Volatile Components of Limburger Cheese

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The volatile constituents of Limburger cheese were obtained by vacuum distillation–extraction, separated by capillary gas chromatography, and subjected to mass spectral analysis. More than 20 neutral and acidic compounds were identified. The major component present is phenol, which can arise from the microbial decomposition of tyrosine. Also at relatively high levels are the odorous compounds dimethyl disulfide and indole. Other compounds identified include a homologous series of methyl ketones, acetophenone, and higher dimethyl polysulfides. In the acidic fraction, even-carbon fatty acids as well as the branched four- and five-carbon acids were identified. These contribute to the strong characteristic aroma of Limburger.

Limburger cheese is the usual name for a cheese of characteristic aroma made from whole cow's milk. It is classified as a semisoft cheese and possesses a typical composition of 45% water, 28% fat, 21% protein, 2% carbohydrate, and 3% ash (Kosikowski, 1978). Limburger is further classified as a surface-ripened cheese. Surface-ripened cheeses are those that have microbial growth on the surface that contributes to the development of the characteristic flavor of the cheese. A progressive growth of yeast, micrococci, and *Brevibacterium linens* occurs on Limburger cheese, with the latter microorganism primarily responsible for the development of the typical aroma (Olson, 1969).

The volatile components of various cheeses have received a great deal of investigation. Most of these studies have centered on blue, cheddar, and Swiss cheeses. Less work has been devoted to the aroma of surface-ripened cheeses, although some results have been reported.

In the mid-fifties, Bassett studied the acidic and neutral carbonyl compounds in various cheeses including Limburger (Bassett, 1956). He found a number of α-keto acids (derived from the deamination of amino acids) including pyruvic and (*p*-hydroxyphenyl)pyruvic acid. In addition, several neutral compounds such as diacetyl, acetoin, acetaldehyde, and acetone were also identified. About the same time, European workers identified (*p*-hydroxyphenyl)propionic and phenylpropionic acids in Limburger cheese (Simonart and Mayaudon, 1956). The important contribution of volatile sulfur compounds to Limburger aroma has been known for some time (Grill et al., 1966). They reported that methyl mercaptan and hydrogen sulfide contributed to the putrid odor and that the former was more important. In another series of experiments, Japanese workers studied the compounds produced by incubating *B. linens* with whole milk (Hosono and Tokita, 1970). Hydrogen sulfide, acetic acid, and *n*- and isobutyric and valeric acids as well as acetaldehyde, acetone, 2-pentanone, and 2-heptanone were identified. This microorganism has also been shown to liberate methyl mercaptan from dairy products (Sharpe et al., 1977).

The purpose of this paper is to study the volatile aroma chemicals of Limburger cheese and to relate these to the characteristic aroma of Limburger.

MATERIALS AND METHODS

Three different samples of imported (German) Limburger cheese were obtained from a single commericial source. Four hundred gram portions of each were analyzed individually as described below, and the analytical results were averaged. The sample was placed in a Waring Blendor with 200 mL of water, the container was flushed with nitrogen for 3 min and sealed, and the system was homogenized for 30 s. The resultant slurry was vacuum steam distilled at 49 °C for 1 h, and the volatiles were trapped in a dry ice–acetone bath. This yielded 300 g of aqueous material that was saturated with sodium chloride and continuously extracted with 100 mL of diethyl ether for 2 h.

The ether was washed with 5% sodium bicarbonate to remove the free acids. This alkaline solution was acidified (pH 2) with dilute HCl and extracted with ether. The ethereal solutions were dried and concentrated by slow distillation to a volume of about 1 mL through a 1 cm × 25 cm Vigreux column.

So that further insights into the higher fatty acid composition could be obtained, the oil fraction was isolated and analyzed by the procedure of Biede and Hammond (1979).

The most volatile components were analyzed by direct headspace methods. In this case, 4 g of Limburger cheese was placed in an 8-mL septum-sealed vial and the system was equilibrated at 50 °C for 5 min. Two-milliliter portions of the headspace were analyzed by gas chromatography.

Separation and Identification. The neutral ethereal concentrate was analyzed by capillary gas chromatography using a Perkin-Elmer Sigma II with a split-type injector. The fused silica capillary column measured 30 m × 0.32 mm and was coated with SE-30 (Supelco, Inc., Bellefonte, PA). The helium carrier gas velocity was 32 cm/s. The column was held 4 min at 40 °C and then temperature programmed to 220 °C at 6 °C/min. Simultaneous detection utilizing FID/sulfur-specific/nitrogen-specific detectors was employed (Parliment and Spencer, 1981).

Mass spectra were obtained by using tandem gas chromatography–mass spectrometry. The column effluent was passed directly into the ion source of a Du Pont Model 21-491 mass spectrometer. Mass spectra were obtained at 70 eV and a source temperature of 210 °C.

The acidic fractions were analyzed by gas chromatography using a glass column measuring 2 mm i.d. × 6 ft and packed with 10% DEGS/1% phosphoric acid on Chromosorb WAW. A Perkin-Elmer Model 3920 chromatograph was used, and it was temperature programmed from 70 to 220 °C at 4 °C/min.

The headspace samples were also analyzed on the Perkin-Elmer 3920 chromatograph by using a 2 mm i.d. × 6 ft glass column packed with 10% SP-1000 on 100/120 Chromosorb WAW.

Sample quantitation was performed on a Perkin-Elmer Sigma 10 data system by using a response factor of unity.

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0021-8561/82/1430-1006$01.25/0

Table I. Composition of Neutral Limburger Volatiles

identification	RT	% composition
diacetyl	2.44	0.9
ethyl formate	2.92	0.5
2-pentanone	3.18	0.7
methyl thioacetate	3.38	0.5
dimethyl disulfide	4.24	13.2
2-hexanone	5.54	0.4
methional	8.30	0.1
2-heptanone	8.47	3.8
dimethyl trisulfide	10.70	0.8
phenol	11.83	54.3
2-octanone	12.00	0.1
phenylacetaldehyde	13.07	tr[a]
acetophenone	13.66	0.7
p-cresol	14.43	0.7
2-nonanone	14.89	1.4
phenylethanol	15.47	tr
dimethyl tetrasulfide	17.90	tr
ethyl octanoate	18.05	tr
indole	19.92	7.3
2-undecanone	20.4	0.9
Δ-decalactone	24.70	tr
2-tridecanone	25.19	0.2
γ-dodecalactone	28.64	tr
2-pentadecanone	29.50	0.1

[a] Trace.

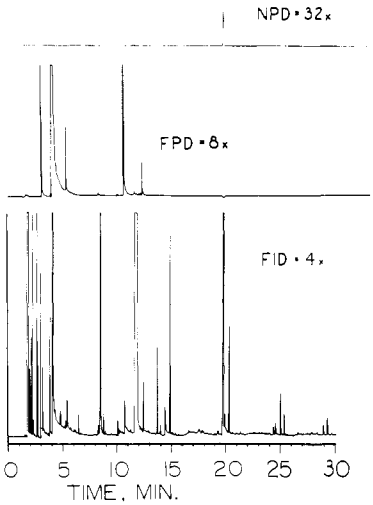

Figure 1. Capillary gas chromatogram of Limburger essence.

RESULTS AND DISCUSSION

The identification of the major neutral compounds of Limburger cheese is presented in Table I and the chromatogram is shown in Figure 1.

B. linens is the primary microorganism involved in Limburger cheese ripening. It possesses active proteolytic enzymes that can degrade casein (Foster et al., 1957), and these reactions produce many of the aromatic chemicals.

The most striking feature of this sample is the high concentration of phenol that is observed. Phenol is formed during the microbiological breakdown of tyrosine by the loss of the alanine moeity. In a similar fashion, indole can arise from the breakdown of tryptophan. Acetophenone is believed to originate via β-oxidation of phenylpropionic acid followed by decarboxylation of the keto acid. Both this compound and indole have pungent–sweet floral aromas in dilution; the latter is more tarry–repulsive when concentrated. These three aromatic compounds have been reported previously in the aroma of other soft ripened cheeses, and pathways for their formation have been discussed (Dumont and Adda, 1979).

The methyl ketones that we identified are commonly found in mold-ripened cheeses, such as blue, to which they impart a characteristic aroma. These compounds may arise by β-oxidation of the appropriate fatty acids (Gehrig and Knight, 1958) or by decomposition of β-keto acids.

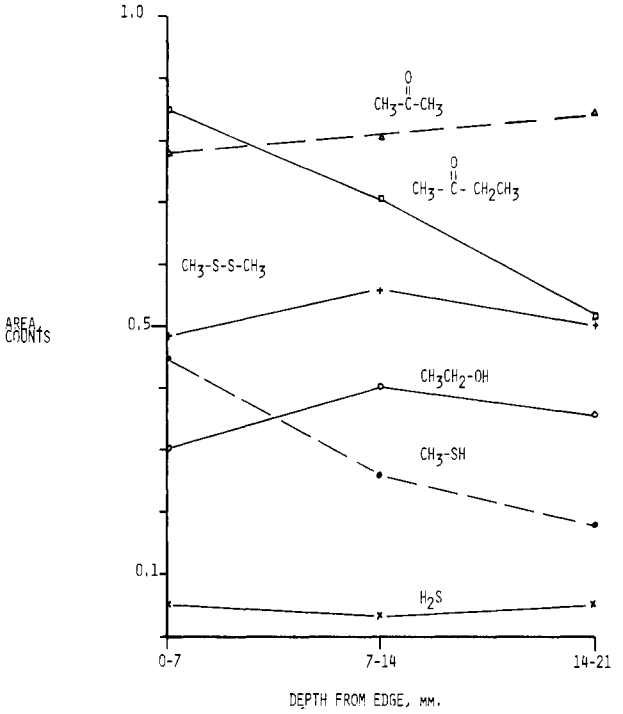

Figure 2. Volatile composition of Limburger cheese at various depths.

Table II. Fatty Acid Composition of Steam Distillate of Limburger

acid	%
propionic	2.7
isobutyric	1.0
n-butyric	19.2
isovaleric	6.1
n-hexanoic	9.0
n-octanoic	29.3
n-decanoic	26.3
n-dodecanoic	5.0

Decomposition of the sulfur amino acids cysteine and methionine produces the volatile sulfur compounds such as hydrogen sulfide and methyl mercaptan during cheese ripening. Oxidative reactions can then convert the latter to dimethyl disulfide and higher polysulfides. The importance of sulfur chemicals to cheese aroma has been known for many years. For example, methional and its degradation product dimethyl disulfide have been implicated as important factors in cheddar cheese flavor (Keeney and Day, 1957; Patton et al., 1958). The flavor of the former is cheesy or potato-like while the latter is onion- or tomato-like on dilution. Methylthioesters have been identified in smear-coated cheeses (Dumont et al., 1976) so the characterization of methyl thioacetate in Limburger is not unexpected. This latter compound has an aroma reminiscent of cooking cauliflower and an odor threshold of 5 ppb (Adda et al., 1978).

The headspace analysis results are shown in Figure 2. On this figure are plotted the relative area counts for each of the major components vs. depth into the cheese. In this case, 0–7 mm represents the rind while 14–21 mm represents the center of the cheese assayed. It is interesting to note that the majority of the chemicals are uniform throughout the cheese; only methyl mercaptan and 2-butanone decrease with depth.

Table III. Free Fatty Acid Content in Oil-Soluble Fraction of Limburger

acid	mg of acid/g of oil
3:0	1.1
*i*4:0	0.2
4:0	3.0
*i*5:0	0.6
6:0	0.5
8:0	0.4
10:0	0.8
12:0	0.9
14:0	2.9
16:0	5.8
18:0	1.4
18:1	10.2

The fatty acid composition of Limburger cheese was analyzed in two different methods. The composition of the steam-distillable free fatty acids is given in Table II. It has been reported that lower free fatty acids are important in cheddar and blue cheese flavor (Day, 1966), and this is also the case with Limburger. Of particular importance are the relatively large amounts of *n*-butyric and isovaleric acids, which possess strong rancid butter, cheesy notes. The six-, eight-, and ten-carbon acids contribute to the sweaty, fatty–rancid character of the cheese. The composition of oil-soluble fatty acids is presented in Table III. This table provides information on the higher molecular weight fatty acids.

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Received for review December 17, 1981. Accepted May 7, 1982. Presented at the 182nd National Meeting of the American Chemical Society, New York, Aug 1981, AGFD 42.