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Research Article

Microbiological, rheological properties and aroma composition of flower and pine honey-added ayran

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ABSTRACT

The objective of this study is to increase the functional qualities of Ayran by using honey from various sources. The goal of the research is to ascertain the physicochemical, rheological, and aroma composition of Ayran made with honey addition, as well as to assess the effect of different types of honey added to Ayran. Two types of honey (flower and pine) and an equal blend of these honey at a mixing ratio of 10% were utilized in making of Ayran samples. The pH values of the Ayran samples were higher in the honey-added samples compared to the control group. According to the statistical data obtained, the difference between L*, a* and b* values in Ayran samples was significantly different ($p<0.05$). Adding honey to Ayran samples increased the number of *Lactobacillus delbrueckii* subps. *bulgaricus* towards the end of storage. The floral and combination honey samples were found to be more viscous than the samples of pine honey. It was determined that the flower honey sample had a total hydrocarbon content close to the control group and the sample containing the highest total terpene among the other samples. Higher amounts of aldehydes, ketone, and carboxylic acids were found in Ayran samples of pine honey. The Ayran with mixed honey had the greatest levels of total ester and alcohol. Honey enhances the qualities of Ayran; thus, both flower and pine honey can be added to Ayran. Although Ayran's smoothness and sweetness had no adverse effects on sensory characteristics, it was decided that 10% or a little less honey could be added.

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INTRODUCTION

Almost every nation in the world produces drinkable fermented milk products. They are widely produced in Asia and the Middle East (with different names such as lassi, doogh, mast, and Ayran) and Scandinavian countries (such as acidophilus milk, viili, täfil and filmjölk). To pique consumer interest, fermented drinkable products frequently include sugar, fruit syrup, sweeteners, flavorings, and colorings. Ayran is the most common drinkable fermented product in Turkey. Unlike other fermented drink items, Ayran has a salty and sour flavor [1]. Ayran can be made to yogurt by adding water (household type) or to milk with standardized dry matter for fermentation. *Streptococcus*

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thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* are used for industrial production [2]. Its high vitamin and calcium content makes it is very beneficial and easily digestible [3]. In addition to their health advantages, fermented milk products like Ayran are renowned for their flavor and odor [4,5]. Additives such as flavors and sweeteners are used to offer a more appealing product to the consumer. According to several studies, numerous ingredients are added to Ayran to taste and sweeten it. Researchers have examined the properties of Ayran by adding apple [6], plum nectar [7], date and fig syrup [8] to Ayran. In other studies, changes in product quality have been studied by adding honey to kefir beverage, which are fermented products, though the addition of honey to Ayran was not reported earlier [5,9–12].

One of the most crucial characteristics of dairy products is their odor, which influences consumer preference and happiness and establishes a crucial quality element in product selection. Volatile chemicals, mostly created during fermentation, are crucial to how we perceive the smell of dairy products [13]. The primary flavoring components of Ayran are acetaldehyde, acetoin, acetone, and acetoin [14]. Ayran's aroma profile has been described by Sarhir et al. [13], but no study in the literature describes the olfactory profile of Ayran that has been sweetened with honey.

Honey is a natural sweetener that is widely used in fermented milk products (such as Ayran, kefir, lassi, and yogurt) to reduce sourness and improve consumer acceptability [15]. Honey is defined as a liquid produced by bees (*Apis mellifera*). pH value of honey is around 3.90, making it compatible with many food products in terms of acidity. In addition to this feature, it can reduce the sourness of solutions [16]. Due to its high antioxidant and anti-inflammatory characteristics, honey's flavonoids and phenolics play a significant role in human health. The cardiovascular, neurological, and gastrointestinal systems are indicated to be protected by honey's antibacterial and anticancer properties [9,17].

The subject of this study was to increase the functional properties of Ayran by adding a foodstuff such as honey, which is important for human health with its many properties, to Ayran and to offer a different alternative for consumers who do not like a sour taste. Since different types of honey have different properties, determining the change in Ayran properties at the determined addition rate revealed the necessity of examining the use of honey in single or mixture form when used industrially. This study aimed to evaluate how different types of honey affect the physicochemical, rheological, and flavor profiles of Ayran and how honeys of different origins modify the properties of Ayran.

MATERIALS AND METHODS

Materials

Raw cow milk used in Ayran production was obtained from Isparta Unsut dairy. For three replicated productions, milk was supplied at three-day intervals. Ayran culture used in production (YF-L903, Chr Hansen™) was supplied by Chr. Hansen San. Trade Inc. Istanbul – Turkey. Honey (pine honey and flower honey) were obtained from Sezen Gıda Ltd Co (Istanbul) which is available in the market (Anavarza Honey™).

Methods

Honey-added Ayran production

Raw cow's milk was pasteurized at 90 °C and then cooled to 45 °C. Then, 2% Ayran culture (It was added from cultures containing live microorganisms at a level of approximately 10^8 cfu/mL) was inoculated into the milk. The fermentation process was terminated when the pH reached 4.4 – 4.5. Ayran dry matter was standardized (with water to be 7.5%). Then, 0.3% salt was added and mixed until it became homogeneous. The fat content of honey-added Ayran is standardized to be 17% fat in dry matter [18,19]. It was stated that adding 10% honey produced honey Ayran-like lassi with high scores that could be evaluated in terms of taste, color, appearance and overall acceptability. It was stated that 10% honey consumption resulted in higher lactobacillus and streptococcus counts, and therefore, 10% honey capacity was chosen for the production and shelf life term of Herbal Honey Lassi [20]. Ayran produced was divided into 4 equal parts. The first group was prepared without the addition of honey as the control group (sample AA). The remaining groups are as follows: 10% (w/v) flower honey (Sample BB), 10% (w/v) pine honey (Sample CC), 10% (w/v) mixture (pine and flower honey; 1:1) (Sample DD) honey. Ayrans were mixed homogeneously with a homogenizer (IKA T 18 Digital Ultra-Turrax, Germany). Afterward, the produced Ayran samples were stored in a refrigerator (+4 °C) to perform their physicochemical, aroma, microbiological and sensory analyses on the 1st, 10th and 21st days. The study was produced in three replications.

Physicochemical properties of honey and Ayran

The pH values of the Ayran with honey added were determined with a digital pH meter (WTW pH 315, Weilheim, Germany). The % salt amount in Ayran samples added honey was determined according to the Mohr method [21].

Color and rheological properties of Ayran samples

The color characteristics of all the samples were determined according to Hunter [22]. The measured L*, a*, and b* values were determined with the Chroma Meter (CR400 MINOLTA, Japan, CIE Lab system). To determine the rheological properties of Ayran, flow type was determined together with a viscometer (Brookfield DV-II+Pro Extra model, Brookfield Engineering Laboratories Inc., USA) and a small sample adapter. SC4-27 and LV-4 cylindrical spindle tip was used. Shear stress, shear rate, and apparent viscosity values of samples were recorded, and the consistency index and the power law index were determined using the power law model for interpreting the shear stress–shear rate relationship of the Ayran samples using RHEOCALC® 32 Application Software (Brookfield Engineering Laboratories Inc.). The RHEOCAL® 32 Application (Brookfield Engineering Laboratories Inc., USA) software was used to draw the graphics. 10 mL of sample was placed in the chamber and measured according to the flow test method for 2 minutes at a constant shear rate of 60 s^{-1}.

The Power Law Model given in the Equation 1 was used to determine the rheological properties of the samples.

$$\sigma = K\gamma^2 \quad (1)$$

where σ, K, γ and n are the shear stress, the consistency coefficient, the shear rate and the flow index values respectively.

Microbiological properties of Ayran

Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus* content was determined according to the method of Mossel et al. [23]. The sample for microbiological cultivation was prepared by adding 10 mL of sample to 90 mL of sterile Ringer's solution. Dilutions were prepared from this prepared solution. For enumeration of *Lactobacillus delbrueckii* subsp. *bulgaricus* 1 mL Ayran samples were taken into a sterile petri dish and poured onto de Man Rogosa and Sharpe (MRS) agar medium. Petri dishes were incubated at 37 °C for 72 hours under anaerobic conditions. To determine the number of *Streptococcus thermophilus*, a 1 mL sample from the prepared dilution was taken into a sterile petri dish and poured into an M17 agar medium. Petri dishes were incubated at 37 °C for 48 hours. At the end of the incubation, counts were made from petri dishes containing between 30 and 300 colonies and the counts were expressed as log 10 cfu mL^{-1}. Yeast, mold and total coliform bacteria results of the Ayrans in the study were evaluated and given [24]. Also chemical, sensory and microbiological analysis results are given [24].

Analysis of aroma components of honey-added Ayran

Analysis of aroma components in Ayran with honey added was determined by gas chromatography (GC-MS) and solid-phase microextraction (SPME) [25]. Shimadzu (Japan) GC-2010 Plus Shimadzu GCMS-QP2010 SE (Detector) was kept in the oven at 40 °C for 2 minutes, reached 250 °C with an increase of 4 °C per minute and kept at 250 °C for 5 minutes. Restek Rx-5Sil MS (catalog no: Restek 13623 CP) 30 m * 0.25 mm, 0.25 µm was used as a column. Injection block and detector temperatures were adjusted to 250 °C. Headspace conditions; fused silica SPME (Solid Phase Microextraction) fiber CAR/PDMS 3 g sample was kept at 45 °C without fiber for 15 minutes with fiber for 30 minutes and desorbed at 250 °C [25].

Sensory analysis of honey-added Ayran

Sensory analyses of Ayrans were applied according to the method given by Uysal et al. [26]. On the 1st, 10th and 21st days of Ayrans, the panelists were given training on evaluation methods, sensory testing techniques and product features. Evaluation was carried out by 15 panelists (12 females and 3 males) according to the scoring method. Ayran samples were presented to the panelists in 20-25 mL portions with plain biscuits and water. The panelists scored on the hedonic scale from 1 to 10 according to the characteristics of yellowness, fluidity, honey-like odor, sweetness, and saltiness. In the evaluation, 1 point was interpreted as I did not like, and 10 points were interpreted as I liked it very much.

Statistical Analysis

Duncan Multiple Comparison Test was applied by performing variance analysis on Ayran samples [27]. Statistical analyses were performed using the SPSS 17.0 program. In this study, the principal component analysis (PCA) method was applied to the first day of sensory analysis and aroma (specific aroma compound for Ayran) results using Xlstat trial version (2022.4.1). It was applied to visualize the relationship between data and reduce the data size.

RESULTS AND DISCUSSION

Properties of Raw Milk and Honeys

The characteristics of the raw cow's milk used to make Ayran were in accordance with the Turkish Food Codex Raw Milk Communiqué [2]. The total soluble solids content (brix) of the honey was found to be 80.5±0.5% in flower honey and 82±0.2% in pine honey [24].

Physicochemical Analysis Results of Honey-added Ayran

Physicochemical analysis results of Ayran produced with the addition of honey from different floral origins are given in Table 1.

pH in Ayran samples exhibited a consistent decline during storage. The sample from the control group had the lowest pH value (4.35). Compared to the control group, the samples with honey addition had higher pH values. The pH values of the DD group (10% mixed honey) and CC group (10% pine honey) samples were higher than those of the other samples. Between the 10th and 21st days of storage, it can be noted that there is little difference in the pH values of the samples containing honey. Anwar et al. [9], in their study, reported that while the pH value of kefir in the control group was 4.20, the sample containing 9% honey had a pH value of 4.50. The lactic acid reacts with the active free hydroxyl groups and active carbohydrate groups (glucose and fructose) in honey. As a result, more organic compounds are created (ester compounds). The lactic acid value is reduced because of this esterification reaction [9].

Ayrans have a salt level that ranges from 0.55 to 0.63%. The control group (sample AA) had the greatest salt concentration (0.63%). BB group sample, to which 10% floral honey were added, had the lowest salt concentration (0.55%). Ayran samples' % salt readings were determined to be reasonably close to one another. Salt increases the perception of taste, and salt is traditionally added to Ayran. Since honey was added, a smaller amount of salt was added.

Luminance L* (0 black, 100 white), a* (- green, + red), and b* (- blue, + yellow) are used to express the findings for color parameters. The L* value of the honey-added Ayran samples (BB, CC, and DD) was lower than the control group. The natural honey color was linked to a decline in L* value. The brightness value (L* value) of honey-added Ayran increased at the end of the storage time. The change in L* value during storage was determined to be statistically insignificant in the control group Ayran samples. Machado et al. [28] found that the effects of honey addition on the L* values of goat yogurts were similar to this study.

Examining the samples' a* values revealed that the honey-added Ayran samples (BB, CC, and DD) had lower a* values than the control. Additionally, it was discovered that the Ayran sample (BB) with 10% flower honey had a lower a* value than the sample (CC) with 10% pine honey. The b* values of Ayran samples range from 4.54 to 6.94. The samples in the control group that did not have any honey added had a lower b* value than those that did. It was discovered that the b* values of group CC's Ayran samples, which contained 10% pine honey, and group DD's samples, which contained 10% mixed honey, were greater than those of group BB's Ayran samples, which contained 10% floral honey. According to Dogan [11], when pine and flower honey were added to the kefir samples, the a* and b* values of the groups were higher than when flower honey was applied to the kefir samples. When pine honey was supplied as in this study, the a* and b* values increased more than those of floral honey.

Rheological Characterization Of Honey-added Ayran

Table 2 provides the apparent viscosity findings of Ayran samples with pine and floral honey added, evaluated at 60 RPM. At the end of storage, honey Ayran with 10% floral honey had the highest viscosity values (22.62 mPas), while the lowest value was measured as 18.50 mPas in the first- and tenth-day control group Ayrans. The viscosity of Ayrans was raised more by adding floral honey than by adding pine honey. Due to its high carbohydrate content, honey has the capacity to bind water. As a result, honey is a substance that can make a substance it is added to thicken or become more viscous [9].

The consistency coefficient (K), flow index (n) and correlation coefficient (R^2) of Ayran samples during storage are given in Table 2. To ensure the flow behavior of Ayran samples with varied floral origin honey added, the shear stress and deformation rate in Ayran samples were calculated. The rheological characteristics of Ayran samples were determined in the study using the power law model. This is due to the non-Newtonian flow behavior of the fluid type and the zero-threshold shear stress. While the flow index (n) is n=1 in the Newtonian flow type, n<1 in the non-Newtonian flow behavior type is considered as pseudoplastic flow [18]. Since the flow index values (n) of all Ayran samples were below one, they showed shear thinning behavior. Köksoy & Kiliç [29] found the flow index of the Ayran samples below one and reported that they showed a similar non-Newtonian flow.

Table 1. Physicochemical analysis results of Ayran samples during the storage period

Analyses	Day	Samples			
		AA*	BB	CC	DD
pH	1	4.53±0.15^{a**}	4.69±0.11^{a}	4.74±0.12^{a}	4.74±0.06^{a}
	10	4.41±0.23^{a}	4.61±0.17^{a}	4.67±0.17^{a}	4.68±0.13^{a}
	21	4.35±0.22^{a}	4.55±0.21^{a}	4.66±0.17^{a}	4.64±0.16^{a}
Salt (%)	1	0.58±0.11^{a}	0.55±0.14^{a}	0.56±0.13^{a}	0.59±0.16^{a}
	10	0.63±0.16^{a}	0.55±0.12^{a}	0.56±0.13^{a}	0.56±0.11^{a}
	21	0.63±0.16^{a}	0.56±0.13^{a}	0.57±0.10^{a}	0.60±0.15^{a}
L*	1	81.14±0.14^{a}	78.31±0.61^{cd}	77.77±0.67^{d}	78.05±0.23^{cd}
	10	81.24±0.23^{a}	78.98±0.18^{b}	78.19±0.31^{cd}	78.04±0.16^{cd}
	21	81.20±0.19^{a}	78.93±0.16^{b}	78.29±0.28^{cd}	78.42±0.21^{bc}
a*	1	-2.81±0.01^{gh}	-2.66±0.06^{cd}	-2.46±0.05^{b}	-2.55±0.05^{c}
	10	-2.77±0.03^{fg}	-2.65±0.01^{de}	-2.37±0.04^{a}	-2.43±0.02^{ab}
	21	-2.88±0.02^{g}	-2.70±0.05^{ef}	-2.42±0.05^{ab}	-2.55±0.07^{c}
b*	1	4.54±0.10^{f}	6.42±0.11^{de}	6.62±0.05b^{cd}	6.52±0.18^{cde}
	10	4.62±0.06^{f}	6.33±0.03^{e}	6.74±0.01^{b}	6.94±0.22^{d}
	21	4.54±0.01^{f}	6.35±0.11^{e}	6.66±0.01^{bc}	6.57±0.13b^{cd}

*AA: Control; BB: 10% (w/v) flower honey; CC: 10% (w/v) pine honey; DD: 10% (w/v) mixture (flower and pine honey; ratio 1:1).
** The letters indicate that the difference between days and samples is significant (p<0.05).

Table 2. Rheological properties of Ayran samples during the storage period

Sample	Day	*K*	*n*	*R2*	Apparent viscosity (mPas, 60 s-1)
AA	1	1679	0.54	0.974	18.50±1.25^{aBC*}
	10	1661	0.28	0.995	18.50±1.25^{aBC}
	21	1991	0.23	0.991	19.25±1.50^{aB}
BB	1	1740	0.41	0.990	19.25±1.50^{aB}
	10	1802	0.41	0.987	20.37±2.75^{aAB}
	21	2064	0.22	0.997	22.62±2.12^{aA}
CC	1	2230	0.31	0.994	19.00±3.00^{aB}
	10	2543	0.38	0.993	19.75±1.00^{aB}
	21	2618	0.38	0.996	20.87±3.37^{aB}
DD	1	1893	0.47	0.993	20.12±1.25^{aAB}
	10	1929	036	0.982	20.87±3.12^{aAB}
	21	2581	0.37	0.969	21.12±3.37^{aAB}

K: Consistency index, n: flow behaviour index; AA: control; BB: 10% (w/v) flower honey; CC: 10% (w/v) pine honey; DD: 10% (w/v) mixture (flower and pine honey ratio; 1:1).

*Difference between groups have determined that significant showed as capital letter. Difference between times have demonstrated that significant showed as small letter.

The ratio of viscosity to shear rate is computed depending on time to ascertain the time-dependent flow behavior of Ayran samples in terms of rheological parameters. Under constant shear rate conditions, some liquids may exhibit a change in apparent viscosity over time. The apparent viscosity of a sample was plotted as a function of shear rate to determine the time-dependent flow behavior of Ayran samples. Apparent viscosity values of the samples decreased as the shear rate increased exhibiting thixotropy. The findings of Bourne [30] and Kok Taş [18] were similar.

As the samples' shear rate rises, it is evident that the apparent viscosity does as well (Figure 1). The samples were found to have thixotropic flow as a result. Kök-Taş et al. [31] determined that the flow type of kefir samples, which they investigated with different fermentation parameters, was similarly thixotropic. It has been suggested that loose physical interactions between proteins cause thixotropic behavior in liquid fermented milk because of weak electrostatic and hydrophobic connections [31].

Microbiological Analysis Results of Honey-added Ayran

Figure 2A displays the numbers of *Lactobacillus delbrueckii* subps. *bulgaricus* found in samples of honey-added-Ayran. The control Ayran sample's *Lactobacillus delbrueckii* subps. *bulgaricus* count was greater on the first day of storage (5.7 log cfu mL^{-1}) than those of the Ayran samples that had been added honey (BB; 3.60, CC and DD; 4.16 log cfu mL^{-1}). After 21 days of storage, the honey-added Ayran samples (BB; 5.53, CC and DD) had greater levels of *Lactobacillus delbrueckii* subps. *bulgaricus* than the control group (4.30 log cfu mL^{-1}). Adding honey to Ayran samples boosted the concentration of *Lactobacillus delbrueckii* subps. *bulgaricus* towards the end of storage. The total number of viable lactobacilli was initially 9.07 log cfu g^{-1} in control Lassi, while 8.72 log cfu g-1 in Herbal Honey Lassi [20]. Figure 2B displays the quantity of *Streptococcus thermophilus* found in samples of honey-added Ayran. The control group Ayran sample had 7.09 log cfu mL^{-1} *Streptococcus thermophilus* numbers on the 1st day of storage that reached 8.37 log cfu mL^{-1} at the end of the storage. *Streptococcus thermophilus* (7.07 log cfu mL^{-1}) concentrations were found to be comparable to the control group in all Ayran samples (except sample DD), even though honey was added to those samples on the first day of storage. *Streptococcus thermophilus* levels in the control group were 8.37 log cfu mL^{-1} on the 21st day of storage, whereas they ranged from 8.60 to 8.97 log cfu mL^{-1} in the honey-added Ayran samples. At the end of storage, Ayran samples included more *Streptococcus thermophilus* due to the honey addition. According to Sert et al. [32], the presence of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subps. *bulgaricus* was increased when honey was added to set-type yogurts at various concentrations. It has been stated that the number of lactobacilli and streptococci is higher in the presence of 10% honey of Herbal Honey Lassi [20]. With the outcomes that were found, it is reasonable.

Total coliform bacteria were not detected in Ayran samples. Considering the yeast-mold numbers, it was determined to be less than 1 log cfu/mL for all samples [24].

Volatile Aroma Component Results of Honey and Honey-added Ayran Samples

A total of 19 volatile substances were found in flower honey. Several chemicals have been found, including aldehydes (3), ketones (1), alcohols (2), carboxylic acids (1), esters (2), hydrocarbons (5), terpenes (2), carbonyl compounds (1), sulfur compounds (1) and furan compounds

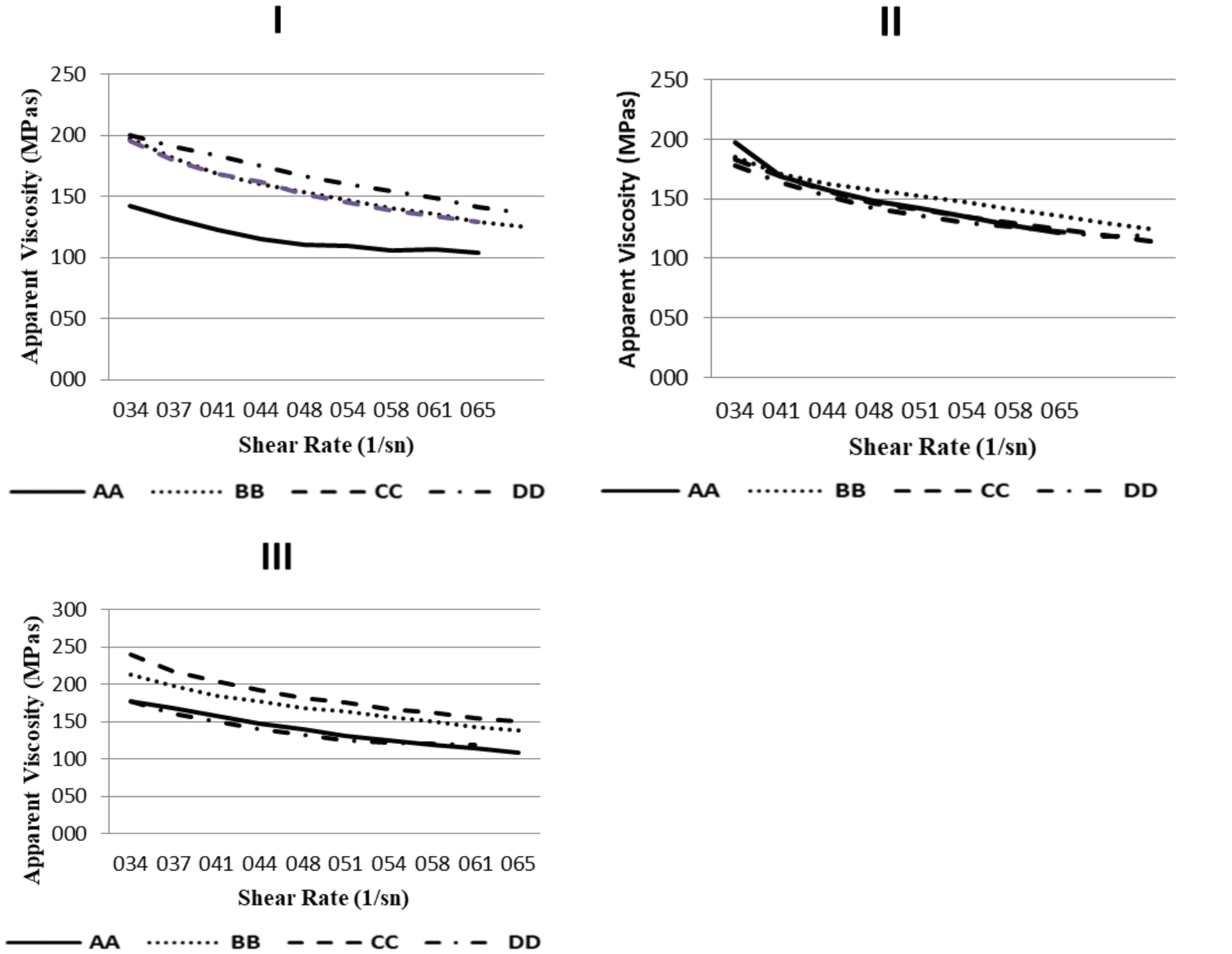

Figure 1. Apparent viscosity/shear rate rheograms of ayran samples. (I: 1st day of storage, II; 10th day of storage; III: 21st day of storage. AA: control; BB: 10% (w/v) flower honey; CC: 10% (w/v) pine honey; DD: 10% (w/v) mixture of flower and pine honey ratio; 1:1).

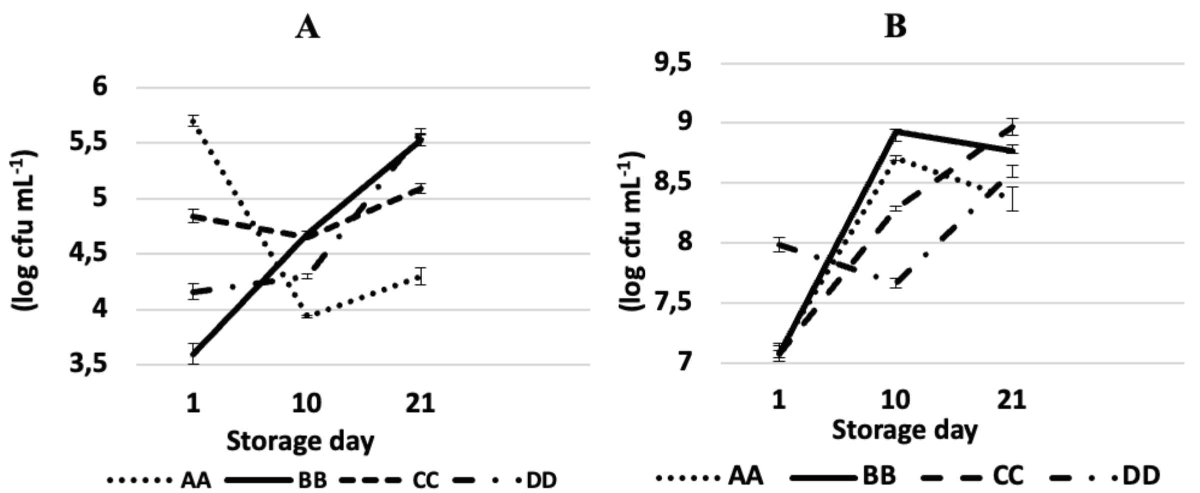

Figure 2. A: Number of *Lactobacillus delbrueckii* subsp. *bulgaricus* of Ayran samples; B: Number of *Streptococcus thermophilus* of Ayran samples (AA; control, BB; 10% (w/v) flower honey; CC: 10% (w/v) pine honey; DD: 10% (w/v) mixture of flower and pine honey ratio; 1:1).

(1). Molecules that make up most of the aroma of pine honey are aldehydes (15.26%), ketones (12.82%), alcohols (9.33%), carboxylic acids (6.33%), esters (11.15%), hydrocarbons (24.62%), terpenes (2.37%), sulfur compounds (7.52%), furan compounds (8.15%) and other compounds (2.14%) were present in the samples.

All honey Ayran samples had a total of 63 volatile components identified on the first day of storage (Table 3 and Table 3 continue). Aldehydes (5), ketones (9), alcohols (7), carboxylic acids (5), esters (6), hydrocarbons (16), terpenes (6), amines (1), carbonyl compounds (1), sulfur compounds (2), furan compounds (1), and other compounds (4) were found on the first day of storage.

Table 3. Volatile aroma components of Ayran samples on the 1st day of storage

Volatile compounds	Samples (area %)					
	Flower Honey	Pine Honey	AA*	BB	CC	DD
Aldehydes						
Acetaldehyde (CAS) Ethanal	nd	nd	11.77	12.99	13.10	10.92
Butanal, 2-methyl- (CAS) 2-Methylbutanal	nd	5.69	nd	nd	nd	nd
2-Phenylacetaldehyde (Hyacinthin)	1.60	nd	nd	nd	nd	nd
Benzaldehyde (CAS) Phenylmethanal	2.88	nd	nd	nd	nd	nd
Nonanal (CAS) n-Nonanal	10.88	9.57	nd	nd	1.07	nd
Ketones						
2-Butanone, 3-hydroxy- (CAS) Acetoin	nd	nd	10.80	8.11	12.39	3.18
2,3-Butanedione (CAS) Diacetyl	nd	nd	4.75	6.80	14.06	6.89
2-Dodecanone (CAS) Dodecan-2-one	nd	nd	nd	nd	1.33	nd
2-Heptanone (CAS) Heptan-2-one	nd	nd	4.54	5.11	4.65	3.32
2-Nonanone (CAS) Methyl heptyl ketone	nd	nd	2.67	2.24	2.82	2.14
4-Octen-3-one (CAS) 3-oxy-4-Octene	nd	nd	nd	nd	nd	8.64
2-Propanone (CAS) Acetone	10.96	12.82	8.93	11.07	14.06	nd
2-Pentanone (CAS) Methyl propyl ketone	nd	nd	nd	nd	0.89	0.44
2-Undecanone	nd	nd	1.72	nd	nd	1.22
Alcohols						
2-Butanol, 3-(2,2-dimethylpropoxy)-	nd	4.02	nd	nd	nd	nd
(2-(2-butoxyisopropoxy)-2-isopropanol	nd	3.69	nd	nd	nd	nd
Ethanol (CAS) Ethyl alcohol	nd	nd	nd	nd	nd	7.24
2,4-bis(1,1 –dimethylethyly)-phenol	4.16	nd	nd	nd	nd	nd
2,6-bis(1,1 –dimethylethyl)-4-(1-methylpropyl)-phenol	1.87	nd	nd	nd	nd	nd
2-Butoxyethanol	nd	1.62	nd	nd	nd	nd
7-Octen-2-ol, 2,6-dimethyl- (CAS) 2,6-Dimethyl-7-octen-	nd	nd	nd	3.95	nd	5.31
Carboxylic Acids						
Acetic acid (CAS) Ethylic acid	nd	6.63	5.07	4.05	5.44	4.93
Acetic acid, anhydride with formic acid	4.14	nd	nd	nd	nd	nd
Hexanoic acid (CAS) n-Hexanoic acid	nd	nd	1.54	nd	3.31	0.88
Octanoic acid (CAS) Caprylic acid	nd	nd	nd	2.65	nd	nd
Propanoic acid, 2-methyl- (CAS) Isobutyric acid	nd	nd	nd	nd	1.03	nd
Esters						
Acetic acid, ethyl ester (CAS) Ethyl acetate	3.24	2.17	1.67	nd	nd	0.73
Butyl citrate (CAS) 1,2,3-Propanetricarboxylic acid, 2-hydroxy-, tributyl ester	nd	1.43	nd	nd	nd	nd
Butyl isocyanatoacetate	nd	nd	nd	nd	nd	5.76
Linalyl acetate	1.90	2.82	nd	nd	nd	nd
3-Hydroxy-2,2,4-trimethylpentyl ester of isobutanoic acid	nd	2.03	nd	nd	nd	nd
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	nd	2.70	nd	nd	nd	nd

*AA; control, BB; 10% (w/v) flower honey, CC; 10% (w/v) pine honey, DD; 10% (w/v) mixture (flower and pine honey ratio; 1:1)

Aldehydes

Aldehydes are defined as the simplest carbohydrate molecules produced because of transamination and decarboxylation of amino acids, Strecker degradation or lipid metabolism by microorganisms. This secondary metabolite of lactic acid bacteria significantly influences the aroma of fermented milk products. Accumulation of acetaldehyde during fermentation can occur when the specific activities of acetaldehyde-forming enzymes are higher than those capable of converting it to ethanol (alcohol dehydrogenase). However excessive aldehyde concentrations cause a sharp (bitter) taste, and they have little effect on overall flavor due to low threshold values. Acetaldehyde provides the desired aroma with a fresh, green apple, slightly creamy and slightly sweet taste [33,34].

Both flower and pine honey include 15.36 and 15.26% of the total aldehydes, respectively. The branched aldehydes 2-methylbutanal (5.69%) found in pine honey are thought to have been generated via Strecker breakdown esters from isoleucine [35].

The pine honey-added Ayran sample had the highest acetaldehyde ratio (13.10%), while the Ayran sample with mixed honey (1:1) had the lowest acetaldehyde ratio (10.92%). Nonanal, a different aldehyde, was found in the pine-added Ayran sample (1.07%). Nonanal is a byproduct of lipid metabolism, and has an aroma that is like green citrus, flowery, cheesy, soapy, and oily [34].

Ketones

Ketones are byproducts of the breakdown of sugar (lactose) and fat by microorganisms. They frequently have low thresholds for detection. Particular ketones, including acetoin, are known to contribute to several advantageous flavor attributes [34,36]. The primary ketone group constituents found in honey-added Ayran samples include acetone, diacetly, 2-heptanone, 2-nonanone, and acetoin. While the acetoin component was discovered in the Ayran sample (12.39%) with a maximum addition of 10% pine honey, it was discovered in the sample with at least 10% mixed honey (3.18%). According to Larosa et al. [37], they discovered an acetoin component in kefir samples that they had sweetened with honey. It adds to the distinctive flavor of yogurt together with acetoin, acetaldehyde, acetone, and diacetyl compounds. Diacetyl and acetoin are produced when lactic acid bacteria ferment citrate during the pyruvate metabolic process [38]. Ayran samples' diacetyl ratios ranged between 4.75 and 14.06%. The Ayran sample from the control group had the lowest diacetyl ratio, whereas the Ayran with 10% pine honey added had the highest ratio. The ketone group's 2-heptanone, 2-nonanone, and 2-undecanone have a fruity aroma [39]. In Ayran samples, the ketone group chemical 2-heptanone was found in concentrations ranging from 3.32 to 5.11%. The sample with 10% flower honey added showed the most significant rate. The Ayran sample with 10% blended honey contained the lowest rate. Similar outcomes were obtained regardless of the sample's 2-nonanone content. The Ayran sample containing 10% mixed honey contained the least level of 2-nonanone.

Alcohols

The sum of the aroma components of the alcohol group in flower honey and pine honey was determined as 6.03 and 9.33%, respectively. Alcohol group aroma components were not found in the control sample and 10% pine-added honey samples. Ayran samples that had 10% blended honey also contained 7.24% ethanol. According to Larosa et al. [37], ethanol was found in kefir, which contained honey. The primary method for producing ethanol is lactose fermentation. However, Strecker decomposition can also make it from alanine. Since ethanol has an extremely high detection threshold (200 ppm), its flavor-enhancing effects are typically not given much weight. Its reaction with free fatty acids can created esters. Additionally, due to citrate metabolism, *Lactococcus* strains are the primary source of ethanol [35,40].

Carboxylic acids

Milk lipid lipolysis or protein breakdown can yield carboxylic acids with more than four carbon atoms, which is crucial for fermented dairy products. The oxidation of esters, ketones, and aldehydes can also result in the formation of short-chain fatty acids. They act on the distinctive olfactory notes and low odor thresholds of dairy products, giving them a highly significant aroma. Hexanoic acid is said to have an acrid, cheesy, sour, rotten, heavy, and putrid taste. Acetic acid is said to have an acidic, sour, vinegar, and harsh flavor [34,41].

The carboxylic acid group's acetic acid content in floral and pine honey was found to be 4.14% and 6.63%, respectively. Escriche et al. [42] identified the fragrance components of flower and pine bamboo honeys in their investigation. They discovered 2.0 and 6.42%, respectively, of acetic acid in floral and pine honey. Pine honey exhibits its similarities to the findings of this study, although flower honey performs somewhat worse.

The range of the total quantities of chemicals from the carboxylic acid group in the Ayran samples was between 5.81 and 9.78%. Sample CC contains 10% pine honey and has the highest acetic acid content (5.44%) of all the Ayran samples. The sample with the lowest level of acetic acid (4.05%) was sample BB, which included 10% floral honey. Additionally, in the Ayran sample, hexanoic and octanoic acids were identified. Larosa et al. [37] reported that kefir samples produced using different sugars and honey, contained acetic acid, hexanoic acid and octanoic acid.

Esters

Esters are defined as substances produced either by fermentation-related de-novo microbial synthesis employing alcohol acetyltransferase on acetyl-CoA and alcohol molecules or by esterifying fatty acids and alcohols. The natural fruit and flower flavors found in food come from aromatic esters. Fruity flavors have reportedly been linked to high

Table 3. Volatile aroma components of Ayran samples on the 1st day of storage (continue)

Volatile compounds	Samples (area %)					
	Flower Honey	Pine Honey	AA*	BB	CC	DD
Hydrocarbons						
Benzene, 1,3-dichloro- (CAS) m-Dichlorobenzene	10.91	nd	14.55	7.50	nd	nd
Benzene, 1,2-dichloro- (CAS) o-Dichlorobenzene	nd	nd	nd	nd	5.38	11.69
Benzene, 1,4-dichloro- (CAS) p-Dichlorobenzene	nd	11.94	nd	nd	nd	nd
Benzene, methyl- (CAS) Toluene	nd	nd	nd	nd	nd	0.75
2,6-di-tert-Butylquinone	nd	1.39	nd	nd	nd	nd
2,4-Di-tert-butylphenol	nd	2.22	nd	nd	nd	nd
Decane, 3,6-dimethyl-	nd	nd	nd	nd	nd	0.36
Dodecane (CAS) n-Dodecane	nd	nd	nd	nd	nd	0.63
Heptane (CAS) n-Heptane	5.20	3.37	5.20	5.6	4.4	4.19
Hexane, 2,4-dimethyl-	nd	nd	nd	nd	nd	3.09
Hexadecane, 1,1-dimethoxy-	3.43	nd	nd	nd	nd	nd
Methane, oxybis- (CAS) Dimethyl ether	nd	nd	6.91	10.79	6.86	nd
Octane (CAS) n-Octane	2.83	3.97	nd	nd	3.72	nd
Pentane, 3-methyl- (CAS) 3-Methylpentane	nd	nd	7.04	5.49	nd	nd
Pentane, 2,4-dimethyl- (CAS) 2,4-Dimethylpentane	7.26	nd	nd	nd	nd	nd
Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methylpropyl)-	nd	1.73	nd	nd	nd	nd
Terpenes						
1,7-Octadiene, 2,6-Dimethyl-	nd	nd	1.44	3.05	1.87	2.67
R(-)3,7-Dimethyl-1,6-octadiene	nd	nd	2.66	4.28	3.63	4.04
dl-Limonene	nd	nd	1.62	nd	nd	nd
Linalool oxide	3.25	nd	nd	nd	nd	nd
Linalool	nd	2.37	nd	nd	nd	nd
Carvacrol	3.56	nd	nd	nd	nd	nd
Other Compounds						
Acetone-oxime	nd	nd	nd	nd	nd	9.80
Chloroform	nd	2.14	nd	nd	nd	nd
2,2,4-Trimethyl-3-hydroxy-n-valeronitrile	nd	nd	nd	nd	nd	1.21
1-(3,5-Dimethyl-1-adamantanoyl) semicarbazide	nd	nd	7.14	nd	nd	nd
2-Heptanamine, 5-methyl- (CAS) 2-Amino-5-Methyl	nd	nd	nd	6.30	nd	nd
Cyclopentadecanone, 2-hydroxy-	1.89	nd	nd	nd	nd	nd
Borane-methyl sulfide complex	7.46	nd	nd	nd	nd	nd
2-Thiapropane (CAS) Dimethyl sulfide	nd	7.52	nd	nd	nd	nd
Furfural	12.57	8.15	nd	nd	nd	nd

*AA: Control; BB: 10% (w/v) flower honey; CC: 10% (w/v) pine honey; DD: 10% (w/v) mixture (flower and pine honey ratio; 1:1).

ethyl ester levels [43]. In floral and pine honey, the amounts of acetic acid and ethyl ester were found to be 3.24% and 2.17%, respectively. In the 10% blended honey sample, 0.73% esters were found, while they were 1.67% in the control group.

Hydrocarbons

Aromatic hydrocarbons can act as building blocks for other aromatic chemicals; however, because of their high threshold values, they have little effect on aroma. Strecker degradation can convert amino acids like Phe and Tyr into phenyl compounds and styrene. Toluene and benzene can, however, naturally occur in milk. Carotene breakdown in milk can also contribute to it [44,45]. In samples containing flower honey, benzene, 1,3-dichloro- (7.50%), dimethyl ether (10.79%), and pentane 3-methyl (5.49%) were found. The sample with blended honey (DD) had the highest benzene, 1,2-dichloro-value. Only in the pine honey (CC) sample was n-octane (3.72%) found.

Terpenes

Animal feed contains plants that are the source of the terpenes present in milk and other dairy products [13]. Group BB's sample, which contains 10% flower honey, has the greatest overall terpene level of all the Ayran samples including 1,7-octodiene, 2,6-Dimethyl-, dl-Limonene, and R(-)3,7-diemethyl-1,6-octadiene. 1,7-octodiene, 2,6-Dimethyl- (1.87%) and R(-)3,7-diemethyl-1,6-octadiene (3.63%) were found in the Ayran sample (CC) that had pine honey added to it. 1,7-octodiene, 2,6-Dimethyl- (2.67%), and R(-)3,7-diemethyl-1,6-octadiene (4.04%) were discovered in the sample of mixed honey.

Sensory analysis results

Sensory analysis results of Ayran samples during storage are given in Table 4. It can be noticed that the Ayran sample from the control group has a greater salinity rating than the samples that contained honey. On the 21st day of storage, sample CC had the greatest yellowness rate (4.80). The panelists gave the sample DD the highest flowability and honey-like odor scores on the first day of storage. The sample containing flower honey was found to have the greatest sweetness score on the first day of storage.

On the first and twenty-first days of storage, it was found that the control group's sample was saltier than the other

Table 4. Sensory properties of Ayran samples during storage

Sample	Day	Yellowness	Fluidity	Honey-like odour	Sweetness	Saltiness
AA*	1	1.73^{aB**}	2.80^{aA}	1.80^{aA}	1.70^{aB}	6.40^{bA}
	10	1.70^{aB}	5.63^{abB}	1.83^{aA}	1.90^{aB}	7.03^{bA}
	21	1.73^{aA}	2.83^{aA}	1.40^{aA}	1.40^{aA}	7.43^{bA}
BB	1	3.60^{bcA}	5.10^{bB}	4.13^{bcA}	5.93^{cA}	3.93^{aA}
	10	3.40^{bA}	4.73^{aB}	4.20^{bA}	5.03^{bA}	4.00^{aA}
	21	3.20^{bA}	3.33^{aA}	4.00^{cA}	4.70^{bA}	4.33^{aA}
CC	1	3.23^{bA}	6.50^{bcB}	3.43^{bAB}	4.60^{bA}	4.60^{aA}
	10	4.40^{cC}	5.43^{bB}	4.03^{bB}	4.53^{bB}	4.83^{aA}
	21	4.80b^{cB}	3.73^{abA}	3.00^{bA}	4.10^{bAB}	5.20^{aA}
DD	1	4.23^{cA}	6.53^{cB}	5.00^{cA}	5.10^{cA}	4.40^{aA}
	10	4.40^{cA}	5.20^{abAB}	4.40^{bA}	4.83^{bA}	5.10^{aA}
	21	4.15^{cA}	4.60^{bA}	4.40^{cA}	4.23^{bA}	4.83^{aA}

*AA: Control; BB: 10% (w/v) flower honey; CC: 10% (w/v) pine honey; DD: 10% (w/v) mixture (flower and pine honey ratio; 1:1).
** Capital letters indicate the difference between storage time, lowercase letters indicate that the difference between samples is significant ($p<0.05$).

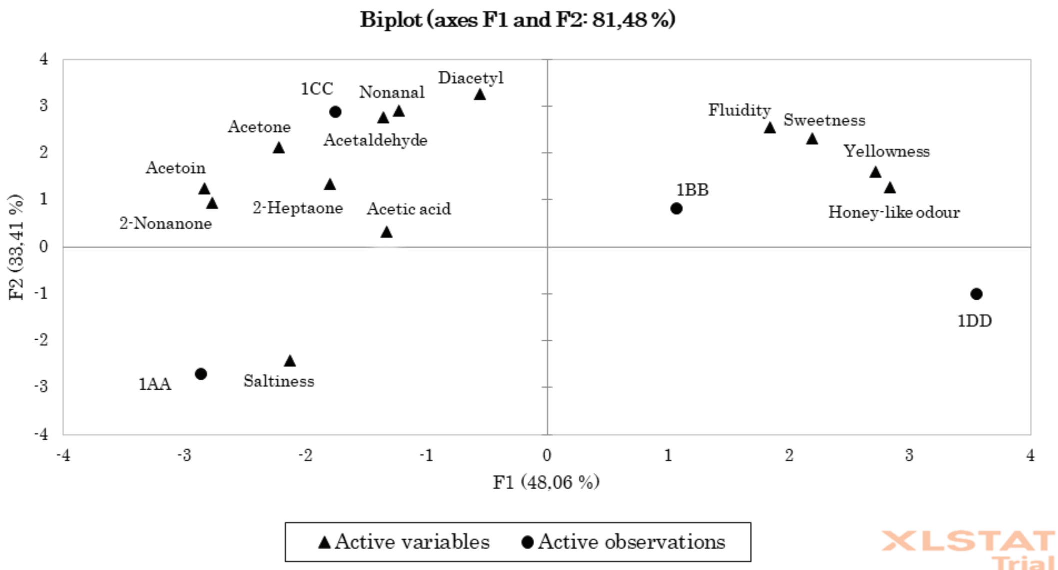

Figure 3. Evaluation of Ayran samples by principal component analysis (PCA). (1AA, 1BB, 1CC and 1DD samples are 1st day of storage. AA: Control; BB: 10% (w/v) flower honey; CC: 10% (w/v) pine honey; DD: 10% (w/v) mixture of flower and pine honey; ratio 1:1).

samples that the flowability of the honey-added samples (BB, CC, and DD) was higher on the first day of storage. After storage, it was found that the Ayran samples (BB, CC, and DD) had a more noticeable honey-like odor, sweetness, and yellowness on the twenty-first days of storage.

Principle Component Analysis

Principal component analysis was used to assess the sensory analysis and aroma findings for Ayran samples (PCA). The model developed for principal component analysis was applied to 4 components in this investigation. These elements account for 81.48% of the variance overall. The first principal component explains 48.06% of the entire variance, and 33.41% by the second component. The second component's value was found as 4.34, while that of the first primary component's eigenvalue was 6.24 (Figure 3).

Since honey was not added to the control group samples, panelists identified the samples as salty. It was found that the Ayran samples (1BB) had a more noticeable flowability, honey-like odor, sweetness, and yellowness. Pine honey added Ayran (1CC) was a higher amount of aroma compounds such as acetoin, acetone, acetaldehyde, diacetyl, nonanal, acetic acid, 2-heptaone, 2- nonanone than other samples. It was also observed that the 1DD sample containing mixed honey was the closest sample to the characteristics of the sample containing flower honey (1BB). (Figure 3).

CONCLUSION

Ayran is a product with a unique acidic salty character. By including a product with high nutritional value and functional qualities, it was believed that Ayran could be made more appealing to customers in general and kids by doing away with this taste. The qualities of Ayran benefited from the addition of honey. It was found that the samples containing honey had less of a honey-like smell, sweetness, and yellowness by the end of storage than they had on the first day.

This study examined the microbiological, physicochemical, rheological, and volatile aroma components of Ayrans throughout storage after adding 10% of flowers and pine honey, and a combination of the two to Ayrans (1st, 10th, and 2st days). While there was a significant difference between samples for L*, a*, and b* values as well as microbiological outcomes, pH and % salt were not significantly different. It was noted that the samples with additional honey had higher viscosity ratings. When floral honey was added to the sample (BB), it was seen that the sample had a thicker consistency than when pine honey was added. Additionally, it was found that the mixed honey sample (DD) had a higher viscosity value than that of pine honey. The pine honey sample (CC) had higher concentrations of acetaldehyde diacetyl, acetoin, acetone, and acetic acid than the other samples. It was discovered that (BB) contained floral honey, octanoic acid, heptane, dimethyl ether, and 1,7-octadiene, 2,6-dimethyl-ratios were greater than the other samples among the Ayran samples. Although ethanol was found in the sample containing blended honey (DD), ethanol was absent in the other samples.

It can be noticed that Ayran with floral honey and a mixture of honey added has greater apparent viscosity values than Ayran with pine honey and the control group. Additionally, combining several types of honey with Ayran to diversify the product and create a new one with useful features. Both flower and pine honey are recognized to have beneficial qualities. Both flower honey and pine honey can be used to make Ayran, it has been discovered, since these honey can enhance the qualities of Ayran. Adding 10% or less honey to Ayran may be appropriate because the ratio being utilized increases viscosity and negatively affects the sensory characteristics. Since Ayran samples that have a mixture of honey added have greater sensory qualities than flower honey, such as a honey-like odor and sweetness, it is projected that a mixture of pine and flower honey can also be employed in the manufacturing of Ayran.

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CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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