Identification of Allyl Esters in Garlic Cheese

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This study describes the identification of six allyl esters in a garlic cheese preparation and in a commercial cream cheese. The extracts were prepared by liquid/liquid extraction and concentrated by the SAFE process. The identification of the allyl esters of acetic, butyric, hexanoic, heptanoic, octanoic, and decanoic acids is based on the correlation of their mass spectrometric data and chromatographic retention time data obtained from the extracts with those of authentic standards. In addition to the gas chromatography (GC)/mass spectrometry analysis, the flavor ingredients were characterized by GC sniffing by a trained flavorist. Some of the esters were isolated by preparative GC.

INTRODUCTION

In flavor chemistry, culinary targets are playing an increasingly important role for the understanding of complex flavor sensations. Besides the simple combination of the flavor compounds, which are introduced from the different ingredients, also the interaction between the individual flavor compounds is of high interest. In the past, the knowledge about the formation of new reaction products was driven by investigations of off-notes (*1*). This study is focused on the culinary target garlic cheese preparation to learn about possible reaction products between cheese constituents and the highly reactive garlic flavor ingredients. It is well-known from the literature that cheese contains high concentrations of free fatty acids (*2, 3*). Also, the formation of esters in cheese is reported, but the mechanism of their synthesis is still largely unknown (*4*) and it is not yet proven whether enzymes are involved or not (*5*). Excessive levels of ethyl esters can cause a fruity flavor defect in cheddar cheese (*6*). On the other hand, in extracts of garlic bulbs, allyl alcohol was identified (*7, 8*). Although the ingredients of many cheese brands and garlic are well-investigated, one can not find publications about the volatiles of garlic cheese.

The purpose of this study was to investigate whether allyl esters are formed in kitchen-prepared garlic cheese. It was expected that the compounds of interest would occur, if at all, only in trace-level concentrations. The allyl esters of short-chain fatty acids are potent flavor chemicals (*9*), so that, even in trace concentrations, they would contribute to the perceived flavor of garlic cheese preparations. One finds such cheese preparations especially in the Mediterranian diet, for instance, the Roman

garlic cheese based on the milk cheese of sheep (*10*) or soft cream cheese. After having identified allyl esters in the homemade preparation, these findings were verified by analyzing a commercially available garlic cheese, namely, Boursin.

MATERIALS AND METHODS

On the basis of a formula given by Pütz and Kirschner, a garlic cheese preparation was made using pickled cheese (*10*). A garlic bulb contains about 15 cloves; on average each clove weighed between 6 and 8 g. Therefore, the formula resulted in a ratio of two parts of cheese and one part of garlic. Using a Grindomix (Retsch, Haan, Germany), 1000 g of cheese (German cheese from cow milk in salt brine matured, 45% fat, GUT and GÜNSTIG) was mixed with 500 g of fresh garlic cloves from France and 200 g of olive oil (native olive oil extra vergine, the fruity; Bertolli). All of these materials were purchased at the local market. Spices, as often mentioned in recipes, were not added to facilitate the analysis and to get clearer information on reaction pathways occurring during maceration. The mixture was homogenized by an Ultra Turrax (IKA, Staufen, Germany) to get a creamy paste. This was allowed to stand for 4 days at a temperature between 20 and 30 °C.

The commercial product Boursin Ail and Fines Herbes (Unilever) was chosen as a second starting material, which was to be checked for the occurrence of the above-mentioned reaction products. This is a full fat cheese produced from cream from the milk of cows and originates from Normandy, France. This cheese is flavored by garlic, salt, pepper, and fine herbs, e.g., parsley and chives. A total of 5 kg of Boursin cheese was bought at a local supermarket. When stored between +2 and +6 °C, it still had a residual shelf life of 4 weeks.

Isolation of Volatiles. For a standard solution, 105.9 mg of 2-nonanol was dissolved in 100 mL of ethanol. After the maturation of the self-prepared garlic cheese, 1.0 mL of this solution was added to the paste and the mixture was homogenized by the Ultra Turrax for 10 min. Subsequently, the paste was extracted 4 times using 500 mL of diethyl ether/pentane (1:1) for 15 min each time. Then, the solvent

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mixture was decanted, combined, and concentrated by a TurboVap (Zymark, Rüßelsheim, Germany) apparatus at 25 °C under a nitrogen atmosphere to yield a 650 mL extract. By the SAFE process, the volatiles were separated from the nonvolatiles at 40 °C and at a pressure of 10^{-4} mbar (*11*) and finally concentrated by a TurboVap to yield 500 μL. Because only trace concentrations of reaction products were expected, comprehensive, with regard to allyl esters and possible precursors, blank experiments were conducted. The raw materials (cheese, garlic, and olive oil) were checked for the occurrence of sulfur compounds and especially allyl derivatives by the same procedure as was applied for the garlic cheese preparation. The components identified in these gas chromatography/mass spectrometry (GC/MS) analyses were those already known for cheese, garlic, and olive oil. A cross-contamination by allyl esters of any origin could be excluded. The internal standard 2-nonanol was checked directly by GC/MS and was free of any impurities.

The Boursin cheese was divided into 6 equal parts of about 800 g. To each of these parts, 0.44 mL of the 2-nonanol standard solution was added. Then, the 800 g of cheese were extracted 4 times using 500 mL of diethyl ether/pentane (1:1) for 15 min each time. The solvent mixture was decanted, combined, and further concentrated as described for the garlic cheese preparation. After the concentration in the SAFE apparatus, a modification of the isolation procedure was introduced. The extract was washed 3 times with 100 mL of 0.5 M Na_2CO_3 solution to remove the acids. The organic layer was washed 3 times with saturated NaCl solution until neutral, dried with anhydrous Na_2SO_4, and concentrated using the TurboVap to a final volume of 1.16 mL.

Chemicals. 2-Nonanol was purchased from Merck (Darmstadt, Germany). Allyl acetate, allyl butyrate, allyl hexanoate, allyl heptanoate, and allyl octanoate were bought from Aldrich (Taufkirchen, Germany). Allyl decanoate was obtained from ABCR (Karlsruhe, Germany).

All chemicals were characterized by nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and MS and chromatographed on two columns of different polarity to check their purity (see below).

Analysis of Volatiles. The qualitative and quantitative analysis of the volatiles was carried out on a GC/MS system, which consists of two GC 6890 (Agilent, Palo Alto, CA) and a SSQ 7000 quadrupole mass spectrometer (Thermo Electron Corporation, San Jose, CA). The first gas chromatograph contained a ZB-1 column (Phenomenex, Aschaffenburg, Germany), which is directly connected to the mass spectrometer; the second gas chromatograph contained a ZB-Wax column (Phenomenex, Aschaffenburg, Germany). This column was fitted to the mass spectrometer by a cross-piece. One exit leads to the flame ionization detector (FID), and the other exit leads to an odor detection port (ODP of Gerstel, Mülheim/Ruhr, Germany). A total of 50% of the column effluent was led to the mass spectrometer; 40% was led to the odor detection port; and 10% was led to the FID.

The ZB-Wax column (60 m × 0.32 mm i.d.; film thickness of 0.25 μm) was operated from 60 to 240 °C at 3 °C/min, with a carrier gas of He and velocity of 33 cm/s. The ZB-1 column (60 m × 0.25 mm i.d.; film thickness of 0.25 μm) was operated from 60 to 280 °C at 3 °C/min, with a carrier gas of He and velocity of 31 cm/s. The injection on both columns was done by using a cold injection system (CIS 3 of Gerstel, Mülheim/Ruhr, Germany). The CIS was operated from 80 °C with 10 °C/s to 280 °C for the ZB-1 column and from 40 °C with 10 °C/s to 240 °C for the ZB-Wax column.

The results from the GC/FID were used for the quantitative work. For calculations, a response factor of 1 was applied. The qualitative work was done by running GC/MS analyses and simultaneous olfactorial assessment. The mass spectrometer was operated at 70 eV ionization energy (EI mode, mass range of 25–450 amu). Linear retention indices were determined after injection of a series of *n*-alkanes on both columns, ZB-1 and ZB-Wax, under the a.m. conditions. For the qualitative analysis, mass spectra as well as linear retention indices were compared to those of authentic components and to the in-house database.

The GC/MS system MAT 8200 (Thermo Electron Corporation, San Jose, CA) equipped with a gas chromatograph Carlo Erba 5300 (Carlo Erba, Milano, Italy) with an on-column injection port was used in addition to using the following capillary column: DB-Wax (60 m × 0.32 mm i.d.; 0.32 μm of film thickness; J&W Scientific, Folsom, CA).

The temperature program used was as follows: 40–230 °C at 4 °C/min. The injection port had a temperature of 40 °C. The carrier gas of He was used at a velocity of 40 cm/s. The mass spectrometer was operated at 70 eV of ionization energy (EI mode, mass range of 25–200 amu).

Preparative GC was performed by means of a Hewlett Packard HP 5890 Series II gas chromatograph equipped with multicolumn switching and a preparative fraction collector (Gerstel, Mülheim/Ruhr, Germany). A capillary column DB-1 (30 m × 0.53 mm i.d; 3 μm of film thickness; J&W Scientific) was used applying the following temperature program: 60–230 °C at 3 °C/min with He as the carrier gas at 5 mL/min.

GC/Fourier transform infrared spectroscopy (FTIR) spectra of the reference substances were measured on a Biorad 575C FTIR spectrometer or on a Hewlett Packard 5890 B gas chromatograph (ZB-1 or ZB-Wax; 60 m × 0.32 mm; film thickness of 0.50 μm; from 60 to 240 °C at 4 °C/min, with a carrier gas of He and velocity of 33 cm/s with a Hewlett Packard 5965B IR detector (lightpipe). The NMR spectra of the reference substances were registered on a Varian Gemini 2000 with 200 MHz (^{1}H NMR) or 50 MHz (^{13}C NMR) or on a Varian Unity 400 MHz (^{1}H NMR) or 100 MHz (^{13}C NMR) in CDCl$_3$ using tetramethylsilane (TMS) as the internal standard.

Spectral Data. *Allyl Acetate.* ^{1}H NMR (200 MHz, CDCl$_3$) δ: 2.09 (s, 3, –CO–C$\underline{H}_3$), 4.58 (dt, 2, J = 5.7, 1.5 Hz, –O–C$\underline{H}_2$–CH–), 5.25 (ddt, 1, J = 10.5, 1.6, 1.6 Hz, C$\underline{H}_2$=CH–), 5.32 (ddt, 1, J = 17.2, 1.6, 1.6 Hz, C$\underline{H}_2$=CH–), 5.93 (ddt, 1, J = 17.2, 10.5, 5.7 Hz, CH$_2$=C$\underline{H}$–). ^{13}C NMR (50 MHz, CDCl$_3$) δ: 20.9 (qd, –CO–$\underline{C}$H$_3$), 65.2 (t, O–$\underline{C}$H$_2$–), 118.2 (t, $\underline{C}$H$_2$=CH–), 132.2 (d, CH$_2$=$\underline{C}$H–), 170.7 (s, CH$_3$–$\underline{C}$O–O–). FTIR (lightpipe) cm^{-1}: 3095 (w), 2956 (w), 1764 (s), 1377 (m), 1230 (vs), 1027 (m), 930 (w). MS M$^+$ ion *m/z*: 100 (0.2), 27 (4), 29 (5), 39 (10), 40 (3), 41 (12), 42 (4), 43 (100), 44 (3), 57 (8), 58 (13).

Allyl Butyrate. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 0.96 (t, 3, J = 7.4 Hz, C$\underline{H}_3$–CH$_2$–), 1.67 (m, 2, J = 7.4 Hz, CH$_3$–C$\underline{H}_2$–CH$_2$–), 2.32 (t, 2, J = 7.4 Hz, –CH$_2$–C$\underline{H}_2$–CO–), 4.58 (dt, 2, J = 5.7, 1.5 Hz, –O–C$\underline{H}_2$–CH–), 5.23 (ddt, 2, J = 10.4, 1.4, 1.4 Hz, C$\underline{H}_2$=CH–), 5.32 (ddt, 1, J = 17.2, 1.4, 1.5 Hz, C$\underline{H}_2$=CH–), 5.92 (ddt, 1, J = 17.2, 10.4, 5.7 Hz, CH$_2$=C$\underline{H}$–). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 13.7 (qd, $\underline{C}$H$_3$–CH$_2$–), 18.5 (t, CH$_3$–$\underline{C}$H$_2$–), 36.2 (t, –$\underline{C}$H$_2$–CO–), 64.9 (t, –O–$\underline{C}$H$_2$–), 118.1 (t, $\underline{C}$H$_2$=CH–), 132.4 (d, CH$_2$=$\underline{C}$H–), 173.4 (s, –CH$_2$–$\underline{C}$O–). FTIR (lightpipe) cm^{-1}: 3096 (w), 2973 (m), 2891 (w), 1757 (vs), 1455 (w), 1370 (w), 1249 (m), 1174 (vs), 1097 (w), 993 (m), 930 (w). MS M$^+$ ion *m/z*: 128 (1), 27 (10), 29 (4), 39 (12), 41 (39), 42 (7), 43 (71), 55 (4), 57 (6), 71 (100), 72 (5). Further, the mass spectrum shows a fragment ion of *m/z* 100 with an intensity of 3%; this ion is formed by a McLafferty rearrangement and is a key fragment for allyl esters of straight-chain acids with a chain length longer than three carbon atoms. The *m/z* 100 ion is always accompanied by a *m/z* 113 fragment.

Allyl Hexanoate. ^{1}H NMR (200 MHz, CDCl$_3$) δ: 0.90 (t, 3, J = 6.8 Hz, C$\underline{H}_3$–CH$_2$–), 1.43 (m, 4, CH$_3$–C$\underline{H}_2$–C$\underline{H}_2$–), 1.65 (m, 2, –C$\underline{H}_2$–CH$_2$–CO–), 2.34 (t, 2, J = 7.5 Hz, –C$\underline{H}_2$–CO–), 4.58 (dd, 2, J = 5.8, 1.5 Hz, –O–C$\underline{H}_2$–CH–), 5.23 (ddt, 2, J = 10.3, 1.4, 1.4 Hz, C$\underline{H}_2$=CH–), 5.32 (ddt, 1, J = 17.2, 1.5, 1.5 Hz, C$\underline{H}_2$=CH–), 5.92 (ddt, 1, J = 17.2, 10.3, 5.8 Hz, CH$_2$=C$\underline{H}$–). ^{13}C NMR (50 MHz, CDCl$_3$) δ: 13.9 (qd, $\underline{C}$H$_3$–CH$_2$–), 22.4 (t, CH$_3$–$\underline{C}$H$_2$–), 24.8 (t, –$\underline{C}$H$_2$–CH$_2$–CO–), 31.5 (t, CH$_3$–CH$_2$–$\underline{C}$H$_2$–), 34.4 (t, –$\underline{C}$H$_2$–CO–), 65.1 (t, –O–$\underline{C}$H$_2$–), 118.3 (t, $\underline{C}$H$_2$=CH–), 132.9 (d, CH$_2$=$\underline{C}$H–), 173.9 (s, –CH$_2$–$\underline{C}$O–). FTIR (lightpipe) cm^{-1}: 3096 (w), 2944 (m), 2881 (w), 1756 (vs), 1455 (w), 1371 (w), 1232 (m), 1166 (s), 1107 (m), 996 (m), 930 (w). MS M$^+$ ion *m/z*: 156 (0.2), 29 (8), 39 (9), 41 (40), 43 (74), 55 (21), 69 (17), 71 (53), 99 (100), 100 (33), 113 (12).

Allyl Heptanoate. ^{1}H NMR (200 MHz, CDCl$_3$) δ: 0.88 (t, 3, J = 6.8 Hz, C$\underline{H}_3$–CH$_2$–), 1.30 (m, 6, CH$_3$–C$\underline{H}_2$–C$\underline{H}_2$–CH$_2$–), 1.63 (m, 2, –C$\underline{H}_2$–CH$_2$–CO–), 2.34 (t, 2, J = 7.7 Hz, –C$\underline{H}_2$–CO–), 4.59 (dt, 2, J = 5.7, 1.5 Hz, –O–C$\underline{H}_2$–CH–), 5.23 (ddt, 2, J = 10.1, 1.4, 1.4 Hz, C$\underline{H}_2$=CH–), 5.32 (ddt, 1, J = 17.1, 1.4, 1.4 Hz, C$\underline{H}_2$=CH–), 5.93 (ddt, 1, J = 17.1, 10.1, 5.7 Hz, CH$_2$=C$\underline{H}$–). ^{13}C NMR (50 MHz, CDCl$_3$) δ: 14.1 (qd, $\underline{C}$H$_3$–CH$_2$–), 22.5 (t, CH$_3$–$\underline{C}$H$_2$–), 25.0 (t, –$\underline{C}$H$_2$–CH$_2$–CO–), 28.9 (t, CH$_3$–CH$_2$–CH$_2$–$\underline{C}$H$_2$–), 31.5 (t, CH$_3$–CH$_2$–$\underline{C}$H$_2$–), 34.3 (t, –$\underline{C}$H$_2$–CO–), 64.9 (t, –O–$\underline{C}$H$_2$–), 118.0 (t, $\underline{C}$H$_2$=CH–), 132.4 (d, CH$_2$=$\underline{C}$H–), 173.5 (s, –CH$_2$–$\underline{C}$O–). FTIR (lightpipe) cm^{-1}: 3096 (w), 2941 (m), 2876 (w), 1756 (vs), 1457

Table 1. Allyl Esters Identified in a Garlic Cheese Preparation and in Boursin Cheese by GC/MS and GC–O

component	concentration[a] (mg/kg)	odor description by flavorist	linear retention index ZB-Wax	linear retention index ZB-1	identification by
allyl alcohol[b]	1.1	pungent, mustard-like	1109	<600	b
allyl acetate	1.4	pungent, rum, slightly fruity, fresh onion character, radish, over-ripe	1023	680	a, c
allyl butyrate[b]	5.6	fruity, slightly green, over-ripe, wine character	1180	866	b, c
allyl hexanoate[b]	6.1	canned pineapple, sweet, fruity, juicy, ripe fruit	1370	1061	b, c
allyl heptanoate	<0.01	canned pineapple, fruity, sweet, over-ripe, ripe pear	1468	1160	b
allyl octanoate[b]	0.4	heavy fruity, soapy, slightly pineapple, wine character, fatty, waxy, over-ripe	1571	1261	b
allyl decanoate	0.08	soapy, winey, fermented, fatty, heavy fruity, brandy, waxy	1765	1458	a

[a] Related to internal standard 2-nonanol. [b] Also identified in Boursin cheese, concentration: trace < 0.1 mg/kg; a = identification by GC/MS on one column; b = identification by GC/MS on two columns; c = identification by ODP.

(w), 1371 (w), 1228 (m), 1163 (s), 1109 (m), 995 (w), 930 (w). MS M$^+$ ion *m/z*: 170 (0.1), 29 (16), 39 (14), 41 (59), 43 (100), 55 (28), 57 (18), 69 (17), 85 (18), 100 (25), 113 (65).

Allyl Octanoate. ^{1}H NMR (200 MHz, CDCl$_3$) δ: 0.88 (t, 3, *J* = 6.8 Hz, C$\underline{H}_3$–CH$_2$), 1,3 (m, 8, CH$_3$–C$\underline{H}_2$–C$\underline{H}_2$–C$\underline{H}_2$–C$\underline{H}_2$–), 1.62 (m, 2, –C$\underline{H}_2$–CH$_2$–CO–), 2,34 (t, 2, *J* = 7.5 Hz, –C$\underline{H}_2$–CO–), 4.58 (dt, 2, *J* = 5.6, 1.4 Hz, –O–C$\underline{H}_2$–CH–), 5.24 (ddd, 2, *J* = 10.3, 2.9, 1.4 Hz, C$\underline{H}_2$=CH–), 5.32 (ddd, 1, *J* = 17.2, 2.9, 1.4 Hz, C$\underline{H}_2$=CH–), 5.93 (ddt, 1, *J* = 17.2, 10.3, 5.6 Hz, CH$_2$=C$\underline{H}$–). ^{13}C NMR (50 MHz, CDCl$_3$) δ: 14.1 (qd, $\underline{C}$H$_3$–CH$_2$–), 22.7 (t, CH$_3$–$\underline{C}$H$_2$–), 25.1 (t, –$\underline{C}$H$_2$–CH$_2$–CO–), 29.1/29.3 (2× t, CH$_3$–CH$_2$–CH$_2$–$\underline{C}$H$_2$–$\underline{C}$H$_2$–), 31.8 (t, CH$_3$–CH$_2$–$\underline{C}$H$_2$–), 34.4 (t, –$\underline{C}$H$_2$–CO–), 65.1 (t, –O–$\underline{C}$H$_2$–), 118.3 (t, $\underline{C}$H$_2$=CH–), 132.9 (d, CH$_2$=$\underline{C}$H–), 173.8 (s, –CH$_2$–$\underline{C}$O–). FTIR (lightpipe) cm^{-1}: 3095 (w), 2937 (s), 2871 (m), 1755 (vs), 1459 (w), 1370 (w), 1239 (w), 1161 (m), 1111 (w), 993 (w), 930 (w). MS M$^+$ ion *m/z*: 184 (0.6), 29 (12), 41 (55), 43 (41), 55 (44), 57 (100), 58 (13), 83 (19), 100 (39), 113 (16), 127 (77).

Allyl Decanoate. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 0.88 (t, 3, *J* = 6.9 Hz, C$\underline{H}_3$–CH$_2$), 1,3 (m, 12, CH$_3$–C$\underline{H}_2$–C$\underline{H}_2$–C$\underline{H}_2$–C$\underline{H}_2$–C$\underline{H}_2$–), 1.63 (m, 2, –C$\underline{H}_2$–CH$_2$–CO–), 2,32 (t, 2, *J*= 7.5 Hz, –C$\underline{H}_2$–CO–), 4.58 (dt, 2, *J* = 5.7, 1.4 Hz, –O–C$\underline{H}_2$–CH–), 5.23 (ddd, 2, *J* = 10.3, 2.8, 1.3 Hz, C$\underline{H}_2$=CH–), 5.31 (ddd, 1, *J* = 17.2, 2.8, 1.5 Hz, C$\underline{H}_2$=CH–), 5.92 (ddt, 1, *J* = 17.2, 10.3, 5.7 Hz, CH$_2$=C$\underline{H}$–). ^{13}C NMR (100 MHz, CDCl$_3$) δ: 14.11 (q, $\underline{C}$H$_3$–CH$_2$–), 22.68 (t, CH$_3$–$\underline{C}$H$_2$–), 24.98 (t, –$\underline{C}$H$_2$–CH$_2$–CO–), 29.16, 29.26, 29.27, 2942 (4× t, CH$_3$–CH$_2$–CH$_2$–$\underline{C}$H$_2$–$\underline{C}$H$_2$–$\underline{C}$H$_2$–), 31.88 (t, CH$_3$–CH$_2$–$\underline{C}$H$_2$–), 34.30 (t, –$\underline{C}$H$_2$–CO–), 64.92 (t, –O–$\underline{C}$H$_2$–), 118.06 (t, $\underline{C}$H$_2$=CH–), 132.38 (d, CH$_2$=$\underline{C}$H–), 173.54 (s, CH$_2$–$\underline{C}$O–). FTIR (lightpipe) cm^{-1}: 3095 (w), 2935 (vs), 2867 (m), 1756 (s), 1459 (w), 1372 (w), 1162 (m), 1116 (w), 994 (w), 930 (w). MS M$^+$ ion *m/z*: 208 (0), 29 (37), 41 (100), 43 (79), 55 (43), 57 (39), 69 (43), 71 (52), 100 (42), 113 (42), 155 (72).

RESULTS AND DISCUSSION

In the extract of the garlic cheese preparation, more than 70 compounds were identified. Most of them are already described to occur in cheese or garlic. It should be mentioned here: allyl alcohol (1 mg/kg), butyric acid (70 mg/kg), pentanoic acid (0.6 mg/kg), hexanoic acid (34 mg/kg), heptanoic acid (0.2 mg/kg), benzoic acid (0.4 mg/kg), octanoic acid (0.6 mg/kg), decanoic acid (0.7 mg/kg), and dodecanoic acid (0.2 mg/kg). In addition to these substances, six allyl esters were identified (**Table 1**) using the described GC/MS conditions and a CIS. They were not found in the starting materials of the garlic cheese preparation as was shown by the blank experiments. Because the final temperature of the CIS is 240 and 280 °C depending upon the column used, theoretically allyl esters could be formed as analytical artifacts in the injection system. To rule out the possibility that the allyl esters were generated during injection, a blank experiment was conducted. A total of 3 µg of allyl alcohol and 3 µg of hexanoic acid, dissolved in methyl tert-butyl ether, were injected on the ZB-Wax and ZB-1 columns using the CIS, applying the above-mentioned temperature programs for the CIS and columns. In the chromatograms, the ranges of the retention indices were checked whether an allyl
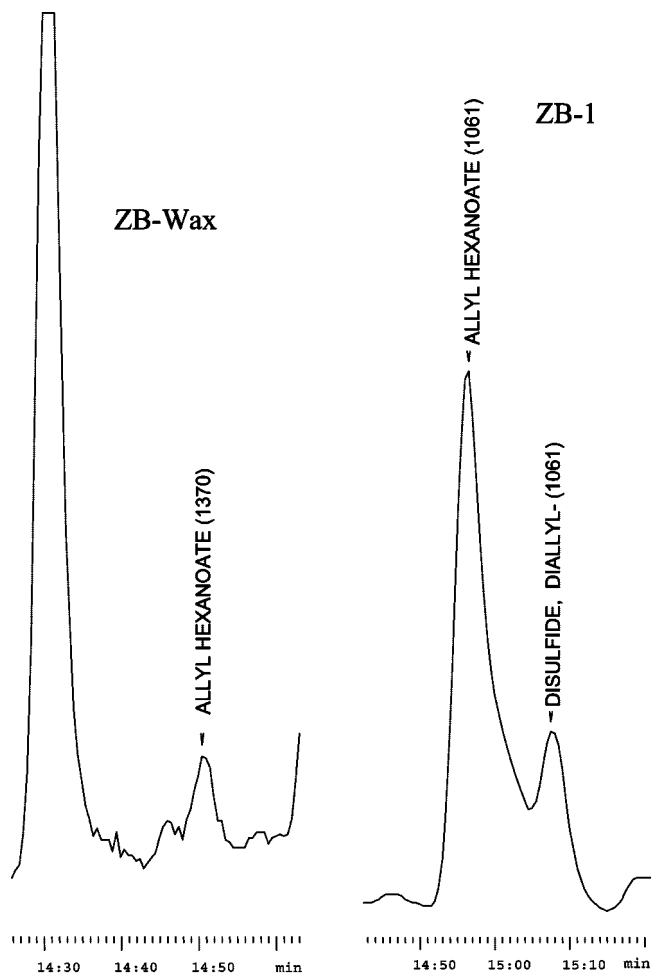

Figure 1. Sections of the GC/MS runs of the aroma extract of the garlic cheese preparation.

hexanoate peak was appearing. Neither with the mass spectrometer when set for selected ion monitoring at *m/z* 99 amu nor with the FID could hints for the formation of allyl hexanoate be found. The finding that the allyl esters originate from the cheese extracts was supported by the analyses using an on-column injection. Also, in these analyses, the allyl esters of acetic, butyric, and hexanoic acids could be unambiguously identified.

During the clean-up of the Boursin cheese, the acids were washed out to avoid their hypothetical reaction with allyl alcohol in the injection port. In the extract of Boursin cheese, the search was concentrated on allyl alcohol and the allyl esters. Mass spectra of components originating from the fine herbs or spices were detected but not further analyzed for this study. Allyl esters are highly flavor-active components (9). The odor qualities of the target compounds given in **Table 1** were determined in a
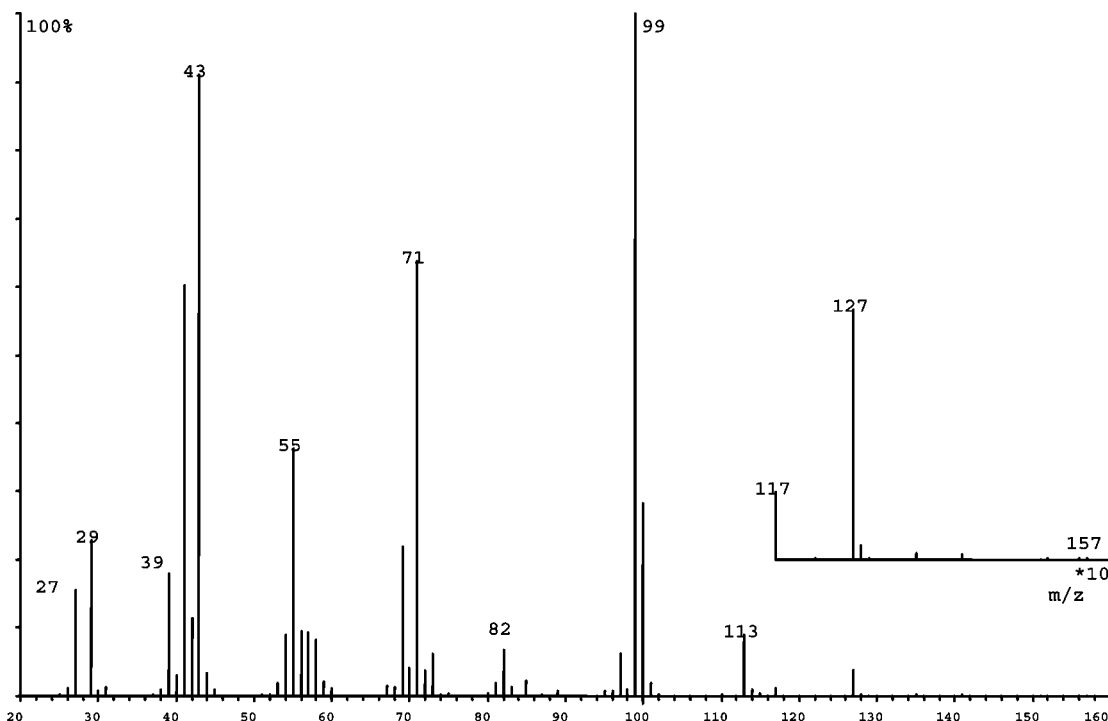
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Figure 2. Mass spectrum of the unknown peak of the garlic cheese preparation at the linear retention index 1370 (ZB-Wax column) after purifying by preparative GC.

1% solution in 1,2-propanediol. Knowing the smell of the esters and their retention indices on the ZB-Wax column, it was possible to detect the allyl esters of acetic, butyric, and hexanoic acids also by sniffing at the ODP. It is published that some of the allyl esters occur in fruits and food. This is the first report of allyl esters in garlic cheese or a garlic cheese preparation.

Allyl alcohol was identified in garlic extract as published already in 1971 by Brodnitz et al. (*7*). In the garlic cheese preparation, about 1 mg/kg was found; also, in the Boursin cheese, it is present. Allyl acetate has not yet been reported to occur in consumed natural products. Because of its ethereal-winey and rum odor, it is mentioned to be useful for rum, pineapple, and arrak flavors as described, for example, by S. Arctander (*9*). It was identified in the analysis using the ZB-1 column. The same author describes that allyl butyrate is used for the creation of fruit flavors, such as apple, apricot, pineapple, and in traces, in butter flavors. This ester is mentioned to occur in the Spanish Roncal cheese, a hard cheese prepared from the raw milk (*12*) of ewes. Another identification of this ester is published by Awad in 2002, who found it in the green algae *Caulerpa racemosa* (*13*). Wan et al. reported its occurrence in kiwi fruit; this identification is possibly incorrect because the retention time mentioned in the publication is much too high compared to other esters given in the same table (*14*). In the garlic cheese preparation reported here, allyl butyrate could be identified on ZB-1 and ZB-Wax columns. Additionally, it was isolated by preparative GC. Also, in the Boursin cheese, it was clearly seen on the ZB-1 column. Allyl hexanoate is a flavor chemical, which is highly appreciated by flavorists because of its fruitiness. In 1982, Nitz et al. described the occurrence of allyl hexanoate in pineapple fruit (*15*). However, neither in the publication of 1982 nor in later research papers did the authors show the mass spectrum or the retention indices on different columns (*16, 17*). In a recent study, Ara and Heil could not identify allyl hexanoate in pineapples using a methodology that had a limit of detection of 0.1 *μ*g/L (*18*). Other publications exist that report the detection of allyl hexanoate in morels (*19*),

the essential oil of *Cymbopogon flexuosus* (*20*), baked Oolong tea (*21*), and acerola fruits (*22*). In this culinary preparation of garlic cheese, allyl hexanoate was found in a concentration of about 6 mg/kg (**Figure 1**); in the Boursin cheese, it could be identified as a trace component < 0.1 mg/kg. The mass spectrum of the allyl hexanoate peak obtained from the extract of garlic cheese additionally purified by preparative GC is presented in **Figure 2**. The linear retention indices of allyl hexanoate were found to be 1061 on a ZB-1 column and 1370 on a ZB-Wax column.

Allyl heptanoate occurs only in a concentration < 0.01 mg/kg in the extract of the garlic cheese preparation. The identification was possible when the GC eluate of the range of the retention index was enriched by preparative GC using a DB-1 column. This concentrate was rechromatographed on an analytical ZB-Wax column. At the index value of 1468, the most important fragment ions of the allyl heptanoate mass spectrum were detected. Therefore, it was tentatively identified. Audouin had found a trace of this ester in the morel *Morchella conica*, besides a larger concentration of allyl hexanoate (*19*).

Another ester with fruity odor occurring in the extract of garlic cheese is allyl octanoate; in 2003, Pino et al. published its identification in papaya fruit (*23*). It is found in the extract of the garlic cheese preparation in a concentration of 0.4 mg/kg and in the Boursin cheese in a concentration < 0.1 mg/kg. Its identification is based on the mass spectra and the linear retention indices on a ZB-Wax and ZB-1 column. Arctander describes the ester allyl decanoate as having a somewhat fatty-sweet and powerful odor without being pungent. The taste is very sweet, fruity, and slightly fatty, with an initial sharpness reminiscent of pineapple. In 1997, Sagrero-Nieves et al. had published that they identified this ester in the essential oil of *Aristolochia brevipes* (*24*). In the extract from the garlic cheese, it occurred in a concentration of 0.08 mg/kg on the ZB-1 column.

It is well-known that cheese contains large amounts of short-chain fatty acids. In a review article about flavor changes, Sinki

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et al. (*25*) mentioned esterification as the most common interaction among components. For cheese, this reaction is described by Thierry et al. (*5*). Because allyl alcohol occurs in garlic, a maceration period of 4 days at 20–30 °C of a garlic cheese preparation is obviously sufficient for this reaction to give rise to allyl esters in analytically and sensorially detectable concentrations. For Boursin cheese, the producer recommends a storage temperature from +2 to +6 °C. Even at this temperature, traces of allyl esters had already been formed. Whether enzymes present in cheese or garlic were involved in the ester formation was not assayed in this study.

ABBREVIATIONS USED

GC–O, gas chromatography olfaction; CIS, cold injection system.

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Supporting Information Available: Mass spectra of 5 unknown peaks of the garlic cheese preparation, each followed by the reference mass spectrum of the respective allyl ester and the reference mass spectrum of allyl hexanoate. This material is available free of charge via the Internet at http://pubs.acs.org.

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