The Use of Sour and Sweet Whey in Producing Compositions with Pleasant Aromas Using the Mold *Galactomyces geotrichum*: Identification of Key Odorants

Kamila Szudera-Kończal, Kamila Myszka, Piotr Kubiak, and Małgorzata A. Majcher*

**ABSTRACT:** Fermented products with a pleasant aroma and with strong honey, rose, and fruit odor notes were developed through the biotransformation of a medium containing sour or sweet whey with the addition of L-phenylalanine by the *Galactomyces geotrichum* mold. In order to obtain the strong honey-rose aroma, *G. geotrichum* strains were screened and fermentation conditions were optimized to achieve a preferable ratio (>1) of phenylacetaldehyde to 2-phenylethanol by the Ehrlich pathway. This allowed post-fermentation products with the ratio of concentrations of phenylacetaldehyde to 2-phenylethanol being 1.7:1. Additionally, the use of gas chromatography–olfactometry (GC–O) analysis and the calculation of odor activity values (OAVs) allowed 10 key odorants to be identified in post-fermentation products. The highest OAVs were found for phenylacetaldehyde with a honey odor in both sour and sweet whey cultures (3010 and 1776, respectively). In the variant with sour whey, the following compounds with the odorants to be identified in post-fermentation products. The highest OAVs were 3-(methylthio)-propanal (112), dimethyl trisulfide (37), and 2-phenylethanol (29). In the post-fermentation product with sweet whey, the following compounds with the highest OAVs were 3-methyl-1-butanol (131), 3-(methylthio)-propanal (119), 3-methylbutanal (90), dimethyl trisulfide (71), 2,3-butanedione (37), and 2-phenylethanol (29). In the post-fermentation product with sweet whey, the following compounds with the highest OAVs were 3-(methylthio)-propanal (112), dimethyl trisulfide (69), and 2,3-butanedione (41).

**KEYWORDS:** *Galactomyces geotrichum*, whey, fermentation, aroma-active compounds, GC–O, SIDA, OAV

**INTRODUCTION**

There has, in recent years, been a growing demand for flavorings in industry, especially in food production.¹⁻³ These compounds enrich the aroma of food products, which is especially important given that modern food production technologies often simplify and shorten fermentation processes. Chemical synthesis of aroma compounds is highly efficient and results in a relatively inexpensive product, but these have low acceptability among consumers, who prefer foods containing additives of natural origin. This means that there is a need for relatively efficient and straightforward technologies that can produce volatile compounds of natural origin. Particular attention should be paid to biotechnological processes that do not suffer from the difficulties attendant on the extraction of aroma compounds from natural materials (including seasonality and effect of climate change on the acquisition of the raw materials), which can make use of industrial byproducts.

*Galactomyces geotrichum* is a mold that occurs naturally in dairy products, which, according to our recent studies, is responsible for the formation of rose-like aroma compounds, such as phenylacetaldehyde, 2-phenylethanol, and phenylacetic acid.⁴⁻⁷ We have, moreover, noted that the concentration ratio of phenylacetaldehyde and 2-phenylethanol is unusually close to 1:1 in the fried cottage cheese we tested. Phenylacetaldehyde has a lower odor threshold (OT) than 2-phenylethanol in a range of food matrices (60 times lower in water, 10 times lower in oil, and 4 times lower in starch), which is why its highest content in the produced aroma is desirable.⁶,⁷

Whey is a yellowish liquid obtained during cheese production by separating the curd. About 160 million tons of whey is produced globally each year. The whey produced during cheese production is the most contaminated waste in this sector, which is why it is so important to develop new methods for managing it. During dairy production, sweet whey is obtained after enzymatic coagulation and sour whey comes from cottage cheese production. Depending on the type, whey contains 93–94% water, 4.5–6.0% lactose, 0.6–1.1% protein, 0.8–1.0% minerals, 0.05–0.9% lactic acid, and 0.06–0.5% fat.¹²,¹³,¹⁵ It should be noted that whey contains L-phenylalanine, which is a precursor to the rose-like aroma compounds formed in the Ehrlich pathway.¹⁴ In this study, both types of whey were analyzed, but sour whey is preferred to sweet on account of how it is produced: sour whey is formed during the production of cottage cheese when coagulation occurs by acidifying the milk to a pH of 5.1 or less. Fermentation by lactic acid bacteria results in more lactic acid than is produced in sweet whey, which affects the growth of *G. geotrichum*.³,¹²,¹³,¹⁵ In addition, sour whey appears to be a more natural environment for the development of *G. geotrichum* from fried cottage cheese. During the production of this cheese, the
environment is acidified by lactic acid bacteria, as with any kind of cottage cheese. Sour whey is formed in these types of processes, which is why it may also contain low-molecular-weight metabolites of lactic acid bacteria that can affect the metabolism of G. geotrichum. Furthermore, sour whey is more difficult to utilize than sweet whey, which is why we give special attention to new means of managing this byproduct.16

At present, there is increasing interest in the biotechnological production of aroma mixtures, rather than of individual aroma compounds,17 as mixtures can more closely resemble the products of natural processes that occur during food fermentation.3,17,18 In the present study, the final fermentation product has thus been treated as an aroma composition of several compounds, and all its key odorants have been identified, in order to find the compounds responsible not only for the honey-like and rose-like aroma notes but also for the fruit-like, caramel-like, or butter-like. Potential applications of this type of aroma are food products using fermentation processes such baking, brewing, and dairy industries. In this type of product, it is possible to utilize whey, and it is also desirable to enrich the aroma with honey-like and rose-like odor notes.19

The aim of this study was to determine the optimal conditions for the biotransformation by G. geotrichum of sour and sweet whey into post-fermentation products with an intense, honey-rose pleasant aroma. This is achieved by obtaining preferable ratio (>1) of phenylacetaldehyde to 2-phenylethanol through the Ehrlich pathway by screening of G. geotrichum strains and adjusting culture conditions. The post-fermentation product with the most intense, pleasant honey-rose-fruit aroma then underwent sensosomics analysis,17 which included identification of the key aroma compounds by gas chromatography–olfactometry (GC–O) and gas chromatography–mass spectrometry (GC–MS) and quantitation of the most important aroma compounds by the stable isotope dilution assay (SIDA) and by calculation of odor activity values (OAVs).

### MATERIALS AND METHODS

**Chemicals.** Yeast extract, sucrose, glucose, and medium with chloramphenicol were obtained from RITL (Lódź, Poland). Galactose was purchased from Acros Organics (Geel, Belgium). Citric acid, Na2HPO4·2H2O, and MgCl2 were obtained from POCH (Gliwice, Poland). L-Phenylalanine, lactic acid, sodium sulfate, dichloromethane, and diethyl ether were purchased from Sigma-Aldrich (Oschadzyn, Poland): the following reference aroma compounds were added per liter to the cultures: 1-phenylalanine, lactic acid, sodium sulfate, dichloromethane, and ethyl ether were purchased from Sigma-Aldrich (Poznań, Poland). Inulin was obtained from Hortimex Plus (Konin, Poland). Spray-dried sour and sweet whey products were purchased from Laktopol (Suwałki, Poland). The following reference aroma compounds were purchased from Sigma-Aldrich (Poznań, Poland): 2,3-butanedione, acetic acid, 3-methylbutanal, 3-methyl-1-butanol, butanoic acid, 3-(methylthio)-propanal, dimethyl trisulfide, phenylacetaldehyde, 2-phenylethanol, and phenylacetic acid. The following stable isotopes were obtained from aromaLAB (Freising, Germany): [13C2] 2,3-butanedione, [13C] acetic acid, [13C] 2-methylbutanal, [2H5] 3-methyl-1-butanol, [2H5] butanoic acid, [2H4] 3-(methylthio)-propanal, [2H6] dimethyl trisulfide, [2H6] phenylacetaldehyde, [2H4] 2-phenylethanol, and [2H6] naphthalene.

**Microorganisms.** Thirty nine G. geotrichum strains were isolated during the ripening stage of Wielkopolski fried cheese produced in the vicinity of Poznań in Poland. The isolated strains were identified by amplification of the 18S rRNA coding sequence, lyophilized, and deposited in the microbial culture collection of the Department of Food Chemistry and Instrumental Analysis.

**Screening of 39 G. geotrichum Strains.** Shake flask cultivations were carried out in 300 mL Erlenmeyer flasks with 100 mL of medium containing per liter (modified, based on Grygier et al.22) 22.8 g of Na2HPO4·2H2O, 10.3 g of citric acid, 0.5 g of MgCl2, and 0.17 g of yeast extract. After sterilizing the medium, 60 g of sucrose and 21 g of 1-phenylalanine were added per liter; these had been exposed to UV radiation for 30 min before use. This procedure was intended to prevent the formation of aroma compounds due to high temperatures. This medium was employed in all the subsequent experiments. Each flask was inoculated with 10 μL of a G. geotrichum strain, which had previously been revived by inoculating the agar slant with chloramphenicol (incubation at 30 °C for 72 h under aerobic conditions). Fermentation in flasks with 39 strains of G. geotrichum was carried out in a water bath at 30 °C for 7 days with shaking (150 rpm).

**Lyophilization of the G. geotrichum Strain.** The strain selected during screening was lyophilized to standardize inoculum concentration. Four flasks with the selected mold strain were prepared in the same way as during screening. Fermentation in the flasks was carried out in a water bath at 30 °C for 72 h with shaking (150 rpm). The cultures were centrifuged for 10 min at 2024g (3000 rpm) and 20 °C after fermentation. The G. geotrichum precipitate was resuspended in 400 mL of the mixture of base medium and 10% inulin solution (1:1, v/v). To determine the number of colony-forming units (CFUs), the culture was sequentially diluted and plated onto agar plates with chloramphenicol. The plates were incubated for 72 h in 30 °C, and the CFUs were then counted to calculate the number of colonies per milliliter of sample. The freeze-drying process was conducted in a Beta 1-16 freeze dryer (Martin Christ, Osterode am Harz, Germany). The process was initiated with a freezing step at −35 °C for 2 h, followed by the main drying stage at 15 °C for 20 h, and the final drying at 22 °C (5 h). The dried preparations were collected into glass jars under a nitrogen atmosphere, each of which received the product formed by freeze-drying 3 mL of culture.

**Optimization of Culture Conditions.** We analyzed the effects of the type of sugar, the pH of the medium, and the incubation temperature on the sensory profile of the aroma produced by the selected strain of G. geotrichum mold. Optimization was carried out on two types of media: the first variant was prepared using the base medium with sour whey, while the second used sweet whey. In both cases, spray-dried whey was used at a concentration of 130 g/L. Citric acid as a component of the nutrient was replaced at this stage with lactic acid, as this naturally occurs in whey.23 Once the medium had been prepared, the pH was adjusted to the set value in sterile conditions by adding lactic acid in the presence of litmus papers as pH indicators. Shake flask cultivations were carried out in 300 mL Erlenmeyer flasks with 200 mL of different medium variants. Each flask was inoculated with 3.4 × 104 CFU of lyophilized G. geotrichum mold.

**Type of Sugar.** We analyzed the effects of four types of sugar—sucrose, glucose, galactose, and fructose—on the aroma profile of the culture. Each type of sugar was added to the medium at a concentration of 60 g/L. As before, the UV-treated sugar was added to the sterilized medium. The pH of the media was adjusted to 5.0. Fermentation was carried out in a water bath at 30 °C for 7 days with shaking (150 rpm).

**pH Value.** The effect of pH on the culture was examined by carrying out fermentation on nutrient media with pH values of 3.0, 4.0, and 5.0. The type of sugar in the medium was sucrose. Fermentation was carried out in a water bath at 30 °C for 7 days with shaking (150 rpm).

**Incubation Temperature.** The incubation temperature was optimized by culturing G. geotrichum at three temperatures. The type of sugar in the medium was sucrose, and the pH value was 5.0. Incubation was carried out in water baths at different temperatures (25, 30, and 35 °C) for 7 days with shaking (150 rpm).

**Sensory Evaluation of Cultures.** Ten panelists experienced in descriptive sensory analysis carried out evaluation of the samples. The evaluation was run in triplicate in separate odor profiling sessions. The odor descriptors were selected from the basic flavor descriptive language (Givaudan Roure Flavor)20 and were determined in preliminary tests, which looked for the variant with the highest
possible ratio of phenylacetaldehyde to 2-phenylethanol. The qualities used were honey-like, caramel-like, rose-like, and fruit-like. The sensory panel also evaluated the general desirability of the aroma. Sensory analysis was performed by scoring odor descriptors on a 10 cm linear scale, with the beginning labeled “none” and the end labeled “very strong”. We collected 20 g of G. geotrichum samples from the various culture conditions and placed them in 100 mL glass containers. These were presented to the panelists at room temperature. The sensory evaluation of samples obtained from the bioreactor cultures was carried out in the same way except that, in addition to the previously mentioned odor descriptors, the following notes were also assessed: butter-like, cheese-like, and sour-like. The results were converted to numerical values for data analysis.

**Bioreactor Cultures.** For the biotechnological production of aroma composition, selected G. geotrichum mold culture parameters were used in a laboratory-scale bioreactor. Bioreactor cultures were carried out in a 2.3 L Labfors 5 bioreactor (Infors HT, Bottmingen, Switzerland) with a working volume of 2 L and aerated to 1.5 vvm, with the inlet air sterilized by filtration. The medium prepared in the same way as for previous flask cultures was stirred at a speed of 150 rpm. The batch fermentations were carried out in two variants, with sour whey and with sweet whey, and each variant was inoculated with 3.4 × 10^5 CFU of lyophilized G. geotrichum molds. The incubation temperature, medium pH (determined at the beginning of the culture), and type of sugar were determined in relation to the results obtained during the optimization of the culture parameters. The pH of the medium, as with the flask culture, was determined using lactic acid before inoculation and was not regulated during the experiment. Although the pH value was not actively controlled throughout the fermentation process, it was measured at the end with a value of 4.7. For each variant, also blank tests were performed with the same culture conditions, without inoculation of the medium with G. geotrichum.

**Headspace Solid-Phase Microextraction (HS-SPME).** HS-SPME was used as an isolation method to determine the levels of phenylacetaldehyde and 2-phenylethanol in samples collected during the screening of the 39 G. geotrichum strains. This employed divinylbenzene/Carboxen/polydimethylosiloxane (DVB/CAR/PDMS) fiber (Supelco, Bellefonte, PA, U.S.A.) in combination with an MPS 2XL multipurpose sampler (GERSTEL, Mülheim an der Ruhr, Germany). Samples (10 g) were placed in 20 mL headspace vials, spiked with internal standards (d₆-phenylacetaldehyde and d₂-2-phenylethanol) to a concentration of 500 ppb each, and sealed with septum magnetic caps. The compounds were extracted from headspace of the vials at 40 °C for 30 min. Afterward, the analytes were desorbed in the splitless mode at the GC injection port at 250 °C.

**Solvent-Assisted Flavor Evaporation (SAFE).** Odor-active compounds were isolated from the fermentation broth obtained by biotransformation of the sour and sweet whey in the bioreactor using the SAFE method described by Engel et al.¹¹ Samples (50 g) were mixed with dichloromethane (100 mL) and spiked with the internal standard [²H₅] naphthalene (25 µg). The samples were then extracted for 2 h by shaking in the horizontal shaker, and the volatiles were isolated by SAFE extraction. In the next step, the extracts were dried over anhydrous sodium sulfate. Finally, the extracts were concentrated to approximately 500 µL using a Kuderna Danish concentrator (Sigma-Aldrich, Poznań, Poland).

**GC−O.** SAFE extracts underwent GC−O analysis to identify odor-active compounds, using an HP 5890 chromatograph (Hewlett-Packard, Wilmington, DE, U.S.A.) with two columns of different polarities: SPB-5 (30 m × 0.53 mm × 1.5 µm) and SUPELCOWAX 10 (30 m × 0.53 mm × 1 µm) (Supelco, Bellefonte, PA, U.S.A.). The effluent was divided between the olfactometry port with humidified air as a makeup gas and a flame ionization port using a Y splitter in GC. The operating conditions for the SPB-5 were as follows: an initial oven temperature of 40 °C (1 min) raised at 6 °C/min to 180 °C and at 20 °C/min to 280 °C. For SUPELCOWAX 10 column, the operating conditions were as follows: an initial oven temperature of 40 °C (2 min) raised to 240 °C at a 6 °C/min rate and held for 2 min isothermally. Injection of the SAFE extract (2 µL) into a GC column was using a splitless mode. GC-effluent sniffing (GC−O) was carried out by three panelists who detected odor-active regions and specified notes for the analyzed volatiles. For all peaks and flavor descriptors with specific retention times, retention indices were calculated in order to compare results with those obtained by GC−MS and literature data. For each compound, the retention indices (RIs) were calculated using a homologous series of C₇−C₉ n-alkanes.

**GC−MS.** The analyzed chemical compounds were identified and quantified using a 7890A GC (Agilent Technologies, Santa Clara, CA, U.S.A.) coupled to a 5975C MSD (Agilent Technologies, Santa Clara, CA, U.S.A.).

**GC−MS after SAFE Extraction.** The apparatus was equipped with two columns: SUPELCOWAX 10 (30 m × 0.25 mm × 0.25 µm) and an SLB-5Ms (30 m × 0.25 mm × 0.5 µm) (Supelco, Bellefonte, PA, U.S.A.). Helium was used as a carrier gas with a flow of 32.2 cm/s. The temperature programs were the same as for GC−O. Mass spectra were recorded in an electron impact mode (70 eV) in a scan range of m/z 33−350. Compounds were identified by comparing their mass spectra, RIs, and flavor notes on two columns of different polarities to those of standard compounds, National Institute of Standards and Technology (NIST) 09 Mass Spectral Library and literature data.

**HS−SPME−GC−MS.** This analysis was carried out using an HP-5ms column (30 m × 0.18 mm × 0.3 µm) (Hewlett-Packard, Wilmington, DE, U.S.A.) operating as follows: an initial oven temperature of 40 °C (1 min) raised at 9 °C/min to 200 °C and at 15 °C/min to 280 °C and held for 6 min isothermally. The other GC−MS conditions and the compound identification process were the same as for SAFE extract analysis.

**Quantitation by Stable Isotope Dilution Assays (SIDA).** All compounds identified during GC−O analysis were quantified by the SIDA method. To this end, stock internal standards of the labeled isotopes were prepared in diethyl ether and added to the samples of post-fermentation products after bioreactor culture, in concentrations similar to that present in the post-fermentation broth for each compound. The samples prepared in this way were extracted by the SAFE method. The identified aroma compounds were analyzed by GC−MS, monitoring the intensity of the respective ions presented in Table 1. For all volatiles, response factors were calculated in the standard mixture of labeled and unlabeled compounds at a known concentration of 500 ppb. In the case of 3-methylbutanol, for which the quantitation by stable isotopic dilution was impossible, isotope dilution isotherms were prepared in diethyl ether and added to the samples of post-fermentation products after bioreactor culture, in concentrations similar to those of standard compounds, National Institute of Standards and Technology (NIST) 09 Mass Spectral Library and literature data.

**Table 1. Labeled Standards and Quantitation Ions Used for SIDA (Stable Isotope Dilution Assay) Concentration Calculations of 10 Key Odorants Present in the Post-Fermentation Product from the Bioreactor Culture**

| compound          | quantitation ions (m/z)  | labeled standards (m/z)  | ion IS (m/z)  |
|-------------------|--------------------------|--------------------------|--------------|
| 2,3-butanedione   | 86                       | [¹³C₁₂] 2,3-butanedione  | 90           |
| acetic acid       | 63                       | [¹³C₁₂] acetic acid      | 61           |
| 3-methylbutanal   | 86                       | [²H₁₆] 3-methylbutanal   | 89           |
| 3-methyl-1-butanol| 70                       | [²H₅] 3-methyl-1-butanol| 72           |
| butanoic acid     | 73                       | [¹³C₁₂] butanoic acid    | 77           |
| 3-(methylthio)-propanal | 104                  | [¹³C₁₂] 3-(methylthio)-propanal | 107 |
| dimethyl trisulfide| 126                     | [²H₅] dimethyl trisulfide| 132          |
| phenylacetaldehyde| 122                     | [²H₅] phenylacetaldehyde| 125          |
| 2-phenylethanol   | 127                      | [²H₅] 2-phenylethanol    | 127          |
| phenylacetic acid | 136                      | [²H₅] 2-phenylethanol    | 127          |

*Here, *ions of analytes used for quantitation, *b*ions of internal standard IS (isotopologues) used for quantitation.
no direct isotopologue was available, we used a 2-methylbutanal isotopologue, which in our opinion is the most similar to this compound. We then applied the correction using the response factor. The concentrations of the analyzed volatiles in the samples were calculated using the peak area of the analyte and its corresponding internal labeled standard obtained for selected ions. The odor activity values (OAVs) were then calculated by dividing the concentration of a given analyte by its odor threshold (OT) value determined in water. The results obtained for the blank samples were subtracted from the results obtained for the sour and sweet whey products.

■ RESULTS AND DISCUSSION

Screening of *G. geotrichum* Strains. In the first experiment, 39 strains of *G. geotrichum* molds were characterized in terms of the ratio of phenylacetaldehyde to 2-phenylethanol. This experiment was intended to find those strains with the ability to produce more phenylacetaldehyde than 2-phenylethanol through the Ehrlich pathway. When L-phenylalanine is transformed, both compounds are usually produced and are present at the same time in the fermentation products; however, in most cases, 2-phenylethanol dominates in quantity over phenylacetaldehyde. Reversing the ratio to give higher amounts of phenylacetaldehyde would increase aromatization power. The results in Figure 1 thus illustrate the ratio of phenylacetaldehyde to 2-phenylethanol obtained during fermentation. It can be seen that most strains produced significantly greater amounts of 2-phenylethanol than phenylacetaldehyde. However, for strains 32 and 36, the ratios of phenylacetaldehyde to 2-phenylethanol were 1.6:1 and 1.1:1, respectively. Based on these results, strain 32 was selected for further study.

Optimizing Culture Conditions. The aim of this experiment was to optimize the culture conditions so as to increase the pleasant honey, rose-like aroma produced by the Ehrlich pathway from L-phenylalanine. To this end, the type of sugar added, the pH, and the incubation temperature were recorded. Each culture variant was subjected to a sensory analysis with four target odor descriptors: honey-like, rose-like, fruit-like, and caramel-like and general desirability.

The effect of the type of sugar in the medium on the sensory quality of the post-fermentation product is outlined in Figure 2. Our preliminary studies showed that *G. geotrichum* molds do not ferment lactose, which is why sucrose, glucose, galactose, and fructose were used in the experiment as nutrient ingredients. The results in Figure 2 illustrate that for both fermentation products, the panelists ranked general desirability highest when sucrose was used. In the sour whey culture, the broadest aromatic profile was achieved for the variants with sucrose and with galactose (Figure 2A). Both fermentation products gave strong honey-like and rose-like notes. On the other hand, when using fructose and glucose in a sour whey

---

**Figure 1.** Peak areas of phenylacetaldehyde and 2-phenylethanol extracted from post-fermentation products obtained by biotransformation of the base medium by 39 strains of *G. geotrichum* by SPME.

**Figure 2.** (A, B) Sensory profiles of post-fermentation products from flask cultures with (A) sour whey and (B) sweet whey for various types of sugars.
fermentation, the aroma was the weakest for all descriptors. In the case of the sweet whey culture (Figure 2B), again the sucrose variant had the most diverse odor profile, with strong honey-like, rose-like, and caramel-like notes. The use of glucose and fructose as components of the sour whey medium lowered the intensity of the rose-like and caramel-like notes, while the addition of galactose was the least appreciated by the panelists, who in this case ranked all the descriptors lowest. Taking these results into consideration, sucrose was selected as the carbon source that yielded the most pleasant aroma with strong honey and rose-like odor notes.

The presence of rose-like aroma is typical of certain types of cheeses and fermented food products, such as black tea and pumpernickel bread. It has been well established that during L-phenylalanine transformation, honey-rose aroma compounds are produced, including phenylacetaldehyde, 2-phenylethanol, phenylactic acid, and 2-phenylethylacetate. These compounds are formed in the Ehrlich pathway. Although the formation pathway of these compounds is interdependent, they are rarely all present in one product at a time. It should be noted that there are dependencies between the compounds arising in the Ehrlich pathway that affect the characteristics of the aroma. According to Whetstone et al., phenylacetaldehyde plays a more important role in shaping the rose aroma than does phenylacetic acid. However, it has been shown that phenylacetaldehyde, in combination with even a small amount of phenylacetic acid, is characterized by a much higher intensity of rose aroma than the sum of individual impressions of these compounds. This phenomenon is most pronounced at high concentrations of phenylacetaldehyde in the product. According to Dunkel et al., phenylacetaldehyde and 2-phenylethanol are equally often recognized as key odorants as in 22.5 and 22.9%, respectively, of food products. Numerous studies of fermented products have shown that both those compounds are formed during fermentation and are present in the final product. For example, Schuh and Schieberle identified the phenylacetaldehyde and 2-phenylethanol formed during fermentation of black tea leaves to have a concentration ratio of 0.3:1. On the other hand, in our research on ripened cheese, we found the concentration of phenylacetaldehyde and 2-phenylethanol with a ratio of 0.7:1, which correlated with the sensory analysis and honey-rose flavor. Further analysis confirmed that it is *G. geotrichum* that is responsible for this biotransformation and for the formation of phenylacetaldehyde and 2-phenylethanol. This also reinforces the fact that phenylacetaldehyde has stronger aromatization power than 2-phenylethanol and therefore has higher potential to bring desirable rose-like aroma to fermented products.

In our study, the presence of L-phenylalanine in the medium as a component of whey and as a nutrient ingredient for *G. geotrichum* allows the formation of honey-like and rose-like compounds such as phenylacetaldehyde, 2-phenylethanol, phenylactic acid, and 2-phenylethylacetate. *G. geotrichum* grows over broad ranges of temperature and pH, although 25–30 °C and pH 5.0–5.5 are suggested as optimal. We tested three different incubation temperatures (25, 30, and 35 °C) to find which is optimal for the growth of *G. geotrichum* and for the honey-rose aroma. The sensory profiles obtained were the broadest and had the strongest honey-like note for the cultures grown at 30 °C, for both sour and sweet whey variants. Panelists noted the presence of a strong rotten fruit note in the samples incubated at 25 °C, while at 35 °C, a very poorly perceptible aroma was obtained.

Comparing the effects of pH values of 3.0, 4.0, and 5.0 on the aroma showed that the most intense honey-rose odor profile was obtained by incubation on medium at pH 5.0, again for both types of whey. The aroma produced using the medium of pH 4.0 was about half as intense as at pH 5.0, while the culture at pH 3.0 had the least intense sensory profile.

The results for the individual descriptors are consistent with the assessments of general desirability for the variants we tested. In both sour and sweet whey cultures, samples with medium at pH 5.0 and those with an incubation temperature of 30 °C were most desirable by 6 to 7 points. In comparing the type of sugar in the medium, the highest values of desirability were achieved with sucrose in both whey variants. It should be noted that neither incubation temperature nor pH value caused as large a difference as did sugar type.

Based on the sensory evaluation and quantitative analysis of the aroma compounds in further experiments, the following were selected as the ideal culture conditions: sucrose in the medium, medium pH at 5.0, and an incubation temperature of 30 °C.

**Sensory Evaluation of Bioreactor Cultures.** Having determined the optimal culture parameters, the fermentation process was moved to a larger scale with a 2.3 L bioreactor using media with sour and sweet whey. In the first stage, the aroma compositions we obtained underwent extended sensory evaluation, the results of which are given in Figure 3; these show that both compositions had strong honey and rose aromas, with a slight advantage for the sour whey. In addition, the sour whey culture had a more intense fruit-like aroma, while the sweet whey sample showed a significantly less intense caramel-like note. Butter-like and cheese-like flavor notes were barely perceptible in either variant, whereas the sour whey culture showed a sour-like aroma of the medium intensity. The difference between the odor profiles of the bioreactor cultures and the flask cultures may be associated with the more carefully controlled conditions in the bioreactor, such as regarding aeration.

**Identification of Key Aroma Compounds in a Post-Fermentation Product by Means of GC–O Analysis and...**

---

**Figure 3.** Sensory profile of post-fermentation products from bioreactor cultures with sour and sweet whey.
Table 2. Key Odorants Identified in Post-Fermentation Products from Sour and Sweet Whey from the Bioreactor Culture

| Compound          | Odor            | IR-DB 5 | IR-wax | concentration (μg/kg) | OT in water (μg/kg) | OAV* |
|-------------------|-----------------|---------|--------|----------------------|--------------------|------|
| 2,3-butanedione   | buttery         | 670     | 991    | 550 ± 11             | 610 ± 12           | 15   |
| acetic acid       | vinegar         | 691     | 1445   | 250,120 ± 128        | 5126 ± 89          | 99,000 |
| 3-methylbutanal   | malty           | 695     | 948    | 45 ± 2               | 0.8 ± 0.05         | 0.5  |
| 3-methyl-1-butanol| fruity          | 719     | 1204   | 121,511 ± 498        | 1546 ± 57          | 980  |
| butanonic acid    | cheesy          | 836     | 1620   | 18,523 ± 212         | 17,450 ± 400       | 1000 |
| 3-(methylthio)-propanal | boiled potato | 908     | 1458   | 51 ± 0.8             | 48 ± 0.7           | 0.43 |
| dimethyl trisulfide| cabbage        | 985     | 1377   | 7 ± 0.05             | 6.8 ± 0.05         | 0.099|
| phenylacetaldehyde| honey           | 1080    | 1644   | 12,040 ± 131         | 7105 ± 95          | 4    |
| 2-phenylethanol   | rosy            | 1124    | 1920   | 7090 ± 91            | 4125 ± 52          | 240  |
| phenylacetic acid | honey           | 1260    | 2568   | 1725 ± 32            | 3218 ± 38          | 1000 |

*Compounds identified by comparison with reference compounds on the basis of the following criteria: retention index (RI), mass spectra obtained by MS (EI), and odor quality at the sniffing port. Odor perceived at the sniffing port. Retention indices on BD-5 and SUPELCOWAX 10 columns. Mean of triplicates ± standard deviations. OT: odor thresholds in water. OAV: odor activity values calculated by dividing the concentration of an analyte by its odor threshold value.

Calculation of OAVs. In the SAFE extracts prepared from samples obtained after bioreactor fermentation of sour and sweet whey mediums by *G. geotrichum*, 10 compounds were identified by GC–O analysis. All were then quantified, and the OAVs were calculated for them by dividing the concentration of the analyte by its odor threshold value. The results in Table 2 show that for both whey variants, the highest OAVs were calculated for sweet whey, the concentration of 2-phenylethanol at 1892 μg/kg, whereas in fermented cocoa beans, this ratio was 0.7:1:0.2 after 4 days of ripening, with the concentration of 2-phenylethanol at 1892 μg/kg, whereas in fermented cocoa beans, this ratio was 0.03:1:2.1 and 2-phenylethanol was at a concentration of 2100 μg/kg.\(^\text{13}\) In the drink produced by fermentation of the wort by *Trametes versicolor*, all three compounds were found in a ratio of 5.2:1:2.7. The concentration of 2-phenylethanol in the analyzed product was 31 μg/L, and despite the highest OAV content in the aroma tested for phenylacetaldehyde, it was characterized by only a slight floral aroma.\(^\text{29}\) It should be noted that phenylacetaldehyde, 2-phenylethanol, and phenylacetic acid are found in fermented products together but that their levels may vary greatly, depending on the type of the substrate and the conditions of the fermentation process.\(^\text{27}\) An example is the traditional Chinese fermented red pepper paste that contains phenylacetaldehyde and 2-phenylethanol in a ratio of 0.05:1 (at a 2-phenylethanol concentration in the product of 129.22 μg/kg) and no phenylacetic acid at all.\(^\text{34}\) However, it should be noted that in the case of both phenylacetaldehyde and 2-phenylethanol, the post-fermentation product with sour whey contained 1.7 times more of these compounds than in the case of sweet whey.

The aroma compounds discussed earlier are important odorants in the aroma produced by *G. geotrichum*; however, it should be noted that human perception of mixtures of various flavor compounds is not just the sum of their individual aroma notes.\(^\text{17,35}\) It has been shown that mixtures containing more than four odorants are characterized by the loss of individual fragrance notes for each compound in order to create a specific perception of the entire aroma.\(^\text{37}\) Most aromas of natural origin are complex mixtures of different odorants, as is the aroma produced by *G. geotrichum* on the test substrates.\(^\text{38}\) Another example is ripe honey in which 2,3-butanedione, with its butter aroma (typical of fermentation processes), is formed as a result of natural transformations; dimethyl trisulfide with its cabbage-like aroma and (E)-β-damascenone with its scent of cooked apples are also produced. These compounds are characterized by a relatively high OAV, but the aroma as a whole is not directly related to any of these descriptors.\(^\text{12,25,26}\) Another compound identified as one of the key odorants in post-fermentation products is 3-methyl-1-butanol, with its fruit aroma. This is the second most abundant compound (121,511 μg/kg) in samples based on sour whey and is characterized by a relatively high OAV (131), whereas in the variant with sweet whey, it is present at 1546 μg/kg, being the sixth most abundant compound. Its formation is associated with fermentation processes, and its concentration increases to a maximum of 5600 μg/kg during three-stage sourdough fermentation, which is part of the preparation of pumpernickel bread.\(^\text{24}\) This compound is present in fermented beverages, such as wine and cider.\(^\text{37–39}\) It is produced by the microorganisms associated with grapes grown for wine
production. It is the substance found in the highest concentration in the aromas produced by Paenibacillus sp. and Aerobacillus pullulans, and the second highest in the aromas is produced by Sporobolomyces roseus. In apple cider, after fermentation by Hanseniaspora osmophila and its mixed culture with Torulaspora quercuum, 3-methyl-1-butanol was found in the concentration from 3.56 to 4.65 μg/L. This compound is also produced by Staphylococcus xylosus, which gives its characteristic aroma to fermented meat products. Other compounds identified in high concentrations in the cultures tested here are acetic acid and butanoic acid with their odors of vinegar and cheese, respectively. Acetic acid has been shown to be formed during enzymatic degradation in the fermentation process of fruit pulp. Butanoic acid is also a characteristic fermentation product formed during the transformation of carbohydrates. In addition, subjecting whey to controlled fermentation processes could be a way of producing acetic or butanoic acid. In samples with sour whey, acetic acid is the key odorant found in the highest concentration, but due to its high OT value, this results in an OAV of 2.5—the lowest value after 2-phenylethanol. In the sweet whey variant, acetic acid is present at a concentration lower than the OT value, which gives OAV < 1. However, butanoic acid is present in a similar concentration in both sweet and sour whey cultures (at 18,523 and 17,450 μg/kg, respectively). Another compound identified as a key odorant is 2,3-butanedione, with its aroma of butter, which is present at 550 and 610 μg/kg in the sour and sweet whey variants, respectively. This compound is present in various types of cheese due to bacterial or mold fermentation, being a key aroma compound in fried cottage cheese, Lazur, Camembert, Cheddar, and Emmental cheeses. The compound with the eighth highest concentration in both culture variants (51 and 48 μg/kg, respectively, for sour and sweet whey) was identified as 3-(methylthio)-propanal, which has a smell of boiled potatoes. This compound is characterized by a low OT value, which resulted in the third and second highest OAVs for the sour and sweet whey variants, respectively. Both 3-(methylthio)-propanal and 3-methylbutanial with its smoky aroma (found at 45 and 0.8 μg/kg in the sour and sweet whey, respectively) are formed during Strecker degradation from methionine and leucine, respectively. This reaction can also be carried out by means of an enzymatic reaction in the Ehrlich pathway. It has been demonstrated during the preparation of bread dough that increasing the amount of yeast and applying proper fermentation conditions (reduced temperature and shorter time) increased the content of 3-(methylthio)-propanal in the bread. 3-Methylbutanal is present in high concentrations in soy sauce due to the formation during the fermentation process of large amounts of free amino acids, which are precursors of this compound. The final key odorant determining the aroma produced by G. geotrichum molds is dimethyl trisulfide, with the fifth highest OAV for the sour whey and the third OAV for the sweet whey. The cabbage odor note of this compound was not strongly perceptible in the aroma studied, but it significantly affects the overall composition. According to the review on key odorants, the following compounds: acetic acid, butanoic acid, 2,3-butanedione, 3-(methylthio)-propanal, and 3-methylbutanial identified as key odorants in the post-fermentation product analyzed here contributed to the aroma of more than 25% of 227 food samples and therefore belong to the “generalist” group. Combined analysis of the results from GC–O analysis, from the OAVs, and from sensory evaluation show that phenylacetaldehyde is responsible for the honey note and 2-phenylethanol for the rose note in the aroma produced by the G. geotrichum mold. The occurrence of a delicate buttery note can be associated with 2,3-butanedione. 3-Methyl-1-butanol is a compound that is present in a higher concentration in cultures with sour whey, with this variant also characterized by a more intense fruity note, so the presence of this compound should be associated with the aroma. Acetic acid is responsible for the more intense sour aroma note in the bioreactor culture with sour whey, present in this case in a higher concentration than in the sweet whey. It is hard to link the rest of the odor descriptors with the specific aroma compounds responsible for their appearance, probably as a result of the combination of several compounds, individually described by other notes.

In summary, we determined the optimal conditions for culturing G. geotrichum, resulting in post-fermentation products with an intense honey-like, pleasant aroma. The screening of G. geotrichum strains and optimization of fermentation conditions were conducted with the aim of achieving the preferred ratio (>1) of phenylacetaldehyde to 2-phenylethanol in the Ehrlich pathway. Strain 32 was chosen in the case of both medium supplements, and the selected culture conditions were sucrose as the medium component, a pH of 5.0, and an incubation temperature of 30 °C. Further experiments in a 2.3 L bioreactor gave fermented products with strong honey-like, rose-like, and fruit-like aromas. The application of GC–O analysis and the calculation of OAVs allowed 10 key aroma compounds to be identified in the post-fermentation culture. Phenylacetaldehyde with its honey aroma had the highest OAV among all the key odorants in both the sour and sweet whey variants. In addition, in both culture variants, we determined the presence of phenylacetaldehyde and 2-phenylethanol, responsible for honey and rose odor notes, respectively, in a ratio of 1.7:1, which resulted in a much more intense aroma. However, the concentration of these compounds was 1.7 times higher in the product with sour whey than with sweet whey. In conclusion, our results show that fermentation with G. geotrichum on a medium with sour or sweet whey gives the possibility to obtain a post-fermentation product with strong honey-like and rose-like aromas due to the high ratio of phenylacetaldehyde to 2-phenylethanol formed by the Ehrlich pathway.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.0c03979.

TIC chromatogram of SAFE extract obtained from sour whey fermentation by G. geotrichum in a 2L bioreactor (PDF)

AUTHOR INFORMATION

Corresponding Author

Malgorzata A. Majcher – Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Poznań 60-624, Poland; orcid.org/0000-0003-4234-7881; Phone: +48618487398; Email: malgorzata.majcher@up.poznan.pl; Fax: +48618487314
Journal of Agricultural and Food Chemistry

Authors
Kamila Szudera-Kończal – Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Poznań 60-624, Poland
Kamila Myszka – Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Poznań 60-624, Poland
Piotr Kubiak – Faculty of Food Science and Nutrition, Poznań University of Life Sciences, Poznań 60-624, Poland

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.0c03979

Funding
This research was financed by the National Science Center, Poland (Project 2017/25/B/NZ9/01520).

Notes
The authors declare no competing financial interest.

ABBREVIATIONS
GC–O, gas chromatography–olfactometry; HS-SPME, headspace solid-phase microextraction; OAV, odor activity value; OT, odor threshold; GC–MS, gas chromatography–mass spectrometry; SIDA, stable isotope dilution assay

REFERENCES
(1) Krings, U.; Berger, R. G. Biotechnological Production of Flavours and Fragrances. Appl. Microbiol. Biotechnol. 1998, 49, 1–8.
(2) Celisista, E.; Olkowicz, M.; Grajek, W. L-Phenylalanine Catabolism and 2-Phenylethanol Synthesis in Yarrowia Lipolytica: Mapping Molecular Identities through Whole-Proteome Quantitative Mass Spectrometry Analysis. FEMS Yeast Res. 2015, 15, 1–18.
(3) Sales, A.; Paulino, B. N.; Pastore, G. M.; Bicas, J. L. Biogeneration of Aroma Compounds. Curr. Opin. Food Sci. 2018, 19, 77–84.
(4) Etschmann, M.; Bluemke, W.; Sell, D.; Schrader, J. Biotechnological Production of 2-Phenylethanol. Appl. Microbiol. Biotechnol. 2002, 59, 1–8.
(5) Bicas, J. L.; Silva, J. C.; Dionisio, A. P.; Pastore, G. M. Biotechnological Production of Bioflavors and Functional Sugars. Cínc. Tecnol. Aliment. 2010, 30, 7–18.
(6) Grygiér, A.; Majcher, M.; Myszka, K. Analysis of the Ability to Form 2-Phenylethanol by Galactomyces Geotrichum MK017. Zynom. Nauk. Technol. Zakop. 2015, 21, 74–83.
(7) Majcher, M. A.; Myszka, K.; Kubiak, J.; Jelen, H. H. Identification of Key Odorants of Fried Cottage Cheese and Contribution of Galactomyces Geotrichum MK017 to the Formation of 2-Phenylethanol and Related Rose-like Aroma Compounds. Int. Dairy J. 2014, 39, 324–329.
(8) Grygiér, A. Malo Poznany Grzyb Pleśniowy Galactomyces Geotrichum w Wyrobach Mlecznych. Przem. SPOŻYWCA 2016, 1, 30–34.
(9) Majcher, M. A.; Myszka, K.; Gracka, A.; Grygiér, A.; Jelen, H. H. Key Odorants of Lazur, a Polish Mold-Ripened Cheese. J. Agric. Food Chem. 2018, 66, 2443–2448.
(10) Pollner, G.; Schieberle, P. Characterization of the Key Odorants in Commercial Cold-Pressed Oils from Unpeeled and Peeled Rapeseeds by the Sensomics Approach. J. Agric. Food Chem. 2016, 64, 627–636.
(11) Sahin, B.; Schieberle, P. Characterization of the Key Aroma Compounds in Yeast Dumplings by Means of the Sensomics Concept. J. Agric. Food Chem. 2019, 67, 2973–2979.
(12) Frazeres, A. R.; Carvalho, F.; Rivas, J. Cheese Whey Management: A Review. J. Environ. Manage. 2012, 110, 48–68.
(13) Conde-Báez, L.; Castro-Rosas, J.; Villagómez-Ibarra, J. R.; Páez-Lerma, J. B.; Gómez-Aldapa, C. Evaluation of Waste of the Cheese Industry for the Production of Aroma of Roses (Phenylethyl Alcohol). Waste Biomass Valoriz. 2017, 8, 1343–1350.
(14) Nakada, H.; Ohata, M.; Hosaka, M.; Ochi, H.; Abe, F.; Arita, K. Investigation of Potent Odorants Generated during the Production of Whey Protein Hydrolysates. Anim. Sci. J. 2018, 89, 1348–1354.
(15) Chaves-López, C.; Serio, A.; Rossi, C.; Pepe, A.; Compagnone, E.; Paparella, A. Interaction between Galactomyces Geotrichum KL208, Lactobacillus Plantarum LAT3 and Enterococcus Faecalis KE06 during Milk Fermentation. Fermentation 2017, 3, 52.
(16) Tsai, S.-B.; Yuan, Z.; Yu, J.; Liu, X. Waste Management Techniques for Improved Environmental and Public Health: Emerging Research and Opportunities; IGI Global: Hershey, 2020, DOI: 10.4018/978-1-7998-1966-0.
(17) Dunkel, A.; Steinhaus, M.; Kotthoff, M.; Nowak, B.; Krautwurst, D.; Schieberle, P.; Hofmann, T. Nature’s Chemical Signatures in Human Olfaction: A Foodborne Perspective for Future Biotechnology. Angew. Chem., Int. Ed. 2014, 53, 7124–7143.
(18) Longo, M. A.; Sanromán, M. A. Production of Food Aroma Compounds: Microbial and Enzymatic Methodologies. Food Technol. Biotechnol. 2006, 44, 335–353.
(19) Krolczyk, J. B.; Dawidziuk, T.; Janiszewska-Turak, E.; Solowiej, B. Use of Whey and Whey Preparations in the Food Industry - A Review. Pol. J. Food Nutr. Sci. 2016, 66, 157–165.
(20) Stampanoni, C. R. The Quantitative Flavor Profiling Technique. Perfect Flavor. 1993, 18, 19–24.
(21) Engel, W.; Bahr, W.; Schieberle, P. Solvent Assisted Evaporation - A New and Versatile Technique for the Careful and Direct Isolation of Aroma Compounds from Complex Food Matrices. Eur. Food Res. Technol. 1999, 237.
(22) Whetstone, M. E. C.; Cadwallader, K. R.; Drake, M. Characterization of Aroma Compounds Responsible for the Roxy/ Floral Flavor in Cheddar Cheese. J. Agric. Food Chem. 2005, 53, 3126–3132.
(23) Schuh, C.; Schieberle, P. Characterization of the Key Aroma Compounds in the Beverage Prepared from Darjeeling Black Tea: Quantitative Differences between Tea Leaves and Infusion. J. Agric. Food Chem. 2006, 54, 916–924.
(24) Majcher, M. A.; Olszak-Ossowska, D.; Szudera-Kończal, K.; Jelen, H. H. Formation of Key Aroma Compounds during Preparation of Pumpernickel Bread. J. Agric. Food Chem. 2019, DOI: 10.1021/acs.jafc.9b06220.
(25) Rivasio, D.; Wendland, J.; Walther, A. Major Contribution of the Ehrlich Pathway for 2-Phenylethanol/Rose Flavor Production in Asbya Gossypii. FEMS Yeast Res. 2014, 14, 833–844.
(26) Ehrlich, F. Über Die Bedingungen Der Fuselölbildung Und Über Ihren Zusammenhang Mit Dem Eiweißaufbau Der Hefe. Bcr. Dtsch. Chem. Ges. 1907, 40, 1027.
(27) Zhang, L.; Liu, Q.; Pan, H.; Li, X.; Guo, D. Metabolic Engineering of Escherichia Coli to High Efficient Synthesis Phenylacetic Acid from Phenylalanine. AMB Express 2017, 7, 1.
(28) Skora, M.; Witalis, J.; Krzyściak, P.; Macura, A. B. Grazyb z Rodzaju Geotrichum Jako Oportunistyczny Patogen Czlowieka. Postepy Mikrob. 2009, 48, 125–132.
(29) Zhang, Y.; Fraatz, M. A.; Müller, J.; Schmitz, H. J.; Bisk, F.; Schrenk, D.; Zorn, H. Aroma Characterization and Safety Assessment of a Beverage Fermented by Trametes Versicolor. J. Agric. Food Chem. 2015, 63, 6915–6921.
(30) Nsogning Dongmo, S.; Sacher, B.; Kollmannsberger, H.; Becker, T. Key Volatile Aroma Compounds of Lactic Acid Fermented Malt Based Beverages – Impact of Lactic Acid Bacteria Strains. Food Chem. 2017, 229, 565–573.
(31) Zhang, Y.; Fraatz, M. A.; Horlamus, F.; Quittmann, H.; Zorn, H. Identification of Potent Odorants in a Novel Nonalcoholic Beverage Produced by Fermentation of Wort with Shiitake (Lentinula Edoxes). J. Agric. Food Chem. 2014, 62, 4195–4203.
(32) Steinhaus, P.; Schieberle, P. Characterization of the Key Aroma Compounds in Soy Sauce Using Approaches of Molecular Sensory Science. J. Agric. Food Chem. 2007, 55, 6262–6269.
(33) Frauendorfer, F.; Schieberle, P. Key Aroma Compounds in Fermented Forastero Cocoa Beans and Changes Induced by Roasting. Eur. Food Res. Technol. 2019, 245, 1907–1915.
(34) Li, Z.; Dong, L.; Jeon, J.; Kwon, S. Y.; Zhao, C.; Baek, H. H. Characterization and Evaluation of Aroma Quality in Doubanjiang, a Chinese Traditional Fermented Red Pepper Paste, Using Aroma Extract Dilution Analysis and a Sensory Profile. *Molecules* 2019, 24, 7–9.

(35) Gottfried, J. A. Central Mechanisms of Odour Object Perception. *Nat. Rev. Neurosci.* 2010, 11, 628–641.

(36) Ruisinger, B.; Schieberle, P. Characterization of the Key Aroma Compounds in Rape Honey by Means of the Molecular Sensory Science Concept. *J. Agric. Food Chem.* 2012, 60, 4186–4194.

(37) Verginer, M.; Leitner, E.; Berg, G. Production of Volatile Metabolites by Grape-Associated Microorganisms. *J. Agric. Food Chem.* 2010, 58, 8344–8350.

(38) Wei, J.; Zhang, Y.; Qiu, Y.; Guo, H.; Ju, H.; Wang, Y.; Yuan, Y.; Yue, T. Chemical Composition, Sensorial Properties, and Aroma-Active Compounds of Ciders Fermented with Hanseniaspora Osmyphila and Torulaspora Quercuum in Co- and Sequential Fermentations. *Food Chem.* 2020, 306, 125623.

(39) Rita, R.-D.; Zanda, K.; Daina, K.; Dalija, S. Composition of Aroma Compounds in Fermented Apple Juice: Effect of Apple Variety, Fermentation Temperature and Inoculated Yeast Concentration. *Procedia Food Sci.* 2011, 1, 1709–1716.

(40) Li, X.; Zhao, C.; Zheng, C.; Liu, J.; Vu, V. H.; Wang, X.; Sun, Q. Characteristics of Microbial Community and Aroma Compounds in Traditional Fermentation of Pixian Broad Bean Paste as Compared to Industrial Fermentation. *Int. J. Food Prop.* 2018, 20, S2520–S2531.

(41) Deetae, P.; Bonnarme, P.; Spinnler, H. E.; Helinck, S. Production of Volatile Aroma Compounds by Bacterial Strains Isolated from Different Surface-Ripened French Cheeses. *Appl. Microbiol. Biotechnol.* 2007, 76, 1161–1171.

(42) Di, R.; Kim, J.; Martin, M. N.; Leustek, T.; Jhoo, J.; Ho, C. T.; Turner, N. E. Enhancement of the Primary Flavor Compound Methional in Potato by Increasing the Level of Soluble Methionine. *J. Agric. Food Chem.* 2003, 51, 5695–5702.

(43) Cho, I. H.; Peterson, D. G. Chemistry of Bread Aroma: A Review. *Food Sci. Biotechnol.* 2010, 19, 575–582.

(44) Van Gemert, L. J. Odour Thresholds; Oliemans Punter & Partners: Utrecht, Netherlands, 2011.