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Chemical characterization and adulteration risk evaluation of commercially available sour pomegranate concentrates from different regions

Farklı bölgelerden temin edilen ticari ekşi nar konsantrelerinin kimyasal karakterizasyonu ve tağşiş riskinin değerlendirilmesi

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Abstract

Sour pomegranate concentrate is a traditional food product valued for its distinctive taste and potential health benefits. However, concerns about adulteration have necessitated detailed chemical characterization. This study analyzed the chemical composition of traditionally produced sour pomegranate concentrate samples from different regions. The samples were evaluated for °Brix, pH, titratable acidity, sugar content, amino acid profile, and Fourier Transform Infrared (FTIR) spectroscopy. The results revealed that most samples had a °Brix value between 70.46% and 74.36%, with corresponding glucose and fructose levels ranging from 2.32 to 2.99 g/L and 2.09 to 2.73 g/L, respectively. One sample exhibited an unusual °Brix value of 72.7% but had significantly lower glucose (0.64 g/L) and fructose (0.43 g/L) content, suggesting possible adulteration. FTIR spectra showed deviations in the carbohydrate fingerprint region, supporting this observation. Additionally, amino acid profiling revealed notable regional differences. The Suruç sample exhibited the highest total essential amino acid content (126.2 µM), including valine (18.7 µM), lysine (17.4 µM), and threonine (15.9 µM), while the Silifke sample had the lowest levels overall. These findings emphasize the importance of standardizing traditional production processes and implementing advanced analytical techniques to detect adulteration. Further studies incorporating a broader sample set and additional methods could enhance the detection of fraudulent practices and contribute to the protection of traditional food products.

Keywords: Sour pomegranate Concentrate, Traditional production, Chemical analysis, FTIR, Adulteration detection

Özet

Ekşi nar konsantresi, kendine özgü tadı ve potansiyel sağlık yararları nedeniyle değerli bir geleneksel gıda ürünüdür. Ancak tağşişe yönelik artan endişeler, ayrıntılı kimyasal karakterizasyonu gerekli kılmıştır. Bu çalışmada, farklı bölgelerden geleneksel yöntemlerle üretilmiş ekşi nar konsantresi örneklerinin kimyasal bileşimi analiz edilmiştir. Örnekler; °Brix, pH, titrasyon asitliği, şeker içeriği, amino asit profili ve Fourier Dönüşümlü Kızılötesi (FTIR) spektroskopisi açısından değerlendirilmiştir. Sonuçlar, örneklerin °Brix değerlerinin %70.46 ile %74.36 arasında değiştiğini, glikoz ve fruktoz seviyelerinin ise sırasıyla 2.32–2.99 g/L ve 2.09–2.73 g/L aralığında olduğunu göstermiştir. Bir örnekte °Brix değeri yüksek olmasına rağmen (72.7%) şeker içeriği düşük bulunmuştur (glikoz: 0.64 g/L, fruktoz: 0.43 g/L); bu durum olası tağşişi işaret etmektedir. FTIR analizleri de karbonhidrat parmak izi bölgesinde sapmalar göstererek bu bulguyu desteklemiştir. Ayrıca, amino asit profillemesi örnekler arasında belirgin farklılıklar göstermiştir. Suruç örneği toplam esansiyel amino asit açısından en yüksek değere sahipti (126.2 µM) ve özellikle valin (18.7 µM), lizin (17.4 µM) ve treonin (15.9 µM) konsantrasyonları dikkat çekmiştir. Elde edilen bulgular, geleneksel üretim süreçlerinin standardizasyonu ve tağşişin tespitinde ileri analitik tekniklerin kullanımının önemini ortaya koymaktadır. Genişletilmiş örnek gruplarıyla yapılacak ileri çalışmalar, gıda sahteciliğinin önlenmesine ve geleneksel ürünlerin korunmasına katkı sağlayacaktır.

Anahtar kelimeler: Ekşi nar konsantresi, Geleneksel üretim, Kimyasal analiz, FTIR, Tağşiş tespiti

1 Introduction

Pomegranate (*Punica granatum* L.) has held significant cultural importance throughout history due to its high nutritional value and various health benefits. The anatomical structure of the fruit consists of different parts, including the flower, leaves, peel, arils, and seeds. Approximately half of the fruit's weight is composed of peel, while the remaining portion consists of seeds and arils. Pomegranate is rich in bioactive compounds, including vitamins, minerals, organic acids, polyphenols, and anthocyanins, which contribute to its strong antioxidant properties. The arils primarily contain water along with carbohydrates, pectin, and organic acids. Additionally, pomegranate arils are abundant in anthocyanins and flavonoids, which significantly enhance the fruit's antioxidant capacity. Pomegranate juice also contains tannin compounds, particularly gallic acid and punicalagin, which are notable bioactive components [1]. Research has shown that pomegranate exhibits higher antioxidant activity compared to many other fruits. Due to these properties, pomegranate stands out as a valuable fruit in both the nutrition and health fields [2, 3].

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Pomegranate, due to its rich bioactive compound content, stands out as a functional food and has found widespread applications in the food industry. Pomegranate-based products exhibit a wide variety, including pomegranate juice, pomegranate juice concentrate, pomegranate jam, pomegranate jelly, sour pomegranate concentrate, pomegranate vinegar, pomegranate wine, pomegranate tea, and pomegranate seed oil [1, 4]. Although variations in antioxidant activity can be observed depending on the production processes, pomegranate juice and sour pomegranate concentrate are particularly notable for their high antioxidant capacity [5-8]. Traditional methods of producing sour pomegranate concentrate, in particular, contribute to its rich nutritional value and strong antioxidant properties. Due to these characteristics, the inclusion of sour pomegranate concentrate in a healthy diet is increasingly recommended [5].

Sour pomegranate concentrate is a traditional product obtained by concentrating pomegranate juice (*Punica granatum* L.), widely used in Middle Eastern cuisine [9, 10]. This thick, sweet-and-sour syrup can be produced using either traditional or commercial methods. The traditional production process involves washing, crushing, pressing, boiling, cooling, filtering, and bottling steps without the use of any additives, resulting in a completely natural product. In contrast, commercial production involves additional processes such as pasteurization, enzyme addition, clarification, filtration, and evaporation. Furthermore, commercial products often contain additives such as glucose/fructose syrup, citric acid, antioxidants, colorants, and preservatives to enhance taste, texture, and shelf life [9-11].

Sour pomegranate concentrate is rich in minerals, phenolic compounds, and flavonoids, which contribute to its high antioxidant capacity and potential anti-diabetic effects [9, 12]. Studies have shown that sour pomegranate concentrate exhibits stronger antioxidant activity compared to fresh pomegranate juice. In particular, homemade sour pomegranate concentrate has been reported to generally have higher antioxidant activity than commercial products [12, 13]. Animal studies suggest that the consumption of sour pomegranate concentrate is associated with improved antioxidant status and enhanced immune functions [14]. Additionally, a study conducted on mice revealed that sour pomegranate concentrates reduced weight gain in various organs, lowered triglyceride levels, and inhibited lipid peroxidation [13].

The physicochemical properties of sour pomegranate concentrate can vary significantly depending on the production method [13]. Notable differences between commercial and traditional products are observed, particularly in parameters such as pH, soluble solid content, and viscosity. However, in some commercial products, the addition of inexpensive ingredients like glucose syrup to reduce costs negatively impacts the product's authenticity and nutritional value. Advanced analytical techniques such as spectroscopic (UV-Vis, ATR-FTIR), chromatographic (HPLC), and hyperspectral imaging methods have been widely used to detect such adulteration [15, 16]. In particular, FTIR analysis provides valuable insights into product purity and compositional differences by examining spectral shifts in the carbohydrate region [15, 17-19].

In recent years, cases of adulteration and fraud in commercially produced sour pomegranate concentrate have increased due to the use of sugar syrups, synthetic acids, and other additives. This has made it essential to determine the chemical composition and authentic characteristics of traditionally produced sour pomegranate concentrate. The literature includes various studies that examine the physical, chemical, and antimicrobial properties of sour pomegranate concentrate produced in different regions, as well as comparisons between traditional and commercial products [5, 7, 17, 18, 20-22]. Expanding research on the detection of adulteration is crucial for the standardization of traditional production processes. However, most studies have focused on the general composition, organic acid content, phenolic profile, and antioxidant capacity of sour pomegranate concentrate. While there are studies on determining the protein content of sour pomegranate concentrate [9, 11, 14, 23], no detailed research has been found specifically analyzing its amino acid composition. Existing research primarily focuses on the amino acid profile of pomegranate juice [24-27]. Determining the amino acid composition of traditionally produced sour pomegranate concentrate not only contributes to understanding its nutritional value but also helps reveal compositional differences based on geographical variations. The findings obtained in this context are of great importance for both the standardization of traditional production processes and the protection of consumers.

Although there are various studies in the literature examining the chemical characteristics of pomegranate juice and pomegranate syrup, the present research distinguishes itself by conducting a comprehensive comparative analysis of commercially available products marketed as traditional sour pomegranate concentrate from different geographical regions, namely Siverek, Suruç, and Silifke. Unlike previous studies that primarily focus on samples produced under controlled laboratory conditions, this study evaluates real-market products, thereby reflecting the actual variability and potential risks associated with traditional production practices.

Importantly, this study integrates multiple analytical approaches, including FTIR spectroscopy and amino acid profiling, to assess both compositional differences and possible adulteration. The use of FTIR spectroscopy enabled the identification of spectral shifts indicative of ingredient modification, while the amino acid profile, particularly the levels of asparagine and glutamic acid, provided insight into the extent of thermal processing and the likelihood of Maillard reaction intermediates.

Moreover, since the products were not manufactured under the direct supervision of the researchers, the study serves as a market-oriented investigation into the authenticity and quality parameters of traditional sour pomegranate concentrates. This approach offers practical implications for quality control, consumer protection, and the traceability of regional food products.

The aim of this study is to determine the chemical composition of traditionally produced sour pomegranate concentrate, identify chemical differences among samples obtained from different production regions, and detect possible adulterations. The study includes analyses based on °Brix, pH, titratable acidity, sugar content, amino acid profile, and FTIR spectroscopy data of sour pomegranate concentrate samples.

2 Material and methods

2.1 Sour pomegranate concentrate samples

The sour pomegranate concentrate samples used in this study were obtained between May 5–16, 2024. The samples from Siverek and Suruç were sourced from local producers who reported using traditional methods, typically involving the concentration of sour pomegranate juice through prolonged boiling without added sugars or thickeners. The sample from Silifke was purchased from a boutique shop selling artisanal food products, and although labeled as "natural sour pomegranate concentrate," it was not produced under controlled conditions.

In this study, the term *sour pomegranate concentrate* refers to traditional pomegranate syrup produced solely by thermal evaporation of sour pomegranate juice. However, chemical composition and FTIR analysis revealed that the Silifke sample may have undergone adulteration or modification with added substances, potentially resembling a molasses-like product. All samples were stored in glass bottles under refrigerated conditions until analysis.

2.2 Determination of pH, titratable acidity, and Brix

The pH measurements of the sour pomegranate concentrate samples were performed using a benchtop pH meter (Isolab, Germany), which was calibrated daily using standard buffer solutions at pH 4.00 and 7.00. Measurements were carried out at room temperature (20 ± 2 °C), and results were recorded after stabilization of the electrode reading.

The soluble solid content (°Brix) of each sample was determined using a digital refractometer (Isolab, Germany) with an accuracy of ±0.1 °Brix. Prior to measurement, the refractometer was calibrated with distilled water. A few drops of the homogenized sample were placed on the prism, and the measurement was taken at 20 °C.

For titratable acidity analysis, the method described by Cemeroğlu [28] was followed. Samples were diluted at a ratio of 1:2 (w/w) with distilled water. After the addition of 3 drops of phenolphthalein indicator, titration was performed using 0.1 N NaOH solution until a light pink endpoint persisted for at least 30 seconds. The amount of NaOH used was recorded, and titratable acidity was calculated as citric acid equivalent using Equation (1):

$$\text{Titratable acidity, \% citric acid} = \frac{V \times f \times E}{M} \times 100 \quad (1)$$

V: Volume of 0.1 N NaOH used (mL),
f: Normality factor of the NaOH used in the solution,
E: Equivalent acid content per mL of 0.1 N NaOH (0.0064 g),
M: Volume or mass of the titrated sample (mL and g).

2.3 Sugar analysis

The samples were diluted with distilled water to achieve appropriate concentrations and homogenized using a vortex mixer for 1 minute. The prepared solutions were filtered through a 0.45 µm PTFE membrane filter and injected into the HPLC system for analysis.

The analyses were performed using a CARBOSep CHO-682 carbohydrate column (300 × 7.8 mm, Transgenomic, USA) and a refractive index (RI) detector (Shimadzu LC RID-10A, Japan). Ultra-pure water was used as the mobile phase, and the analysis was conducted under isocratic elution conditions with a flow rate of 0.4 mL/min. The column temperature was maintained at 80°C.

The identification of sugar components was carried out by comparing the retention times of the sample peaks with those of prepared standard sugar solutions.

2.4 Amino acid profile analysis

The amino acid composition of sour pomegranate concentrate samples was determined using liquid chromatography–tandem mass spectrometry (LC-MS/MS). For this purpose, the samples were first diluted in distilled water and homogenized. Proteins were precipitated by adding a solution of methanol: water (80:20, v/v), followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant was filtered through a 0.22 µm PTFE membrane filter prior to injection.

Chromatographic separation was carried out on a C18 reverse-phase column (2.1 × 100 mm, 1.7 µm particle size) using gradient elution. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), at a flow rate of 0.3 mL/min. Detection and quantification were performed in multiple reaction monitoring (MRM) mode with positive electrospray ionization (ESI+). The identification of amino acids was based on the comparison of retention times and mass transitions with those of analytical standards. Quantification was carried out using external calibration curves generated with standard solutions of amino acids. Results were expressed in micromolar (µM) concentrations. This method was adapted and optimized based on previous studies conducted on fruit-based matrices such as pomegranate juice and molasses [25,26].

2.5 Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy (IRTracer-100, Shimadzu Corporation, Kyoto, Japan) was used to characterize the structural bonds of the samples. Spectral analyses were conducted in the 500–4000 cm^{-1} wavelength range with a 1 cm^{-1} spectral resolution.

2.6 Statistical analysis

All experimental analyses were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). The normality of the data distribution was verified using the Shapiro–Wilk test. One-way analysis of variance (ANOVA) was employed to determine statistically significant differences between the sour pomegranate concentrate samples from different regions. When ANOVA indicated significance ($p < 0.05$), mean separation was

performed using Tukey's Honest Significant Difference (Tukey HSD) test.

Statistical analyses were conducted using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). In the tables, different lowercase letters (e.g., a, b, c) next to the means indicate statistically significant differences ($p < 0.05$) among the samples within the same row.

3 Results and discussions

The results of pH, titratable acidity, °Brix, glucose, and fructose content are presented in Table 1 as mean ± SD values based on three replicates. One-way ANOVA followed by Tukey HSD test revealed that the differences in titratable acidity and sugar content among the samples were statistically significant ($p < 0.05$). Particularly, the Silifke sample showed significantly lower glucose and fructose levels compared to the others, while no significant difference was observed in °Brix values.

Table 1. The average pH, titratable acidity (% citric acid), °Brix (%), glucose, and fructose values of the three sour pomegranate concentrate samples

Sample name	pH	Titratable acidity (% citric acid)	°Brix (%)	Glucose (g/L)	Fructose (g/L)
Siverek	3.33±0.02^a	7.41±0.1^a	74.36±0.18^a	2.99±0.05^a	2.73±0.04^a
Suruç	3.47±0.03^a	7.20±0.12^a	70.46±0.23^a	2.32±0.03^b	2.09±0.02^b
Silifke	3.11±0.01^b	5.32±0.14^b	72.7±0.21^a	0.64±0.02^c	0.43±0.01^c

Note: Values are given as mean ± standard deviation (n = 3). Different superscript letters in the same column indicate statistically significant differences ($p < 0.05$, ANOVA and Tukey HSD).

3.1 pH and titratable acidity

The acidic characteristics of sour pomegranate concentrate are primarily attributed to the natural organic acids present in the fruit, as reflected by its pH and titratable acidity values. In this study, the average pH of the analyzed samples was found to be 3.30 ± 0.21, while the total titratable acidity was 6.64 ± 1.04%, expressed as citric acid equivalent.

The pH and titratable acidity of sour pomegranate concentrate are important indicators of its chemical quality. Previous studies on both commercial and homemade products emphasize the importance of these parameters in defining the product's acidic profile. These properties may also vary depending on production methods and the geographical origin of the concentrate.

Yilmaz et al. [9] reported the pH of commercial sour pomegranate concentrate as 1.74 ± 0.09. Similarly, Vardin et al. [18] analyzed seventeen commercial pomegranate juice concentrates from various regions in Türkiye (Şanlıurfa, Gaziantep, Hatay, and Adana), finding pH values between 1.34 and 2.78, and titratable acidity values ranging from 6.11% to 14.27%. In the same study, laboratory-produced samples from Şanlıurfa had pH values between 2.1 and 2.9, with titratable acidity ranging from 5.8% to 13.3%. İncedayı et al. [23] reported pH values of commercial samples between 0.87 and 1.98, and titratable acidity values between 5.76% and 9.83%. Similarly, İncedayı [5] found pH values of 1.69 and 2.83 in two different commercial samples, with corresponding acidity values of 10.25% and 7.14%, respectively.

Orak [7] analyzed a traditional pomegranate juice concentrate from Denizli (Aegean region, Türkiye) and reported a pH of 3.02 and a total organic acid content of 3.46%. Similarly, Karabıyıklı and Kışla [29] evaluated traditional sour pomegranate concentrate from Aydın, with pH values ranging from 2.51 to 2.64 and titratable acidity levels between 12.6% and 18%. Akpınar-Bayizit et al. [21] examined commercial samples obtained from local markets in Bursa and found pH values between 1.71 and 2.96. The total acidity, expressed as citric acid, ranged from 4.70 to 9.73 g/100 g.

Turkmen et al. [22] analyzed homemade pomegranate concentrates from Kilis (Eastern Mediterranean, Türkiye) and reported a pH of 3.51 ± 0.00 and a titratable acidity of 13.19 ± 0.12%. In contrast, commercial samples showed a lower pH of 3.06 ± 0.05 and a titratable acidity of 3.58 ± 0.02%. Hepsağ et al. [30] evaluated six traditionally produced homemade sour pomegranate concentrate samples from Osmaniye and nearby regions, finding pH values between 2.93 and 2.99 (mean: 2.96) and titratable acidity values ranging from 5.27% to 8.03%. Similarly, Oğuz et al. [20] reported that commercial sour pomegranate concentrate samples had pH values between 2.00 and 2.88, with titratable acidity ranging from 2.91% to 8.60%. In a laboratory-produced control sample from Gümüşhane, the pH was 3.03 ± 0.06, and titratable acidity was 8.75 ± 0.06%.

According to the quality criteria established by the Turkish Standards Institute (TS 12720) [31], the pH of sour pomegranate concentrate should range between 2.4 and 4.0, and its titratable acidity must be at least 6% (expressed as citric acid equivalent). In this study, the pH values of the analyzed sour pomegranate concentrate samples were found to be within the standard range. However, the Silifke sample had a titratable acidity of 5.32%, which falls below the minimum required value set by the standard. The apparent inconsistency between the pH and titratable acidity values in the Silifke sample may be attributed to the fact that there is no direct correlation between pH and total acidity. While pH reflects the concentration of free hydrogen ions (H^+), titratable acidity encompasses both free and bound organic acids. In the Silifke sample, although the pH was low, the total acidity remained relatively limited. This could be explained by the types of organic acids present, the buffering capacity of the sample, or the possible addition of acidulants (e.g., citric acid). Similar cases of low pH combined with low titratable acidity have been reported in the literature, particularly in commercial products with added components [18,15].

These findings indicate that the chemical properties of sour pomegranate concentrate can vary significantly depending on the production region and production method. The impact of traditional (homemade) versus commercial production techniques on acidity levels is particularly evident. In general, products produced using traditional methods tend to exhibit higher titratable acidity values, likely due to the absence of dilution or additive agents, compared to their commercially produced counterparts.

3.2 Soluble solid content (°Brix)

The Brix value, which indicates the total soluble solid content, is commonly used as a quality parameter for various agricultural products [20]. In this study, the average Brix value of the analyzed sour pomegranate concentrate samples was 72.51 ± 3.57, with relatively small variations among samples. This finding is consistent with the literature, which reports that the soluble solid content of sour pomegranate concentrate may vary depending on production region and processing methods.

Vardin et al. [18] analyzed seventeen commercial pomegranate juice concentrates collected from different regions of Türkiye (Şanlıurfa, Gaziantep, Hatay, and Adana) and reported soluble solid content ranging from 50.1% to 77.3%. In the same study, laboratory-produced samples had soluble solid content values between 60.6% and 70.2%. Similarly, Zor et al. [17] examined twenty-seven commercial and homemade sour pomegranate concentrate samples collected from three different regions of Türkiye (Ağrı, Gaziantep, and Hatay) and found total soluble solid content (°Brix) ranging from 60.60 to 76.20. İncedayi et al. [23] conducted a study on seven commercial sour pomegranate concentrate samples, reporting soluble solid content values ranging from 58.25 to 74.50 g/100 g. Likewise, İncedayi [5] determined the Brix values of two commercial sour pomegranate concentrate samples as 75.17 ± 0.33 and 73.8 ± 0.06, respectively.

Akpınar-Bayizit et al. [21] analyzed commercial sour pomegranate concentrate samples from local markets in Bursa and reported total soluble solid contents ranging from 62.40 to 75.00 g/100 g. Orak [7] examined a traditional pomegranate juice concentrate from Denizli and found a total soluble solid content of 61.24%. In a study by Türkmen et al. [22], homemade pomegranate concentrates from Kilis (Eastern Mediterranean, Türkiye) had a Brix value of 75.53 ± 0.35, while commercial samples exhibited a slightly higher value of 78.20 ± 1.04.

Hepsağ et al. [30] analyzed six homemade sour pomegranate concentrate samples produced using traditional methods in Osmaniye and nearby regions, reporting Brix values ranging from 58.0% to 69.5%, with an average of 63.41%. In a study by Özmert Ergin [10], the Brix values of three commercially produced samples from Kahramanmaraş, Antalya, and Burdur ranged from 69.90% to 86.23%. In contrast, three traditionally produced samples obtained from a local supermarket in Burdur had Brix values between 81.30% and 84.13%. Yilmaz et al. [9] reported a Brix value of 73.90 ± 2.30 for commercial sour pomegranate concentrate. Similarly, Oğuz et al. [20] analyzed fifteen commercial samples and found Brix values ranging from 59.20% to 75.70%. In their laboratory-produced control sample, the Brix value was measured as 72.33 ± 0.10.

Considering the TS 12720 standard [31], which stipulates that the soluble solid content of sour pomegranate concentrate must be at least 68%, it is evident that all analyzed samples meet this quality criterion.

Studies in the literature indicate that the Brix values of sour pomegranate concentrate vary within a wide range. This variation can be attributed to factors such as the pomegranate variety used, geographical location, production method, and seasonal differences.

3.3 Glucose and fructose

The sugar composition of pomegranate juice is influenced by various factors, including geographical origin, genetic differences, processing methods, and the ripening stage of the fruit. Literature reports indicate substantial variation in sugar content among pomegranate varieties grown in different countries. For example, in several Turkish cultivars, sucrose levels have been reported as very low or even undetectable [32,33]. Similarly, pomegranate varieties from Saudi Arabia [34] and Iran [35] were found to contain no sucrose. In contrast, studies on Spanish pomegranate juices identified glucose as the dominant sugar, followed by fructose, maltose, and sucrose [36]. In Indian varieties, fructose was reported as the major sugar, followed by glucose and mannitol [37]. These findings highlight the diversity in sugar profiles across pomegranate varieties, with glucose and fructose consistently recognized as the principal sugars in pomegranate juice.

During the concentration of pomegranate juice, levels of reducing sugars such as glucose and fructose are known to increase significantly [7]. In the present study, the glucose, fructose, xylose, mannose, and sucrose contents of sour pomegranate concentrate samples from Siverek, Suruç, and Silifke were analyzed. However, sucrose, xylose, and mannose were not detected in any of the samples. This result suggests that the natural sugar profile of the sour pomegranate concentrate was maintained. Moreover, according to the TS 12720 standard [31], sour pomegranate concentrate should not contain sucrose, making this absence a key indicator of compliance with quality standards.

Based on the results, the Siverek sample exhibited the highest concentrations of glucose (2.99 g/L) and fructose (2.73 g/L), followed by the Suruç sample with lower levels of glucose (2.32 g/L) and fructose (2.09 g/L). In contrast, the Silifke sample contained the lowest sugar concentrations, with only 0.64 g/L of glucose and 0.43 g/L of fructose. When these values are compared with the corresponding Brix measurements, which represent total soluble solid content, a notable discrepancy is observed. The Siverek and Suruç samples demonstrated a consistent relationship between their sugar content and Brix values (74.36% and 70.46%, respectively). However, despite its significantly lower glucose and fructose levels, the Silifke sample showed a disproportionately high Brix value (72.7%).

In the Silifke sample, the significantly lower glucose and fructose levels, despite a relatively high °Brix value, may also be attributed to thermal degradation processes. Extended heat treatment during production could have triggered Maillard reactions or caramelization, leading to the transformation of reducing sugars into intermediate or final browning products such as furfural or hydroxymethylfurfural. This interpretation is further supported by FTIR findings. In particular, the carbohydrate fingerprint region showed a shift from 1029/1031 cm^{-1} to 1022 cm^{-1}, and a distinct peak at 918 cm^{-1} was observed, which may indicate pyranose ring disruption or formation of

new glycosidic structures. The broadening of the C=O stretching peak at 1710 cm^{-1} also aligns with the presence of Maillard reaction products. These spectroscopic patterns collectively suggest that the sugar loss in the Silifke sample may be linked to intense thermal processing rather than dilution alone.

In addition, 5-hydroxymethylfurfural (HMF) is a critical indicator of thermal degradation in high-sugar, low-pH fruit products such as pomegranate concentrate. Although HMF and furfural derivatives were not assessed in this study, their inclusion in future analyses would provide deeper insight into the progression of Maillard and caramelization reactions and support more comprehensive quality evaluations.

3.4 Amino acid profile

Amino acid profile analyses revealed that sour pomegranate concentrate samples produced in different geographical regions exhibit distinct compositions (Figure 1). These differences suggest that the nutritional value and potential health benefits of sour pomegranate concentrate may vary by region of origin. In the Siverek sample, higher levels of beta-aminoisobutyric acid, cystathionine, serotonin, histamine, and phosphoethanolamine were detected. The Suruç sample generally showed the highest overall amino acid concentrations, with alanine, arginine, asparagine, serine, glutamic acid, and aspartic acid being predominant. In contrast, the Silifke sample exhibited lower levels of several amino acids compared to the other two regions, although it contained higher concentrations of glutamine and glycine.

The essential amino acid profiles of sour pomegranate concentrate samples from Siverek, Suruç, and Silifke revealed notable regional differences, underscoring their potential nutritional value. Among the identified essential amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), the Suruç sample contained the highest concentrations, particularly of methionine, valine, threonine, and lysine. The Siverek sample exhibited moderate levels of essential amino acids, with relatively higher amounts of valine and threonine. In contrast, the Silifke sample showed the lowest levels of most essential amino acids, though it still provided a meaningful contribution to dietary intake. These findings suggest that sour pomegranate concentrate may serve as a functional food component, supplying essential nutrients important for protein synthesis, immune function, and metabolic health.

Although essential amino acids are not directly involved in flavonoid biosynthesis, they participate in metabolic pathways that affect flavonoid stability and production. For example, phenylalanine and its derivative tyrosine (a non-essential amino acid) serve as precursors in flavonoid synthesis [38]. Additionally, hydrophilic amino acids such as lysine have a high binding affinity for flavonoid compounds, forming stable complexes that may influence their bioavailability [39]. Previous studies have shown that the amino acid content of plant-based products is influenced by environmental factors, cultivar differences, and processing techniques. Since sour pomegranate concentrate is produced through water evaporation, it typically exhibits higher amino acid concentrations compared to fresh pomegranate juice.

To date, no specific study has been found on the amino acid profile of sour pomegranate concentrate; however, several studies have investigated the amino acid composition of pomegranate juice and fruit. For example, Abdygapparova et al. [24] analyzed the amino acid profiles of Ashyk-anar and Shah-Nar pomegranate varieties grown in the South Kazakhstan region. The study reported that the juice of the Ashyk-anar variety contained 17 amino acids, including 8 essential ones. Glutamic acid and aspartic acid were identified as the dominant amino acids, while phenylalanine levels ranged from 0.013% to 0.015%.

In a study conducted by Li et al. [26], the juices of six pomegranate varieties obtained from two different regions in China (Shandong and Xinjiang) were analyzed. The findings revealed that glutamine, serine, aspartate, and alanine were the most abundant amino acids in these samples. Additionally, the study reported that the total aromatic amino acid content varied based on regional differences, with pomegranate juice from the Shandong region exhibiting a higher total amino acid content compared to that from Xinjiang.

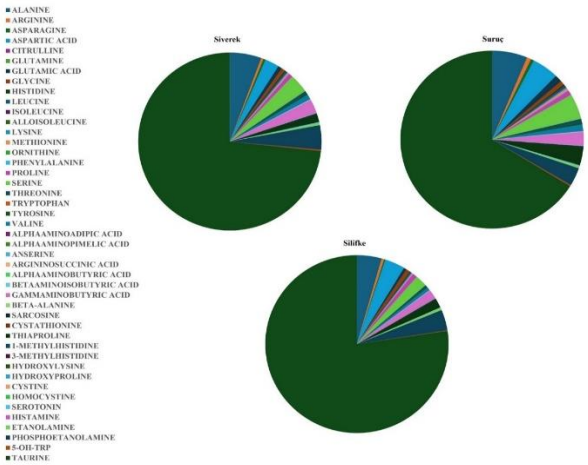

Figure 1. Average amino acid values (µM) of three different sour pomegranate concentrate varieties

Villa-Ruano et al. [27] conducted a ^1H NMR-based metabolomic study comparing organically and conventionally grown pomegranates. The results showed that organically grown pomegranates had higher levels of arginine, alanine, glutamine, methionine, phenylalanine, and proline, while conventionally grown ones contained more aspartic acid.

A study on commercial pomegranate varieties from Tunisia and China [40] analyzed their amino acid profiles and found that the seeds of both varieties were rich in storage proteins. The amino acid composition was independent of the flavor characteristics of the fruit. Glutamine, serine, and asparagine were particularly abundant in the seeds. These results indicate that pomegranate can be considered not only as a fruit but also as a valuable source of dietary protein.

Additionally, in a study conducted by Tezcan et al. [25], the amino acid profile of pomegranate juice was analyzed in detail. The findings revealed that serine, proline, and alanine were the dominant amino acids present in pomegranate juice.

The elevated taurine levels observed in sour pomegranate concentrate samples from Siverek, Suruç, and Silifke may be influenced by a combination of environmental and biochemical factors. Taurine (2-aminoethanesulfonic acid) is a sulfur-containing amino acid commonly found in animal-derived foods but can also accumulate in plants and microorganisms in response to osmotic stress, oxidative defense, and secondary metabolism [41,42]. Studies have shown that high salinity can stimulate taurine synthesis, particularly in microalgae such as Tetraselmis sp., suggesting that pomegranate trees in these regions may increase taurine production as an adaptive response to saline soils or drought conditions [43].

As a sulfonic acid, taurine biosynthesis is closely linked to sulfur metabolism, particularly through pathways involving sulfur-containing amino acids like cysteine and methionine [41,44]. Thus, variations in soil sulfur content may enhance taurine accumulation in pomegranate fruit. Taurine also plays a protective role against oxidative stress and helps regulate metabolic responses under environmental pressures [45]. Given the harsh climatic conditions in Siverek, Suruç, and Silifke, characterized by drought, high temperatures, intense UV radiation, and mineral-rich soils, pomegranate plants may upregulate taurine synthesis as part of their stress-response mechanisms.

Additionally, the traditional concentration process used in sour pomegranate production, which involves extended heating, may influence taurine levels. While many amino acids degrade with prolonged heat exposure, taurine is known to be thermally stable [46], potentially allowing it to accumulate at higher concentrations. Genetic variability among pomegranate cultivars may also contribute to differences in taurine levels, as certain genotypes may exhibit enhanced metabolic resilience under stress [47]. In summary, the high taurine content in sour pomegranate concentrate likely results from the interplay of plant physiology, environmental stressors, and processing methods.

In light of these findings, the amino acid profile analyses of sour pomegranate concentrate reveal both similarities and differences compared to the existing literature on pomegranate juice and seed composition. Further studies examining environmental influences and varietal differences would provide deeper insights into the factors contributing to these variations. Notably, sour pomegranate concentrate from the Suruç region appears to have a more nutritionally rich profile due to its higher amino acid content. Additionally, the presence of neurotransmitter-related amino acids (e.g., taurine, gamma-aminobutyric acid (GABA), and serotonin) suggests that sour pomegranate concentrate may offer potential neurological health benefits. Overall, these findings provide valuable insights into both the nutritional value and the potential functional food properties of sour pomegranate concentrate.

3.5 FTIR

FTIR analysis is a crucial method for determining the chemical composition of food products. Figure 2 presents the FTIR spectra of the sour pomegranate concentrate samples. In the Siverek, Suruç, and Silifke sour pomegranate concentrate samples, the FTIR peaks observed between 3310–3320 cm^{-1} are attributed to the stretching vibrations of hydroxyl (O–H) functional groups [48].

The FTIR peaks observed in the 2930–2940 cm^{-1} range across all samples correspond to C–H stretching vibrations of aromatic and aliphatic hydrocarbon structures. Additionally, due to the presence of carbohydrates, the C–H stretching vibrations identified between 3050–2800 cm^{-1} are specifically associated with CH and CH_2 groups [49]. These findings suggest that sour pomegranate concentrate possesses a diverse carbohydrate composition, comprising both simple and complex carbohydrates. In the case of carboxylic acids, the spectral region around 2900 cm^{-1} exhibits C–H stretching bands characteristic of $–CH_3$ (methyl) and $–CH_2$ (methylene) groups [50].

The FTIR peaks observed in the 1710–1715 cm^{-1} range correspond to C=O (carbonyl) stretching vibrations, typically indicating the presence of esters and other carbonyl-containing functional groups [15,17,51]. These compounds may originate from the organic acids naturally present in sour pomegranate concentrate or from thermal reactions, such as the Maillard reaction. Peaks in the 1640–1645 cm^{-1} region are attributed to C=C stretching vibrations of aromatic rings [52], but may also correspond to N–H bending vibrations in amides and amines. The Amide I band, located between 1600–1700 cm^{-1}, is particularly characteristic of C=O stretching and N–H bending in proteins [51,53], suggesting a link to the amino acid content of the samples. Furthermore, the Maillard reaction resulting from interactions between sugars and amino acids during heating may cause structural changes in proteins, leading to the appearance of specific FTIR peaks within this region.

Heating and concentration processes involved in sour pomegranate concentrate production promote the formation of carbonyl compounds. Non-enzymatic browning reactions, such as the Maillard reaction and caramelization, are

essential for the development of its characteristic color and aroma. FTIR peaks in the 1700–1600 cm^{-1} region correspond to carbonyl (C=O) stretching, conjugated double bonds (C=C), and imine (C=N) groups, reflecting compounds formed at various stages of these reactions [7, 54, 55]. Formation of melanoidins, Amadori compounds, and Schiff bases during these processes contributes to the dark color and enhances antioxidant properties of the concentrate [56, 57].

The FTIR peaks observed in the 1400–1200 cm^{-1} range can be attributed to C–O stretching, O–H bending, and C–H deformation vibrations, which are associated with carbohydrates, phenolic compounds, and organic acids [58-61].

Located between 950 and 1200 cm^{-1}, the so-called 'fingerprint region' for carbohydrates is important for identifying polysaccharide structures [62]. FTIR peaks at 1143 cm^{-1}, 1145 cm^{-1}, and 1147 cm^{-1} were observed in the Siverek, Suruç, and Silifke samples, respectively. These peaks correspond to C–H and C–O stretching vibrations, typically present in sugars like fructose and glucose. Peaks detected around 1029–1045 cm^{-1} are specifically associated with C–O stretching in glucose molecules [63].

The presence of a 918 cm^{-1} peak in the FTIR spectrum suggests its association with C–C stretching vibrations and C–H bending, which are characteristic of carbohydrate structures [48]. Additionally, the peaks observed in the 860–700 cm^{-1} range are related to the anomeric region of carbohydrates and may represent different ring deformations due to C–H bending [48, 64].

Comparison of FTIR spectra for sour pomegranate concentrate samples from Siverek, Suruç, and Silifke revealed that Siverek and Suruç shared similar spectral features, while the Silifke sample exhibited distinct differences. A broad absorption band between 3313–3319 cm^{-1} appeared in all samples, corresponding to hydroxyl (–OH) groups. These signals are likely related to water content, organic acids, and carbohydrates [48, 61]. Given that sour pomegranate concentrate is produced by thermal evaporation of juice, this broad band may also indicate the presence of bound water within the molasses-like matrix [60]. Entrapped water molecules could influence both the viscosity and the physical stability of the product. In the region around 1712–1710 cm^{-1}, carbonyl (C=O) stretching vibrations were observed, primarily associated with organic acids and esterified compounds. A slight shift toward 1710 cm^{-1} in the Silifke sample suggests possible differences in pH or in the composition of acidic constituents.

In the carbohydrate region (1022–1031 cm^{-1}), the Siverek and Suruç samples showed distinct peaks at 1029 cm^{-1} and 1031 cm^{-1}, respectively. In contrast, the Silifke sample exhibited a peak at 1022 cm^{-1}, which may suggest a structural change in carbohydrate content. Additionally, strong peaks were observed at 862, 813, and 771 cm^{-1} in the Siverek and Suruç samples, while these peaks were significantly weaker or absent in the Silifke sample. These peaks are linked to C–O–C glycosidic bonds and pyranose ring vibrations, commonly associated with natural polysaccharides like pectin [49]. Their absence in the Silifke sample, especially at 862, 813, and 771 cm^{-1}, may indicate dilution with sweeteners, acids, or other non-sugar components. Such changes could result from degradation or replacement of polysaccharide structures during processing or through the addition of different ingredients.

The distinct peak observed at 918 cm^{-1} in the Silifke sample was either very weak or entirely absent in the Siverek and Suruç samples. In infrared spectroscopy, the 900–950 cm^{-1} region is associated with C–O–C glycosidic bonds, pyranose ring vibrations, and β-glycosidic linkages [49]. One possible reason for the appearance of this peak in the Silifke sample could be the hydrolysis of polysaccharides and the breakdown of pectin. This suggests that structural modifications in carbohydrate composition may have occurred, possibly due to processing conditions or the addition of certain ingredients.

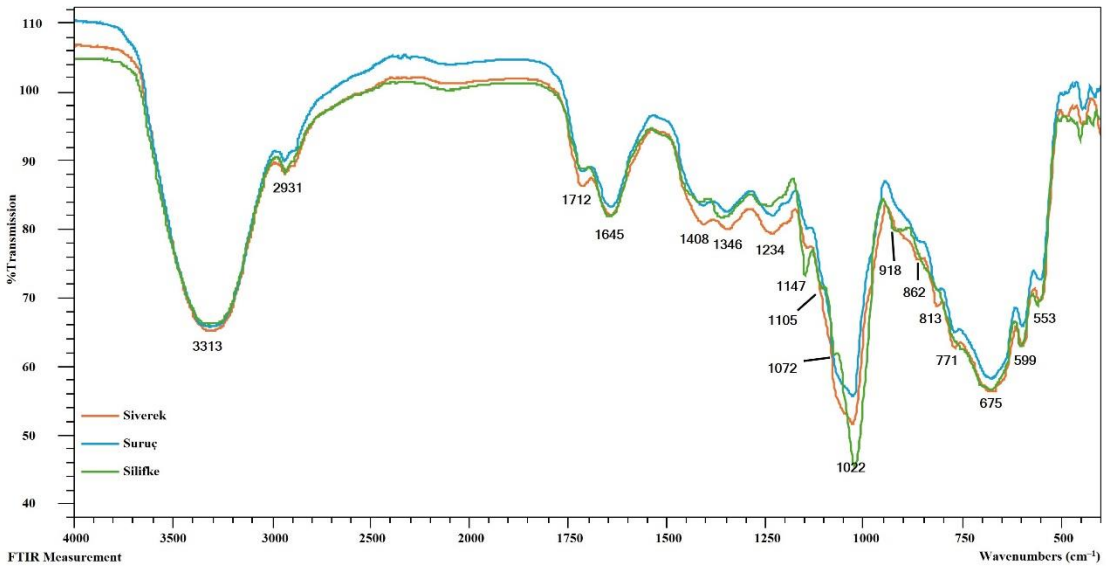

Figure 2. Comparative FTIR spectra of Siverek, Suruç, and Silifke sour pomegranate concentrate samples

Acid hydrolysis, potentially caused by acid addition, may degrade pectin into smaller oligosaccharides, leading to the appearance of a new FTIR peak around 918 cm^{-1}. The presence of this peak in the Silifke sample, but not in the Siverek or Suruç samples, suggests that another fruit juice or syrup may have been added. This modification could result in a distinct glycosidic spectral fingerprint, indicating a compositional deviation from traditionally produced sour pomegranate concentrate.

Additionally, the discrepancy between the high °Brix value and the low sugar content in the Silifke sample provides strong evidence of dilution or the addition of different components. When all these findings are considered, it can be concluded that the Siverek and Suruç samples exhibit the characteristics of natural sour pomegranate concentrate, whereas the Silifke (Mersin) sample may have been diluted, contained thickening agents, or undergone acidic modification.

Furthermore, the FTIR spectral characteristics of the Silifke sample suggest a more advanced stage of the Maillard reaction. Specifically, the broadened carbonyl (C=O) stretching band near 1710 cm^{-1} and the shift of the carbohydrate fingerprint region to 1022 cm^{-1}, along with the presence of a unique absorption band at 918 cm^{-1}, are indicative of sugar degradation and protein–sugar interactions. These spectral features, which were not observed in the Siverek and Suruç samples, imply that the Silifke concentrate may have been subjected to more intense thermal treatment, thereby promoting the formation of Maillard intermediates and final reaction products.

Despite showing the lowest reducing sugar content among the samples, the Silifke concentrate exhibited the highest °Brix value, which may indicate the use of non-reducing sugars or alternative carbohydrate sources. Although significant spectral differences were not observed in the 1700–1600 cm^{-1} region typically associated with Maillard reaction products, the shift observed in the carbohydrate fingerprint region (particularly at 1022 cm^{-1} and 918 cm^{-1}) suggests compositional modifications. These changes may not necessarily result from advanced Maillard products but rather from added carbohydrate-based thickeners or the degradation of sugars due to intense thermal processing. In contrast, the higher glucose and fructose levels detected in the Siverek and Suruç samples align with traditional production methods that preserve natural reducing sugars more effectively.

4 Conclusions

The chemical compositions of traditional sour pomegranate concentrate samples obtained from different regions were analyzed in detail. While the Siverek and Suruç samples exhibited similar chemical parameters, a notable discrepancy between sugar content and °Brix value was identified in the Silifke sample. FTIR spectroscopy analyses indicated potential compositional differences in the Silifke sample, suggesting the presence of additives. In particular, spectral shifts in the carbohydrate region pointed to the possible addition of sugar or the incorporation of different components. The discrepancy between the °Brix value and the reducing sugar content in the Silifke sample, along with the characteristic FTIR spectral shifts observed in the carbohydrate region, strongly suggests the possible addition of non-reducing sugars or other carbohydrate-based additives. While direct evidence of sugar adulteration was not quantified, the analytical findings point toward compositional modifications inconsistent with traditional sour pomegranate concentrate. These findings contribute to the understanding of quality parameters and potential adulteration risks in the production of traditional sour pomegranate concentrate. The use of advanced analytical techniques for detecting adulteration and fraud is crucial for protecting consumer rights and ensuring product safety. Future studies should incorporate broader chemical and sensory analyses of samples from various geographical regions and different harvest years, contributing to the standardization of traditional production processes. Additionally, the inclusion of specific adulteration markers such as hydroxymethylfurfural (HMF) and the use of isotope or NMR-based techniques could further enhance authenticity assessments.

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Conflict of interest

The authors declare that there is no conflict of interest.

Similarity rate (iThenticate): 8%

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