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Identification of Off-Odor Compounds in Turkish White Cheese with Putrid Defects by SPME-GC/MS

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Abstract

Spoilage problem in Turkish white cheese characterized by off-odor occurs occasionally and leads to serious economic losses. It is called as "putrid" by manufacturer and occurs in the later stages of ripening. This study was carried out to identify key odorant compounds associated with main off-odor (putrid) in Turkish white cheese and to estimate the possible causal factors. A total of 14 putrid and 5 normal cheese (control) samples were obtained from local cheese manufacturers from Kırklareli province, Turkey. A total of 65 volatile compounds were identified by employing solid-phase microextraction-gas chromatography-mass spectrometry method. They were composed of carboxylic acids (19), alcohols (13), esters (12), ketones (4), aldehydes (5), and miscellaneous (12). Thirty-five of them were not found in control cheese samples. Carboxylic acids, such as isovaleric acid (42.72 ppm), hexanoic acid (71.17 ppm), octanoic acid (48.81 ppm), and decanoic acid (38.37 ppm), and sulfur compounds, such as methanethiol (0.33 ppm), mercaptoethanol (0.45 ppm), dimethyl disulfide (0.75 ppm), and dimethyl trisulfide (0.38 ppm), were remarkable volatiles in samples of putrid cheese. The results indicated that the off-odor problem in Turkish white cheese probably originated from sulfur compounds that are only found in putrid cheese samples and are characterized by odor of a rotten, boiled cabbage-cauliflower and over-ripened cheese, and carboxylic acids that arise by metabolism of undesirable microorganisms.

Keywords: Cheese, isovaleric acid, sulfur compounds, odors, volatile organic compounds

Introduction

Turkish white cheese is widely produced in the Thrace region and is one of the most popular cheese varieties in Turkey. It is a full-fat pickled cheese that is ripened in tinplate containers for at least 3 months, and it has a soft texture and salty and sour taste (Hayaloglu et al., 2002). This cheese comes as soft, semisoft, or hard. It has a grainy appearance and its varieties include high, medium, and low-fat content. A large part of the white cheese production is made in small dairy plants known as "mandıra."

Chemical, microbial, and enzymatic transformations of milk protein, fat, lactose, and citrate form the cheese flavor (McSweeney and Fox, 2004). Lipolysis, fermentation of lactose, and proteolysis play an important role in the formation of the flavor compounds (Engels et al. 1997). These biochemical events lead to the development of volatile and nonvolatile compounds, such as fatty acids (e.g., butyric acid, acetic acid), alcohols (e.g., ethanol, 2-heptanol), aldehydes (e.g., 3-methylbutanal), ketones (e.g., 2-butanone, 2-pentanone), and esters (e.g., ethyl acetate), that have great effect on the aroma. Some of these reactions may affect the flavor and aroma favorably, but they can also lead to undesirable changes in taste and odor (Fox et al., 1995).

In general, deteriorative reactions such as putrification are associated with microorganisms. Spoilage microflora in dairy products is reported in various studies. Late blowing defect

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caused by *Clostridium* spp. is the primary reason of spoilage in semihard and hard varieties of cheese. It causes defects in flavor and texture (McSweeney and Fox, 2004). In Swiss-type cheese samples, the salt-tolerant lactobacilli give rise to undesirable phenolic putridity caused by hydrogen-sulfur flavors during ripening by metabolizing amino acids. The psychrotrophs *Pseudomonas, Aeromonas*, and *Acinetobacter* can cause surface discoloration, off-odors, and off-flavors in fresh soft cheese samples (Farkye, 2014).

Branched chain fatty acids are an important characteristic of goat and sheep cheese. The 3-methylbutanoic acid (isovaleric) is associated with excessive degradation of the proteins and may cause a putrid off-odor in cheese (Yvon and Rijnen, 2001). However, factors such as changes in animal feeding methods, use of different feed sources, changes in milking and storage techniques, and new developments in the modern food technology can alter the indigenous microflora; different microorganisms can become a current issue.

The aim of this work was to identify key odorant compounds related to the main off-odor (putrid) in Turkish white cheese and to estimate the causal factors from the data we obtained. Former studies focused on the characterization of active aroma components in Turkish white cheese. Although it is frequently seen, no article has been published about the reason and formation of off-odor in Turkish white cheese.

Materials and Methods

Cheese samples

Nineteen cheese samples maturated for 6 months were obtained from different local cheese makers in Kırklareli province, Turkey. Fourteen cheese samples were cheese samples returned from markets with odor defects (putrid) and five samples were standard cheese samples (control). Samples were transferred directly to the laboratory under refrigeration.

Physicochemical analyses

The cheese samples were analyzed for chemical properties. The total solids were determined by oven drying at 102±2°C to a constant weight (IDF, 1982). Cheese samples were diluted (10 g cheese sample:90 mL distilled water) and pH levels were measured by a pH meter (Mettler Toledo, Seven Compact pH S210). Titratable acidity (as % of lactic acid) was determined according to the AOAC (2012). The fat content was determined by the Gerber-Van Gulik method (IDF, 2008). The Kjeldahl method was used to determine the protein content of the cheese samples. Water-soluble nitrogen (WSN) content was determined by micro-Kjeldahl method as described by the AOAC (2012). The *Mohr* method was used for *determination* of *salt content* (IDF, 1988), and the procedure described by the AOAC (2012) was used for determination of ash (%).

Volatile compound analyses

For extracting volatile compounds, the method modified by Ozturkoglu-Budak et al. (2016) was used. Headspace volatile compound analyses were performed with the solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) method. Five grams of cheese, homogeneously prepared, were weighed in a 20-mL vial, and 10 µL of Internal Standard (2 methyl-3-heptanone and 2-methylpentanoic acid) was added. The vial was put in a magnetic stirrer at 60°C for 30 min for trapping volatile compounds in the headspace. A carboxen/polydimethylsiloxane solid-phase microextraction (SPME) fiber (Supelco, Bellefonte, PA) of 75 µm was injected into the vial and exposed to the headspace for 30 min at 60°C. The fiber was inserted (split less mode at 250°C for 3 min) into the injection port of the GC equipped with a DB-Wax column (30 m, 0.25 mm i.d., and 0.25 µm film thickness). The GC/MS systems consisted of Agilent 7890 GC system (Agilent Technologies, Santa Clara, CA) fitted with a mass spectrometer (Agilent 5975C VL MSD with Triple-Axis detector). Helium was used as carrier gas at a linear velocity of 1 mL/min. The initial temperature of the oven was programmed at 40°C for 10 min. The temperature was increased to 110°C at a rate of 5°C/min and 240°C at a rate of 10°C/min with a final hold time of 5 min at 250°C, respectively. Wiley National Institute of Standards and Technology (NIST), and Flavor (Agilent MSD Chemstation, Santa Clara, CA) libraries and n-alkanes (C4-C20) standards (Sigma-Aldrich, St. Louis, MO) were used for the identification of volatile compounds by comparison of mass spectra. A comparison of peak area of the volatile compounds with those of the internal standard was used for the calculation of the concentrations (Ozturkoglu-Budak et al., 2016). The experiment was replicated twice.

Statistical analyses

Data from chemical analysis were analyzed using an independent samples *t*-test procedure of SPSS Statistics 22 to determine if there was a significant difference ($p<0.05$) between putrid and control samples (Statistics, 2015). Principal component analysis (PCA) was carried out using a covariance matrix and varimax rotation between the putrid and control cheese samples (Ozturkoglu-Budak et al., 2016). The concentration of volatile compounds was used as variable. Minitab Release 17 statistical software was used for PCA.

Results and Discussion

Chemical composition

The chemical compositions of the cheese samples are given in Table 1. There were no significant differences between the cheese samples in terms of pH, acidity (lactic acid %), and total nitrogen. Titratable acidity and pH values of the control cheese samples were in line with those reported in the literature for ripened white cheese samples (Kavas et al., 2004; Öner et al., 2006; Tuncel et al., 2010). But the pH value of the putrid cheese samples was higher than the given values. The pH value of the cheese may vary depending on many factors. The lactic acid concentration can be affected by the initial acid production of starter bacteria, the amount of lactose lost in whey, and the

Table 1. Some chemical properties and pH values of control and putrid Turkish white cheese samples

Variables	pH	Acidity (% lactic acid)	Dry matter (%)	Fat in dry matter (%)	Protein (%)	Total nitrogen (g/100 g)	Water-soluble nitrogen (%)
Control cheese samples	5.17±0.98[a]	1.37±0.10[a]	50.90±0.51[b]	52.86±1.39[a]	21.2±0.58[a]	3.32±0.09[a]	0.46±0.15[a]
Putrid cheese samples	5.33±0.17[a]	1.26±0.18[a]	48.34±0.88[a]	55.47±2.42[b]	24.79±1.08[b]	3.89±0.17[a]	0.67±0.12[b]

All values are in mean±SD.
[a,b]Means in the same column followed by different superscript letters represent significant differences between cheese samples. $p<0.05$.

secondary flora in cheese. Consequently, the amount of lactic acid affects the pH of the cheese. pH increase is prevalent in cheese samples ripened with mold and yeast. Assimilation of acids by molds or yeasts, deamination of amino acids (Schlesse et al., 1992), amphoteric proteolysis products (Kurt and Çağlar, 1993), and the breakdown of fatty acids to methyl ketones by ammonia formation (Kaminarides et al., 1990; McSweeney, 2004) may cause an increase in pH.

The putrid cheese samples are significantly different from control cheese samples in terms of total protein and WSN ($p<0.05$). The WSN value of putrid cheese samples was found to be higher than former studies (Cinbaş and Kılıç, 2005; Şahingil et al., 2014; Tuncel et al., 2010). The WSN value is commonly used as the proteolysis index. The extracts contain small and medium peptides, amino acids, and their degradation products. The WSN value varies with cheese type and increases during ripening (Fox et al., 2017). As a result of proteolysis the protein network breaks down and undesirable flavor components can also occur through secondary catabolic changes (McSweeney, 2004; Sousa et al., 2001). The high WSN value can be associated with the putrid odor in the cheese samples.

Volatile compounds

Sixty-five volatile compounds were identified from the cheese samples (Table 2). They were composed of 19 carboxylic acids, 13 alcohols, 12 esters, 4 ketones, 5 aldehyde, and 12 miscellaneous.

Carboxylic acids are a contributor of the volatile fraction of cheese and they serve as precursors of lactones, alcohols, methyl ketones, and esters (McSweeney and Sousa, 2000). Carboxylic acids are generated from lipolysis, lactose fermentation, and amino acid catabolism. They affect the aroma of cheese depending on the concentration (Zabaleta et al., 2016). An increase in the concentration of carboxylic acids may result in rancid odor and taste (Bontinis et al., 2012). As a result of deamination of free amino acids, branched chain α-keto acids are released. The acids are metabolized by oxidative decarboxylation to branched chain carboxylic acids such as 3-methylpropanoic acid, 3-methylbutanoic acid, 2-methyl pentanoic acid, 2-methyl hexanoic acid, and 2-methyl-butanoic acid (Brennand et al., 1989).

In this study, the putrid cheese samples had higher levels of carboxylic acids than the control cheese samples. Propionic, isovaleric, hexanoic, octanoic, and decanoic acids were not detected in the control cheese samples. Hexanoic acid was the first highest abundant acid in the putrid cheese samples. The acid has been associated with a cheesy and oily odor in cheese. Hexanoic acid has a nauseous, sweaty, stinky, sour, sharp, greasy, and unpleasant smell. It has a sharp taste (Burdock, 2019). In the former studies on Turkish white cheese, hexanoic acid concentration was reported to be 13 ppm, 1.51 ppm, 0.6 ppm, and 16.5 ppm, respectively (Demirci, 2012; Özer et al., 2011; Salum et al., 2018).

The 2-methyl hexanoic acid (57.82 ppm) is the second most abundant carboxylic acid in the putrid cheese samples. It is a branched chain fatty acid. It has sweat and oily smell when its concentration is 50 ppm (Brennand et al., 1989). Branched chain fatty acids are characteristics of sheep and goat cheese samples. They are formed as a result of the breakdown of isoleucine and leucine amino acids formed by the advanced degradation of proteins (Demirci, 2012).

Valeric acid was the third highest abundant acid in the putrid cheese samples. Isovaleric acid is known for its bad smell (putrid, sweaty, rotten, etc.) and may cause a putrid off-odor in cheese (Yvon and Rijnen, 2001). Frank et al. (2004) also reported that isovaleric acid gives a rotten cheesy aroma in cheese samples. Isovaleric acid is generated from amino acid degradation containing catalytic deamination (Ganesan et al., 2007). The acids are expected to be produced by microorganisms from branched chain amino acids such as valine and leucine. In a study on rotten-odoring natto, it was reported that the problem is caused by the isovaleric acid, isobutyric acid, and 2-methylbutyric acid produced due to the proteolytic activity of the *Bacillus subtilis natto* (Takemura et al., 2000). Isovaleric acid concentration was determined to be 43.72 ppm in the putrid cheese samples. In a former study, the amount of isovaleric acid was reported to be 0.34 ppm in Turkish white cheese (Demirci, 2012). This component was not found in other studies in Turkish white cheese. In our study, the high level of isovaleric acid in the putrid cheese samples indicates that it may be the cause of bad odor (Table 2).

In total, 13 alcohols were determined. The alcohols 3-methyl-2-butanol and 3-Methylthio-1-propanol (methionol) were

Table 2. Mean volatile compound concentrations of control and putrid Turkish white cheese samples

Volatiles	Control	Putrid	Volatiles	Control	Putrid
Alcohols			Ethyl butanoate	0.67±0.05[b]	4.98±0.47[a]
Ethyl alcohol	9.77±1.54	5.39±1.57	Ethanethioic acid, S-methyl ester	nd	1.60±0.32
2-butanol	7.91±0.98	6.80±4.11	Ethyl hexanoate	5.41±0.24[b]	13.76±1.21[a]
1-propanol	0.23±0.08	0.33±0.09	Propyl hexanoate	nd	0.30±0.09
1-butanol	0.16±0.05	0.24±0.05	Malonic acid, bis(2-trimethylsilyl)ethyl ester	nd	0.82±0.18
2-heptanol	0.05±0.02[b]	0.14±0.05[a]	Ethyl octanoate	3.06±0.29[b]	11.50±2.44[a]
3-methyl-2-butanol	nd	2.19±0.18	Ethyl nonanoate	2.17±0.15[b]	6.58±1.60[a]
1-hexanol	0.13±0.01[b]	0.79±0.13[a]	Ethyl 9-decenoate	0.62±0.12	1.10±0.23
2,3-butanediol	0.17±0.01	0.20±0.05	Propyl decanoate	nd	0.22±0.07
1-octanol	0.11±0.01[b]	0.60±0.21[a]	Ethyl myristate	0.52±0.12	0.80±0.29
3-methylthio-1-propanol	nd	0.09±0.02	Ethyl palmitate	nd	1.09±0.27
1,3-propanediol	1.01±0.01[b]	0.08±0.02[a]	**Ketones**		
Phenylethyl alcohol	2.39±1.57[b]	0.07±0.01[a]	Methyl ethyl ketone	4.22±0.84	5.91±0.95
2-pentanol	0.96±0.13[b]	0.26±0.08[a]	2-Undecanone	nd	0.20±0.07
Carboxylic acids			2-tridecanone	nd	0.09±0.01
2-propenoic acid	nd	0.98±0.16	2-Pentadecanone	nd	0.08±0.01
Acetic acid	22.56±2.28	23.50±3.90	**Aldehydes**		
Propanoic acid	nd	3.60±1.99	Hexanal	0.08±0.01	0.10±0.01
Butyric acid	44.70±3.84[a]	28.38±4.23[b]	Butanal	0.08±0.03	0.12±0.03
Valeric acid	44.96±2.86	52.82±9.06	3-methylbutanal	0.06±0.01	0.12±0.01
Isovaleric acid	nd	43.72±1.86	Nonanal	0.12±0.01	0.11±0.02
Hexanoic acid	nd	71.17±3.88	Pentanal	0.08±0.01	0.09±0.11
Heptanoic acid	0.12±0.03[b]	0.67±0.13[a]	**Miscellaneous**		
Adipic acid	nd	0.59±0.11	Methanethiol	nd	0.33±0.07
Octanoic acid	3.31±0.95[b]	48.81±7.10[a]	Mercaptoethanol	nd	0.45±0.13
Nonanoic acid	nd	3.78±0.73	Dimethyl disulfide	nd	0.75±0.12
Decanoic acid	nd	38.37±0.28	Dimethyl trisulfide	nd	0.38±0.10
9-decanoic acid	nd	3.70±0.91	Phenol	nd	0.08±0.01
Dodecanoic acid	0.04±0.01[b]	7.81±1.43[a]	m-cresol	nd	0.61±0.12
Tetradecanoic acid	nd	3.87±0.71	delta-decalactone	0.6±0.10	1.01±0.11
Myristic acid	nd	3.83±0.26	n-dodecane	nd	0.13±0.01
Hexadecanoic acid	nd	1.89±0.23	Dodecane	nd	0.16±0.03
Hexanoic acid, 2-methyl	12.50±1.50[b]	57.82±6.50[a]	n-eicosane	nd	0.16±0.02
Butanoic acid, 2-methyl	nd	1.16±0.29	Tetradecane	nd	0.25±0.02
Esters			Hexadecane	nd	0.08±0.01
Ethyl acetate	0.25±0.04[b]	2.7±0.55[a]	All values are in mg/kg±SD.		

[a,b]Means in the same column followed by different superscript letters represent significant differences between cheese samples. p<0.05. nd: not detected.

detected only in the putrid cheese samples (Table 2). Zabaleta et al. (2016) reported that there is an association between fecal off-flavor and the presence of 3-methyl-1-butanol and methionol in ewe's raw milk commercial cheese samples. Methionol (3-Methylthio-1-propanol) may have a role in the formation of undesirable odor in the putrid cheese samples in our study.

Twelve esters were detected in the putrid cheese samples (Table 2). Ethyl esters made up the majority of the identified esters and their amounts were not remarkable. On the other hand, close amounts have been reported in studies on white brined cheese (Kondyli et al., 2012; Ozer et al., 2011; Sahingil et al., 2014). Therefore, esters cannot be associated with the odor problem.

Ketones originate from fatty acids by enzymatic oxidation and contribute to the aroma of cheese samples (Curionia and Bosset, 2002). In this study, four ketones were identified in putrid cheese samples (Table 2). Curionia and Bosset (2002) reported that 2-undecano, 2-tridecanone, and 2-pentadecanone have musty, goaty, and moisture odors, respectively. There is no study that ketones cause an undesirable change in cheese samples. Therefore, the bad odor in the putrid cheese samples cannot be caused by ketones.

Aldehydes are formed by the catabolism of amino acids (Fox et al., 1995). Only five aldehydes were determined in both types of cheese (Table 2). The levels of butanal and 3-methylbutanal were significantly ($p<0.05$) higher in the putrid cheese samples. Straight-chain aldehydes, such as butanal, nonanal, hexanal, and pentanal, that have green grass and herbaceous aromas are reported as active odorants in cheese samples (Curionia and Bosset, 2002). No relation was found between aldehydes and off-odor.

Twelve miscellaneous compounds, including sulfurs, phenols, hydrocarbons, and lactone, were identified in putrid cheese samples (Table 2). Delta-decalactone was only present in control cheese samples. Volatile sulfur compounds (VSCs), such as hydrogen sulfide, methanethiol, dimethyl sulfide, dimethyl disulfide (DMDS), dimethyl trisulfide (DMTS), methional, are important contributors to the flavor of fermented dairy products, especially cheese (Landaud et al., 2008). VSCs generally have low threshold values and are found in trace amounts in cheese (Sable and Cottenceau, 1999). Sulfur compounds may have a putrid, fecal-like aroma, and are commonly produced by microbial enzymes from amino acids (Curionia and Bosset, 2002; Liu and Crow, 2010; McSweeney and Sousa, 2000; Weimer et al., 1999). They give strong odor in many fermented and ripe foods, have boiled cabbage and cauliflower, meat, garlic, eggs, and rotten odors, and are produced from sulfur-containing amino acids methionine and cysteine. Although these components are found in parts per billion concentrations in cheese, they can give garlic, broccoli, and cooked cabbage odors (Bontinis et al., 2012). Methionine degradation occurs by cleaving the bond between carbon and sulfur with a methionine demethylase enzyme (Yvon and Rijnen, 2001). Methanethiol is formed by the degradation of methionine-containing peptides or by direct separation from the side chain of amino acid. Methanethiol is a precursor in the formation of sulfur components (McSweeney and Sousa, 2000; Molimard and Spinnler, 1996). Methanethiol can be converted to DMDS and DMTS via oxidative reactions, and it can react with carboxylic acids to form thioesters (Ardö, 2006).

It has been stated in the review by Curioni and Bosset (2002) that DMDS and DMTS components have sulfur, rotten, cooked cabbage-cauliflower odors in many studies. American Industrial Hygiene Association (2013) related these components with rotten odor in its publication about odor threshold values and determined the range of odor values of DMDS between 29×10^{-5} and 1.45 ppm. Frank et al. (2004) related methanethiol to fermented cabbage odor in their study about aroma components in cheese. The results of our study indicate that the off-odor may be associated with sulfur compounds.

Table 3. Rotated factor loadings for the first 2 principal component and commonalities varimax rotation

Variables	Factor 1	Factor 2	Commonality
Carboxylic acids	0.960	0.080	0.928
Miscellaneous	0.933	0.220	0.919
Esters	0.928	0.178	0.893
Aldehydes	0.870	0.163	0.783
Ketones	0.010	0.923	0.852
Alcohols	−0.443	−0.593	0.548

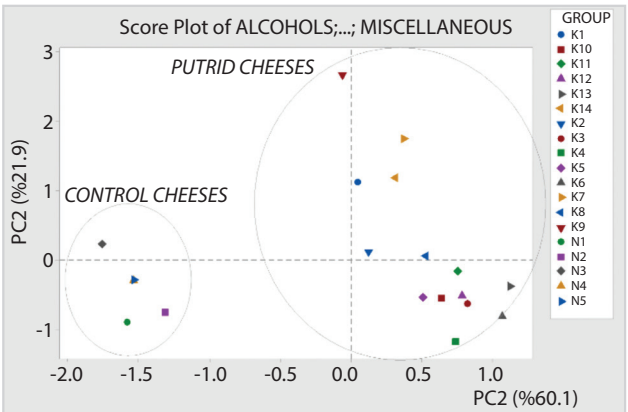

Figure 1. Score plot of the 2 first PC (PC1 and PC2) on putrid and control cheese samples

Principal component analysis

PCA was performed to differentiate the volatile profile of control and putrid cheese samples (Figure 1). Total concentrations of principle groups (acids, alcohols, esters, ketones, aldehydes, and various compounds) were used as variables.

The percentage of variability explained by component 1 (PC1) is 60.1%, and PC1 was defined by total carboxylic acids (0.960), esters (0.928), aldehydes (0.870), and miscellaneous compounds (0.933). All these compounds were located close on the chart and showed a positive correlation with each other (Figure 2). The principal component 2 (PC2) explained 21.9% of the total variability; ketones have large positive (0.923) and alcohols (−0.593) have large negative loadings on PC2 (Table 3). PC1 can be considered as the representative of putrid cheese samples because they are clustered on the positive side of PC1. PC2 is the representative of the control group cheese samples that were rich in alcohols and ketones because they are clustered on the negative side of PC2 (Figure 2).

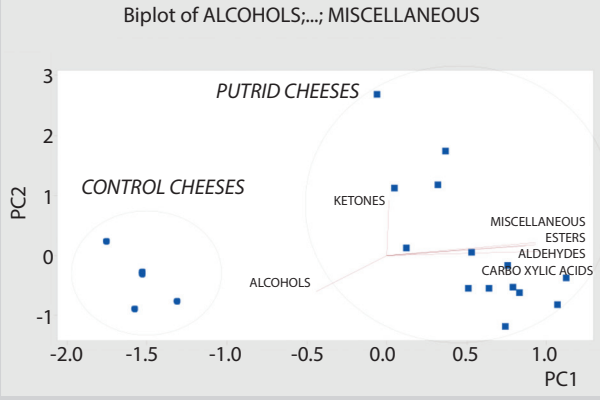

Figure 2. Biplot graph of the PCA

Conclusion

The work presented here provides first information on the off-odors of Turkish white cheese. The results of this study indicate that primary contributors to the off-odor were carboxylic acids (especially isovaleric acid) and sulfur compounds. The carboxylic acids derived from amino acids point out that there is an advanced level of proteolysis metabolism in the putrid cheese sample. These compounds, which are responsible for odors, such as sulfury, cabbage-like, and putrid, are formed through degradation of proteins as a result of metabolism of proteolytic bacteria. Thus, the malodor in cheese is directly related to the presence of undesirable microorganisms. Subsequent studies should focus on the presence of microorganisms that are responsible for spoilage in cheese and the development of methods to destroy these microorganisms.

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