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Evaluation of Flavor and Aroma Compounds Present in Kefir

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ABSTRACT: Kefir is fermented milk that has a unique flavor because of acetaldehyde, diacetyl, acetoin and ethanol. The aim of this study was to evaluate flavor and aroma compounds present in kefir. Two types of starter (Christian Hansen and Sacco) and three levels of temperatures (30, 33 and 35°C) were used for the preparation of kefir samples. The samples were analyzed for acetaldehyde, diacetyl, acetoin and ethanol by GC equipped with FID detector. Levels of acetaldehyde, diacetyl, acetoin and ethanol were increased with raising the temperature and decreased during cold storage from the first day until the 14th day. pH was decreased and acidity was increased in all samples during cold storage. After organoleptic evaluation, the prepared sample by Sacco at 35°C was known as the best sample and then the prepared samples by Sacco at 33 and 30°C were recognized as the favorable samples.

Keywords: *Aroma and Flavor Compounds, Fermentation, Kefir, Starter.*

Introduction

Kefir is fermented milk that has an acidic flavor, yeasty aroma, creamy consistency and slightly alcoholic odor (Witthuhn *et al*., 2005). Kefir is derived from "kef" which means pleasant taste in Turkey (Kurman *et al*., 1992). This beverage was produced in Russia for the first time (Plessas *et al*., 2007). Kefir is made by inoculating milk with kefir grains. Kefir grains are like cauliflower blossoms, yellowish-white, small and hard. They are made from every kind of milk: cow, goat, sheep, camel, buffalo, coconut, rice and soybean; but it is common to use cows milk (Otles & Cagindi, 2003). A Kefir grain consists of numbers of bacterial and yeast species that have symbiotic metabolic activity (Vedamuthu, 1977). There is a complex mixture of lactic acid bacteria, *acetobacter* and yeast species in kefir (Piermaria *et al.*, 2008; Plessas *et al.,* 2008). Kefir microbial population produces lactic acid and other metabolites that increases milk maintenance potentiality and prevent the growth of pathogenic microorganisms (Witthuhn *et al.,* 2005).

Kefir unique flavor is because of lactic acid, ethanol, carbon dioxide and other flavor products, such as acetaldehyde, acetoin and diacetyl (Beshkova *et al*., 2003). Production of vitamin B1, B12, calcium, amino acids, folic acid and vitamin K is increased during the fermentation (Irigoyen *et al.,* 2005).

In the preparation of industrial kefir, starter culture was added to pasteurized milk at the rate of 2-3% (V/V) and incubated at 24- 26 °C until the pH was decreased to 4.6. After incubation, kefir was refrigerated (Guzel-Seydim *et al*., 2000).

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The objective of this study was to evaluate flavor and aroma compounds content in kefir.

Materials and Methods

Materials

Kefir starter cultures were purchased from Christian Hansen and Sacco companies.

a) DVS starter made by Christian Hansen was a mixture of CHN-22, ABT-2 and LAF-4.

CHN-22 included *Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Leuconostoc mesenteroides subsp. cremoris* and *Lactococcus lactis subsp. lactis biovar diacetylactis.*

ABT-2 included *Lactobacillus acidophilus, Bifidobacterium bifidum* and *Streptococcus thermophilus.*

LAF-4 included *Kluyveromyces marxianus subsp. marxianus.*

b) DVS starter made by Sacco (Lyofast MT 030 N) included *Lactococcus lactis subsp. lacis, Lactococcus lactis subsp. lactis biovar diacetylactis, Lactobacillus brevis, Leuconostoc* and *Saccharomyces cerevisiae.*

Standards including acetaldehyde and diacetyl were purchased from Sigma Chemical Co, acetone was purchased from Fluka, and finally ethanol was purchased from Merck Chemical Co.

Kefir Sample Preparation

Hansen package was 50 units and it was suitable for 500 L milk. First it was added into 1 L milk and vortexed for 30- 45min (150rpm). 8ml of a mixture of milk and starter was added into 4 L pasteurized and homogenized milk with 2.5% fat. Sacco package was 10 units and it was suitable for 1000 L milk. Similar to Hansen, it was added into 1 L milk and vortexed for 30-45min (150rpm). 4ml of a mixture of milk and starter was added into 4 L pasteurized and homogenized milk with 2.5% fat. Immediately after inoculation, milk was incubated at one of the temperatures (30, 33 and 35^{o}C). The desired final pH of the product was 4.6. After that, the samples were transferred to a refrigerator and stored for two weeks at 4^{o}C. Physicochemical experiments were carried out on days 1, 7 and 14 during cold storage.

Determination of pH and acidity

pH was measured by CRISON pH meter and acidity was measured by AOAC method (2002).

Determination of Flavor and Aroma Compounds

Twenty grams of each sample was diluted with 30 mL distilled deionized water, HCl was added until the pH was decreased to 2.5 and vortexed for 1h at 25^{o}C. The Samples were left for 2h to coagulate, centrifuged for 10min (4000 rpm) to separate coagulations, collected at the upper solutions and defatted with normal hexane. They were passed through Sephadex column (G 75 – 15×1 cm) to remove proteins and other polymers (Dean, 1974). Again, the samples were passed through XAD-2 column (10cm×1cm) to remove sugars and non-polar compounds (Grabarczyk & Korolczuk, 2010). The passed solution was extracted with 15 mL diethyl ether two times and cooled at 0^{o}C.

For the analysis of volatile component analysis, 8mL sample was transferred into GC vials,and injected onto a 3m Propac Q column (1/6 inch diameter) maintained at 100^{o}C. The column temperature was programmed at 150^{o}C and the temperature of FID detector set at 250^{o}C. Argon (flow of 20 mL/min) was used as the carrier gas (Determann, 1972).

Standard solutions of acetaldehyde, acetoin, diacetyl and ethanol were prepared with distilled deionized water. To remove error, the standard addition was carried out for all samples and the analysis was repeated. The qualification of the volatile

components in the experimental samples was accomplished by comparison between the relative retention time of the samples and standard solutions.

Sensory Evaluation

Sensory evaluation was performed by ranking method (Hubard, 1990). 10 persons were selected as panelists. Color, odour, flavor, consistency, acidity and CO2 of the samples were evaluated.

Statistical Analysis

The data were analyzed using the two-way analysis of variance (ANOVA) by SPSS 16. Duncan's multiple range tests were employed compare the means when a significant variation was established by ANOVA at the significance level 0.05 ($\alpha = 0.05$) All samples were analyzed three times.

Results and Discussion

Both homofermentative and heterofermentative pathways were employed by the bacteria and led to an acidic product. The process of acidification is reported in Figure 1, showing a drop in pH values when the temperature or time of the storage was increased in all samples, meaning an increase in acidity as presented in Figure 2.

Organic acids may occur in dairy products as a result of hydrolysis of fatty acids, biochemical metabolic processes, or bacterial metabolism (Guzel-Seydim *et al*., 2000).

Guzel– Seydim *et al* (2005) also indicated that during cold storage, acidity was increased with a consequent drop-in pH (Fig. 2).

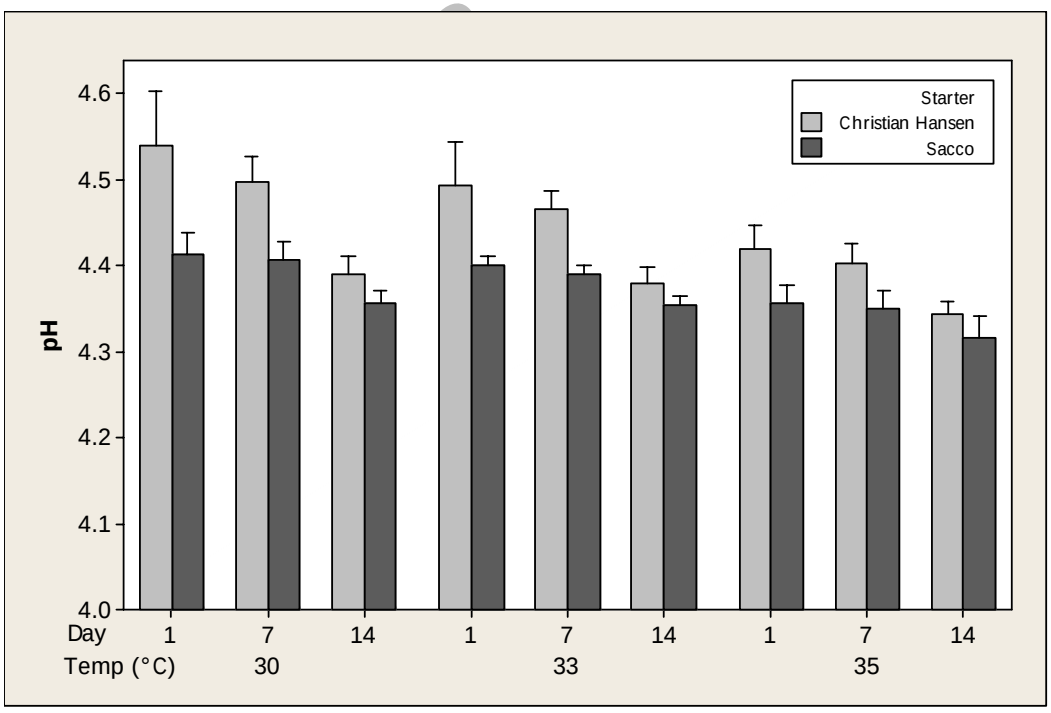

Fig. 1. pH comparison between kefir samples produced by Christian Hansen and Sacco starters at different times and temperatures

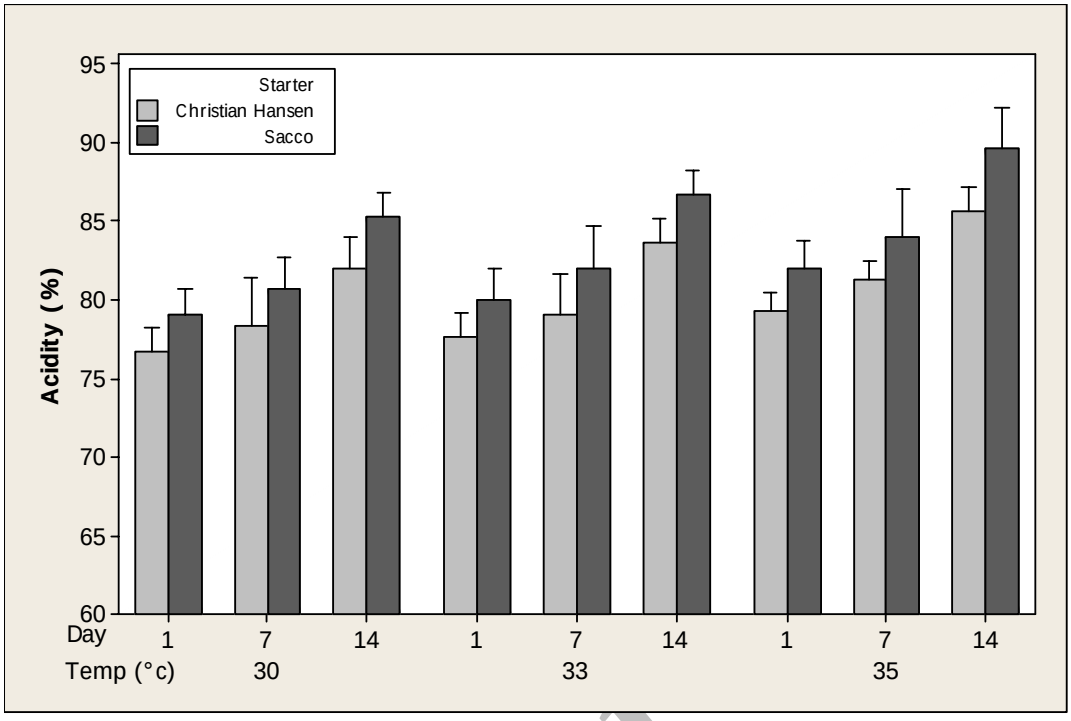

Fig. 2. Acidity comparison between kefir samples produced by Christian Hansen and Sacco starters at different times and temperatures

Acetaldehyde, diacetyl, acetoin and ethanol are important flavor substances for many types of cultured dairy products.

In all samples, acetaldehyde concentration was increased significantly ($P<0.05$) with raising the temperature and decreased during cold storage ($P<0.05$) Highest concentration of acetaldehyde was detected at 0.933 µg/g in the sample produced by Sacco starter at 35°C, on the first day and the least was at 0.186 µg/g in the sample produced by Christian Hansen starter at 30°C, on the 14th day (Fig. 3). Beshkova *et al* (2003) showed that acetaldehyde concentration was decreased during cold storage within 7 days. Acetaldehyde can be converted to ethanol by alcohol dehydrogenase (Tamime & Robinson, 1983).

Yeasts are primarily responsible for the alcohol production in kefir (Guzel-Seydim *et al*., 2000). The yeasts used in this study are *Kluyvermyces marxianus* (Rohm *et al*., 1992) and *Saccharomyces cerevisiae* (Rohm *et al*., 1992; Angulo *et al*., 1993). Although yeasts are commonly recognized for ethanol-producing ability, the bacterium, *L. kefir*, is a heterofermentative lactobacillus capable of producing ethanol (up to 0.25%) and CO2 (Marshall *et al*., 1984). There are notable variations among the reported ethanol contents of kefir (0.01 – 1.0%) (Kurman *et al.,* 1992; Marshall & Cole, 1985; Vedamuthu, 1977). In all samples, ethanol concentration was increased significantly ($P<0.05$) with raising the temperature and decreased during cold storage ($P<0.05$) The most amount of ethanol was 1863.3 µg/g in the sample produced by Sacco starter at 35 °C on the first day and its least amount was 298 µg/g in the sample produced by Christian Hansen starter at 30 °C, on the 14th day (Fig. 4). Higher alcohol content may be associated with a yeasty flavor and, in fact, authentic kefir does have a very slight yeasty flavor (Kroger, 1993; Vedamuthu, 1977).

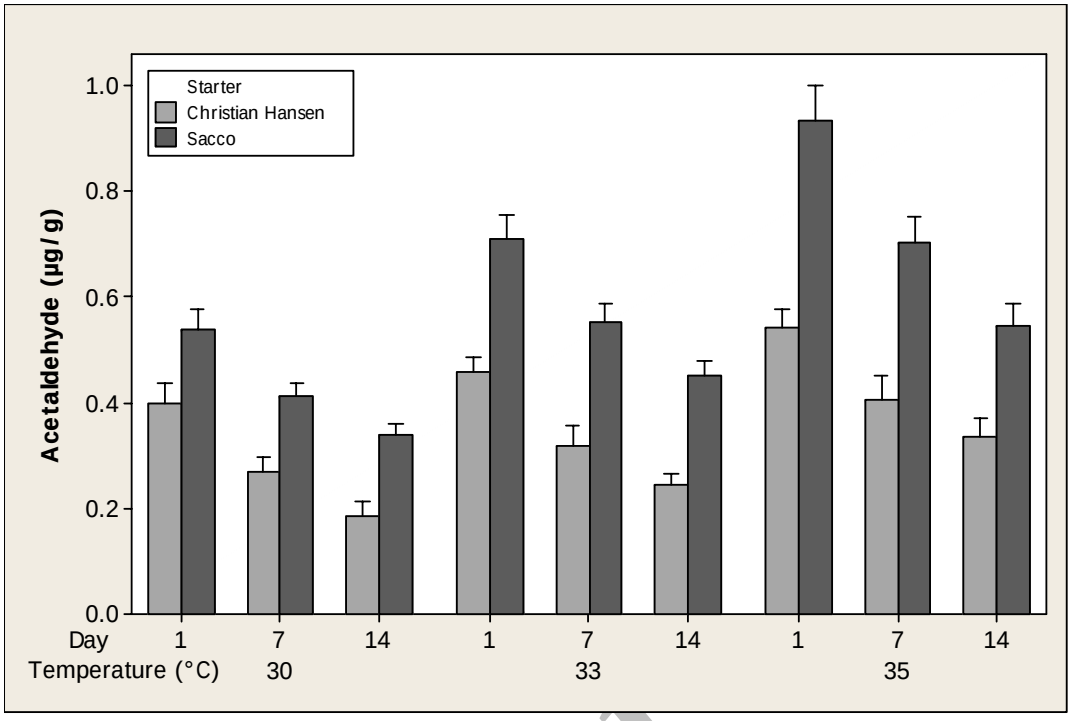

Fig. 3. Acetaldehyde comparison between kefir samples produced by Christian Hansen and Sacco starters at different times and temperatures

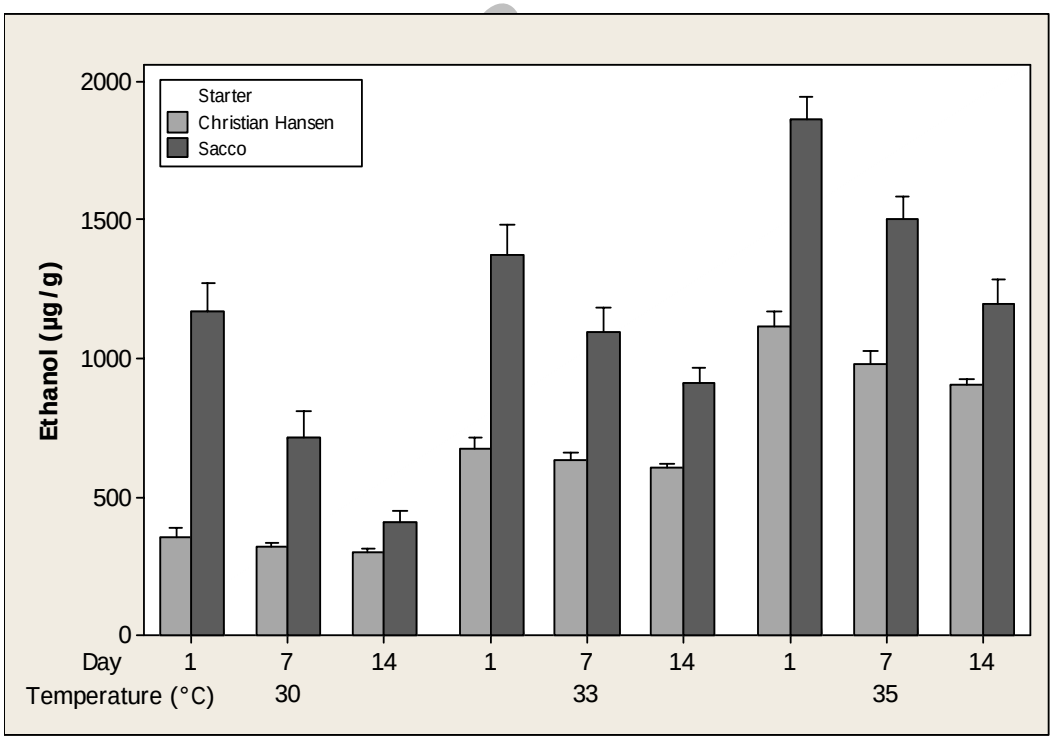

Fig. 4. Ethanol comparison between kefir samples produced by Christian Hansen and Sacco starters at different times and temperatures

Samples prapered by Christian Hansen starter didn't produce acetoin and diacetyl at all. In samples produced by Sacco, acetoin concentration was increased significantly ($P<0.05$) with raising the temperature and decreased during cold storage ($P<0.05$) The most amount of acetoin was 0.396 µg/g at 35°C, on the first day and its least amount was 0.050 µg/g at 30 °C, on the 14th day (Fig. 5). Beshkova *et al* (2003) showed that acetoin concentration was decreased during cold storage in 7 days. Production of acetoin by yeasts was stimulated by acetaldehyde addition (Chuang & Collins, 1968).

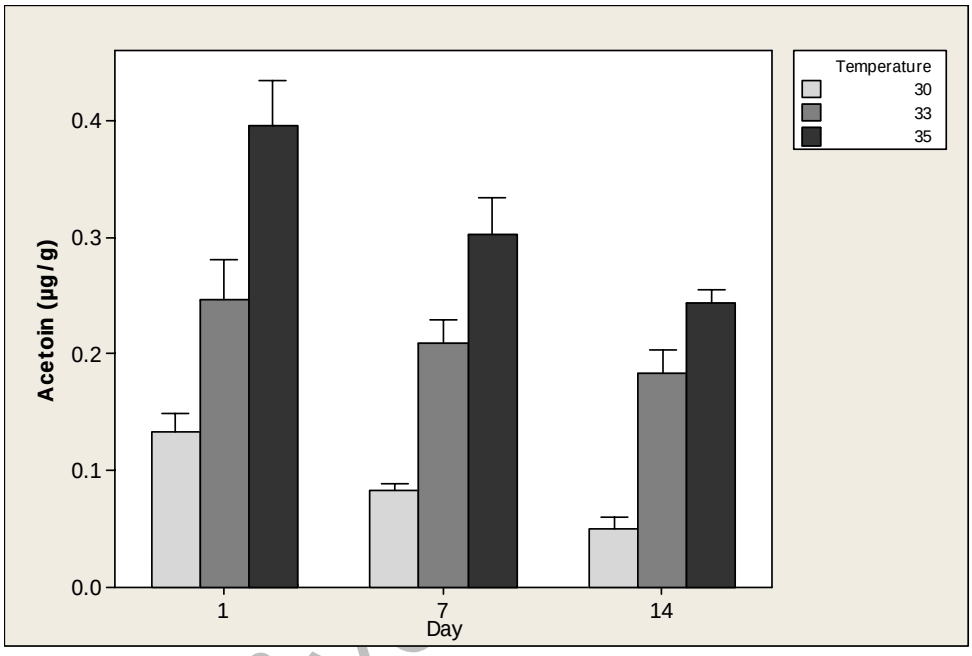

Fig. 5. Acetoin comparison between kefir samples produced by Sacco starter at different times and temperatures

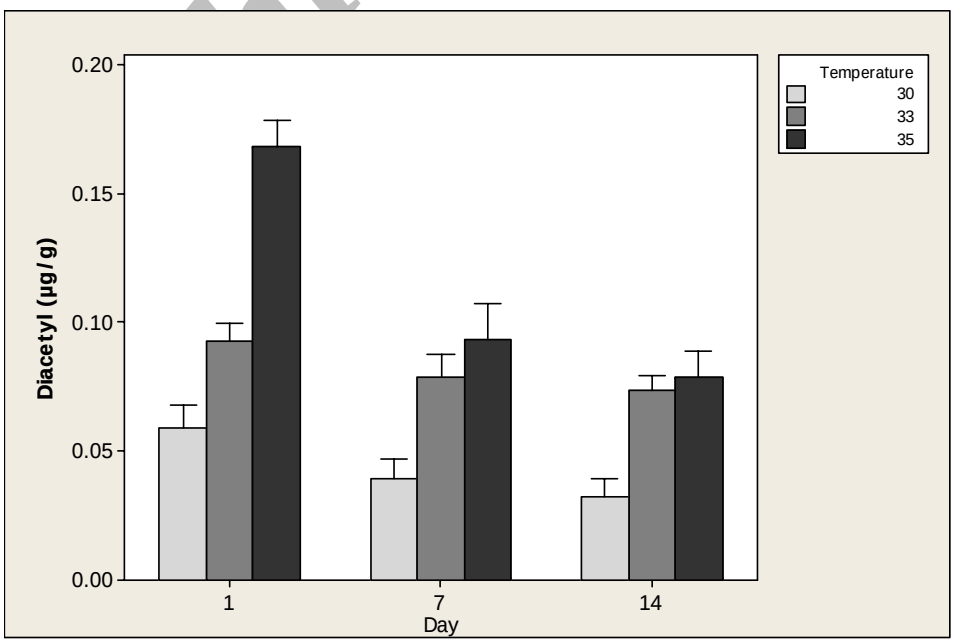

Fig. 6. Diacetyl comparison between kefir samples produced by Sacco starter in different times and temperatures

In the samples produced by Sacco, diacetyl concentration was increased significantly ($P<0.05$) with raising of the temperature and decreased during cold storage ($P<0.05$). The most amount of diacetyl was 0.168 µg/g at 35 ^{o}C, on the first day and the least was 0.032 µg/g at 30 ^{o}C, on the 14th day (Fig. 6). Beshkova *et al* (2003) showed that diacetyl concentration was decreased during cold storage in 7 days.

Conclusion

The samples prepared by Sacco produced acetaldehyde, acetoin, diacetyl and ethanol but the samples prepared by Christian Hansen just produced acetaldehyde and ethanol. Production of flavor and aroma compounds at 35 ^{o}C was higher than other temperatures. The amount of flavor and aroma compounds in the samples prepared by Sacco was more than the samples prepared by Christian Hansen. In all samples, when the temperature was increased, pH was decreased and consequently the acidity was increased. During cold storage, pH in all samples was decreased and acidity was increased. Organoleptic evaluation of the samples prepared by Sacco at 35 ^{o}C were identified as the best samples and the samples prepared by Sacco at 33 and 30 ^{o}C were recognized as favorable samples.

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