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DETERMINATION OF FREE HYDROCYANIC ACID IN HOMEMADE FRUIT BRANDIES

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Abstract

Fruit brandy is a traditional alcoholic beverage widely consumed in the Republic of N. Macedonia and other Balkan countries, produced by distillation of fermented fruits such as plum, apricot, quince and apple, using either homemade or industrial technology. This study aimed to evaluate the safety of 24 homemade fruit brandy samples by determining the content of free hydrocyanic acid (HCN), a potentially toxic compound. HCN is formed during alcoholic fermentation as a result of enzymatic hydrolysis of cyanogenic glycosides naturally present in fruit seeds. The quantification of free HCN was performed spectrophotometrically using König reaction, a colorimetric method based on the formation of cyanogen chloride, which reacts with pyridine and barbituric acid to form a stable pink complex with maximum absorbance at 580 nm. Results were recalculated to a 100% v/v ethanol basis to allowed comparison with the EU legal limit of 70 mg/L. All samples were within the permissible safety threshold. The highest HCN concentration was found in apricot and apple brandies (up to 9.81 mg/L), while plum and quince brandies contained significantly lower levels. A moderate correlation was observed between HCN levels and several chemical parameters, including methanol, aldehydes, ethanol, total esters, furfural and fusel alcohols. These results suggest that fruit type, fermentation conditions and the duration of seed contact during the preparation of the fruit mash before the fermentation play a critical role in HCN formation. This highlights the importance of controlled processing practices to ensure the safety of traditional fruit brandies.

Key words: *fruit brandies, free hydrocyanic acid, spectrophotometry.*

INTRODUCTION

The production of fruit brandies by distilling fermented fruit is a long-standing tradition throughout the Balkans, especially in North Macedonia, where it plays an important role in rural life and cultural identity (Petrova et al., 2024). In this study, analyses of homemade brandies made from different types of fruit were performed, including brandies produced from plum (*Prunus domestica*), apricot (*Prunus armeniaca*), apple (*Malus domestica*), and quince (*Cydonia oblonga*). Today, both homemade and commercial production continue to develop, helping to preserve traditional knowledge and support the local economy. However, homemade brandy production also comes with some safety concerns, especially when it comes to the final product.

One of the main risks of fruit brandies is the possible presence of hydrogen cyanide (HCN), a highly toxic and volatile compound. HCN is formed as a degradation by-product of cyanogenic glycosides, mainly amygdalin and prunasin, which are naturally present in seeds and stone fruits. During alcoholic fermentation, these glycosides are enzymatically hydrolyzed by *β-glucosidases* coming either from the fruit itself or from microorganisms involved in the fermentation process. The process releases

benzaldehyde, glucose and free HCN (Kuca et al., 2024). These reactions become more intense if the fruit seeds are damaged during processing or if the pulp remains in contact with the seeds for a long time (Ballhorn, 2005; Lee et al., 2021). Grinding or crushing the fruit activates certain natural enzymes such as linamarase or dhurrinase, which accelerate the degradation of these glycosides (Voldřich & Kyzlink, 1992). The steps of this process are shown in Figure. 1 (Nyirenda, 2020).

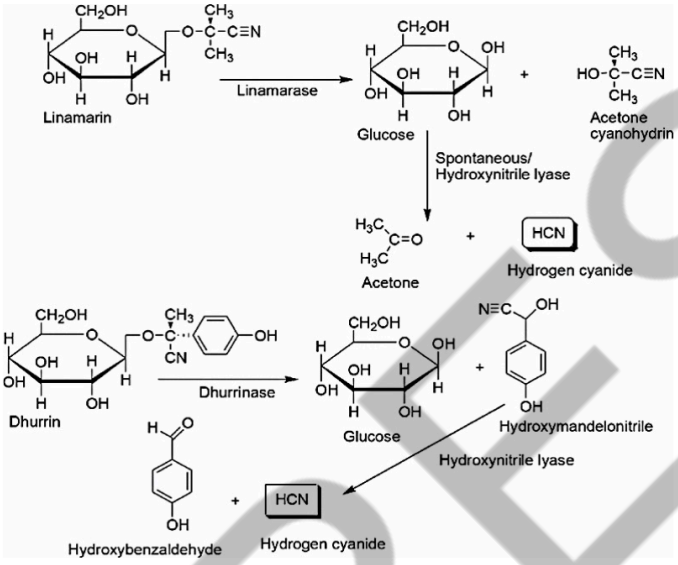

Figure 1. Mechanism of cyanogenic glycosides hydrolysis and spontaneous breakdown to hydrogen cyanide in stone fruit fermentation.

The process begins with enzymatic degradation of the sugar component, followed by degradation of the resulting nitrile compounds into free HCN. Aromatic aldehydes such as benzaldehyde are also formed as by-products (Novak et al., 2016). Considering the relatively low boiling point of HCN (26°C), it exhibits high volatility during distillation and can be co-distilled together with other volatile components (Liu et al., 2024). In domestic distillation, there are limited technological possibilities, the efficient separation of volatile fractions is at a low level and as a result there is a possibility of the appearance of toxic volatile components in the final distillate. One of these components is free HCN. From a health perspective, HCN is particularly dangerous because it blocks the activity of cytochrome oxidase in mitochondria, disrupting cellular respiration and causing oxygen deficiency at the tissue level (Claus & Berglund, 2005; Smith & Jernigan, 2019). The excess amount of HCN can lead to respiratory arrest, cardiovascular failure, and even death. Even at lower levels, long-term exposure to HCN has been associated with nervous system problems, hormonal imbalances that particularly affect thyroid function, and other cumulative toxic effects (Shmerling, 2025). In contrast, industrial distillation technologies typically involve fractionating columns and precise thermal control, which improves the removal of volatile contaminants and minimizes toxicological risk (Claus & Berglund, 2005; Bolarinwa et al., 2014). Regulatory frameworks, such as those of the European Commission, have subsequently imposed strict thresholds for the permissible concentration of HCN in alcoholic beverages. According to Regulation (EU) 2019/787 of the European Parliament and of the Council, the maximum permitted level of HCN in brandies produced from stone fruit is 7 g/hL (100% v/v) alcohol, which is equivalent to 70 mg/L. This regulation aims to protect consumers by limiting the concentration of free HCN. In view of the above risk, the analytical determination of free hydrogen cyanide in fruit distillates is of the utmost importance. Among the various available methodologies, spectrophotometric quantification using pyridine - barbituric acid reagent has emerged as the method of choice for

routine analysis due to its operational simplicity, cost-effectiveness and sufficient sensitivity (Sriprapat et al., 2014). This technique is based on the formation of a chromogenic complex with HCN, which gives a measurable absorption signal within the visible spectrum at 580 nm (Epstein, 1947). In this study, the content of free HCN was analyzed in 24 samples of home-made fruit brandies collected from the eastern region of the Republic of North Macedonia, including Berovo, Štip, Kočani and Veles and Kavadarci as part of the Tikveš region. The primary objectives were to determine the concentration of free HCN in these homemade fruit brandies and to assess their safety for human consumption based on the maximum permitted limits set by the EU. In addition to the determination of HCN, basic chemical analysis of fruit brandy samples was performed, including quantification of methanol, furfural, higher alcohols, total esters, sulfites, dry extract and ethyl acetate. The aim was not only to assess compliance with regulatory standards, but also to provide a comprehensive chemical characterization of the samples. Furthermore, this research aims to introduce a rapid and cost-effective spectrophotometric method for the routine determination of HCN, intended for use in both academic research and quality control laboratories.

MATERIAL AND METHODS

Sample collection

A total 24 samples of homemade fruit brandies were collected for the purpose of this study. All samples were traditionally produced through spontaneous fermentation of crushed fruit followed by classical distillation in copper pot stills. According to on-site interviews with local producers, the fermentation period lasted approximately 24 days and was carried out in plastic fermentation vessels under uncontrolled temperature conditions. In several cases, producers removing the stone and seeds prior the fermentation to reduce bitterness and potential cyanide release. However, other producers fermented the entire mush including pulp and seeds, which may have increased the probability of HCN formation due to enzymatic breakdown of cyanogenic glycosides. The collected samples originated from two district geographic regions from North Macedonia:

- The Eastern Region, which included samples from Štip, Kocani, and Berovo (Maleshevia region) – a region characterized by diverse microclimatic condition and traditional fruit-growing practices.
- The Tikveš Region, represented by Kavadarci and Veles, known for its rich viticultural heritage and long-standing tradition of fruit brandies production.

All brandy samples were transferred in the laboratory in 1L glass bottles, properly sealed and labeled with information regarding their geographical origin and fruit type. The samples were stored under appropriate conditions prior to analysis. Information on the location and type of fruit for each samples is provided in Table 1.

Sample preparation for analysis

Prior to analysis, the collected homemade fruit brandies sample were subjected to a redestilation step. This was necessary because several of the original samples were yellow colored, attributed to aging in oak barrels, a traditional practice used by local producers to enhance aroma and flavor characteristics. Coloration and matrix complexity could interfere spectrophotometric measurements too. A preliminary predestination was performed to obtain colorless distillates suitable for analytical determination. The redestilation was carried out using a standard simple distillation apparatus, without fractionation, to preserve the original volatile profile. For each 100 ml of sample was distilled, and 100 ml of distillate was collected for analysis. For the determination of free HCN, distillation was performed in the presence of H_3PO_4. The acidic environment facilitates release of HCN, as cyanogenic compounds and their anionic forms (such as CN-) are more readily converted to volatile molecular HCN under low pH conditions. This step is critical to ensure quantitative recovery of cyanide from the sample matrix during distillation (Claus & Böhme, 1980; Kawakami & Konno, 1980).

Table 1. Geographical location and fruit type of the analyzed homemade fruit brandy samples from North Macedonia.

Sample	Location (Municipality)	Fruit Type
S1	Berovo	Yellow Plum
S2	Berovo	Yellow Plum
S3	Berovo	Yellow Plum
S4	Berovo	Yellow Plum
S5	Štip	Yellow Plum
S6	Štip	Yellow Plum
S7	Berovo	Blue Plum
S8	Berovo	Blue Plum
S9	Štip	Blue Plum
S10	Kavadarci	Blue Plum
S11	Kavadarci	Blue Plum
S12	Veles	Blue Plum
S13	Kavadarci	Apricot
S14	Kavadarci	Apricot
S15	Kavadarci	Apricot
S16	Kavadarci	Apricot
S17	Berovo	Apricot
S18	Veles	Apricot
S19	Kavadarci	Quince
S20	Kavadarci	Quince
S21	Berovo	Quince
S22	Berovo	Apple
S23	Berovo	Apple
S24	Kavadarci	Apple

Chemical analysis

The chemical analysis of the fruit brandies samples was conducted in accordance with internationally recognized methods established by the International Organization of Vine (OIV). Methanol was determined spectrophotometrically using the chromotropic acid method, following preliminary oxidation of methanol to formaldehyde. This colorimetric method allows for quantitative detection based on chromogen formation, as described OIV-MA-AS312-03B Ethanol (alcoholic strength) was measured using the picnometric method in accordance with OIV-MA-AS312-01. Furfural content was determined according to OIV-MA-AS315-27. Total esters were quantified via acid-base titration, as described in OIV-MA-AS315-03. Ethyl acetate was determined spectrophotometrically, utilizing its absorption characteristics in the UV/Vis spectrum. The method complies with OIV-MA-AS315-03. Sulphur dioxide (SO_2) was determined using Iodometric titration method, in line with OIV-MA-AS323-04B, allowing quantification of total SO_2. Total dry extract was measured gravimetrically, based on the evaporation of a known sample volume and weighing of the residual non-volatile solids, according to OIV-MA-AS2-03.

Analysis of free HCN

The quantification of free hydrocyanic acid in the fruit brandies samples was performed using a spectrophotometric method based on the König reaction, in accordance with the standardized procedures described in OIV-MA-AS315-06A. This two-step colorimetric method involves the initial oxidation of cyanide ions (CN-) by Chloramine-T, forming cyanogen chloride (ClCN), which than reacts with pyridine and barbituric acid to form a stable pink-colored polymethine complex. The complex exhibits maximum absorbance at 580 nm, enabling sensitive and specific quantification of free HCN.

Each sample (100 mL of brandy) was redistilled in the presence of H_3PO_4 which provides an acidic environment to enhance the release of free HCN. A total of 100 mL of distillate was collected and used for the analisys. From the distillate, 25 mL was pipetted into a volumetric flask of 50 mL. Than 1 mL 3% Chloramine-T was added. After 1 min 10 mL of phosphate buffer (pH 7.6) was added, followed by 3 mL of barbituric reagent (3.65 g barbituric acid in 15 mL pyridine, diluted to 50 mL with distillated water). The mixture was left for 10 min at room temperature, diluted to 50 ml water and the absorbance was measured at 580 nm using UV/Vis spectrophotometer. A stock standard solution of KCN with concentration of 1000 mg/L CN- was used to prepare a series of working standards in the range of 1-20 mg/L. Each standard was processed identically to the samples. The obtained absorbance's values were used to construction of calibration curve, which used to linearity over the tested range and enabled the quantification of free HCN in the analyzed samples.

The free HCN concentrations obtained from the calibration curve were expressed as mg/L of the analyzed distillate. However, in accordance with EU Regulation 2019/787, which defines the maximum permitted HCN concentration as 70 mg/L of ethanol (100% v/v), it was necessary to standardize the results to enable accurate safety.

In addition, for each sample, the maximum allowed HCN concentration was calculated based on its ethanol strength using the following formula:

HCN (limit) = 70 mg/L × Ethanol (% v/v)/100%

These corrections allowed for precise comparison with regulatory thresholds and were used to assess whether the samples complied with legally permitted safety limits for free cyanide in fruit brandies.

Therefore, for each sample, the measured HCN values was recalculated to its equivalent per (100% v/v) ethanol using the following formula:

HCN [100 % (v/v) EtOH] = HCN (measured mg/L) / Ethanol (% v/v) ×100%

- HCN (measured) represent the concentration determined from the calibration curve in mg/L of the sample
- Ethanol (% v/v) is the ethanol content of the sample, determined by picnometric analysis.

RESULTS AND DISCUSSION

Basic chemical parameters

The overall chemical characteristics of the analyzed fruit brandy samples (S1-S24) revealed different compositional trends that were largely influenced by the type of fruit, as well as the specific technological practices applied during fermentation and distillation. The chemical composition of the analyzed fruit brandy samples is presented in Table 2. The chemical parameters were examined with a special emphasis on their impact on the aromatic quality, toxicological safety and overall sensory potential of the brandy. Among the analyzed samples, sample S1 showed the highest ethanol content (51.9% v/v). The higher ethanol content of this brandy sample is most likely because traditional producers prefer to produce brandy with a higher alcohol content. In contrast, sample S15 and sample S6 showed significantly lower ethanol levels (38.85 and 39.68% v/v respectively). This is potentially due to incomplete sugar conversion or premature termination of fermentation. Overall, yellow plum distillates (S1-S6) consistently showed increased alcohol levels compared to quince (S19-S21) and apple brandies (S22-S24). Methanol, a toxic by-product of pectin degradation (Lachenmeier et al., 2008), was present in varying concentrations across the samples. The highest value was detected in sample S3 (1.14% v/v) and S15 (1.12% v/v), most likely due to inclusion of crushed fruits stones during fermentation, rich in pectin substances.

Table 2. Chemical composition of the analyzed homemade fruit brandy samples.

Sample	Ethanol (% v/v)	Methanol (% v/v)	Aldehydes (mg/L)	Fusel Alcohols (mg/L)	Total Esters (mg/L)	Ethyl Acetate (mg/L)	Dry Extract (g/L)	Furfural (mg/L)	Free SO_2 (mg/L)
S1	51.91	0.99	305.2	3750.2	3119.1	1214.2	4.05	27.2	6.41
S2	42.25	0.98	147.9	2854.1	3420.3	1584.2	1.85	8.98	6.42
S3	41.32	1.14	197.4	3201.6	1711.3	920.11	1.12	40.6	6.44
S4	45.31	0.61	112.4	3017.6	1325.4	617.31	5.11	40.1	6.41
S5	40.28	0.36	395.1	2421.2	1668.2	826.12	4.21	90.7	6.41
S6	39.68	0.31	209.3	3750.4	739.81	202.11	4.11	2.51	12.8
S7	41.11	0.94	192.6	2692.8	3553.6	1820.2	1.92	9.62	7.68
S8	40.11	0.72	109.7	3252.3	1223.4	616.23	1.25	10.2	6.41
S9	42.51	0.44	222.1	2954.1	1428.3	512.42	1.15	7.65	7.41
S10	45.22	0.65	147.2	3214.2	2358.7	985.61	2.11	9.65	6.41
S11	40.55	0.32	154.7	1988.2	2012.2	1111.2	3.22	4.21	6.41
S12	41.25	0.62	241.7	2845.6	1325.2	625.32	2.22	5.21	7.68
S13	42.58	0.81	165.3	3428.6	3720.1	1860.2	2.12	13.9	6.41
S14	41.88	0.72	504.2	3780.9	2689.3	1344.5	2.11	16.1	6.41
S15	38.85	1.12	634.2	2187.1	235.51	100.21	1.17	4.62	7.41
S16	40.09	0.64	197.5	2861.1	1580.4	780.11	4.71	26.2	6.41
S17	42.61	0.52	144.6	1120.1	1115.4	626.21	1.07	12.2	6.41
S18	41.25	0.62	241.7	2845.6	1325.2	625.32	2.22	5.21	7.68
S19	43.52	0.81	283.1	1817.8	889.72	428.13	0.92	7.41	6.41
S20	40.12	0.31	354.2	2587.1	925.44	421.31	1.15	5.22	7.68
S21	40.25	0.22	428.7	3241.1	452.81	198.24	2.25	4.22	6.41
S22	41.22	0.32	444.1	1985.2	1369.2	624.54	1.11	8.99	6.41
S23	40.08	0.41	847.5	2477.7	2151.2	1111.8	2.25	10.4	6.41
S24	40.11	0.24	725.3	3547.2	1487.3	541.23	1.47	11.2	6.41

Samples S21 and S6 showed lower methanol levels 0.22–0.31% v/v respectively, likely reflecting de-seeding practices or milder fermentation conditions. These findings support the observation that apple brandies generally maintain a safer methanol concentration (Zhang et al., 2012). Fusel alcohols are crucial for mouthfeel and aromatic complexity, but excessive concentrations can impair sensory quality (Lachenmeier et al., 2008; Hazelwood et al., 2008). Samples such as S1 and S14 recorded exceptionally high levels (>3700 mg/L), indicating vigorous amino acid metabolism and uncontrolled distillation conditions. In contrast, sample S17 showed a significantly lower concentration (1120.1 mg/L), indicating a more controlled fermentation and improved distillation refinement. Quince samples (S19-S21) showed intermediate values for methanol, which consist of moderate metabolic activity and balanced fermentation profiles. Esters, especially ethyl acetate, are vital factors contributing to the fruity and floral aroma of brandies (Coldea et al., 2014). Sample S13 showed exceptionally high ester content (3720.1 mg/L) along with elevated ethyl acetate (1860.2 mg/L), indicating robust esterification processes and favorable enzymatic activity. In contrast, S21 showed minimal ester presence (452.8 mg/L) and low ethyl acetate (198.2 mg/L), reflecting the more neutral aromatic profile typical of fruits such as quince (Risticevic et al., 2001; Znang, 2019). Elevated levels of aldehydes, especially acetaldehyde, can result in harsh sensory attributes. The highest concentrations were found in S23 and S24 847.5–725.3 mg/L respectively, exceeding the usual sensory thresholds. In contrast, sample S4 and sample S8 contained significantly improved oxidative control and optimized distillation protocols. The formation of furfural, often associated with thermal degradation of sugars, serves as an indicator of the intensity of the distillation. Sample S5 showed the highest concentration of furfural (90.7 mg/L), indicating excessive thermal load during distillation. Conversely, samples S6 and S11 had minimal levels 2.51–4.22 mg/L respectively, indicating efficient temperature management during the distillation process. Dry extract reflects the concentration of non-volatile residual solids, including organic acids, sugars and polyphenols. Notably, sample S4 and S16 displayed high values (>4.7 g/L), possible due to partial carryover of non-volatile components or aging in oak barrels. In contrast, samples S19 and S23 had low extract levels (<1.2 g/L), suggesting cleaner distillation and minimal matrix interference. Sulfur dioxide concentrations remained within acceptable enological limits in all samples. The highest value was recorded in sample S6 (12.8 mg/L), while most other samples, such S1, S4 and S10, ranged between 6.4 and 7.6 mg/L. These values indicate minimal preservative use, consistent with traditional homemade production practices.

From the obtained results for the basic chemical analysis of fruit brandy samples, it could be concluded that the type of fruit, together with the accompanying fermentation and distillation technique, had a major impact on the chemical safety and sensory attributes of homemade fruit brandies. Stone fruits (plum and apricot) produce brandies with greater aromatic complexity, although accompanied by an increased risk of methanol and high alcohol (Zhao et al., 2014). In contrast, pome fruits (quince and apple) yield more natural and chemically stable distillates, sometimes at the expense of higher aldehyde content. These findings highlight the importance of raw materias selection, fermentation management and precise distillation control in the production of high-quality brandy.

Free HCN analysis

In addition to the general chemical profiling of the fruit brandy samples, particular attention was given to the determination and interpretation of free HCN concentrations, due to its toxicological relevance and regulatory importance. The quantitative results obtained from spectrophotometric analysis are presented in Table 3, along with ethanol-corrected values and legal threshold calculation. To better visualize the comparison between observed values and safety thresholds, Figure 2 illustrates both the measured and corrected HCN concentrations, alongside two reference lines: a fixed EU regulatory limit of 70 mg/L and an individualized limit calculated for each sample based on its ethanol content. The presence of free HCN

in fruit brandies is of significant toxicological concern, especially in brandies produced from stone and seeds fruits known to contain cyanogenic glycosides such amygdalin (Velíšek & Cepjek, 2012). In the present study, HCN concentration was determined using a validated spectrophotometric method, and the results ranged from 1.11 to 9.81 mg/L in the final product (Table 3). To ensure regulatory, all measured values were recalculated to their equivalent per liter of absolute ethanol (100% v/v), in accordance with the EU Regulation 2019/787, which imposes a maximum permitted limit of 70 mg/L free HCN per liter of pure alcohol. The recalculated HCN values ranged between 2.70 and 24.1 mg/L (Figure 2), with none of the samples exceeding the regulatory threshold. Furthermore, sample-specific thresholds were calculated based on individual ethanol content, ranging from 27.20 to 36.34 mg/L, depending on alcohol strength, and none of the samples exceeded their respective thresholds. Samples with highest absolute HCN concentrations S22 (Apple, 9.81 mg/L), S15 (Apricot, 9.37 mg/L) and S23 (Apple, 8.11 mg/L) remained well below both standardized and ethanol-adjusted limits. These findings demonstrate that, despite the use of traditional, non-standardized fermentation and distillation processes, the analyzed brandies are toxicologically safe under current European regulations.

Fruit type was identified as a major factor influencing HCN concentration. Samples produced from apricot and apple (S13-S17 and S22-S24) exhibited consistently higher levels of HCN averaging above 7.5 mg/L. These fruit types are known for their high amygdalin content, particularly in seeds and stones (Balarinwa et al., 2014). In traditional brandy production, whole fruits are often fermented without seed removal, allowing enzymatic hydrolysis of cyanogenic glycosides, by endogenous β-glucosidases. In contrast, plum-based distillates (S1-S12) had markedly lower HCN values, mostly under 2.5 mg/L regardless of ethanol content or region. The lower values may be attribute to lower glycoside content in plum seeds, or to more frequent removal of pits before fermentation, a common practice in homemade distillates. Quince-based samples (S19-S21) showed intermediate values, consistent with their limited but present cyanogenic potential. This fruit-specific distribution is in line with previous studies, and confirms that raw material selection and seed treatment are primary contributes to cyanide risk in traditional fruit brandies

To better understand the technological and biochemical dynamics of cyanide formation, HCN levels were evaluated in relation to a set of basic chemical quality parameters. This includes comparison with the basic chemical parameters of the fruit brandy samples such as methanol, aldehydes, fusel alcohols, furfural, dry extract and SO2. Methanol, a by-product of pectin hydrolysis, shares a metabolic pathway with cyanide release and was found to moderately correlate with HCN levels. Fruits rich in pectin and processed with extended maceration may release both methanol and cyanogenic glycosides from seeds and skin of the fruit. Samples S23 (0.41% v/v) and S14 (0.72% v/v) (Table 2) also exhibited high HCN values 8.11 mg/L and 7.88 mg/L respectively (Table 3). This suggests that a processing method involving prolonged contact with the skin and seeds may induce the formation of both toxic alcohols and cyanide. An inverse relationship was generally observed between ethanol concentration and HCN levels. Samples with lower ethanol content (S15: 38.85% v/v) tended to have higher HCN concentrations (9.37 mg/L), indicating that uncontrolled distillation allowed toxic compounds from early distillation, such as HCN, to remain in the final product. Conversely, high ethanol concentrations often indicate better distillation control, leading to reduced HCN formation. This correlation highlights the role of distillation efficiency as a critical factor in limiting cyanide content. Total aldehydes, which reflect oxidative processes during fermentation and storage, also showed a significant relationship with HCN content. High aldehyde concentration were observed in several samples with elevated HCN levels (S23: 847.5 mg/L and 8.11 mg/L HCN), suggesting that oxidative stress and microbial imbalance during fermentation may promote both aldehyde formation and enzymatic hydrolysis of cyanogenic glucosides (Niedźwiedź-Siegień, 1998).

Table 3. Free HCN concentration, ethanol content and ethanol-corrected HCN values in homemade fruit brandies.

Sample	Free HCN mg/L	Ethanol % v/v	HCN mg/L 100% v/v alcohol	Legal Limit mg/L
S1	1.76	51.91	3.39	36.34
S2	1.14	42.25	2.70	29.58
S3	1.32	41.32	3.19	28.92
S4	1,57	45.31	3.47	31.72
S5	1,41	40.28	3.50	28.20
S6	2.21	39.68	3.57	27.78
S7	1.18	41.11	2.87	28.78
S8	1.45	40.11	3.62	28.08
S9	2.14	42.51	5.03	29.76
S10	1.84	45.22	4.07	31.65
S11	1.11	40.55	2.74	28.39
S12	1.12	41.25	2.71	28.88
S13	8.78	42.58	20.6	29.81
S14	7.88	41.88	18.8	29.32
S15	9.37	38.85	24.1	27.20
S16	7.88	40.09	19.6	28.06
S17	2.46	42.61	5.77	29.83
S18	1.12	41.25	2.71	28.88
S19	1.91	43.52	4.39	30.46
S20	2.01	40.12	5.01	28.08
S21	1.54	40.25	3.83	28.18
S22	9.81	41.22	23.8	28.85
S23	8.11	40.08	20.2	28.06

The table shows the measurement HCN concentrations (mg/L) the ethanol content (v/v %) and the HCN values recalculated to 100% alcohol for comparability. The legal limit for HCN was individually adjusted for each sample based on ethanol content using the formula (Limit = 70 mg/L × Ethanol (% v/v)/100 %.

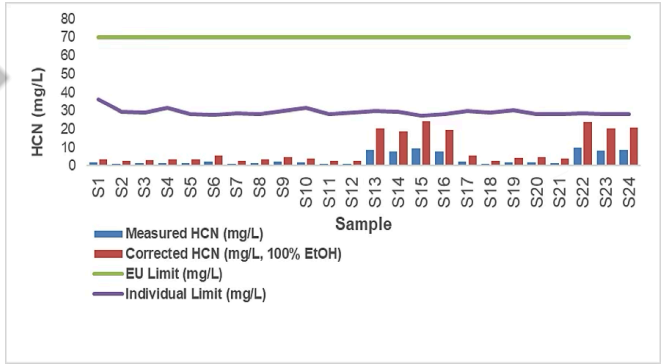

Figure 2. Measured and ethanol-corrected HCN concentration in 24 homemade fruit brandy samples. The green line indicates the EU regulatory limit (70 mg/L of Absolut ethanol), while the blue line represents individualized limits based on the ethanol content of each sample.

This indicate that oxidative fermentation conditions could be a co-factor in cyanide formation. The presence of fusel alcohols and total esters was more variable but occasionally consistent with elevated HCN levels. These compounds are often associate with fermentation intensity and microbial activity (Ough et al., 1998). For example, samples with intense esterification reactions, such S13 and S14, exhibited both high ester levels and increased cyanide. This could be due to enhanced enzymatic activity under vigorous fermentation, indirectly favoring the breakdown of cyanogenic precursors (Kósnáčová et al., 2009). High levels of ethyl acetate, a marker of fermentation stress and volatile acidity, were higher HCN concentration. Samples S13 and S23 not only had high content of ethyl acetate (1860.2 and 111.8 mg/L respectively), but also high HCN values. This indicated a fermentation imbalances or contamination, which may favor enzymatic release of cyanide. Furfural, derived from the decomposition of sugar under heat and the dry extract content representing non-volatile solids, did not show a strong statistical correlation with HCN levels. However, the elevated furfural in some samples with high HCN content suggests that the higher distillation temperature and use of direct fire may be associated with the increased retention or formation of volatile cyanides. This observation requires further controlled investigation.

A factor analysis was performed to investigate the underlying relationships between HCN levels and other chemical parameters in the brandy samples. This multivariate statistical method was employed to reduce the dimensionality of the dataset and to identify latent structures (factors) that explain shared variability among the measured parameters. Based on the E-values and the Kaiser criterion (E-value >1), four factors were extracted. Together, these four factors explain over 85 % of the total variance in the dataset, indicating a strong and comprehensive representation of the chemical relationships among the variables. The results of the factor analisys are presented in Table 4.

Factor 1: This factor is moderately influenced by methanol (loading = 0.40) and ethanol (loading = 0.36), with a smaller contribution from fusel alcohols (loading = 0.25) (Table.4; Figure 3). These compounds are typically formed during alcoholic fermentation. Factor 1 reflects the general alcohol production potential of the row material and fermentation conditions. While HCN does not load significant here, this factor describes the baseline ethanol-methanol profile that may indirectly affect the behavior of cyanogenic precursors in fruit brandies.

Factor 2: This factor is strongly defined by ethanol (loading = 0.85), with supporting influence from fusel alcohols (loading = 0.34) and methanol (loading = 0.14) (Table 4; Figure 3). This factor likely reflects the distillation efficiency, particularly the effect of rectification and dilution on volatile compounds. A high ethanol score indicates well purified distillates, while the presence of fusel alcohols hints at retention of some fermentation volatiles. HCN shows no meaning loading here, suggesting it is less influenced by distillation strength than by precursor breakdown.

Factor 3: Factor 3 shows strong loadings for HCN (loadings = 0.65) and aldehydes (loadings = 0.70) (Figure 4). This is key factor for toxicological and technological concern. It suggests that HCN and aldehydes are co-related or co-formed possibly due to cyanogenic glucoside hydrolysis, thermal degradation of sugars or suboptimal fermentation and distillation. The strong coupling of HCN and aldehydes indicate that this factor represents risky bio product formation, relevant for both flavor deterioration and safety monitoring.

Factor 4: This factor has moderate positive loading for fusel alcohols (loadings = 0.41), negative loadings for HCN (loadings = -0.37) and aldehydes (loadings = - 0.33). This factor likely represents a counterbalance between favorable aroma active compounds and toxic by-products. Higher fusel alcohols are often associate with aromatic complexity, while negative contributions from HCN and aldehydes suggest that more aromatic distillates may also be cleaner in terms of toxic content. This factor may reflect quality perception and aromatic profile development.

Table 4. Factors loadings, Communalities, E-values and explained variance from the factor analisys of brandy samples parameters.

Parameter	F 1	F 2	F 3	F 4	Comm.
HCN (mg/L)	0.04	-0.29	0.65	-0.37	0.65
Ethanol (% v/v)	0.36	0.85	-0.16	-0.21	0.91
Methanol (% v/v)	0.40	0.14	-0.20	-0.15	0.25
Aldehydes (mg/L)	-0.20	-0.21	0.70	-0.33	0.68
Fusel Alcohols (mg/L)	0.25	0.34	0.37	0.41	0.48
Total Esters (mg/L)	0.99	0.08	0.05	0.03	1.00
Ethyl Acetate (mg/L)	0.99	-0.10	-0.04	-0.02	1.00
Furfural (mg/L)	0.11	0.05	-0.15	-0.10	0.05
Eigenvalue (E-value)	2.38	0.99	1.15	0.49	0.75
Explained Variance %	47.5	19.8	22.9	9.79	

F1-loading of Factor 1, F2-loading of Factor 2, F3-loading of Factor 3, F4-loading of Factor 4, E-Eingene value, Comm-Communality.

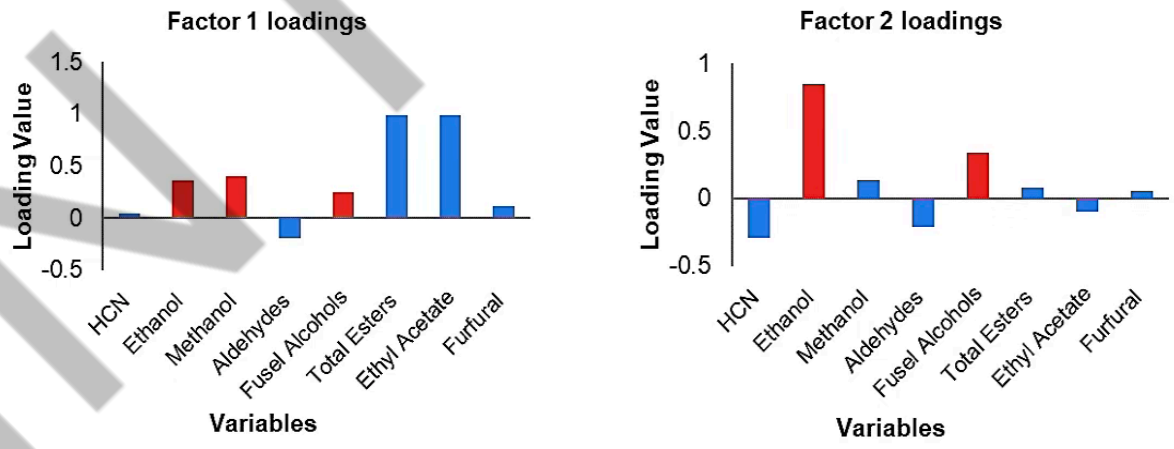

Figure 3. Variable loadings of Factor 1 and Factor 2.

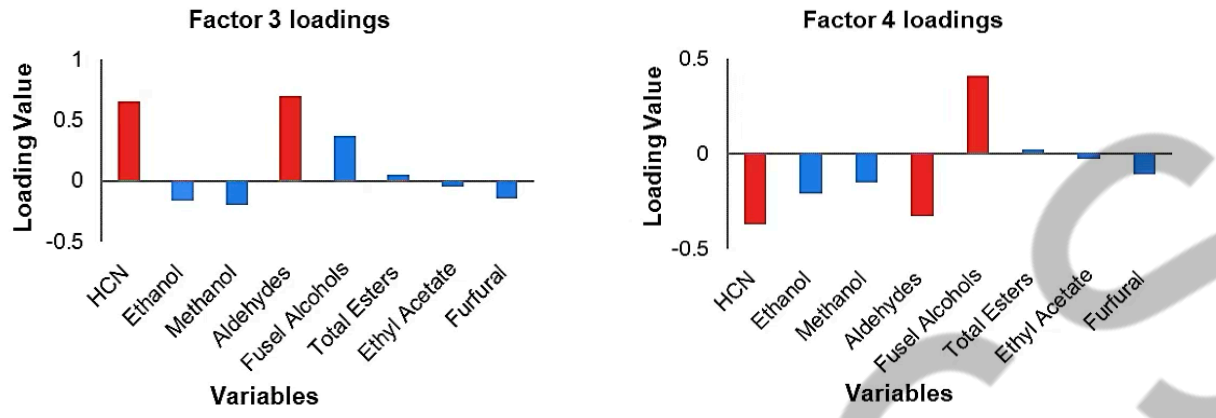

Figure 4. Variable loadings of Factor 3 and Factor 4.

Sulfur dioxide (SO_2) was not included in the factor analysis due to its very low concentrations in the analyzed samples and the absence of common practice for its intentional addition in traditional fruit brandy production. Furthermore, dry extract was excluded as it represents a non-volatile stable residue that is chemically and functionally distinct from the volatile and reactive compounds examined in this study. Its inclusion could have introduced statistical heterogeneity and reduced the interpretability of the extracted factors related to aroma, fermentation and toxicological parameters.

CONCLUDING REMARKS

The results of this study showed that all analyzed samples of homemade fruit brandies met the regulatory threshold for free hydrogen cyanide (HCN), as established by EU Regulation 2019/787 (70 mg/L absolute alcohol). Even in the samples with relatively high HCN content, especially those obtained from apricot and apple, the ethanol-corrected values were within the permitted limits, confirming the toxicological safety of the distillates. The obtained results showed that the type of fruit has a major influence on the free HCN content in fruit brandies. Apricot and apple brandies show a higher cyanogenic potential. This is attributed to the enzymatic degradation of cyanogenic glycosides present in the seeds and peel of the fruit, especially when the seeds were not removed before fermentation. In contrast, plum and quince brandies showed a lower cyanogenic potential. This was due to the practice of removing seeds from the fruit before fermentation.

Given the observed variability, the study highlights the need for routine analytical monitoring and targeted education of domestic producers. Particular emphasis should be placed on the impact of seed management, fermentation duration and distillation efficiency on HCN formation and its transfer to the final distillate. To ensure the safety and quality of traditional fruit spirits, it is recommended that small-scale producers be provided with access to accredited laboratories and receive practical training in safe and hygienic distillation techniques. These interventions are essential to protect public health and promote responsible production practices within the domestic distillation.

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ОПРЕДЕЛУВАЊЕ НА СЛОБОДНА ЦИЈАНОВОДОРОДНА КИСЕЛИНА ВО ДОМАШНИ ОВОШНИ РАКИИ

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Резиме

Ракијата од овошје прерставува традиционален алкхолен пијалак што широко се консумира во Република С. Македонија и другите балкански земји, а се произведува со дестилација на ферментирано овошје како слива, кајсија, дуња и јаболко, користејќи традиционални (домашни) или индустриски методи. Целта на ова истражување е да се процени безбедноста на 24 домашно произведени примероци на овошна ракија преку определување на содржината на слободна цијановодородна киселина (HCN), потенцијално токсично соединение. HCN се формира за време на алкохолната ферментација како резултат на ензимска хидролиза на цијаногените гликозиди кои природно се присутни во семките на овошјето. Определувањето на слободната HCN беше извршено користејќи ја Кениговата реакција, колориметриска метода базирана на формирање на цијаноген хлорид, кој реагира со пиридин и барбитурна киселина и формира стабилен розов комплекс со максимална апсорпција од 580 nm. Вредностите беа пресметани на основа на 100% v/v етанол за да се овозможи споредба со законскиот лимит на ЕУ од 70 mg/L. Највисока концентрации беа измерени во ракија од кајсија и јаболко (до 9,81 mg/L), додека оние од слива и дуња покажаа пониски нивоа на слободна HCN. Умерена корелација беше забележана помеѓу HCN и неколку хемиски параметри како што се метанол, алдехиди, етанол, вкупни естри, фурфурал и фузелни алкохоли. Резултатите укажуваат дека видот на овошје, условите на ферментацијата и степенот на контакт со семките при подготовката на овошната каша пред ферментацијата играат клучна улога во формирањето на слободна HCN.

Клучни зборови: *овошни ракии, слободна цијановодородна киселина, спектрофотометрија.*