GIDA (2009)34(3): 137-142 *Research* / Araştırma

THE DETERMINATION OF VOLATILE COMPOUNDS IN SET-TYPE YOGURTS USING STATIC HEADSPACE GAS CHROMATOGRAPHIC METHOD

Zehra Güler*, Abdulkadir Taşdelen, Hasan Şenol, Nurhan Kerimoğlu, Uğur Temel

Department of Food Engineering, Faculty of Agriculture,
University of Mustafa Kemal, Tayfur Sökmen Campus, Antakya

Received / Geliş tarihi: 07.03.2008
Received in revised form / Düzeltilerek geliş tarihi: 06.04.2009
Accepted / Kabul tarihi: 15.04.2009

Abstract
In this study, cows' milk and aromatic cultures such as CH-1, YF-3331 and YC-350 used in production of yogurts. The volatile compounds in the samples were determined on the 1st, 7 th, 14th and 21st days of storage by using static headspace gas chromatographic method. During storage, the amount of individual volatile compounds varied significantly in yogurts depending on type of culture used. The volatile compounds acetaldehyde, acetone, ethanol, acetoin, 2-furanmethanol and ethyl phthalate were determined in all samples. On day 1, acetaldehyde (82 mg/kg) was the highest compound in yogurt made using culture CH-1 which followed by yoghurts with YC-350 (66 mg/kg) and YF-3331 (54 mg/kg). Even though the amounts of acetaldehyde and ethanol decreased at the end of storage, acetoin increased in all the samples. While the amount of acetone in yogurt made using culture CH-1 showed fluctuations during storage, its levels in yogurts with cultures YF-3331 and YC-350 steadily increased and decreased, respectively.

Keywords: Yogurt cultures, yogurt, volatile compounds

STATİK TEPE BOŞLUĞU-GAZ KROMATOGRAFİK METOT KULLANILARAK SET –TİP YOĞURTLARDA UÇUCU BİLEŞENLERİN BELİRLENMESİ

Özet
Bu çalışmada, yoğurt üretimleri inek sütüne CH-1, YF-3331 ve YC-350 aromatik yoğurt kültürleri ilave edilerek gerçekleştirilmiştir. Uçucu bileşenler, statik tepe boşluğu metodu kullanılarak gaz kromatografisinde depolamanın 1, 7, 14 ve 21. günlerinde belirlenmiştir. Depolama süresince her bir uçucu bileşen kullanılan kültürün çeşidine bağlı olarak istatistiksel olarak önemli bir değişim göstermiştir. Uçucu bileşenlerinden, asetaldehit, aseton, etanol, asetoin, 2-furan methanol ve etil fitalat tüm örneklerde tespit edilmiştir. Depolamanın 1. gününde, CH-1 kültürü ile yapılan yoğurtta en yüksek seviyede asetaldehit (82 mg/kg) belirlenmiş, onu YC-350 (66 mg/kg) ve YF-3331 (54 mg/kg) kültürleri ile yapılan yoğurtlar izlemiştir. Tüm örneklerde asetaldehit ve etanol depolamanın sonunda azalma göstermesine rağmen asetoin artmıştır. Asetoin miktarı, CH-1 kültürü ile yapılan yoğurtta depolama süresince dalgalanmalar göstermesine karşın, YF-3331 kültürlü yoğurtta artma ve YC-350 kültürlü yoğurtta ise azalma eğilimi göstermiştir.

Anahtar kelimeler: Yoğurt kültürleri, yoğurt ve uçucu bileşenler

* *Corresponding author* / Sorumlu yazar
zguler@mku.edu.tr, (+90) 326 245 5845/1055, (+90) 326 245 5832

INTRODUCTION

Consumption of yogurt and related products are very popular in Europe, North America and the Middle Eastern countries (1). Its popularity and high consumption is due to nutritional value and the beneficial effects of yogurt starter bacteria during the fermentation of milk into yogurt (2, 3). Besides the sour nature of yogurt, the characteristic aroma is highly appreciated by consumers. The most important compounds produced by lactic starter cultures are known to be acetaldehyde, diacetyl, acetone, acetic acid and lactic acid and it is the balance between them which is thought to determine acceptability (4-6). The starter culture used may be responsible for the presence of a pleasant balanced flavour or of certain off-flavours. Acetaldehyde is considered as the major flavour component of yogurt and the proportion of carbonyl compounds, especially acetaldehyde and acetone, is important to establishing a well-balanced flavour in yogurt (7).

Headspace gas chromatography (HSGC) is a well-known technique used in flavour researches and has also been successfully applied in the studies on yogurt volatiles (8-10).

Many studies on volatile compounds in yogurt (11-13) have identified major components; however, studies on volatile compounds of fermented–milks produced using different yogurt starter cultures are limited. Thus, this study aimed to identify and quantify major volatile compounds and their concentration in set-type yogurts made using different types of starter culture such as CH-1, YF-3331 and YC-350.

MATERIALS AND METHODS

This study was carried out with cows' milk collected from Güzelburç village in Hatay province. The yogurts were manufactured in duplicate according to the protocol proposed by Tamime and Robinson (6). Milk heated to 85 °C for 30 min using batch pasteurization and cooled to 45 °C in a water bath. The treated milk was divided into three equal portions (10 L) and inoculated with CH-1 (A), YF-3331(B) and YC-350 (C) types cultures (Chr. Hansen-Peyma, Istanbul, Turkey). The inoculation rate was 20g/100L milk for all samples. Then each portion of milk was dispensed into plastic cups, incubated at 43 °C. When yogurt pH reached to 4.65, samples were removed from the incubator, and transferred to a cold store at 4 °C. Analyses were carried out at 1st, 7 th, 14th and 21st days of storage. The samples were analyzed in quadruplicate using two yogurt sample at each sampling time, and the analysis was repeated if necessary.

Volatile Compound Analysis

Ten grams of sample transferred in 20 mL headspace vial (Agilent, USA), which was sealed with PTFE/BYTL headspace septa (Agilent, Germany) and aluminum cap (Agilent, USA). Samples were kept at –20 °C until analyses. Prior to analysis, frozen samples of yogurt (10 g) were thawed at 4 °C overnight. The vials with samples were held at 60 °C for 1hour, then at 75 °C for 10 min and stirred 5 times, and subsequently kept for 5 min. Headspace (250 µL) was injected with gas-tight syringe onto the GC column using split mode (10:1). The temperature of syringe was kept at 80 °C. All glass materials (HS vials, volumetric flasks) were sterilized before use. Double distilled water to be used for preparation of standard solutions was boiled for 20 minutes to remove residual volatiles and was subsequently stored in a stopped glass container. All chemicals were analytical grade supplied by Merck Ltd. (Merck House Poole, Dorset England BH15 ITD, UK).

The volatile compounds were separated on a HP-INNOWAX capillary column (30 m x 0.32 mm id x 0.25 µm film thickness) under the following conditions: injector temperature 200 °C; carrier gas helium at a flow rate of 1.4 mL/min; oven temperature program initially held at 50 °C for 6 minutes and then programmed from 50 °C to 180 °C at 8 °C/min and held at 180 °C for 5 minutes. The GC column was connected to the Agilent 5973N model mass selective (MS) detector (Agilent, USA) which was operating in the scan mode within a mass range of 33 to 330 m/z at 1 scan /s. The interface line to MS was set at 250 °C. The MS was operated in an electron impact mode at electron energy of 70 eV. The compounds were identified by a computer-matching of their mass spectral data with those of known compounds from the Nist 02.L. Mass Spectral Database. Based on the peak resolution, their areas were estimated

from the integrations performed on selected ions. The resulting peak areas were expressed in the arbitrary area units. Quantification of constituents was calculated by external standard technique. For this purpose, authentic standards of acetaldehyde, acetoin, diacetyl, acetone and ethanol were accurately weighed and dissolved in 10 mL of double distilled water. Five sets of concentrations were prepared in the range of 1 to 30 ppm. Results achieved from the collections of standards were used to calculate a mean peak area for each standard compound. All collections were made in triplicate and consequently the amounts of each compound in sample were calculated over known amount of standard and its peak area.

Statistical Analysis

The data for chemical composition was subjected to one-way analysis of variance (ANOVA) using SPSS computer program (version 9.05) to test the differences among the three yogurts at each sampling time and to determine the effects of storage days. Duncan's multiple range test was used to compare the means when a significant variation was established by ANOVA at the significance level 0.05.

RESULTS AND DISCUSSION

The volatile compounds in the yogurt samples are presented in Figures (1-4). It was observed that acetaldehyde, acetone, acetoin and ethanol were found in substantial amounts in all the samples during storage. Some researchers found that acetaldehyde (2.0 to 41.0 mg/kg), diacetyl (0.2 to 2.3 mg/kg), acetoin (2.2 to 28.2 mg/kg), ethanol (0.2 to 9.9 mg/kg), acetone (1.8 to 3.4 mg/kg), and 2-butanone (0.1 to 0.6 mg/kg) are the most important flavour compounds in yogurt (4, 5, 14). According to the results, diacetyl and 2-butanone were not detected in any of yogurt samples. This finding is also confirmed by another report (15). This could be attributed to low diacetyl and 2-butanone production capacity of cultures used and/or being below of detection limit of diacetyl and 2-butanone levels in all yogurts. Marshall, Cole and Mabbit (16) found that the content of diacetyl in yogurt rarely reached to 0.5 ppm which was a very low concentration.

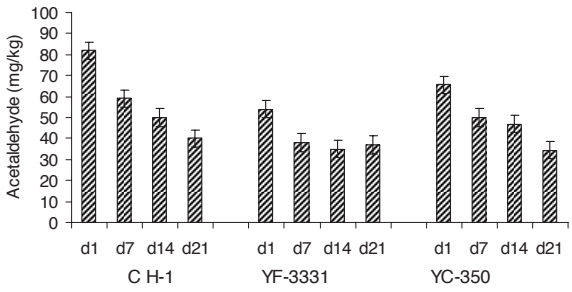

Figure 1. Variations in the acetaldehyde contents of yogurts produced with different cultures (n=2, $P<0.01$).

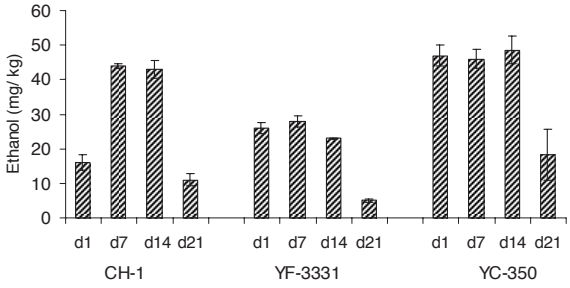

Figure 2. Variations in the ethanol contents of yogurts produced with different cultures (n=2, $P<0.01$).

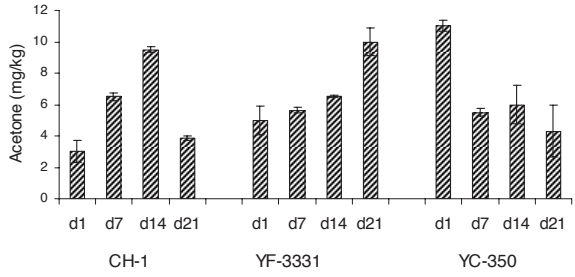

Figure 3. Variations in the acetone contents of yogurts produced with different cultures (n=2, $P<0.01$).

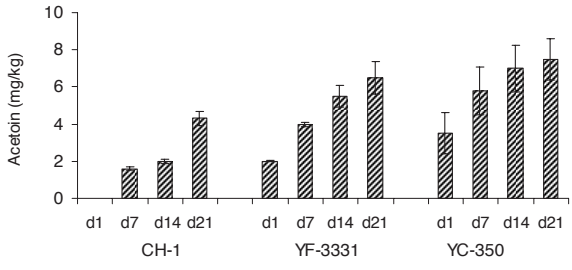

Figure 4. Variations in the acetoin contents of yogurts produced with different cultures (n=2, $P<0.01$).

Although the yogurts were manufactured under the same conditions, the concentration of volatile compounds varied significantly from sample to sample ($P<0.01$). Additionally, volatile compounds in yogurts were significantly influenced by storage period ($P<0.01$). Yogurt made using culture CH-1 had higher acetaldehyde and lower acetoin during the storage than those in yogurts made using cultures YF-3331 and YC-350 (Fig. 1 and Fig. 4). Acetaldehyde levels of all the yogurts were the highest on day 1. Acetaldehyde level was significantly unchanged in yogurt produced with culture YF-3331 from the 7th day up to the end of storage, but decrease in other yogurts continued during cold-storage.

Eventually, it was observed that the yogurt samples produced with culture CH-1 had the highest mean of acetaldehyde (57.5 mg/kg) whereas the yogurt sample produced with culture YF-3331 had the lowest mean level of acetaldehyde (39.12 mg/kg). The higher level of acetaldehyde in yogurt with culture CH-1 is thought to be due to the fast metabolic activity of starter bacteria. At lower pH values, acetaldehyde can easily be oxidized to acetate and, therefore, during storage, the level of acetaldehyde decreases (6). Additionally, acetaldehyde can easily be degraded to ethanol by alcohol dehydrogenase synthesized by *Str. thermophilus* and this is more likely at the later stages of storage since alcohol dehydrogenase is more active at lower pH values (17). This point was further highlighted by the variation of ethanol levels in the experimental yogurts during storage. The mean level of ethanol was the highest in yogurt with culture YC-350 compared with other yogurts (Fig. 2). Ethanol increased in all yogurt samples up to 14th day of storage and decreased significantly at the end of storage ($P<0.01$) (Fig. 2).

In general, when acetaldehyde level significantly decreased in yogurt samples, ethanol increased during storage. Diethyl phthalate was determined in all the samples. Diethyl phtalate in yogurts produced with cultures YF-3331 and YC-350 detected only on day 21 at levels of 18.66 and 16.50 mg/kg, respectively whereas it was detected in yogurt with CH-1 on the 14th (4.30 mg/kg) and 21st (15.20 mg/kg) days of storage. Phthalates are ester of benzene-1,2-dicarboxylic acid which is an aromatic dicarboxylic acid. However, phthalates are used as plasticizers and probably originate from the plastic containers in which the yogurts were produced (18, 19). There is limited information on pathways of exposure to phthalates (20). In another study, pH values were lower in yogurts produced with culture CH-1 than those in yogurts with cultures YF-3331 and YC-350 (21). Therefore, the migration of diethyl phthalate from plastic material into the yogurt samples may be faster at the yogurts with CH-1 culture over the other yogurt samples during storage. Among the volatile compounds, acetone showed the most interesting variation during storage. Even though acetone levels were showed fluctuations in yogurt with culture YC-350 during storage, it increased steadily in yogurt with culture YF-3331 towards the end of storage. Acetone increased noticeably in yogurt with culture CH-1 up to day 14 and decreased again at the end of storage (Fig. 3). In contrast to acetaldehyde, acetoin increased steadily in all yogurt samples during storage (Fig. 4). Interestingly, although yogurt produced with culture CH-1 contained acetaldehyde at high levels, acetoin was not detected in the same yogurt on day 1, and was at the low levels at the later days of storage, when compared with other yogurts. In a previous study using ether extract and dynamic head space techniques, acetoin was not detected in yogurts (9). Acetoin is mainly produced from citrate and lactose by the activity of lactic acid bacteria (22). In the present study, acetoin content was less in yogurt with CH-1 culture compared with yogurts produced with the other cultures. On the contrary acetaldehyde, ethanol and acetone, the levels of acetoin increased in all the yogurts during storage and reached the highest level at the end of storage (Fig. 4).

Another volatile compound 2-furan methanol was detected in all yogurts at 1st and 7th days of storage. From day 1 to day 7, the content of 2-furanmethanol varied in the range of 5.30-7.50 mg kg^{-1} in yogurt with CH-1, 1.28-5.65 mg/kg in yogurt with YF-3331, and 2-4.46 mg/kg in yogurt with YC-350. Glucose, in the presence of L-alanine, can lose either C-1 atom to produce a pentitol moiety that is responsible for the formation of furan methanol or the C-6 atom to produce a pentose moiety which is responsible for the formation of furfural at high temperatures and acidic conditions (especially at pH 5 or lower) (23). On the other hand, among the above-mentioned fermentation metabolites of furfural, 2-furanmethanol has been reported to be

major product. 2-furanmethanol may be obtained by the direct reduction of furfural 1 by the action of alcohol dehydrogenase (24). 2-furanmethanol causes baked flavour in dairy products (25). After 7th day, 2-furanmethanol was not detected in any of the yogurt samples. It can be suggested that it was degraded due to oxidizing agent and acidic conditions since the organic acids can promote polymerisation.

Overall, it should be remarked that the concentration of acetaldehyde and ethanol in all samples was far higher than that of acetone, acetoin and other volatile compounds during storage. This result was similar to the results obtained by Gyosheva (26). The most remarkable changes in all volatile compounds occurred at the end of storage. This may be due to the prolonged storage as a result of enzymatic reactions.

Some researchers insisted that a ratio of 1:1 to 2.8:1 of acetaldehyde to acetone is required to procedure desired texture or "fullness" of yogurt flavour (4, 26). The results obtained from present study were exceeded in all cases (Table 1), however they were consistent with these obtained by Kneifel, Ulbert, Erhard and Jaros (5) for yogurts produced using mixed yogurt starter. The mean ratios of acetaldehyde to acetone and acetaldehyde to ethanol were the highest in yogurt produced with culture CH-1 which was followed by yogurts with YF-3331 and YC-350 cultures (Table 1).

Table 1. The ratios of acetaldehyde to ethanol and acetaldehyde to acetone in yogurts produced with different yogurt starter cultures

	Storage days				
Ratio	1	7	14	21	Mean
		Culture CH-1			
A	5.13	1.34	1.16	3.64	2.03
B	27.33	9.08	5.26	10.36	10.11
		Culture YF-3331			
A	2.08	1.36	1.52	7.40	2.00
B	10.80	6.74	5.38	3.70	6.04
		Culture YC-350			
A	1.39	1.09	0.97	1.88	0.82
B	5.95	9.09	7.83	8.02	4.91

A: acetaldehyde: ethanol, B: acetaldehyde: acetone

CONCLUSIONS

It was concluded that the differences in the results obtained from yogurt samples produced with three different starter cultures should be attributed to discrepancies in the metabolic processes of the individual cultures and enzymatic activity during the fermentation process and storage of the product. During storage, the mean levels (mg kg^{-1}) of acetaldehyde (57.75), ethanol (28.5), 2-furanmethanol (3.2) and ethyl phthalate (4.88) were the highest in yogurt made using culture CH-1, the levels of acetone (6.79) and acetoin (6.13) were high in yogurt made using culture YF-3331.

REFERENCES

1. Anon. 1995. *Consumption Statistics for Milk and Milk Products 1993*. International Dairy Federation, Documant no 301, pp 4-6, Brussels.

2. Hamann WT, Marth EH. 1984. Survival of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in commercial and experimental yoghurts. *J Food Protec*, 47: 781-786.

3. McGroger JU, White C H. 1987. Effect of sweeteners on major volatile compounds and flavour of yoghurt. *J Dairy Sci*, 70: 1828-1834.

4. Rasic JL, Kurman JA. 1978. *Yoghurt*. Volume I, Technical Dairy Publishing House, Copenhagen, Denmark, 466p.

5. Kneifel W, Ulbert F, Erhard F, Jaros D. 1992. Aroma profiles and sensory properties of yoghurt and yoghurt related products. I. Screening of commercially available starter cultures. *Milchwiss*, 47: 362-365.

6. Tamime AY, Robinson RK. 2001. *Yoghurt Science and Technology*. CRC Press, New York, USA, 619 p.

7. Zourari A, Accolas JP, Desmazeaud MJ. 1992. Metabolism and biochemical characteristics of yoghurt bacteria. A review: *Lait*, 72: 1-34.

8. Ulbert F. 1991. Headspace gas chromatographic estimation of some yoghurt volatiles. J. *Assoc Official Chem*, 74: 630-634.

9. Kang Y, Frank JF, Lillard DA. 1988. Gas chromatographic detection of yoghurt flavour compounds and changes during refrigerated storage. *Cult Dairy Prod J*, 11: 6-9.

10. Ott A, Fay LB, Chaintreau A. 1997. Determination and origin of the aroma impact compounds of flavour. *J Agric Food Chem,* 45, 850-858.

11. Dumont JP, Adda J. 1973. Rapid method for highly volatile flavour compounds in dairy products. Application to yoghurt. *Lait*, 23:12-22.

12. Gosheva B. 1984. The organoleptic and gas chromatographic charateristics of the flavour of Bulgarian yoghurt. *Khranit Promishlnt*, 33: 36-38.

13. Kondrstenko M, G'osheva B. 1984. Volatile substances forming yoghurt aroma. *Moloch Promysh*, 7: 45-46.

14. Xanthopoulos V, Picque D, Basit N, Boquien CY, Corrieu G. 1994. Methods for the determination of aroma compounds in dairy products: a comparative study. *J Dairy Res*, 61: 289-297.

15. Xanthopoulos V, Petridis D, Tzanetakis N. 2001. Characterization and classification of *Streptecoccus thermophilus* and *Lactobacillus bulgaricus* strains isolated from traditional greek yogurts. *Food Micand Safety*, 66: 747-752.

16. Marshall VM E, Cole WM, Mabbit LA. 1984. Yoghurt made from single starter organisms using heta- or enzyme-treated mil kor milk to which casein hydrolysate or sodium formate is added. *J Dairy Res,* 49: 147-152.

17. Varga F. 1998. The origine, production and control of acetaldehyde in yoghurt. Ph. D. thesis, The University of Reading, 112 p, UK.

18. Mikula P, Suobodava z, Smutna M. 2005. Phthalates: Toxicology and food safety. *Czech J Food Sci,* 3: 217-223.

19. Chou K, Wright RO. 2006. Phthalates in food and medical devices. *J Med Toxi*, 2: 126-135.

20. Hauser R, Williams P, Altshul L, Calafat AM. 2005. Evidence of interaction between polychlorinated biphenyls and phthalates in relation to human sperm motility. *Env Health Perspect,* 113 : 425–430

21. Güler Z. 2008. Evaluation of lipolysis in set-type fermented milks made with different commercial yogurt starter cultures during storage. *Milchwiss,* 33: 73-77.

22. Molimard P, Spinnler HE. 1996. Compound Involved in the Flavor of Surface Mold-Ripened Cheeses: Origins and Properties. *J Dairy Sci*, 79: 169-184.

23. Bemiller JN, Huber KC. 2008. Carbohydrates. In: *Fennema's Food Chemistry,* Damodaran S, Parkin KL, Fennema, OR (eds), CRC Press, New York, US, pp. 83-155.

24. Machizuki N, Kitabatake K. 1997. Analysis of 1-(2-furyl) propane-1, 2-diol, a furfural metabolite in beer. *J Ferment and Bio*, 83: 401-403.

25. Quian M, Reineccius G. 2002. Identification of aroma compounds in Permigiano-Reggiona cheese by gas chromatography /olfactometry. *J Dairy Sci,* 85: 1362-1369

26. Gyosheva H. 1982. Compounds forming the aroma complex of Bulgarian sour milk. *Milchwiss,* 37: 267-269.