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FTIR-ATR Spectroscopy as a Tool for Quality Control and Authentication of Apple Juice

Renad Riyad Mahmoud Ramadan

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Prepared By:
Renad Riyad Mahmoud Ramadan

B.Sc. Biology/ Science and Technology Department
Al-Quds University-Palestine

Supervisor: Dr. Amer Marei

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Thesis Approval

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Prepared by: Renad Riyad Mahmoud Ramadan
Registration Number: 21820371

Supervisor: Prof. Amer Marei

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The Names and signatures of the examining committee are as follows:

1. **Prof. Dr. Amer Marei, … Head of committee, . Signature:**
2. **Dr. Mahmoud Alkhatib, Internal examiner, Signature:**
3. **Dr. Subhi Samhan, External examiner, Signature:** Subhi Samhan

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Dedication

I dedicate this work to my parents, whose love, support, and guidance have shaped every step of my journey—I am forever grateful to them.
To my dear brother and sisters, with love.
To my husband, whose unwavering support means the world to me.
And to the exceptional professors who have been a continual source of wisdom and inspiration.

Renad Ramadan

Declaration

I certify that this thesis submitted for the degree of Master in environmental science is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not be submitted for a higher degree to any other university or institution.

Signature: 

Name: Renad Riyad Mahmoud Ramadan

Date: 18 / 05 /2025

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Renad Ramadan

Abstract

Apple juice's sweet taste, nutritional value, and health advantages make it one of the most widely consumed fruit drinks. However, the possibility of adulteration has increased due to the juice industry's growing demand and financial incentives, particularly when sugars are added or less expensive ingredients are substituted. Consumer trust and product quality are both compromised by these actions. The use of FTIR-ATR spectroscopy as a quick, inexpensive, and non-destructive method to identify adulteration and evaluate the quality of apple juice is examined in this work.

Four apple varieties—Red Delicious, Gala, Golden Delicious, and Granny Smith—as well as fourteen dilution levels of each, standard solutions of the main constituents of apple juice, which are the sugar and malic acid. Moreover, seven commercial juice items from the Palestinian market were used to prepare fresh juice samples for the study. At the special mid-infrared peaks of 1062 cm^{-1} (sugar), 1281 cm^{-1} (malic acid), and 1634 cm^{-1} (water reference), absorbance measurements were taken.

Significant differences were found when commercial samples were compared to these reference values. The majority of commercial juices had noticeably lower absorbance values at the acid and sugar peaks, suggesting that the sugar may have been enhanced or diluted. Different levels of adulteration were proven by calculated percentages of sugar and malic acid. The product with the greatest estimated natural juice percentage, 98.20%, was (A) Juice, an industrial brand rather than a natural product. However, the lowest natural juice content was found in (D) and (E) juices, which had estimated adulteration levels of 33.17% and 29.39%, respectively.

These findings provide credence to FTIR-ATR spectroscopy's usage in the juice business as a useful instrument for quick quality control. It has a useful edge over conventional laboratory techniques due to its capacity to identify minute chemical variations. With this strategy, manufacturers, regulators, and food safety authorities can guarantee authenticity, stop fraud, and safeguard consumer health in an efficient and approachable manner.

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List of Abbreviations

EMA	Economically Motivated Adulteration
IR	Infrared
FTIR-ATR	Fourier-Transform Infrared Spectroscopy with Attenuated Total Reflectance
NHANES	National Health and Nutrition Examination Survey
JAMA	Journal of the American Medical Association
FDA	Food and Drug Administration
PCBS	Palestinian Central Bureau of Statistics
HPLC	High-Performance Liquid Chromatography
PSI	Palestinian Standards Institution
HFCS	High Fructose Corn Syrup
NIR	Near-Infrared
FTIR	Fourier Transform Infrared
Mid-FTIR	Mid-Fourier Transform Infrared Spectroscopy
LDA	Linear Discriminant Analysis
PLS	Partial Least Squares
PCA	Principle Component Analysis

Chapter One:

Introduction

1.1 General Introduction

A healthy lifestyle entails consuming food and beverages in a nutritious manner, prioritizing natural items whenever feasible, hence enhancing overall human productivity. As the population grows rapidly, the need for food has risen, resulting in an expansion of the food industry (Black et al., 2016). One hurdle that has emerged is that imposters have persistently exploited the food market to maximize economic gains and satisfy the elevated demand for food (Dhaulaniya et al., 2020). One strategy employed by these imposters involves the incorporation of inferior ingredients, which yields substantial economic gains, alongside the fraudulent labeling of substandard products as premium ones. The application of product authentication and quality control serves to mitigate hurdles of this nature (Dhaulaniya et al., 2020).

The intentionally incorporated low-cost substances are referred to as Economically Motivated Adulteration (EMA). This category of substances generally exerts a negligible influence on the physical qualities of food products, exemplified by the addition of sugars to fruit juices. Consequently, this may result in significant deterioration of product quality and might pose various health risks (Manning et al., 2016). Food products may unintentionally harbor pollution dangers, including the presence of heavy metals, pesticides, and antibiotics (Aghamirlou et al., 2015).

One of the most critical aspects of the food industry is food authenticity (Andrade et al., 2019). To address economically driven adulteration and unintended contaminants, numerous countries have established regulatory authorities to uphold standards and oversee traceability systems. Neglecting these regulatory authorities may result in significant health risks due to such adulteration (Dhaulaniya et al., 2020).

A prevalent approach for modifying the quality of fruit juices is the adulteration of higher-quality juice with inferior juice, such as incorporating pear juice into apple juice. Furthermore, the incorporation of inexpensive sugars yields a similar outcome. Given the expansion of food trade and the intricacies of food supply chains, it has become imperative

to cultivate an interest in and necessity for enhanced quality control systems to protect customers from adulteration and contamination in their food. Consequently, the pursuit of these technologies emerges as a paramount concern for the authenticity and traceability of food products (Dhaulaniya et al., 2020).

The predominant soluble solids in fruit juices are sugars, rendering them a significant quality indicator of fruit juices (Cassani et al., 2018). The predominant soluble solids in fruit juices are sugars, rendering them a significant quality indicator of fruit juices As known that simple sugars (glucose, fructose and sucrose) are stable in fruit juices, which makes them direct indicators of fruit product quality (Fügel et al., 2005), (Rodriguez-Saona et al., 2001).

In order to estimate the total sugar content found in fruit juices, there were classical methods such as the volumetric procedures or refractive index measurements (Cassani et al., 2018). On the other hand, chromatographic method and enzymatic analysis method can be used to quantify the individual sugars (Fuleki et al., 1994), (Karadeniz et al., 2002).

Physical criteria, including density, color, texture, and solubility, are not significant indicators of fruit juice quality and are hence deemed unreliable for detecting food adulteration (Dhaulaniya et al., 2020).

The scientific community uses vibrational spectroscopy in conjunction with chemometric techniques to establish rapid, straightforward, non-destructive, and cost-effective ways for extracting optimal information from intricate multivariate data (Moore et al., 2012), (Nunes, 2014). Infrared (IR) spectroscopy has been used for food quality analysis. The most commonly used spectroscopy method is Fourier-Transform Infrared Spectroscopy with Attenuated Total Reflectance(FTIR-ATR), which has been used for different purposes, such as the quantification of sugars, organic acids, polyphenols, etc., in food products (Sivakesava et al., 2001).

Due to its palatable flavor, substantial nutritional value, and potential health benefits, apple juice is a widely consumed beverage. It is derived from apples (Malus Domestica) and contains a plethora of bioactive compounds, including vitamins, flavonoids, and polyphenols, which contribute to its anti-inflammatory and antioxidant properties (Koutsos et al., 2020).

Water makes up 70–97% of apple juice, making it its primary ingredient (Awolu et al., 2013). Glucose, fructose, and sucrose are examples of carbohydrates, which make up the second largest component (Marc et al., 2012). The sugar content varies from 3 to 25%. Additionally, there are 770 mg of fiber, 740 mg of organic fruit acids, 70 mg of protein, 116 mg of potassium, 7.0 mg of phosphorus, 6.9 mg of magnesium, 4.2 mg of calcium, and 1.4 mg of vitamin C in 100 mL of apple juice (Rydzak et al., 2020).

The composition and health effects of fresh and manufactured apple juices vary greatly. Due to less processing, fresh apple juice has a higher concentration of bioactive substances like polyphenols and antioxidants than manufactured apple juice, which is subjected to filtration and clarity procedures that lessen these advantageous substances. Research shows that less processed, unclear apple juice has more polyphenols than highly processed, clear apple juice (Vallée Marcotte et al., 2022).

Unprocessed or minimally processed apple juice possesses a greater polyphenol concentration, enhancing antioxidant efficacy and reducing inflammation and oxidative stress. Research indicates that the high polyphenol content in cloudy apple juice can enhance cardiovascular health markers. Conversely, clarified, processed apple juice may possess diminished health benefits (Vallée Marcotte et al., 2022).

Fresh and manufactured apple juices might differ in their mineral content and organic acid compositions. These elements are influenced by variables like apple variety, farming practices, and processing processes, which may have an impact on the juice's nutritional value and flavor (Dobrowolska-Iwanek et al., 2015). See Table 1.

Table 1. Comparison of fresh and manufactured apple juice based on composition and health impact.

Aspect	Fresh Apple Juice	Manufactured Apple Juice	Reference
Processing	Minimally processed, retains most natural compounds	Undergoes clarification and filtration, reducing bioactive compounds	(Vallée Marcotte et al., 2022)
Polyphenol Content	High, especially in cloudy juice	Lower, particularly in clear juice due to processing	(Vallée Marcotte et al., 2022)
Mineral & Organic Acid Composition	Retains natural mineral content and organic acids depending on apple variety	Mineral and organic acid composition may be altered due to processing methods	(Dobrowolska-Iwanek et al., 2015)
Antioxidant Activity	Higher, helps reduce oxidative stress and inflammation	Lower, leading to reduced antioxidant benefits	(Vallée Marcotte et al., 2022)
Cardiovascular Health Impact	May positively affect cardiovascular markers due to higher polyphenol content	Offers fewer cardiovascular benefits due to reduced polyphenols	(Vallée Marcotte et al., 2022)
Overall Health Benefits	More beneficial due to higher retention of bioactive compounds	Less beneficial compared to fresh juice, especially in clear varieties	(Vallée Marcotte et al., 2022)

Excessive consumption of certain commercially produced apple juices may contain added sugars, increasing calorie content and potentially leading to weight gain and metabolic issues. To mitigate these negative effects, it is recommended to consume 100% apple juice devoid of added (Link, 2023).

Moreover, regulatory bodies have set maximum allowable values to mitigate health risks; yet, commercially produced apple juices may sporadically include toxins such as patulin, a mycotoxin produced by certain fungi (Zhong et al., 2018).

In contrast, manufactured apple juice may have lower nutritional value and may contain additional sugars and pollutants, whereas raw apple juice has a higher antioxidant content

and may have cardiovascular advantages. Therefore, to optimize health advantages, choose fresh, unfiltered apple juice without added sugars. See Table 2.

Table 2. Comparison the negative health effects of fresh and manufactured apple juice.

Aspect	Fresh Apple Juice	Manufactured Apple Juice	Reference
Negative Effects			
Added Sugars and Calories	No added sugars, retains natural sugars from apples	May contain added sugars, increasing calorie content and contributing to weight gain	(Link, 2023)
Nutrient Loss	Retains most nutrients and beneficial compounds	Nutrient loss due to pasteurization and processing (lower vitamins and polyphenols)	(Vallée Marcotte et al., 2022)
Contaminant Risks	Lower risk of contaminants if freshly pressed and handled properly	Can contain contaminants like patulin (mycotoxins) from improper storage or processing	(Zhong et al., 2018)

Children frequently drink manufactured apple juice since it's a convenient way to get some nutrients and hydration. Research has shown that children who moderately consume 100% fruit juice, particularly apple juice, have higher intakes of vital nutrients and better food quality. For example, studies examining data from the National Health And Nutrition Examination Survey (NHANES) from 2003 to 2010 discovered that eating apples was associated with a healthier diet overall and a lower risk of childhood obesity(O'Neil et al., 2015).

Concerns have been raised regarding the potential health risks associated with excessive consumption of fruit juice. Research indicates that children who ingest substantial quantities of fruit juice may experience weight gain and have an elevated risk of obesity. In 2021, Journal of the American Medical Association(JAMA) Pediatrics published a systematic review and meta-analysis that looked at the connection between children's obesity and consuming 100% fruit juice, emphasizing the need for moderation (Nguyen et al., 2024).

Children frequently drink manufactured apple juice, however a number of studies have shown that there may be health dangers involved. Its high sugar content is one of the main issues, as it leads to excessive calorie intake and raises the risk of children obesity, a disease associated with diabetes and hypertension (Health, 2019). Furthermore, studies indicate that children's asthma risk may double due to apple juice's high fructose-to-glucose ratio, suggesting possible respiratory health consequences (DeChristopher et al., 2020). Additionally, unpasteurized apple juice may contain dangerous bacteria that raises the possibility of foodborne infections, especially in young children. To reduce possible health hazards, the U.S. Food and Drug Administration (FDA) recommends that children under one

year old not drink fruit juice and that those between the ages of one and three should only drink four ounces of 100% fruit juice daily ((AAP), 2023). See Table 3.

Children's diets can be enhanced by moderate consumption of processed apple juice. However, it is essential to monitor intake levels to prevent adverse consequences such as weight gain. Moreover, it is imperative to ensure the quality and safety of these products to protect children from hazardous contaminants.

Table 3. Disadvantages of Children's Consumption of Manufactured Apple Juice.

Disadvantage	Description	Reference
High Sugar Content and Obesity Risk	Manufactured apple juice often contains high sugar levels, contributing to excessive calorie intake and increasing the risk of obesity in children. Childhood obesity is linked to various health issues like hypertension and diabetes.	(Health, 2019)
Asthma Risk	The high fructose-to-glucose ratio in apple juice has been associated with a two-fold increase in asthma risk among children, suggesting a potential negative impact on respiratory health.	(DeChristopher et al., 2020)
Potential for Foodborne Illness	Unpasteurized apple juice can harbor harmful bacteria, increasing the risk of foodborne illness, particularly in young children. The FDA advises limiting juice intake in children under three years old.	((AAP), 2023)

Israel, Jordan, and other adjacent states provide Palestine with a substantial volume of processed juices. These comprise branded products such as ready-to-drink beverages, concentrated juices, and carbonated beverages. Local Palestinian enterprises also create a range of manufactured juices. Conversely, in Palestine, natural juices, including freshly squeezed varieties, are readily available, especially in local markets, juice bars, and restaurants. Oranges, pomegranates, lemons, and apples—fruits abundant in the region—are commonly utilized to produce,

The Palestinian Central Bureau of Statistics (PCBS) estimates that in 2022, fruit juice imports totaled about $21 million for Palestine. Israel ($13.1 million), Egypt ($4.61 million), the United Arab Emirates ($2.88 million), Jordan ($127,000), and Thailand ($56,400) were the main importers ((OEC)).

In particular, 19,020 kg of orange juice, worth $25,000, were imported in 2022. A total of 4,750 kg of apple juice, valued at $19,000, were imported (Times, 2022a, 2022b).

According to projections, Palestinian juice imports are anticipated to expand at an average yearly rate of 3.7% to reach about $23 million by 2028 (ReportLinker). See Table 4.

Table 4. Structured table summarizing the imported juice statistics for Palestine in 2022.

Year	Product Quantity	Imported (kg)	Value (USD)	Sources
2022	Fruit Juices (Total)	Not Available	21,000,000	((OEC))
2022	Orange Juice	19,020	25,000	(Times, 2022b)
2022	Apple Juice	4,750	19,000	(Times, 2022a)
2022	Israel (Supplier)	Not Available	13,100,000	((OEC))
2022	Egypt (Supplier)	Not Available	4,610,000	((OEC))
2022	UAE (Supplier)	Not Available	2,880,000	((OEC))
2022	Jordan (Supplier)	Not Available	127,000	((OEC))
2022	Thailand (Supplier)	Not Available	56,400	((OEC))
2028	(Projected)	Not Available	23,000,000	(ReportLinker)

1.2 Problem Description

Commercial apple juices are frequently subjected to dilution, artificial sweetening, and the addition of chemical preservatives. These modifications significantly diminish the natural nutritional value of the juice, particularly reducing essential compounds such as polyphenols, antioxidants, and vitamin C. Excessive sugar content in such products has been linked to serious public health concerns, including obesity, type 2 diabetes, and other metabolic disorders. Despite these risks, advanced analytical techniques such as High-Performance Liquid Chromatography (HPLC) are not widely accessible in regions like Palestine, where monitoring systems remain limited. This lack of effective quality control increases the potential for mislabeling and consumer deception. Furthermore, the Palestinian Standards Institution (PSI) faces ongoing difficulties in enforcing consistent quality standards across the market. In light of these challenges, there is a pressing need for a fast, cost-effective, and reliable method to assess the authenticity of apple juices.

1.3 Research Objectives

This study aims to establish a rapid, reliable, and cost-effective analytical method for evaluating the authenticity of commercial apple juice and detecting potential adulteration. Specifically, the research focuses on employing (FTIR-ATR) to capture the chemical fingerprint of the samples, enabling the identification of added sugars, dilution, and other forms of quality manipulation. The goals of this work include:

1. Understanding how fresh apple juice appears in FTIR-ATR spectral scans, and comparing these results with those of various industrial juice products found in the local market.
2. Identifying key markers the main constituents of apple juice, particularly sugars and malic acid that show up clearly in pure juice, and noting their spectral locations (wavenumbers) as reference points for comparison.

3. Measuring how much these key indicators (like sugar and acid levels) differ between pure juice and commercial brands, using changes in absorbance values at specific infrared regions.

Chapter Two:

Literature review

This chapter contains the literature review that were read and cited during the study.

2.1 EMA in Apple Juice

The deliberate modification of food products for financial advantage, frequently at the expense of quality and safety, is known as EMA. When it comes to apple juice, EMA usually entails adding less expensive ingredients like sucrose, High Fructose Corn Syrup (HFCS), or other fruit juices to simulate real apple juice, misleading customers and sometimes endangering their health. This review looks at prominent instances, detection techniques, mitigation strategies, and prevalent adulteration procedures in apple juice.

Studies have found unlisted additions of HFCS, grape juice, pear juice, and other sweeteners in commercial apple juice products. Common adulteration practices in apple juice usually involve the addition of low-cost sweeteners like sucrose or HFCS to improve sweetness and increase volume (Johnson, 2014). Another typical method is dilution with water, where manufacturers combine apple juice with water and then add sugars to restore the original taste, allowing them to lower production costs while retaining a similar flavor profile (Banach et al., 2021). Furthermore, some manufacturers mislead consumers about the true quantity and quality of their products by blending apple juice with less expensive fruit liquids like grape, pear, or pineapple juice without properly labelling the product (Johnson, 2014).

The frequency of apple juice adulteration has been brought to light by a number of occurrences, which has raised questions over consumer safety and regulatory monitoring. An FDA inquiry revealed that several apple sauce and puree products included lead-contaminated components in one notable example involving lead contamination in apple goods. These components were added for financial reasons because they were an inexpensive way to add flavor, but they also presented significant health hazards, especially to young children (Formaspace, 2023). Furthermore, reports have shown cases of apple juice containing unlisted ingredients like grape juice, HFCS, and other sweeteners, misleading customers and breaking food labelling laws (Johnson, 2014). These incidents highlight the continuous difficulties in guaranteeing the safety and authenticity of apple juice products.

Various analytical techniques have been established to combat EMA in apple juice. One technique is Near-Infrared (NIR) spectroscopy, which utilizes the spectral data of apple juice to predict adulteration. Studies suggest it can detect adulteration in samples with HFCS at a

threshold of 9.5% (León et al., 2005). Studies have shown that Fourier Transform Infrared (FTIR) spectroscopy may quickly and accurately identify sugar adulterants in apple juice when used in conjunction with chemometric techniques (Kelly et al., 2005). Additionally, certain adulterants, such d-malate, which is not naturally present in apples and acts as a sign of adulteration, can be detected using HPLC (Yamamoto et al., 2008). These methods play an important role in making sure that apple juice products are safe and genuine.

2.2 Fresh and Manufactured Apple Juice

A popular beverage that comes in both fresh and processed varieties is apple juice. The juice's nutritional composition and health benefits may be changed by the manufacturing process, which frequently involves pasteurization and occasionally the inclusion of preservatives. The nutritional value, health effects, and consumer factors of fresh and produced apple juice are the main topics of this review.

Fresh apple juice, typically unpasteurized, contains a higher concentration of vitamins, enzymes, and antioxidants among its natural constituents. Conversely, to extend shelf life, commercially produced apple juice undergoes pasteurization, which may lead to the degradation of certain components, such as vitamin C and certain antioxidants. Moreover, fresh juices often possess a pH that is more neutral compared to their commercial equivalents, potentially influencing their acidity and flavor (Densupsoontorn et al., 2002). Drinking apple juice in moderation may provide several health advantages, including better heart health and lower chances of chronic illnesses. Research indicates that apple juice can have beneficial impacts on indicators associated with the risk of heart disease, especially in reducing oxidative stress (Vallée Marcotte et al., 2022). Drinking too much apple juice, particularly the manufacture kind, may result in higher calorie and sugar intake, which could lead to gaining weight and having metabolic problems. In addition, the elevated sugar levels in certain commercial apple juices can lead to quick increases in blood sugar levels, which might be a worry for those who have diabetes (Liji Thomas, 2019).

When determining whether to purchase fresh or processed apple juice, consumers must acknowledge the distinctions in nutritional content and potential health implications. Fresh apple juice contains higher concentrations of certain nutrients and delivers a more genuine flavor profile. Conversely, commercially produced apple juice is more convenient to store and has a longer shelf life, however it may contain additional sugars and preservatives that could diminish its health advantages. Moreover, the heating method employed in the production of manufactured juices might diminish the levels of beneficial compounds such as vitamin C and antioxidants (UKEssays, 2018).

2.3 Detection of Sugar Adulterants in Apple Juice Using Mid-Fourier Transform Infrared Spectroscopy (Mid-FTIR) Spectroscopy and Chemometrics

Maintaining apple juice's authenticity is crucial for both consumer confidence and legal compliance. There are serious problems with apple juice adulteration, especially when less expensive sugars like cane or beetroot syrups are used. When paired with chemometric analysis, mid-infrared (mid-FTIR) spectroscopy has become a potent tool for identifying these adulterations (Sivakesava et al., 2001).

In order to create a spectral fingerprint specific to a sample's composition, mid-FTIR spectroscopy measures the absorption of infrared light by molecular bonds inside the sample. This technique can be used to detect adulterants in fruit juices because it is very sensitive to the vibrational modes of the functional groups found in sugars (Sivakesava et al., 2001).

The effectiveness of mid-FTIR spectroscopy in identifying beetroot and cane invert sugar adulteration in apple juice was shown by Sivakesava et al. (2001). They used Linear Discriminant Analysis (LDA) to analyse the spectrum area between 800 and 1500 cm^{-1} and obtained 100% and 96.2% classification accuracies for beet and cane invert sugars, respectively. Additionally, standard errors of prediction for Partial Least Squares (PLS) regression models ranged from 1.3% to 1.8% (w/w), with a Coefficient of Determination (R^2) of roughly 0.98, demonstrating accurate quantification capabilities (Sivakesava et al., 2001).

Mid-FTIR spectroscopy combined with chemometric approaches, which are multivariate statistical techniques, improves the interpretation of complex spectral data and makes it easier to identify and measure adulterants.

In order to detect and measure cane sugar adulteration in apple juice, Dhaulaniya et al. (2020) created a chemometric model utilizing Principle Component Analysis (PCA) and PLS regression, which are statistical programs. Using the fingerprint range of 1200 to 900 cm^{-1} as their focus, they found that contaminated samples had a noticeable peak at 997 cm^{-1} that was not present in pure juices. Their model accurately measured adulterant levels as low as 5% and successfully distinguished contaminated samples from pure ones (Dhaulaniya et al., 2020).

There are a number of advantages and disadvantages to using mid-FTIR spectroscopy in conjunction with chemometric analysis for adulterant detection. Rapid analysis is one of its main benefits; it enables prompt screening without requiring a lot of sample preparation, making it a time-efficient technique (Sivakesava et al., 2001). It is also a non-destructive testing method, guaranteeing that samples stay intact for any necessary additional analysis (Meza-Márquez el al., 2010). Additionally, the method's high sensitivity and specificity allow for the precise detection and measurement of trace amounts of adulterants (Dhaulaniya et al., 2020). Nevertheless, there are disadvantages, such as the expense of instruments, as good FTIR spectrometers and chemometric software can be costly, making them inaccessible to certain labs (Sivakesava et al., 2001). Additionally, the technique needs experience because it takes certain skills to evaluate spectrum data and create reliable chemometric models (Meza-Márquez et al., 2010). Despite these difficulties, mid-FTIR spectroscopy is still an effective method for verifying the authenticity of food and identifying commercially driven adulteration.

Several studies have demonstrated the sensitivity and applicability of mid-FTIR spectroscopy combined with chemometric techniques for detecting sugar adulterants in fruit juices. For instance, Dhaulaniya et al. (2020) successfully detected cane sugar adulteration in apple juice at levels as low as 5% using ATR-FTIR and PLS regression, while Meza-Márquez et al. (2010) detected adulteration at 10% and higher with multivariate analysis. Sivakesava et al. (2001) reported high classification accuracy using FTIR and LDA for invert beet and cane sugars, with predictive error margins of 1.3–1.8% (w/w). Based on detection thresholds and classification accuracies reported in these studies, a semi-quantitative scale for interpreting sugar adulteration in apple juice using mid-FTIR can be proposed as shown in Table 5. This scale offers practical guidance for routine screening and authenticity assessment of apple juice samples.

Table 5. Classification of Sugar Adulteration Levels in Apple Juice Based on Mid-FTIR Spectroscopic Analysis.

Adulteration Level	Range (%)	Interpretation	Detection Feasibility (FTIR)	References
Authentic / Pure	0	No adulteration	Not detectable	—
Very Low	1–5	Minimal substitution; difficult to detect without strong calibration	Possible with advanced chemometrics	(Dhaulaniya et al., 2020)
Low	6–10	Low-level adulteration; detectable with sensitive models	Detectable	(Meza-Márquez et al., 2010)
Moderate	11–25	Adulteration clearly affecting composition	Easily detectable	(Dhaulaniya et al., 2020)
High	26–50	Major sugar substitution	Strong spectral deviation	(Sivakesava et al., 2001)
Severe	>50	Heavy adulteration; major quality loss	Very strong deviation	Sivakesava et al., 2001)

Chapter Three:

Methodology

3.1 Introduction

This chapter aims to determine the percentage of fresh apple juice in industrial apple juice using Mid-FTIR, See Figure 1. The methodology includes analyzing different apple varieties, preparing fresh juice samples, measuring transmission values, and comparing them with industrial apple juice samples. Additionally, solutions of malic acid and sugars were prepared and analyzed to understand their spectral behavior.

Figure 1. Mid-FTIR Spectroscopy instrument.

To provide a clear understanding of the experimental design, Figure 2 presents a visual summary of the overall workflow, outlining the key stages from sample collection and

preparation to FTIR-ATR analysis and adulteration detection. This schematic serves as a guide to the detailed procedures described throughout this chapter, ensuring clarity and coherence in the methodology.

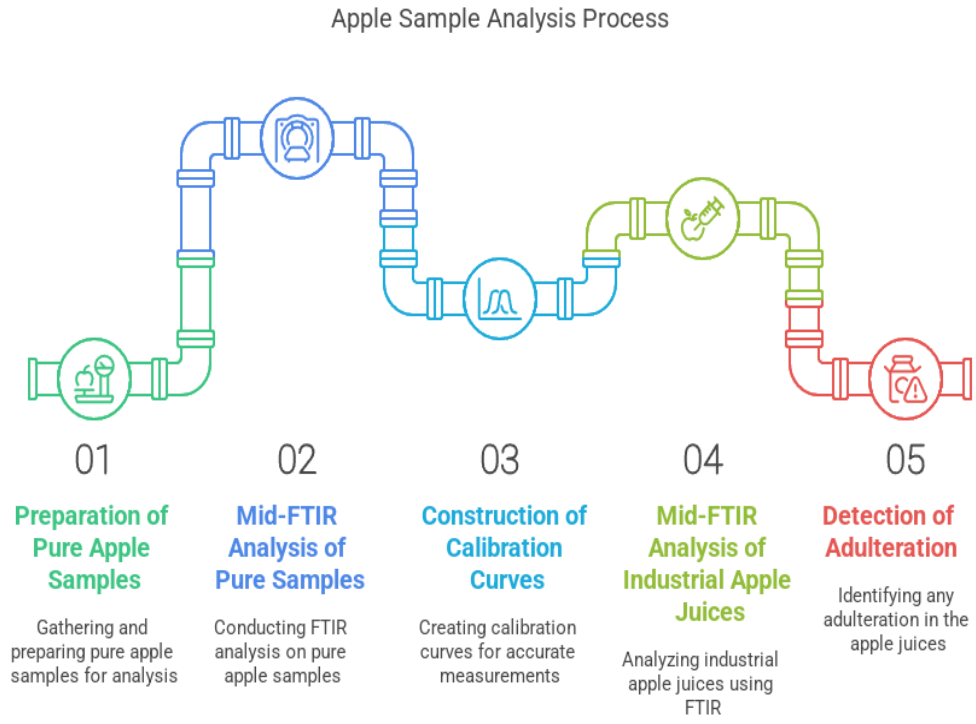


Figure 2. Workflow for detecting apple juice adulteration using Mid-FTIR spectroscopy.

3.2 Sample Collection and Preparation

The following subsections outline the detailed procedures used for preparing and analyzing various juice samples and reference solutions to ensure accurate and reproducible FTIR-based measurements.

3.2.1 Fresh Apple Juice Preparation:

- Four different fresh apple varieties were collected from the local market. See Table 6.
- For each variety, three apples were tested on different days to ensure the reliability of the results.
- The apples were thoroughly washed, cut into small pieces, and juiced using a household juicer.
- The extracted juice was transferred into test tubes and centrifuged at 4000 RPM for one hour at room temperature (25 ± 2°C) to separate the pure apple juice from the pulp.
- The clear supernatant (pure apple juice) was carefully collected for further analysis.
- The clear juice was analyzed within 2 hours of extraction using Mid-FTIR to avoid degradation of sugars and organic acids, because juice contains chemicals easily oxidized with air.

Table 6. Comparison of Apple Varieties.

Image	Scientific Name	Local Name	Color	Taste	Nutrients	References
	Malus domestica	Granny Smith	Green	Tart, crisp	High in fiber, vitamin C, and antioxidants	(Aprifel)
	Malus domestica	Red Delicious	Dark red	Sweet, mildly crisp	Good source of fiber, vitamin C, and polyphenols	(Foundation)
	Malus domestica	Gala	Red with yellow streaks	Sweet, juicy	Contains vitamin C, fiber, and natural sugars	(Food)
	Malus domestica	Golden Delicious	Golden-yellow	Sweet, soft, and mildly tart	High in Potassium, Vitamin C, and Dietary Fibe	(Food)

3.2.2 Preparation of Different Apple Juice Concentrations:

To simulate different dilution levels of fresh apple juice, the pure juice was mixed with distilled water to create the following concentrations:

(3%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% and 100% fresh apple juice).

3.3.3 Malic Acid Solution Preparation

- A 1% (w/v) malic acid stock solution was prepared in distilled water.
- Diluted solutions were made with concentrations ranging from 0.1% to 0.9%.

- Transmission values were measured using Mid-FTIR, and absorbance was calculated using the formula: $A = -\log(T)$

  Where A represents absorbance and T represents transmission.

- The transmission of solid malic acid was measured using FTIR. The transmission data points were then converted to absorbance values in order to plot the spectrum based on the relationship between absorbance and wavelength, see Figure 3. This allowed for the identification of the specific peaks at which malic acid absorbs, and consequently, the determination of the characteristic wavelength(s) of malic acid absorption.
- Solid malic acid was used to obtain clear peaks because liquid malic acid is present in low concentrations in natural apple juice, making it difficult to distinguish its absorption wavelengths due to overlapping signals from water, sugars, and other compounds.
- From this spectrum, the characteristic absorption of malic acid was identified at 1281 cm^{-1}, which serves as a distinguishing wavenumber for malic acid.

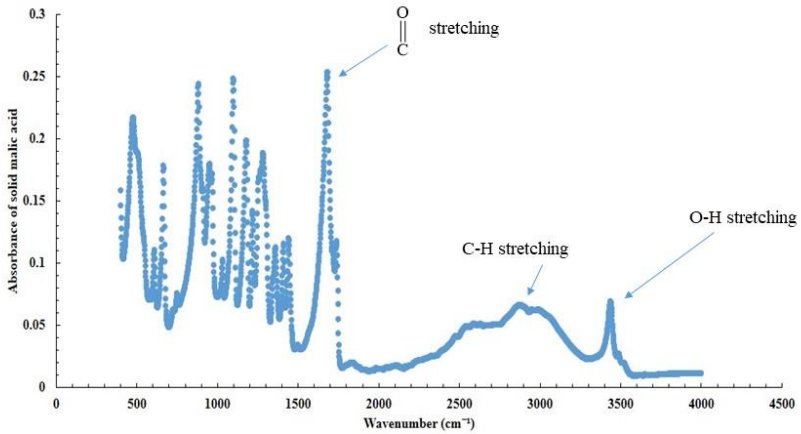


Figure 3. Spectrum of solid malic acid.

3.3.4 Sugar Solution Preparation

- A 25 mL solution containing the sugars fructose, glucose, and sucrose was prepared.
- The concentration of each sugar was based on the mean values reported in a previously published study (Eisele et al., 2005).
- According to the study, the mean concentrations were as follows:
  - Fructose:5690 mg/100 mL
  - Glucose:2.010 mg/100 mL
  - Sucrose: 2.160 mg/100 mL
- These concentrations were used to calculate the appropriate amount of each sugar to be dissolved in the 25 mL solution.
- The prepared solution was then analyzed using the Mid-FTIR spectrometer, and the transmission values were measured.

- The absorbance was calculated using the formula: A= −log (T)

- The transmission values were used to plot the spectrum based on the relationship between transmission and wavelength, see Figure 4. This allowed for the identification of the specific peaks at which sugar absorbs, and consequently, the determination of the characteristic wavelength(s) of sugar absorption.
- From this spectrum, the characteristic absorption of sugars was identified at 1062 cm^{-1}, which serves as a distinguishing wavenumber for sugars.

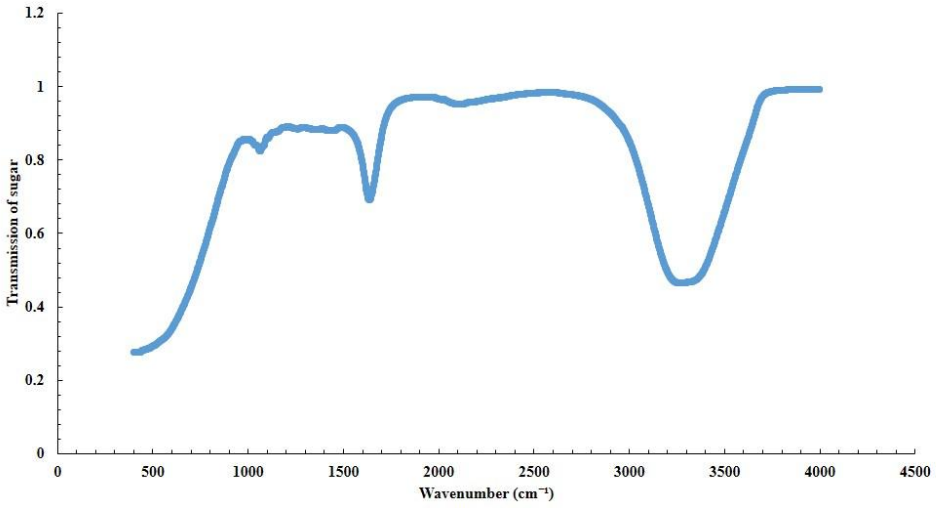


Figure 4. Spectrum of sugar.

3.3.5 Industrial Apple Juice Analysis

- Industrial apple juice samples were collected from the market. See Figure 5.
- Each sample was tested 10 times, using 1 µL per measurement on the Mid-FTIR device.
- Transmission values were recorded and converted to absorbance.

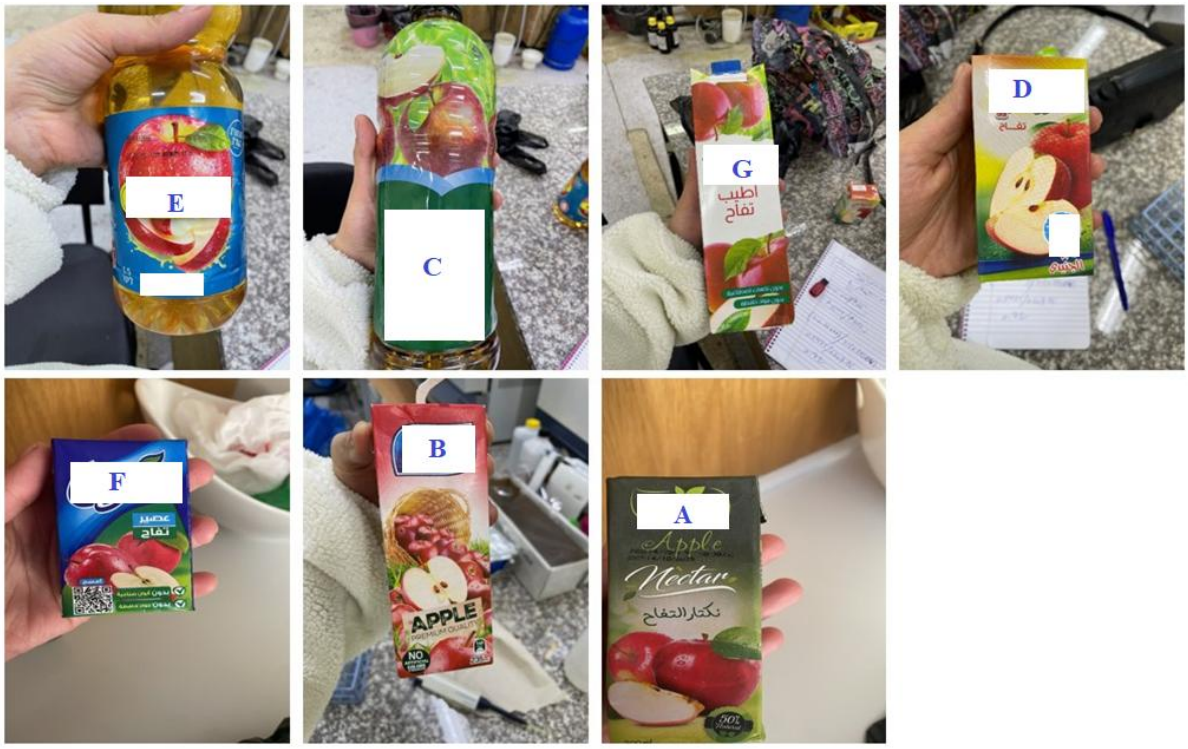


Figure 5. Industrial apple juice samples.

3.3.6 Mid-FTIR Spectral Data Collection

- **Instrument:** Mid-FTIR spectrometer
- **Procedure:**
  - 1 µL of each fresh juice dilution, industrial juice sample, malic acid solution, and sugar solution was analyzed.
  - Transmission (T) was measured at four specific wavelengths: 1634 cm^{-1} (water reference), 1062 cm^{-1} (sugars) and 1281 cm^{-1} (malic acid).
  - Absorbance (A) was calculated using the formula: $A = -\log(T)$

3.4 Data Analysis

- The absorbance values at the selected wavelengths were analyzed to determine correlations between concentration levels and spectral response.
- A calibration curve was developed to quantify the percentage of fresh juice in industrial samples.

3.5 Storage Conditions

- All apple juice samples, both fresh and industrial, were stored at room temperature throughout the study.

3.6 Ensuring Accuracy and Reliability

- Each fresh apple variety was tested on different days for consistency.
- Industrial juice samples were tested 10 times to ensure precise readings.
- Standardized procedures were followed to minimize errors.
- FTIR was calibrated daily using a background scan of pure water.

3.7 Limitations

- Additional ingredients in industrial apple juice could affect the spectral readings.
- Variations in apple juice composition based on apple type and processing methods may impact the results.
- Sugar/malic acid levels fluctuate with apple ripeness and storage.

Chapter Four:

Results & Discussion:

4.1 Overview of Methodology

This study investigated potential adulteration in commercial apple juices using mid-FTIR spectroscopy. Four pure apple juice samples (Red Delicious, Gala, Golden Delicious, Granny Smith) were analyzed to establish calibration curves for sugars (1062 cm^{-1}) and malic acid (1281 cm^{-1}), normalized against a water reference peak (1634 cm^{-1}).These curves served as reference models to assess seven commercial apple juices.

4.2 Absorbance Data

In this study, pure apple juice from four different apple varieties was analyzed using mid-FTIR spectroscopy to identify differences in their spectral profiles.

4.2.1 Red Delicious Apple Juice:

The following table presents the absorbance readings for Red Delicious apple juice at key infrared wavelengths used for sugar (at 1062 cm^{-1}) and for malic acid (at 1281 cm^{-1}). The absorbance was normalized using the water reference peak at 1634 cm^{-1}. These values were used to construct the calibration curve. See Table 7.

Table 7. Absorbance readings for Red Delicious apple juice.

Concentration (mg/L)	Absorbance at 1634 cm^{-1} (water)	Absorbance at 1281 cm^{-1} (malic acid)	Absorbance at 1062 cm^{-1} (sugar)
0	0.15	0.04	0.04
3	0.16	0.04	0.04
5	0.16	0.04	0.05
10	0.16	0.04	0.05
15	0.16	0.04	0.05
20	0.16	0.04	0.05
30	0.16	0.04	0.06
40	0.16	0.05	0.06
50	0.15	0.05	0.07
60	0.15	0.05	0.07
70	0.15	0.05	0.08
80	0.15	0.05	0.09
90	0.15	0.05	0.09
95	0.15	0.05	0.10
100	0.15	0.05	0.10

4.2.2 Gala Apple Juice:

The following table presents the absorbance readings for Gala apple juice at key infrared wavelengths used for sugar (at 1062 cm^{-1}) and for malic acid (at 1281 cm^{-1}). The absorbance was normalized using the water reference peak at 1634 cm^{-1}. These values were used to construct the calibration curve. See Table 8.

Table 8. Absorbance readings for Gala apple juice.

Concentration (mg/L)	Absorbance at 1634 cm^{-1} (water)	Absorbance at 1281 cm^{-1} (malic acid)	Absorbance at 1062 cm^{-1} (sugar)
0	0.16	0.04	0.04
3	0.16	0.04	0.04
5	0.16	0.04	0.05
10	0.16	0.04	0.05
15	0.15	0.04	0.05
20	0.15	0.04	0.05
30	0.15	0.04	0.06
40	0.15	0.05	0.06
50	0.15	0.05	0.07
60	0.15	0.05	0.07
70	0.15	0.05	0.08
80	0.15	0.05	0.09
90	0.15	0.05	0.09
95	0.15	0.05	0.10
100	0.15	0.05	0.10

4.2.3 Golden Delicious Apple Juice:

The following table presents the absorbance readings for Golden Delicious apple juice at key infrared wavelengths used for sugar (at 1062 cm^{-1}) and for malic acid (at 1281 cm^{-1}). The absorbance was normalized using the water reference peak at 1634 cm^{-1}. These values were used to construct the calibration curve. See Table 9.

Table 9. Absorbance readings for Golden Delicious apple juice.

Concentration (mg/L)	Absorbance at 1634 cm^{-1} (water)	Absorbance at 1281 cm^{-1} (malic acid)	Absorbance at 1062 cm^{-1} (sugar)
0	0.16	0.04	0.04
3	0.15	0.04	0.04
5	0.16	0.04	0.05
10	0.15	0.04	0.05
15	0.16	0.04	0.05
20	0.16	0.04	0.05
30	0.16	0.04	0.06
40	0.15	0.05	0.06
50	0.15	0.05	0.07
60	0.15	0.05	0.07
70	0.15	0.05	0.08
80	0.15	0.05	0.08
90	0.15	0.05	0.09
95	0.15	0.05	0.09
100	0.09	0.03	0.06

4.2.4 Granny Smith apple juice:

The following table presents the absorbance readings for Granny Smith apple juice at key infrared wavelengths used for sugar (at 1062 cm^{-1}) and for malic acid (at 1281 cm^{-1}). The absorbance was normalized using the water reference peak at 1634 cm^{-1}. These values were used to construct the calibration curve. See Table 10.

Table 10. Absorbance readings for Granny Smith apple juice.

Concentration (mg/L)	Absorbance at 1634 cm^{-1} (water)	Absorbance at 1281 cm^{-1} (malic acid)	Absorbance at 1062 cm^{-1} (sugar)
0	0.16	0.04	0.04
3	0.16	0.04	0.04
5	0.15	0.04	0.04
10	0.15	0.04	0.05
15	0.15	0.04	0.05
20	0.15	0.04	0.05
30	0.15	0.04	0.05
40	0.15	0.04	0.06
50	0.15	0.05	0.06
60	0.15	0.05	0.06
70	0.15	0.05	0.07
80	0.15	0.05	0.07
90	0.15	0.05	0.07
95	0.15	0.05	0.08
100	0.15	0.05	0.08

4.3 Selection of Apple Samples for Calibration Curves

To construct calibration curves for both sugars and malic acid using FTIR, apple sample number (1) from each of the four apple types (Red Delicious, Gala, Golden Delicious, and Granny Smith) was selected. This selection was based on scientific criteria ensuring representative and reliable calibration, as detailed below:

- Statistical Representativeness:

  The sugar and malic acid concentrations of sample #1 in each group were close to the average values of the three samples per type, making them good representatives of their respective apple types.

- Absence of Outliers:

  None of the selected apples exhibited extreme high or low concentrations, which helps avoid introducing anomalies that could negatively impact the accuracy of the calibration curve.

- Consistency and Stability:

  The selected samples showed consistent values and minimal variation when compared to the other two apples in each group, indicating stable and uniform chemical composition.

- Adequate Coverage of Concentration Range:

  Although values within each type were relatively close, the selection of sample #1 from each group ensured sufficient variability across the four types to cover a suitable concentration range for both sugar and malic acid calibration.

Accordingly, the use of these samples provides a sound and representative basis for accurate calibration of sugar and malic acid concentrations in apple juice using FTIR. See Table 11 and Table 12.

Table 11. Sugar Concentration Data of Apple Juice Samples Used for Calibration Curve Construction (All Varieties).

Juice Sample	RD #1	RD #2	RD #3	G #1	G #2	G #3	GD #1	GD #2	GD #3	GS #1	GS #2	GS #3
A	90.21	106.71	86.48	90.81	88.60	87.39	98.37	99.86	97.34	120.09	104.14	111.15
B	92.48	109.34	88.63	93.04	90.76	89.50	100.82	102.12	99.46	122.90	106.35	113.43
C	79.57	94.34	76.37	80.32	78.47	77.48	86.83	89.05	87.39	106.85	93.53	99.70
D	68.09	80.98	65.45	68.99	67.54	66.77	74.38	77.44	76.72	92.55	81.94	87.65
E	67.43	80.20	64.83	68.35	66.92	66.17	73.67	76.85	76.08	91.74	80.81	86.32
F	75.36	89.45	72.37	76.17	74.47	73.56	82.27	84.87	83.60	101.61	89.40	95.08
G	81.15	96.17	77.87	81.88	79.98	78.95	88.54	90.62	88.98	108.81	95.17	101.30

Note. “RD” represents the Red Delicious apple juice. “G” represents the Gala apple juice. “GD” represents the Golden Delicious apple juice. “GS” represents the Granny Smith apple juice. “#1, #2, #3” represents the number of apple.

Table 12. Sugar Concentration Data of Apple Juice Samples Used for Calibration Curve Construction (All Varieties).

Juice Sample	RD #1	RD #2	RD #3	G #1	G #2	G #3	GD #1	GD #2	GD #3	GS #1	GS #2	GS #3
A	94.34	110.84	88.26	94.35	97.55	85.30	98.59	110.21	97.35	98.85	111.50	112.31
B	103.35	121.34	96.94	103.13	108.42	94.67	108.01	120.48	103.91	107.66	123.62	121.74
C	85.21	99.32	79.32	84.63	88.66	77.68	88.75	98.72	90.26	89.65	99.06	102.61
D	56.61	64.27	51.23	55.16	58.74	51.12	58.01	63.48	68.51	60.87	64.25	71.98
E	64.30	74.42	58.93	63.55	66.98	59.49	66.76	74.16	74.13	69.06	74.19	80.73
F	79.26	92.43	74.03	78.65	82.40	72.48	82.52	91.94	85.40	83.81	92.13	96.91
G	91.34	106.26	84.97	90.54	94.41	82.58	94.92	105.90	94.43	95.42	107.54	109.10

Note. “RD” represents the Red Delicious apple juice. “G” represents the Gala apple juice. “GD” represents the Golden Delicious apple juice. “GS” represents the Granny Smith apple juice. “#1, #2, #3” represents the number of apple.

4.4 Calibration Curves

Calibration curves for both sugar and malic acid were developed using pure apple juice extracted from four different apple varieties. For each variety, juice was prepared and analyzed using mid-FTIR spectroscopy. The absorbance values were measured at specific wavelengths corresponding to the functional groups (C-H stretching, O-H stretching and C double bond O) of sugars and malic acid.

For sugar, absorbance was measured at 1062 cm^{-1}, which is characteristic of carbohydrate functional groups, and normalized against 1634 cm^{-1}, the reference peak for water. For malic acid, absorbance was measured at 1281 cm^{-1}, a specific peak associated with malic acid, and normalized against 1634 cm^{-1}, the reference peak for water. The ratio of absorbance at 1634 to 1281 cm^{-1} and the ratio of absorbance at 1634 to 1062 cm^{-1} were calculated for each pure apple sample and used to construct the calibration curve. See Figure 6 and Figure 7.

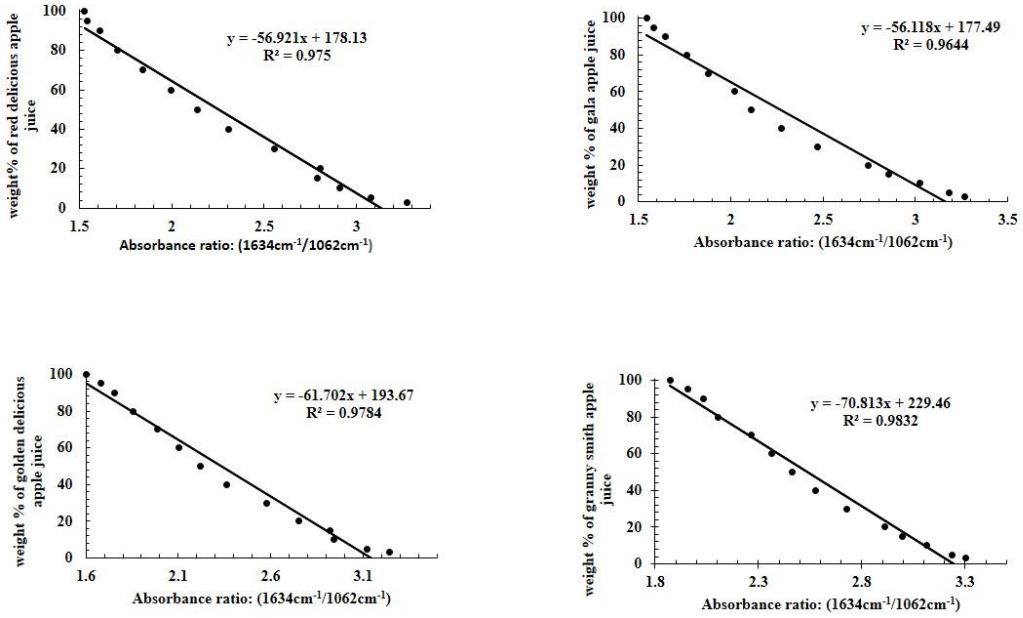


Figure 6. Calibration curves for sugars.

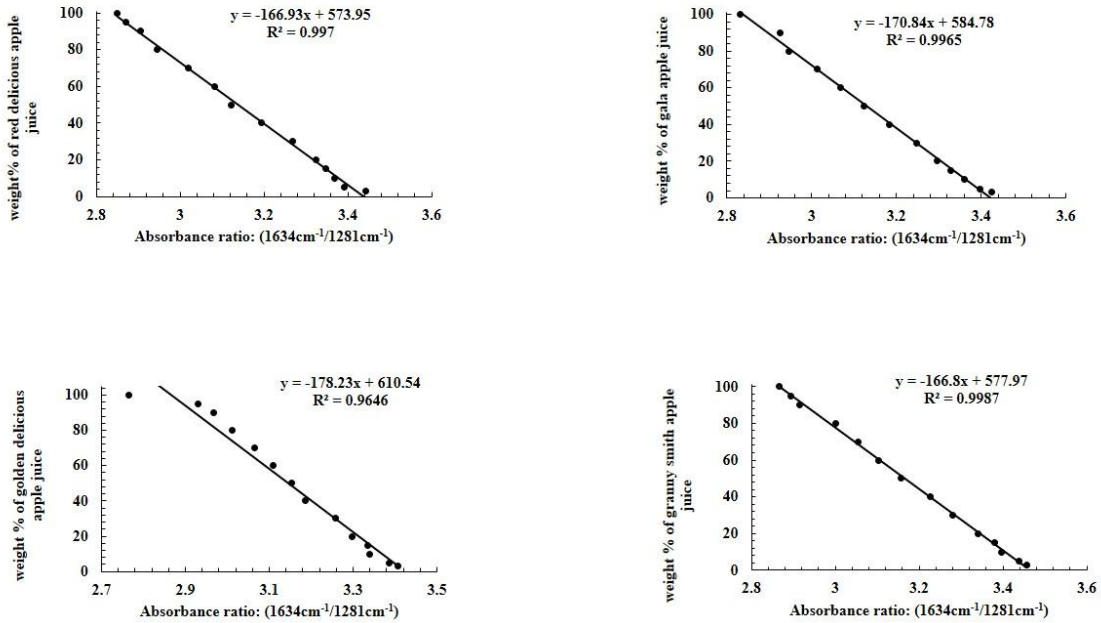


Figure 7. Calibration curves for malic acid.

Each apple variety was analyzed using three individual apples to ensure reliability and reproducibility. From each set, the curve generated from Apple #1 was selected for use in industrial juice evaluation based on its consistency, linearity, and minimal variation. These calibration curves were later applied to determine sugar and malic acid concentrations in commercial apple juice samples and to assess potential adulteration.

It is important to keep in mind that each commercial sample's estimated natural juice content is not determined by comparing it directly to a different pure juice sample. Instead, the degree of alignment between the calibration curves made from pure apple juices and the sample's FTIR absorbance ratios is used to infer it. This offers a consistent and repeatable method for determining the juice content's validity.

The workflow for detecting adulteration Figure 8 combines calibration curves derived from pure juices with FTIR analysis at key wavelengths - 1062 cm^{-1} (sugars) and 1281 cm^{-1} (malic acid), normalized to the water peak (1634 cm^{-1}) - to estimate adulteration levels in commercial samples.

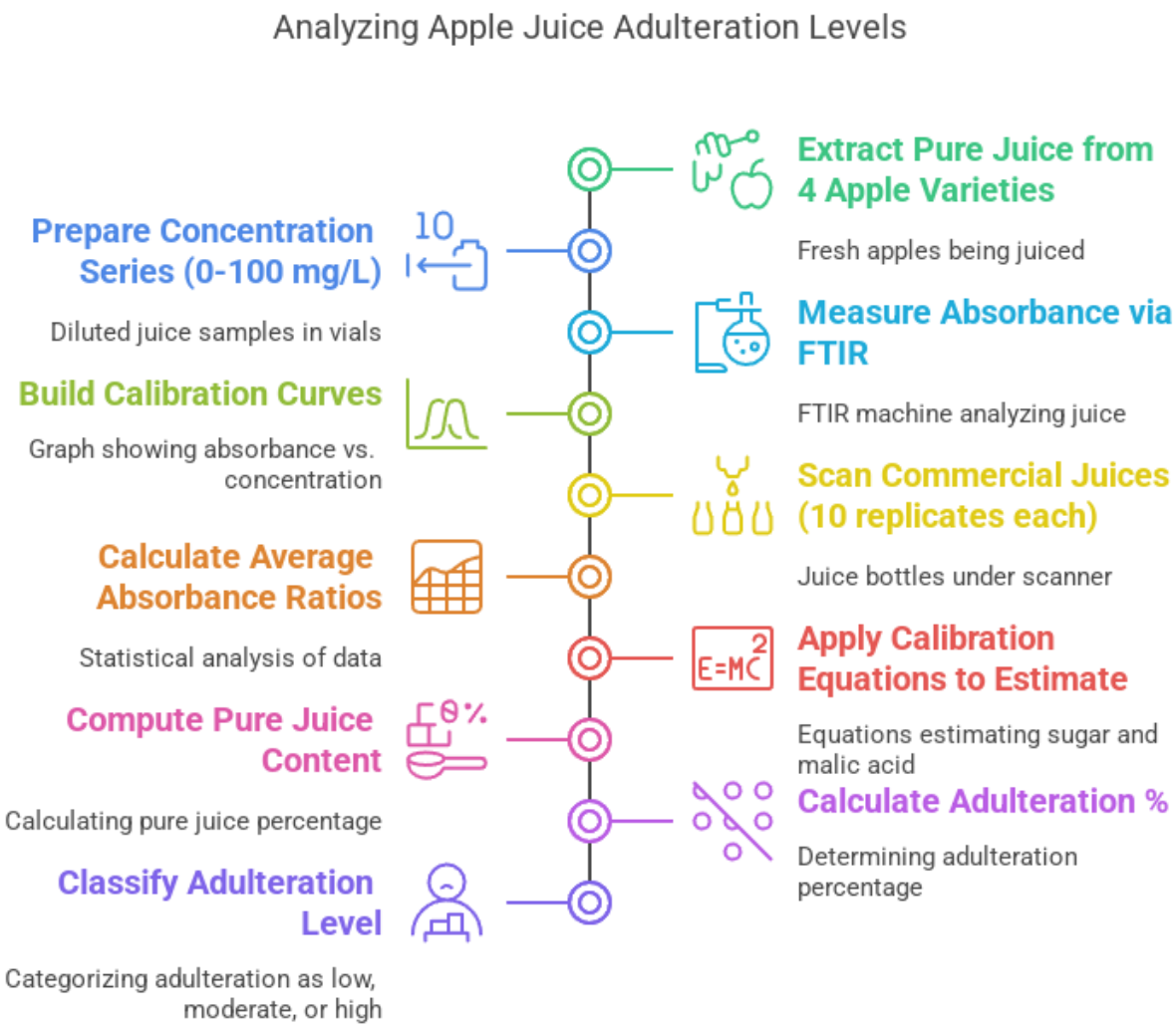


Figure 8. Workflow for detecting adulteration in apple juice, from pure juice extraction to FTIR analysis and adulteration calculation.

The same FTIR procedure was used to quantify the sugar and malic acid content of commercial juices once calibration curves had been constructed.

4.5 FTIR Analysis of Commercial Apple Juices

Each of the seven commercial juice samples was scanned ten times using the FTIR device. The transmission spectra were recorded and converted to absorbance values for each juice, absorbance values were collected at the relevant wavelengths for sugars (1062 cm^{-1}) and for malic acid (1281 cm^{-1}), as well as at 1634 cm^{-1}, which was used as a water reference band. See Figure 9 and Figure 10.

Table 1: Absorbance values for A apple juice.

No.	A 1634 cm^{-1}	A 1281 cm^{-1}	A 1062 cm^{-1}
1	0.1572	0.0551	0.1033
2	0.1575	0.0551	0.1034
3	0.1576	0.0551	0.1035
4	0.1574	0.0551	0.1036
5	0.1567	0.0551	0.1033
6	0.1564	0.0541	0.0995
7	0.1566	0.0541	0.0998
8	0.1563	0.0541	0.0999
9	0.1572	0.0541	0.1000
10	0.1568	0.0541	0.1001

Note. "No." refers to the number of times the juice sample was tested. "A" represents absorbance values at the specified wavenumbers.

Table 2: Absorbance values for B apple juice.

No.	A 1634 cm^{-1}	A 1281 cm^{-1}	A 1062 cm^{-1}
1	0.1585	0.0558	0.1033
2	0.1572	0.0554	0.1023
3	0.1513	0.0538	0.1006
4	0.1586	0.0560	0.1048
5	0.1581	0.0562	0.1058
6	0.1572	0.0555	0.1032
7	0.1561	0.0558	0.1065
8	0.1586	0.0567	0.1081
9	0.1581	0.0560	0.1050
10	0.1581	0.0560	0.1051

Note. "No." refers to the number of times the juice sample was tested. "A" represents absorbance values at the specified wavenumbers.

Table 3: Absorbance values for C juice.

No.	A 1634 cm^{-1}	A 1281 cm^{-1}	A 1062 cm^{-1}
1	0.1576	0.0533	0.0907
2	0.1585	0.0540	0.0913
3	0.1579	0.0533	0.0905
4	0.1556	0.0523	0.0884
5	0.1583	0.0539	0.0913
6	0.1591	0.0542	0.0926
7	0.1587	0.0539	0.0914
8	0.1581	0.0536	0.0905
9	0.1581	0.0538	0.0913
10	0.0751	0.0272	0.0448

Note. "No." refers to the number of times the juice sample was tested. "A" represents absorbance values at the specified wavenumbers.

Table 4: Absorbance values for D apple juice.

No.	A 1634 cm^{-1}	A 1281 cm^{-1}	A 1062 cm^{-1}
1	0.1564	0.0505	0.0810
2	0.1579	0.0516	0.0841
3	0.1533	0.0478	0.0744
4	0.1524	0.0465	0.0711
5	0.1572	0.0511	0.0824
6	0.1570	0.0509	0.0822
7	0.1590	0.0523	0.0858
8	0.1566	0.0513	0.0835
9	0.1566	0.0511	0.0830
10	0.1570	0.0512	0.0829

Note. "No." refers to the number of times the juice sample was tested. "A" represents absorbance values at the specified wavenumbers.

Figure 9. Absorbance values for industrial apple juices brands (A, B, C, and D).

Table 5: Absorbance values for E apple juice.

No.	A 1634 cm^{-1}	A 1281 cm^{-1}	A 1062 cm^{-1}
1	0.1588	0.0525	0.0832
2	0.1590	0.0525	0.0829
3	0.1595	0.0527	0.0841
4	0.1572	0.0516	0.0815
5	0.1583	0.0524	0.0834
6	0.1593	0.0524	0.0830
7	0.1597	0.0526	0.0838
8	0.1589	0.0525	0.0832
9	0.1562	0.0490	0.0726
10	0.1587	0.0515	0.0787

Note. "No." refers to the number of times the juice sample was tested. "A" represents absorbance values at the specified wavenumbers.

Table 6: Absorbance values for F apple juice.

No.	A 1634 cm^{-1}	A 1281 cm^{-1}	A 1062 cm^{-1}
1	0.1601	0.0536	0.0871
2	0.1597	0.0535	0.0871
3	0.1597	0.0535	0.0871
4	0.1595	0.0535	0.0872
5	0.1594	0.0536	0.0875
6	0.1598	0.0538	0.0883
7	0.1598	0.0539	0.0887
8	0.1584	0.0539	0.0890
9	0.1581	0.0539	0.0897
10	0.1585	0.0541	0.0907

Note. "No." refers to the number of times the juice sample was tested. "A" represents absorbance values at the specified wavenumbers.

Table 7: Absorbance values for G apple juice.

No.	A 1634 cm^{-1}	A 1281 cm^{-1}	A 1062 cm^{-1}
1	0.1575	0.0541	0.0923
2	0.1234	0.0436	0.0745
3	0.1593	0.0548	0.0944
4	0.1552	0.0528	0.0895
5	0.1585	0.0545	0.0938
6	0.1589	0.0545	0.0934
7	0.0768	0.0281	0.0451
8	0.1572	0.0536	0.0913
9	0.1590	0.0545	0.0937
10	0.1566	0.0530	0.0899

Note. "No." refers to the number of times the juice sample was tested. "A" represents absorbance values at the specified wavenumbers.

Figure 10. Absorbance values for industrial apple juices brands (E, F and G).

4.6 Determination of Sugar and Malic Acid Content

The absorbance ratio was calculated by dividing the absorbance at 1634 cm^{-1} by the absorbance at each target wavelength, and then calculated the average of absorbance ratio. See Table 13.

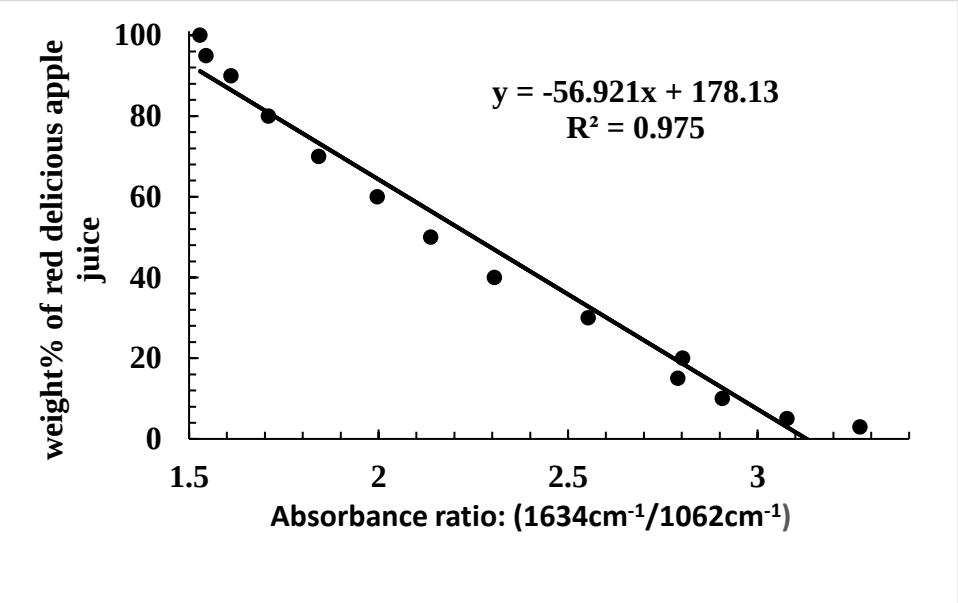
Table 13. The average of the absorbance ratio of the industrial juices.

Industrial apple juice	Avg. of Absorbance Ratio (1634/1281 cm^{-1})	Avg. of Absorbance Ratio (1634/ 1062 cm^{-1})
A apple juice	2.87	1.54
B apple juice	2.81	1.50
C apple juice	2.92	1.73
D apple juice	3.10	1.93
E apple juice	3.05	1.94
F apple juice	2.96	1.80
G apple juice	2.89	1.70

Note. "Avg." represents the average of absorbance ratio.

The average ratio from ten readings was substituted into the corresponding calibration curve equations to determine the concentration of sugars and malic acid in each commercial juice. See Example 1 and Example 2.

- Example 1: calculation for the sugar content in A apple juice according to the Red Delicious apple juice.



y= -56.921x + 178.13

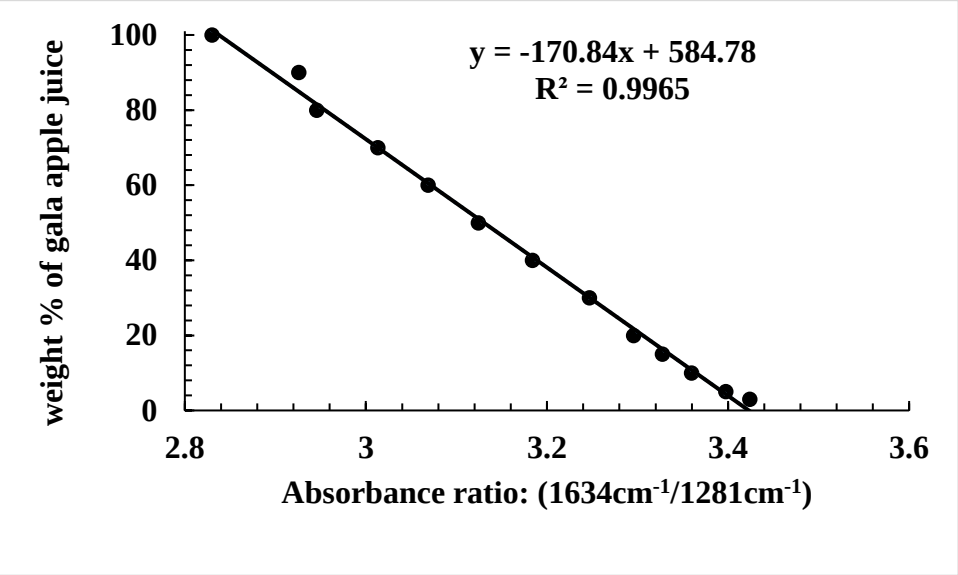
Where x represents the average of absorbance ratio at 1634/1062 cm^{-1} for the A juice which equal 1.54.

y = -56.921* 1.54+ 178.13

y= 90.17

These calculations were applied to each industrial apple juice sample, based on the calibration curves of the four apple varieties, to determine the sugar content. The obtained results are summarized in a table presenting the corresponding sugar percentages. See Table 14.

- Example 2: calculation for the malic acid content in E apple juice according to the Gala apple juice.



y = -170.84x + 584.78

Where x represents the average of absorbance ratio at 1634/1281 cm^{-1} for the E juice which equal 3.05.

y = -170.84*3.05+ 584.78

y = 63.55

These calculations were applied to each industrial apple juice sample, based on the calibration curves of malic acid at 1281 cm^{-1}, to determine the malic acid content. The obtained results are summarized in a table presenting the corresponding malic acid percentages. See Table 14.

Table 14. Summarized table presenting the corresponding sugar and malic acid percentages in the industrial juices.

Industrial apple juice sample	Sugar–Red Delicious (%)	Sugar-Gala (%)	Sugar-Golden Delicious (%)	Sugar-Granny Smith (%)	Malic-Red Delicious (%)	Malic-Gala (%)	Malic-Golden Delicious (%)	Malic-Granny smith (%)
A juice	90.21	90.81	98.37	120.09	94.34	94.35	98.59	98.85
B juice	92.48	93.04	100.82	122.90	103.35	103.13	108.01	107.66
C juice	79.57	80.32	86.83	106.85	85.21	84.63	88.75	89.65
D juice	68.09	68.99	74.38	92.55	56.61	55.16	58.01	60.87
E juice	67.43	68.35	73.67	91.74	64.30	63.55	66.76	69.06
F juice	75.36	76.17	82.27	101.61	79.26	78.65	82.52	83.81
G juice	81.15	81.88	88.54	108.81	91.34	90.54	94.92	95.42

4.7 Estimation of Adulteration Percentage Based on FTIR Calibration

This study used Mid-FTIR spectroscopy to determine the amount of sugar and malic acid in commercial apple juices in order to identify possible adulteration. The assessment was carried out in two steps: first, the concentration of sugar was estimated as an initial indicator, and then the concentration of malic acid was determined as a secondary and more reliable indicator.

Due to its widespread and low-cost availability in the market, sugar was chosen as the first signal. It is a popular ingredient used in commercial juice manufacturing to simulate natural sweetness and confuse consumers. Malic acid, the main organic acid found naturally in apples, is rarely added chemically since it is far more costly and less widely available than sugar. As a result, its presence offers a more accurate estimate of the true amount of natural juice.

This method offers a simple, low-cost, and easily accessible strategy that does not require complicated tools or processes. It can be used as an initial screening method to spot potential adulteration and decide whether more complex and accurate studies, such as HPLC, are necessary.

4.8 Calculation Method

To estimate the percentage of adulteration in each industrial apple sample juice, the following steps were taken:

1. For each juice sample, the sugar content (%) was measured using four types of apples: Red Delicious, Gala, Golden Delicious and Granny Smith.
2. The malic acid content (%) was also measured using the same four apple types.
3. The average sugar percentage and the average malic acid percentage were calculated for each sample:

- Avg. sugar = mean (sugar from Red, sugar from Gala, sugar from Golden and sugar from Granny).
- Avg. malic acid = mean (malic from Red, malic from Gala, malic from Golden and malic from Granny).

4. Then, the average pure juice content was estimated as the mean of both average:
   - Avg. pure juice content = (Avg. sugar + Avg. malic acid) / 2
5. Finally, the percentage of adulteration was calculated as:
   - Adulteration (%) = 100 – Avg. pure juice content. See Example 3 for more details.

To detect potential adulteration in commercial apple juices, this study applied a two-step strategy based on Mid-FTIR spectral analysis. The first indicator was sugar concentration, selected due to its widespread and inexpensive use in juice manufacturing to simulate sweetness and compensate for dilution. While sugar is often added artificially, it is not a definitive marker of natural juice content, as its levels can be manipulated.

As a second and more reliable indicator, malic acid concentration was measured. Malic acid is the dominant organic acid naturally present in apples and is rarely added during manufacturing due to its higher cost and limited commercial availability. Thus, the malic acid profile offers a more accurate reflection of the true natural juice content.

By comparing the sugar and malic acid levels in each commercial sample against calibration curves built from pure apple juices, the study was able to estimate the actual juice content and calculate the degree of adulteration. This dual-indicator approach balances sensitivity and specificity, enabling effective detection of economic adulteration.

Example 3: The calculation process using the data of C juice:

1. Extract sugar data for four apple types:
   - Red Delicious: 79.57%
   - Gala: 80.32%
   - Golden Delicious: 86.83%
   - Granny Smith: 106.85%

Avg. sugar = (79.57 + 80.32 + 86.83 + 106.85) / 4

= 353.57 / 4

= 88.39%

2. Extract malic acid data:
   - Red Delicious: 85.21%
   - Gala: 84.63%
   - Golden Delicious: 88.75%
   - Granny Smith: 89.65%

Avg. malic acid = (85.21 + 84.63 + 88.75 + 89.65) / 4

= 348.24 / 4

= 87.06%

3. Calculate average pure juice content:

Avg. pure juice content = (88.39 + 87.06) / 2

= 87.73%

4. Calculate adulteration percentage:
Adulteration % = 100 – 87.73
=12.27%

The same steps were followed for the remaining industrial juice samples, and the final results were included in the following table. See Table 15.

Table 15. Results of Adulteration Analysis in Industrial Apple Juices.

Juice Sample	Avg. pure Juice Content (%)	Adulteration (%)
A	98.20	1.80
B	103.92	-3.92
C	87.73	12.27
D	66.83	33.17
E	70.61	29.39
F	82.46	17.54
G	91.58	8.42

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4.9 Interpretation of Adulteration Results

- A Juice
  - Estimated Natural Juice Content: 98.20%

- Adulteration Percentage: 1.80%

Interpretation: The sugar and malic acid content in this juice is very close to natural levels, indicating a high proportion of real apple juice or a carefully formulated blend of additives. The low adulteration percentage suggests that the product was not significantly diluted and may contain apple concentrate or a well-balanced formulation mimicking natural juice.

- B Juice
  - Estimated Natural Juice Content: 103.92%
  - Adulteration Percentage: -3.92%

Interpretation: B juice had values above 100% (103.92%), which could mean that either (1) extremely concentrated apple extract was used in excess of the usual single-strength juice levels, or (2) isolated apple components (such as sugar fractions or refined malic acid) were added to improve flavour profiles. Instead of only dilution, this artificial elevation above natural composition raises the possibility of technological processing.

Such enhancement, while not traditional adulteration, deviates from freshly pressed juice composition. This discrepancy could conflict with labeling regulations in certain jurisdictions, particularly those governing 'natural' claims or concentrate disclosure.

- C Juice
  - Estimated Natural Juice Content: 87.73%
  - Adulteration Percentage: 12.27%

Interpretation: Relatively high sugar content combined with slightly lower malic acid levels may indicate that sugar was added to enhance sweetness, while no effort was made to match the natural acid profile. This points to a partial imitation of natural juice, resulting in a moderate adulteration level.

- D Juice
  - Estimated Natural Juice Content: 66.83%
  - Adulteration Percentage: 33.17%

Interpretation: Low levels of both sugar and malic acid suggest significant dilution or use of synthetic flavoring and sweeteners with minimal natural apple content. This sample represents the highest adulteration among all tested juices, likely due to the replacement of apple juice with cheaper alternatives.

- E Juice
  - Estimated Natural Juice Content: 70.61%
  - Adulteration Percentage: 29.39%

Interpretation: Similar to D juice, this juice shows reduced sugar and malic acid levels, indicating substantial dilution or minimal use of natural apple ingredients. This high adulteration percentage suggests a lower-quality product with limited authentic content.

- F Juice
    - Estimated Natural Juice Content: 82.46%
    - Adulteration Percentage: 17.54%

  Interpretation: Moderate sugar and malic acid levels suggest partial use of natural apple content or diluted concentrate. The medium adulteration level implies a cost-reduction strategy, likely by minimizing real fruit content while maintaining acceptable taste.

- G Juice
    - Estimated Natural Juice Content: 91.58%
    - Adulteration Percentage: 8.42%

      Interpretation: This juice is relatively close to natural levels, with a balanced sugar and malic acid profile. It may contain a high percentage of apple concentrate or be slightly diluted. The low adulteration percentage indicates an overall good quality compared to others.

4.10 Limitations

While this technique can detect sugar-malic acid differences to screen for adulteration, it cannot identify specific adulterants (e.g., HFCS). FTIR and HPLC may be combined in future research to accurately characterise adulterants.

Chapter five:

Conclusion And Recommendations

5.1 Conclusion

In conclusion, FTIR spectroscopy has proven to be an efficient, rapid, and straightforward technique for the quantitative analysis of apple juice adulteration. It offers a valuable preliminary estimation of adulteration levels without requiring complex sample preparation. However, for more precise and confirmatory quantification, advanced analytical techniques such as HPLC are recommended.

5.2 Recommendations

Based on the findings of this study, several recommendations can be made to support better practices in juice production, quality control, and food safety:

1. Looking at whether FTIR-ATR is a practical choice for everyday use in food labs — especially in places that may not have access to more expensive or time-consuming techniques like chromatography.
2. Adopt FTIR-ATR spectroscopy in routine food analysis labs, especially those focused on fruit juice authentication. Its speed, simplicity, and accuracy make it a valuable tool for detecting sugar adulteration and assessing product quality without the need for expensive or complex procedures.
3. Regulatory authorities such as the Palestinian Standards Institution (PSI**)** should consider integrating spectroscopic methods into their inspection protocols for fruit juices and other processed beverages. This would strengthen food monitoring systems and help prevent adulterated products from entering the market.
4. Raise consumer awareness about juice authenticity and labeling. Educational campaigns can help customers understand what makes a juice “natural” and how to recognize signs of excessive processing or sugar addition.
5. Future research should expand this work to include a wider range of juice types and adulterants, such as artificial sweeteners, acids, or colorants. This will help build a more complete database of spectral markers that can support broader applications in food authentication.
6. Collaboration between universities, research centers, and industry is encouraged to improve access to analytical technologies and support the training of specialists in modern food quality testing.

By applying these recommendations, food producers, regulators, and researchers can work together to ensure that what reaches consumers is safe, natural, and truly what the label claims.

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استخدام التحليل الطيفي بتقنية FTIR-ATR لضبط نوعية ومصداقية والتحقق من أصالة عصير التفاح

إعداد: رناد رياض محمود رمضان

إشراف: د. عامر مرعي

ملخص

فاكهة التفاح وعصيرها من أشهر وأكثر الفواكه شهرة واستهلاك، وذلك بسبب مذاقها الحلو وقيمتها الغذائية والصحية. ولكن، للأسف ازدياد الطلب والحوافز لزيادة المكاسب المالية في صناعة العصائر أدى إلى زيادة إمكانية الغش سواء بإضافة السكر أو استبدال المكونات بمكونات أقل تكلفة، وبالتالي يؤثر سلبا على جودة المنتج وثقة المستهلك.

الهدف من هذا العمل، هو دراسة استخدام تقنية FTIR-ATR كوسيلة سريعة، بسيطة وغير مكلفة لتقييم جودة عصير التفاح الصناعي وكشف الغش في هذه العصائر إن وجد.

هذه الدراسة اعتمدت على تحضير عينات من أربع أنواع من التفاح

(Red Delicious, Gala, Golden Delicious, and Granny Smith) من أجل الحصول على تراكيز مختلفة من هذه العينات (تم التخفيف باستخدام الماء المقطر). وأيضاً تم تحضير محاليل معيارية للسكر وحمض الماليك، بالإضافة لاستخدام سبع منتجات من عصائر التفاح الصناعية من السوق المحلي.

تم اعتماد الأطوال الموجية للأشعة تحت الحمراء عند 1062 سم$^{-1}$(الطول الموجي الخاص بالسكر)، 1281 سم$^{-1}$ (الطول الموجي الخاص بحمض الماليك) و1634 سم$^{-1}$(الطول الموجي الخاص بالماء) من اجل قياس امتصاصية العينات.

ظهرت اختلافات ملحوظة عند مقارنة عينات العصائر الصناعية وقيم العصير النقي بالاعتماد على المنحنيات المعيارية الناتجة من تراكيز عصير التفاح النقي استناداً على نسبة السكر وحمض الماليك. هذا الأمر قد يشير إلى إحتمالية زيادة السكر للمنتج الصناعي أو أنه تم تخفيفه.

من خلال حساب نسبة العصير الطبيعي في العينات الصناعية استناداً على السكر وحمض الماليك تم إثبات أن هناك نسبة غش بمستويات مختلفة، حيث أظهر عصير "A" وهو منتج صناعي أعلى نسبة مقدرة من عصير التفاح الطبيعي بنسبة 98.20%, عماً بأن "فريش" هو أسم المنتج التجاري وليس دليلاً على الجودة الطبيعية للمنتج. في المقابل، أظهرت النتائج أن كل من عصيري "D" و "E" حققا أقل نسبة من العصر الطبيعي بنسبتي غش مقدرتين 33.17% و29.39% على التوالي.

تدعم هذه النتائج إمكانية استخدام تقنية FTIR-ATR في صناعة العصائر كوسيلة سريعة وفعالة للرقابة السريعة على الجودة. حيث إن هذه التقنية تتميز بميزة مهمة بالمقارنة بالتقنيات المخبرية التقليدية الأخرى، وهي قدرتها على الكشف عن الفروقات الكيميائية الدقيقة. وبالتالي، تتيح للمصنعين وجهات الرقابية وسلطات سلامة الغذاء من ضمان أصالة المنتجات والحد من إمكانية الغش وبالتأكيد حماية صحة المستهلك بطريقة سهلة وفعالة التطبيق.