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AROMATIC COMPOUNDS VALUABLE IN IDENTIFYING LOCAL VARIETIES OF KEFIR

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Abstract: *This paper attempts to analyze the range of five types of kefir aromatic presence in Romania, particularly aiming at the concentration of higher alcohols, aldehydes, ketones, esters and carboxylic acids. GC-MS methods used have led to results which gives the aromatic characteristics of these products vary according to the manufacturer, the resulting values being situated between 230.4567 mg / L and 345.2267 mg / L higher alcohol, 235.6722 mg / L and 398.9604 mg / L to aldehydes, 130.9861 mg / L and 162.2452 mg / L ketones, 439.0863 mg / L and 463.2015 mg / L esters and 291.7619 mg / L and 333 1917 mg / L for carboxylic acids. Flavorings results in these samples are conferred majority of the metabolic activity of the microorganisms involved in the production of Kefir and the recipe used by the manufacturer default fat concentration of the product.*

Key words: *Kefir, aroma compounds, GS-MS, bacteria, yeasts.*

1. Introduction

Kefir is a fermented milk product obtained by bringing the temperature of the milk kefir microflora of specific granule. Kefir grains microflora is defined as being formed of different species of lactic acid bacteria and yeasts [3, 9]. The production of kefir is characterized by a mixed lactic and alcoholic fermentation of the lactose [17]. Although the main micro-organisms in the product are the major metabolite of lactic acid bacteria and lactic acid fermentation, the yeast can provide a number of features such as kefir can be defined as a carbonated milk beverage containing varying amounts of ethanol, acetic acid and flavour compounds [8, 10]. Kefir grains have a size of 0.2-2 cm, a no uniform irregular shape and a wrinkled surface, cauliflower-like elastic consistency, white colour is the colour that turns into a cream after washing

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and drying. When washed with cold water and then stored in a saline solution, the granules remain intact at least one month [23].

Traditionally Kefir granules produced directly from kefir may contain up to 2% ethanol, more than cumulus, but the product achieved in industrial ethyl alcohol is between 0.01 and 0.1%. Kefir contains 0.04-0.15% industrial acetic acid, up to 0.4% CO2, 1-4 mg / L diacetyl and a few mg / l acetaldehyde [23]. Kefir can be made with whole milk, semi-skimmed or skimmed. Preliminary treatment of the milk is identical to the one obtained by other acidic dairy products. Of the two types of kefir products in Europe, kefir fluid is more common than coagulated kefir. Like other fermented milk products, kefir has been associated with a number of health benefits such as cholesterol metabolism [14] and inhibition of the angiotensin converting (ACE) inhibitors, antimicrobial activity [18], tumour suppression [16], increasing the rate of wound healing and immune system modulation, alleviation of allergy and asthma. These reports have led to increased interest in kefir as a focus of research as potential product containing probiotics [1-3], [11].

Kefir microbiota lead to improved intestinal infections, acting with significant results on the bacteria *Escherichia coli*, *Yersinia enterocolitica* [4, 5], [7]. The anti-inflammatory and anti-allergic it has been found by Lee et al. in 2007 [12], and the potential of probiotic kefir has been shown Leite et al. [13] different samples from Brazil. The amount of inoculum starter culture and the type of influencing the sensory characteristics of the final product {15]. Flavour and consistency can be adjusted as desired attributes of kefir is appreciated creamy texture, uniform throughout the mass, but the intensity is variable taste of yeast. Kefir is dominated by bacteria such as the genera *Lactobacillus, Lactococcus, Acetobacter, Leuconostoc* and kazachstan fungi of the genus *Kluyveromyces, Naumovozyma Saccharomyces* [9]. Kefir is a dairy beverage that involves health is produced when kefir grains, consisting of a consortium of microorganisms is added to start the fermentation of milk natural. Metagenomics sequencing method revealed that the bacterial species Lactobacillus kefiranofaciens was dominant kefir during the early stages of fermentation, but *Leuconostoc mesenteroides* accumulated in the later stages. Significant changes in the structure of the communities which lead to the diversification of the volatiles bacterin.

The species listed below are generally related to specific flavour compounds such as *Acetobacter* spp. Correlated with acetic acid; *Lactobacillus* spp. Correlated with carboxylic acids, esters and ketones; *Leuconostoc* spp. Correlated with acetic acid and 2,3-butanedione; and *Saccharomyces* spp. correlated with esters. The results illustrate the dynamic nature of kefir fermentation and microbial succession models and can be applied to optimize the fermentation processes, flavours and health-related attributes of this type of food and other fermented foods [25]. Kefir has a content of vitamins B1, B2, folic acid greater than milk. In general, kefir assigned healing powers and therapeutic and nutritional properties, not always confirmed. Mineral composition was determined to kefir produced from cow's milk and goat's milk. The macro minerals (Ca, P, K, Na and Mg) and trace (Cu, Fe and Zn) were determined in both the samples of milk, kefir and the.

Ratio Ca / P in the kefir produced from cow's milk and goat was 1.32. The levels of Ca, P, Mg, Cu and Fe were significantly higher in the kefir produced from cow's milk than in cow's milk; K and Na concentrations were low in the product of cow's milk kefir ($P < 0.05$), while there were no changes in the levels of zinc. The concentrations of Ca, P, Na and Mg produced increased goat milk kefir, while K and Fe levels decreased significantly ($P < 0.05$). There were no changes to levels of Cu and Zn in goat milk and goat milk kefir product. Kefir is produced from goat milk was the better source for minerals Ca, P, K, Na, and Mg [24].

2. Materials and Methods

In this paper were selected to analyze five samples of kefir from producers with original recipes manufacturing Romania:

1. Kefir 3% Lactis- is a symbiotic made from pasteurized cow's milk with the addition of oligofructose and specific lactic acid bacteria culture, yeast and probiotic bacteria (*Bifidobacterium* spp. and *Lactobacillus acidophilus*) (K1);
2. Light Kefir 0.1%, Paco - is a dietary product with 0.1% fat obtained from cow's milk and contains fibrulina which is a natural source of fiber in the paper product being denoted by K2;
3. Kefir 3.5% Napolact -obtained from cow's milk with a fat content of 3.5% (K3);
4. Kefir 3% Zuzu- obtained from pasteurized cow's milk and the standardized milk 3% fat and to increase the dry matter content was added to milk and milk protein powder (K4);
5. Kefir Caucasian, min 2.5%, Clever, obtained from cow's milk with a fat content of 2.5%, which skimmed milk powder was added and the culture of kefir (K5).

Currently it applies a series of modern methods of analysis of aroma compounds in food (wine, juices, meat and meat products, milk and dairy products, etc.) focusing particularly on systems GC-MS, GC-FID [19-22] complex systems with very precise and well-defined procedures [19-22]. In order to determine the selected method of aroma compounds was GC-MS, aiming at higher alcohols, aldehydes, ketones, esters and carboxylic acids. GC-MS standard solutions were prepared by chromatographic purity of the reagents from Sigma-Aldrich, Germany. The working method applied was according to the procedure set of Walsh et al. [25] adapted, which involves placing one gram of the sample to be analyzed into a vial microextractie, centrifuged for 5 seconds at 400 revolutions / minute at a temperature of 750°C. The samples were then injected into the column device type Agilent 1200 (Agilent Technologies, Germany), gas chromatograph, mass spectrometer, coupled with the headspace setting a regimen of temperature and time increased from 2300 C to 3250 C according to the procedure This carrier gas is helium. The results were then compared with standard values using known equipment available to the software.

3. Results and Discussions

As it is known in literatures which form volatile compounds in many of the food products based on the activity of microorganisms, especially bacteria and yeasts [8], [10], [25]. The alcohols from amino acid metabolism, lipid and carbohydrate effects consisting of fruit flavors, alcohol, fusel oil, or flowers. From Figure 1 it is seen that higher alcohols formed by ethanol, 1-pentanol, 2-butanol,

2-methyl-1-butanol, hexanol had values between 230.4567 mg / L and 345.2267 mg / L minima and maxima is K2 samples respectively found to K5.

Intermediate values presented evidence K1, K3 and K4, their average hovering around 314.9600 mg / L. Aldehydes is frequently found in dairy products which are the result of the action of microorganisms involved in lipid and amino acid metabolism, giving gentle flavours of green apple and floral, fruit or vegetable, sometimes rough and wild. The concentrations of the aldehydes identified in five samples (Figure 2) showed values between 235.6722 mg / L to 398.9604 mg sample K2 / K4 L sample. Samples K1, K3 and K5 had amounts that were located between 313.1245 mg / L, 336.7345 mg / L, respectively 342.6721 mg / L, values of which acetaldehyde was between 2.0912 mg / L and 5.1119 mg / L.

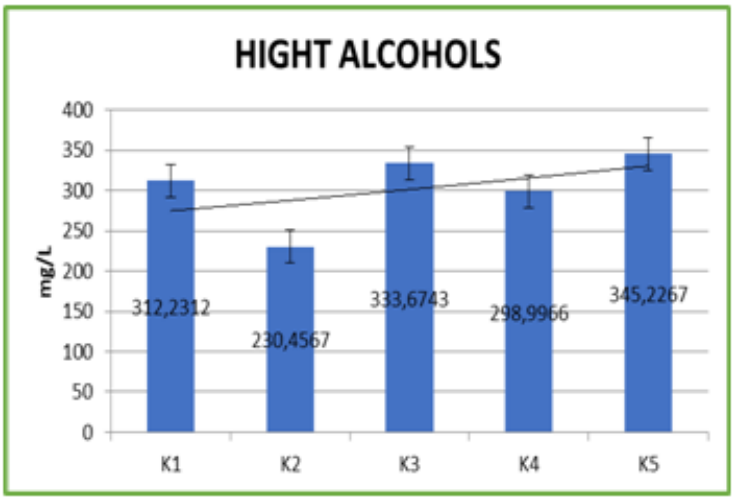

Fig. 1. *The amount of higher alcohol resulting in five samples of kefir (K1, K2, K3, K4, K5)*

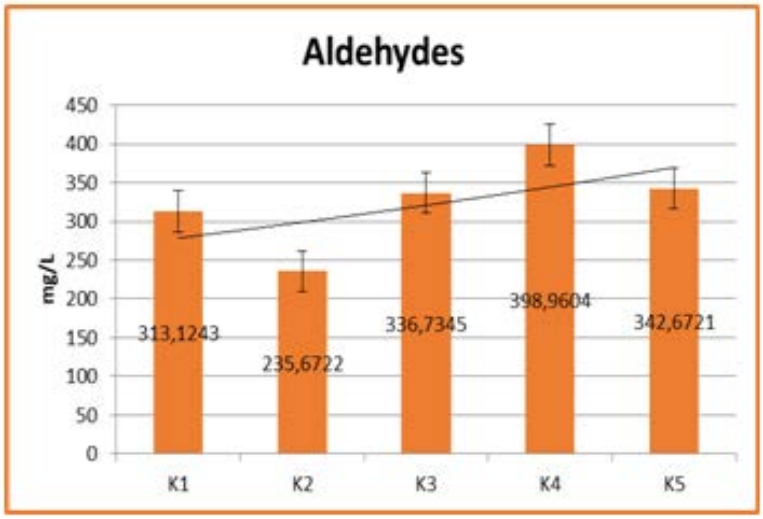

Fig. 2. *The amount of the aldehydes resulting in five samples of kefir (K1, K2, K3, K4, K5)*

Ketones (Figure 3) are also compounds of the complete flavour profile kefir, which is specific lipid and carbohydrate metabolism. Strong flavours are generally notified of butter (2,3 butanedione, 2-butanone, 2,3 pentanedione, 2,3-hexanedione) smoked cheese (2-nonanone, 2,3 - pentanedione) or ether (2- butanone), floral (2-undecanone, 2-pentanone), the floor (acetone), fruity (2-undecanone, 2-pentanone, acetone). K1 values were determined to 162.2452 mg / L, 20% lower in the sample K2 and K3 sample 153.6413 mg / L. K4 samples exhibited values close to 148.0916 mg / L and K5 to 145.9207 mg / L values of about 9.4% lower than the maximum detectable 162.2452 mg / L in the K1 sample. The resulting ketone is presented in Figure 3.

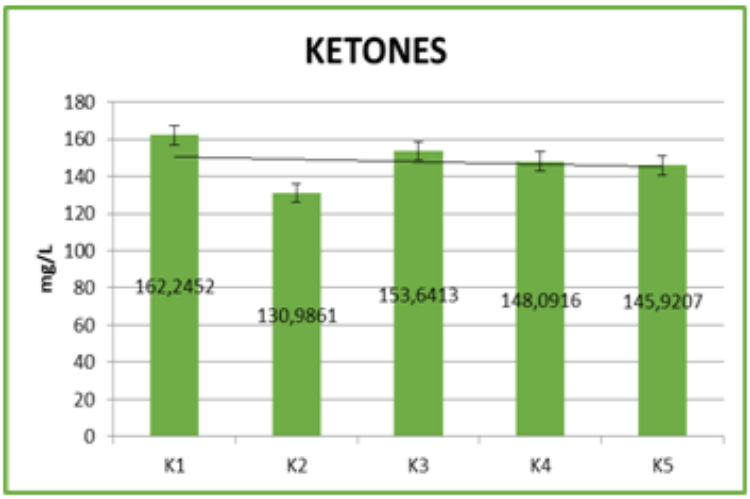

Fig. 3. *The amount of five samples of kefir (K1, K2, K3, K4, K5)*

Actively participate in the formation of esters taste and flavour kefir, so that some of them such as ethyl acetate, butanatul ethyl acetate and hexane giving scores from carbohydrate metabolism gentle fruit, pineapple, green apple or banana. But other esters (ethyl octanatul, the dean acetate or ethyl 3-methyl-1-butanol) identifiable as a shade of orange, banana, brandy or grape). Their amount ranged from between 439.0863 mg / L and 463.2015 mg / L, the average being set 451.5534 mg / L. Samples K1 and K3 exhibited amounts 461.1552 mg / L, respectively 463.2015 mg / L, with 3.6% higher than samples K4 and K5 according to Figure 4.

The carboxylic acids are mainly in carbohydrate metabolism or lipid of the micro-organisms so that the flavours results are vinegar, pepper, floral, fruity for acetic acid, cheese, sweet, sour for hexanoic acid, cheese, sweet soap octanoic acid or soap, waxy, butter, fruit or n-decanoic acid grass. The resulting values are close to those of the higher alcohols being observed in the sample minima and maxima in the case of the sample K4 K5. The determined values were not significantly different between each other, they do not exceed 12%.

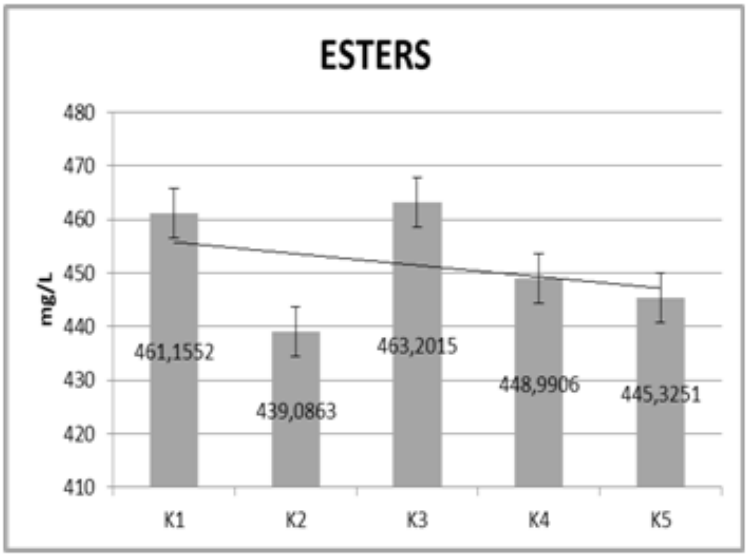

Fig. 4. *The amount of the esters resulting in five samples of kefir (K1, K2, K3, K4, K5)*

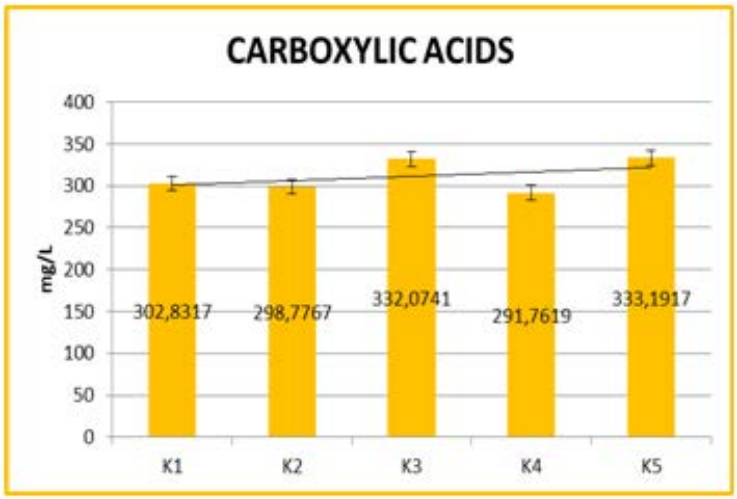

Fig. 5. *Amount of carboxylic acids resulting in five samples of kefir (K1, K2, K3, K4, K5*

4. Conclusions

Kefir flavours are dependent on the association of microorganisms used in its manufacture by the manufacturer, but also the percentage of fat in these products is. It has been observed that lipid metabolism was more pronounced for samples with particularly high levels of fat, so that the microorganisms involved in the fermentation process resulted in the accumulation of compounds such as aldehydes, ketones and carboxylic acids in amounts higher than in the case of the sample lith.

The resulting esters have the majority of the carbohydrate, so that the values determined were consistent with bacterial

cultures used and higher alcohols varied depending on the recipe used for the producer. Consequently, the flavors found in kefir cultures depend largely on the microorganisms used by the manufacturer of the concentration of fat in the milk used and the quality.

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