Arch Lebensmittelhyg 75, 13–23 (2024) DOI 10.53194/0003-925X-75-13

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Vinegar production from different cherry laurel fruits and investigation of some of their physicochemical properties

Essigherstellung aus verschiedenen Kirschlorbeerfrüchten und Untersuchung einiger ihrer physikochemischen Eigenschaften

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Summary In this study, seven types of traditional cherry laurel fruit vinegars (CLFV) belonging to seven types of traditional cherry laurel fruit vinegars (CLFV) belonging to three different species were produced. Vinegars, including those produced during the study and supplied from the market, were analyzed for total acidity, volatile acidity and non-volatile acidity, pH, ash, oxidation number, iodine number, ester, mineral substance, alcohol, total solids, and total sugar-free solids. Analyses and the ranges of the results that were found in vinegar samples were as: Acidity percentage (as acetic acid) 1.68–4.13 %, volatile acidity (as acetic acid) 5.70–18.27 g/L, non-volatile acidity (as tartaric acid) 3.31–36.10 g/L, alcohol percentage 0.01–0.48, pH 2.24–3.57, total sugar 6.54–283.56 g/L, total solids 22.01–486.56 g/L, total sugar-free solids 14.11–217.73 g/L, ash 0.22–3.84 g/L, ester 16.80–61.14, oxidation number 389.60–394.05 and iodine number 35.20–386.88. For the color analysis, the values were found to be between 10.91 and 25.79 for L*, 5.15 and 15.08 for a*, between –4.83 and 8.72 for b*, between 3.54 and 16.83 for ΔE^* . Based on their physicochemical properties, the vinegars numbered N4, N5, N6, N8, and N9 are considered suitable for vinegar production compared to the samples numbered N7 and N10. The raw material contents of N7 and N10 vinegars differ from the others and inhibit the development of acidity. Additionally, it has been determined that the physicochemical properties of N4, N5, N6, N8, and N9 vinegar samples are superior to those of apple and grape vinegars. According to the study result, cherry laurel fruits (CLF) are suitable for natural vinegar production under optimum conditions, and further field studies should be carried out to apply these findings at the industrial level.

Keywords: vinegar, cherry laurel fruit, honey, physicochemical property

Introduction

Numerous research studies have demonstrated that vinegar may hold promise in mitigating conditions for many health problems and diseases. Vinegar exhibits antimicrobial (Budak et al., 2014), antiobesity (Cho et al., 2010), antioxidant (Yun et al., 2007; Budak et al., 2014), antidiabetic (Petsiou et al., 2014) and antihyperlipidemic (Lee et al., 2013) properties due to the acetic acid and phenolic substances it possesses. It has been noted that the daily consumption of a beverage containing 15 mL of vinegar (equivalent to 750 mg of acetic acid) can lead to improvements in lifestyle-related conditions, including hypertension, high cholesterol levels, obesity (Samad et al., 2016). These positive effects on health are evidence that vinegar is a powerful food source for humans (Samad et al., 2016; Budak et al., 2014).

Vinegar is subjected to two-stage fermentation during its production. The first stage is the conversion of fermentable sugars into ethanol by yeasts, which are usually Saccharomyces species. The second stage includes the formation of acetic acid through the oxidation of ethanol by bacteria, which is usually Acetobacter species (Luzón-Quintana et al., 2021). As a raw material of vinegar around the world, various products such as sugar cane, barley, rice, grapes, apples, figs, currants, raspberries, mulberries, dates, coconuts, cherries, pears are used. One of the important points in vinegar production is whether the raw material to be used is suitable for alcohol fermentation. If the raw material has a low sugar content, the sugar content should be adjusted by adding sugar (Giudici et al., 2017). Therefore, the raw material to be used in vinegar is one of the crucial factors in the composition of vinegar. CLF is also considered suitable for vinegar production with its features as a raw material (Yılmaz et al., 2023; Yikmis et al., 2021).

CLF's nutritional content can fluctuate based on factors like its growth location, the season in which it's cultivated, its level of ripeness, changes in color (Celik et al., 2011; Halilova et al., 2010; Yildiz et al., 2014). It comprises components such as water, proteins, carbohydrates, pectin, as well as various phenolic compounds including flavonoids, anthocyanins, lignin and tannins. Additionally, it contains vitamins A, C, D, as well as minerals (Karahalil and Sahin, 2011). In fully ripe CLFs, you can find phenolic acid in the form of vanillic acid and an unsaturated fatty acid known as linoleic acid Furthermore, its composition includes arabinose, xylose, glucose, fructose, (Ayaz et al., 1997; Akkol et al., 2012).

CLFs have distinct darker colors and shapes and are a type of berry grown on cherry laurel trees which remain green throughout the year. CLF is grown in multiple areas, spanning, Western Europe, Balkan nations, Black Sea Region, Eastern Marmara Sea region, Iran, Southern and Western Caucasus, some other Mediterranean countries. Notably, the consumption of CLF is particularly prevalent in Turkey's Eastern Black Sea region, as reported by Vahapoglu et al. (2018). While CLF is grown in many countries, its production and usage on a large scale are still limited. There are about 20 varied species in terms of different growing conditions (soil, sun, etc.), growth patterns, leaf size and shape, winter hardiness. The fruits of the Cherry laurel (*Laurocerasus officinalis* Roem., syn: *Prunus laurocerasus* L.) belong to the family of *Rosaeceae* and the subfamily of *Prunoideae* (Aktas, 2012; Talih and Dirim, 2018).

CLF offers versatility in consumption, with options ranging from fresh and dried fruits to marmalade, jam, pickles, molasses, jam. Moreover, it serves as an enhancer for flavor and fluidity in fruit juices and cakes. Liyana-Pathirana et al. (2006) concentrated juice from the kiraz-cherry laurel variety, examining its antioxidant features. Additionally, a marmalade from CLF was produced and its effects on yogurt storage duration were investigated (Temiz et al., 2014). In their research, Alasalvar et al. (2005) focused on developing pekmez products using different CLF types. Chemical composition disparities were also revealed by Alasalvar et al. (2005) between kiraz and pointed CLF varieties. Additionally, Ayaz et al. (1997) delved into the phenolic and fatty acid profiles of wild, kiraz, pointed CLF variants.

The choice of wild (Yabani), pointed (Sivri), kiraz CLF types were primarily driven by their abundant presence in Trabzon province of Turkey and the surrounding area. These CLF variants are associated with distinct cherry laurel cultivar trees, characterized by varying phylogenetic, cytological, morphological attributes. Their classification relies on physical features such as plant structure, shape of leaf, the appearance of young leaves, the appearance and taste of the fruit. From a phylogenetic standpoint, the Oxygemmis and Globigemmis cherry laurels share close genetic ties but differ from the wild cherry laurel, which has smaller leaves and fruits (Sandalli et al., 2005; Yilmaz et al., 2023).

The kiraz CLF, which is named according to its close resemblance to cherry fruit, derived from Globigemmis cherry laurels, boasts a thinner mesocarp and tastes sweeter than the pointed CLFs. Unripe kiraz CLFs display a red hue, while mature ones turn blackish and develop a slight bitterness. In contrast, pointed CLFs, originating from Oxygemmis cherry laurels, are larger, black, more astringent. The wild CLFs utilized in this research are attributed to the cultivar of Angustifolia cherry laurel (Yazici et al., 2011; Yilmaz et al., 2023). Kiraz CLF is the preferred choice due to its sweet flavor among the locals of Trabzon and the neighboring provinces in Turkey.

Various vinegars are produced all over the world using different raw materials and production methods. Through this study, CLFV was produced with a different approach in terms of raw materials as an alternative to the vinegars available in the market. In line with this target, seven types of traditional CLFV belonging to three different types were produced. In our earlier study with the same vinegars and production procedure, we found that CLFVs have a high antioxidant capacity, with significantly higher levels of phenolic content than control vinegars (Yilmaz et al., 2023). There has not been any study on the physicochemical properties of CLFV within the scientific literature. Quality characteristics of vinegars produced in this work were evaluated by performing total acid, volatile acid and non-volatile acid, pH, ash, number of oxidations, iodine number, ester, mineral substance, alcohol, total solids, total sugar-free solids, total sugar analyses.

Materials and method

Materials and design of experiment

Between August 1 and 15, 2020, CLFV was produced using fruits from four different cherry laurel trees belonging to three species – kiraz cherry laurel, wild cherry laurel, pointed cherry laurel. These trees were sourced from Kirazlik village, Vakfikebir, located in the Trabzon province of Turkey (41°03'13.9"K 39°19'10.8"E, 600 m). The samples were carefully handpicked without causing any harm to the inflorescences or fruit bunch structure. The plant identification was carried out by Assistant Professor Mehmet Oz from the Department of Forestry at Gumushane University, Turkey.

To produce the vinegar, honey and various CLF sources were used, including kiraz-CLFV, pointed CLF, wild CLF, unripe CLF, kiraz-pointed CLF, mixed CLF, CLF kernel mixture. Honey (Balparmak Brand, Blossom Honey, Cekmekoy, Istanbul, Turkey), Grape and apple cider vinegars (Kemal Kukrer Vinegars, Tepebası, Eskisehir, Turkey) were obtained from a local store. All chemicals and solvents, whether of HPLC or analytical grade, were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Merck (Darmstadt, Germany).

The harvested CLF samples were preserved at a temperature of 15°C and then transported to the laboratory for bioactivity analyses. The analyses were conducted in the food engineering department laboratory of Gumushane University (Gumushane, Turkey), the samples underwent two repetitions and three parallel studies.

Production of vinegars

After the CLFs were thoroughly washed, they were separated from the stems, the kernels were removed. Then, the size reduction of the fruits was done with a blender (Waring Corporation, 1967 Broadway, New York, NY, USA) till reaching to a marmalade-like consistency. To obtain more information on the raw materials, fruit extracts were obtained using water extraction, dry matter and bioactivity-related analyses were performed. The CLF samples were then prepared according to the proportions given in Table 1 and placed in sterilized jars (8 L) for fermentation. The incubators were maintained at temperatures ranging from 25 to 28 °C. During the fermentation process, the raw materials were mixed twice daily, ensuring homogeneity. The mixing process was continued until the fruits settled to the bottom. After approximately three and a half months, the vinegar mother was formed, the precipitation process was completed (Fig 1). The thick layer of extracellular cellulose formed by acetic acid bacteria on the surface of vinegar is called the mother of vinegar. The total acidity was analyzed weekly during this period, incubation was halted when the acidity exceeded 4%. Following filtration, the vinegar was filled into jars. Honey vinegar, which was produced using the same procedure, was used as the control sample, grape and apple vinegars from the market were used as additional control samples (Table 1).

FIGURE 1: *Stages of production of cherry laurel fruit vinegars.*

TABLE 1: *Samples used in the analyses and their formulations.*

Determination of total soluble solids (TSS), ash, total sugar-free solids (TSFS)

TSS analysis was conducted in an oven with a setting of 103 \pm 2 °C (Dahian brand oven, Dahian, Korea), while the ash content analysis was carried out according to the Turkish Standards (TS) 1880 method in an ash furnace (Protherm PLF115M, Turkey) with a rating of 525 ± 10 °C (TS 1880, 2004). TSFS was obtained by subtracting the total sugar from TSS.

Determination of total sugar content (TSC)

TSC analyses were completed according to the Lane-Eynon general volumetric method (AOAC Official Method 923.09, 2000). TSC was calculated in invert sugar as a mass percentage.

Determination of total acid amount (TAA), volatile acid (VA), non-volatile acid values (NVA), and alcohol content (AC)

The TAA, VA, NVA and AC analyses were performed according to the TS 522 which includes analysis methods of wines (TS 522, 1976). The amount of NVA was calculated in acetic acid (g/L) by subtracting the total amount of VA from the TA (TS 522, 1976).

Determination of pH value

For pH measurement, the pH meter electrode (Hannah, Hi, 2211-02, USA) was immersed in vinegars at 20 ± 2 °C, the pH value was recorded (TS 1748, 2001).

Determination of oxidation, iodine number, ester

The oxidation number, iodine number, ester analyses were done according to the method of TS 1880 (TS 1880, 2004).

Determination of color values

The L*, a*, b* values were determined using the Konica Minolta CR-300 (Minolta Osaka, Japan) color meter. The a* value indicates the redness or greenness of the food, the b* value indicates the yellowness or blueness, the L* value indicates the degrees of luminosity between 0 and 100 (black and white) (Quek et al., 2007).

In addition, the ΔE^* value was calculated by using the L, a, b values (Eq. 1).

$$
\Delta E \, * = \sqrt{\left(L^* - L_{ref}^*\right)^2 + \left(a^* - a_{ref}^*\right)^2 + \left(b^* - b_{ref}^*\right)^2} \quad \text{(Eq. 1)}
$$

Determination of mineral substances

The Nordic-Baltic Committee on Food Analysis (NMKL) 161 method was used to determine the mineral substances (NMKL 161, 1998). For this purpose, MP/AES (MP-AES 4200, Agilent Technologies, Melbourne, Australia) was used for this purpose. The determined minerals were Fe, Mn, Cu, Zn, Co, Ni, Cd, Pb, Na, K, Ca, Mg.

Statistical analysis

All analyses were performed for at least three parallel studies. The results were given with mean and standard deviation. Principal component analysis (PCA) was performed using Microsoft Excel software with XLSTAT (Addinsoft, Version 2020 New York, USA).

Results and discussion

Findings of TSS, TSFS, ash content

The findings of TSS, TSFS, ash content are given in Table 2. Vinegar is mainly composed of water and acetic acid. Additionally, it contains dissolved substances such as minerals, sugars, possibly small amounts of other organic compounds like amino acids, reducing sugar, gluconic acid, protein, etc. These substances collectively form the TSS. According to the outcome of the analysis of the TSS, the highest value was observed in honey vinegar, the lowest value was observed in grape vinegar. The TSS of grape and apple cider vinegar purchased from the market were determined as 22.18 g/L and 31.63 g/L, respectively. This ratio is lower than the vinegars produced and statistically different ($p < 0.05$). N7 had the highest (143.91 g/L) and N6 (60.53 g/L) had the lowest TSS among CLFVs ($p <$ 0.05). The TSSs of CLFVs are lower than the honey vinegar produced ($p < 0.05$). The different results for TSS may be due to the raw material composition of 8 samples (including honey vinegar as a control) used in the study. For instance, the honey vinegar used for the control contained 1 kg honey, whereas only 400 g honey was used in the other samples. As a result, the TSS amount was higher in the control sample. Since the study samples also contained high amounts of CLF, their TSS was higher compared to grape and apple vinegar. The TS 1880 vinegar standard requires the TSS value of vinegars to be at least 8 g/L. However, no limit value for TSS is specified in the TS 1880 for the vinegars (TS 1880, 2004). When comparing the TSS contents of our produced vinegars with those of others, we obtained similar as well as different results. This indicates that the TSS content depends on various factors such as the raw material composition used in vinegar production and the acetification system. In a different study, it was stated that the TSS values of apple cider vinegars ranged from 15.20 g/L to 85.10 g/L (Kara et al., 2021). Kan (2021) showed the minimum and maximum amount ranges of TSS of natural and industrially produced vinegars in apple cider vinegars, respectively: 9.77–10.48 g/L, 9.63–9.99 g/L, in grape vinegars; 9.66–9.68 g/L, 7.47–10.15 g/L, in hawthorn vinegars; $9.58-9.94$ g/L, $9.85-10.06$ g/L, in pomegranate vinegars; 10.00–10.12 g/L, 9.94–10.04 g/L, in rosehip vinegars; 9.62–10.00 g/L, 9.87–10.18 g/L.

The amount of ash in vinegar indicates the quantity of inorganic substances that remain unburned. According to the data, N8 vinegar sample had the lowest ash value of 1.55 g/L, while N10 had the highest value of 3.14 g/L ($p < 0.05$). The high amount of ash in CLF kernel vinegars may be due to the use of kernel and CLF pulp as raw materials during vinegar production. However, there was no significant statistical difference between N10 ash and N7, N6, N1 ($p > 0.05$). The ash content of grape and apple vinegars purchased from the market, honey vinegar produced as a control, were 2.59 g/L, 2.12 g/L, 0.47 g/L, respectively. The minimum ash limit for vinegars produced in Turkey is 0.8 g/L (TS 1880, 2004), which

TABLE 2: *Results of the vinegars for the analyses of TSS, TSFS, ash, TSC, AC, and acidities.*

	TSS g/L	TSFS g/L	Ash g/L	TSC g/L	VA g/L(AA)	NVA g/L(AA)	NVA g/L(TA)	TAA g/L(AA)	% TAA (AA)	pH	% AC	
N ₁	22.18h ±0.17	15.62 ⁶ ±0.19	2.59^{ab} ±0.21	6.56 ^h ±0.02	16.75^{bc} ±0.96	23.37de ±1.14	29.21 ^{de} ±1.43	40.12^{bc} ±0.30	4.01^{bc} ±0.03	3.11 ^e ±0.01	0.02 ^b ±0.01	
N ₂	31.63 ⁹ ±0.28	14.88 ^f ±0.77	2.12^{bc} ± 0.02	16.759 ±0.49	18.17a ±0.10	20.75 ^f ± 0.35	25.94f ±0.44	38.92 ^e ±0.30	3.89e ±0.03	3.09 ^e ±0.02	0.02 ^b ±0.01	
N ₃	478.47 ^a ±8.09	202.34 ^a ±15.46	0.47 ^d ± 0.25	276.13a ±7.34	14.55c ± 0.13	25.17 ^b ±0.24	31.46 ^b ±0.31	39.72bcd ± 0.35	3.97bcd ±0.03	2.28 ^h ±0.05	0.37 ^b ± 0.13	
N ₄	116.63c ±1.34	65.88c ±0.09	1.87c ± 0.42	50.76 ^d ±1.25	16.35^{b} ±0.31	23.17 ^e ±0.31	28.97 ^e ±0.38	39.52a ±0.30	3.95a ±0.03	3.39 ^c ±0.04	0.06 ^b ±0.00	
N ₅	100.26 ^d ±0.50	67.53c ±0.89	2.04 ^{bc} ±0.38	32.73f ±0.39	5.81 ^d ±0.05	28.63a ±0.28	35.78 ^a ±0.35	34.43 ^f ±0.30	3.44 [†] ±0.03	2.98 ^f ±0.04	0.34a ± 0.13	
N ₆	60.53 ^f ±0.18	28.73 ^e ±0.28	3.09a ± 0.11	31.80 ^f ±0.10	16.75 ^b ±0.71	24.27c ±0.62	30.34c ±0.77	41.02a ±0.30	4.10 ^a ±0.03	3.54 ^a ±0.03	0.34a ±0.24	
N7	143.91 ^b ± 0.75	71.53c ±0.01	2.74a ±0.09	72.38 ^b ±0.76	14.12c ±0.25	2.95 ^h ±0.48	3.69 ^h ±0.59	17.07h ±0.30	1.75 ^h ±0.03	3.25 ^d ±0.02	0.17 ^{ab} ±0.10	
N ₈	99.87 ^d ±1.25	54.97 ^d ±1.06	1.55c ±0.06	44.90 ^e ±0.20	16.53 ^b ±0.06	23.19 ^e ±0.18	28.99 ^e ±0.23	39.72cd ± 0.17	3.97cd ±0.02	3.46 ^b ±0.02	0.17 ^{ab} ± 0.13	
N9	140.86 ^b ±0.00	84.53 ^b ±0.23	1.75c ±0.09	56.33c ±0.23	16.04 ^b ±0.25	24.18 ^{cd} ± 0.13	30.23 ^{cd} ±0.16	40.22 ^b ± 0.17	4.02 ^b ±.02	2.90 ⁹ ±0.03	0.14 ^{ab} ±0.09	
N ₁₀	87.60e ±0.40	69.43c ±0.53	3.14 ^a ±0.70	18.179 ±0.18	5.73 ^d ±0.03	14.539 ±0.20	18.179 ±0.25	20.269 ± 0.17	2.039 ±0.02	3.50 ^{ab} ±0.01	0.31 ^a ± 0.17	

a–c: *n=3, ± standard deviation, a, b, c… shows the notable differences within the same colon at p<005, AA: Acetic acid, TA: Tartaric acid.

means that all the samples met the standard for ash content. The low ash content in the honey vinegar control sample may be due to honey's low mineral content. Total solids content exhibited a strong negative correlation with ash and pH analyses, but showed a noticeable positive correlation between total sugar and total non-sugar solids, as well as a relatively weak positive correlation with other analyses (Table 3).

It was stated in a different study for ash content ranges as for grape vinegars 0.74–3.56 g/L, honey vinegars between 0.11–2.72 g/L, wine vinegars between 2.03–2.61 g/L (Akbas and Cabaroglu, 2010; Alak, 2015; Gerbi et al., 1998). The ash content in the vinegars we produced closely matched the values found in different fruit vinegars available in the market and also aligned with values reported in the literature. Honey vinegar has a low ash content. This signifies that the quality of vinegar is entirely dependent on the type of raw materials used.

The analyses of TSS, TSC, TSFS are interconnected. As TSS increases, TSC decreases and TSFS increases. The content of TSFS was determined to be 15.62 g/L and 14.88 g/L in grape and apple vinegars purchased from the market, respectively. Honey vinegar produced as a control had a TSFS content of 202.34 g/L. The CLFV values vary between 54.96 g/L and 84.54 g/L. The ratio of TSFS depends on various factors such as the raw material composition variable used in vinegar production and the acetification system. From Table 3, it can be concluded that total solids have a strong positive correlation with total sugar and total sugar-free solids.

Findings of TSC

The TAA and TSC are important indicators during the production of vinegar. These indicators give crucial information for both microbial growth and metabolite accumulation during vinegar fermentation. As can be seen in Table 2, the TSC values of commercial grape vinegar, apple cider vinegar, honey vinegar were determined as 6.56 g/L, 16.75 g/L, 276.13 g/L, respectively. Among the TSCs, grape vinegar showed the lowest value, the highest value belonged to honey vinegar, as expected ($p < 0.05$). The TSC of the CLFVs was between 18.16 g/L and 72.38 g/L. The TSC of CLFV was significantly lower compared with the TSC of honey vinegar ($p < 0.05$). The highest value within the CLFVs was N7, with a value of 72.38 g/L. The next highest values were found to be N9 and N4, respectively. N10 has the lowest TSC among CLFVs due to the raw material used. It is important to note that there are no legal regulations in Turkey regarding the allowable amount of sugar. As a conclusion of evaluating the earlier studies, the total sugar contents of vinegars used and produced in this work were found to be compatible with the existing literature. Ashanti et al. (2019) conducted a study on the TSC values of red wine vinegars derived from different grape varieties, they found that the values ranged from 3.3 to 11.6 g/L, except for one sample's high value of 148.8 g/L. The same study also revealed that two apple vinegars had sugar contents of 0.3 and 2.1 g/L. In a separate research, apples and red grapes had sugar contents of 7.17 and 5.00 °Bx, respectively (Kim et al., 2013). Furthermore, a study on jaboticaba berries found that the TSC values for fresh and dried vinegars were 3.0 and 1.1 °Bx, respectively. The TSC value for Korean traditional black raspberry vinegar was 6.6 °Bx, according to the research conducted by Song et al. (2016).

Since CLF has low sugar compared to many other fruits, honey was added to vinegars for the fermentation process. Hence, the sugar found as a result of the sugar analysis is due to the honey added to the media to make the fermentation progress during the start of the vinegar production.

TSC results showed a strong negative correlation with ash and pH analyses, whereas they had a weak positive correlation with other analyses (Table 3).

Findings of TAA, VA, NVA, pH and AC

The findings of TAA, VA, NVA, pH and AC are given in Table 2. The TAA value of honey vinegar was found to be 39.72 g/L. The highest TAA values (as AA) were observed in N6 and N4 with the results of 41.02 g/L and 39.52 g/L, respectively, the lowest value was observed in N7 with a value of 17.07 g/L ($p < 0.05$). The TAA values of grape and apple cider vinegar were 40.12 g/L and 38.92 g/L , respectively ($p < 0.05$). During the period of vinegar production, the acidity development of samples N7, N10, N5 was low. This result may depend on the type of CLF. The TAA values of N3, N4, N6, N8, N9 samples were in accordance with TS 1880 (The minimum acidity value limit for vinegar is 40 g / L) (TS 1880, 2004). Low TAA indicates low organic acids.

During the weekly controls, it was noticed that sample N3 had completed its acidity development in around one month, whereas other samples took more than three months. The slow progress in other samples can be attributed to the presence of phenolic and flavonoid-type compounds in the CLFs used. It is believed that the use of only unripe cherries in N7 production and the use of unripe cherries and CLF kernels in N10 production had an impact on the acidity development.

Determining the specific reasons for a lack of acid development during vinegar fermentation requires careful observation and analysis of the process and conditions. There may be several factors that contribute to a lack of acid development. Inadequate fermentation time, insufficient alcohol content, poor quality alcohol sources, incorrect temperature, poor ventilation, contamination, weak or inactive starter cultures, pH levels, lack of nutrients, environmental factors can all cause low acidity. All the necessary conditions were maintained, careful measurements were taken during vinegar production. We concluded that the low acidity of our vinegars is most probably due to the CLFs used as raw materials. By optimizing and making improvements to production techniques and procedures, some progress in acidity levels may be gained.

The lowest VA (g/L) values in vinegar samples were 5.73 and 5.81, respectively, within the samples of N10 and N5 $(p > 0.05)$. The highest value of VA (g/L) was observed in apple cider vinegar sample with a value of 18.17 g/L. The VAs of CLFVs vary between 5.73 g/L and 16.75 g/L, whereas the values of honey and grape vinegar were determined as 14.55 g/L and 16.75 g/L, respectively. Researchers reported that the NVA of some grape vinegars produced in Turkey ranged from 0.7 to 4.5 g/L, the VA ranged from 35.6 g/L to 52.1 g/L (Akbas and Cabaroglu, 2010).

The lowest value of NVA as acetic acid was detected within the sample of N7 with the value of 2.95 g/L, the highest amount was 28.63 (p<0.05), belonging to the sample of N5. NVA as acetic acid was 20.75 g/L in apple cider vinegar, 23.37 g/L in grape vinegar, 25.17 g/L in honey vinegar. The NVA of CLFVs varies between 2.95 g/L and 28.63 g/L as acetic acid and between 3.69 g/L and 35.78 g/L as TA (Table 2). NVA (as TA) was positively correlated with acidity percentage and acidity (as AA).

According to the pH measurements made in CLFVs, the lowest value was observed in N5 and N9, with results of 2.98 and 2.90, respectively. N6 and N10 samples have the

highest values with pH values of 3.54 and 3.50, respectively. The pH results were determined as 3.11, 3.09, 2.28 in grape, apple, honey vinegars, respectively. The pH values for the CLFVs were between 2.90 and 3.54. Kara (2021) stated that different apple cider vinegars had a pH of between 3.70 and 5.33 and AC ranging from 0.0% to 1.5%. It is shown that ranges of the pH values of grape and apple cider vinegars in Turkey vary between 2.70–3.90 and 2.71–3.56, respectively, the titration acidity percentage values vary between 0.32–5.72 and 0.66–7.20 respectively (Ousaaid et al., 2021; Ozturk et al., 2015).

Foods such as vinegar and fruit juice contain volatile organic acids (C2–C12) and non-volatile acids such as malic and citric. The total acidity in vinegar is the sum of these two types of acids. The correlation map of all the acidity data is presented in Table 3. The pH value results demonstrate a negative correlation with other acidity data (Table 3). Additionally, the total acidity shows a strong positive correlation with both volatile and non-volatile acidity results.

CLFV, with the lowest AC, was found to be the N4 sample with 0.06%. The AC of CLFV varies between 0.06% and 0.34%. The AC was determined to be 0.37% in honey vinegar and 0.02% in grape and apple cider vinegars.

In accordance with TS 1880, it is stated that the residual AC should not be more than 0.50% by volume in vinegars other than wine vinegars and 1.50% by volume in wine vinegars (TS 1880, 2004). According to all the alcohol analyses, the AC was below 0.5% by volume among the results of the produced CLFVs.

There are specific standards in regional areas for vinegars produced or sold in European countries. Unlike the US, the EU has established standards for both TAA (at least 5.0% w/v) and AC (maximum of 0.5% v/v). Wine vinegar should have a TAA of at least 6.0% (w/v) and a maximum of 1.5% (v/v) ethanol only when obtained by acetic acid fermentation of wine (EC No. 1493/1999). Within the U.S., the Food and Drug Administration (FDA) has defined that vinegar products must have at least 4.0% TAA. This limit refers to the minimum TAA of all vinegars sold in the retail market (TS 1880, 2004).

Findings for the oxidation number, iodine number, ester analyses

According to the results for the CLFVs, the oxidation numbers were between 392.71 and 394.02. The oxidation numbers in apple and grape vinegars were 391.60 and 390.00 (p>0.05). The iodine numbers were between 384.68 and 385.80 (Table 4), the iodine numbers of apple and grape vinegars were 51.20 and 62.40 (p<0.05), respectively. The iodine number measures the amount of unsaturation in a substance, particularly in fats and oils. It is determined by the amount of iodine (in grams) that is consumed by 100 grams of a substance. The high iodine numbers of our vinegars indicate they contain high amounts of unsaturated organic compounds. The iodine number in vinegar is mostly affected by acetylmethylcarbinol and diacetyl (Macrae et al., 1993). In addition, Cline (2003) reported that oxygen-induced oxidation processes occur in vinegar as a result of chemical changes in polyphenolic compounds.

The results of ester analysis in CLF ranged from 17.39 to 60.14 (Table 4). Among the CLFVs, N6 showed the highest ester count value of 60.87, N9 showed the lowest value of 17.39 ($p < 0.05$). The other values were observed as 21.09 in apple cider vinegar, 19.71 in grape vinegar, 21.41 in the honey vinegar as the control sample.

The ester helps to create flavor aroma substances in vinegar. The amount of ester obtained is an indicator of how much the alcohol formed during the analysis interacts with the carboxylic acid (Kobya, 2018). The flavor components in vinegar affect taste and quality, such as esters, acids and total reducing sugars. In our study, except for samples N7 and N9, the ester numbers of other vinegars where only one type of CLF was used. The number of esters is high in N4, N5 and N6 vinegars where direct CLFs are used. This may be thought to be due to the abundance of ester-type organic compounds found in CLFs.

Findings for color values

CIE (International Commission on Illumination) Lab system, which sets standards for lighting and color, was used to define the color of vinegar. The L* value indicates light

TABLE 3: *Correlation values for all analyses.*

*n=3, AA: Acetic acid, TA: Tartaric acid

TABLE 4: *Oxidation number, iodine number, ester, and color values of vinegar samples.*

 $*$ n=3, ± standard deviation, a, b, c, , shows the notable differences within the same colon at p<0.05

to dark, 0–100, the larger the value, the greater the brightness. The a* red/green value represents positive partial red and negative partial green values. The value b* represents the yellow/blue value, positive yellow and negative blue. The L* values of the CLFVs range from 13.29 to 25.78, the a* value from 8.10 to 15.06, the b* values from –2.55 to -1.28 (Table 4). The highest L^* and a* values were detected in the sample of N8 vinegar. The L* and a* values were 21.25 and 5.26 in apple cider vinegar, 10.98 and 9.26 in grape vinegar, 15.66 and 9.91 in honey vinegar. A positive a* value in CLFVs (red color) coincides with the observable color of the samples. The a* value has a weak positive correlation with the L* value. L* has a strong positive correlation with b* and ΔE^* values (Table 3).

N4 sample gave the highest b* value among the CLFVs. The b^* value was -4.32 in apple cider vinegar, -4.65 in grape vinegar, -0.35 in the control sample ($p < 0.05$). Among b*

values, –b denotes yellow, +b denotes blue. The b* value of all vinegars was determined as a negative value.

The color of vinegar can be affected by a number of factors, as mentioned in a study by Liu et al. (2008). These factors include the color of the raw materials used, chemical reactions that occur during preparation, pigments produced by chemical or enzymatic reactions during fermentation, the addition of caramel colorants. The polyphenolic compound of each raw material is also influential on the color of vinegars (Mas et al., 2014). During the fermentation of CLFs, it was observed that the colors became more intense while the brightness decreased (Fig. 2). The CLFVs had a reddish appearance, in contrast to honey vinegar, which retained its yellowish hue. This suggests that CLFVs have more biological properties than honey vinegar. Variations in color during fruit processing are expected but can indicate changes in chemical composition due to raw materials or problems during production.

∆E* values of vinegar products were calculated according to the control sample. The highest ∆E* value was observed in N7 sample with 6.51 and N8 sample with 5.81, the lowest in N4 sample with 0.72 ($p < 0.05$). The ΔE^* value was 2.90 in apple cider vinegar and 1.23 in grape vinegar ($p < 0.05$). L^{*}, a*, b* values showed positive correlations with each other, according to the statistical analysis of ∆E* results related to color analysis. Except for the N4 sample, the ∆E* values of the other CLFV samples, as well as the grape and apple cider vinegars, were found to be greater than 1 ($p < 0.05$). A standard observer accepts the color difference results as follows: "0 < ∆E < 1 – the observer does not notice the difference, 1 < $\Delta E < 2$ – only the experienced observer can notice the difference, 2 < ∆E < 3.5 – the inexperienced observer also notices the difference, $3.5 < \Delta E < 5$ – the clear color difference is noticeable, 5 < observer ∆E – observer notices two different colors." (Mokrzycki and Tatol, 2011).

FIGURE 2: *Color appearances of vinegar samples.*

Findings of mineral substance analysis

The study of mineral elements in vinegar is essential due to its potential toxicity, possible adverse effects, its use as an indicator. The mineral content in vinegars can come from the source of raw materials, contact with materials during processing, or environmental contamination. The results determined by using MP-AES in the study indicated that the vinegars are generally richer in Na, K, Mn, Mg, Fe, Ca than other minerals. The results of the ranges of the mineral substances in CLFV are as follows in mg/L: Fe; 1.27–5.08, Ca; 25.00–26.08, Zn; 0.63–1.34, Mg; 13.26–15.95, Cu; 0.17–1.00, Ni; 0.06–0.93, Mn; 0.82–6.23, Na; 17.92–18.78 N; 146.2–150.28 (Table 5). There are no legal limitations for Na, K, Mg, Ca in vinegar.

Because of their toxic effects, the heavy metals Pb and Cd require special attention (Rahman & Singh, 2019). The Pb and Cd contents in vinegars were lower than their LOQ (Table 5). The Turkish Government regulates the maximum permitted contents of some heavy metals in vinegars. Accordingly, toxic metals such as Cd and Pb should not exceed 0.02 mg/L (Cd) and 0.2 mg/L (Pb) (Anonymus, 2002).

Zinc is a mineral that is important in many aspects of our health. Daily 15–30 mg of elemental zinc can improve blood sugar levels, immunity, eye, heart, skin health. Consuming more than 40 mg of zinc daily can cause digestive problems and flu-like symptoms, reduce the effectiveness of some antibiotics, reduce copper absorption. High zinc intake may cause severe neurological diseases due to copper deficiency. (Hedera et al. 2009). Our study determined that the Zn level in vinegars did not comply with the legislation. However, it is below EFSA's daily intake rates (EFSA, 2014). In Codex Alimentarius CODEX STAN 162-1987, the limit for zinc was specified as the total of zinc and copper with the amount of 10 mg/kg (Anonymous, 2002). In this sense, the totals of zinc and copper amounts are at an acceptable level.

Mg content in the produced vinegars was between 13.26 and 15.95 mg/L. Therefore, there is no notable difference between vinegars for Mg amounts. In Turkey, the legal legislation regarding the total amount of copper and iron in vinegar is regulated as 30 mg/L at most (TS 1880, 1988), above our study's results.

Manganese is a naturally occurring element found in Earth's crust, water, the atmosphere. It is essential for normal body function and the recommended safe daily intake is between 2.5 to 5.0 mg. There is no legal limit on the Ni content in vinegar. The lowest adverse effect level observed by EFSA, 4.3 mg Ni/kg body weight, was chosen as the reference point (EFSA, 2020).

PCA analysis of physicochemical properties

Principal component analysis (PCA) was applied to evaluate the physicochemical properties of vinegars. PCA is the most popular method for investigating the relationship between variables and observations. It can be analyzed graphically, considering the data and all variables related to this method simultaneously. In the PCA analysis of vinegar samples, the PCA1 and PCA2 diagrams explained 55.38% of the cumulative variance. About 91% is explained in PCA5. As can be seen in Fig 3, there are five groups, including a group in the purple circle (N1, N2), a green $(N4, N6, N8, N9)$ group, a black $(N5)$ group, a yellow $(N3)$ group, a red (N7, N10) group. The scores are arranged in four areas. The clear distinction between vinegar samples indicated differences in some of the physical and chemical parameters examined. The score plot also showed a clear separation between control samples and CLFVs' samples. N1 and N2 coded samples in the purple group are grape and apple cider vinegars used as control samples. While N1 and N2 samples have high values in terms of acidity, nonvolatile acidity, volatile acidity, N3, N7, N10 vinegars have lower values in terms of these analyses. Sample N6 is close to this group in terms of acidity parameter.

The sample of N3 in the yellow group is honey vinegar, which stands out in terms of TSC, TSS, TSFS. In addition, N3 is close to the samples in the purple group regarding acidity. N7 and N10 samples in the red group are at the forefront in terms of pH and ash analyses according to PCA analysis. Ash and pH analysis results in N10 and N7 samples were higher than other samples. On the contrary, the acidity, non-volatile acidity, TS, TSS, TS-FS were low. In honey vinegar number N3, used as control, ash, pH analysis results were low, while acidity, non-volatile acidity, TS, TSS, TS-FS amounts were high.

> Samples N1 and N2 are similar to samples N7 and N10 regarding the analysis. The closest N6 example shows similar features to this group. N5 sample in the black group stands out in terms of ester, oxidation number, iodine number, % alcohol. The closest samples to this group are N6 and N8, as seen from the PCA graph in Fig 3. The sample N8 in the green group is different than others in terms of color values. It is observed that N2, N4, N9 samples are close to N8 sample in terms of color values. Based on the analysis results, it was determined that samples N8 and N9 shared some similarities with sample N3. Samples N5, N6, N8 had high ester numbers while vinegar numbers N3, N7, N9 had low vinegar numbers. The oxidation numbers were the same in all samples. Vinegar sample number N8 had higher L* and a* values. The L* value was found to be low in sample number N5. All vinegar samples had almost the same iodine numbers except for N1 and N2, which were used as controls.

TABLE 5: *Mineral substance analysis results in vinegar samples (mg/L).*

*n=3, ± standard deviation, a, b, c… shows the notable differences within the same colon at p<0.05 LOQ= 0.01 mg/L

FIGURE 3: *PCA analysis of physicochemical properties of vinegars.*

Conclusion

With the increasing health problems in recent years, people started preferring fermented products increasingly (Šikic´ Pogačar et al., 2022). Through this trend, vinegar has been recently produced more from raw materials such as hawthorn, pomegranate, kiwi, lemon, honey, cherry, orange, artichoke, carob, in addition to vinegars such as apples and grapes (Ozturk et al., 2023). The objective of this study was to produce new natural vinegar using Cherry Laurel Fruits, which are readily available in Turkey. The resulting vinegar has high phytochemical content and effective antioxidant activity, making it a valuable food product. A proposed process for vinegar production from CLF has been initiated to facilitate the industrialization of this fermentation process.

According to the results of this study, vinegars produced from CLF have lower total sugar-free solids and total sugar values, have higher values of ash and pH analyses, have a similar outcome for the iodine number compared to the vinegars produced from control samples (apple, grape and honey vinegars). In addition, volatile acidity, oxidation numbers, the number of esters for CLFVs were mainly determined to be higher than the control vinegars' values. The results of the total solids, the total sugar-free solids, the oxidations number, the iodine number for the CLFVs were consistently higher, the total sugar and ester were generally higher than the results for the commercial grape and apple cider vinegar.

In our study, the ester numbers of vinegars except N7 and N9 samples were higher than grape and apple cider vinegar. The number of esters is high in N4, N5, N6 vinegars, where only CLFs are used. Flavor components in vinegar, such as esters, acids, total reducing sugars, affect taste and quality. They contribute to the aroma and flavor of many fruits and are found in various types of vinegar, contributing to their characteristic odor and taste. The high amount of iodine in the produced CLFVs and honey vinegar shows the presence of high amounts of unsaturated organic compounds. Total soluble solids (TSS) in vinegar refers to the total concentration of dissolved solids in the liquid. In the context of vinegar, this primarily includes sugars, organic acids, other dissolved compounds. In our study, the values of the TSS, TSFS, TSC amounts of the vinegars, which were found to be high, will positively contribute to CLFVs' taste and aroma. It is seen that the vinegars numbered N4, N5, N6, N8, N9 are suitable for vinegar production because their physicochemical properties are higher than the samples numbered N7 and N10.

As detected in one of our previous studies which investigated the same CLFV products, the newly produced vinegars are rich in phenolic compounds and flavonoids. The phytochemicals in the vinegars often mirrored those in the original fruits. Consumers can incorporate fermented vinegars from various plants into their cooking and benefit from the small amounts of phytochemicals provided by these vinegars as an additional source of antioxidants in their diet. The abundance of phenolic content in vinegars causes higher oxidation and iodine numbers as observed in the results of this study.

The findings of this research clearly show that the CLF chosen as the raw material for vinegar production has a signifi-

cant effect on the final product. There have yet to be studies on the production and development of CLFV. Therefore, working with more optimized biotechnological processes and determining the ideal conditions is necessary.

In conclusion, this study shows that CLFVs have the potential to obtain a value-added product that will increase the functional product diversity in the market.

Funding

This study was supported by Gumushane University Scientific Research Project Unit (GUBAP). Project number: 21.E3107.07.01

Conflict of interest

The authors declare no competing interest.

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