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RESEARCH ARTICLE

Integrated Evaluation of NADES for Green Extraction of Bioactive Compounds from Strawberries: A Study on Anthocyanin, Antioxidant Activity, Chemometric FTIR Profiling

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Abstract

Strawberries (*Fragaria x ananassa*) are a rich source of anthocyanins and phenolic compounds with strong antioxidant properties. This study introduces an integrated approach combining quantitative analysis, including total anthocyanin content, total phenolic content, and antioxidant activity via DPPH assay, along with qualitative profiling using Fourier Transform Infrared (FTIR) spectroscopy and Principal Component Analysis (PCA). The aim was to thoroughly assess the extraction efficiency of 5 Natural Deep Eutectic Solvents (NADES), synthesized from choline chloride and various organic acids (citric, oxalic, malic, tartaric and lactic), in comparison to ethanol. Among these, NADES E (choline chloride:lactic acid) showed the highest extraction performance, yielding 125.57 ± 12.22 mg/100 g anthocyanins, 11,032.02 ± 785.13 mg GAE/g phenolics, and 89.15 ± 6.1% antioxidant activity. Interestingly, NADES A (choline chloride:citric acid) produced similar bioactive yields but exhibited the most intense FTIR absorbance in regions associated with phenolic functional groups (-OH, C=O and C=C), indicating a different compound affinity or preservation capacity. This suggests that while NADES E is superior for quantitative yield, NADES A might better retain specific chemical fingerprints. Moreover, PCA demonstrated clear separation of NADES A, C, and E from ethanol, highlighting the superior compound selectivity and profile retention of NADES. These findings highlight the importance of integrating multiple analytical techniques to evaluate both the efficiency and selectivity of green solvent systems for extracting bioactive compounds from plant matrices.

Keywords: Anthocyanins, Chemometric, FTIR, NADES, PCA

Introduction

Strawberry (*Fragaria x ananassa*) is a type of berry plant currently categorized as a "functional component" due to its high nutritional value, particularly its richness in phenolic compounds, flavonoids, vitamins, minerals, and dietary fiber [1]. This is evidenced by strawberries ranking No.9 on the list of the 100 richest food sources of phenols, providing 390 mg of total phenols per serving [2]. Strawberry extracts contribute positively to human health due to their phenolic content, with anthocyanins being the primary components responsible for anti-inflammatory, anticancer, antidiabetic, and anticarcinogenic activities [3]. Considering the high anthocyanin content in strawberries, but its sensitive and easily degradable nature, it is essential to discover effective extraction methods for their application in the food sector.

Anthocyanins are a type of secondary metabolite responsible for the red to purplish-blue coloration observed in various types of flowers, fruits, and vegetables [4]. Strawberries contain anthocyanins in the range of 200 to 800 mg/kg, making them compounds utilized as phytoalexins, anti-inflammatory agents, antioxidants, and other health-promoting components [5]. However, the anthocyanin content in strawberries is relatively lower compared to that of some other berries. For comparison, bilberries contain between 29,000 and 65,000 mg/kg of anthocyanins, blueberries range from 630 to 4,840 mg/kg, and raspberries contain between 300 and 500 mg/kg. These differences in anthocyanin levels are influenced by various factors such as species, cultivar, fruit size, climatic conditions, ripeness level, growing environment, and storage methods [2,6,7].

Anthocyanins have a major drawback due to their sensitive and unstable nature. These pigment compounds are highly prone to degradation caused by various factors such as pH changes, storage temperature, light exposure, the presence of oxygen, and other environmental conditions that can affect their stability [8]. Therefore, an appropriate approach is required to effectively extract anthocyanins from strawberries. One of the strategies that can be applied is the use of extraction methods that consider solvent efficiency, in order to maximize the optimal recovery of anthocyanins.

Anthocyanin extraction is commonly carried out using organic solvents such as methanol, ethanol, or other conventional organic solvents. However, the use of these solvents presents several drawbacks, including high volatility, significant toxicity, and negative environmental impacts [9]. With the growing emphasis on health, food safety, and the principles of green chemistry in the modern food industry, there is a pressing need for extraction methods that utilize low-toxicity, safe, and environmentally friendly solvents [10]. One of the eco-friendly solvent alternatives currently being developed for anthocyanin extraction is Natural Deep Eutectic Solvents (NADES).

Natural Deep Eutectic Solvents (NADES) are solutions formed by combining 2 or more components that act as hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD). The constituents of NADES are generally derived from primary metabolites such as amino acids, organic acids, glucose, alcohols, or urea, which are renewable, biodegradable, and exhibit low toxicity [11]. In addition to being environmentally friendly, NADES are easy to synthesize and demonstrate high solubility for various compounds due to their ability to form hydrogen bonds. The use of NADES in anthocyanin extraction has shown greater potential compared to conventional solvents. This is supported by the findings who reported that organic acid-based NADES possess high polarity, which can significantly enhance the efficiency of anthocyanin extraction [12].

In addition to the choice of solvent, the extraction method also plays a crucial role in determining the successful recovery of target compounds. One increasingly popular technique is Ultrasound-Assisted Extraction (UAE), which is known for accelerating the extraction process by utilizing high-frequency ultrasonic energy. The working principle of UAE is based on the cavitation effect with the formation and collapse of microbubbles within the solvent, which mechanically disrupts the structure of cell walls, thereby enhancing the interaction between the solvent and active compounds. The combination of UAE and NADES offers a superior approach compared to conventional extraction methods, as it significantly improves extraction efficiency while simultaneously reducing processing time.

The extraction method not only influences the quantity of the extract but also plays an important role in understanding the chemical characteristics of the extracted compounds. One approach used to identify the functional groups in extracted compounds is Fourier Transform Infrared Spectroscopy (FTIR). This method allows for the identification of the main functional groups in a compound based on specific bond vibrations. FTIR is useful for comparing the extraction results from different solvents, as it can reveal changes or differences in absorption intensity of functional groups such as -OH (hydroxyl), C=O (carbonyl), and C=C (aromatic), which are commonly found in phenolic and anthocyanin compounds. Thus, FTIR analysis provides a preliminary overview of the functional groups present in strawberry extracts derived from both conventional solvents and NADES [13].

However, FTIR spectra produce complex multivariate data with overlapping peaks, requiring advanced statistical techniques such as chemometric analysis using Principal Component Analysis (PCA). PCA is employed to simplify the variability in FTIR data and extract key information responsible for differences among samples. With PCA, chemical pattern variations between extracts from conventional solvents and NADES can be visualized more systematically and objectively in the form of a score plot that illustrates the relationships among principal components. This analysis not only helps distinguish extracts based on solvent type but also validates the extent to which NADES influence the chemical spectrum of strawberry extracts [14].

Previous studies have conducted strawberry extraction using NADES composed of 5 types of organic acid combinations: Citric acid, oxalic acid, malic acid, tartaric acid, and lactic acid. These studies demonstrated that strawberry extracts based on NADES produced higher yields in antioxidant activity, total anthocyanin, and total phenolic content compared to extracts using conventional solvents as control treatments Therefore, to gain deeper insight, further research was conducted using PCA chemometric analysis and the identification and quantification of anthocyanin compounds in strawberry extracts based on NADES and comparing them with extracts from conventional solvents.

This study aims to explore the metabolite profile of NADES based strawberry extracts. A deeper understanding of the bioactive compounds contained in strawberry extracts through chemometric approaches contributes significantly to enhancing food safety and health quality. This research may serve as a publication that applies chemometric methods to compare the metabolite profiles of strawberry extracts using NADES with those using conventional solvents.

Materials and methods

Materials

The material used in this study was strawberry fruit obtained from the Alahan Panjang region, Solok Regency, West Sumatra, Indonesia. The chemicals used in this research included Choline Chloride p.a (Choline Chloride, Hi-LR^{TM} 99%) obtained from HiMedia Laboratories Pvt. Ltd. (Thane, India), Citric Acid p.a (Citric acid anhydrous for synthesis 99%), Oxalic Acid p.a (Oxalic acid dihydrate for analysis 99%), Malic Acid p.a (Malic acid for biochemistry 99%), Tartaric Acid p.a (Tartaric acid for analysis 99%), Lactic Acid p.a (Lactic acid 90%), Acetic Acid (Acetic Acid Glacial 100%), Ethanol 96% p.a, Methanol p.a, Analytical Standard Anthocyanin Cyanidin 3-Glucoside, and Analytical Standard Flavonoid Naringin, as well as Acetonitrile p.a, all obtained from Merck KGaA (Darmstadt, Germany), and distilled water (Aquabidest) from PT. IKAPHARMINDO PUTRAMAS.

Research design

To evaluate the effect of different solvents on the extraction efficiency of anthocyanins, phenolic compounds, and antioxidant activity from strawberries, the experimental data were subjected to statistical analysis using IBM SPSS Statistics version 31.0.0.0. The design of the experiment consisted of 6 solvent treatments, including ethanol (as control) and 5 Natural Deep Eutectic Solvents (NADES A to E), each performed in triplicate.

Prior to analysis, the data were assessed for normality and homogeneity of variance using the and Levene's tests, respectively, to ensure the assumptions of parametric analysis were met. Upon confirming the assumptions, a 1-way Analysis of Variance (ANOVA) was applied to determine whether the type of solvent had a statistically significant effect ($\alpha < 0.05$) on the total anthocyanin content, total phenolic content, and antioxidant activity.

When the ANOVA showed significant differences, Duncan's Multiple Range Test (DMRT) was employed as a post-hoc test to identify which pairs of treatments differed significantly. DMRT was selected for its sensitivity in comparing means across multiple treatment groups, particularly suitable for agricultural and food-based research with moderate sample sizes.

To assess the chemical profile variations of strawberry extracts obtained using various Natural Deep Eutectic Solvents (NADES), multivariate data analysis was conducted using Principal Component Analysis (PCA). PCA was chosen as an unsupervised pattern recognition method to reduce the dimensionality of the FTIR spectral data and to identify clustering patterns and similarities among the extract samples based on their absorbance features.

The FTIR spectra were collected in the wavenumber range of 4,000 - 400 cm^{-1}, with a specific focus on the fingerprint region of 1,400 - 1,700 cm^{-1}, which corresponds to characteristic vibrations of phenolic compounds, including –OH stretching, C=O stretching, and aromatic C=C bending. All spectra were preprocessed using baseline correction and normalization to ensure uniformity and to minimize instrumental noise prior to statistical analysis.

The resulting absorbance matrix (samples as rows, wavenumbers as columns) was imported into OriginLab Pro 2025, which supports advanced chemometric tools for PCA. In Origin, the matrix was standardized (mean-centered and auto-scaled), and PCA was performed using covariance matrix computation. The software automatically generated the score plots to visualize the distribution and clustering of the samples in the new principal component space, as well as loading plots to evaluate the contribution of each variable (wavenumber) to the principal components.

Preparation of NADES

The preparation of NADES was carried out based on previous studies with several modifications [12,15,16]. All chemical components used in the NADES formulations were placed in an oven at 45 °C for 1 h prior to synthesis. The NADES components consisted of choline chloride as the hydrogen bond acceptor (HBA), and the hydrogen bond donors (HBD) included citric acid, malic acid, oxalic acid, tartaric acid, and lactic acid, with a ratio of 1:2 (g/g). These components were melted together using a hotplate stirrer at 80 °C until a clear and homogeneous solution was obtained. Then, 50 %(v/v) of double-distilled water (aquabidest) was added. The mixture was stirred using a hotplate stirrer at 50 °C and 200 rpm for 30 min, then filtered to obtain a homogeneous solution. The resulting NADES solvents were stored in tightly sealed bottles. The formulation of NADES preparation are presented in **Table 1**.

Table 1 The formulation of NADES preparation.

Type	Compounds	Ratio
NADES A	Choline chloride and Citric Acid	1:2
NADES B	Choline Chloride and Oxalic Acid	
NADES C	Choline Chloride and Malic Acid	
NADES D	Choline Chloride and Tartaric Acid	
NADES E	Choline Chloride and Lactic Acid	

Strawberry extraction using UAE

The extraction of strawberries using Ultrasound-Assisted Extraction (UAE) was carried out based on the previous study with several modifications [12]. The extraction process employed an ultrasonic-assisted method. A total of 10 g of pre-prepared strawberry powder was weighed and mixed with 100 mL of NADES solvent in a 500 mL Erlenmeyer flask. The extraction was conducted at 50 °C for 30 min under UAE conditions. The mixture was then filtered using Whatman filter paper to obtain the filtrate. The resulting extract was stored in a sealed container, protected from light exposure to maintain its quality. Storage was carried out at 4 °C (in a refrigerator) prior to further analysis.

pH analysis

The pH analysis of the strawberry extract was conducted based on the method described, aiming to measure the acidity or alkalinity level of the strawberries [17]. The pH measurement began with the calibration of the pH meter using standard buffer solutions at pH 4, 7 and 10. Once the pH meter was calibrated and set, the sample measurement was performed. The strawberry sample was prepared in a container, ensuring that the sample temperature was at room temperature. The sample was then immersed in the electrode, which had been previously rinsed and dried. The measurement was allowed to stabilize, and the pH value was recorded from the pH meter display.

Total anthocyanin analysis

The total anthocyanin content was determined using the pH differential method with the aim of quantifying the total anthocyanins present in strawberries [18]. A 1 g sample was weighed and placed into a 25 mL volumetric flask using pH 1.0 and 4.5 buffer solutions. The sample in pH 1.0 buffer was incubated for 15 min, while the sample in pH 4.5 buffer was incubated for 5 min. Subsequently, the samples were transferred into cuvettes, and their absorbance was measured using a UV-Vis spectrophotometer at wavelengths of 510 and 700 nm.

Antioxidant activity analysis using DPPH method

The antioxidant activity analysis was conducted using the DPPH method, based on the study [19]. This method relies on the principle of color change in the free radical compound (2,2-diphenyl-1-picrylhydrazyl or DPPH) upon reduction by the presence of antioxidants. The antioxidant activity of the strawberry extract was measured through the radical scavenging activity method using DPPH radicals and spectrophotometric analysis.

A 1 mg sample of the strawberry extract was weighed and mixed with a DPPH solution (25 mg/100 mL). The mixture was vortexed and incubated in the dark for 60 min at room temperature. Prior to absorbance measurement, the sample-DPPH mixture was diluted 10-fold using methanol. The absorbance was then measured at a wavelength of 517 nm against a blank solution.

Total phenolic content analysis using Folin-Ciocalteu method

The total phenolic content was analyzed using the Folin-Ciocalteu method based on the study [20]. The strawberry extract obtained was tested for its total phenolic content using the Folin-Ciocalteu reducing reagent, which is associated with a color change from yellow to blue. Gallic acid was used as a reference standard for the calibration curve, with concentrations ranging from 100 to 1,000 ppm.

A 0.4 mL aliquot of the strawberry extract was mixed with 1.5 mL of Folin-Ciocalteu reagent and allowed to stand for 5 min at room temperature. Then, 1.6 mL of 20% sodium carbonate solution was added, and the mixture was homogenized using a vortex mixer. The mixture was incubated for 30 min at room temperature. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 765 nm. The total phenolic content was calculated based on the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of sample.

Analysis of FTIR-ATR

FTIR analysis was conducted based on the previous study [12]. The FTIR analysis was performed on both NADES-based strawberry extracts and conventional solvent-based strawberry extracts to identify the specific functional groups present in the compound molecules. The Attenuated Total Reflectance (ATR) crystal was cleaned using methanol and dried with tissue paper. A background scan was performed prior to measurement and before each sample change. The sample to be analyzed was placed on the ATR crystal and scanned in the IR region from 4,000 to 400 cm^{-1}, with a resolution of 32 cm^{-1} and 4 scans.

Results and discussion

pH (degree of acidity)

The pH is a value that provides information on the acidity or alkalinity level of the strawberry extract, which may influence the efficiency of the extraction process and the desired outcomes. Based on the analysis of variance (ANOVA) at a 5% significance level (α = 0.05), the extraction process of strawberries using NADES had a significant effect on the pH of the resulting extract. pH of strawberry extract in various solvents are presented in **Figure 1**.

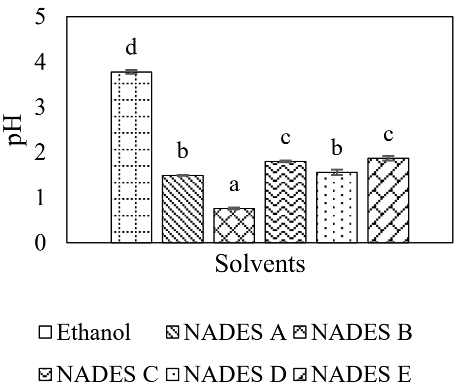

Figure 1 pH of strawberry extract in various solvents. Vertical bars represent SD of means. Different letters indicate significant differences (α = 0.05).

Based on **Figure 1**, the pH values of the strawberry extracts obtained using NADES solvents ranged from 0.76 ± 0.02 to 1.87 ± 0.05, while the extract using ethanol showed a higher pH value of 3.78 ± 0.04. The treatment with NADES E (choline chloride-lactic acid), produced the highest pH at 1.87. In contrast, the lowest pH was recorded in NADES B (choline chloride-oxalic acid) with a value of 0.76 ± 0.02. These variations in pH directly affect the appearance of pigment color in the material, as anthocyanins typically appear red under acidic conditions, blue under alkaline conditions, and purple in neutral environments.

The color variation in anthocyanins is also influenced by several factors, particularly the type and concentration of anthocyanins present. The functional groups within the anthocyanin structure play a critical role on high levels of hydroxyl groups tend to produce bluish hues, while a predominance of methoxyl groups results in more reddish tones. In addition, anthocyanins can form complexes with flavonol compounds, which further contribute to the appearance of blue coloration. This complex formation occurs through hydrogen bonding interactions between the carbonyl group in the anthocyanin's anhydrobase structure and the aromatic hydroxyl groups of flavonoids [21].

The pH has a significant impact on the stability of anthocyanins. Under highly acidic conditions, particularly at pH levels between 1 and 2, anthocyanins exist in the stable, red-colored flavylium cation form. However, as the pH rises to around 4 to 5, anthocyanins shift to a pseudobase form, and at approximately pH 6, they transition into the anhydrobase form. These transformations are generally reversible, except under strongly alkaline conditions, which can lead to irreversible structural changes. When the pH of the extract exceeds 4 or continues to increase, it tends to compromise the stability of anthocyanins, thereby reducing their effectiveness [22].

The study demonstrated that the use of NADES with a pH below 3 can enhance the efficiency of anthocyanin extraction, as anthocyanins are more stable in their flavylium cation form under acidic conditions [23]. Furthermore, extraction under low pH conditions not only increases the total anthocyanin content but also helps maintain color intensity and enhances the bioactive properties of the resulting extract. These findings align with their research results, in which the use of choline chloride and lactic acid-based NADES yielded higher anthocyanin content compared to 75% ethanol [24].

Total anthocyanin

The analysis of total anthocyanin content using the pH differential method was conducted to compare the total anthocyanin levels in strawberry extracts obtained using ethanol and NADES. Based on the analysis of variance (ANOVA) at a 5% significance level (α = 0.05), the extraction of strawberries using NADES had a significant effect on the total anthocyanin content of the extract. Total anthocyanin content of strawberry extract in various solvents. are presented in **Figure 2**.

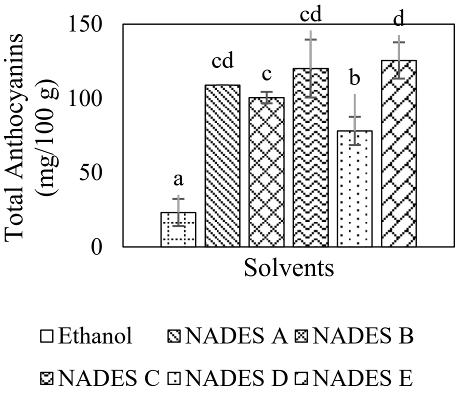

Figure 2 Total anthocyanin content of strawberry extract in various solvents. Vertical bars represent SD of means. Different letters indicate significant differences (α = 0.05).

Based on **Figure 2**, the total anthocyanin content in strawberry extracts obtained using NADES ranged from 78.09 ± 9.51 to 125.57 ± 12.22 mg/100 g, whereas the extract obtained using ethanol contained only 23.20 ± 9.15 mg/100 g. The highest anthocyanin content was found in treatment with NADES E (choline chloride-lactic acid), reaching 125.57 ± 12.22 mg/100 g. In contrast, the lowest content was observed in NADES D (choline chloride-tartaric acid), with a value of 78.09 ± 9.51 mg/100 g. The high levels of anthocyanins extracted using NADES are attributed to the highly acidic and polar nature of the solvent environment, conditions that favor the stability of anthocyanins during the extraction process [15].

The extraction of anthocyanin compounds is generally more effective under acidic conditions, as anthocyanins exhibit their highest stability at low pH. In this study, the use of 5 types of organic acids as components of NADES solvents resulted in variations in the total anthocyanin content extracted, which is closely related to the differences in the dissociation constant (Ka) values of each acid. Acids with higher Ka values have stronger acidity, enabling them to release more H^+ ions and thus create a more acidic environment. This acidic condition promotes the formation of stable anthocyanin pigments in the form of flavylium cations, which is reflected by higher absorbance values in spectrophotometric analysis. The increase in absorbance correlates with a higher detected concentration of anthocyanins in the samples [4]. The color of strawberry extract in various solvents are shown in **Figure 3**.

Figure 3 The color of strawberry extract in various solvents.

Oxalic acid is known as one of the strongest organic acids due to its classification as a dicarboxylic acid, while lactic acid is considered a weak organic acid [25]. However, the results of this study showed that the highest total anthocyanin content was obtained from the extraction using NADES E, which consists of a combination of choline chloride and lactic acid. This finding indicates that high acidity and polarity of the solvent are not the sole factors determining the success of anthocyanin extraction. Extraction efficiency is also strongly influenced by the solvent's ability to form hydrogen bonding interactions, which facilitates the release and solubilization of anthocyanins from the plant matrix more effectively.

The high anthocyanin content in NADES E, composed of choline chloride and lactic acid, is also attributed to the strong hydrogen bonding interactions between the solvent and the target compounds. These hydrogen bonds are formed between the carboxyl and hydroxyl groups on the cyanidin structure, thereby enhancing the stability of anthocyanins in solution [26]. Such interactions play a crucial role in preserving and protecting the bioactive compounds from external factors that may lead to anthocyanin degradation [27]. Moreover, these bonds help reduce the likelihood of oxygen exposure to the extract, ultimately minimizing oxidative damage and improving the overall stability of anthocyanin compounds [28].

In a study, several types of anthocyanins identified in strawberries include pelargonidin 3-O-glucoside, cyanidin 3-O-glucoside, and pelargonidin 3-O-rutinoside. Among these, pelargonidin is the dominant anthocyanin component, accounting for approximately 70% to 90% of the total anthocyanin content, with concentrations reaching up to 199.49 mg/kg of fresh weight [2]. Pelargonidin is responsible for the bright red color characteristic of strawberries, while cyanidin 3-O-glucoside contributes to the deeper red hues of the fruit [19]. The content and types of anthocyanins in strawberries are greatly influenced by various factors such as species, variety, fruit size, environmental conditions, climate, as well as storage methods and duration.

Antioxidant activity

The antioxidant activity analysis using the DPPH method was carried out to evaluate the ability of antioxidants to scavenge free radicals in strawberry extracts obtained using ethanol and NADES. Based on the Analysis of Variance (ANOVA) at a 5% significance level (α = 0.05), the extraction process using ethanol and NADES had a significant effect on the antioxidant activity of the strawberry extracts. Antioxidant activity of strawberry extract in various solvents are presented in **Figure 4**.

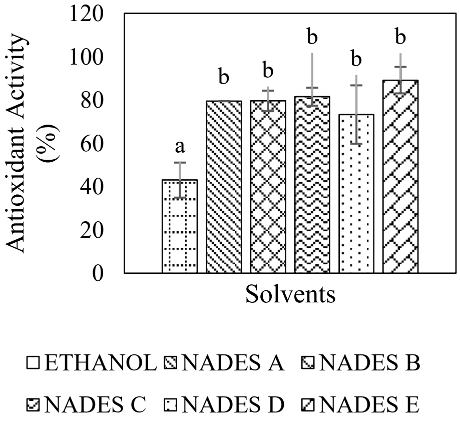

Figure 4 Antioxidant activity of strawberry extract in various solvents. Vertical bars represent SD of means. Different letters indicate significant differences (α = 0.05).

Based on **Figure 4**, the antioxidant activity of strawberry extracts obtained using NADES ranged from 73.28 ± 13.47% to 89.15 ± 6.1%, while the extract obtained using ethanol showed an activity of only 43.00 ± 8.09%. The highest value was recorded for the treatment with NADES E (choline chloride-lactic acid), with antioxidant activity reaching 89.15%. Conversely, the lowest antioxidant activity was observed in NADES D (choline chloride-tartaric acid) at 73.28 ± 13.47%. These findings are consistent with the study which reported that the high antioxidant activity in strawberry extracts is closely related to the high content of phenolic compounds, which effectively neutralize free radicals [16].

There is a strong correlation between antioxidant activity and anthocyanin content, both in aglycone and glycosylated forms. The chemical structure of anthocyanins aligns with the characteristics of antioxidant compounds, particularly due to the presence of hydroxyl (-OH) groups at the 3’ and 4’ positions on the B-ring, as well as the double bond between carbon atoms 2 and 3 on the C-ring. In glycosylated anthocyanins, the abundance of -OH groups on the B-ring significantly contributes to enhanced antioxidant activity, although a similar effect is not always observed in the aglycone forms. The -OH groups at positions 3' and 4' also play a crucial role in protecting vitamin C from oxidation, through their ability to chelate pro-oxidant metal ions [29].

Recent studies have shown that the high antioxidant activity in strawberry extracts is not only related to the total anthocyanin content but is also influenced by other phenolic profiles such as flavonoids, phenolic acids, and vitamin C. This finding is consistent with the research which reported that the intensity of the red color in strawberries indicative of pelargonidin anthocyanin content is also closely associated with increased antioxidant activity. This is because anthocyanins act synergistically with other phenolic compounds in neutralizing free radicals [30].

In addition, the molecular structure of anthocyanins, particularly the glycosylated forms with multiple hydroxyl groups and complex glycosides, enhances their stability and effectiveness as antioxidants through direct scavenging mechanisms against ROS/RNS and interactions with metal ions. Therefore, the presence of hydroxyl groups in the ortho position on the B-ring is crucial for increasing metal chelation capacity and overall antioxidant activity [31].

A study which utilized NADES in combination with UAE on strawberry waste, demonstrated significantly higher yields of phenolic extracts with markedly enhanced antioxidant activity compared to conventional methods (ethanol) [32]. This finding is further supported by the fact that the NADES-UAE combination enables optimal extraction efficiency in a short time and preserves high bioactive freshness without requiring further processing.

Total phenolic content

The analysis of total phenolic content was carried out using the Folin-Ciocalteu method to identify differences in total phenolics in strawberry extracts obtained using ethanol and NADES. Based on the analysis of variance (ANOVA) at a 5% significance level (α = 0.05), the extraction process using NADES showed a statistically significant effect on the total phenolic content of the strawberry extracts. Total phenolic content of strawberry extract in various solvents are presented in **Figure 5**.

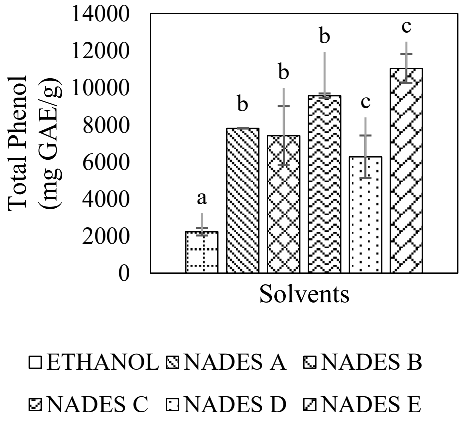

Figure 5 Total phenolic content of strawberry extract in various solvents. Vertical bars represent SD of means. Different letters indicate significant differences (α = 0.05).

Based on **Figure 5**, the total phenolic content of strawberry extracts using NADES ranged from 6,271.01 ± 1,151.15 mg GAE/g to 11,032.02 ± 785.13 mg GAE/g, whereas the total phenolic content obtained from ethanol-extracted strawberries was 2,230.43 ± 200.98 mg GAE/g. The highest total phenolic content was observed in treatment with NADES E (choline chloride-lactic acid), amount 11,046.37 ± 762.56 mg GAE/g. In contrast, the lowest phenolic content was found in NADES D (choline chloride-tartaric acid) at 6,271.01 ± 1,151.15 mg GAE/g. The high phenolic content in

NADES E is attributed to strong hydrogen bonding interactions between the solvent and target compounds.

The high total phenolic content in strawberry extracts using NADES E, composed of choline chloride and lactic acid, indicates a more efficient extraction process compared to other solvents. This can be attributed to the chemical structure of NADES E, which provides a solvent environment rich in hydrogen bond interactions between phenolic compounds and the -OH and C=O groups in the solvent. The hydroxyl and carbonyl groups present in lactic acid play a crucial role in enhancing the solubility of phenolic compounds by forming bonds with the polar groups of the target molecules. Moreover, the high polarity of NADES E allows for deeper penetration into the plant matrix, thereby facilitating the optimal release of phenolic compounds [33].

The high total phenolic content observed in the NADES E treatment is not only attributed to the presence of hydroxyl (-OH) and ether (C-O) groups but also influenced by the physicochemical characteristics of the NADES solvent itself. NADES composed of choline chloride and lactic acid exhibits relatively lower viscosity compared to NADES based on dicarboxylic acids, thereby accelerating the diffusion of phenolic compounds from the plant matrix into the solvent. The lower viscosity enhances the mobility of solvent molecules and facilitates the dissolution of polar compounds such as phenolics [34].

In addition, the polarity of NADES E is highly compatible with that of phenolic compounds, thereby enhancing both the solubility and extraction selectivity for these compounds. The tunable polarity characteristic of NADES allows for the adjustment of affinity between the solvent and target compounds, and in this case, NADES E has been shown to exhibit high affinity toward phenolics [35]. Moreover, the presence of lactic acid as a hydrogen bond donor plays a crucial role in forming strong interactions with the hydroxyl groups of phenolic compounds, thereby preventing oxidative degradation during the extraction process [36].

Furthermore, NADES E also possesses the ability to stabilize phenolic compounds after extraction due to its acidic solvent environment, which offers protection against degradation reactions caused by heat, light, and oxygen. Within this system, phenolic compounds remain in a chemically active dissolved form and are less prone to oxidation [37]. Therefore, the combination of low viscosity, polarity compatibility, stabilization capacity, and strong hydrogen bonding interactions makes NADES E a highly effective solvent for extracting phenolic compounds from strawberries.

A similar finding was reported that strong hydrogen bonding interactions are formed between NADES components and solutes due to the increased availability of hydrogen bond donors with higher molar ratios. This facilitates the induction of phenolic compounds into the NADES matrix, thereby enhancing extraction efficiency. Such interactions help stabilize phenolic compounds in a relatively mild solvent environment that is protected from oxidative degradation. Furthermore, the success of total phenolic extraction is also influenced by the use of auxiliary techniques such as ultrasound [38].

Ultrasonic extraction plays a crucial role in enhancing mass transfer rates and disrupting plant cell walls, thereby facilitating the release of active compounds, including phenolics, into the solvent. The cavitation phenomenon generated by ultrasonic waves produces momentary high-pressure conditions that effectively improve the solubilization and penetration of NADES into plant tissues, as reported by Fu *et al.* [39]. The combination of optimal NADES composition and ultrasound-assisted extraction technology resulted in the highest total phenolic content observed in the NADES E treatment compared to other solvents.

The study also confirmed that lactic acid-based solvents exhibit a high affinity for phenolic compounds due to their polarity compatibility and the ability to form stable hydrogen bonds. This condition not only maximizes the extraction process but also helps maintain the stability of phenolic compounds against degradation caused by oxidation or unfavorable pH [40]. Additionally, NADES containing strong hydrogen bond donors can significantly enhance the total phenolic content by preventing thermal and oxidative degradation during the extraction process [41].

Analysis of functional group

The identification of functional groups in strawberry extract was analyzed using Fourier Transform Infrared Spectroscopy (FTIR) with the aim of identifying changes in functional groups based on the differences in solvents used during the extraction process. The samples analyzed included strawberry extracts prepared using several types of NADES with different organic acids as main components, as well as ethanol as a comparison. The FTIR spectra represent the characteristic functional group profiles of each NADES-based strawberry extract, marked by specific absorption at certain wavelengths. Transmittance against wavenumber was used to identify the presence of key functional groups such as -OH, C=O, C-O, and others, indicating hydrogen bonding interactions and the formation of eutectic complexes between the NADES components [16]. The FTIR spectra of strawberry extract in various solvents can be seen in **Figure 6**.

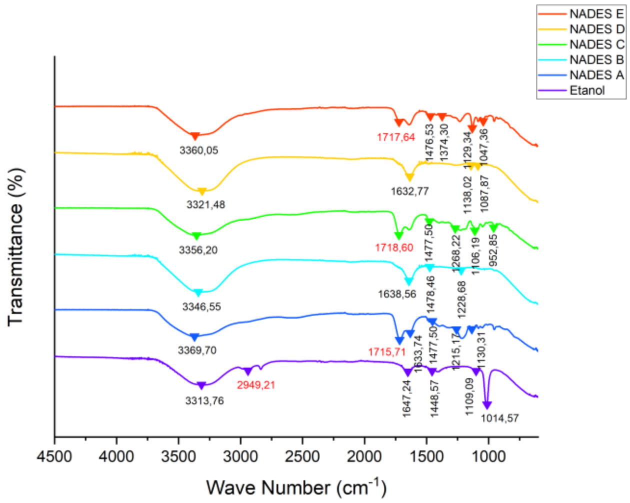

Figure 6 The FTIR spectra of strawberry extract in various solvents.

Based on **Figure 6**, it can be observed that in the wavenumber range of 3,600 - 3,200 cm^{-1}, there is a broad stretching vibration peak in all treatments. This indicates the presence of hydroxyl (-OH) group stretching, which originates from phenolic compounds found in the strawberry extract, such as anthocyanins. The -OH stretching typically produces a very broad absorption band due to hydrogen bonding interactions, both intra- and intermolecular. This explanation is consistent with literature stating that polar groups such as -OH exhibit a characteristic intense absorption band in this region due to the large dipole moment change occurring during the stretching vibration [13].

Furthermore, the wavenumber range of 3,300 - 2,700 cm^{-1} indicates the presence of hydrocarbon (C-H) stretching vibrations in methyl ($-CH_3$) and methylene ($-CH_2-$) groups derived from aliphatic hydrocarbon compounds. These stretching vibrations occur in 2 types: Symmetric and asymmetric, typically within the wavenumber range of 2,960 - 2,850 cm^{-1}. The spectrum shows that only the ethanol solvent displays a peak at 2,949.21 cm^{-1}, while the NADES spectra do not exhibit significant peaks in this region. This suggests that ethanol tends to extract compounds with similar polarity or those containing non-polar and semi-polar aliphatic groups. In contrast, NADES demonstrates greater selectivity toward polar compounds such as anthocyanins, which contain fewer free aliphatic groups [42,43].

In the wavenumber range of 2,000 - 1,700 cm^{-1}, stretching vibrations of carbonyl (C=O) groups and aromatic compounds (C=C) are observed, originating from NADES components based on organic acids such as carboxylic acids. These peaks may also arise from extracted compounds, such as phenolic compounds containing ketone or aldehyde groups. These stretching vibrations are commonly associated with the characteristic structures of anthocyanins and aromatic compounds within substituted benzene rings. Anthocyanins possess a flavylium core structure composed of 3 rings: Ring A (benzene), ring B (substituted benzene), and ring C (a heterocyclic pyrimidine ring) that connects the 2 [39].

In the wavenumber range of 1,650 - 1,430 cm^{-1}, stretching vibrations of aromatic carbon-carbon double bonds (C=C) and in-plane bending of -OH and aromatic C-H groups are observed. The wavenumber at 1,600 cm^{-1} represents the C=O stretching of carboxylic acid groups with polar moieties such as -OH or -COOH, as well as possible contributions from conjugated C=O vibrations. The shift in this wavenumber indicates changes in hydrogen bonding and ionic interactions occurring during the formation of NADES components. This is related to the hydrogen atom in the -COOH group forming hydrogen bonds with Cl^- ions in choline chloride, which in turn affects the C=O group within the carboxylic acid [44].

Analysis of PCA

Principal Component Analysis (PCA) is a multivariate statistical method that transforms a large number of variables into a few principal components that can significantly explain the variability in the data [45]. In practice, PCA can identify patterns and data clusters within extracts, thus facilitating classification based on solvent type, extraction level, or variations in chemical composition. This process not only reduces data complexity but also reveals the relationship between compound profiles and extraction conditions in an objective and quantitative manner [46]. The FTIR transmittance spectra of strawberry extract in various solvents are shown in **Figure 7**.

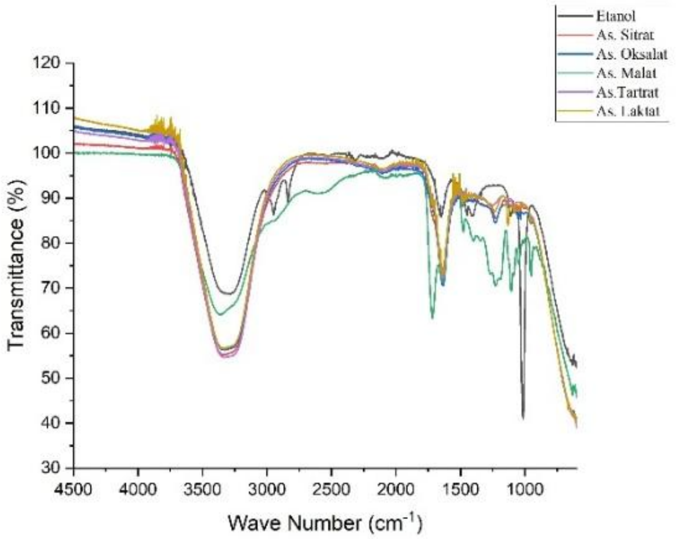

Figure 7 The FTIR transmittance spectra of strawberry extract in various solvents.

Based on **Figure 7**, the FTIR spectra display variations in the chemical profiles of strawberry extracts obtained using ethanol and 5 types of NADES based on organic acids. These differences are evident from the shapes of the curves and the intensity of the absorption peaks, represented as transmittance percentage (%T) at each wavenumber. The lower the %T value at a given wavenumber, the stronger the infrared radiation absorption at that band, indicating a higher concentration or activity of specific functional groups in the extract sample. Furthermore, the sharper and deeper the peak, the greater the quantity or intensity of compounds absorbed in that region [47].

To gain a deeper understanding of the chemical variations among strawberry extracts using different solvents, further PCA analysis was conducted by visualizing the score plot. The score plot is used to illustrate the distribution or separation of samples based on the principal components generated by PCA, typically PC1 and PC2. Each point on the score plot represents a single sample, and the distance between points reflects the degree of similarity or difference among samples based on their overall wavenumber profiles. The farther apart 2 points are on the score plot, the greater the chemical difference reflected in the wavenumber data between those samples [14]. The score plot of strawberry extract in various solvents are shown in **Figure 8**.

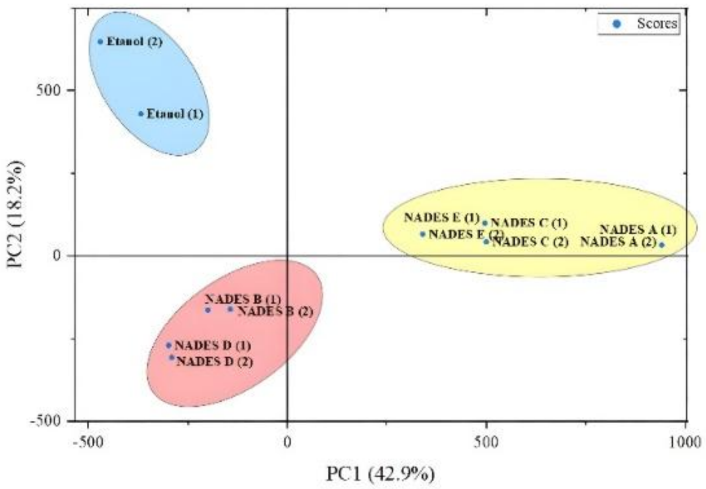

Figure 8 The score plot of strawberry extract in various solvents.

Based on **Figure 8**, it can be seen that the first principal component (PC1) explains 42.9% of the total variation, while the second component (PC2) explains 18.2%. The ethanol group shows a clear separation from the other NADES groups. This is confirmed by the higher %T values at several important bands, particularly in the -OH group region (3,400 - 3,200 cm^{-1}) and the fingerprint region (<1,500 cm^{-1}). Higher %T values indicate that ethanol extracts a lower amount of compounds with polar groups compared to NADES. Therefore, its position on the PCA plot is located far from the NADES solvent group, which is more effective in dissolving phenolic and anthocyanin compounds.

On the other hand, the NADES B and NADES D groups form a distinct cluster in the lower left quadrant, indicating that they have a different functional group profile compared to NADES A, NADES C, and NADES E. The %T values of NADES B and NADES D tend to fall between those of ethanol and the other NADES groups, reflecting moderate extraction capacity, particularly for O-H and C=O groups. The chemical characteristics of the components used in NADES B and NADES D may influence the polarity and viscosity of the solvents, thereby resulting in more limited extraction effectiveness for complex aromatic compounds [48].

Meanwhile, the NADES A, NADES C, and NADES E groups, located on the right side, form a cluster in the first quadrant, indicating a high similarity in their wavenumber profiles. These solvents exhibit lower %T values at several characteristic bands, such as -OH stretching (around 3,300 cm^{-1}), C=O and aromatic C=C (1,700 - 1,600 cm^{-1}), as well as C-O and glycosidic vibrations (1,200 - 1,000 cm^{-1}). Citric acid and malic acid are acids with additional hydroxyl groups that enhance hydrogen bonding capacity, while lactic acid contains only 1 carboxylic group but also a strong hydroxyl group. This trend suggests that NADES with acid structures balancing hydroxyl and carboxyl groups can provide a synergistic effect in extracting bioactive compounds [49].

To further investigate the factors contributing to the formation of these clusters, an analysis of the loading plot was conducted. The loading plot helps identify which wavenumbers have the most influence on the separation of sample groups in the score plot. Thus, the loading plot can explain the origin of the wavenumber differences and link them directly to the chemical structure of the compounds present in the samples [50]. In this study, the loading plot can be used to determine whether functional groups such as -OH, C=O, or C-O are responsible for the differences among solvents in extracting specific bioactive compounds. The loading plot of strawberry extract in various solvents are shown in **Figure 9**.

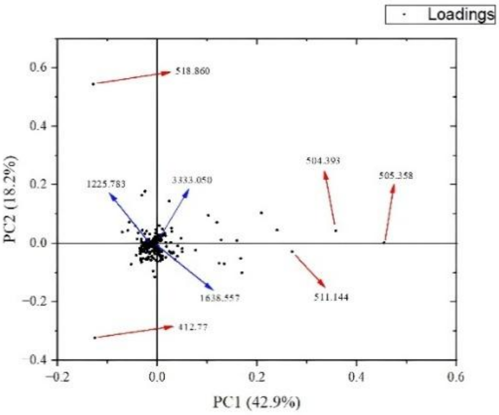

Figure 9 The loading plot of strawberry extract in various solvents.

Based on **Figure 9**, the PCA loading plot identifies several wavenumbers, such as 3,333, 1,638 and 1,225 cm^{-1} as the main contributors to the separation of sample scores. These 3 bands represent the key bonds within the anthocyanin structure: -OH, aromatic C=C, and C=O from glycosides [12]. This indicates that the wavenumber variations detected by PCA indeed originate from the core structure of anthocyanins themselves, rather than from contaminants or residual solvents. Therefore, FTIR analysis combined with PCA not only differentiates between solvents but also reveals changes in the molecular environment of the bioactive compounds within those solvents. This serves as validation that NADES not only extracts anthocyanins but also interacts with and alters the molecular microstructure of these compounds.

After conducting a comprehensive FTIR spectral analysis within the wavenumber range of 4,400 - 400 cm^{-1}, further chemometric analysis was focused on the 1,400 - 1,700 cm^{-1} region, which is recognized as a crucial area for identifying carbonyl and aromatic functional groups closely associated with the presence of phenolic compounds, including anthocyanins. This region includes characteristic absorption bands for aromatic C=C stretching vibrations between 1,450 - 1,600 cm^{-1} and carbonyl (C=O) stretching vibrations between 1,650 - 1,700 cm^{-1}, both of which are defining features of anthocyanin structures [34]. Therefore, this study focused specifically on NADES A, NADES C, and NADES E, as these 3 showed the most significant transmittance profiles in this region. The aim of this analysis was to evaluate differences in intensity and absorption patterns as indicators of successful anthocyanin extraction using different solvents. The FTIR spectra in the 1,400 - 1,700 cm^{-1} region are presented in **Figure 10**.

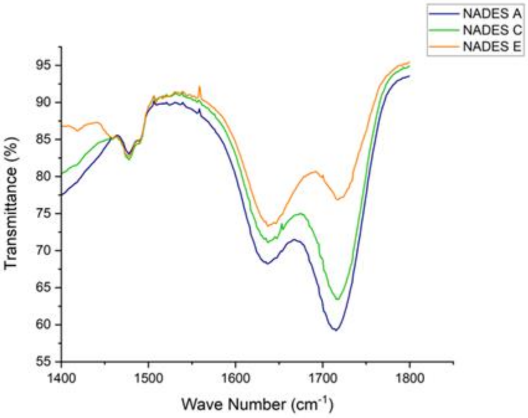

Figure 10 The FTIR spectra in the 1,400 - 1,700 cm^{-1} region.

Based on **Figure 10**, the FTIR spectrum of NADES A shows the lowest transmittance peak around 1,700 cm^{-1} compared to NADES C and NADES E. This peak is associated with the stretching vibration of the carbonyl (C=O) group from carboxylic acids, which also overlaps with the carbonyl vibrations from anthocyanins [33]. This indicates that NADES A has a high capacity for anthocyanin extraction due to its richness in carbonyl groups. Citric acid is a tricarboxylic acid containing 3 carboxyl groups and 1 hydroxyl group, thus providing a very high ability to form hydrogen bonds with the hydroxyl and carbonyl groups in anthocyanins. In addition, the relatively high acidity of NADES A creates an optimal acidic environment for stabilizing the flavylium form of anthocyanins, which is the active red-colored form. This combination of high polarity and acidity allows NADES A to form strong interaction bonds that can protect and enhance the solubility of anthocyanins during the extraction process [51].

Meanwhile, NADES C still shows a strong spectrum in the 1,700 cm^{-1} region, though with a slightly higher transmittance peak compared to NADES A. This suggests that anthocyanin extraction is also effective but slightly lower than that of NADES A. Malic acid contains 2 carboxyl groups and 1 hydroxyl group, resulting in more limited hydrogen bonding capacity compared to citric acid. The simpler structure and moderate acidity of malic acid create a solvent environment that is fairly effective in extracting anthocyanins, although not as optimal as NADES A. This is evident from the absorption of the carbonyl (C=O) group, which remains prominent, indicating the presence of anthocyanins, but with lower intensity [51].

In contrast, NADES E shows a spectrum with a higher transmittance peak in the 1,700 cm^{-1} region, indicating the weakest absorption intensity among the 3. Lactic acid contains only 1 carboxyl group and 1 hydroxyl group, which limits its ability to form hydrogen bonds with anthocyanins. The relatively low acidity of lactic acid also does not support the stabilization of the flavylium form of anthocyanins, making the anthocyanins more prone to degradation or transformation into inactive forms such as chalcone or quinonoid, which are not detected in the FTIR spectrum. Therefore, the low absorption intensity in NADES E may be due to the low solubility and stability of anthocyanins in this solvent [40].

To statistically and systematically understand spectral variation, PCA analysis was conducted on the FTIR data in the 1,400 - 1,700 cm^{-1} region. PCA works by transforming spectral data into 2 principal components: PC1 and PC2. PC1 accounts for 97.3% of the data variance, while PC2 explains 2.7%. This indicates that most of the information or differences among samples can be explained by the horizontal dimension, PC1. The horizontal axis (PC1) represents the main contribution of the most dominant functional groups affecting the spectrum, particularly the carbonyl and aromatic groups from phenolic compounds. Meanwhile, the vertical axis (PC2) captures minor or secondary variations that may arise from subtle structural differences, noise, or the presence of minor compounds in the extracts. The correlation between the X-axis (PC1) and Y-axis (PC2) can be interpreted as the relationship between major and minor variations among the samples. The score plot of strawberry extracts in the 1,400 - 1,700 cm^{-1} region is shown in **Figure 11**.

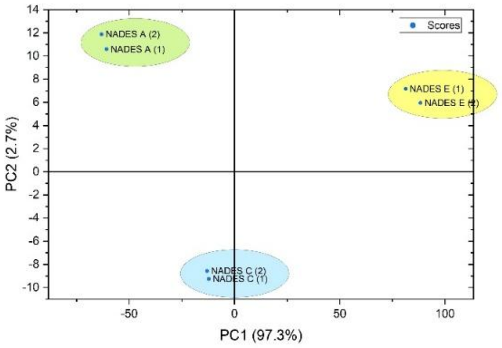

Figure 11 The score plot of strawberry extracts in the 1,400 - 1,700 cm^{-1} region.

Based on **Figure 11**, a clear separation is observed among the NADES A, NADES C, and NADES E groups. NADES A is located in the upper left quadrant (negative PC1 and positive PC2), NADES C in the lower left quadrant (negative PC1 and PC2), and NADES E in the upper right quadrant (positive PC1 and PC2). The horizontal position (PC1) serves as a key indicator in identifying the extent to which each sample's spectrum is influenced by the presence of carbonyl groups and phenolic compounds. NADES A, positioned far to the left on PC1, indicates that its extract contains a higher concentration of dominant carbonyl-containing compounds, including anthocyanins. NADES E, located far to the right, suggests that the FTIR spectrum is more influenced by other compounds or the solvent components themselves. NADES C, relatively close to the vertical axis, indicates a more balanced spectral profile between the 2. Although the vertical dimension (PC2) accounts for only 2.7%, it remains important as it reinforces that each NADES has a unique and reproducible spectral fingerprint.

The PC1 reflects the dominant sources of variance in the dataset, largely attributed to the presence and intensity of carbonyl stretching vibrations from both the target analysis of anthocyanins and phenolics and the solvent matrix [52,53]. NADES A and C, located on the negative side of PC1, indicate stronger signals associated with conjugated C=O and aromatic C=C vibrations, characteristic of anthocyanins and polyphenols. In contrast, NADES E, situated on the positive side of PC1, reflects higher contributions from non-conjugated C=O groups, likely arising from lactic acid components, suggesting a different chemical environment or less interaction with anthocyanins [54].

The PC2, although capturing a smaller portion of variance (2.7%), still contributes to understanding secondary differences between extracts. These may be associated with minor spectral shifts, instrumental noise, or the presence of less dominant compounds. The vertical positioning reinforces the uniqueness of each NADES system's chemical fingerprint, as no 2 groups overlap in the score space. These differences reflect not only the chemical properties of the solvents themselves, but also their selectivity and efficiency in extracting functional groups relevant to antioxidant and phenolic profiles [55]. The loading plot of strawberry extracts in the 1,400 - 1,700 cm^{-1} region is shown in **Figure 12**.

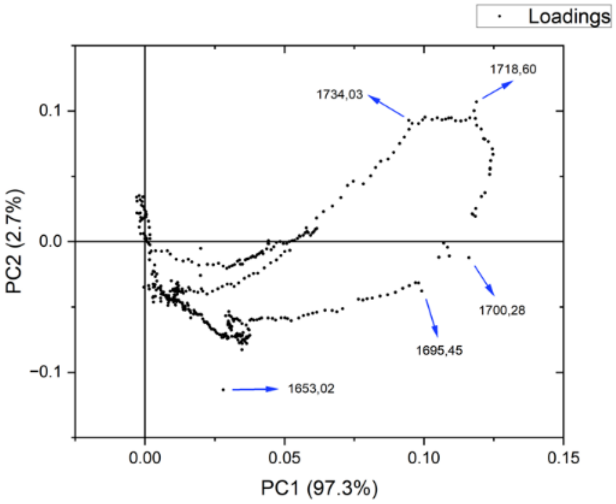

Figure 12 The loading plot of strawberry extracts in the 1,400 - 1,700 cm^{-1} region.

Based on **Figure 12**, the wavenumbers that contribute most significantly to the separation of data along PC1 and PC2 are identified. Wavenumbers such as 1,734.03 and 1,718.60 cm^{-1} have high positive loadings on PC1 and indicate a dominant contribution from NADES E, which is positioned at the high end of the PC1 score. These wavenumbers can be associated with strong C=O vibrations, most likely originating from lactic acid or degraded compounds. Meanwhile, wavenumbers at 1,653.02 and 1,695.45 cm^{-1} show negative loadings and are correlated with NADES A and NADES C, indicating the presence of conjugated aromatic carbonyl groups, which are characteristic of anthocyanin structures. This suggests that differences in spectral composition among NADES solvents arise not only from the extracted compounds, but also from the types of dominant functional groups that contribute to solvent-compound interactions.

The high loading values at these wavenumbers explain why NADES E is positioned on the far right (positive side) of PC1 in the score plot, indicating a dominant spectral contribution from free carbonyl groups and possibly solvent matrix effects [26]. In contrast, wavenumbers such as 1,695.45 and 1,653.02 cm^{-1} exhibit negative loadings on PC1, corresponding to the spectral features observed in NADES A and C. These regions are commonly attributed to conjugated carbonyl groups and aromatic C=C stretching, which are key structural features of anthocyanins and other phenolic compounds [54].

The presence of these bands indicates stronger interactions between NADES A and C with phenolic compounds during the extraction process, leading to spectra enriched in conjugated structures and shifting their PCA positions toward the negative PC1 axis. This observation is further supported who reported that the 1,600 - 1,700 cm^{-1} region serves as a major spectral fingerprint for NADES discrimination using PCA [55].

Conclusions

This study integrated quantitative measurements, including total anthocyanin content, total phenolic content, and antioxidant activity, with qualitative profiling using FTIR spectroscopy and PCA, to evaluate the extraction efficiency of 5 NADES based on choline chloride and various organic acids compared to conventional ethanol. The results showed that NADES E, composed of choline chloride and lactic acid, achieved the highest extraction yield. However, NADES A, containing choline chloride and citric acid, also exhibited statistically comparable results in quantitative analysis. In addition, FTIR spectra revealed that NADES A had the strongest absorbance in key phenolic-related regions, suggesting a higher affinity or preservation of diverse chemical structures. PCA further confirmed distinct clustering of NADES A, C, and E extracts apart from ethanol, reflecting the influence of solvent composition on the chemical profile of the extracts. These findings confirm that NADES are not only environmentally friendly but also technically efficient for the selective extraction of bioactive compounds. Therefore, the multi-analytical approach employed in this study is essential to assess both the yield and quality of extracted compounds, and highlights the potential of NADES in the development of functional food products based on green extraction technology.

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Declaration of Generative AI in Scientific Writing

The author acknowledge the use of generative AI tools (ChatGPT by Open AI and Grammarly) in the preparation of this paper, specially for the language editing and grammar correction. No content generation or data interpretation was performed by AI. The authors take full responsibility for the content and conclusions of this paper.

CRediT Author Statement

Annisa Ul Karimah: Conceptualization, Methodology, Formal Analysis, Writing Original Draft. **Kesuma Sayuti**: Supervision, Data curation, Investigation, Validation, and Visualization. **Novelina**:

Data curation, Investigation, Validation, and Visualization. **Daimon Syukri**: Conceptualization, Methodology, Data Curation, Investigation, Validation, and Visualization.

References

[1] P Padmanabhan, A Mizran, JA Sullivan and G Paliyath. *Strawberries*. *In*: B Caballero, PM Finglas and F Toldrá (Eds.). Encyclopedia of food and health. Academic Press, Oxford, England, 2016, p. 193-198.

[2] AM Mustafa, S Angeloni, D Abouelenein, L Acquaticci, J Xiao, G Sagratini, F Maggi, S Vittori and G Caprioli. A new HPLC-MS/MS method for the simultaneous determination of 36 polyphenols

in blueberry, strawberry and their commercial products and determination of antioxidant activity. *Food Chemistry* 2022; **367**, 130743.

[3] M Huang, Y Han, L Li, K Rakariyatham, X Wu, Z Gao and H Xiao. Protective effects of non-extractable phenolics from strawberry against inflammation and colon cancer *in vitro*. *Food Chemistry* 2022; **374**, 131759.

[4] X Sui, Y Zhang, L Jiang and W Zhou. *Anthocyanins in food*. *In*: L Melton, F Shahidi and P Varelis (Eds.). Encyclopedia of food chemistry. Academic Press, Oxford, England, 2019, p. 10-17.

[5] D Li, X Zhang, L Li, MS Aghdam, X Wei, J Liu, Y Xu and Z Luo. Elevated CO_2 delayed the chlorophyll degradation and anthocyanin accumulation in postharvest strawberry fruit. *Food Chemistry* 2019; **285**, 163-170.

[6] J Hellström, S Karhu, J Karhu, E Järvenpää and AL Välimaa. Phenolic profiles differentiate wild bilberry and cultivated blueberry fruit. *LWT* 2024; **199**, 116080.

[7] Y Yuan, Y Tian, S Gao, X Zhang, X Gao and J He. Effects of environmental factors and fermentation on red raspberry anthocyanins stability. *LWT* 2023; **173**, 114252.

[8] GAB Canuto, DR Oliveira, LSM da Conceição, JPS Farah and MFM Tavares. Development and validation of a liquid chromatography method for anthocyanins in strawberry (*Fragaria spp.*) and complementary studies on stability, kinetics and antioxidant power. *Food Chemistry* 2016; **192**, 566-574.

[9] MU Kousar, A Jabeen, T Fatima, SZ Hussain, IA Zargar, T Amin and M Yaseen. Optimization of ultrasonic-assisted extraction of eugenol-rich fraction from basil leaves: Characterization of extract for phenols, flavonoids and antioxidant activity. *Food Chemistry Advances* 2023; **3**, 100374.

[10] Y Wang, Y Ye, L Wang, W Yin and J Liang. Antioxidant activity and subcritical water extraction of anthocyanin from raspberry process optimization by response surface methodology. *Food Bioscience* 2021; **44**, 101394.

[11] F Pena-Pereira and IDL Calle. *Solvents and eutectic solvents*. *In*: P Worsfold, C Poole, A Townshend and M Miró (Eds.). Encyclopedia of analytical science. Academic Press, Oxford, England, 2019, p. 184-190.

[12] DTD Silva, R Pauletto, SDS Cavalheiro, VC Bochi, E Rodrigues, J Weber, CDBD Silva, FDP Morisso, MT Barcia and T Emanuelli. Natural deep eutectic solvents as a biocompatible tool for the extraction of blueberry anthocyanins. *Journal of Food Composition and Analysis* 2020; **89**, 103470.

[13] BH Stuart. *Organic molecules*. *In*: J Wiley and L Sons (Eds.). Infrared spectroscopy: Fundamentals and applications. Wiley, Queensland, Australia, 2004.

[14] IT Jolliffe and J Cadima. Principal component analysis: A review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 2016; **374(2065)**, 20150202.

[15] MS Jovanović, N Krgović, J Živković, T Stević, G Zdunić, D Bigović and K Šavikin. Ultrasound-assisted natural deep eutectic solvents extraction of bilberry anthocyanins: Optimization, bioactivities, and storage stability. *Plants* 2022; **11(20)**, 2680.

[16] O Zannou and I Koca. Greener extraction of anthocyanins and antioxidant activity from blackberry (*Rubus* spp) using natural deep eutectic solvents. *LWT* 2022; **158**, 113184.

[17] AR Pereira, VC Fernandes, C Delerue-Matos, VD Freitas, N Mateus and J Oliveira. Exploring acylated anthocyanin-based extracts as a natural alternative to synthetic food dyes: Stability and application insights. *Food Chemistry* 2024; **461**, 140945.

[18] T Taghavi, H Patel and R Rafie. Anthocyanin extraction method and sample preparation affect anthocyanin yield of strawberries. *Natural Product Communications* 2022; **17(5)**, 1934578X2210999.

[19] T Dzhanfezova, G Barba-Espín, R Mülle, B Joernsgaard, JN Hegelund, B Madsen, DH Larsen, MM Vega and TB Toldam-Andersen. Anthocyanin profile, antioxidant activity and total phenolic content of a strawberry (*Fragaria* × *ananassa* Duch) genetic resource collection. *Food Bioscience* 2020; **36**, 100620.

[20] MZ Alam, MSR Alhebsi, S Ghnimi and A Kamal-Eldin. Inability of total antioxidant activity assays to accurately assess the phenolic compounds of date palm fruit (*Phoenix dactylifera* L.). *NFS Journal* 2021; **22**, 32-40.

[21] B Enaru, G Drețcanu, TD Pop, A Stănilă and Z Diaconeasa. Anthocyanins: Factors affecting their stability and degradation. *Antioxidants* 2021; **10(12)**, 1967.

[22] S Zhang, S Lin, J Zhang and W Liu. Ultrasound-assisted natural deep eutectic solvent extraction of anthocyanin from *Vitis davidii* Foex. pomace: Optimization, identification, antioxidant activity and stability. *Heliyon* 2024; **10(12)**, e33066.

[23] P Velásquez, D Bustos, G Montenegro and A Giordano. Ultrasound-assisted extraction of anthocyanins using natural deep eutectic solvents and their incorporation in edible films. *Molecules* 2021; **26(4)**, 984.

[24] AN Nunes, A Borges, AA Matias, MR Bronze and J Oliveira. Alternative extraction and downstream purification processes for anthocyanins. *Molecules* 2022; **27(2)**, 368.

[25] ME Alañón, M Ivanović, S Pimentel-Mora, I Borrás-Linares, D Arráez-Román and A Segura-Carretero. A novel sustainable approach for the extraction of value-added compounds from *Hibiscus sabdariffa* L. calyces by natural deep eutectic solvents. *Food Research International* 2020; **137**, 109646.

[26] Y Dai, E Rozema, R Verpoorte and YH Choi. Application of natural deep eutectic solvents to the extraction of anthocyanins from *Catharanthus roseus* with high extractability and stability replacing conventional organic solvents. *Journal of Chromatography A* 2016; **1434**, 50-56.

[27] Y Bi, X Chi, R Zhang, Y Lu, Z Wang, Q Dong, C Ding, R Yang and L Jiang. Highly efficient extraction of mulberry anthocyanins in deep eutectic solvents: Insights of degradation kinetics and stability evaluation. *Innovative Food Science & Emerging Technologies* 2020; **66**, 102512.

[28] S Prakash, A Goswami, R Patil, A Mitra and NN Kutty. An eco-friendly approach to extract anthocyanins from rose flowers using natural deep eutectic solvents. *Industrial Crops and Products* 2024; **210**, 118059.

[29] M Lakshmikanthan, S Muthu, K Krishnan, AB Altemimi, NN Haider, L Govindan, J Selvakumari, ZT Alkanan, F Cacciola and YM Francis. A comprehensive review on anthocyanin-rich foods: Insights into extraction, medicinal potential, and sustainable applications. *Journal of Agriculture and Food Research* 2024; **17**, 101245.

[30] NR Hernández-Martínez, C Blanchard, D Wells and MR Salazar-Gutiérrez. Current state and future perspectives of commercial strawberry production: A review. *Scientia Horticulturae* 2023; **312**, 111893.

[31] I Sadowska-Bartosz and G Bartosz. Antioxidant activity of anthocyanins and anthocyanidins: A critical review. *International Journal of Molecular Sciences* 2024; **25(22)**, 12001.

[32] QQ Koh, ZL Chew, Y Zhao, YL Kua, S Gan, KW Tan, TZE Lee and HLN Lau. Formulation and characterization of natural deep eutectic solvents (NADES) for simultaneous phenolics and carotenes extraction from fresh oil palm leaf. *Food and Bioproducts Processing* 2024; **147**, 459-473.

[33] A Paiva, R Craveiro, I Aroso, M Martins, RL Reis and ARC Duarte. Natural deep eutectic solvents - solvents for the 21st century. *ACS Sustainable Chemistry & Engineering* 2014; **2(5)**, 1063-1071.

[34] NPE Hikmawanti, D Ramadon, I Jantan and A Mun'Im. Natural Deep Eutectic Solvents (NADES): Phytochemical extraction performance enhancer for pharmaceutical and nutraceutical product development. *Plants* 2021; **10(10)**, 2091.

[35] K Radošević, N Ćurko, VG Srček, MC Bubalo, M Tomašević, KK Ganić and IR Redovniković. Natural deep eutectic solvents as beneficial extractants for enhancement of plant extracts bioactivity. *LWT* 2016; **73**, 45-51.

[36] S Milošević, AB Markovinović, N Teslić, A Mišan, M Pojić, IB Karačonji, K Jurica, D Lasić, P Putnik, DB Kovačević and B Pavlić. Use of natural deep eutectic solvent (NADES) as a green extraction of antioxidant polyphenols from strawberry tree fruit (*Arbutus unedo* L.): An optimization study. *Microchemical Journal* 2024; **200**, 110284.

[37] H Du, W Wu, Y Wang, P Tang, Y Wu, J Qiu, C Xu, L Li and M Yang. Natural deep eutectic solvent-ultrasound for the extraction of flavonoids

from *Fructus aurantii*: Theoretical screening, experimental and mechanism. *Arabian Journal of Chemistry* 2024; **17(9)**, 105886.

[38] K Pusty, KK Dash, S Giri, GVSB Raj, A Tiwari, AM Shaikh and K Béla. Ultrasound assisted phytochemical extraction of red cabbage by using deep eutectic solvent: Modelling using ANFIS and optimization by genetic algorithms. *Ultrasonics Sonochemistry* 2024; **102**, 106762.

[39] X Fu, D Wang, T Belwal, J Xie, Y Xu, L Li, L Zou, L Zhang and Z Luo. Natural deep eutectic solvent enhanced pulse-ultrasonication assisted extraction as a multi-stability protective and efficient green strategy to extract anthocyanin from blueberry pomace. *LWT* 2021; **144**, 111220.

[40] R Thakur, V Gupta, T Ghosh and AB Das. Effect of anthocyanin-natural deep eutectic solvent (lactic acid/fructose) on mechanical, thermal, barrier, and pH-sensitive properties of polyvinyl alcohol based edible films. *Food Packaging and Shelf Life* 2022; **33**, 100914.

[41] A Palos-Hernandez, MYG Fernández, JE Burrieza, JL Pérez-Iglesias and AM González-Paramás. Obtaining green extracts rich in phenolic compounds from underexploited food by-products using natural deep eutectic solvents. Opportunities and challenges. *Sustainable Chemistry and Pharmacy* 2022; **29**, 100773.

[42] O Kapitan, D Kusumawardhani, F Nitti and G Kedjo. Profil senyawa polar dan semi polar daun tembelekan (*Lantana camara*) asal pulau timor (*in Indonesian*). *Indigenous Biologi: Jurnal Pendidikan Dan Sains Biologi* 2024; **7(3)**, 111-121.

[43] IL Tarigan, JAN Sinurat, II Ramadhan, DE Wijaya, RD Puspitasari and MS Sowe. Green extraction of bioactive compounds from *Peronema canescens* Jack. using sodium acetate/glycerol-based natural deep eutectic solvents as a source of natural antioxidants. *Jurnal Kartika Kimia* 2025; **8(1)**, 60-70.

[44] R Mogale, YW Abraha, M Schutte-Smith, HG Visser and E Erasmus. Natural deep eutectic solvents (NADES) derived from choline chloride and indole-3-butyric acid for optimized CO_2 utilization through cycloaddition with epichlorohydrin. *Journal of Ionic Liquids* 2025; **5**, 100142.

[45] M El Mouftari, I Essafi, A Khalidi, F Kzaiber, GAM Ali, FZ Mahjoubi and A Oussama. Applications of FTIR and chemometrics methods in authenticity analysis of walnut oil. *Emergent Materials* 2022; **5(1)**, 167-174.

[46] D Granato, JS Santos, GB Escher, BL Ferreira and RM Maggio. Use of principal component analysis (PCA) and hierarchical cluster analysis (HCA) for multivariate association between bioactive compounds and functional properties in foods: A critical perspective. *Trends in Food Science & Technology* 2018; **72**, 83-90.

[47] ABD Nandiyanto, R Oktiani and R Ragadhita. How to read and interpret FTIR spectroscope of organic material. *Indonesian Journal of Science and Technology* 2019; **4(1)**, 97-118.

[48] JO Airouyuwa, H Mostafa, A Riaz and S Maqsood. Utilization of natural deep eutectic solvents and ultrasound-assisted extraction as green extraction technique for the recovery of bioactive compounds from date palm (*Phoenix dactylifera* L.) seeds: An investigation into optimization of process parameters. *Ultrasonics Sonochemistry* 2022; **91**, 106233.

[49] T Jeliński, M Przybyłek and P Cysewski. Natural deep eutectic solvents as agents for improving solubility, stability and delivery of curcumin. *Pharmaceutical Research* 2019; **36(8)**, 116.

[50] M Mayonu, S Babaee, L Jiang, D Wilson and B Wang. Evaluation of a new approach for principal component analysis application in metabolomics studies. *Analytical Letters* 2025. https://doi.org/10.1080/00032719.2025.2513548

[51] R Qin, H Chen, R Wen, G Li and Z Meng. Effect of boric acid on the ionization equilibrium of α-hydroxy carboxylic acids and the study of its applications. *Molecules* 2023; **28(12)**, 4723.

[52] P Kalhor and K Ghandi. Deep eutectic solvents for pretreatment, extraction, and catalysis of biomass and food waste. *Molecules* 2019; **24**, E4012.

[53] C Florindo, LC Branco and IM Marrucho. Quest for green-solvent design: From hydrophilic to hydrophobic (deep) eutectic solvents. *ChemSusChem* 2019; **12(8)**, 1549-1559.

[54] ML Picchio, D Minudri, D Mantione, M Criado-Gonzalez, G Guzmán-González, R Schmarsow, AJ Müller, LC Tomé, RJ Minari and D Mecerreyes. Natural deep eutectic solvents based on choline chloride and phenolic compounds as efficient bioadhesives and corrosion protectors. *ACS Sustainable Chemistry & Engineering* 2022; **10(25)**, 8135-8142.

[55] ON Pozharitskaya, ED Obluchinskaya, VA Shikova, EV Flisyuk, EV Vishnyakov, EV Makarevich and AN Shikov. Physicochemical and antimicrobial properties of lactic acid-based natural deep eutectic solvents as a function of water content. *Applied Sciences* 2024; **14(22)**, 10409.