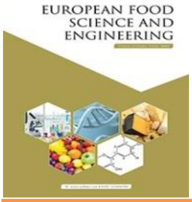

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Investigation properties of Ayran (yoghurt drink) produced from different ratio of cow and hemp seed milk mixtures

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ABSTRACT

Ayran, the fermented product has been produced from animal milk, mixed different levels (0, 25, 50, 75, and 100%) of hemp seed milk. Effects of using hemp seed milk on quality parameters as chemical, physicochemical and microbiological were studied. Among the ayran samples containing hemp milk, the ayran sample containing 25% hemp seed milk has the highest general acceptability. While the total phenolic content of the control sample decreased at the end of the storage period, ayran samples with hemp seed milk showed an increase significantly in total phenolic content ($p<0.05$). At the end of the storage period (14th day), while the lowest serum separation rate (13.64%) belonged to the cow milk-based sample, the hemp milk-based sample (100%) had the highest level as 63.04%. The rheological behavior of the ayran samples were fitted the power-law model (0.98-0.99). These results provide useful information for the production possibilities of hemp seed milk-based ayran.

1. Introduction

The *Cannabis* genus has been used as food, fiber, psychoactive/religious drug, and medicine since ancient times (Cromack, 1998). Historically, the cultivation and production of hemp have been limited because of the psychoactive compound known as tetrahydrocannabinol (THC) (Wang, Jiang, & Xiong, 2018). The mentioned compound can be trace amount in hemp seed, and commercial hemp seed can be used for hemp seed-based food products (Rodriguez-Leyva & Pierce, 2010) with its nutritive value and low allergenicity (Wang et al., 2018). The whole hemp seeds contain oil (25-35%), protein (20-25%), carbohydrates (20-30%), and insoluble fibers (10-15%); in addition to macronutrients, the seeds have vitamins and minerals such as potassium, phosphorus, magnesium, sulfur, calcium, iron and zinc (Callaway, 2004; House, Neufeld, & Leson, 2010). The seeds can be consumed as raw, cooked, or roasted for different food products with the mentioned properties. The roasted seeds are ground into flour and added to different cooked dishes to improve the flavor in Nepal and India (Clarke, 2007). In addition to that, the dried hemp seeds can also be mixed with other grains and honey to produce a kind of snack. The hemp seed oil can be used as a butter substitute in Eastern European countries, and hemp seed-based commercial products including hemp seed oil, dehulled hemp seeds, milk, flour, coffee, protein powders, and butter can be sold at markets in Canada (Aluko, 2017).

Hemp seed milk is plant-based vegetable milk that is fast gaining attention on the global scale cause of lactose intolerance, allergenic constituents, diet selection, social and religious concerns. For these reasons, although the nutritional benefits and recommendations for the consumption of animal-based milk, its consumption in North America has decreased over the past decade (Centre, 2015). Bovine milk alternative beverage market place has a $6 billion scale at the US retail market sales in 2016, and it is 25% of the total US dairy and milk alternative beverage industry. In addition, It is estimated that the milk alternative beverage sector's scale will become $28 billion in total US retail market sales in 2021 (Markets, 2017). Although hemp milk and hemp milk-based products can contribute to market expansion as other non-dairy vegetable-based products, there is limited research about hemp seed milk and its products in literature (Astolfi, Marconi, Protano, & Canepari, 2020; Jeske, Zannini, & Arendt, 2017; Wang et al., 2018).

One of the milk products is ayran, a special kind of acidic milk drink, and it is popular in Asia and the Middle East countries (Erkaya, Baslar, Sengul, & Ertugay, 2015). It is traditionally prepared by diluting yoghurt with water at a level of 30–50% (Koksoy & Kilic, 2004); it is also be industrially produced by the fermentation of the milk standardized to 8% dry matter content, the addition of yoghurt starter culture and table salt at a maximum level of 1% (Erkaya, Baslar, Sengul, & Ertugay, 2015). There are studies about its textural stabilization (Koksoy & Kilic, 2004), physico-chemical changes by adding different components (Akkoyun & Arslan, 2020; Çelekli,

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Alslibi, & Bozkurt, 2019), fermentation process (Çelekli et al., 2019), different types of milk (Shunekeyeva, Alimardanova, & Majorov, 2021).

Although different types of milk have been used for ayran production and their effects on fermentation and final product characterization were investigated, there is no research about hemp seed milk for ayran production. In this study, we investigated the properties of ayran produced from different cow and hemp seed milk mixtures.

2. Materials and methods

2.1. Materials

Hemp seeds *(Cannabis sativa L.)* were obtained from Cannabis Research Institute of Ondokuz Mayıs University, and semi-fat ultra-high-temperature (UHT) cow milk (CM) was purchased from a local market in Samsun, Turkey. The characteristics of semi-fat cow milk were 6.74 pH, 1.5 g/100 g fat, 4.7 g/100 g carbohydrate, and 3.01 g/100 g protein. The ayran starter culture (YF-L902 500U) was obtained from Danisco Biolacta (Olsztyn, Poland). All reagents and solvents (analytical grade or HPLC grade) were obtained from Merck (Darmstadt, Germany). Lactic acid standard and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of hemp seed milk

For the preparation of hemp seed milk (HM), hemp seeds were grounded using a blender for 10 min and grounded hempseed (100 g) was treated with 1000 mL of water in order to obtain similar total solid content with semi-fat cow milk for 10 min using an Ultra-Turrax homogenizer (IKA-Werke GmbH & Co. KG, Germany) at 10.000 rpm. The resulting slurry was filtered through a double-layered cheesecloth.

The characteristics of hemp seed milk were as follows: 6.90 pH, 10.28 g/100 g total solid, 3.50 g/100 g protein, 5.95 g/100 g fat, and 0.63 g/100 g ash.

2.3. Ayran production and storage

For ayran production, firstly, CM and HM were mixed at five different concentrations. The milk mixtures were pasteurization at 90 °C for 5 min and quickly cooled down to 43°C before inoculation of ayran starter culture (YF-L902 500 U) according to inoculation standards as 0.02%. These concentrations were chosen as whole cow milk ayran 100% (CM), 75/25% (CM/HM), 50/50% (CM/HM) and 25/75% (CM/HM) and whole hemp seed milk ayran 100% (HM) and codded as C, 3CH, 2C2H, C3H, and H respectively. Samples were incubated at 40°C until the pH of 4.5 was reached. Ayran samples were then cooled down (10 °C) and salt at a concentration of 0.5% in the final product was added to each sample. Samples were mixed thoroughly and filled into 1000 ml of cups and stored at 4 °C.

2.4. Physicochemical analyses

Moisture, protein (6.38 was used as a conversion factor), and ash content were measured according to AOAC (2000) standard methods. Fat content is determined according to the Gerber method (Kleyn, Iynch, Barbano, Bloom, & Mitchell, 2001). The pH values of the samples were measured with a calibrated pH meter at 25 °C (Eutech, Singapore), and titratable acidity (Akcay, Besir, & Yazici, 2020) was determined for ayran samples.

2.5. Lactic acid composition

Lactic acid content (%) was determined according to Atalar (2019). Five grams of each ayran sample were diluted with 25 ml H_2SO_4 (0.01 N) and centrifuged (Sigma 3K30, Germany) at 7000g 4 °C for 15 min. The supernatant was removed and filtered with a nylon disc filter (Supelco Iso-DiscTM Filters, N-25-4 Nylon, 25 mm x 0.45 µm) and transferred into vials. HPLC (Agilent, 1260, Germany) system was used with Shim-pack 150 mm, 4.6 mm i.d ODS-3 column and UV detector. 0.01N perchloric acid was used as a mobile phase. Analysis conditions were set up as 0.7 ml/min isocratic flow rate; 35 °C oven temperature; 210 nm wavelength; 20 µl sample injection volume. Lactic acid standard (Sigma-Aldrich, USA) was used to obtain a calibration curve to determine lactic acid content.

2.6. Microbiological analysis

Ayran samples (10 mL) were diluted with 90 mL of sterile peptone water, and serial dilutions were prepared. Total viable count (TVC) was counted on PCA-Agar medium after incubation at 30°C for 48 to 72 hours. Total yeasts and molds were obtained on YGC-Agar at 25 °C for three days (all the media were purchased from Merck, Germany). Colony-forming units (CFU) were counted on petri dishes expressed as log CFU/mL of ayran.

2.7. Rheological analyses

Rheological analyses of ayran samples were carried out using Haake Mars III rheometer (Thermo Scientific, Germany) with a cone and plate (35 mm diameter, 0.105 mm gap, 2° angle). To obtain rheological behavior, the shear rate range of 1–100 s^{-1} was selected. Measurements were performed at 4 °C. The apparent viscosity values of ayran samples were evaluated at the specified shear rate of 50 s^{-1}.

The rheological behavior of the ayran samples was fitted the power-law model described in Eq. (1):

$$\sigma = K\gamma^{n} \tag{1}$$

Where σ is shear stress, K is the consistency coefficient, γ is the shear rate, and n is the flow behavior index.

2.8. Syneresis and volumetric serum separation

Syneresis and volumetric serum separation analyses were carried out as an indication of instability. To determine the level of syneresis, ayran samples were weighed (*ma*) and centrifuged at 6000 rpm for 15 min at 4°C. Following the centrifugation process, the weight of the supernatant (*ms*) was obtained, and the syneresis (%) was calculated with the following Equation 2:

$$S = \frac{ms}{ma} \times 100 \tag{2}$$

The volumetric serum separation was determined at the 1st, 7th, and 14th days, storing the samples in measuring cylinders of 100 mL at 4 °C.

2.9. Total phenolic content (TPC) and antioxidant activity (DPPH)

Bioactive compounds of ayran samples were extracted according to the procedure carried out by Akcay et al. (2020). Briefly, each Ayran sample (2 ml) was mixed with an appropriate amount (8 ml) of methanol (80:20, methanol: distilled water) and centrifuged at 7200 rpm for 10 min at 4°C. The supernatant was filtered and stored at 4°C for DPPH and TPC analysis. TPC was calculated as µg gallic acid equivalents (GAE) per gram of sample using the calibration curve (R^2 = 0.998) in Equation 3.

$$TPC\ (\mu g/ml) = [Absorbance - 0.0102)/\ 0.0109] \times \text{Dilution Factor} \quad (3)$$

To determine DPPH free radical scavenging activity was expressed according to Equation (4):

$$ARA(\%) = ((\ A_c - A_s)/A_c\ ) \times 100 \quad (4)$$

Where antiradical activity as *ARA (%)*, control absorbance as A_c, and sample absorbance as A_s.

2.10. Sensory evaluation

The sensory properties of ayrans were organoleptically evaluated with maximum scores of 10 for appearance and color, texture, flavor, mouthfeel, and general acceptability. Ayran samples in 150 mL plastic cups with three-digit random numbers were randomly presented to 15 sensory panelists selected from the Department of Food Engineering at Ondokuz Mayis University. The evaluations were carried out after the first and 14th days of storage at 3-5°C.

2.11. Statistical analysis

Data obtained from the ayran samples were statistically evaluated using analysis of variance (ANOVA). The differences among the means were compared with Duncan's multiple range tests (SPSS 15.0, SPSS Inc., Chicago, IL, USA). A significance level of 0.05 was used.

3. Results and Discussion

3.1. Proximate and acidity based results of the ayran samples

For the first day of the storage, Table 1 shows some chemical analysis results of ayran samples.

Table 1. Proximate analysis results of the ayran samples

Sample code	Total solids (%)	Ash (%)	Protein (%)	Fat (%)
C	9.61±0.01^c	1.18±0.01^d	1.34±0.02^d	1.34±0.0^a
3CH	8.64±0.00^b	1.09±0.00^c	0.94±0.04^c	2.0±0.00^b
2C2H	7.85±0.00^{ab}	0.87±0.01^b	0.81±0.03^{bc}	2.10±0.14^b
C3H	7.44±0.49^a	0.85±0.03^{ab}	0.77±0.03^b	2.5±0.14^c
H	7.15±0.03^a	0.81±0.01^a	0.59±0.03^a	3.0±0.00^d

*C (100% CM), 3CH (75:25%, CM:HM), 2C2H (50:50%, CM:HM), C3H (25:75%, CM:HM) and H (100% HM). CM: Cow milk, HM: hemp seed milk.
Different letters show differences between the samples (P < 0.05).

Total solid contents of ayran samples ranged between 7.15-9.61% and the total solid content was found as the lowest in-sample H (7.15 g/100 g), while the highest concentration was found in sample C (9.61 g/100 g). Lai et al. (2021) reported that after fermentation, the contents of total solids in the hemp kernel milk were 6.08±0.22 g/100 g, which were significantly lower than those before fermentation. Since the basic component distribution of cow's milk and hemp milk used was different, the samples' dry matter, ash, protein, and fat ratios were significantly different from each other (P<0.05). The protein content decreased as the hemp milk ratio increased. This may be due to the possibility that proteins are metabolized by bacteria to form volatile compounds during fermentation (Çabuk et al., 2018). As the rate of hemp milk increased, the fat content of ayran samples was significantly increased because of the higher fat content in hemp seed milk (5.95%) according to cow milk used (1.5 %) (P<0.05). While ayran sample C was fitted in the semi-skimmed ayran group (0.8%–1.2% milk fat), according to the Turkish Food Codex on fermented milk products, others were included in the full-fat ayran group (≥ 1.8%) (Anonymous, 2009). It was normal for adding any different component or raw materials to show different proximate results, and it was found similar to other research about ayran, including spirulina (Çelekli et al., 2019). When the total solids, ash, protein, and fat contents of the samples were compared, the fat content of the H sample was found higher because the hemp seed has approximately 30-35% fat. pH was measured every thirty minutes until the pH reached around 4.5 (Figure 1). The acidification rate was calculated as the time variation of ph (dpH/dt) and expressed as ph units/min. The sample whose fermentation was completed in the shortest time (4 hours) with the highest acidification rate of 0.0082 ph units/min was the 3CH-coded ayran. This represents a decrease in fermentation time of 72% compared to the control sample (C). As shown in Figure 1, the H sample had the longest fermentation time (7.5 hours) and the lowest acidification rate (0.0035 ph units/min). Acidification rate-fermentation time of C, 2C2H and C3H samples were determined as 0.0057 ph units/min-5.5 hour, 0.0070 ph units/min-4.5 hour and 0.0060 units/min-5 hour, respectively. Compared to the C sample consisted of 100% cow milk, the higher acidification rate in the samples with hemp milk added was evidence that the addition of hemp milk created a synergistic effect on pH decreasing rate. Adding hemp seed milk showed a stimulating effect on the acidification capacity of samples, similar to these results. In a study milk samples enriched with thyme and grape seed extracts exhibited the highest values of acidification capacity with 0.0065 and 0.0068 pH unit/min (Markets, 2017). In another study where the fermentation process of cow milk and/or pea milk mixtures by different lactic acid bacteria cultures was carried out, an increase in pea concentration leads to products with higher acidity (Yousseef, Lafarge, Valentin, Lubbers, & Husson, 2016).

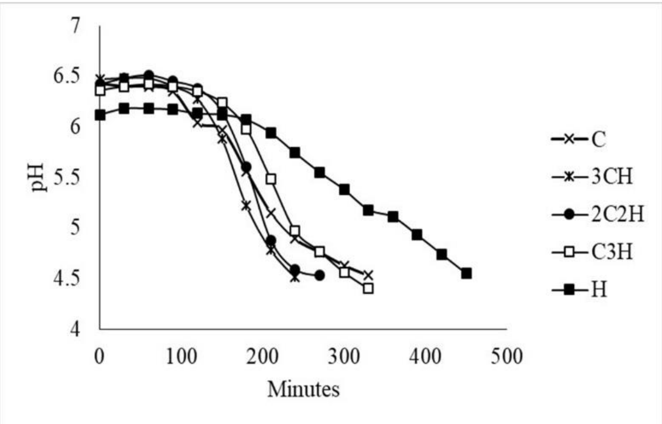

Figure 1: Changes in pH until the pH reach to around 4.5

pH values on the 1st and 14th days of storage were not statistically significant except for the control sample, as shown in Table 2. It can be explained that hemp seed matrices have a buffering capability (Nissen, di Carlo, & Gianotti, 2020). The percentages of titratable acid experienced a significant increase throughout the storage period ($P<0.05$) (Table 2). However, as the hemp seed milk ratio in the milk mixture increased, the titration acidity and lactic acid concentration in the ayran samples decreased. Because of being lactose-free, increasing hemp seed milk concentration resulted in lower lactic acid concentration and higher pH value (Table 2) (Wang et al., 2018). Except C sample lactic Acid (%) values of the samples were not changed during 14 days of storage similar to literature (Avsar, Kocak, & Tamucay, 2008). In same storage day, there is a significant difference in the lactic acid content of the samples ($P<0.05$) (Table 2).

Table 2. Acidity and microbiological properties of the samples

Analysis	Sample code	Storage days 1	7	14
pH	C	4.39±0.03^{bB}	4.25± 0.01^{aB}	4.21±0.01^{aA}
	3CH	4.25±0.01^{bA}	4.18± 0.00^{aA}	4.23±0.01^{bA}
	2C2H	4.26±0.01^{aA}	4.21± 0.01^{aAB}	4.20±0.04^{aA}
	C3H	4.28±0.01^{aA}	4.22± 0.02^{aAB}	4.27±0.01^{aA}
	H	4.49±0.00^{bC}	4.44± 0.00^{aC}	4.45±0.00^{bB}
Titratable acid, %	C	1.64±0.02^{aC}	1.78±0.00^{bB}	1.83±0.01^{bC}
	3CH	1.55±0.00^{aBC}	1.71±0.08^{abB}	1.75±0.04^{bC}
	2C2H	1.34±0.04^{aAB}	1.51±0.02^{bA}	1.62±0.02^{bB}
	C3H	1.13± 0.00^{aA}	1.35±0.02^{bA}	1.50±0.01^{cA}
	H	1.16± 0.11^{aA}	1.38±0.04^{abA}	1.60±0.02^{bAB}
Lactic acid, %	C	9.79± 0.26^{aD}	11.01±0.30^{bE}	10.79±0.19^{abE}
	3CH	9.25± 0.15^{aD}	8.90±0.13^{aD}	8.87±0.11^{aD}
	2C2H	7.00± 0.07^{bC}	6.55±0.05^{aC}	6.85±0.05^{bC}
	C3H	4.80± 0.03^{bB}	4.56±0.01^{aB}	4.57±0.01^{aB}
	H	3.85± 0.01^{bA}	3.70±0.00^{aA}	4.09±0.01^{cA}
Total viable counts, cfu/mL	C	2.20±0.19^{aA}	3.05±0.32^{bA}	3.54±0.22^{bA}
	3CH	4.47±0.09^{aB}	5.74±0.35^{bB}	4.51±0.02^{aB}
	2C2H	5.04±0.06^{aC}	4.95±0.19^{aB}	4.81±0.15^{aC}
	C3H	5.58±0.15^{aD}	5.41±0.73^{aB}	6.05±0.00^{aD}
	H	7.71±0.16^{aE}	7.57±0.01^{aC}	7.72±0.03^{aE}
Total yeasts and molds, cfu/ mL	C	0.85±0.14^{aA}	0.97±0.10^{aA}	1.02±0.08^{aA}
	3CH	2.89±0.25^{aB}	2.58±0.01^{aB}	2.70±0.19^{aB}
	2C2H	3.14±0.20^{aBC}	3.53±0.01^{aC}	3.22±0.35^{aBC}
	C3H	3.42±0.18^{aC}	3.54±0.01^{aC}	3.55±0.04^{aC}
	H	4.65±0.03^{aD}	4.22±0.03^{bD}	3.28±0.42^{cC}

*C (100% CM), 3CH (75:25%, CM:HM), 2C2H (50:50%, CM:HM), C3H (25:75%, CM:HM) and H (100% HM). CM: Cow milk, HM: hemp seed milk. Different uppercase superscript letters show differences between the samples within the same day.
Different lowercase superscript letters show differences between days within the same sample ($P < 0.05$).

3.2. Microbiological Results

There was no significant change in the total viable count for all samples during the storage period (Table 2). The total viable count of the samples prepared with hemp seed milk ranged from 4.47 cfu/ml to 7.72 cfu/mL, whereas the C sample prepared with only cow milk range was found as 2.20-3.54 cfu/mL. Similarly, the total viable count of almond milk was found as 2.24 cfu/mL (Manzoor, 2017). The yeast and mold count was increased with hemp seed milk amount in milk mixture ($P<0.05$) and handmade production of hemp seed can be reason of this increasing. Total yeast and molds values of the hemp seed milk and its mixtures were found 2.89 and 4.65 cfu/mL interval and it was similar with the value of the tiger nut beverage was found 2.87±0.32 cfu/mL (Codina-Torrella, Guamis, Zamora, Quevedo, & Trujillo, 2018). In addition to that the value was found 1.22 cfu/mL at almond milk (Manzoor, 2017).

3.3. Rheological properties of the samples

The ayran samples showed non-Newtonian flow behavior, as shown in Figure 2, and the power-law model well described the flow behavior of the samples (R^2 = 0.9865–0.9996) (Table 3). In addition, ayran samples showed non-Newtonian behavior, as shown in Table 3, because the flow behavior index (n) results were below 1. While ayran samples were considered a pseudoplastic fluid, because of the flow behavior index (n), results ranged between 0 and 1.

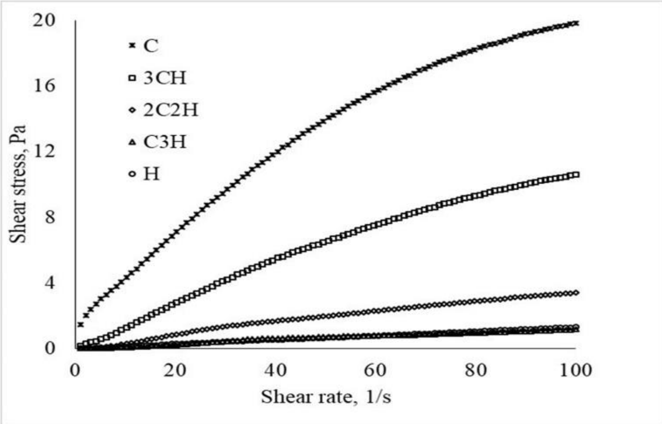

Figure 2: Changes in the shear stress with shear rate in ayrans

These findings follow the other studies related to fermented milk-ayran (Erkaya et al., 2015; Koksoy & Kilic, 2004) H coded samples approached nearly Newtonian flow behavior with a 0.96 flow behavior index (n). Many researchers reported that the flow behavior of cultured dairy products was satisfactorily characterized by the power-law model (Uzay, Ozturk, Buzrul, & Maskan, 2021). Increasing hemp seed milk ratio increased the flow behavior index of ayran, approaching it to Newtonian behavior and decreased apparent viscosity at 50 s^{-1} shear rate. Similar to our results, a decrease in viscosity was obtained in a study that added Spirulina platensis into ayran to enhance the growth of probiotic bacteria and the nutritional content (Çelekli et al., 2019). When the H sample was held out of the comparison, adding hemp seed milk to cow milk resulted in the highest acidification rate (0.0082 and belonged to the 3CH-coded sample) and decreased viscosity.

Table 3. Viscosity parameters of ayran samples

Sample code	Consistency coefficient, K (mPa s^{n})	Flow behaviour index, n	Apparent viscosity (mPa s)	R^2
C	1128±52.32^{c}	0.63±0.01^{a}	278±2.83^{d}	0.9968
3CH	261.35±31.32^{b}	0.81±0.01^{b}	130±8.48^{c}	0.997
2C2H	69.91±12.21^{a}	0.85±0.04^{b}	39.45±1.31^{b}	0.9965
C3H	24.78±2.51^{a}	0.84±0.02^{b}	14.31±0.05^{a}	0.9865
H	15.28±1.46^{a}	0.96±0.02^{c}	13.18±0.02^{a}	0.9996

*C (100% CM), 3CH (75:25%, CM:HM), 2C2H (50:50%, CM:HM), C3H (25:75%, CM:HM) and H (100% HM). CM: Cow milk, HM: hemp seed milk.
Different letters show differences between the samples ($P < 0.05$).

It can be explained by the fact that a faster rate of pH reduction can affect the aggregation behavior of milk proteins, resulting in a weak gel, low viscosity (McCann, Fabre, & Day, 2011). An increase in the hemp seed milk level decreased consistency coefficients (K) dramatically in ayran samples (Table 3). The consistency coefficient of the control ayran coded C was highest.

3.4. Results of syneresis and volumetric serum separation

Syneresis or serum separation is one of the quality factors, allowing to interpret about the product stability. Although both terms are used as the same meaning in the literature, however, in this study, while syneresis was determined by centrifugation method, serum separation was carried out under gravitational force throughout the storage period. Throughout the storage period, while the percentage of syneresis for both the C3H and H ayran samples showed significant change ($P<0.05$), there were not any significant changes in C ($P>0.841$), 3CH ($P>0.086$), and 2C2H ($P>0.394$) samples (Appendix A). It can be inferred from Figure 3 that an increase in the ratio of hemp seed milk used in ayran production caused an increase in syneresis percentage at the 1st,7th, and 14th days of storage ($p<0.05$) (Appendix A). H samples had the highest separation rate, similar to the syneresis rate among the other samples, while the lowest value for both analyses belonged to the C sample. Furthermore, the serum separation grew from the first day to the 14th storage day (Figure 3). According to the syneresis and serum separation results, it was evident that using hemp seed milk showed an adverse effect on the syneresis and serum separation of ayran samples. Compared to the C sample, a lower percentage of the solid content of samples that of adding hemp seed milk can be the one reason for this situation (Amatayakul, Sherkat, & Shah, 2006). At the end of the storage period (14th day), while the lowest serum separation rate (13.64%) belonged to the C sample, the H sample had the highest level as 63.04%. In addition, serum separation levels of 3CH, 2C2H, and C3H samples were determined as 35.56%, 43.75%, and 54.35%, respectively. After two week storage period, 24% and 27% are the serum separation rate of control ayran samples which was carried out in some studies (Erkaya et al., 2015; Koksoy & Kilic, 2004).

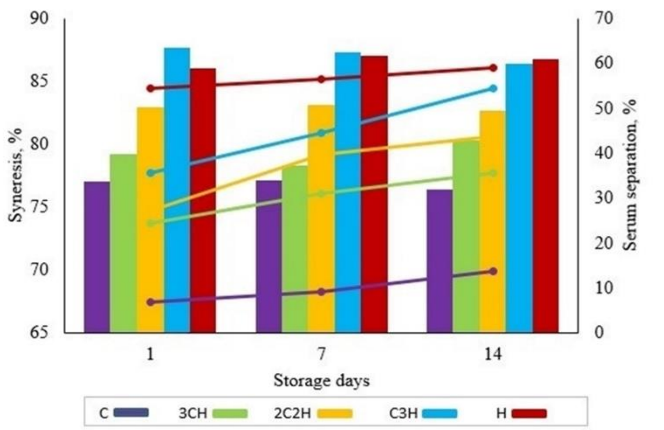

Figure 3: Syneresis and serum separation of ayran samples (%). (The same colors represents the same samples. While y-axis on the left side depicts syneresis rate with bar chart, y-axis on the right side shows serum separation results with line graph)

3.5. Total phenolic and antioxidant properties of the samples

Figure 4 shows both total phenolic content as gallic acid and antioxidant properties based on the percentage of inhibition. Ayran samples consisting of the cow-hemp seed milk mixture showed higher phenolic content than those made of only cow or only hemp seed milk. Proteolysis of hemp seed proteins may release amino acids with phenolic side chains, contributing to the increase in TPC (Muniandy, Shori, & Baba, 2016). Interestingly, the antioxidant activity of 3CH (contains minimum hemp seed milk ratio) had a lower rate than the control sample. Nevertheless, as hemp seed milk rate increased in the mixture, the percentage of inhibition was increased. It can be related about that hemp seeds are rich in terpenes and terpenoid compounds, such as 1-(R)-α-pinene, Δ-3-carene, β-myrcene, and β-caryophillene, which are antioxidant (Nissen et. al., 2020). The highest percentage of inhibition belonged to H-coded ayran made of only hemp seed milk. While the total phenolic content of the control sample decreased at the end of the storage period, ayran samples with hemp seed milk showed an increase significantly in total phenolic content ($P<0.05$) (Appendix B). Antioxidant activity of ayran samples decreased with storage time. This result can be explained probably associated with the formation of a complex between polyphenols and milk proteins (Pelaes Vital et al., 2015).

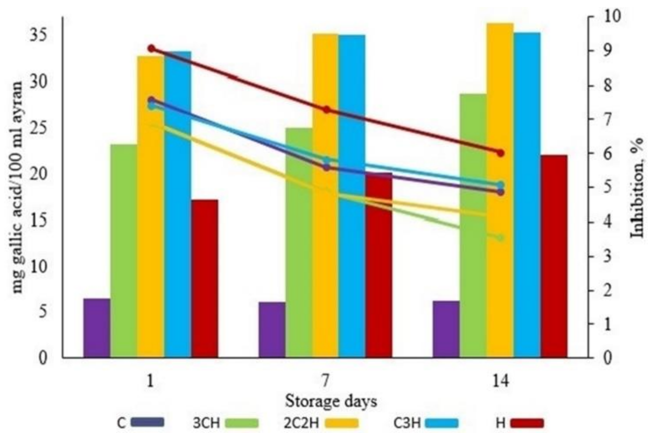

Figure 4: Total phenolic and antioxidant activity values of ayran samples. (%). (The same colors represents the same samples. While y-axis on the left side depicts total phenolic content, y-axis on the right side shows the inhibition percentage with line graph)

3.6. Sensory Evaluation

Sensory analysis is considered a determinant analysis, especially in the case of developing a new product. However, the appearance scores of 3CH, 2C2H, and C3H were not significantly different, and the situation is good for the material substitution of food products (Table 4).

Texture scores of both 3CH and C were not significantly different ($P>0.05$). It means that 3CH can be preferred to C samples regarding the texture aspects. When ten is considered the top point of the scale of scores, a general acceptability score of 6.07 belonged to 3CH can be a satisfying score for a new product (Table 4). The substitution of 50% or more cow milk with hemp seed milk in ayran resulted in a decrease in liking scores, which might be since consumers were expecting the fermented milk product to taste like a regular ayran (Yousseef et al., 2016).

4. Conclusion

Developing a new alternative product for vegan consumption is the main reason for this study. Increasing the nutritional value of traditional ayran products can be ensured by adding different amounts of hemp seed milk. This study was the first study for hemp seed milk-based fermented milk product in terms of its production facility based on physical,

Table 3. Sensory properties of ayran samples

Quality factor	Samples codes				
	C	3CH	2C2H	C3H	H
Appearance	8.47±0.91^{c}	7.2±1.37^{b}	6.60±1.68^{b}	6.07±1.39^{b}	4.07±2.31^{a}
Texture	7.60±1.45^{b}	6.60±1.80^{ab}	5.80±1.74^{a}	5.80±2.04^{a}	5.53±1.96^{a}
Flavour	8.13±1.30^{d}	6.00±2.03^{c}	4.40±1.96^{b}	4.20±1.57^{b}	2.87±1.55^{a}
Mouthfeel	7.53±1.35^{c}	5.60±2.06^{b}	4.73±1.83^{ab}	4.47±1.92^{ab}	3.67±2.53^{a}
General acceptability	7.60±1.72^{d}	6.07±1.98^{c}	4.67±1.72^{b}	4.13±1.77^{b}	2.73±1.75^{a}

*C (100% CM), 3CH (75:25%, CM:HM), 2C2H (50:50%, CM:HM), C3H (25:75%, CM:HM) and H (100% HM). CM: Cow milk, HM: hemp seed milk.
*Different letters show differences between the samples ($P < 0.05$).

chemical, and microbiological properties, and it demonstrated that ayran samples with hemp seed milk showed an increase significantly in total phenolic content (P<0.05). Moreover, the ayran sample containing 25% of hemp seed milk has the highest general acceptability after cow milk (100%) based one. "As a result, hemp milk added ayran at 25% concentration level can be recommended as an alternative to yogurt-like products."

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