RESEARCH OF ACETALDEHYDE QUANTITIES IN COMMERCIAL AND LABORATORY PRODUCED FERMENTED PRODUCTS

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Abstract: The main purpose of this research study is the determination of volatile flavoring substance acetaldehyde in some fermented, industrially manufactured dairy products, offered on the Bitola's market and the comparison with acetaldehyde quantities measured in fermented dairy products produced in laboratory conditions. Six samples of fermented dairy products (yoghurt and sour milk) were purchased from the local supermarkets and four samples were "homemade" manufactured in laboratory conditions. Results of the acetaldehyde quantities in different fermented dairy products were obtained through the observation of acetaldehyde values successively in a two week period and, in order to confirm the mutual correlation of variables, i.e. of the absorbance and concentration, calibration curve was created. The highest acetaldehyde quantities in all fermented dairy samples were measured on the first day of this research study, while after the fifteenth day of examination acetaldehyde concentration in each sample was equal to zero. Undoubtedly, certain conditions like pH, temperature, strain ratio, etc. need to be met.

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Introduction

Fermented dairy products are produced by adding cultures with thermophilic and mesophilic lactic acid bacteria (LAB) into milk. Generally, these cultures consist of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains, which ferment the lactose into the lactic acid (Gelinas and Lachance, 1995; Shah, 1997; Gandhi 2006; Sfakianakis and Tzia, 2014) and produce volatile aromatic compounds (acetaldehyde, diacetyl, acetic acid and esters). They are produced in minimal quantities during biochemical ripening processes and are actually aroma and flavor carriers in fermented dairy products that deliver rich, rounded and characteristic taste of the fermented dairy products (Zonji, 1971; Marshall, 1987; Berković, 1998).

Acetaldehyde is an active carbonyl compound, which can react with amino acids in order to produce aromatic combinations. Hamdan et al. (1971) reported that acetaldehyde importance for yoghurt aroma was for the first time suggested by Pette and Lolkema in 1950, and later in 1968 by Keenan and Bills, who in their review stated that "high concentrations of acetaldehyde are necessary to produce a desirable flavor in yoghurt." In literature, a broad range of optimal acetaldehyde level exists, suggesting the characteristic aroma of the plain yoghurt. There are statements where acetaldehyde levels from 10 to 20 mg kg⁻¹ are necessary for optimal taste and aroma, while there are reports implying acetaldehyde values from 21 to 41 mg kg⁻¹, are required for typical yoghurt flavor (Senel et al. 2009).

Yoghurt flavor formation occurs in three main stages; glycolysis, lipolysis and proteolysis, and the first phase of glycolysis is in fact the transformation phase in which the key aromatic yoghurt compounds like acetaldehyde, diacetyl, acetone and ethanol, are produced (Van Hylckama Vlieg et al. 2007; Baran, 2012). On the other hand, Ledenbach and Marshall (2009) examined factors influencing the relationship of aromatic compounds in fermented dairy products and concluded that the culture disbalance, inappropriate temperature or ripening time, culture infection with bacteriophages, the presence of inhibitors and microbe contamination could lead to products with undesired characteristics. This was also confirmed by Rašić and Kurmann (1978) and Baranowska et al. (2006) who suggested that yoghurt need to be fermented from a pH of 3.8 – 4.4, and optimal yoghurt taste could be obtained at pH 4.0 – 4.4.

Considering above statements the main purpose of this research study is the determination of the volatile flavoring substance acetaldehyde in some fermented, industrially manufactured dairy products, offered on the Bitola's market and compared with the acetaldehyde quantities measured in fermented dairy products produced in laboratory conditions.

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Materials and methods

Research material

Six samples of fermented dairy products (yoghurt and sour milk) were purchased from the local supermarkets and four samples were “homemade” manufactured in laboratory conditions. As a starter culture for laboratory produced samples *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* strains were used, as well as certain quantities of already purchased fermented products. Laboratory samples were produced from 1 liter of fresh milk, first pasteurized at 89 – 100° C for 5 minutes, then with stirring cooled to 37 – 40° C, and “inoculated” with 80 – 100 ml of previously purchased yoghurt or sour milk. To initiate the fermentation, milk samples were left at room temperature for 4 to 6 hours. When the fermentation process was finished, samples were stored in a refrigerator at 4° C.

Chemicals and reagents

All chemicals used for this examination had p.a. grade. The standard solution of acetaldehyde – C₂H₄O was prepared through dilution of the ampule quantity in dH₂O, in 1:100000 ratio. Different concentrations of working solutions, later used for the calibration curve creation, were prepared with additional dilution of the standard solution. Reagents were prepared appropriately to the instructions given in the methods.

Method and sample preparation

The determination of the acetaldehyde quantity in fermented dairy products in this paper was based on the mechanism described by Sawicki et al. (1961). This procedure, previously applied to formaldehyde quantification, includes the following steps: reaction of the aldehyde with 3-methyl-2-benzothiazolone hydrazone (A), to form the azine (B), oxidation of A to a reactive cation (C) and formation of the blue cation (D), (Figure 1).

This method underwent certain modifications by Pack et al. (1964), who have used the reaction with 3-methyl-2-benzothiazolone hydrazone to determine diacetyl, while their modification one year later was adapted for routine analysis of acetaldehyde in dairy cultures by Lindsay and Day (1965).

Figure 1: Aldehyde reaction with 3-methyl-2-benzothiazolone hydrazone monohydrate

Source: Sawicki et al. (1961)

Samples were prepared according to the method reported by Carić et al. (2000). 5 to 15 g of research material were measured in a reagent glass (25 x 250 mm). HCl was added into the glass in order to prevent foam formation. The next step included the addition of the following mixture to the glass content: 2.5 ml H₂O + 2.5 ml water solution of 3-methyl-2-benzothiazolone hydrazone hydrochloride acid + 0.5 ml dimethyl sulfoxide. The glass was closed with a rubber seal and placed in a water bath at 65° C for 60 minutes, with N₂ insufflation at the same time (100-125 ml per minute). After 60 minutes, insufflation was interrupted and the glass pipe was rinsed with small quantities of dH₂O, inside the glass. The content was left at room temperature for 25 minutes and then 12.5 ml solution of iron (III) chloride was added to it, thoroughly mixed and left for another 25 minutes at room temperature. In order to stop oxidation, 20 ml of acetone was added into the reagent glass, and the content was quantitatively transferred into the volumetric flask of 50 ml and filled with acetone to the mark. The

flask content was centrifuged at room temperature, 3 minutes and 9000 RPM, and the supernatant was filtered (Figure 2). At the end, the filtrate was analyzed with a spectrophotometer and the color change was read at 666 nm. The result was compared with the absorbance value obtained for a blank sample (for blank sample appropriate quantity of dH₂O was used).

Figure 2: Colour changes after oxidation interruption with acetone

Source: Authors

Results and discussion

Results of acetaldehyde quantities in different fermented dairy products were obtained by observation of acetaldehyde values, successively in a two week period (Table 1), and in order to confirm the mutual correlation of variables, i.e. of the absorbance and concentration, calibration curve was created (Figure 3), indicating high positive interaction, visible from the value of coefficient square, $R^2 = 0.9578$ (the closest value to 1, the stronger the correlation among the variables). Dependence of acetaldehyde concentrations on examination period can be observed from Figure 4.

A general suggestion is that the aroma and flavor formation occur in the first 24 hours of the production process and this is supported by values collected in this research. The highest acetaldehyde quantities in all fermented dairy samples were measured on the first day of this research, after the fifteenth day of examination acetaldehyde concentration in each sample was equal to zero.

Many authors have also confirmed this suggestion, demonstrating that acetaldehyde formation during yoghurt production develops in the first 6 hours and this is probably due to the fast metabolic activity of the starter. According to Imhof et al. (1994); Samet-Bali et al. (2012) and Chandan (2014), formation of acetaldehyde in yoghurt takes place predominantly in the first 1-2 hours of incubation, and its level decreases in later phases. They also note that combined cultures produce higher quantities of acetaldehyde, due to proto-cooperative growth of yoghurt cultures.

Table 1: Acetaldehyde quantities in examining samples (samples purchased in supermarkets are marked with S and the ones produced in the laboratory are marked with L)

Period/ Days	C ₂ H ₄ O (µg/ml)									
	c									
	1S	2S	3S	4S	5S	6S	1L	2L	3L	4L
I	0.70	2.56	1.53	1.12	0.92	2.34	1.37	1.28	1.62	1.09
II	0.53	1.28	0.80	0.79	0.34	1.33	0.84	1.06	1.11	0.79
III	0.34	1.02	0.34	0.08	0.15	1.14	0.24	0.13	0.65	0.22
VII	0.24	0.61	0.11	0	0.14	1.08	0.03	0	0.04	0.05
X	0	0.50	0	0	0.05	0.95	0	0	0	0
XIII	0	0.27	0	0	0	0.53	0	0	0	0
XV	0	0	0	0	0	0	0	0	0	0

Source: Authors

It is important to make a parallel comparison among the values of samples purchased from supermarkets and acetaldehyde quantities of those produced in the laboratory. It is obvious that after the seventh day of analysis no concentrations were detected in laboratory manufactured samples. This could be connected to the storage period and fermentation conditions.

Güler et al. (2009) has reported that volatile compounds found in yoghurt to a large degree depend on the storage period. Acetaldehyde levels in yoghurt samples were the highest on the first day, but decreased during the period of cold storage. Considering the prolonged term, the most meaningful

changes in all volatile compounds appeared at the end of the storage period, probably as a result of enzymatic reactions. Many authors connected (Tamime and Robinson, 2007; Güzel-Seydim et al. 2005) the lowering of acetaldehyde values during the storage period with the decrease of pH and increased oxidation of acetaldehyde to acetate. Özer et al. (2007) suggested that reduction of acetaldehyde concentrations during storage is induced by alcohol dehydrogenase, produced by yoghurt cultures, which converts acetaldehyde into ethanol during the storage.

Figure 3: Positive correlation of concentration and absorbance

Source: Authors

Figure 4: Acetaldehyde quantities in examining fermented dairy products

Source: Authors

The only concern that arose regarding the storage period of samples purchased in supermarkets was the interval between the date of production and the date of delivery to the local shops.

Furthermore, when samples were sensory tested, aroma, flavor and taste of industrially produced samples were stronger and more expressive in comparison with laboratory manufactured samples. However, it is relevant that industrially manufactured samples are produced under precise and controlled conditions, with employment of sophisticated technology, which is not the case with the samples produced in the laboratory. Undoubtedly, certain conditions like pH, temperature, strain ratio, etc. need to be met.

Proper fermentation with yoghurt culture leads to a typical aromatic compound formation and obviously acetaldehyde and other volatile aromatic compounds cannot be discussed separately,

considering the symbiosis among LAB. Xu et al. (2015) in their research indicated that acetaldehyde quantities and other flavoring compounds are connected with the fermentation time. The longer the fermentation time, the higher concentrations of acetaldehyde and other aroma compounds in the yoghurt. According to them, sustainability of *Streptococcus thermophilus* has double positive effect in flavoring compounds production; on the one hand it stimulates *Lactobacillus bulgaricus* to produce more acetaldehyde, and on the other, it produces more diacetyl itself.

Conclusion

Summarized the results illustrate that with further research of symbiosis among *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and other bacteria strains, as well as with the investigation of the balancing ratio among acetaldehyde and other aromatic volatile compounds, new possibilities for creating fermented dairy products with desired aroma and flavor characteristics are accessible. Furthermore, this includes experimentation with factors affecting the fermentation process itself. Controlled conditions in an industrial production environment, utilization of sophisticated technology and regulated storage conditions, both in plants and supermarkets, will provide fermented dairy products with high quality, positively accepted by the consumers.

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