved in bottled water: 2,3-butanedione (buttery), (*E*,*E*)-2,4-decadienal (fatty), acetic acid (sour), 2-acetyl-2-thiazoline (roasty/popcorn-like), *trans*-4,5-epoxy-(*E*)-2-decenal (metallic), ethyl butanoate (fruity), butanoic acid (sweaty), (3-methylthio)propanal (cooked potato-like), dimethyl trisulfide (sulfury), δ-octalactone (coconut-like), and hexanoic acid (rancid). For training of taste and mouthfeel attributes, 12 assessors from the Institute of Food Chemistry, Muenster, were trained to recognize and quantify the taste of aqueous solutions (5 mL each) of the following standard compounds dissolved in bottled water (Evian; low mineralization = 500 mg/L) by means of a triangle test: sucrose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, NaCl (12 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, monosodium glutamate (8 mmol/L, pH 5.7) for umami taste, an aqueous emulsion of oleic acid (1 mmol/L, containing 0.02% Emultop) for mouth-coating, a water/sunflower oil emulsion (4/6, w/w; containing 0.5% Span 65) for fatty mouthfeel, and an aqueous emulsion of stearic acid (1 mmol/L; containing 0.02% Emultop) for a grainy, powdery mouthfeel. Sensory analyses were performed in a sensory panel room at 19–22 °C in three different sessions.

Three-Alternative Forced-Choice Test. Tests were carried out according to section 35 LMBG, method 00.90-7. This method was used to study the similarity of the aroma of two samples. Three glasses, two of them containing sample A and the third one containing sample B, were labeled with a three-digit code and presented to the panelists. Using a forced-choice methodology, the assessor had to identify the differing sample. Each sensory experiment was repeated three times, and the data are given as means of replicates.

Aroma Profile Analysis. For orthonasal aroma profile analysis, cream samples (20 g) were filled in closed sensory vials (total volume = 45 mL), coded, and randomly presented to the trained sensory panel. The assessors evaluated the samples by scoring the odor intensity for different aroma attributes on a half-point scale from 0 (not detectable) to 3 (intensely detectable).

Sensory Profiling of Cream Fat Fractions in W/O Emulsion. The fractions (6 g each) isolated from the cream fat were emulsified in table water (Evian, 3 mL) containing 0.5% Span 65 (0.045 g); upon ultrasonification at 40 °C, the samples were coded and then randomly presented to the trained sensory panel, which was asked to score the intensity of the sensory descriptors mouth-coating, fatty mouthfeel, and grainy mouthfeel on a scale from 0 (none at all) to 5 (extremely strong). To achieve this, the samples (2 g) were briefly swirled in the mouth and then expectorated.

Sensory Evaluation of Cream Fat Fractions in Whipped Cream. Aliquots (70 mg) of the fractions isolated from cream fat were intimately mixed with cream (70 g) and, then, whipped with a kitchen mixer. The samples were coded and randomly presented to the trained sensory panel, which was asked to score the difference of the intensity of three sensory descriptors, namely, mouth-coating, fatty mouthfeel, and grainy mouthfeel, as well as the intensity of the overall creaminess perception between the test sample and a whipped cream as the control on a scale from 0 (none at all) to 5 (extremely strong). The samples (2 g) were briefly swirled in the mouth and then expectorated.

Taste Recognition Thresholds of Lactones in O/W Emulsions. The taste thresholds were determined by means of a three-alternative forced-choice test using aqueous solutions (5 mL each) of the lactones in bottled water (Evian; low mineralization = 309 mg/L) containing 0.02% Emultop. The samples (5 mL each) were coded and randomly presented in serial 1:1 dilutions in order of ascending concentrations. At the start of each session, and before each trial, the subject rinsed the mouth with tap water and expectorated. The samples, blanks as well as taste solutions, were briefly swirled in the mouth and expectorated. The panel was asked to evaluate threshold concentrations for the four sensory qualities bitter, mouth-coating, fatty mouthfeel, and grainy mouthfeel using a nose clip. Retronasal odor threshold concentrations were determined without the use of nose clips. After indicating which glass vial contained the flavorant, the panelist received another set of two blanks and one taste sample. To prevent fatigue, tasting began at a concentration level two steps below the individual threshold concentration, which had been determined in a preliminary sensory experiment. The threshold values evaluated in three different sessions by eight panelists were averaged. The values between individuals and separate sessions differed by not more than one dilution step; that is, a threshold value of 340 μmol/L for γ-decalactone represents a range of 170–680 μmol/L.

Taste Recognition Thresholds of Lactones in Whipped Cream. Aliquots (10–20 mg) of the purified lactones were intimately mixed with cream (140 g). Aliquots (70 g) of the spiked cream sample were

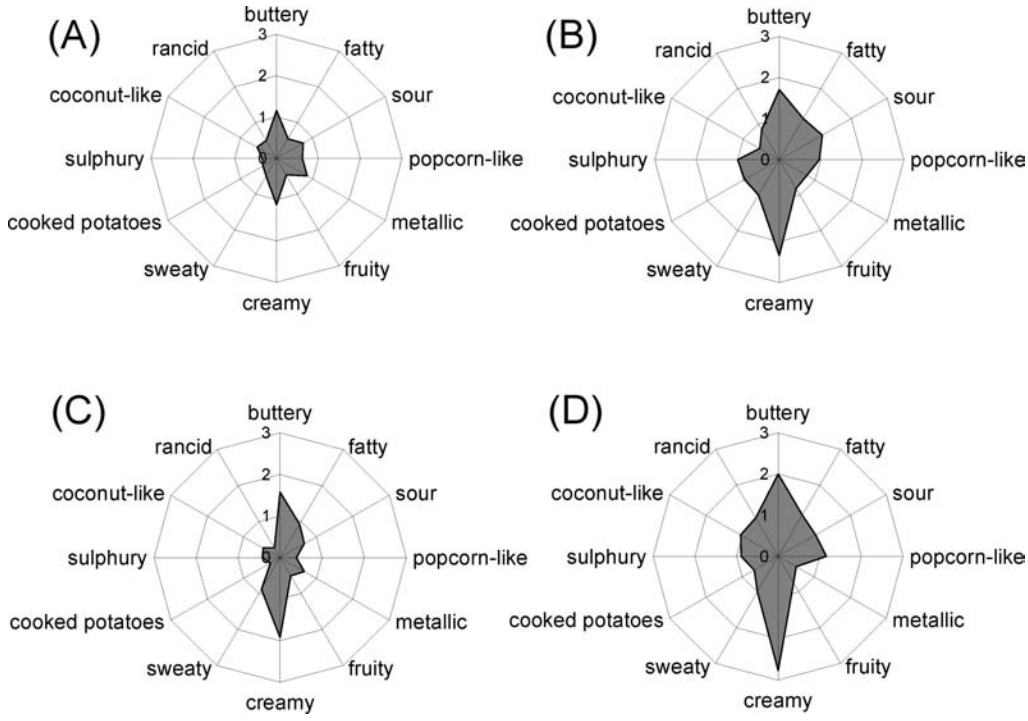

Figure 1. Aroma profile analysis of (**A**) nonheated cream (NHC), (**B**) high-heat-treated cream (HHC), (**C**) whipped nonheated cream (WNHC), and (**D**) whipped high-heat-treated cream (WHHC).

diluted 1:1 with cream to obtain different concentrations levels. The spiked samples as well as the blank cream (control) were whipped following exactly the same procedure and were then presented to the sensory panel in order of ascending concentrations. The samples were briefly swirled in the mouth and then expectorated. The panel was asked to evaluate the threshold concentrations for a change in mouthfeel and retronasal perception, respectively, compared to two controls following a three-alternative forced-choice protocol as given above. The values between individuals and separate sessions differed by not more than one dilution step, that is, a threshold value of 66 μmol/L for the creaminess-inducing sensation of δ-tetradecalactone represents a range of 33–132 μmol/L.

RESULTS AND DISCUSSION

A full-fat cream sample was selected to sensorially characterize the molecular inducers of creaminess in dairy products. To study the influence of a mechanical as well as a thermal treatment on the sensory profile of cream, a nonheated and a high-heat-treated cream sample was presented to a trained sensory panel before and after whipping, respectively. In a preliminary experiment, a series of three-alternative forced-choice tests was carried out to evaluate the similarity of the orthonasal aroma of four cream samples, that is, a nonheated cream (NHC), a high-heat-treated cream (HHC), a whipped nonheated cream (WNHC), and a whipped high-heat-treated cream (WHHC).

The assessors were able to distinguish between samples NHC and HHC as well as between samples WNHC and WHHC, thus giving first evidence that both the thermal treatment and the whipping procedure induced changes in the orthonasal aroma of cream (data not shown).

To further investigate the individual aroma profiles of the four cream samples, aroma profile analyses were performed. The nonheated cream NHC exhibited a rather weak overall aroma centering around a buttery and creamy note as well as a slight metallic modality (**Figure 1**). A comparison of the aroma profile with the whipped cream WNHC implied that already whipping of the cream led to a more intense creamy aroma with increased intensities of the aroma notes creamy, buttery, fatty, and sweaty. However, the overall aroma intensity of the cream was strongly enhanced when the sample was thermally treated (**Figure 1**). The strongest effects were observed for the intensitiy of the descriptors creamy, buttery, popcorn-like, and sulfury in the thermally treated cream HHC; for example, the intensity of the creamy note increased from 1.1 to 2.3 by heat treatment. Finally, whipping of the thermally treated cream was found to induce an additional increase of the creamy note, because a maximum intensity of 2.8 for creaminess was detected (**Figure 1**). In addition, the thermally treated, whipped sample was also described as more coconut-like and buttery than the heat-treated cream prior to whipping, thus confirming that also the mechanical treatment affects the sensory perception of cream. On the basis of these data, the conclusion was drawn that, in particular, the thermal treatment is a key step in enhancing the perceived creamy flavor. Because the sensory evaluation revealed whipped heat-treated cream as the most creamy product, this sample was selected for the analysis of the odor-active volatiles.

Identification of Aroma-Active Volatiles. With the aim of identifying the odorants contributing to the creamy aroma of WHHC, an aroma extract was prepared by solvent extraction and SAFE distillation and, after concentration by boiling off the solvent under vacuum, was analyzed by means of HRGC-O. Thirty-four odor-active regions were detected, but it is interesting to note that none of the odorants exhibited a creamy odor quality. To rate the volatiles in their relative aroma impact, an AEDA was performed by HRGC-O of serial dilutions of the aroma extract (**Figure 2**). Among the 34 odorants detected with FD factors ranging from 4 to 1024, two peach-like smelling odorants (nos. **27** and **28**) were evaluated with by far the highest FD factor of 1024 (**Figure 2**).

For the identification of the aroma-active volatiles detected at the sniffing port, the aroma volatiles were fractionated into a neutral/basic fraction (NBF) and an acidic fraction (AF), and the odorants were located in both fractions by means of HRGC-O. The unequivoval identification of the odorants was then

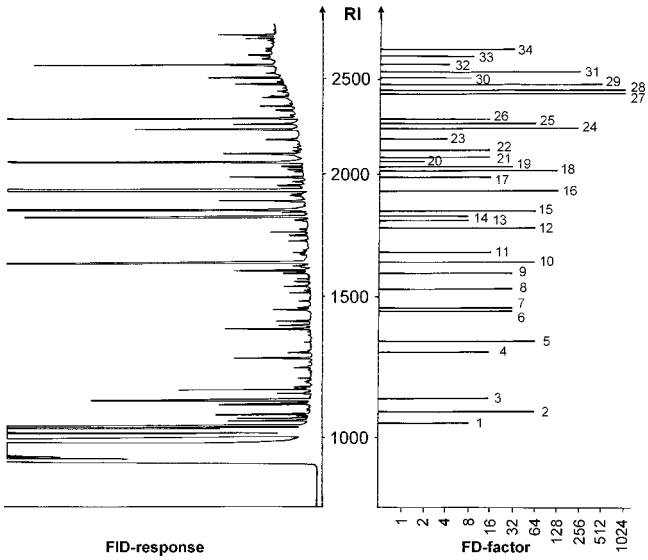

Figure 2. Gas chromatogram and flavor dilution (FD) chromatogram of an aroma extract isolated from whipped high-heat-treated cream (WHHC).

achieved by comparison of the aroma qualities perceived at the sniffing port and the retention indices at three capillary columns, as well as the mass spectra (MS-EI; MS-CI) with those measured for the corresponding reference compounds. On the basis of this approach, the aroma compounds judged with the highest FD factors were identified as the peach-like smelling compounds γ-dodecalactone (no. **27**), (*Z*)-6-dodeceno-γ-lactone (no. **28**), and δ-dodecalactone (no. **29**), the coconut-like smelling δ-decalactone (no. **24**), and 3-methylindole (no. **31**), exhibiting a fecal odor quality (**Table 1**). The identification of these compounds confirmed earlier reports on lactones as key odorants in milk products, such as fresh and heated butter (*26–29*) as well as butter oil (*30*).

To understand the molecular basis for the sensory differences in the aroma of cream induced by whipping and thermal treatment, a comparative AEDA was performed on all four cream samples. As shown in **Table 2**, a comparison of the AEDA results obtained for nonheated and thermally treated cream clearly demonstrates that the popcorn-like smelling 2-acetyl-2-thiazoline (no. **12**) is formed upon pasteurization; for example, its FD factor rose by a factor of more than 64 when NHC was compared with HHC. This odorant is well-known as a Maillard reaction product involving the amino acid cysteine and has already been identified as an important odorant in various heat-treated food products (*31, 32*), including dairy products (*33*). Furthermore, the thermal treatment was found to also induce an increase in the FD factors of 1-hexen-3-one (no. **2**) and (*E,Z*)-2,6-nonadienal (no. **9**) as well as of the lactones γ-dodecalactone (no. **27**) and δ-dodecalactone (no. **29**) (**Table 2**). In contrast, short- and medium-chain fatty acids (nos. **10**, **11**, **14**, **20**, **26**) were evaluated with higher FD factors in the nonheated cream samples than in the thermally treated ones (**Table 2**). This might be explained by inactivation of lipases during heat treatment, thus leading to lower amounts of these acids in the heated samples. The results obtained for the nonheated cream samples before and after whipping did not reveal major differences in the spectrum of odorants, thus clearly demonstrating that whipping did not induce any major qualitative changes in the odorant composition.

As highly volatile compounds are known to be lost during workup procedures used for the AEDA, the cream samples were analyzed by means of static headspace-olfactometry (SHO) to detect such odorants. By means of this method methanethiol, dimethyl sulfide, and dimethyl disulfide were identified as additional odorants in the whipped high-heat-treated cream (WHHC). However, whereas dimethyl sulfide and dimethyl disulfide were found in both the nonheated and the thermally treated cream, methanethiol was detected exclusively in the heat-treated cream (data not shown). It can, therefore, be assumed that the thiol is formed by a thermal degradation of free methionine in the cream during thermal processing. Sensory experiments showed that methanethiol may play a role in creaminess perception, but in particular the amounts have to be present in a distinct concentration range (data not shown). Whipping of the cream samples did not induce any clear changes in the qualitative composition of the cream headspace.

These data indicate that the attractive fresh cream aroma might be due to an interplay of a group of odorants, none of which presented a creamy odor quality on their own, thus strengthening the theory that the aroma of milk products is based on a distinct balance of concentrations of individual aroma components. To investigate whether, besides the odor-active volatiles reported above, additional compounds contribute to the creaminess perception of dairy products, the following studies were performed.

Identification of Sensory Active Semivolatiles/Nonvolatiles. With the aim of identifying such compounds in cream, first, the fat fraction was isolated from heat-treated cream (HHC) by means of *n*-pentane extraction, and the fat phase was examined by means of a sensory-directed fractionation as follows.

To gain first insight into the chemical compounds contributing to a creaminess sensation, the lipids were fractionated by means of column chromatography on silica gel using *n*-pentane/diethyl ether mixtures of increasing polarity. The five fractions obtained were sensorially evaluated in oil-in-water emulsions. To achieve this, the emulsified lipid fractions were presented to the trained sensory panel, which was asked to score the intensity of the four sensory descriptors mouth-coating, fatty mouthfeel, grainy mouthfeel, and creaminess on a scale from 0 to 5. As shown in **Figure 3**, the fatty mouthfeel was evaluated with the highest score of 3.5 in fractions B and C, followed by fraction A, which was judged with a score of 2.5. In comparison, the more polar fraction D was evaluated with an intensity of only 1.5 for the fatty mouthfeel. Furthermore, a grainy mouthfeel was observed for fraction A, and a long-lasting mouth-coating sensation was detectable in fraction D. In contrast, fraction E did not elicit any oral sensation, and the sensory panel did not detect any creaminess sensation in all emulsified fractions.

To answer the question as to how these lipid fractions would perform in cream as the matrix as compared to an aqueous emulsion, cream samples were spiked individually with each of the five fractions and, after whipping, the sensory panel was asked to score the difference of the intensity of the three sensory descriptors mouth-coating, fatty mouthfeel, and grainy as well as the intensity of the overall creaminess perception between the test sample and whipped cream (control). Compared to the blank, the cream spiked with fraction A and, to a smaller extent, also fraction B was evaluated with a grainy mouthfeel, thus fitting well with the above data found in aqueous emulsion (**Figure 4**). In contrast, a fatty mouthfeel was detectable when the whipped cream contained fractions C and D, respectively. However, the sample spiked with fraction D was evaluated as exhibiting significantly higher scores for creaminess as the control (**Figure 4**). Additionally, a persistent mouth-coating sensation was induced by this sample.

Table 1. Identification of Important Odor-Active Compounds (FD ≥ 2) in Whipped High-Heat-Treated Cream (WHHC)

no.[b]	odorant[c]	odor quality[d]	RI[a] FFAP	RI[a] SE-54	RI[a] OV-1701	FD[e]
1	hexanal	green, grassy	1070	796	890	4
2	1-hexen-3-one	plastic	1095	790	870	64
3	(*Z*)-3-hexenal	green, grassy	1135	810	885	16
4	1-octen-3-one	mushroom-like	1285	985	1073	16
5	2-acetyl-1-pyrroline	popcorn-like	1328	930	1023	64
6	(*Z*)-4-nonenal	fatty	1427	1105	1195	32
7	3-(methylthio) propanal	cooked potatoes	1452	920	1050	32
8	(*E*)-2-nonenal	fatty, cucumber-like	1525	1170	1285	32
9	(*E*,*Z*)-2,6-nonadienal	fatty, cucumber-like	1570	1160	1279	32
10	butanoic acid	sweaty	1620	823	996	64
11	2- and 3-methylbutanoic acid	sweaty	1660	875	1043	16
12	2-acetyl-2-thiazoline	popcorn-like	1753	1115	1260	64
13	(*E*,*E*)-2,4-decadienal	fatty	1800	1325	1467	8
14	hexanoic acid	rancid	1830	1019	1186	8
15	(*E*)-2-dodecenal	citrus-like	1835	1471	1597	64
16	2-phenylethanol	honey-like	1935	1500	1293	128
17	δ-octalactone[f]	coconut-like	1980	1298	1527	16
18	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	2010	1385	1576	128
19	γ-nonalactone[f]	coconut-like	2035	1370	1625	32
20	octanoic acid	sweaty	2050	1279	1370	2
21	4-methylphenol	cowshed-like	2085	1082	1305	16
22	unknown	eucalyptus-like	2120	1363	1512	16
23	3-ethylphenol	leather-like	2195	1166	1405	4
24	δ-decalactone[f]	coconut-like	2225	1510	1760	256
25	*o*-aminoacetophenone	foxy	2240	1318	1505	64
26	decanoic acid	soapy	2270	1373	1464	16
27	γ-dodecalactone[f]	peach-like	2415	1680	1950	1024
28	(*Z*)-6-dodeceno-γ-lactone[f]	peach-like	2435	1670	1925	1024
29	δ-dodecalactone[f]	peach-like	2470	1725	1990	512
30	dodecanoic acid	soapy	2475	2169	2260	8
31	3-methylindole	faecal	2520	1410	1650	256
32	phenylacetic acid	honey-like	2585	1276	1519	4
33	4-hydroxy-3-methoxybenzaldehyde	vanilla-like	2600	1402	1655	8
34	phenylpropanoic acid	honey-like	2635	nd[g]	nd[g]	32

[a] Linear retention index. [b] Aroma compounds were numbered consecutively in the order of elution from the GC column. [c] The compound was identified by comparing it with the reference substance on the basis of its odor quality and odor intensity perceived at the sniffing port, retention index on three capillary columns (FFAP, SE-54, and DB-1701), and mass spectrometry (MS-EI; MS-CI). [d] Odor quality perceived at the sniffing port. [e] FD factor of the aroma compound determined on a FFAP capillary column. [f] The enantiomeric distribution of the compounds was not determined. [g] Not determined.

To locate the compounds responsible for the increased creaminess sensation in fraction D, this fraction was further separated by RP-HPLC coupled to an evaporative light scattering detector (ELSD). Comparison of the HPLC chromatogram obtained for the total cream fat (**Figure 5A**) with that recorded for fraction D (**Figure 5B**) revealed that only the early eluting, less hydrophobic compounds of the fat are present in the creaminess-inducing fraction D, whereas the triglycerides containing long-chain fatty acids eluting at retention times between 50 and 160 min were separated. LC-MS-MS measurements indicated that the peaks eluting between 20 and 50 min are triglycerides containing at least one short-chain fatty acid, such as butanoic acid or hexanoic acid, respectively.

To remove these triglycerides from the putative creaminess-inducing components, fraction D was extracted with acetonitrile, and the triglycerides were separated by crystallization at −20 °C. Then, the volatiles were removed from the extract by high-vacuum distillation at 50 °C. To further separate the residue into a semivolatile and a nonvolatile subfraction, an additional high-vacuum distillation was performed at 150 °C. The semivolatiles as well as the nonvolatiles obtained were then individually added to cream samples and, after whipping, the creaminess of both samples was sensorially evaluated. The panel judged the cream sample spiked with the semivolatiles as being significantly creamier when evaluated without the use of a nose clip (**Figure 6**). In contrast, no creaminess increase was detectable when the sensory evaluation was done with nose clips. These data clearly showed that the target molecules are semivolatile.

To identify the active molecules, the isolate was analyzed by HRGC-MS operating in the electron impact (EI) ionization mode. In the chromatogram (**Figure 7A**) peaks a and d–f showed a fragment ion with *m*/*z* 99 and peaks b and c exhibited a fragment ion with *m*/*z* 85 as the base ions. Because the ions *m*/*z* 85 and 99 are known as characteristic fragment ions of γ-lactones and δ-lactones, respectively (*33*), the isolate was analyzed by HRGC-MS-CI to visualize the pseudomolecular ions of these compounds. These experiments unequivocally demonstrated the $[M + 1]^+$ ion of peaks a (*m*/*z* 171), b (*m*/*z* 197), c (*m*/*z* 199), d (*m*/*z* 199), e (*m*/*z* 227), and f (*m*/*z* 255) and suggested their structures as δ-decalactone (a), (*Z*)-6-dodecen-γ-lactone (b), γ-dodecalactone (c), δ-dodecalactone (d), δ-tetradecalactone (e), and δ-hexadecalactone (f). Comparison of the retention times, mass spectra (MS-EI; MS-CI), and sensory activity detected with data obtained for the synthesized lactones, δ-decalactone (no. **24**), (*Z*)-6-dodeceno-γ-lactone (no. **28**), γ-dodecalactone (no. **27**), δ-dodecalactone (no. **29**), and δ-tetradecalactone (no. **35**) finally confirmed the structures of these compounds. After synthesis of the commercially unavailable C_{16}-homologues, also the presence of δ-hexadecalactone (no. **36**) could be unequivocally confirmed. The identification of these lactones is very well in agreement with earlier literature studies reporting these compounds as constituents of heated milk, milk fat, and butter (*34*–*36*).

Table 2. Comparison of Odor-Active Compounds in Nonheated Cream (NHC), Whipped Nonheated Cream (WNHC), High-Heat-Treated Cream (HHC), and Whipped High-Heat-Treated Cream (WHHC) Based on Flavor Dilution (FD) Factors

			FD factor[a]			
no.[b]	odorant[c]	odor quality[d]	NHC	WNHC	HHC	WHHC
1	hexanal	green, grassy	4	1	32	4
2	1-hexen-3-one	plastic	8	8	64	64
3	(*Z*)-3-hexenal	green, grassy	2	8	32	16
4	1-octen-3-one	mushroom-like	16	16	32	16
5	2-acetyl-1-pyrroline	popcorn-like	64	64	128	64
6	(*Z*)-4-nonenal	fatty	4	1	4	32
7	3-(methylthio)propanal	cooked potatoes	32	16	32	32
8	(*E*)-2-nonenal	fatty, cucumber-like	8	8	8	32
9	(*E*,*Z*)-2,6-nonadienal	fatty, cucumber-like	<1	4	16	32
10	butanoic acid	sweaty	128	128	64	64
11	2-/3-methylbutanoic acid[e]	sweaty	32	64	8	16
12	2-acetyl-2-thiazoline	popcorn-like	<1	<1	64	64
13	(*E*,*E*)-2,4-decadienal	fatty	8	8	16	8
14	hexanoic acid	rancid	16	16	8	8
15	(*E*)-2-dodecenal	citrus-like	<1	16	16	64
16	2-phenylethanol	honey-like	8	8	16	128
17	Δ-octalactone[f]	coconut-like	16	8	64	16
18	*trans*-4,5-epoxy-(*E*)-2-decenal	metallic	32	16	64	128
19	γ-nonalactone[f]	coconut-like	2	4	2	32
20	octanoic acid	sweaty	16	16	2	2
21	4-methylphenol	cowshed-like	16	32	16	16
22	unknown	eucalyptus-like	16	16	16	16
23	3-ethylphenol	leather-like	8	8	32	4
24	δ-decalactone[f]	coconut-like	1024	512	256	256
25	*o*-aminoacetophenone	foxy	32	32	64	64
26	decanoic acid	soapy	64	64	64	16
27	γ-dodecalactone[f]	peach-like	64	128	1024	1024
28	(*Z*)-6-dodeceno-γ-lactone[f]	peach-like	1024	1024	1024	1024
29	δ-dodecalactone[f]	peach-like	64	128	1024	512
30	dodecanoic acid	soapy	16	32	4	8
31	3-methylindole	fecal	512	512	1024	256
32	phenylacetic acid	honey-like	32	32	2	4
33	3-methoxy-4-hydroxybenzaldehyde	vanilla-like	8	8	16	8
34	phenylpropanoic acid	honey-like	32	16	32	32

[a] Linear retention index. [b] Aroma compounds were numbered consecutively in the order of elution from the GC column. [c] The compound was identified by comparing it with the reference substance on the basis of its odor quality and odor intensity perceived at the sniffing port, retention index on three capillary columns (FFAP, SE-54, and DB-1701), and mass spectrometry (MS-EI; MS-CI). [d] Odor quality perceived at the sniffing port. [e] FD factor of the aroma compound determined on a FFAP capillary column. [f] The enantiomeric distribution of the compounds was not determined.

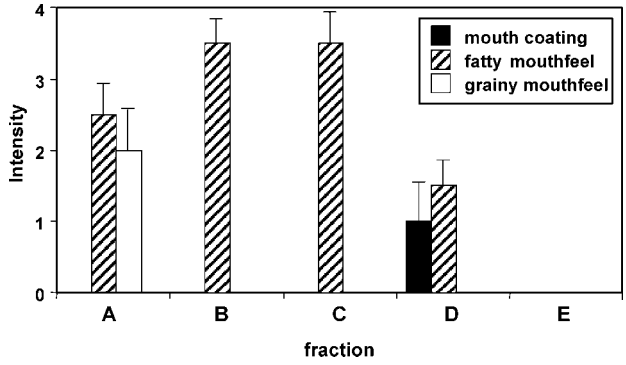

Figure 3. Sensory profile of cream fat fractions emulsified in water containing 0.5% Span 65 at 40 °C.

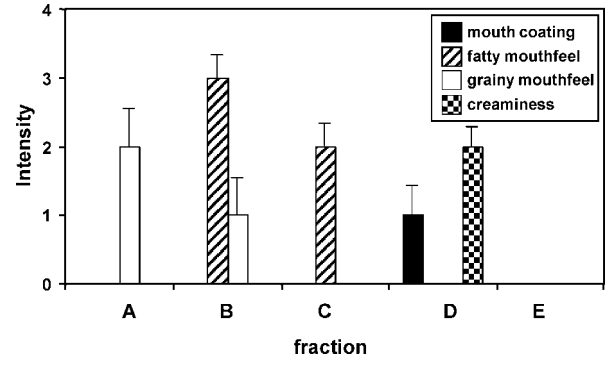

Figure 4. Sensory profile of whipped cream spiked with individual fat fractions.

To screen for lactones with longer carbon skeletons, the semivolatiles were isolated in higher amounts by high-vacuum distillation of the total cream lipids without prior column chromatographic separation. To achieve this, first, the highly volatile compounds were removed from the lipids by high-vacuum distillation at 50 °C and, then, after the temperature had been increased to 150 °C, the semivolatiles were trapped by means of a cryofocusing device in a yield of about 0.25%. A sensory experiment on whipped cream spiked with this distillate showed a clearly creamier flavor. HRGC-MS-CI analysis of the semivolatile distillate confirmed lactones **24**, **27**–**29**, **35**, and **36**, and in addition $[M + 1]^+$ ions with *m*/*z* 227, 255, and, 283 for peaks g, h, and i (**Figure 7B**), thus suggesting the presence of γ-tetradecalactone, γ-hexadecalactone, and δ-octadecalactone. After synthesis of these lactones, a comparison of retention times, spectral data (MS-EI, MS-CI), and sensory activity as well as cochromatography with the reference compounds unequivocally identified γ-tetradecalactone (no. **37**), γ-hexadecalactone (no. **38**), and δ-octadecalactone (no. **39**) in the cream fat, confirming earlier reports on the presence of these lactones in heated butter fat (*37*), milk fat (*38*), and cheese (*25*). A previous study on the stereodifferentiation of

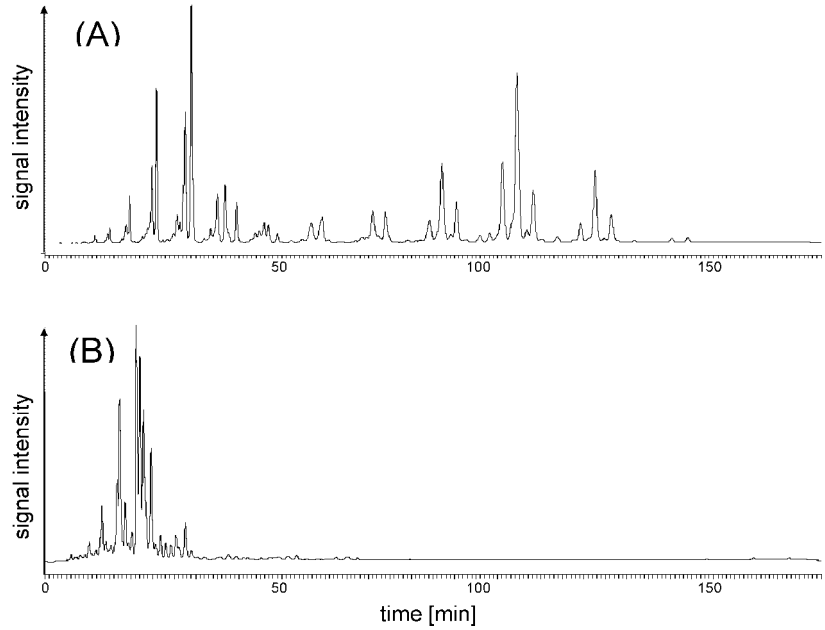

Figure 5. RP-HPLC/ELSD chromatograms of (**A**) total cream fat and (**B**) fraction D isolated from the fat.

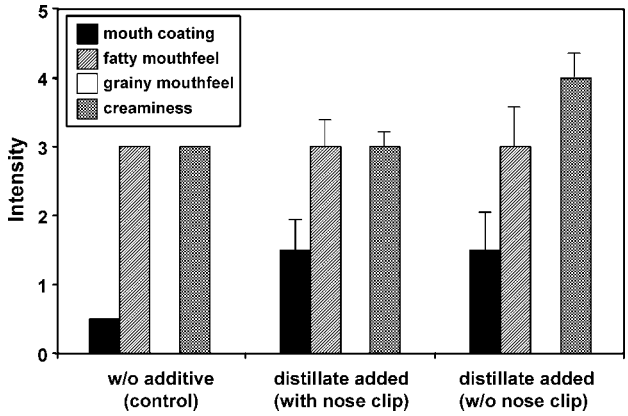

Figure 6. Influence of the semivolatile isolate obtained by acetonitrile extraction of fraction D and high-vacuum distillation at 150 °C on the mouthfeel and creaminess perception of cream. The isolate (0.1%) was added to the cream prior to whipping.

lactones by means of enantioselective multidimensional gas chromatography revealed that the genuine lactones of dairy products occur preferably as (*R*)-enantiomers (*39*).

Sensory Properties of C_{10}–C_{20}-Lactones. Because the flavor attributes of the individual lactones with C_{10}–C_{18}-carbon skeletons have as yet not been systematically studied, the synthesized γ- and δ-decalactone, γ- and δ-dodecalactone, γ- and δ-tetradecalactone, γ- and δ-hexadecalactone, and γ- and δ-octadecalactone as well as γ- and δ-eicosalactone were sensorially evaluated in aqueous emulsions as well as in whipped cream.

First, the recognition thresholds for bitter taste and mouthfeel as well as retronasal and orthonasal odor of these lactones were sensorially evaluated in aqueous emulsions. The data revealed the lowest bitter thresholds for γ- and δ-decalactone at concentration levels of 340 or 420 μmol/L, respectively (**Table 3**). Independently from the γ- or δ-lactone ring, the threshold for bitterness increased with an elongation of the aliphatic chain; for example, the bitterness threshold of 340 or 420 μmol/L for γ- or δ- decalactone increased to 2040 or 2480 μmol/L for γ- or δ-tetradecalactone, whereas the hexadeca-, octadeca-, and eicosadecalactones did not show any bitter taste up to a concentration of 10000 μmol/L. At concentrations below the bitter threshold levels, all lactones imparted a persistent mouth-feeling, which was described as a dry mouth-coating sensation. The recognition thresholds of 10 and 50 μmol/L for the γ- and δ-decalactones increased upon elongation of the carbon chain to 250 and 150 μmol/L for γ- and δ- tetradecalactones (**Table 3**). It is interesting to note that further elongation of the side chain in C_{16}-, C_{18}-, and C_{20}-lactones induced a change of the drying sensation into a fatty and oily mouthfeel. In particular, the two octadecalactones as well as the eicosalactones were described with a rather strong fatty and oily mouthfeel.

In addition, the retronasal as well as the orthonasal odor thresholds were determined. The data revealed that also the odor thresholds as well as the sensory quality of the lactones were strongly influenced by their chain length. The lowest thresholds were found for γ- and δ-decalactone as well as for γ- and δ-dodecalactones, all of which were evaluated with orthonasal and retronasal threshold concentrations below 1.0 μmol/L. However, an increase of the chain length of the lactones induced a drastic increase in the threshold concentrations that was even more pronounced for the orthonasal thresholds. For example, in the series of the γ-lactones, the retronasal thresholds of tetradecalactone and octadecalactone were 215 and 237 times above the thresholds found for the dodecalactones, whereas the orthonasal threshold of the dodecalactone was 1093 and 2406 times below the orthonasal thresholds of the tetradeca- and octadecalactones (**Table 3**). The eicosalactones, however, did not exhibit any retronasal or orthonasal odor. Moreover, the sensory quality of the lactones was found to be strongly dependent on the chain length of the lactone; for example, within the δ-lactones the deca- and dodecalactone exhibited a coconut-like, peach-like, and fruity character, whereas the tetradecalactone was evaluated with the descriptors mushroom-like, waxy, and fatty, respectively, and the hexadecalactone and the octadecalactone were judged as perfume-like and fatty, respectively (**Table 3**).

It is, however, interesting to note that none of these lactones imparted any creamy sensation, thus fitting well with the above experiments showing that an aqueous emulsion of the lactone-containing fraction D (**Figure 3**) did not show any creaminess. As this fraction induced a significant increase in the intensity

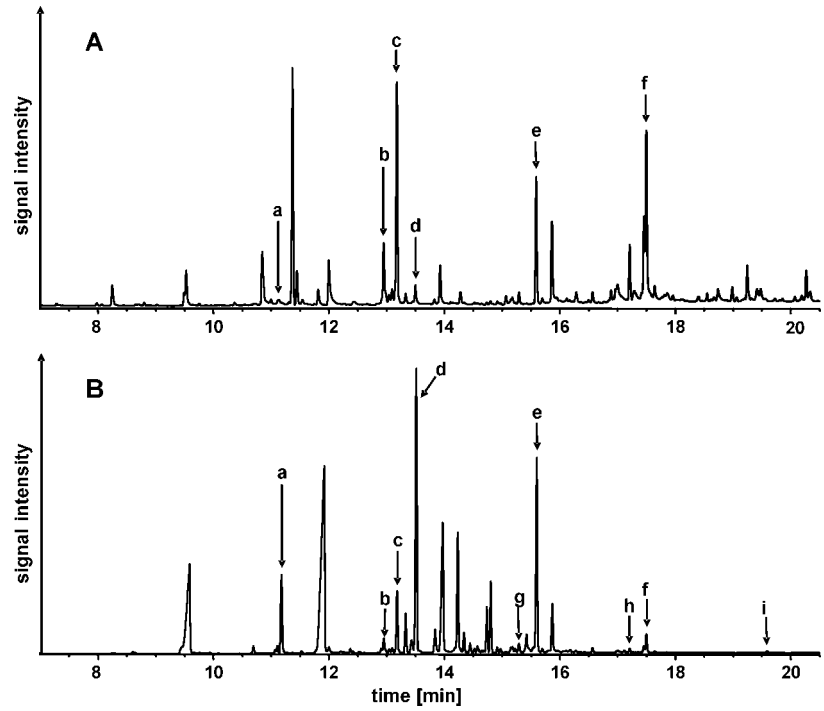

Figure 7. HRGC-MS-CI analysis of the semivolatile isolate obtained by high-vacuum distillation (150 °C) of (**A**) the acetonitrile solubles of fraction D and (**B**) the total cream lipids: a, δ-decalactone (no. **24**); b, (*Z*)-6-dodecen-γ-lactone (no. **28**); c, γ-dodecalactone (no. **27**); d, δ-dodecalactone (no. **29**); e, δ-tetradecalactone (no. **35**); f, δ-hexadecalactone (no. **36**); g, γ-tetradecalactone (no. **37**); h, γ-hexadecalactone (no. **38**); i, δ-octadecalactone (no. **39**).

Table 3. Taste and Odor Threshold of Lactones in an Oil/Water Emulsion

		threshold concentration (μmol/L) for			
lactone	bitterness	mouthfeel	odor (retronasal)	odor (orthonasal)	odor quality
γ-C_{10}	340	10[a]	0.30	0.30	fruity, peach
γ-C_{12}	380	20[a]	0.32	0.32	fruity
γ-C_{14}	2040	250[a]	26.0	52.0	soapy
γ-C_{16}	>10000	690[b]	69.0	350.0	fatty
γ-C_{18}	>10000	380[b]	76.0	770.0	fatty
γ-C_{20}	>10000	420[b]	>10000	>10000	
δ-C_{10}	420	50[a]	0.45	0.45	coconut, peach
δ-C_{12}	450	56[a]	0.74	0.74	fruity, coconut
δ-C_{14}	2480	150[a]	63.0	126.0	mushroom, waxy, fatty
δ-C_{16}	>10000	190[b]	100.0	170.0	perfume-like
δ-C_{18}	>10000	70[b]	140.0	830.0	fatty
δ-C_{20}	>10000	90[b]	>10000	>10000	

[a] Eliciting a dry mouth-coating. [b] Eliciting a fatty mouthfeel.

Table 4. Recognition Thresholds (Micromoles per Kilogram) of Lactones in Whipped Cream

lactone	retronasal	aroma quality	detection threshold for an accelerated melting behavior
γ-decalactone	38	fruity	
	>76	sweetish	
γ-dodecalactone	148	fruity	
γ-tetradecalactone	190	like refrigerator-stored cream	
γ-hexadecalactone	290	soapy	
γ-octadecalactone	>10000		29
γ-eicosalactone	>10000		54
δ-decalactone	13	coconut-like, fruity	
δ-dodecalactone	47	fruity, perfume-like	
δ-tetradecalactone	66	creamy	
	>470	mushroom-like	
δ-hexadecalactone	360	melted butter-like	
δ-octadecalactone	>10000		19
δ-eicosalactone	>10000		20

of the creaminess perception when applied to whipped cream, this prompted us to investigate how these lactones would perform in cream instead of in an aqueous emulsion. Thus, cream samples were spiked individually with the purified lactones and, after whipping, the sensory panel was asked to describe the sensory impression induced by lactone addition and to determine the recognition threshold by means of a three-alternative forced-choice test (**Table 4**). Compared to whipped cream (control), the whipped cream spiked with γ- and δ-decalactone or γ- and δ-dodecalactone, respectively, was evaluated with a strong fruity and coconut-like flavor at thresholds of 38 and 13 μmol/kg for the γ- and δ-decalactones and 148 and 47 μmol/kg for the γ- and δ-dodecalactones. In addition, γ-decalactone was found to increase the sweetness of the cream at levels of above 72 μmol/kg. However, after the addition of γ-tetradecalactone at threshold levels of 190 μmol/kg, the flavor of the cream was reminiscent of the off-flavor of a cream stored open in a refrigerator (**Table 4**). However, spiking the whipped cream with the corresponding δ-tetradecalactone did not induce a similar nondesirable flavor, but resulted in a highly pleasant creaminess sensation at a threshold concentration of 66 μmol/kg. When this lactone was added in concentrations of more than 470 μmol/kg, however, the creaminess sensation was covered by a mushroom-like flavor. Although sensory studies of lactones have been performed earlier using synthetic butter as the matrix (*40*), this creaminess-inducing activity of the δ-tetradecalactone was not decribed earlier. Substitution of tetradecalactone by γ- or δ-hexadecalactone revealed a soapy or buttery sensation at threshold levels of 290 and 360 μmol/kg, respectively, but did not change the intensity of the creaminess of the whipped cream.

In contrast to all other lactones investigated so far, neither the octadecalactones nor the eicosalactones exhibited any

retronasal flavor sensation in whipped cream (**Table 4**). However, when the whipped cream was spiked with one of the δ-octadecalactones or the eicosalactones, respectively, a significantly accelerated melting behavior in the oral cavity was perceived as compared to whipped cream. This effect was detected above threshold concentrations of 29 and 19 μmol/kg for the γ- and δ-octadecalactones and 54 and 20 μmol/kg for the γ- and δ-eicosalactones, respectively, but was not perceived for the other lactones (**Table 4**).

To study whether the influence of these long-chain lactones on the in-mouth melting behavior of cream is just due to a physicochemical effect, the experiment was repeated, but substituting δ-octadecalactone by stearic acid (568 μmol/kg). As the sensory panel was not able to detect any difference in melting behavior between the blank and the cream sample spiked with stearic acid, it is probably not just the chain length of the compounds imparting this effect. Further studies are currently in progress to investigate the mechanism behind this phenomenon.

In conclusion, a series of lactones was found to contribute to the overall flavor of cream, but spiking experiments with individual lactones revealed that it is the δ-tetradecalactone which is able to impart the typical creaminess character to cream. Rather than contributing to the retronasal aroma of cream, first evidence was found that γ- and δ-octadecalactones influence the melting behavior of cream in the oral cavity. In addition, the formation of methanethiol in the pasteurized cream, which showed a more pronounced creaminess, also suggests a contribution of this odorant to creaminess perception. Quantitative studies are currently in progress to evaluate the influence of thermal processing on the formation of lactones in dairy products and, also, to confirm the contribution of these lactones as well as that of methanethiol to the creaminess of several milk products.

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