E. Ü. FEN BİLİMLERİ ENSTİTÜSÜ

EGE UNIVERSITY

MASTER THESIS

IDENTIFICATION STUDIES FOR HONEY, GRAPE SYRUP AND FRUIT JUICE PRODUCED IN TURKEY BY USING HPLC AND $^{13}C/^{12}C$ ISOTOPE RATIO MASS SPECTROMETRIC METHODS

Barış GÜMÜŞTAŞ

Supervisors : Prof. Dr. F. Nil ERTAŞ

Asist. Prof. Dr. Hasan ERTAŞ

Department of Chemistry

Code of Dicipline : 405.03.01
Date of Presentation : 04.12.2009

Bornova-İZMİR
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Barış GÜMÜŞTAŞ tarafından **Yüksek Lisans Tezi** olarak sunulan **"Identification studies for honey, grape syrup and fruit juice produced in Turkey by using HPLC and $^{13}C/^{12}C$ Isotope Ratio Mass Spectrometric Methods"** başlıklı bu çalışma E.Ü. Lisansüstü Eğitim ve Öğretim Yönetmeliği ile E.Ü. Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 04.12.2009 tarihinde yapılan tez savunma sınavında aday oybirliği/oyçokluğu ile başarılı bulunmuştur.

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Jüri Başkanı	**:** Prof. Dr. F. Nil ERTAŞ	**.....**
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Üye	**:** Prof. Dr. Ali ÇELİK	**.....**

ÖZET

TÜRKİYE'DE ÜRETİLEN BAL, PEKMEZ VE MEYVE SUYU ÖRNEKLERİNİN HPLC VE $^{13}C/^{12}C$ İZOTOP ORANLARI KÜTLE SPEKTROMETRİSİ YÖNTEMLERİ İLE KİMLİK ANALİZİ

GÜMÜŞTAŞ, Barış

Yüksek Lisans Tezi, Kimya Bölümü-Analitik Kimya
Tez Yöneticileri: Prof. Dr. F. Nil ERTAŞ veYrd. Doç. Dr. Hasan ERTAŞ
Aralık 2009, 44 sayfa

Dünyada bal, pekmez ve meyve suyu üretiminde önemli rol oynayan ülkemizde, hileli ürün üretiminin yaygınlaşması nedeniyle son yıllarda ihracat sorunları yaşanmaktadır. Bala hile amacıyla katılan maddeler geleneksel mısır şurubu, yüksek fruktozlu mısır şurubu ve asitle dönüştürülen şuruplardır. Benzer yolla ülkemizde büyük çapta üretimi yapılan pekmez, meyve suyu ürünlerinin de saflık analizi giderek önem kazanmıştır. Bu tez kapsamında bu ürünlerin $^{13}C/^{12}C$ oranının IRMS, şeker bileşenleri miktarının ise HPLC kırılma indisi dedektörü ile saptanarak, bir veri tabanının oluşturulması ve hile konusunda derinlemesine bilgi sahibi olunabilmesi amaçlanmıştır.

Bu çalışma Türkiye'de ilk kez laboratuvar ortamında yürütülen kalite kontrol çalışmalarına ışık tutacak parametre üretimine ilişkin veriler içermektedir. Bu yöntem valide edilmiş ve uygulamada güvenilirliğin sağlanması için ölçüm belirsizliği hesapları yapılmıştır.

Anahtar sözcükler: Bal, Meyve suyu, Hile, IRMS, HPLC, Şeker.

ABSTRACT

IDENTIFICATION STUDIES FOR HONEY, GRAPE SYRUP AND FRUIT JUICE PRODUCED IN TURKEY BY USING HPLC AND 13C/12C ISOTOPE RATIO MASS SPECTROMETRIC METHODS

GÜMÜŞTAŞ, Barış

MSc in Chemistry

Supervisors: Prof. Dr. F. Nil ERTAŞ and Yrd. Doç. Dr. Hasan ERTAŞ

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Being one of the major producers in honey, grape syrup and fruit juices, Turkey has recently faced to marketing and export problems caused by quality and authenticity these products. Fraud in honey by spiking with syrups with similar oligosaccaride composition was common in last decades. Similarly, the authenticity of grape syrup and fruit juices is also searched. In the context of the thesis, in addition to the $^{13}C/^{12}C$ ratios, the sugar contents of these products will be determined by HPLC with refractive index detector and IRMS. The results will be compared in order to create a data bank.

This study was carried out in Turkey first time to reveal and establish such indication associated with food quality control in laboratory site. The method was validated and uncertainty of measurement was calculated which constitutes a reliable basis for such applications.

Keywords: Honey, Fruit Juice, Adulteration, IRMS, HPLC, Sugar

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İzmir / Aralık 2009 **Barış GÜMÜŞTAŞ**

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ABBREVIATIONS

Amu	Atomic Mass Unit
ACN	Acetonitrile
AIJN	European Fruit Juice Association
C_3	Calvin-Benson Photosynthetic Cycle
C_4	Hatch-Slack Photosynthetic Cycle
CAM	Crassulacean Acid Metabolism
CRM	Certified Reference Material
FAPAS	Food Analysis Performance Assessment Scheme
GC/MS	Gas Chromatography Mass Spectrometer
HFCS	High-Fructose Corn Syrup
HMF	Hydroxymethylfurfural
HPLC	High-Performance Liquid Chromatography
IAEA	International Ayomic Energy Agency
IRMS	Isotope Ratio Mass Spectrometer
ISCIRA	Internal Standard Isotope Ratio Analysis
LOD	Limit of Detection
LOQ	Limit of Quantification
PPM	Parts Per Million

PDB	Pee Dee Belemnite
MeOH	Methanol

ABBREVIATIONS (continued)

RID	Refractive Index Dedector
RuBp	Ribulose Biphosphate
SCIRA	Stable Carbon Isotope Ratio Analysis
SMOW	Standart Mean Ocean Water
SNIF-NMR	Site-Specific Natural Isotope Fractionation-Nuclear Magnetic Resonance
TSE	Turkish Standard Institute

1. INTRODUCTION

1.1. Food Adulteration

Turkey is one of the main importers in honey, grape syrup and fruit juices sector. Like other many sector food is affected by fraud. Besides the economic aspect, fraud in food sector have a series consequences on public health at certain added components may be toxic (Cordella, 2002).

From legislative point of view, quality standards have been established through the requirement of quality labels those specify the chemical composition of each product. From the economic point of view, product authenticity is essential to avoid unfair competition that can create destabilized market and disrupt the regional economy, even the national economy. All food products targeted for adulteration are high commercial value products and or produced in high tonnage around the world.

The addition of syrups from a variety of sources like sugarcane, corn and beets is required to be tested. Besides false declaration of the floral origin, mixture of several origins can be encountered. Fraud by spiking syrups increased considerably in the last three decades as a result of massive production of these syrups.

Low cost products with similar oligosaccharide compositions to native honey were marketed. Therefore, the difficulty in this adulteration arises from the differentiating of identical compositions. The solution to this problem lies in the stable isotope ratio of carbon in plants. Photosynthetic carbon dioxide (CO_2) assimilation via the C_3, C_4 and CAM pathways is of primary importance in the use of carbon stable isotope ratio analysis in food authency control (Hibberd, 2002).

C_3 carbon fixation is a metabolic pathway for carbon fixation in photosynthesis.This process converts carbon dioxide and ribulose bisphosphate (RuBP, a 5-carbon sugar) into 3-phosphoglycerate through the following reaction:

$$CO_2 + RuBP \rightarrow 2,3\text{-phosphoglycerate} \qquad \text{Eq 1.1}$$

This reaction occurs in all plants as the first step of the Calvin cycle. In C_4 plants, carbon dioxide is drawn out of malate and into this reaction rather than directly from the air.

Plants that survive solely on C_3 fixation (**C_3 plants**) tend to thrive in areas where sunlight intensity is moderate, temperatures are moderate, carbon dioxide concentrations are around 200 ppm (parts per million) or higher, and ground water is plentiful. The C_3 plants, originating during Mesozoic and Paleozoic era, predate the C_4 plants and still represent approximately 95 % of Earth's plant biomass. C_3 plants lose 97 % of the water taken up through their roots to transpiration.

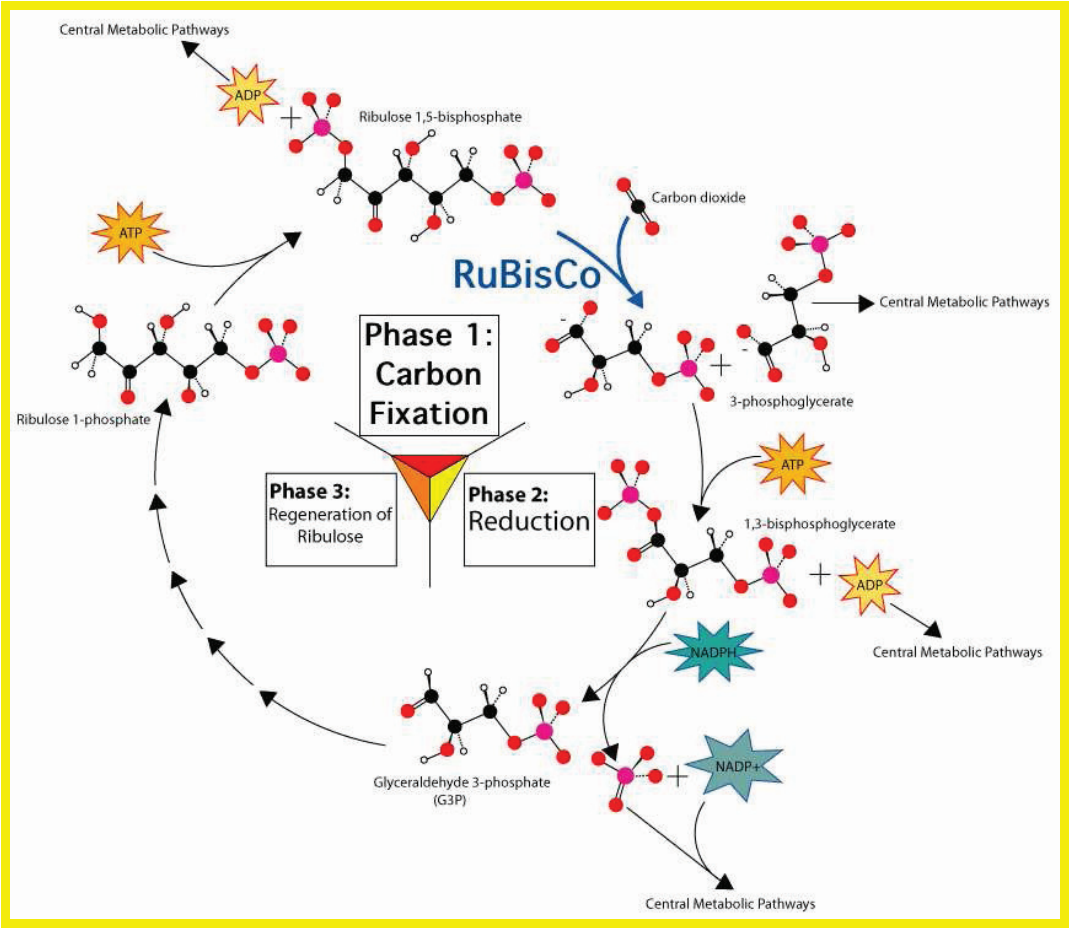

Figure 1.1. C_3 carbon fixation
(C, O and P atoms are represented by black, red and pink dots respectively)

C_3 plants cannot grow in hot areas because RuBisCO incorporates more oxygen into RuBP as temperatures increase. This leads to photorespiration which leads to a net loss of carbon and nitrogen from the plant and can therefore limit growth. In dry areas C_3 plants shut their stomata to reduce water loss but this stops

CO_2 entering the leaves and therefore reduces the concentration of CO_2 in the leaves. This lowers the CO_2:O_2 ratio and therefore also increases photorespiration.

C_4 and CAM plants have adaptations that allow them to survive in hot and dry areas and they can therefore outcompete C_3 plants. The isotopic signature of C_3 plants shows higher degree of ^{13}C depletion than the C_4 plants.

C_4 carbon fixation is one of three biochemical mechanisms, along with C_3 and CAM photosynthesis, functioning in land plants to "fix" carbon dioxide (binding the gaseous molecules to dissolved compounds inside the plant) for sugar production through photosynthesis.

C_4 fixation is an elaboration of C_3 carbon fixation (which operates in most plants), and is believed to have evolved more recently. C_4 and CAM overcome the tendency of RuBisCO (the first enzyme in the Calvin cycle) to fix oxygen rather than carbon dioxide, which leads to a loss of energy and carbon in a process called photorespiration. This is achieved by using a more efficient enzyme to fix CO_2 in mesophyll cells and shuttling the fixed carbon via malate or oxaloacetate to bundle-sheath cells, where Rubisco is sequestered from atmospheric oxygen and can be saturated with CO_2 released by decarboxylation of the malate or oxaloacetate. However, these additional steps require energy in the form of ATP. Because of these tradeoffs, no one of these three photosynthetic pathways is considered superior to the others -- rather, each is best suited to a different set of conditions. The name "C_4" comes from the fact that the first product of CO_2 fixation in these plants has four carbon atoms, rather than three, as is the case in C_3 plants.

The detection of commercial C_4 cane and corn derived sugar syrups in C_3 agricultural products including fruit juices, honey, grape syrup are thus facilitated by characteristic differences in abundance of ^{13}C in these food samples.

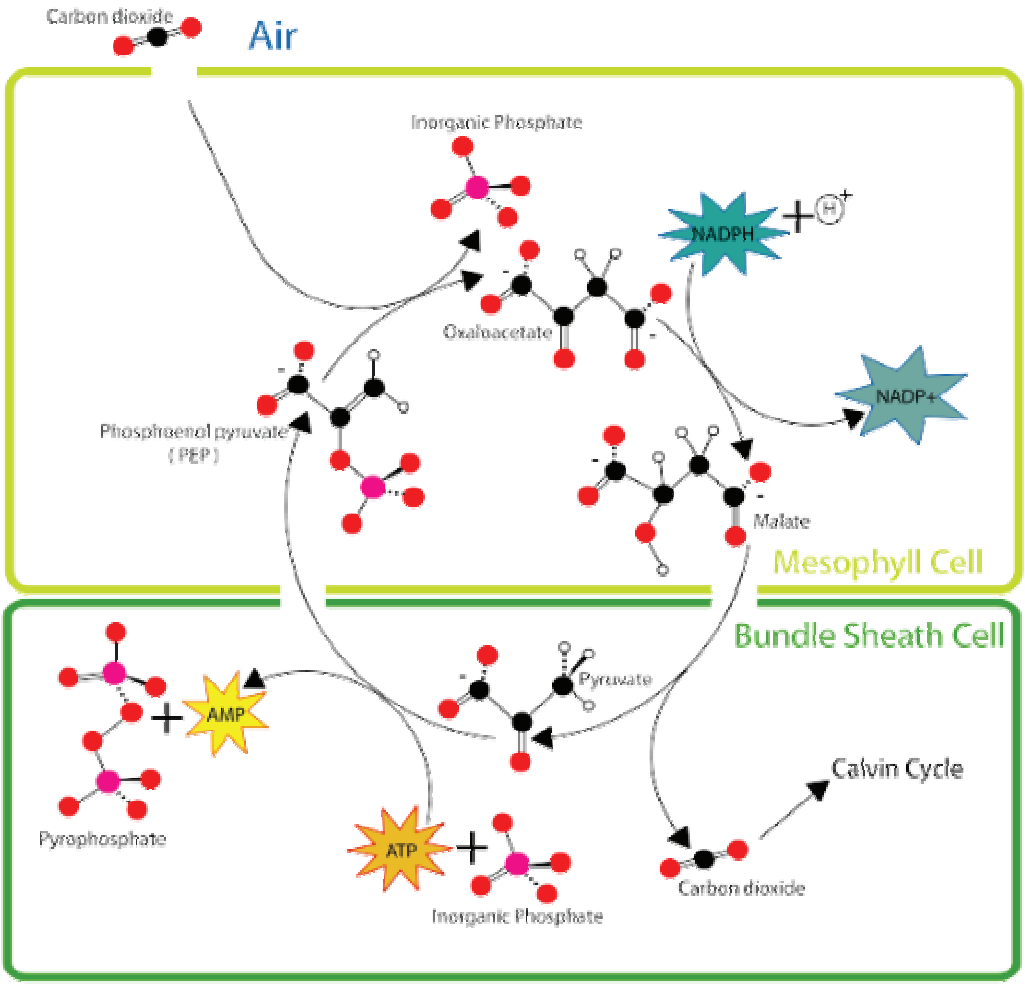

Figure 1.2. C_4 carbon fixation (www.wikipedia.com)

Crassulacean acid metabolism, also known as **CAM photosynthesis**, is an elaborate carbon fixation pathway in some plants. These plants fix CO_2 during the night, storing it as the four carbon acid malate. The CO_2 is released during the day, where it is concentrated around the enzyme RuBisCO, increasing the efficiency of photosynthesis. The CAM pathway allows stomata to remain shut during the day; therefore it is especially common in plants adapted to arid conditions.

1.2. Stable Isotopes

The word isotope originates from the Greek language (isos equal, topos place) and means equal places. This denotes that isotopes occupy the same position in the periodic table of the elements. Thus, isotopes may be defined as atoms possesing the same number of protons but different numbers of neutron in their respective nuclei (Kelly, 2003).

The conventional atomic annotation may therefore be used to describe any given isotope. For example $_6C$ represents the neutral isotope of carbon with a mass number of 12 and an atomic number of 6. Isotopes exist in both stable and unstable (radioactive forms). Table 1.1 lists the mean natural abundance of the principal light bio-elements used in food authenticity studies.

Table 1.1 Mean terrestrial abundance of the stable isotopes- principal elements used in food authenticity studies

Element	Isotope	Abundance (atom %)
Hydrogen	^{1}H	99.985
	^{2}H	0.015
Carbon	^{12}C	98.89
	^{13}C	1.11
Nitrogen	^{14}N	99.63
	^{15}N	0.37
Oxygen	^{16}O	99.759
	^{17}O	0.037
	^{18}O	0.204
Sulfur	^{32}S	95.00
	^{33}S	0.76
	^{34}S	4.22

Differences in isotope effects are usually of the order of a few percent. Consequently, changes in the isotopic ratio at natural abudance levels often occur around the third or fourth significant figure. Isotopic analysis therefore requires very precise measurement and this is achieved by measuring the ratio of the heavy and light stable isotopes in the test material and comparing it to a reference compound of nominal isotope ratio. This is done principally because measurements of absolute isotope ratios are not sufficiently stable over longer periods.

Since the instrumental requirements are so strict for ***Isotope Ratio Mass Spectrometer*** (IRMS), the stability of measurements is affected by, for example, subtle changes in the geometry of the flight tube induced by temperature fluctuations. These changes can however be negated over the long-term if the sample is compared to a reference, as the observed difference between the sample and reference will remain constant as they are equally affected by the instrumental changes.

The approach of using differential comparison between the sample and a suitable reference has been a fundamental part of IRMS since its introduction and formal definition over 50 years ago. Thus, isotopic abundance of a sample relative to a reference is normally expressed by the differential equation 1.2.

$$\delta_{ref} = (R_{samp} - R_{ref}) / R_{ref} \times 1000 \qquad \text{Eq 1.2.}$$

Which can be simplified to equation 1.2.

$$\delta_{ref} = (R_{samp} / R_{ref} - 1) \times 1000 \qquad \text{Eq 1.3.}$$

Where δ_{ref} is the isotope ratio of the sample expressed in delta units relative to the reference materiel. R_{samp} ve R_{ref} are the absolute isotope ratios of the sample and reference material respectively. Multiplying by 1,000 converts the value to parts per thousand (‰), or the more commonly used expression per mil.

In essence the use of equation 1.2. or 1.3. facilitates the comparison of isotope ratios especially at the natural abundance level when the differences being examined are small. For example, the natural flavour chemical vanillin may typically have a $^{13}C/^{12}C$ isotope ratio of 0.010989786 whereas synthetically derived vanilin from petrochemical precursors may be 0.01089989. The difference in the fourth decimal place is masked to some extent by the similarity of the preceding numbers (0.010). However, when these ratios are expressed using the delta notation they become -22.0 ‰ and -30.0 ‰ respectively relative to the ***Pee Dee Belemnite*** reference material, that has a $^{13}C/^{12}C$ ratio of 0.011237. The difference in ^{13}C abundance between the two sources of vanillin is thus much more apparent.

There are four primary isotopic reference materials for the light bio-elements. These are listed in Table 1.2. Neither SMOW nor PDB are available to calibrate isotopic measurements as the original materials have been used up. Consequently, the ***International Atomic Energy Agency*** (IAEA, Vienna) has carefully calibrated alternative reference materials that compare closely to the primary standarts or have defined values relative to the original primary standarts. For example the IAEA prepared VSMOW which has an isotopic composition very close to that of the original primary reference SMOW. Examples of these reference materials are given in Table 1.3.

Table 1.2 Isotopic compositions of the primary reference materials

Primary Reference Material	Isotope Ratio	Accepted Value*
Standart Mean Ocean Water (SMOW)	$^{2}H/^{1}H$	155.76 ± 0.10
	$^{18}O/^{16}O$	2005.20 ± 0.43
	$^{17}O/^{16}O$	373 ± 15.0
Pee De Belemnite (PDB)	$^{13}C/^{12}C$	11237 ± 9.0
	$^{18}O/^{16}O$	2067.1 ± 2.1
	$^{17}O/^{16}O$	379 ± 15.0
Air	$^{15}N/^{14}N$	3676.5 ± 8.1
Canon Diabolo Troilite	$^{34}S/^{32}S$	45004.5
	$^{33}S/^{32}S$	8100.0

*10^{6},ppm with % 95 Confidence Interval

Table 1.3 List of reference materials avaliable for analysis of stable isotope ratios of light elements at environmental levels

Name	Material	Status	δ –value (‰)	δ –value (‰)
VMSMOV	water	CM	$\delta^{2}H = 0$	$\delta^{18}O = 0$
SLAP	water	CM	$\delta^{2}H = -428.0$	$\delta^{18}O = -55.5$
GISP	water	RM	$\delta^{2}H = -189.5 \pm 1.0$	$\delta^{18}O = -24.8 \pm 0.05$
NBS19	limestone	CM	$\delta^{13}C = 1.95$	$\delta^{18}O = -2.2$
IAEA-CH-6	sucrose	RM	$\delta^{13}C = -10.4 \pm 0.2$	
IAEA-CH-6	polyethylene	RM	$\delta^{13}C = -31.8 \pm 0.2$	$\delta^{2}H = -100.3 \pm 2.0$
NBS22	oil	RM	$\delta^{13}C = -29.7 \pm 0.2$	$\delta^{2}H = -118.5 \pm 2.0$
IAEA-S-I	Ag_2S	RM	$\delta^{34}S = -0.30$	
NBS127	$BaSO_4$	CM	$\delta^{34}S = +20.3 \pm 0.4$	$\delta^{18}O = 9.3 \pm 0.4$
IAEA-N-I	$(NH_4)_2SO_4$	RM	$\delta^{15}N = 0.4 \pm 0.2$	
USGS32	KNO_3	RM	$\delta^{15}N = 180 \pm 1.0$	

In practice, routinely used working or laboratory standarts are calibrated against the appropriate reference materials listed in Table 1.3 in order to preserve the limited stocks of these materials. A reference material is then analysed at intervals to control the quality of isotopic measurements. The δ-value measured relative to the laboratory standart may be converted to the appropriate intertional scale using equation 1.4.

$$\delta^{s}_{i} = \delta^{s}_{w} + \delta^{w}_{i} + (\delta^{s}_{w} . \delta^{w}_{i} / 1000) \qquad \text{Eq.1.4.}$$

where :

δ is the δ-value of the sample (s) on the international scale (i)

δ is the δ-value of the sample (s) relative to the working standart (w)

δ is the δ-value of the working standart (w) on the international scale (i).

Again, in practice, the manufacturer's proprietary software supplied with most modern IRMS instruments requires only δ to be entered into the appropriate standart table, for δ to be automatically calculated.

There are two stable isotopes of carbon and $^{13}C/^{12}C$ ratio is characteristic in nature for various carbon reservoirs (Szanto, 2001). In particular, atmospheric CO_2 contains approximately 1.1% of the heavier carbon isotope and 98.9% the lighter one. In the process of photosynthetic CO_2 fixation, discrimination became appearent in the carbon isotope composition: plants favor the fixation $^{12}CO_2$ into their tissues, with the result that all plants contain less ^{13}C than the CO_2 in the atmosphere.

The ranges of $\delta^{13}C$ values for the major carbon reservoirs of importance to the food sciences are shown in Figure 1.3. As can be seen from the Figure above the largest reservoir of carbon is the ocean, where it occurs primarily as dissolved carbonate and bicarbonate. The average $\delta\,^{13}C$ value of the total carbon in the ocean is about 0 ‰; on the PDB scale.

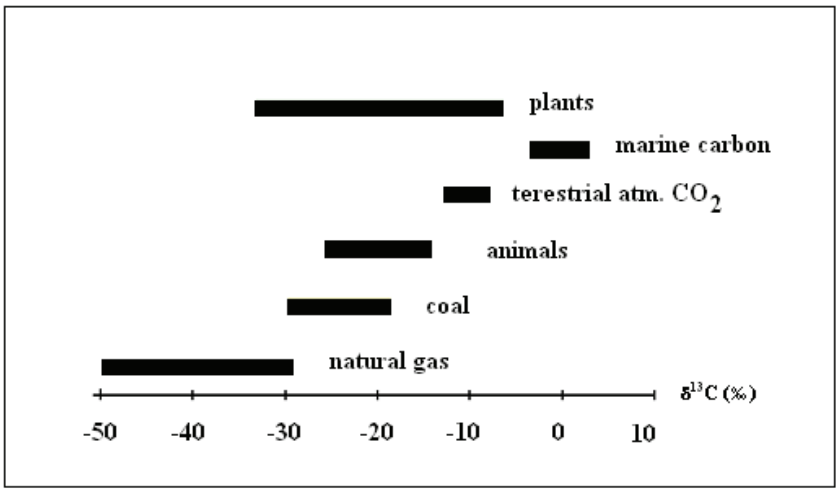

Figure 1.3 Range of carbon isotope variations in selected carbon cycle reservoirs

1.3 The Aim of The Thesis

The main objective of the thesis is to develop an accurate and reliable way to discriminate between original and froud food samples by using IRMS. The samples are honey, fruit juices and grape syrups produced in Turkey. All samples are available in Argefar for authencity analysis.

1.4. The Properties of Selected Food Samples

Honey is a valued sweet and viscous substance produced by bees from flower nectar or from honeydew (Padovana, 2003). Floral honey is composed mainly of the carbohydrates, fructose and glucose; but these sugars can be artificially added to falsify honey. The quality criteria for honey are described as its contents. These contents are; acidity, sugar components ratio, mineral content, diastase activity, hydroxymethylfurfural (HMF), proline.

Chemical techniques have been developed for the detection of honey adulteration. The most widely used is high performance liquid chromatography (HPLC) but this methodology does not detect low levels of adulteration nor is it adequate for the more sophisticated falsifications.

The gas chromatography/mass spectrometer (GC/MS) technique is a precise methodology that can detect low to high levels of adulteration. It is determined by the $^{13}C/^{12}C$ isotope ratio, which is different in monocotyledonous plants (including cane and corn sugar), when compared to dicotyledons (most flowering plants from which bees collect nectar). The different ratios of carbon isotopes are produced by different photosynthesis. Plants with the Calvin–Benson photosynthetic cycle (C_3) have $^{13}C/^{12}C = \delta$ ‰ values from -21 ‰ to -32 ‰ and plants with the Hatch-Slack photosynthetic cycle (C_4) have values from -12 ‰ to -19 ‰ of $^{13}C/^{12}C = \delta$‰; C_4 plants have high ^{13}C when compared to C_3 plants.

This ***stable carbon isotope ratio analysis*** (SCIRA) has been used to detect adulteration in honey (White, 1992). Honey that has $\delta^{13}C$ values less negative than -23.5 ‰ is considered suspect. Companies that produce adulterated honey adapted to this new technique by blending artificial sweeteners with honeys that had $\delta^{13}C$ (^{13}C / ^{12}C) lower than -23.5 ‰. However, by comparing the carbon isotope ratios in the protein and the sugars of honey, which should be the same if they come from the same source, a laboratory can determine if the honey was adulterated, and can estimate the percentage of adulteration by the difference in the $^{13}C/^{12}C$ ratios between the sugar in the honey and its protein. The % adulteration can be calculated by the formula:

$$\% \text{ adulteration} = [(\delta‰ \text{ Protein} - \delta‰ \text{ Honey}) / (\delta‰ \text{ Protein} - \delta‰ \text{ sweetener})] \times 100$$

Eq 1.5

This method is called ***internal standard isotope ratio analysis*** (ISCIRA). Addition of corn or cane sugars from C_4 plants to honey in amounts that result in a delta value ($\delta^{13}C$) more negative than -23.5 ‰ for the mixture can not be detected by the original SCIRA procedure.

Such adulteration however is detected by the ISCIRA procedure from the delta value of the carbon in the protein contained in the honey, which shows the isotopic composition of honey before the addition of C_4 plant sugars.

Table 1.4 Limits of honey in Turkey (TSE, 2005)

Parameter	Flower Honey	Pine Honey
Moisture (max.)	% 20	% 20
Sucrose (max.)	5 g/100 g	5 g/100 g
Fructose + Glucose (min.)	60 g/100 g	45 g/100 g
Fructose / Glucose Ratio	0.9 – 1.4	1.0 – 1.4
Free Acidity (max.)	50 meq/kg	50 meq/kg
Electrical conductivity	0.8 mS/cm (max.)	0.8 mS/cm (min.)
Diastase Number (min.)	8	8
HMF (max.)	40 mg/kg	40 mg/kg
δ‰ Protein - δ‰ Honey	-1.0 or more positive	-1.0 or more positive
% C_4 (max.)	7	7
Proline (min.)	180 mg/kg	180 mg/kg

Fruit Juices are nutritious beverages that have been enjoyed by adults and children for decades. 100 % fruit juices can play an important role in a healthy diet because they offer great taste and a variety of nutrients found naturally in fruits. These juices are fat-free, nutrient-dense beverages that are rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health. Phytonutrients are compounds in fruits, vegetables and other plants that researchers find have disease preventative and disease fighting properties.

Adulteration of orange juice by the addition of high-fructose corn syrup (HFCS) or beet invert syrup plus water is common as sugar represents a major ingredient of juice (Robards, 2001). Adulterations can usually be best detected by the combined use of isotopic and nonisotopic analytical methods.

Among the isotopic techniques, the combination of Site-Specific Natural Isotope Fractionation-Nuclear Magnetic Resonance (SNIF-NMR) and IRMS provides complementary information and improves the reliability of fruit juice authentication methods. However, as the natural variation of the isotopic distribution of fruit juice components is relatively large, the detection of the addition of low amounts of illegal products is usually difficult (Jamin, 1998).

Table 1.5 Limits of several fruit juices (AIJN, 2008)

Metal	**Fructose (g/L)**	**Glucose (g/L)**	**Sucrose (g/L)**	**Sorbitol (g/L)**	**‰ ^{13}C**	**Fructose / Glucose**
Apple	45 -85	15 - 35	5 - 30	2.5 - 7	- 27 - -24	2 - 3.3
Apricot	10 - 45	15 - 50	0 - 55	1.5 - 10	-	0.4 - 1.0
Pomegranate	45 - 100	40 - 80	Not detectable	0.25 (max.)	-	1.0 - 1.25
Strawberry	18 - 40	15 - 35	10 (max.)	0.25 (max.)	-	1.0 – 1.33
Pear	50 - 90	10 - 35	0 - 15	10 - 25	-	2.5 (max.)
Cherry	35 - 70	28 - 60	trace	10 - 35	-	0.7 -0.9
Peach	10 - 32	7.5 - 25	12 - 60	1.5 - 5	-	1 – 1.25
Grape	60-110	60 - 110	trace	Not detectable	- 28 - -23	1.1 – 0.97

Grape Syrup is another important food for our diet. Recently, adulteration of grape syrup is increasingly being recognized as a problem in Turkey (Simsek, 2004). Two kinds of grape syrup are produced, solid and liquid. In order to produce solid grape syrup, starch, egg white, powdered sugar, honey, milk powder and ripe grape syrup are added, which is not considered adulteration. However, according to Turkish Standard Institute (TS-3792) addition of solid grape syrup to liquid syrup is not allowed. Liquid grape syrup should be produced only from fruit extract and should not contain additive material (TSE, 1989). In order to reduce cost, it is however easily and usually adulterated with cheaper carbohydrates such as sucrose, high-fructose corn syrup and glucose syrup.

As the taste of a little cane sugar or corn syrup added to grape syrup is virtually undetectable so fraudulent means can be encountered. However, the ^{13}C signature of corn syrup (mean = -11.29 ‰) and cane sugar (mean = -11.85 ‰) are significantly less negative than that grape syrup (mean = -24.27 ‰), allowing economic adulteration of grape syrup to be detected by ^{13}C analysis.

Table 1.6 Limits of grape syrup in Turkey (TSE, 2007)

Parameter	Liquid Grape Syrup	Liquid Grape Syrup
% Brix (min.)	68	80
HMF (max.)	75 mg/kg	100 mg/kg
% Total Ash (max.)	2.5	3
% Sucrose (max.)	1	1
Fructose / Glucose Ratio	0.9 – 1.1	0.9 – 1.1
Commercial Glucose	-	-
‰ ^{13}C	-1.0 or more negative	-1.0 or more negative
Organic Acids	-	-

1.5. Fundamentals of IRMS

Charged atoms and molecules are separated in a mass spectrometer on the basis of their mass-to-charge ratio ($^{m}/_{z}$). $^{m}/_{z}$ is the common notation used to define a dimensionless quantity derived by dividing the mass number (m) of an ion by its total charge (z). The mass number is the sum of the total number of protons and neutrons in an atom, molecule or ion. The relative abudance of the ions is then determined by measuring the currents produced by the spatially separated ion beams. Most of the mass spectrometers in us efor isotope ratio measurements are based on the design of Nier, whose mass spectrometer design achieved a level of precision and reliability of operation that set the bench- mark for isotope mass spectrometry.

Although subsequent improvements by McKinney *et al.* may be considered to have fully facilitated the precise determination of $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$ isotope ratios in geological and biological samples. The modern Nier-type mass spectrometer has three basic components – an ion source, a mass analyser and an ion detector (collector) system.

The solid or liquid sample is weighed into a small tin capsule and loaded into the Solid AutoSampler. When the AutoSampler is triggered, the sample drops into the combustion reactor that is held at 1020 - 1100°C depending on the elemental species of interest. The sample and capsule melt in an atmosphere temporarily enriched with oxygen, where the tin promotes flash combustion. The combustion products are carried through on oxidation catalyst of CrO_3 by a constant flow of helium.

The oxidation products are then passed through a reduction reactor at 650°C containing copper granules, where oxides of nitrogen (NO, N_2O & N_2O_2) are reduced to N_2 and excess oxygen is removed. The resulting gas species (CO_2, N_2, SO_2, H_2O) are then passed through a magnesium perchlorate filter to remove water. The remaining CO_2, N_2 (if N was present in he original sample) and SO_2 (if S was present in the original sample) then pass through a short chromatographic column where they are time-separated. They then pass through a Thermal Conductivity Detector, and out of the vent on the top of the instrument.

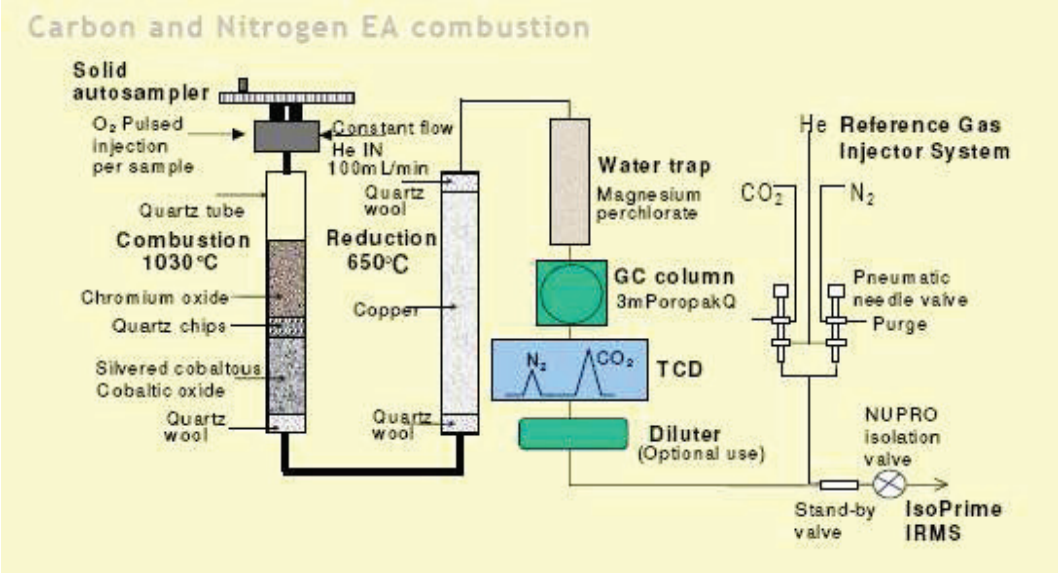

Figure 1.4. Schematic representation of IRMS

When used with the IsoPrime, gas samples, typically H_2, N_2, CO, CO_2 and SO_2 are introduced into the source through a narrow capillary. The gas molecules then undergo ionisation, as a result of impact with a stream of electrons generated by a thoria coated tungsten filament ~70eV .

Excess energy transferred to the newly formed positively charged ion can cause bond dissociation (fragmentation or cracking). For example, during the ionisation of CO_2, significant quantities of CO are formed, which has the same mass as N_2. It is therefore imperative that during the isotope analysis of N_2 the gas is free from all traces of CO_2, otherwise the measurement will be inaccurate.

After ionisation of the sample gas, the positively charged ions are accelerated and collimated (ions moving in a parallel path) by a series of electrode ‘lenses’ to which variable potentials may be applied.

At this stage the ion beam is unresolved and enters the magnetic sector of the flight tube, which is designed to provide low-mass resolution of 1 atomic mass unit (amu) with an average resolution between any two peaks of 10% relative to the peak height.

The Field (B) generated by the permanent or electromagnet deflects the ions into circular trajectories with radii proportional to the mass of the respective isotope, i.e., the heavier gas isotopes (isotopomers) are deflected less than the lighter isotopes. Using carbon dioxide as an example, the major ion $^{12}C^{16}O^{16}O$ (m/z 44) with the relatively lowest mass follows the trajectory with the shortest radius and the heavier isotope m/z 45 ($^{13}C^{16}O^{16}O$) follows a 'central' trajectory and the heaviest isotope $^{12}C^{16}O^{18}O$ (m/z 46) follows the trajectory with the largest radius. The resolved beams then continue along the flight tube to the collector assembly.

The ion beam collides with the inside walls of a metal box (Faraday cup) and all secondary ion emission is suppressed. An accurate measure of the charge deposited by the ion beam is obtained via an electron current that flows through resistors of 10^{9}-10^{12} Ω depending on the relative intensity of the ion beams. The subsequent voltage generated across the resistors is amplified and converted into aa digital output to a personal computer.

The ion beam defining slits are wider than the ion beams, resulting in the characteristic flat top peaks that are insensitive to drift, which may result from temperature variation affecting the geometry of the flight tube and electonic components used in mass selection. The overall result is stable, well-defined beams that can be utilised for precise measurement of the relative abundance of the isotopomers.

1.6. Fundamentals of HPLC

Chromatography is an analytical method that finds wide application for the separation, identification and determination of chemical components complex mixtures. This technique is based on the separation of components in a mixture (solute) due to the difference in migration rates of the components through a stationary phase by a gaseous or liquid mobile phase (Poole, 2003).

Chromatography can be divided into three subsections namely gas, gel and liquid chromatography. Gas chromatography is used for the analysis of volatile samples, gel chromatography for non-volatile samples with a molecular weight larger than 2000 and liquid chromatography for non-volatile samples with a molecular weight smaller than 2000.

HPLC was derived from classical column chromatography and has found an important place in analytical techniques. The major advancement in HPLC was found by the use of efficient separators used small particles high pumping pressures. The main components of an HPLC system are a high pressure pump, a column an injector system as well as detector.

The system works as follows: eluent is filtered and pumped through a chromatographic column, the sample is loaded and injected onto the column and the effluent is monitored using a detector and recorded as peaks.

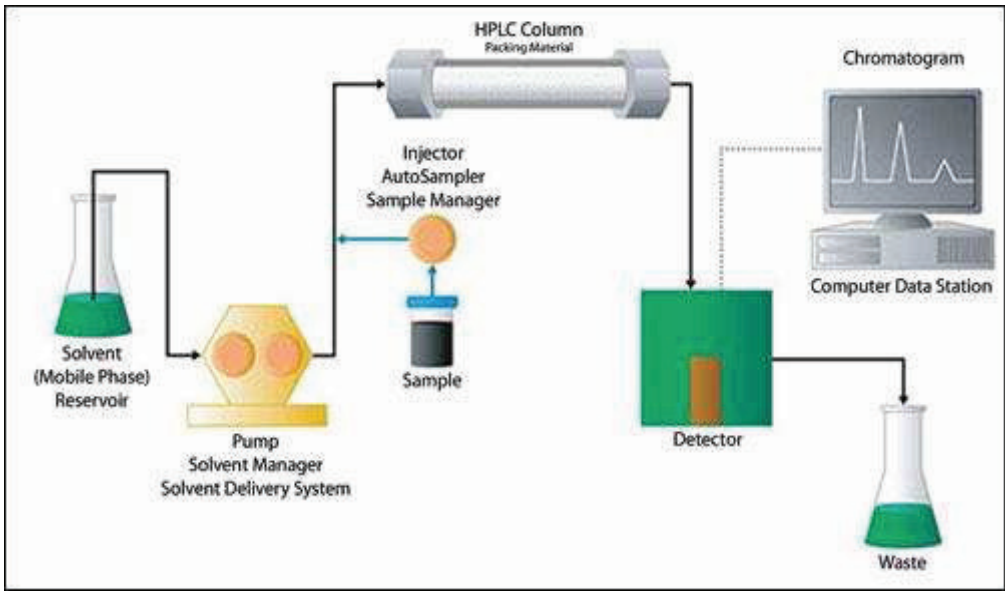

Figure 1.5 Principle components of HPLC System

1.6.1. Analytical Pumps (Solvent Delivery System)

The requirements for HPLC pumps are as follows: they must be able to generate high pressures, have a pulse-free output, deliver flow rates ranging from 0.1 to 10 mL/min, have flow reproducibility's of 0.5 % relative or better and they must be resistant to corrosion by variety of solvents.

Various types of pumping systems exist. These include:

a) Direct Gas-pressure Systems:

This system consists of a cllinder gas pressure, which applied directly to the eluent in a holding coil. Advantages of this pump are that it is reliable and economical although solvent changing is found to be tedious.

b) Syringe-type Pumps

In these pumps an electrically driven lead-screw moves a piston, which is able to pressurize a finite volume of solvent, and thus delivers a pulseless constant flow of solvent to the system. These pumps are found to be reliable although they are expensive, solvent changing is tedious and they have a finite capacity.

c) Pneumatic Intensifier (Constant Pressure) Pumps

Pneumatic ıntensifier pumps are operated via gas pressure. A large area piston drives a small area piston when acted on by pressure from a gas line. The gas pressure is thus amplified in the ratio of the areas of the forces of the pistons and a high pressure liquid at constant pressure is introduced into the system. If a partial blockage occurs in this system a drop in flow rate occurs but the pressure remains constant. Thr flow sensitivity of the detector cell will determine how much pulse damping is requıred in the system to suppress the detector signal caused when the flow stops during the return strike.

d) Reciprocating (Constant Flow) Pumps

A reciprocating pump is the most generally used, as it is economical and allows a wide range of flow rates. With this pump there is no limit on the reservoir size or operating time as is commonly found with other pumps. This type of pump is electrically driven by a motor, which moves back and forth within a hydraulic chamber. On the backward stroke the piston sucks in eluent from the reservoir and due to check valves the outlet to the separation column is closed. During the forward stroke the eluent is pushed onto the column and the inlet from the reservoir is closed. The pumping motion of the piston produces a pulsed flow that requires dampening. These pumps include a high output pressure with constant flow rates and the ability to be used for gradient elution.

1.6.2. Sample Introduction

The ideal method for sample introduction should enable the sample to be injected as a narrow plug onto the column so that peak broadening is negligible. The injection system should contain no void volume, as this would cause a loss of resolution. Syringe injection through an elastomeric septum is often used although it is not very reproducible and is constricted to low pressures.

The most widely used methods are those based on sampling valves and loops. Here the sample loop is filled with sample by means of a syringe. A rotation of the valve rotor causes the eluent stream to pass through thr sample loop thus injecting the sample onto the column without a noticeable change in folw. These valves have interchangeable loops and reproducibility is afew tenths of a percent relative. In stopped-flow injection, the eluent flow is stopped and the sample is injected directly onto the head of the column by means of a syringe. The pump is then switched on again.

1.6.3. Separation Columns

Heavy-wall glass, stainless steel and plastic are among materials that can withstand high pressures and are thus used construct HPLC columns. They must also be able to resist the chemical action of the mobile phase. Wall irregularities will cause a well-packed column to channel near the wall or packing interface thus the tubing must have a smooth, precision bore internal diameter. Channels would cause peak broadening and a decrease in efficiency.

Column connections are made with low dead-volume fittings, which prevent stagnant pockets of eluent. Usually a short guard column is placed in front of the analytical column. This serves to increase the life of the analytical column by removing particulate matter and contaminants from the solvents.

1.6.4. Spectroscopic Detection Methods

a) Molecular Spectroscopic Techniques

UV Detectors: UV detectors measure the change in the UV absorption as the solute passes through a flow cell. In a UV transparent solvent UV detectors are concentration sensitive.

Direct detection has a flaw as not all inorganic ions have appropriate chromophores but this can be compensated for by using the method of derivitisation. This is done by mixing the effluent with a chromogenic reagent in a post column reactor. The formed chelate complex subsequently absorbs at a particular wavelength.

Refractive Index Detectors: The refractive index of a medium is the ratio of the speed of light in a vacuum to the speed in the medium. These detectors measure the change in refractive index in the eluent as the solute passes through the sample cell. This method of detection is less sensitive then UV detection although non-chromatographic compounds can be measured directly without derivitisation.

Fluorometric Detection: In this detection system the solute is exited by UV radiation at a particular wavelength and the emission wavelength is detected. Fluorometric detection has been used with naturally fluorescent compounds but compounds can be reacted to produced fluorescent derivatives.

b) Molecular Spectroscopic Techniques

Atomic spectroscopy includes atomic absorption spectroscopy as well as atomic emission spectroscopy. The spectroscopic determination of atomic species can only be performed on a gaseous medium in which the individual atoms separated from one anather. Thus the first step in atomic spectroscopic techniques is atomization, a process in which the sample is volatilized in such a manner as to produce an atomic gas.

1.7. Method Validation and Measurement Uncertainty

Methods validation is the process of demonstrating that analytical procedures are suitable for their intended use. The methods validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures (Bliesner, 2006). Fundamental terms of validation are given below;

Linearity: The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations.

Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) and/or by using separate weighing of synthetic mixtures of the test product components, using the proposed procedure.

Linearity is determined by a series of 3 to 6 injections of 5 or more standards whose concentrations span 80–120 percent of the expected concentration range. The response should be directly proportional to the concentrations of the analytes or proportional by means of a well-defined mathematical calculation. A linear regression equation applied to the results should have an intercept not significantly different from 0. If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method.

Repeatability is the variation experienced by a single analyst on a single instrument. Repeatability does not distinguish between variation from the instrument or system alone and from the sample preparation process. During the validation, repeatability is performed by analyzing multiple replicates of an assay composite sample by using the analytical method.

Limits of Detection and Quantification: The detection limit (DL) or limit of detection (LOD) is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of detection is frequently confused with the sensitivity of the method. The sensitivity of an analytical method is the capability of the method to discriminate small differences in concentration or mass of the test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting the response against the analyte concentration or mass.

The quantification limit (QL) or limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. The quantization limit is a parameter of quantitative assays for low concentrations of compounds in sample matrices and is used particularly for the determination of impurities. It is usually expressed as the concentration (e.g., percentage, parts per million) of analyte in the sample.

The LOD and LOQ were determined on the basis of signal-to-noise ratio, and formulated below where c_{min} is the minimum concentration of the calibration series, S (b): The signal count of the blank sample, S (s): The signal count of the sample that known concentration;

$$LOD = \frac{3 \times c_{\min} \times S(b)}{S(s)} \qquad \text{Eq. 1.6}$$

$$LOQ = \frac{10 \times c_{\min} \times S(b)}{S(s)} \qquad \text{Eq. 1.7}$$

Accuracy and Recovery: The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found.

The true value for accuracy assessment can be obtained in several ways. One alternative is to compare the results of the method with results from an established reference method. This approach assumes that the uncertainty of the reference method is known. Secondly, accuracy can be assessed by analyzing a sample with known concentrations (e.g., a control sample or certified reference material) and comparing the measured value with the true value as supplied with the material. If certified reference materials or control samples are not available, a blank sample matrix of interest can be spiked with a known concentration by weight or volume.

After extraction of the analyte from the matrix and injection into the analytical instrument, its recovery can be determined by comparing the response of the extract with the response of the reference material dissolved in a pure solvent. Because this accuracy assessment measures the effectiveness of sample preparation, care should be taken to mimic the actual sample preparation as closely as possible. If validated correctly, the recovery factor determined for different concentrations can be used to correct the final results.

Uncertainty: In metrology, measurement uncertainty describes a region about an observed value of a physical quantity, also called a measurand, which is likely to enclose the true value of that quantity.

Uncertainty of a method associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand. Assessing and reporting measurement uncertainty is fundamental in chemistry.

In practice the uncertainty on the result may arise from many possible sources, including examples such as incomplete definition, sampling, matrix effects and interferences, environmental conditions, uncertainties of weights and volumetric equipment, reference values, approximations and assumptions incorporated in the measurement method and procedure, and random variation.The measurement uncertainty tells us what size the measurement error might be. Therefore, the measurement uncertainty is an important part of the reported result.

The basis for the evaluation is a measurement and statistical approach, where the different uncertainty sources are estimated and combined into a single value. Basis for the estimation of measurement uncertainty is the existing knowledge. Existing experimental data should be used quality control charts, validation, interlaboratory comparisons, certified reference material (CRM) etc.

2. EXPERIMENTAL

2.1. Apparatus

The Liquid Chromatography (LC) system used was Agilent 1100 Binary pump liquid chromatography operating at 30^{o}C with a flow rate of 1.5 mL/min for honey and grape syrup samples, at 80^{o}C with a flow rate of 1.0 mL/min for fruit juice samples. The solvents and solutions used for the study were degassed using Agilent 1100 G1379A Degasser. The samples and standarts were injected using Agilent 1100 G1313A autosampler. The column oven was Agilent 1100 G1316A where the columns were kept under a consistent temperature through the study. The detector was a Refractive Index (RID) Agilent 1100 G1362A.

The chromatography was performed using Zorbax NH_2 with a particle size of 5 µ (150 mm x 4.6 mm) column for honey and grape syrup samples; and BIORAD Ca cation exchange with a particle size of 5 µ (250 mm x 4.6 mm) column for fruit juice samples.

Stirring of the samples was carried out using an IKA basic vibratory stirrer (IKA Labortechnik, HS501 digital). For drying purposes, Binder drying-oven was used at 70°C. Weight mesaurement was made by Metler Toledo MT5 balance. Honey samples were heating by using an IKA Werke magnetic stirrer-heater. Centrifuge was made by using Nüve RF-800. Atago Refractometer RX-7000 was used for brix measurements. The IRMS system used was EUROVECTOR Elementar Analysis (EA 8202) with Micromass İsoprime (JB068).

2.2. Chemicals and Reagents

Acetonitrile (ACN), methanol (MeOH) (JT Baker), Sulfuric acid (95–98%), Sodium tungstate dihydrate (99%) (Sigma), and all reagents were HPLC grade. Water was obtained using a USF Purelab and Millipore Elix & Rios Systems combined Milli-Q Synthesis System, including UV radiation and ultra filtration units. Fructose, glucose, sucrose, maltose and sorbitol standards were supplied by Merck. Standard solutions (fructose 2.0 g/100mL, glucose 1.5 g/100mL, sucrose 0.25 g/100mL, maltose 0.25 g/100mL for honey and grape syrup samples; fructose 2.0 g/100mL, glucose 1.0 g/100mL, sucrose 0.5 g/100mL, sorbitol 0.5 g/100mL for fruit juices samples) prepared in water-methanol mixture (75-25%) and kept -20 ^{o}C.

2.3. Analytical Procedures and IRMS&HPLC Conditions

Chromatographic separations were accomplished by using isocratic system at flow rates 1.5 mL/min – 1.0 mL/min and 20 µL of the extract was injected onto the columns as given in Table 2.1 – 2.2. The column temperatures were set to 30°C and 80°C, respectively. Therefore, maximum pressure limit was set to 200 bars. The chromatograms recorded for sugar standard mixtures were given in Figure 2.1 and 2.2.

Table 2.1. HPLC conditions for honey and grape syrup samples

Parameter	Optimized Condition
Flow Rate	1.5 mL/min
Column	Zorbax NH_2, 150 mm x 4.6 mm, 5 µm
Column Temperature	30°C
Mobile Phase	Acetonitrile – Water % 80 - 20
Injection	20 µL

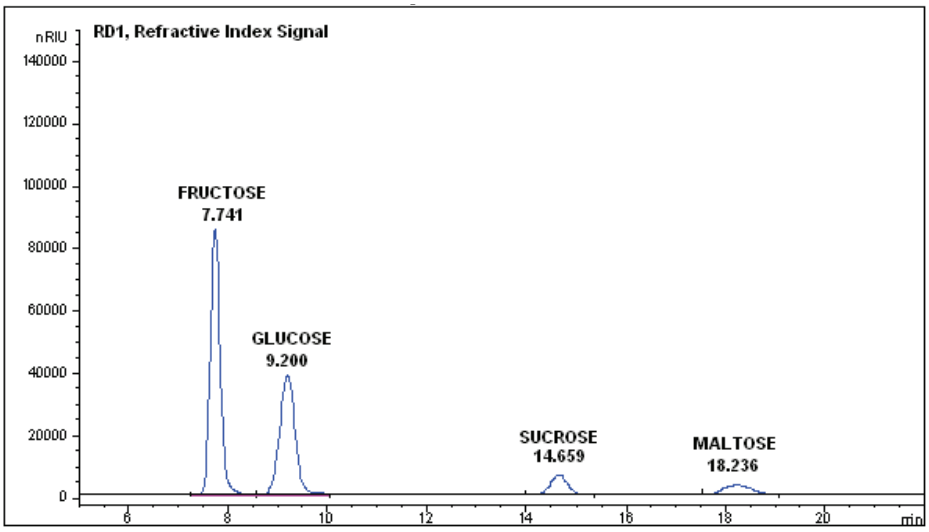

Figure 2.1. HPLC recordings for sugar standards recorded at above conditions (fructose 40.0 g/100g, glucose 30 g/100g, sucrose 5 g/100g, maltose 5 g/100g)

Table 2.2. HPLC conditions for fruit juice samples

Parameter	Condition
Flow Rate	1.0 mL/min
Column	BIORAD Ca cation exchange
Column size	250 mm x 4.6 mm, 5 µm
Column Temperature	80°C
Mobile Phase	Water
Injection	20 µL

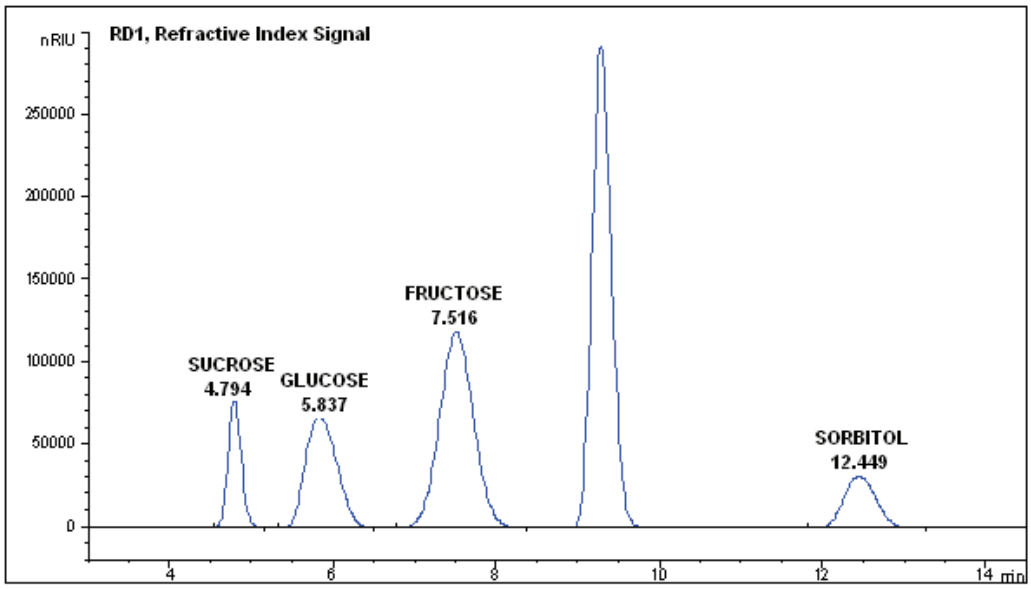

Figure 2.2 HPLC recordings obtained with sugar standards at above conditions (fructose 40.0 g/100g, glucose 20 g/100g, sucrose 10 g/100g, sorbitol 10 g/100g)

In the IRMS system, ultrapure helium (% 99.9995) was used carrier gas and carbondioxide (% 99.995) was used reference gas. Other parameters were shown Table 2.3.

Table 2.3. Working conditions of IRMS system

Parameter		**Parameter**	
Carrier	100 kPa	Purge	80 mL/min
Front Reactor	1030 °C	Rear Reactor	650 °C
Oven	50 °C	Accelerating Voltage	3051.58 V
Extraction Voltage	73.25 % AV	Half Plate Differantial	36.58 V
Z-Plates Voltage	- 35.68 V	Trap Current	361.6 µA
Electron Volts	90.37 eV	Ion Repeller Voltage	- 5.00 V
Magnet Current	3557.39 mA		

2.4. Sample Preparation and Analysis

For the HPLC analysis, sample was weighed into a beaker and dissolve in 40 mL water. The solution was stirred in a vibratory stirrer for 10 minutes. 25 mL methanol was added into a 100 mL volumetric flask and the solution was transferred quantitatively to the flask, and then, diluted to the mark with water. The solution was poured through a membrane filter and collected in a vial. 20 µL of this solution is injected onto the HPLC column.

For the IRMS analysis, 10-12 g honey sample was placed in a clear 50 mL centrifuge tube, 4 mL of water was added and mixed. In another tube, 2 mL 10 % sodium tungstate solution was mixed thoroughly with 2 mL 0.67 N sulfuric acid and then added to honey solution. The tube was agitated in a water bath at 75°C until 1.5 hours and was added 2 mL acid mixture every 30 minutes. The sample was then centrifuged at 1500xg and the supernatant removed. The precipitate was washed by 50 mL water, and this procedure was repeated at least three times. The precipitated protein was transferred to watch glass for drying in an drying-oven set at 70°C. After drying, 150 μg protein and 150 μg honey was placed into tin capsules and injected onto the IRMS.

Grape syrup and fruit juice samples were centrifuged and then, directly injected to IRMS system.

3. RESULTS AND DISCUSSION

3.1. Optimization Studies for Methods Development

The optimization of the IRMS method includes individual parameters that could be expressed in terms of water bath temperature, drying oven temperature and sample weight. The values in Table 3.1 have indicated that the two temperature parameters virtually had no effect on determination of $\delta^{13}C$ values. However, the third parameter related to sample weighing had an affect on $\delta^{13}C$ values. Furthermore, among the three sample weighing amounts, 150 µg sample weight turned out to be the optimum weight since the results stayed much stable when compared to 100 and 200 µg sample weights.

Table 3.1 The effect of selected parameters on the $\delta^{13}C$ values

Parameter		**$\delta^{13}C$ Protein**				
Water Bath Temp. (°C)	**70**	-24.71	-24.56	-24.68	-24.58	-24.62
	75	-24.67	-24.55	-24.51	-24.70	-24.73
	80	-24.76	-24.59	-24.61	-24.60	-24.54
Drying Oven Temp. (°C)	**65**	-24.76	-24.76	-24.53	-24.53	-24.76
	70	-24.68	-24.70	-24.50	-24.57	-24.68
	75	-24.69	-24.52	-24.55	-24.79	-24.72
Sample Weight (µg)	**100**	-24.18	-24.79	-23.86	-24.55	-24.91
	150	-24.58	-24.62	-24.65	-24.60	-24.60
	200	-23.97	-24.64	-25.38	-24.33	-25.03
Parameter		**$\delta^{13}C$ Honey**				
Sample Weight (µg)	**100**	-25.18	-24.25	-25.07	-24.47	-25.54
	150	-24.67	-24.61	-24.69	-24.59	-24.70
	200	-23.76	-24.26	-25.06	-24.76	-24.59

The IRMS method was tested by obtained honey test samples from EUROFINS. As no test material is available for fruit juice and grape syrup, the optimized conditions were also applied for these types of samples.

Table 3.2 Comparison of the results obtained with IRMS to those of honey test sample from EUROFINS

Sample	**Test material value**		**Determined value**	
	$\delta^{13}C$ Honey	**$\delta^{13}C$ Protein**	**$\delta^{13}C$ Honey**	**$\delta^{13}C$ Protein**
2007/1	-26.36±0.3	-26.11±0.3	-26.59	-26.35
2007/2	-26.02±0.3	-25.80±0.3	-25.88	-25.65
2008/1	-25.28±0.3	-25.04±0.3	-25.28	-24.96
2009/1	-24.55±0.3	-24.81±0.3	-24.48	-24.72

The HPLC method was tested by obtained honey test samples from Food Analysis Performance Assessment Scheme (FAPAS). The optimized conditions were also applied for these types of samples.

Table 3.3 Comparison of the results obtained with HPLC to those of honey test sample from FAPAS

Sample	Test material value			Determined value		
	Fructose (g/100g)	Glucose (g/100g)	Sucrose (g/100g)	Fructose (g/100g)	Glucose (g/100g)	Sucrose (g/100g)
FAPAS T2813	30.8±1.3	26.4±1.0	11.3±2.6	30.2	26.2	11.3
FAPAS T2815	32.9±1.4	26.9±1.1	7.6±1.8	32.3	26.4	6.1
FAPAS T2816	36.5±1.5	31.9±1.3	1.7±0.4	36.4	31.4	1.9
FAPAS T2817	29.6±1.2	25.9±1.1	13.0±3.0	29.6	25.9	13.2
FAPAS T2818	39.2±1.6	31.5±1.3	1.7±0.4	38.5	30.4	1.4

3.2. Validation Studies

The validation studies were carried out only for the HPLC method. Under the optimized conditions given above, the validation of the method was accomplished by determining the linearity, repeatability, reproducibility, accuracy and the detection and quantification limits of the method. In addition, uncertainty measurements were calculated for both methods.

3.2.1 Linearity

For calibration studies, sugar components were divided into two groups. The first group includes fructose, glucose, sorbitol and the calibration range is 2.0 to 40.0 g/100g. The second group includes sucrose, maltose and the calibration range is 1.0 to 20.0 g/100g. The response was found to be linear in these concentration ranges (Figure 3.1-3.5).

The linearity of the method was assessed with five standard injections to HPLC as three replicates. Linearity was verified using the value of regression coefficient, R^2, which reaches unity for all the samples.

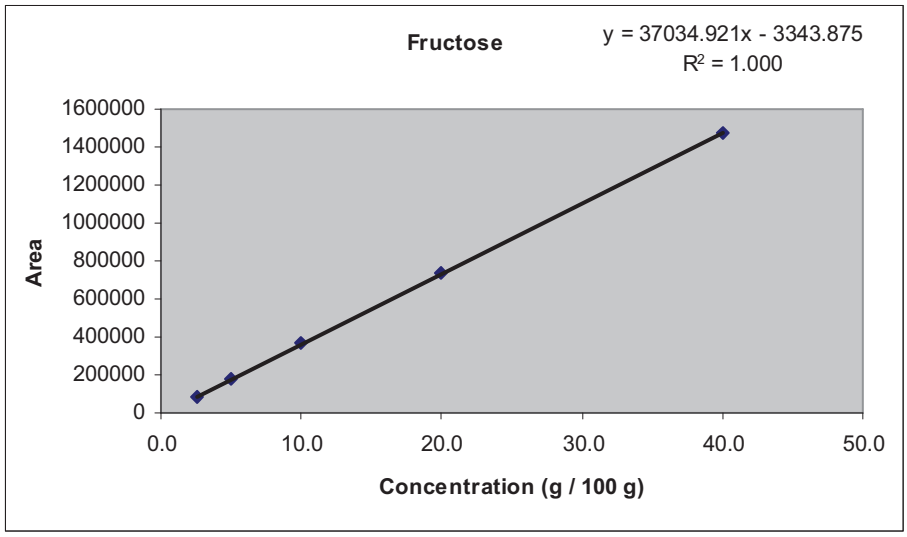

Figure 3.1. Calibration curve for fructose

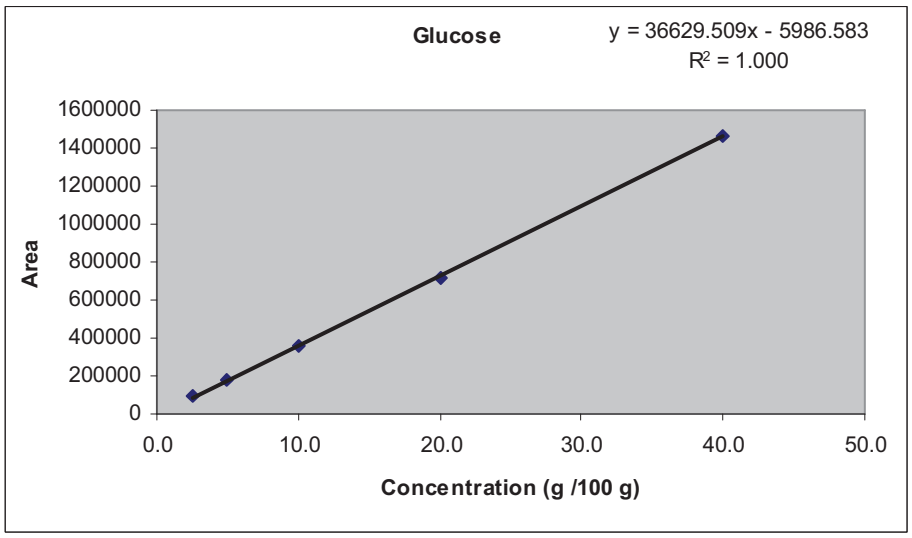

Figure 3.2. Calibration curve for glucose

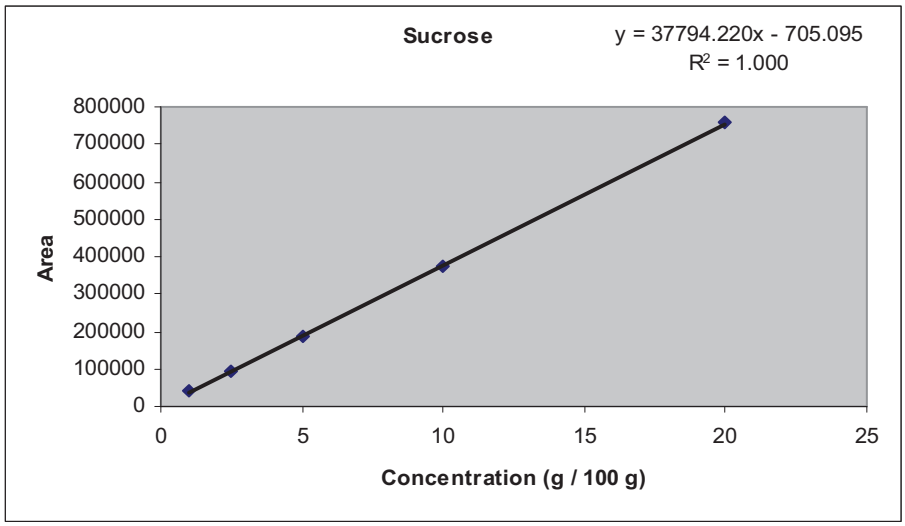

Figure 3.3. Calibration curve for sucrose

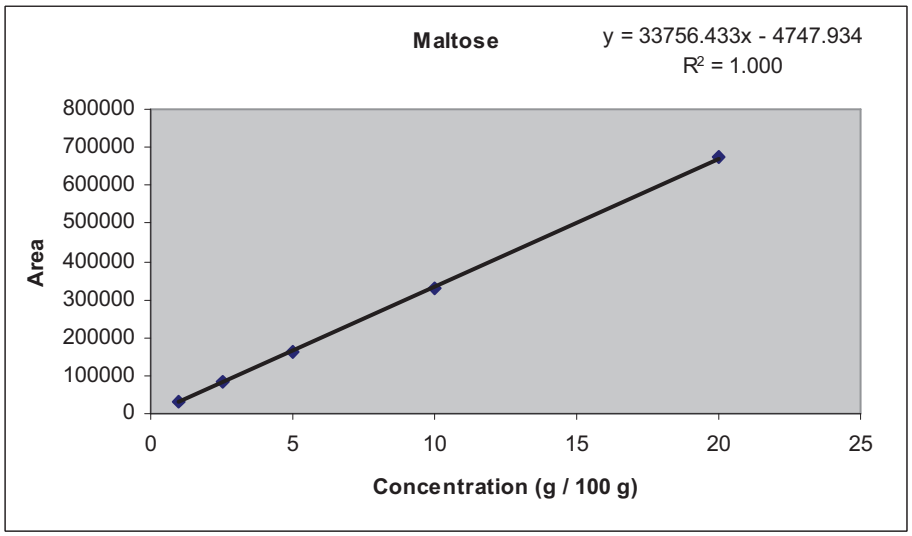

Figure 3.4. Calibration curve for maltose

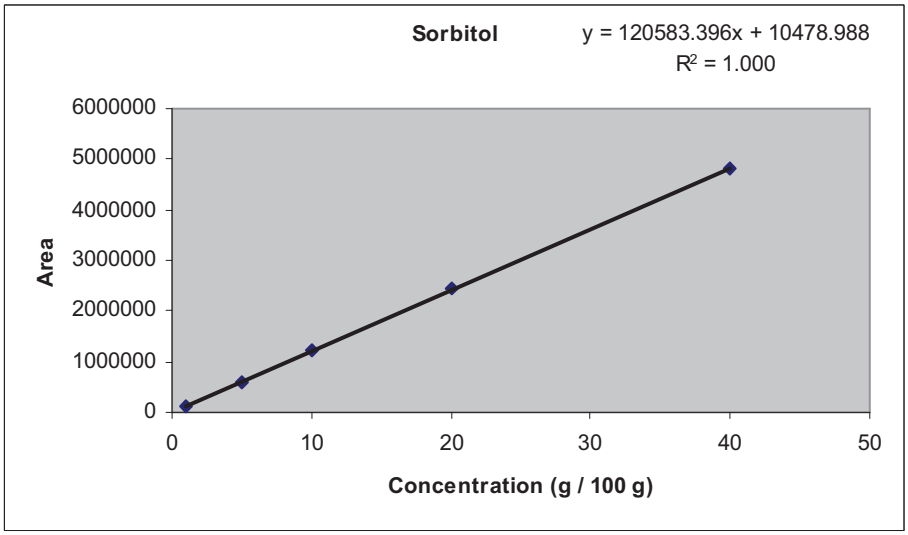

Figure 3.5. Calibration curve for sorbitol

3.2.2. Repeatability

The injection repeatability was calculated by five replicated injections of 20.0 g/100g for the first group and 10.0 g/100g for the second group standard samples. Injection repeatability for individual sugar components as RSD % can be seen in Table 3.4.

Table 3.4. Relative standard deviations of sugars.

Sugar	RSD	Sugar	RSD
Fructose	0.158	Maltose	1.831
Glucose	0.708	Sorbitol	0.139
Sucrose	0.931		

3.2.3. Sensitivity

The detection limits (LOD) and quantification limits (LOQ) were determined on the basis of signal-to-noise ratio, LOQ and LOD for sugar components are listed in Table 3.5.

Table 3.5. LOD and LOQ values of sugar components (g/100g)

Sugar	LOD	LOQ
Fructose	0.158	0.527
Glucose	0.581	1.937
Sucrose	0.276	0.919
Maltose	0.355	1.182
Sorbitol	0.569	1.896

3.2.4. Recovery

The recovery percentages for the sugar components has been determined by preparing spiked honey samples at 2.0, 20.0, 40.0 g/100g concentration levels for the first group and 1.0, 5.0, 20.0 g/100g concentration levels for the second group. The recovery percentages of each sugar components are given in Table 3.6.

Table 3.6. Recovery percentages of the sugar components with HPLC

Sugar	Low Conc. %	Average Conc. %	High Conc. %
Fructose	103.2	103.3	100.1
Glucose	97.7	100.3	98.1
Sucrose	99.3	96.8	97.8
Maltose	96.8	98.5	101.7
Sorbitol	93.8	99.6	101.4

3.2.5. Uncertainty

The uncertainty of the measurement was calculated from quality control charts of each sugar component. The relative uncertainties are given in Table 3.7.

Table.3.7 Relative uncertainty values of sugar components and $\delta^{13}C$

Sugar	Uncertainty
Fructose	0.09
Glucose	0.09
Sucrose	0.22
Maltose	0.18
Sorbitol	0.15

3.3. Application of the Methods to the Samples

The method was applied for a wide range of samples collected from different parts of Turkey. IRMS results for honey, grape syrup and pomegranate juice samples were given in Table 3.8- 3.10. The method has also been applied for Concentrated juice samples including pomegranate, cherry, apple, strawberry, black carrot, pear, quince, grape, concentrates and the results were given in Tables 3.11-3.18 respectively.

Table 3.8 The Results of Honey Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	Protein ‰ ^{13}C	Honey ‰ ^{13}C	% C_4
	Fructose	Glucose	Sucrose	Maltose				
1	41.1	33.3	1.0	-	1.23	-25.5	-25.1	2.5
2	38.5	33.6	1.9	-	1.15	-25.5	-25.3	1.3
3	35.6	31.9	0.6	-	1.11	-25.0	-24.1	5.9
4	37.9	34.1	0.5	-	1.11	-25.7	-25.0	4.4
5	35.5	32.0	1.7	1.2	1.11	-25.2	-24.8	2.6
6	36.3	35.0	0.0	-	1.04	-25.3	-24.6	4.5
7	33.4	29.6	0.3	-	1.13	-24.9	-24.5	2.6
8	36.2	34.6	0.0	-	1.05	-25.2	-25.0	1.3
9	35.5	32.2	0.3	-	1.10	-25.1	-24.6	3.2
10	38.5	34.6	0.0	-	1.11	-25.6	-24.5	6.9
11	32.8	28.5	0.5	-	1.15	-25.1	-24.2	5.8
12	33.9	29.8	0.5	1.8	1.13	-24.8	-24.7	0.7
13	33.1	28.7	0.8	-	1.15	-26.3	-25.9	2.4
14	39.4	35.8	0.0	-	1.10	-24.9	-24.7	1.3
15	30.0	26.5	1.0	-	1.13	-25.1	-24.8	1.9
16	38.1	34.6	0.0	-	1.10	-25.1	-24.9	1.3
17	34.6	27.7	0.4	-	1.23	-25.1	-24.2	5.8
18	38.0	34.4	0.0	-	1.10	-24.6	-24.3	2.0
19	32.9	29.5	0.9	-	1.12	-25.2	-24.6	3.9
20	36.7	33.3	0.0	-	1.10	-25.8	-24.9	5.6
21	37.1	33.1	0.0	-	1.12	-25.4	-24.9	3.2
22	28.3	24.8	0.8	-	1.14	-25.1	-24.2	5.8
23	37.0	32.2	0.0	-	1.15	-24.2	-23.6	4.1
24	31.6	26.8	0.8	-	1.18	-25.2	-24.2	6.5
25	30.7	26.3	0.0	-	1.17	-25.3	-24.6	4.5
26	33.0	26.1	0.9	-	1.26	-25.4	-24.5	5.7
27	35.4	33.2	0.9	-	1.07	-25.3	-25.2	0.6
28	34.2	30.1	0.0	-	1.14	-24.9	-24.7	1.3
29	36.1	32.9	0.0	-	1.10	-25.0	-24.4	3.9
30	35.2	32.1	0.0	-	1.10	-24.7	-24.2	3.3

Table 3.9 The Results of Grape Syrup Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C
	Fructose	Glucose	Sucrose	Maltose		
1	32.4	31.6	-	-	1.03	-25.0
2	32.1	32.3	-	-	0.99	-24.2
3	28.7	28.4	-	-	1.01	-26.0
4	29.5	29.7	-	-	0.99	-23.8
5	30.5	30.0	-	-	1.02	-24.5
6	25.8	25.8	-	-	1.00	-24.9
7	28.7	26.8	-	-	1.07	-25.2
8	30.8	33.0	-	-	0.93	-25.3
9	27.6	27.2	-	-	1.01	-24.7
10	32.1	31.1	-	-	1.03	-23.9
11	30.3	28.6	0.5	-	1.06	-25.0
12	30.8	31.0	-	-	0.99	-25.1
13	29.6	30.7	-	-	0.96	-23.9
14	28.7	26.8	0.6	-	1.07	-24.8
15	31.1	30.7	-	-	1.01	-24.7
16	28.7	27.4	-	-	1.05	-24.5
17	30.9	30.6	-	-	1.01	-24.5
18	24.6	25.1	-	-	0.98	-26.1
19	30.8	31.2	-	-	0.99	-25.0
20	27.5	25.2	-	-	1.09	-25.2
21	30.8	30.6	-	-	1.01	-24.9
22	29.4	29.8	-	-	0.99	-24.7
23	28.7	27.1	-	-	1.06	-24.6
24	32.0	32.5	-	-	0.98	-24.0
25	30.8	29.9	-	-	1.03	-24.1
26	27.9	26.8	1.0	-	1.04	-24.1
27	33.2	33.7	0.7	-	0.99	-25.4
28	28.9	28.5	-	-	1.01	-23.7
29	26.8	28.6	-	-	0.94	-24.8
30	31.4	31.4	-	-	1.00	-25.3

Table 3.10 The Results of Pomegranate Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	7.5	7.3	0.0	0.0	1.03	-26.7	19.4
2	5.2	5.7	0.0	0.0	0.91	-26.9	13.9
3	4.9	5.3	0.0	0.0	0.92	-26.9	12.8
4	5.0	5.3	0.0	0.0	0.94	-26.9	12.3
5	6.5	5.8	0.0	0.0	1.12	-26.4	13.2
6	5.1	5.4	0.0	0.0	0.94	-26.8	12.4
7	4.8	5.0	0.0	0.0	0.96	-26.4	14.5
8	4.4	4.5	0.0	0.0	0.98	-27.3	13.3
9	4.6	4.5	0.0	0.0	1.02	-27.2	13.1
10	4.6	4.6	0.0	0.0	1.00	-27.0	12.8
11	5.0	4.7	0.0	0.0	1.06	-26.7	13.5
12	4.3	4.5	0.0	0.0	0.96	-26.5	13.2
13	4.5	4.8	0.0	0.0	0.94	-27.1	14.5
14	4.6	4.5	0.0	0.0	1.02	-27.2	13.9
15	5.1	4.6	0.0	0.0	1.11	-26.5	14.1
16	5.0	4.7	0.0	0.0	1.06	-26.3	13.9
17	5.2	4.7	0.0	0.0	1.11	-26.3	14.3
18	4.4	4.2	0.0	0.0	1.05	-27.0	13.2
19	4.6	4.6	0.0	0.0	1.00	-27.0	13.6
20	4.4	4.3	0.0	0.0	1.02	-27.0	13.1
21	5.2	5.0	0.0	0.0	1.04	-26.9	13.7
22	5.2	4.9	0.0	0.0	1.06	-27.0	14.2
23	5.4	4.9	0.0	0.0	1.10	-26.3	14.0
24	5.3	4.9	0.0	0.0	1.08	-26.3	13.7
25	5.2	4.7	0.0	0.0	1.11	-26.3	13.5
26	7.4	7.4	0.0	0.0	1.00	-26.0	16.9
27	7.2	7.3	0.0	0.0	0.99	-26.1	16.6
28	6.9	7.2	0.0	0.0	0.96	-26.1	16.1
29	5.3	4.9	0.0	0.0	1.08	-26.4	14.2
30	5.3	5.0	0.0	0.0	1.06	-26.5	14.6

Table 3.11 The Results of Concentrate Pomegranate Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	25.5	22.9	0.0	0.0	1.11	-27.0	65.1
2	25.5	21.9	0.0	0.0	1.16	-27.5	64.8
3	23.8	22.1	2.3	0.0	1.08	-26.9	64.8
4	23.9	24.4	0.0	0.0	0.98	-27.0	65.0
5	26.2	25.9	0.0	0.0	1.01	-26.4	64.7
6	25.6	23.6	0.0	0.0	1.08	-26.6	64.0
7	26.0	24.8	0.0	0.0	1.05	-27.5	64.6
8	25.7	24.9	0.0	0.0	1.03	-27.6	64.6
9	25.9	24.8	0.0	0.0	1.04	-27.5	64.9
10	25.8	25.5	0.0	0.0	1.01	-27.7	64.9
11	24.9	25.9	0.0	0.0	0.96	-27.3	64.0
12	24.7	26.1	0.0	0.0	0.95	-26.4	64.5
13	25.5	26.7	0.0	0.0	0.96	-26.2	64.6
14	24.2	25.2	0.0	0.0	0.96	-26.5	64.8
15	25.2	25.3	0.0	0.0	1.00	-26.1	64.8
16	25.4	25.8	0.0	0.0	0.98	-26.2	64.7
17	24.4	26.1	0.0	0.0	0.93	-26.5	65.0
18	24.4	25.2	0.0	0.0	0.97	-26.5	64.3
19	24.6	25.4	0.0	0.0	0.97	-26.6	64.8
20	23.9	24.5	0.0	0.0	0.98	-26.5	64.4
21	25.2	26.2	0.0	0.0	0.96	-26.3	65.2
22	24.2	25.2	0.0	0.0	0.96	-26.8	65.2
23	23.9	24.6	0.0	0.0	0.97	-26.8	65.3
24	26.0	26.1	0.0	0.0	1.00	-27.0	64.2
25	23.1	25.1	0.0	0.0	0.92	-26.3	64.5
26	24.7	24.5	0.0	0.0	1.01	-26.6	64.6
27	25.5	25.5	0.0	0.0	1.00	-26.3	64.8
28	21.5	22.7	0.0	0.0	0.95	-27.2	65.4
29	23.6	24.6	0.0	0.0	0.96	-26.1	65.1
30	26.0	25.4	0.0	0.0	1.02	-26.2	64.5

Table 3.12 The Results of Concentrate Cherry Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	17.5	24.1	0.0	12.1	0.73	-24.2	65.4
2	17.4	24.3	0.0	11.8	0.72	-24.1	65.4
3	17.2	23.9	0.0	12.3	0.72	-24.0	65.5
4	18.5	24.8	0.0	13.0	0.75	-24.3	65.7
5	18.3	24.2	0.0	13.9	0.76	-24.2	65.2
6	18.5	21.9	0.0	12.3	0.84	-24.4	65.9
7	19.1	22.9	0.0	12.5	0.83	-24.3	65.2
8	18.8	22.5	0.0	12.3	0.84	-24.3	64.1
9	21.1	20.4	0.0	12.0	1.03	-24.1	65.1
10	21.8	20.4	0.0	12.2	1.07	-24.0	65.8
11	20.2	20.1	0.0	12.5	1.00	-24.2	65.7
12	20.2	20.0	0.0	14.3	1.01	-24.1	65.5
13	17.2	22.3	0.0	12.1	0.77	-24.3	64.7
14	17.1	22.0	0.0	12.2	0.78	-24.2	64.7
15	17.2	22.1	0.0	11.7	0.78	-24.7	64.7
16	17.0	22.0	0.0	12.0	0.77	-24.5	64.5
17	17.5	22.5	0.0	11.8	0.78	-24.4	65.2
18	17.8	22.7	0.0	12.4	0.78	-24.3	65
19	17.5	22.3	0.0	12.5	0.78	-24.2	65.1
20	17.7	23.8	0.0	12.2	0.74	-24.2	64.7
21	17.4	23.9	0.0	12.0	0.73	-24.2	64.6
22	17.2	23.2	0.0	12.6	0.74	-24.1	64.7
23	17.6	20.6	0.0	12.0	0.85	-24.1	64.3
24	16.0	21.2	0.0	11.9	0.75	-24.4	65.1
25	16.0	21.2	0.0	12.1	0.75	-24.3	65.2
26	15.8	21.1	0.0	12.1	0.75	-24.2	65.2
27	16.5	21.7	0.0	13.8	0.76	-24.3	65.2
28	16.3	21.6	0.0	12.5	0.75	-24.2	65.1
29	16.2	21.4	0.0	13.4	0.76	-24.4	65.0
30	16.5	22.1	0.0	13.8	0.75	-24.2	65.3

Table 3.13 The Results of Concentrate Apple Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	37.8	15.9	7.5	3.6	2.38	-24.3	70.6
2	34.5	15.2	10.1	3.9	2.27	-24.2	70.0
3	37.5	13.5	11.6	2.1	2.78	-25.8	70.2
4	38.6	14.9	10.5	2.2	2.59	-25.9	70.1
5	38.1	14.6	10.3	2.2	2.61	-26.0	70.1
6	37.8	13.6	11.7	2.0	2.78	-25.9	70.3
7	38.0	14.6	10.2	2.2	2.60	-25.8	70.1
8	37.9	13.7	11.6	2.0	2.77	-25.7	70.6
9	37.3	13.5	11.6	2.1	2.76	-25.7	70.5
10	34.4	13.9	12.1	2.8	2.47	-24.4	70.2
11	34.5	13.8	12.0	2.8	2.50	-24.4	70.3
12	34.7	14.3	11.9	2.8	2.43	-24.5	70.5
13	33.7	13.7	11.5	2.8	2.46	-24.4	70.4
14	34.8	14.9	10.8	2.8	2.34	-24.8	69.4
15	34.4	14.1	12.7	2.7	2.44	-24.7	70.3
16	37.1	14.9	13.0	2.8	2.49	-24.3	70.4
17	38.1	15.8	7.5	3.7	2.41	-24.3	70.4
18	33.7	14.4	13.1	2.9	2.34	-24.7	70.4
19	35.4	15.0	11.3	3.7	2.36	-23.6	70.0
20	34.7	14.7	11.2	3.7	2.36	-23.5	69.9
21	32.6	12.4	11.8	2.3	2.63	-24.6	70.3
22	37.2	16.0	7.2	2.6	2.33	-25.2	70.3
23	37.1	13.2	8.9	2.7	2.81	-25.4	70.4
24	32.4	13.3	10.7	2.5	2.44	-24.9	70.5
25	35.0	13.9	11.0	2.7	2.52	-24.6	70.9
26	35.8	14.3	11.3	3.4	2.50	-23.6	70.0
27	35.4	14.1	11.2	3.4	2.51	-23.6	70.1
28	35.2	13.8	11.4	4.0	2.55	-23.6	70.0
29	36.3	14.8	10.2	3.4	2.45	-24.4	69.6
30	34.4	13.3	13.5	2.4	2.59	-24.8	70.4

Table 3.14 The Results of Concentrate Strawberry Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	23.7	21.5	3.3	0.0	1.10	-25.1	64.8
2	21.6	18.4	2.6	0.0	1.17	-25.6	65.4
3	22.4	19.0	2.6	0.0	1.18	-25.6	65.1
4	22.1	18.6	2.3	0.0	1.19	-25.6	65.2
5	21.3	18.8	4.3	0.0	1.13	-25.3	67.3
6	20.1	17.8	3.0	0.0	1.13	-25.5	58.5
7	21.1	18.0	3.3	0.0	1.17	-25.2	65.2
8	21.8	18.8	3.1	0.0	1.16	-25.2	65.8
9	21.8	18.6	3.4	0.0	1.17	-25.3	65.6
10	23.3	24.5	4.0	0.0	0.95	-25.0	65.5
11	21.6	22.2	3.2	0.0	0.97	-25.4	62.9
12	22.4	22.7	4.4	0.0	0.99	-25.0	65.5
13	21.1	21.2	4.3	0.0	1.00	-25.4	67.3
14	23.3	23.0	3.0	0.0	1.01	-25.3	64.0
15	20.7	24.7	4.4	0.0	0.84	-25.2	67.1
16	21.3	23.6	4.5	0.0	0.90	-25.7	64.7
17	21.4	24.1	4.1	0.0	0.89	-25.7	65.1
18	21.6	22.9	4.9	0.0	0.94	-25.5	65.4
19	21.4	23.2	5.1	0.0	0.92	-26.5	70.4
20	21.1	21.0	4.5	0.0	1.00	-25.4	67.1
21	21.1	20.9	4.6	0.0	1.01	-25.5	67.2
22	24.5	23.4	2.3	0.0	1.05	-25.8	66.0
23	19.2	23.0	4.2	0.0	0.83	-25.5	67.4
24	23.4	20.2	1.7	0.0	1.16	-25.8	66.0
25	20.8	18.8	4.6	0.0	1.11	-25.6	67.2
26	20.9	23.8	3.9	0.0	0.88	-25.8	65.3
27	19.9	24.2	4.0	0.0	0.82	-25.6	64.9
28	23.8	19.9	5.5	0.0	1.20	-25.4	65.7
29	22.1	18.7	5.3	0.0	1.18	-25.4	65.7
30	22.4	20.0	2.1	0.0	1.12	-25.5	64.9

Table 3.15 The Results of Concentrate Black Carrot Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	4.3	4.6	23.5	0.0	0.93	-25.5	62.9
2	4.5	5.0	23.2	0.0	0.90	-25.1	57.2
3	5.4	6.4	23.3	0.0	0.84	-24.7	64.2
4	4.7	5.0	22.2	0.0	0.94	-24.4	64.4
5	4.9	5.5	23.3	0.0	0.89	-24.6	64.6
6	4.0	4.3	25.0	0.0	0.93	-25.6	64.0
7	4.3	4.1	24.6	0.0	1.05	-25.7	64.0
8	3.8	4.0	26.0	0.0	0.95	-25.5	64.1
9	4.2	4.5	25.7	0.0	0.93	-25.6	64.2
10	4.0	4.4	24.8	0.0	0.91	-25.7	64.2
11	4.0	4.2	26.9	0.0	0.95	-25.6	64.2
12	4.0	4.3	26.4	0.0	0.93	-25.8	64.3
13	3.9	3.9	26.2	0.0	1.00	-25.8	64.1
14	4.0	4.1	27.3	0.0	0.98	-25.8	64.1
15	3.9	4.2	26.1	0.0	0.93	-25.8	64.2
16	4.0	4.4	24.7	0.0	0.91	-25.7	64.2
17	4.1	4.1	25.0	0.0	1.00	-25.6	64.2
18	4.0	4.3	26.1	0.0	0.93	-26.1	64.2
19	4.2	4.6	25.2	0.0	0.91	-25.9	64.2
20	4.4	4.7	26.8	0.0	0.94	-26.1	64.3
21	4.0	4.0	24.7	0.0	1.00	-26.0	64.3
22	3.9	4.1	25.2	0.0	0.95	-26.0	64.3
23	3.9	4.3	24.6	0.0	0.91	-26.1	64.2
24	3.9	4.2	24.5	0.0	0.93	-26.1	64.3
25	6.0	6.2	14.0	0.0	0.97	-24.4	64.1
26	6.0	6.9	14.6	0.0	0.87	-24.4	64.3
27	3.9	4.3	25.3	0.0	0.91	-25.6	64.1
28	5.0	6.5	20.1	0.0	0.77	-24.4	64.2
29	5.2	5.8	21.2	0.0	0.90	-24.4	64.2
30	5.3	6.1	20.5	0.0	0.87	-24.4	64.3

Table 3.16 The Results of Concentrate Pear Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	31.7	12.4	11.8	3.7	2.56	-24.9	70.1
2	32.3	13.1	12.5	3.6	2.47	-24.8	70.1
3	32.7	14.4	12.4	4.1	2.27	-24.1	71.0
4	30.6	13.8	0.9	8.4	2.22	-26.2	70.0
5	31.5	14.2	1.3	7.8	2.22	-26.0	71.9
6	27.4	13.0	2.4	9.2	2.11	-26.3	70.3
7	27.3	12.7	2.4	8.9	2.15	-26.2	70.2
8	27.2	12.7	2.2	10.0	2.14	-26.2	69.7
9	27.8	13.0	2.4	7.4	2.14	-26.3	70.0
10	34.0	15.5	2.7	8.0	2.19	-26.3	70.1
11	26.8	12.7	2.0	4.0	2.11	-26.2	69.8
12	27.1	12.1	2.2	4.8	2.24	-26.3	70.1
13	34.5	15.0	12.1	3.5	2.30	-24.6	70.7
14	32.3	13.9	1.7	15.1	2.32	-26.4	70.5
15	30.9	13.6	1.5	15.1	2.27	-23.7	70.2
16	21.4	22.8	4.9	8.1	0.94	-26.5	70.1
17	21.4	23.2	5.1	8.0	0.92	-26.5	70.4
18	27.3	12.0	2.1	18.9	2.28	-26.0	70.3
19	26.9	12.0	2.1	18.6	2.24	-26.0	70.3
20	25.7	11.3	2.3	21.9	2.27	-26.0	71.3
21	27.9	12.2	1.1	13.1	2.29	-26.0	70.4
22	29.7	13.2	6.0	9.3	2.25	-25.0	70.4
23	31.2	15.2	6.2	9.8	2.05	-25.0	70.3
24	31.9	15.3	6.2	10.0	2.08	-25.3	70.5
25	33.8	10.4	1.8	17.9	3.25	-26.5	70.8
26	31.2	12.4	2.4	9.5	2.52	-25.7	71.1
27	30.8	13.6	2.4	8.7	2.26	-25.6	70.7
28	28.6	11.4	3.0	4.9	2.51	-26.0	70.4
29	32.1	12.0	2.7	6.7	2.68	-26.3	70.5
30	29.9	13.7	2.6	10.0	2.18	-26.4	70.8

Table 3.17 The Results of Concentrate Quince Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	28.0	18.8	4.1	7.9	1.49	-26.1	67.9
2	28.1	16.6	3.6	8.0	1.69	-25.7	70.1
3	26.0	15.9	3.8	7.3	1.64	-26.0	70.3
4	25.6	15.9	3.3	9.0	1.61	-24.3	70.3
5	28.1	15.8	4.1	8.0	1.78	-25.9	65.3
6	30.6	16.0	3.2	8.1	1.91	-25.4	66.9
7	25.9	12.8	3.8	8.4	2.02	-24.5	65.6
8	28.5	21.3	4.1	7.5	1.34	-24.8	65.0
9	28.1	21.2	4.1	8.2	1.33	-24.8	65.2
10	29.4	21.0	3.8	7.0	1.40	-26.2	65.1
11	25.7	13.5	3.7	9.1	1.90	-24.8	64.7
12	28.0	15.3	4.2	8.4	1.83	-24.8	65.5
13	28.1	14.9	3.6	9.4	1.89	-24.5	65.8
14	32.4	14.9	3.3	9.4	2.17	-25.7	70.4
15	28.1	14.5	4.2	9.3	1.94	-24.0	65.7
16	31.1	13.4	4.8	8.6	2.32	-26.1	64.9
17	29.4	12.4	4.0	8.5	2.37	-26.4	65.3
18	29.2	12.0	4.1	7.8	2.43	-26.3	65.4
19	30.2	12.7	4.1	8.1	2.38	-26.3	64.3
20	29.9	13.8	4.1	9.6	2.17	-24.4	70.0
21	28.7	14.0	3.7	7.8	2.05	-25.6	66.8
22	29.6	13.8	3.6	8.2	2.14	-26.0	65.7
23	29.5	14.6	3.6	8.3	2.02	-24.9	67.8
24	28.9	13.9	4.2	8.4	2.08	-26.1	68
25	30.0	12.6	4.1	9.0	2.38	-26.0	65.5
26	28.9	12.7	3.5	8.7	2.28	-25.9	65.4
27	27.4	15.1	4.3	8.5	1.81	-24.7	65.6
28	30.3	13.7	3.8	8.4	2.21	-24.8	65.4
29	29.6	15.0	3.6	7.9	1.97	-25.3	66.7
30	28.8	14.2	3.6	9.2	2.03	-24.9	68.2

Table 3.18 The Results of Concentrate Grape Juice Samples

No of sample	Sugar contents (g/100g)				Fructose/ Glucose	‰ ^{13}C	Brix
	Fructose	Glucose	Sucrose	Sorbitol			
1	33.5	32.0	0.0	0.0	1.05	-25.3	66.5
2	29.0	29.7	0.0	0.0	0.98	-24.5	65.1
3	30.4	29.0	0.0	0.0	1.05	-24.5	65.2
4	30.7	29.6	0.0	0.0	1.04	-23.8	66.3
5	31.3	30.9	0.0	0.0	1.01	-23.7	65.4
6	23.0	22.6	0.0	0.0	1.02	-23.4	65.5
7	32.2	30.4	0.0	0.0	1.06	-23.6	65.1
8	29.3	27.7	0.0	0.0	1.06	-25.4	65.2
9	31.5	29.6	0.0	0.0	1.06	-26.9	68.4
10	28.5	29.7	0.0	0.0	0.96	-24.5	65.0
11	28.0	29.3	0.0	0.0	0.96	-24.6	64.8
12	27.5	28.4	0.0	0.0	0.97	-24.9	64.5
13	32.1	30.8	0.0	0.0	1.04	-24.8	65.0
14	28.9	29.8	0.0	0.0	0.97	-23.9	65.3
15	28.7	28.6	0.0	0.0	1.00	-25.0	65.3
16	30.4	31.0	0.0	0.0	0.98	-24.8	66.1
17	29.6	30.4	0.0	0.0	0.97	-24.6	64.8
18	28.7	28.0	0.0	0.0	1.03	-25.1	65.4
19	29.8	32.0	0.0	0.0	0.93	-23.8	65.8
20	31.1	30.0	0.0	0.0	1.04	-24.3	66.2
21	29.6	28.8	0.0	0.0	1.03	-24.7	64.9
22	29.4	29.0	0.0	0.0	1.01	-24.8	64.3
23	25.8	24.8	0.0	0.0	1.04	-25.1	65.8
24	27.9	25.0	0.0	0.0	1.12	-23.7	66.3
25	33.0	31.1	0.0	0.0	1.06	-24.8	65.4
26	30.6	32.0	0.0	0.0	0.96	-24.6	66.2
27	28.5	27.0	0.0	0.0	1.06	-25.2	65.1
28	29.7	29.6	0.0	0.0	1.00	-25.0	66.3
29	30.4	32.5	0.0	0.0	0.94	-24.0	65.2
30	29.8	27.0	0.0	0.0	1.10	-23.7	64

4. **CONCLUSION**

IRMS method offers an accurate and reliable way to discriminate between original and froud honey samples. In this thesis, IRMS method was used for the first time for other food samples including fruit juices, grape syrups and fruit concentrates produced in Turkey.

The developed methodology which includes the parameters such as glycose, fructose, sorbitol,sucrose %, delta ^{13}C value and brix number may be exploited for the examination of the food quality as well as authenticity of the production. It is also equally employed for the assessing whether the food was adulturated or not.

This study was carried out in Turkey first time to reveal and establish such indication associated with food quality control in laboratory site. The method was validated and uncertainty of measurement was calculated which constitutes a reliable basis for such applications.

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