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^{1}H NMR profiling of extracts from common vegetables and fruits

Master´s thesis

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Declaration

I hereby declare that I have authored this master's thesis carrying the name „ 1H NMR profiling of extracts from common vegetables and fruits "independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I futher state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on April 14, 2022 _____

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^{1}H NMR profiling of extracts from common vegetables and fruits

Summary:

The objective of this study was to use nuclear magnetic resonance (NMR) spectroscopy to investigate the some of the nutrients found in fruits and vegetables, such as carbohydrates, and organic acids. ^{1}H NMR spectroscopy is an effective technique for determining structure that requires small sample volumes and a short processing time.

The findings of this study reveal specific information about carbohydrates in study materials, such as sucrose, fructose, glucose, and mono-disaccharides, as well as organic acids, malate, and citric acids. were measured in 35 vegetable and fruit samples, each sample revealing its own chemical characteristic, citrus fruits reveal citric acids in large amounts, which are basic components for their identification and defining their quality, lime, lemon reveals citric acids concentration (135,665 g/L and 148,635 g/L). The citric acid concentration was also found in strawberry, mango, tomato, grapefruit, pomegranate, and oroblanco. Garlic, grapes, and mango all have a high carbohydrate content, according to research. Data were entered into the statistical program Metaboanalist to obtain statistical analysis (Wishart research group at the University of Alberta, Canada, www.metaboanalyst.ca).

This study goes over all of the chemicals mentioned above and their specific roles in food adulteration, authentication, and determining quality. The same rapid mechanism can be used in the aforementioned circumstances of the food industry; there are clear parallels between this research and another identification process revealed by ^{1}H NMR spectroscopy for the identification of food chemicals. ^{1}H NMR enables classification and differentiation not only between species but also within them and reveals limitless scientific capabilities.

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1 Introduction

Nuclear magnetic resonance (NMR) spectroscopy is a renowned investigative technique used by both chemists and biochemists to identify molecular structures and study the progression of chemical reactions. Another type of NMR technology, magnetic resonance imaging (MRI), has been widely used in medical radiology to obtain soft-tissue images for diagnostic purposes. Food scientists have also investigated the use of NMR and MRI in food analysis and processing, and they continue to develop a wide range of applications for food analysis and processing. (NMR) is a sophisticated research technique that provides detailed information about the structure, dynamics, reaction state, and chemical environment of a molecule. NMR spectroscopy is simple to automate, requires little to no sample preparation, is highly repeatable, and is non-destructive. Furthermore, it analyzes all of the constituents in a sample in a single step without knowing the nature of the constituents. As a result, the technique is now used in a wide range of screening and analytical applications. Because of its ability to analyze multiple components of complex mixtures at the same time without causing sample damage, NMR is well suited to the analysis of food and drink. NMR spectroscopy is an analytical chemistry technique used in quality control and research to determine the content and purity of a sample, as well as its molecular structure. All organic compounds are frequently analyzed using ^{1}H NMR and ^{13}C NMR spectroscopies. NMR can reveal the structure of components in complex food systems. The ^{1}H NMR spectroscopy technique can also be used for fingerprinting. These methods can quickly assess the source of chemical variability within food, and the inherent ability of NMR to provide information about the chemical composition of a sample will allow the source of variation to be identified. It delves into the central role of the researcher in qualitative data collection and addresses issues that are particularly relevant to healthcare professionals who conduct their own research, such as dietitians.

2 Scientific hypothesis and aims of the thesis

The aim of the thesis is a screening-profiling of aqueous extracts of common vegetables, such as broccoli, cauliflower, cabbage, spinach, salads with the aim to trace interesting targets for future analytical applications in quality assessment.

Here we hypothesise, that NMR is a method capable of an unpreceded role in the analysis of vegetables' nutritional quality, such as carbohydrates and organic acids.

The aforementioned chemicals are specific components that can provide us with a clear signal for identification, and our research will be a prerequisite to the principles, that hasten the process of exposing authenticity and adulteration.

3 Literature Overview

3.1 Principles of nuclear magnetic resonance spectroscopy

Since its inception in the 1940s, nuclear magnetic resonance (NMR) spectroscopy has evolved into a diverse, influential and Essential Analysis Tool With applications in many areas, including structural biology, pharmaceutical science, material science, medical imaging and more.(Kleinberg, et al., 2001)

NMR has been a fundamental technique, along with single-x-ray diffraction, for determining the structure and dynamics of complex biological molecules, including proteins. NMR spectroscopy techniques use: diagnosing biomarker diseases, food quality control and medical quality, drug discovery and development, materials, and judicial sciences. (Ahmad et al., 2016)

Nuclear magnetic resonance spectroscopy (NMR) has been ubiquitously used for many years to assess the structure of small chemical molecules; This technique is also increasingly applied to study small proteins or protein domains(Ziarek et al., 2018). NMR, unlike x-ray crystallography, does not require a crystalline sample. It is unquestionably necessary to place a small volume of concentrated protein solution in a strong magnetic field. Indeed, it is the primary technique that yields detailed evidence about the three-dimensional structure of molecules in solution. Some atomic nuclei, uniquely hydrogen nuclei, have a magnetic moment or spin: that is, they have intrinsic magnetization, like a magnetic rod. The spin corrects itself along the strong magnetic field but can be transformed into a misaligned excited state in response to applied high-frequency pulses (RF) of electromagnetic radiation.(Boyle, 2008)
When the excited hydrogen nuclei return to their aligned state, they emit RF radiation, that can be measured and displayed as a spectrum. The nature of the emitted radiation depends on the environment of each hydrogen nucleus, and if one molecule is excited, it influences the absorption and emission of radiation by other nuclei that lie close to it. It is consequently possible, by an ingenious elaboration of the basic NMR technique known as two-dimensional NMR, to distinguish the signals from hydrogen nuclei in different amino acid residues and to identify and measure the small shifts in these signals that occur when these hydrogen nuclei lie close enough together to interact. Because the size of such a shift reveals the distance between the interacting pair of hydrogen atoms, NMR can provide information about the spaces between the parts of the protein molecules.(Boyle, 2008) (Braun & Gō, 1985)

NMR can provide information about the distances between the parts of the protein molecule. By combining this information with knowledge of the amino acid sequence, it is possible in principle to compute the three-dimensional structure of a protein.(Alberts et al., 2002)(Havel & Wüthrich, 1984)

NMR spectroscopy is the most practical method for determining the structure of tiny proteins with a molecular weight of fewer than 20,000 daltons. As the size of the macromolecule grows larger, the resolution drops. However, recent technological advancements have increased the limit to around 100,000 daltons, allowing NMR structural investigation of most proteins. NMR investigations are particularly effective for detecting changes in protein structure, such as during protein folding or when the protein links to another molecule, because they are performed in solution.(Boyle, 2008)

NMR is also commonly used to determine molecules other than proteins, and it is essential for evaluating the three-dimensional structures of RNA molecules and the complex carbohydrates side chains of glycoproteins, for example.(Latham et al., 2005)

The resonance state in NMR is satisfied by absorbing in the radio wavelength range (frequency 40 MHz) for 1H nuclear resonance in a few hundred mT external magnetic field. The actual scan field is minimal compared to the field strengths used, and the absorption radio frequencies are clearly indicated on such spectra. The transfer of 'entities' from lower to higher energy levels is aided by energy input in the form of electromagnetic radiation. Nuclear magnetic spins, which occupy energy levels according to quantum chemical laws, are these entities in NMR. The spins will return from the higher to the lower energy level after a set amount of time, a process called relaxing. (Wilson et al., 2010)

Spin– lattice relaxation is the process by which energy produced during the transition of a nuclear spin from a higher to a lower energy state can be expelled as heat into the environment.

NMR spectra are extremely useful for deciphering chemical structures. It is possible to acquire both qualitative and quantitative data. Many more advanced NMR techniques are now possible thanks to advances in computing power. Weak signals can be improved by aggregating data from multiple scans. Baseline noise, which is random, tends to cancel out whereas the signal increases. Computer averaging of transients, or CAT scanning, is a technique that significantly improves the signal-to-noise ratio. Despite the usefulness and continuous use of such "traditional" ^{1}H NMR, significantly more structural information may be gained by using pulsed radio frequency radiation and applying the Fourier transform to the result. (Farrar et al., 2012) (Wilson et al., 2010)

When compared to 1H nuclei, ^{13}C nuclei have a different value of g (magnetogyric ratio), hence their resonance frequencies differ from those of protons in the same applied field. Protons resonate at around 300 MHz in a 7.05 tesla magnet, while carbons resonate at around 75 MHz. This enables us to examine ^{13}C signals via a different 'window' of ratio frequencies. Trimethylsilylpropanoic acid (TSP) is used as a standard compound in ^{13}C NMR investigations to define the 0 ppm, similar to how it is used in ^{1} H NMR. However, the signal from the four equivalent carbon atoms in TSP serves as the standard. Chemical changes in organic compounds for ^{13}C nuclei are spread out across a significantly larger range of roughly 220 ppm. Essentially the same variables that drive the chemical shift of a proton influence the chemical shift of a 13 nucleus: the deshielding action of electronegative atoms and anisotropy effects tend to move signals down field (higher resonance frequency, with higher chemical shifts). Furthermore, sp^2 hybridization causes a significant downfield shift. Due to both sp^2 Hybridization and the double bond to Oxygen, the ^{13}C NMR signals for carbonyl carbons are often the furthest downfield (170-220ppm).(Becker, 2000) (Hatzakis et al., 2019)

3.1.1 ^{13}C-Carbon NMR

Due to the low quantity of the ^{13}C isotope, the chances of discovering two of them in a molecule are extremely slim. As a result, there are no ^{13}C–^{13}C couplings (homonuclear couplings). While ^{1}H–^{13}C interactions (heteronuclear coupling) are feasible, most ^{13}C spectra are decoupled, with all bands representing solely carbon. When compared to ^{1}H spectra, ^{3}C spectra are significantly simpler and cleaner. However, the fundamental drawback is that multiplicities in these spectra are not visible, making it impossible to determine if a given ^{13}C is connected with a methyl (H_3C), methylene (H_2C), or methine (HC) group. During a decoupling experiment, some of this information can be recovered by irradiating with an off-resonance frequency. (Balci et al., 2005.)

The decay of the transverse magnetisation, also known as free induction decay, is observable in pulse-acquired Fourier transform NMR (FID). (Morris, 2017)

Carbon NMR distinguishes itself from proton NMR in that it determines the type and number of carbon atoms in an organic molecule, whereas proton NMR determines the type and number of hydrogen atoms in an organic molecule. (Pelletier et al., 1991)

The most abundant isotope of carbon, ^{12}C (which has a natural abundance of 99 percent), lacks a nuclear magnetic moment and is consequently NMR-inactive. The C NMR is based on

the ^{13}C isotope, which makes up around 1% of all carbon atoms in nature and has the same magnetic dipole moment as a proton. The theories we learnt about ^{1}H NMR spectroscopy apply to ^{13}C NMR as well, although with a few key alterations in the spectrum. (Eckstein et al., 1965-1966)

Because a ^{13}C isotop's magnetic moment is substantially lower than that of a proton, ^{13}C NMR signals are inherently lower than proton signals. This, along with the low natural abundance of ^{13}C, makes detecting carbon signals considerably more challenging. To get the signal-to-noise ratio down to acceptable levels for ^{13}C NMR spectra, a high-concentration sample and a large number of scans (thousands or more) are usually necessary.(K. . & W. J. (Eds.). (2010). P. and techniques of biochemistry and molecular biology. C. university press. Wilson, et al., 2010)

3.1.2 Multidimensional NMR

The decay of the transverse magnetisation, known as free induction decay, is observable in pulse-acquired Fourier transform NMR (FID). As a result, the detected signal is a function of the detection time t2. The time t1 (evolution time) within the pulse sequence describes the time between the first pulse and signal detection. When t1 is varied systematically, the detected signal becomes a function of both t1 and t2, and its Fourier transform has two frequency components. A two-dimensional spectrum is built on these two components. (Wilson et al., 2010)

Chemical shifts on both axes are visible in correlated 2D-NMR spectra. Using different pulse sequences results in various methods, such as correlated spectroscopy (COSY), nuclear overhauser effect spectroscopy (NOESY), and so on. These methods produce homonuclear 1H

couplings. The 1D-NMR spectrum now runs diagonally, and long-range couplings between specific nuclei appear as off-diagonal signals. Although TOCSY and COSY are very similar in their basic principles, TOCSY provides information on the cross-peaks for all protons in the same spin system. In the case of an ABC system with four protons, for example, the COSY spectra reveal cross-peaks for "A to B," "B to C," and "C to D," whereas the TOCSY spectra reveal an additional cross peak for "A to C," "A to D," and "B to D." This means that the TOCSY spectrum reveals a total correlation between the 1 H values of the same spin system. As a result, TOCSY is very useful for identifying natural peptide products because it reveals strong interactions between the spin systems of each amino acid. (Rahman, 2015)(Gouilleux et al., 2018) (Hatzakis et al., 2019)

3.1.3 Phosphorus-31 NMR

Phosphorus-31 NMR spectroscopy is an analytical chemistry technique that uses nuclear magnetic resonance (NMR) to study phosphorus-containing chemical compounds. Because phosphorus is commonly found in organic compounds and coordination complexes (as phosphines), ^{31}P NMR spectra can be measured on a regular basis. Because ^{31}P has a 100 per cent isotopic abundance and a relatively high gyromagnetic ratio, solution ^{31}P-NMR is one of the more common NMR techniques. Because the ^{31}P nucleus has a spin of 1/2, spectra are relatively simple to interpret. 1H and 19F are the only other highly sensitive NMR-active nuclei spin 1/2 that are monoisotopic (or nearly so). (Walker et al., 2019)

Applications in chemistry-^{31}P-NMR spectroscopy is useful to assay purity and to assign structures of phosphorus-containing compounds because these signals are well resolved and often occur at characteristic frequencies. Chemical shifts and coupling constants have a wide range but are not always predictable. The Gutmann-Beckett method assesses the Lewis acidity of molecular species by using Et3PO in conjunction with 31P NMR spectroscopy. (Neu, et al., 2012)

^{31}P-NMR spectroscopy is widely used for studying phospholipid bilayers and biological membranes in their natural state. The analysis of lipid ^{31}P-NMR spectra could reveal a wealth of information about lipid bilayer packing, phase transitions (gel phase, physiological liquid crystal phase, ripple phases, non-bilayer phases), lipid head group orientation/dynamics, and elastic properties of pure lipid bilayers as a result of protein and another biomolecule binding. (McLaughlin et al., 1975)

Operational aspects-With a gyromagnetic ratio of 40.5% of that for 1H, ^{31}P NMR signals are observed near 202 MHz on an 11.7-Tesla magnet (used for 500 MHz ^{1}H NMR measurements). Chemical shifts are referenced to 85% phosphoric acid, which is assigned the chemical shift of 0, with positive shifts to low field/high frequency. Due to the inconsistent nuclear Overhauser effect, integrations are not useful. Most often, spectra are recorded with protons decoupled.(Schott et al., 2012)

3.2 The issue food fraud

In recent years, the incidence of intentional adulteration of food and drink for financial benefit has increased. Unscrupulous food producers deceive their clients by substituting cheaper substitutes for the branded product in order to increase profit margins. With the expansion of trade barriers, the food industry has become a truly global enterprise, making food origins difficult to track and food fraud simpler to conceal. Food adulteration occurs widely and in a variety of forms, affecting nearly all food commodities. Adulteration is not only a significant economic challenge, but it may also cause substantial health problems for users. As food adulteration methods have gotten more comprehensive, highly efficient and dependable approaches for detecting fraudulent alterations are necessary.(Collins, 1993)

Scandals such as the "rapeseed oil" fraud intended for industrial use in Spain (1981), which affected approximately 20.000 people and resulted in between 370 and 835 deaths, dioxin in Belgium, which resulted in massive economic losses (1999), and In China, milk tainted with melamine caused over 50.000 ill infants and six deaths (2008), Methanol poisoning from the sale of illegal spirits, which killed 59 people in the Czech Republic and Poland between 2012 and 2014, horse meat in beef products (2013), fipronil in eggs (2017), and the killing of sick cows (2019) have all garnered international attention.Food fraud has harmed legal businesses, and substantial efforts are being made to eliminate such acts in order to protect the livelihoods of honest suppliers. NMR plays a critical role in all of them. (Sobolev et al., 2019)

The use of nuclear magnetic resonance (NMR) spectroscopy in the field of food authenticity control has grown. Because of its low instrumental variability, it is feasible to collect vast datasets of legitimate spectra. Adulterations in food fraud instances can be discovered, and substances can be quantified for regulatory limit management. The following section discusses the authentication and adulteration of coffee, wine, meat, and dairy products.(Laghi et al., 2014) (Manning, 2016) (Hatzakis et al., 2019)

3.2.1 Honey authentication

Honey has been the primary source of sweet flavor for centuries, and it is also regarded as a healthful food. The taste and aroma of this liquid varies according on the flower source, geographical location, and season. As a result of the abundance of melliferous sources, various unique unifloral and polyfloral nectar honeys can be produced. Each honey is distinct due to the chemistry, quantity, and mix of the many components that give each honey its own distinct

organoleptic profile. In apiculture, quality monitoring and characterisation of unifloral honeys, as well as their botanical origin, are of critical importance and interest.(Mehryar et al., 2011.)

According to paragraphs 7 and 9 of European Regulation (EU) 1169/2011, honey products must be labelled with accurate botanical and geographic information. Several studies have demonstrated that ^{1}H NMR and heteronuclear multiple bond correlation (HMBC) spectroscopy may be used to detect the botanical and geographical origin of honey.(Kuballa et al., 2018)

A combination of principle component analysis (PCA) and linear discriminant analysis (LDA) applied to the chemical fingerprint of ^{1}H NMR spectra obtained from legitimate honey samples can authenticate the provenance of acacia, rapeseed, and forest honey. (Tsagkaris et al., 2021)

Labeling verification of monofloral and multifloral honey varieties was accomplished using ^{1}H NMR profiling combined with chemometric techniques. The scientists demonstrated that sugar addition down to 10% levels may be detected using spiking studies (i.e., adding a pure chemical into real food matrices). Quantification of controlled parameters such as glucose, fructose, sucrose, and hydroxymethylfurfural within the same NMR experiment is attainable when chemometric methods are used. Rice syrup (RS), a byproduct of rice polysaccharide hydrolysis derived from a C3 plant (similar to beet syrup), is one of the most commonly used honey adulterants in China.(Kuballa et al., 2018)

Rice syrup contains three sugars: maltotriose (52 %), maltose (45 %), and glucose (5 %). (3 per cent). Rice syrup acts like 100 per cent glucose inside the body because maltose is two molecules of glucose and maltotriose is three molecules of glucose. Honey tainted with RS has recently appeared on the market. Rice syrup is a C3 syrup adulterant that undergoes photosynthesis in a manner similar to that of natural honey. As an outcome, the use of rice syrup as a honey adulterant is a critical issue affecting quality assurance and food safety. (Fakhlaei et al., 2020)

3.2.2 Coffe authentication

Coffee is one of the most widely consumed food products in the world, and it is critical to the economies of the countries involved in its production and export. Because of its commercial importance, the detection of impurities and foreign matters has been a constant concern in fraud

verification, particularly because adulterations in roasted and ground coffee samples are difficult to detect with the naked eye. Adulteration of roasted coffee, on the other hand, is a common and diversified approach used to cut expenses. It can include the addition of other substances (coffee husks and stems, corn, barley, chicory, wheat middlings, brown sugar, soybean, rye, and triticale) to coffee blends to make them less expensive (considering species, geographical origin, and defective beans), as well as the quality of the beans (considering species, geographical origin, and defective beans). (Daviron et al., 2005)

The proposed ^{1}H NMR spectra as fingerprints revealed that the chemical composition of roasted coffee differed significantly from that of barley, soybean, corn, and coffee husk samples. Some of the compounds identified in coffee, are chlorogenic acid, caffeine, trigonelline, N-methylpyridine, and formic acid in the 6.0 to 9.3 ppm range, and lipid compounds, amino acids, and aliphatic fatty acids in the 0.7 to 3.0 ppm range. The adulterants investigated (soybean, corn, and barley) are seeds with a high carbohydrate content. This feature remained even after the roasting process. It was necessary to find peaks in the adulterant spectra that were not present in the coffee spectrum in order to identify an adulterant using this technique. Because there must be no interfering peaks, the selection of these marker peaks took into account two parameters: intensity and location. Several peaks that could be used as markers after superposition of the adulterant spectra with the fingerprint of roasted coffee. A high intensity singlet chemical shift at 5.03 (s) was chosen to indicate the presence of corn, corresponding to the signal of hydrogen attached to the anomeric carbon of a specific carbohydrate. To indicate the presence of barley, signals at 5.30 (s) and 3.15 (s) were chosen. For soybean, the marker signals were chemical shifts at 5.30 (J = 4.15 Hz) (dd) and 4.87. (t). The coffee and coffee husk spectra were strikingly similar, with several compounds present in the coffee that could also be found in the husk. In the analyses of commercial coffee samples, signals at 5.08 (J = 5.92 Hz) (d) and 4.98 (s) were identified as markers for the presence of coffee husk. (de Moura Ribeiro et al., 2017)

The proposed procedure, which employed the ^{1}H NMR technique, proved to be highly effective for identifying and quantifying the major types of adulterants in roasted coffee. The next step is to identify metabolites that can be used to differentiate between different types of seeds and other adulterants, allowing for the elucidation of the main differences between them. This will pave the way for the development of a broader approach that includes a broader range of seed types that are found less frequently, as well as the impact of geographical variability on defective beans and adulterants.

A quantitative assessment of adulterants was also carried out using adulterant blends and roasted coffee. Using different peak markers, it was discovered that increasing percentages of adulterants (30, 50, and 70%) in coffee blends resulted in increased intensity of the marker compound peaks, ensuring that the analyses were quantitative. The data were visually analyzed before the chemometric analysis to identify differences between the spectra. This NMR data analysis is necessary to confirm that spectral differences are caused by differences in sample composition rather than problems in spectroscopic measurements caused by factors such as shimming, signal suppression due to H_2O/HDO, phase shifts, and baseline corrections. Variations in these parameters must be carefully monitored to ensure that they do not cause errors in the analysis, because when there are differences between the spectra, whether due to differences in sample composition or other factors, the chemometric analysis will distinguish between them, classifying them into many different groups. To begin, the entire spectrum was used in the analysis, with the exception of the region of the TSP signal corresponding to the hydrogens of the reference and water (H_2O/HDO). The coffee extracts were analyzed without being pretreated in any way. Principal component analysis (PCA) was used to analyze the 31 commercial coffee samples and four adulterants, with the spectra autoscaled. Three variables accounted for 47.20 percent of the total variance. To aid in the interpretation of the PCA results, two-dimensional graphs were used. Scores graphs represent sample data projected onto new axes corresponding to the main components, allowing for the identification of similarities and classification of different classes. Loadings graphs show the differences in the chemical compositions of the samples and indicate those variables from the original set that contribute to the discrimination observed in the score's graphs. The scores plot of the samples after standard pretreatment revealed a good separation of main components 1 and 2 (PC1 PC2). Some coffee samples appeared to be mixed in with adulterants barley, corn and soybean. Several Samples, on the other hand, tended to cluster with adulterant (Coffee husk). All of the other pure samples fell into two distinct groups as a result of the roasting processes and geographical origins.(de Moura Ribeiro et al., 2017)

3.2.3 Authentcation Dairy products

The use of NMR to investigate cheeses has been extensively documented in several areas, including determining their geographic origin, molecular water mobility, and water retention capacity. Scientists used ^{1}H NMR to investigate the ripening and regional differentiation of Parmigiano Reggiano Italian cheese. The chemical, physical, and enzymatic characteristics of

the cheese components were shown to change during the ripening process. Experiments have shown that the ripening and production environment may affect amino acid content, with high levels of leucine and isoleucine in the early stages of ripening and high levels of threonine in the late stages. When the lactate, butanoate, acetate, leucine, and isoleucine content of Parmigiano Reggiano cheese was compared to the lactate, butanoate, acetate, leucine, and isoleucine content of Eastern Europe Grana type cheese, the latter was richer in lactate, butanoate, acetate, leucine, and isoleucine, while the first was richer in other compounds, such as threonine In addition to defining the regional origin of these cheeses, these amino acids and fatty acids contributed to their distinctive flavour. (Balthazar et al., 2021)

Another research used ^{1}H NMR to assess water mobility and distinguish between the most mobile bulk water trapped in the casein matrix and a less mobile aqueous phase bound directly to the casein of mozzarella cheese. Furthermore, the ^{1}H NMR data demonstrated that the ice crystals penetrated the protein matrix, triggering the formation of water pockets and the diffusion of water molecules and fat globules during the freezing of mozzarella cheese. (Balthazar et al., 2021)

Using N-acetyl carbohydrate biomarkers to detect bovine milk adulteration in caprine milk: A 115-sample matrix of pure and artificially adulterated pasteurized milk samples was created and used to identify bovine milk biomarkers that are independent of chemical and biological variation caused by factors such as genetics, diet, or seasonality. Analyses of principal components and orthogonal projections to latent structures A discriminant analysis of pure bovine milk and pure caprine milk revealed spectral features associated with four molecules' resonances. The PCA results revealed that the metabolite profiles of these milk samples are classified into two groups. The first two principal components account for 22.2 and 16.0 per cent of the total variance, respectively. The variance value is 38.2 per cent, which is due to many independent factors in the data set. The OPLS-DA score plot revealed a clear separation of caprine and bovine milk, with a goodness of fit R2Y = 0.989 (P 0.001) and predictive ability Q2 = 0.964 (P 0.001.). These results from the random permutation test indicated that the model had a very high explanatory and predictive ability, as well as good data concordance. Based on the p(corr)correlation coefficients, the corresponding coefficient color-coded plot revealed bins that were more abundant in bovine milk and may serve as biomarkers of adulteration (2.06, 2.50–2.66, 5.34, and 6.18 ppm.). Analyses of principal components and orthogonal projections to latent structures A discriminant analysis of pure bovine milk and pure caprine milk revealed spectral features associated with four molecules' resonances. The peaks

corresponding to protons in the N-acetylglucosamine and N-acetylgalactosamine acetyl moieties were particularly useful for their method.

This method differentiated caprine milk adulterated with 5% bovine milk with 84.78 per cent accuracy and caprine milk adulterated with 10% bovine milk with an excellent 95.65 per cent accuracy, demonstrating that N acetyl carbohydrates could be used as biomarkers for the detection of bovine milk in caprine milk.(Rysova et al., 2021)

3.2.4 Authentication of Meat

Many consumers today are concerned about the meat they eat, and accurate labeling is critical to informing consumer choice. The preference for one product over another can reflect aspects of lifestyle (for example, vegetarianism and organic food), religion (for example, the absence of pork in some diets), and diet and health concerns (e.g., absence of allergens). Furthermore, accurate labeling is necessary to support fair trade. Additional descriptive label information can be added as a result of branding, product marketing, and regulatory requirements. While regulations enshrined in national and international law support mandatory label information, they are insufficient to prevent food fraud. Robust analytical tests are required to ensure compliance with regulations and to impose punitive measures when necessary. Meat authenticity has been more popular in recent years. Many customers are concerned about the meat they consume, and correct labeling is necessary to help them make important choices. Meat origin, meat substitution, meat processing treatment, and non-meat ingredient addition can all be classified as areas where fraud is most likely to occur. (Ballin, 2010)

Many DNA-based methods for determining the species present in meat products exist. Animals, on the other hand, differ not just in their DNA; there are other compositional variables that may be measured and used to determine species.

Pork and beef fat, for example, are well known to be quite different from one another. This is owing to variances in the triglyceride compositions of the animals, which are caused by changes in their diets, metabolisms, and digestive systems. H-1 relaxometry is the most studied field of NMR in meat science Relaxometry has been widely used due to its ability to characterize water and structural features in heterogeneous systems such as muscle/meat.(Kirtil et al., 2016)

P-31 NMR spectroscopy has been used to explore the influence of genetic, dietary, and slaughter variables on meat quality through their effects on phosphorous metabolism

postmortem, and these findings are considered and addressed in connection to their contribution to fundamental meat science. (Bertram et al., 2002)

Authentication of beef versus horse meat using 60 MHz ^{1}H NMR spectroscopy-there are typical 60 1H MHz spectra of triglycerides that contain a range of long-chain fatty acids with differing amounts of unsaturation. Based on the assignment for 60 MHz 1H NMR of triglycerides, some of the key spectral regions are indicated. It can be seen that the spectra from horse samples vary more than those from beef samples, and that some of the formers are significantly noisier and thus distinguishable more easily in the overlaid spectra. This is likely a consequence of the generally lower fat content of horses compared to beef. (Jakes et al., 2015)

3.2.5 Authentication of Wine

Wine is a historically significant alcoholic beverage that has grown in commercial importance. It is classified as a premium product and is manufactured and consumed in a variety of nations across the world. Wine is a classic alcoholic beverage with a high economic value that is made from grape must fermentation. The content and variety of grapes are connected to the quality of wine, according to this definition. Furthermore, the geographic location of vineyards, differences within the same vineyard, viticultural practices, and winemaking and aging procedures can all separate wines. Wine is a complex blend of several hundred compounds, many of which are found in very low amounts; yet, they all have a part in the evolution and quality of the wine. Water, 86 percent; ethanol, 12 percent; glycerol and polysaccharides or other trace elements, 1 percent; different types of acids, 0.5 percent; and volatile compounds, 0.5 percent are the typical concentrations of the principal components of wine. Red, white, and rosé wines are classified by their sweetness, alcohol content, carbon dioxide concentration, color, grape variety, fermentation, and maturation procedure, as well as their geographic origin.(Markoski et al., 2016)

NMR has been used to authenticate wine for decades. It is one of the most advanced techniques of spectroscopy for food and beverage classification. Initially, site-specific natural isotopic fractionation NMR (SNIF-NMR) spectroscopy was presented as a method for determining the biological origin of ethanol based on the natural distribution of deuterium, which may disclose the illegal use of chaptalization (sugar addition) in winemaking, for example.

Researcher identified German wines from five locations according to geography, variety, and vintage using the complete ^{1}H NMR spectrum as a fingerprint in combination with LDA, with overall correct classifications of 89 percent (geographical), 95 percent (varietal), and 96–97 percent (vintage) (vintage). (Godelmann et al., 2013)

^{1}H NMR metabolomic data has also been used to quantify a variety of metabolites such as sugars, amino acids, organic acids, alcohols, and phenolic compounds, which have been used to differentiate wines as a function of terroir (which includes biophysical and cultural factors in the production region) and cultivar. Mascellani employed NMR and a random forest (RF) machine learning method to identify over 900 Czech wines by kind (based on colour and residual sweetness) and varietal, with the goal of developing and testing multivariate statistical models and machine learning methods for the classification of 6 types based on colour and residual sugar content, 13 wine grape varieties, and 4 locations based on ^{1}H NMR spectra. With an accuracy of more than 93 per cent, the predictive models correctly classified dry and medium dry, medium and sweet white wines, and dry red wines.

PCA analysis was performed on the data matrix of 911 samples classified by type after 14 strong outliers were removed. The PCA score plot revealed a clear separation between the red and white wine clusters, with the first two principal components accounting for 10.1% and 7% of the variation, respectively. PCA also demonstrated potential for classifying different types of dry and medium dry wines, as well as medium, sweet white and dry red wines. Three main sub-clusters were identified in the PCA cluster describing white wines: dry and medium dry, medium and sweet white wines. there were researched correlations with actual alcoholic strength and residual sugar content-that includes actual alcoholic strength and residual sugar content.

Random forest analyses for type and wine grape variety-First, the RF model was trained to recognize different types of wines; the training and test sets were made up of 635 and 268 randomly assigned ^{1}H NMR spectra, respectively. The test set was not used to build the model to reduce the risk of over-fitting. Internal validation of the RF model yielded a rather satisfactory OOB error (8.82 percent), with the classification error always being higher for rosés and Blancs de noir. The model classified rosés and blancs de noir as dry, medium-dry, or medium white wines. The model correctly classified white dry and medium dry wines, as well as medium and sweet wines, with sensitivity values of 0.95, 0.89, and 1, and specificity values of 0.93, 0.95, and 0.98, respectively. 99.93 percent of dry red wines were correctly classified. Bins containing signals of fructose (bins 3.78, 3.86, 4.06, 3.82, 3.7 ppm), uridine (bin 5.86 ppm), ethanol (bin 1.02 ppm), catechin (bins 5.98 and 6.06 ppm), tyrosine (bin 3.5 ppm), and

glycerol were the most important features for type prediction, according to Mean Decrease Gini (bin 3.5 ppm). (Mascellani et al., 2021)

3.3 NMR in The Determination of Food Quality

The peculiar conditions of production areas, which impart unrepeatable organoleptic and nutritional properties to agro products, have a significant impact on food quality. Beginning in 1992, European legislation established international food labels such as "Protected Geographical Indication" (PGI) to protect the high quality of all typical EU productions (EEC Reg. 2081/92). As a result, the demand for powerful analytical tools for determining food provenance has grown over time. NMR spectroscopy, in particular, when combined with multivariate statistical analysis, is regarded as a powerful and effective method of investigating the metabolic profile of organic samples. High-field NMR spectroscopy is only used with active nuclei (such as 1H, ^{13}C, and ^{31}P) and is widely used in food origin authentication. In particular, proton (1H) liquid NMR spectroscopy is used for metabolite profiling with minimal sample preparation, but it is also used in a non-targeted metabolite fingerprinting approach with very interesting performance in order to detect the peculiar chemical composition and to identify specific markers of foods (target analysis).In fact, using this method, the NMR spectrum is obtained as a complete fingerprint of the sample, rather than identifying the metabolites from the peaks, as is required in a targeted approach. Furthermore, when a significant overlap of signals prevents the complete assignment of spectrum peaks to specific molecules, two-dimensional (2D) NMR experiments are used. (Paccian et al., 2001)

Recently, new automated NMR methodologies have been developed in order to shorten NMR data analysis (metabolite identification and quantitation in complex mixtures) and avoid potential errors due to manual manipulation in baseline, phase, and integration. For example, to trace the geographical origin of dark chocolate, NMR experiments based on a semi-automated NMR approach with completely automated metabolite identification and quantification have been proposed. High-Resolution Magic Angle Spinning (HRMAS) NMR spectroscopy is a precise and fast alternative to traditional liquid NMR spectroscopy because it allows for direct examination of the entire sample without the need for compound extraction. This highly adaptable technique generates high-resolution NMR data from samples containing solutions, gels, and swellable solids, and it has been used in food research to analyze jams, fruit jellies, and other semi-solid foods. (Emwas et al., 2019)

Advances in high-field magnets and probe design, which increase the analytical capabilities of current NMR spectrometers, are primarily responsible for the rapidly rising application of NMR in food research. In food research, several types of NMR investigations have been shown to be very beneficial.

3.3.1 Food composition and physical characteristics

Because of the widespread use of automatic spectrometer setup methods in recent decades, quantitative investigations on current NMR equipment are no longer as reliant on the operator. Furthermore, the consequences of poor instrument setup may be precisely delineated. As a result, the number of quantitative NMR (qNMR) applications aimed at individual molecules is growing, which is especially advantageous when all of the regulatory requirements for food can be examined fully using NMR spectroscopy. This is the case with egg yolk-based liqueurs, where the overall sugar and alcohol content have been measured directly while the egg yolk content has been calculated effectively. Mathematical correlations between NMR spectra and even physical features of food, of key-value for transformation, have been developed, allowing for high percentages of correct predictions. This is the case with beef, where the most significant qualities of meat, tenderness and water-holding capacity, as well as appearance, have been effectively simulated using NMR. This is also true for milk, whose coagulating characteristics have been linked to the metabolite profile as determined by ^{1}H NMR and Principal Component Analysis (PCA).(Trimigno et al., 2015)

In 1956, ^{1}H NMR was first used in a meat science application. The characterization of water's dynamic states by transverse relaxation time (T2) serves as the foundation for evaluating WHC using NMR. The proportion of various water states within muscle tissue indicates the amount of free water available for loss as drip/purge. The nuclear magnetism of hydrogen atoms in molecules allows NMR to characterize these water states. When atomic nuclei exhibit angular momentum and a resulting magnetic moment, they align with the magnetic field force of their surroundings. Certain nuclei will wobble (precess) on their axis at a given magnetic field force. The energy of an applied radio frequency (RF) is absorbed by the nuclei as they precess. The absorption of RF energy by all members of a given atomic species (for example, 1H) precessing together at the specified magnetic field force can be detected, amplified, and measured. In other words, the amount of RF energy absorbed at the specified field force required to precess 1H is a good indicator of the amount of free water in a sample and has been found to correlate with cooking loss. In NMR relaxometry, the nuclei aligned with the magnetic field that has absorbed RF energy will return to their equilibrium/ground state via a relaxation

process. The various states of water found in meat will relax at different rates. These WHC-related NMR techniques make use of the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo technique. This means that the sample is exposed to a single RF pulse ($\pi/2$) followed by equally spaced pulses. The intensity of an equal number of spin echoes can be plotted against the time elapsed between the initial $\pi/2$ pulse and echo detection. Depending on the study, two or three distinct peaks can be used to graphically represent this. Brown et al. (2000) argue that two regions are necessary, whereas Bertram and Andersen (2007) argue that three regions are more appropriate.(Oswell et al., 2021)

3.3.2 ^{31}P NMR spectroscopy in food analysis

Phosphorus is an essential element that accounts for around 1% of an adult's total body weight. Inorganic phosphorus, Pi, and organic phosphorus-containing compounds play a key part in life's metabolic activities and are engaged in a variety of body functions. The main component of bone and teeth is hydroxyapatite, or calcium phosphate. Phospholipids play a crucial role in the structure of cell membranes. Phosphorylation is required for various proteins to become active and execute their functional roles, and nucleotides such as adenosine triphosphate (ATP) are at the center of the energy transfer cascade in all living cells. Because of their influence on the meat's water-holding capacity (WHC) and the loss of flexibility with the start of rigour Mortis, metabolic processes in muscle post-mortem are a crucial element for meat quality. (Vogel, 1989)

3.3.3 ^{31}NMR for Starch

Starch is a carbohydrate polymer functioning as the major energy resource in plants, where it is found mainly in seeds, roots and tubers, and represents the most important polysaccharide for human nutrition. Phosphorus in native starch is found in three major forms: starch phosphate monoester, phospholipids, and inorganic phosphate.Phosphorus is present in the form of covalently bonded monophosphate in potato and taro starch, either at the O-6 or O-3 position of the glucopyranosyl residues of starch, according to an early ^{31}P NMR study.(Spyros et al., 2009)

3.3.4 ^{31}P NMR for Meat

Because of their influence on the meat's water holding capacity (WHC) and the loss of extensibility after the development of rigor mortis, metabolic processes in muscle post-mortem

are a significant determinant for meat quality. Since phosphorus is found in several important metabolites, ^{31}P NMR can be used to investigate both intrinsic factors affecting muscle metabolisms, such as species, age, genetic type, and muscular type, as well as technological processes used during meat production, such as feeding, slaughter conditions, storage temperature, and brine injection. Early research found that ^{31}P NMR spectra of raw intact muscle do indeed provide information on phosphorylation metabolite levels as well as changes post-mortem. The ^{31}P NMR chemical shifts of ATP, PME, and Pi can also be utilized to determine the intracellular pH of the muscle. Because of its favourable pK value, most investigations employ the chemical shift of the inorganic phosphorus Pi peak for pH measurement. The breadth of the same signal serves as a measure of muscle pH heterogeneity. The ^{31}P NMR approach was then utilized to investigate the rate of post-mortem metabolism in muscles from a variety of meat-related animals, including cattle, rabbit, lamb, and hog.(Spyros et al., 2009) (Meyerspeer et al., 2021)(Jastrzębska & Szłyk, 2009)

3.4 Composition of Food and Vegetables

Fat, protein, and carbohydrates are macronutrients that provide energy and essential components for life. Fat is made up of glycerol and fatty acids; protein is an agglomeration of amino acids; and carbohydrate is made up of simple sugars that occur as monosaccharides or chains of connected monosaccharides (e.g., starch) whose bonds are either hydrolyzed to monosaccharides in the human small intestine or are resistant to hydrolysis (dietary fibre). A combination of these macronutrients is required in our diet to maintain longevity and health.(Venn, 2020) (Damodaran et al., 2007)

3.4.1 Fats

Fats, tiny molecules found inside living tissue, are constituted mostly of glycerol esters of fatty acids, triacylglycerols\s(TAGs) being the primary components(Cole & Eastoe, 1988). Nonglyceride compounds such as phospholipids, sterols, and pigments are commonly found in food fats and oils. Fats are often defined depending on their sources, such as fish oil, bird oil, and hog fat (lard), and are part of a larger class of substances known as lipids, which may be categorised based on their content, origin, and nature. Lipids are a varied group of chemicals formed from living organisms that are typically nonpolar or water insoluble. It should be emphasized, however, that certain lipids (albeit only a small fraction) are indeed

water-soluble. Glycerides (such as mono-, di-, and TAGs), phospholipids, prostaglandins, steroids, carotenoids, and waxes are all examples of lipids. Fats, like carbohydrates and proteins, make up a large component of the composition of living things. TAGs constitute the vast majority of the various structural types of fats found in mammals (and indeed in living organisms). Lipids are found in all organs of animals and as depositions in numerous locations inside organisms. Fats in higher concentrations are found in connective tissue, adipose tissue, bone marrow, the brain, liver, and kidneys. Whole milk powder contains a significant amount of fat in addition to protein. At room temperature, fats can exist as liquids or solids and are thus classified as oils or just fats. The latter is the most energy-dense of the three fundamental foods, carbs, proteins, and fats. Fatty acids are undoubtedly the most important form of lipids in terms of health (especially in the form of TAGs). (Bowen-Forbes & Goldson-Barnaby, 2017)

3.4.2 Carbohidrates

Carbohydrates are chemically stable organic molecules that contain carbon, hydrogen, and oxygen [Empirical formula Cx(H2O)y]. Carbohydrates are almost entirely derived from plant-based foods, where they are synthesized from carbon dioxide and water using energy derived from sunlight. Carbohydrates are the main source of energy in the human diet, accounting for 50–70% of total energy intake. They are classified into three types: sugars, starch, and non-starch polysaccharides.(Lunn et al., 2007)

A monosaccharide sugar, such as glucose, fructose, or galactose, is the most basic type of carbohydrate. Disaccharides and sugar alcohols are also included in the sugar classification. Disaccharides, such as sucrose and lactose, occur naturally in foods and are made up of two monosaccharide units (glucose + fructose = sucrose; glucose + galactose = lactose). Sucrose is by far the most common sugar in the human diet, accounting for about 14% of total energy intakes. Sugar alcohols occur naturally, but they are also synthesized commercially for use as sweeteners (e.g., sorbitol or xylitol). In addition, these sugars are not absorbed in the small intestine. (Sturgeon et al., 1990)

Oligosaccharides are made up of 3–15 monosaccharide units that are not digested by enzymes in the human digestive tract but are broken down by bacterial enzymes in the large bowel. These compounds, which include raffinose, stachyose, and verbascose, are found in plant seeds and are regarded as 'unavailable carbohydrate.' Inulins are a type of oligosaccharide that is physiologically important. Inulin is a fructan, or a chain of fructose

molecules, which is a type of carbohydrate (fructo-oligosaccharide). Some plants use inulin to store energy, and it is typically found in roots or rhizomes. The majority of plants that produce and store inulin do not store energy in other carbohydrate forms. (Bell, 2012.)

Polysaccharides or, to use a popular lay term, 'complex' carbohydrates are used to describe the larger carbohydrate polymers. There are two types of polysaccharides: starches and non-starch polysaccharides. Starches are glucose polymers that can be straight-chain (amylose) or branched (amylopectin). Non-starch polysaccharides are a diverse group of compounds that are not broken down by human digestive enzymes. In 1977, the term "complex carbohydrate" was coined for use in a US Senate Select Committee report, and it was used without a formal definition. It was used in the context of the report to distinguish simple sugars from polysaccharides, and this definition has been adopted for general use. There has been some debate about the validity of this term, as it encompasses a group of carbohydrates with very different physiological properties. (Lunn et al., 2007)

Indeed, some argue the at because digestibility is a fundamental difference between carbohydrates, this feature, rather than size, should be used to distinguish them. Because these so-called 'complex' carbohydrates can be digested as quickly as simple sugars, using the term may be considered misleading, as consumers may perceive complex carbohydrates to be more slowly broken down in the gastrointestinal tract. The Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Consultation on Carbohydrates in Human Nutrition (1997) rejected the term "complex" and advised against its use. Sugar Contents in Some Vegetables and Fruit (g/100 g Fresh Weight) Average, Maximum Content, and Minimal Content.

Averge: apple 12.00g, bananas 18,00g, blueberries 4,5g, Grapefruit 6,80, Mango 14.00g, orange 7,90, persimmon 16.00g, strawberries 5,70g, tomatoes 2,80g.

Maximum content: apple 16.60g, bannabas21.70g, blueberries 10.40g, grapefruit 9.96g, mango 14.00

Minimal content: apple 6.00g, bananas 11,40g, blueberries 2.40g, grapefruit 3.30g.(Yahia et al., 2019)

3.3.2.1.1 Mono-and disaccharides

The human diet is high in simple sugars. Glucose is found naturally in fruit and plant juices, and it can be synthesized from starch for use as a food ingredient (glucose syrups). These syrups are made by acidic or enzymatic hydrolysis of maize or wheat starch

and are mostly made of glucose, though other monosaccharides may be present. These syrups are typically less sweet than glucose and are a more cost-effective alternative to sucrose derived from sugar cane and sugar beet. They are found in many manufactured foods, including confectionery, soft drinks, and preserves, and provide foods with better eating quality, improved texture, and a longer shelf life when compared to similar foods that do not contain these sugar syrups (Hanover & White 1993).

Fructose is found naturally in most fruits and vegetables, as well as honey, and because it is the sweetest sugar known, less of it can be used. High-fructose syrups are made by converting some of the glucose in glucose syrups to fructose. It has recently been proposed that the rise in consumption of glucose-fructose syrups in the United States over the last 30 years is partly to blame for the rise in obesity and type 2 diabetes. However, a similar increase in obesity and type 2 diabetes rates has been observed in the United Kingdom, despite the fact that the use of glucose-fructose syrups is not widespread.

The most common disaccharides in the diet are sucrose, maltose, and lactose. They are found naturally in sugar cane (sucrose), sugar beet (sucrose), malt beverages (maltose), and milk (lactose). (Johnson & Conforti, 2003)(Stylianopoulos, 2013)

COMA decided in 1991 to categorize sugars as intrinsic or extrinsic. Intrinsic sugars are those that are bound to the cellular structure of foods (for example, sugars in whole fruits and vegetables), whereas extrinsic sugars are those that are not bound to a cellular structure [for example, lactose (milk sugar) in dairy products]. Honey, fruit juices, table sugar, and confectionery are other foods that contain extrinsic sugars, also known as non-milk extrinsic sugars (NMES), and their consumption should be limited.

3.3.2.1.2 Polysaccharides

Plant foods supply nearly all of the polysaccharides in the human diet. These can be found in a variety of forms. The liver consumes very small amounts of glycogen as well as mucopolysaccharides, which are present at low levels in most animal tissues. When an animal dies, however, most polysaccharides are broken down to glucose.

Starches are the most common dietary polysaccharides. These come in the form of granules that are the size and shape of a plant. Some starches are insoluble in water and indigestible when consumed raw. This includes starches like those found in potatoes and flour. The starch granules swell and gelatinize when the foods are boiled in water.(Schneeman & Tinker, 1995)

3.4.3 Dietary fibre is a form of carbohydrate that the body's enzymes cannot breakdown.

Fibre. It is a complex carbohydrate as well. Because most fibres cannot be broken down by the body, eating fibre-rich foods can help you feel full and reduce your tendency to overeat. Fibre-rich diets have additional health benefits. They may aid in the prevention of stomach or intestinal issues such as constipation. They may also aid in the reduction of cholesterol and blood sugar levels. Fibre can be found in a variety of plant-based foods, including fruits, vegetables, nuts, seeds, beans, and whole grains. Because of its importance in disease prevention and control, fibre is an intriguing subject for food scientists, nutritionists, and food makers. Dietary fibres have been linked to a number of substantial physiological health advantages, including a lower risk of colon cancer, bowel problems, type II diabetes, and coronary heart disease. Dietary fibres are a significant sector of the functional foods industry due to their health advantages. Dietary fibres are divided into two categories based on their solubility in water: soluble dietary fibre and insoluble dietary fibre(Mudgil & Barak, 2019). Soluble dietary fibres include -glucan, pectin, gums, and inulin. Cellulose, hemicellulose, lignin, cutin, suberin, chitin, chitosan, and resistant starches are examples of insoluble dietary fibres. In general, dietary fibre sources contain both forms of dietary fibre, albeit in varying quantities. Cereals, legumes, fruits, and vegetables are excellent sources of dietary fibre. Dietary fibres' physicochemical qualities play an important role in their functioning and are linked to the physiological activities of human metabolism. Dietary fibres have important features such as solubility, viscosity, water-holding and binding capacity, fermentability, mineral and bile acid-binding ability, oil binding ability, particle size, and porosity. (Lunn et al., 2007)(Barber et al., 2020)(Dorcas Adegbaju et al., 2022)

3.4.4 Protein

The nutritional requirement for dietary proteins is widely accepted to consist of three components: indispensable (dietary essential) amino acids, conditionally indispensable amino acids, and nonspecific nitrogen required for the synthesis of dispensable (nonessential) amino acids and other important nitrogenous compounds. (R. P. Wilson, 2003)(Heger, et al., 2003)

Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are the amino acids that are required in all conditions. Cystine, tyrosine, taurine, glycine, arginine, glutamine, and proline are the amino acids that become essential under certain conditions. Nutritionally inactive amino acids include aspartic acid, asparagine,

glutamic acid, alanine, and serine. Essential amino acids, also known as indispensable amino acids, are those that humans and other vertebrates are unable to synthesize from metabolic intermediates. Because the human body lacks the metabolic pathways required to synthesize these amino acids, they must be obtained from an exogenous diet. Amino acids are classified as either essential or non-essential in nutrition. These classifications arose from early studies on human nutrition, which revealed that specific amino acids were required for growth or nitrogen balance even when an adequate supply of alternative amino acids was available. Although variations are possible depending on an individual's metabolic state, the general consensus is that nine essential amino acids are phenylalanine, valine, tryptophan, threonine, isoleucine, methionine, histidine, leucine, and lysine. Because it contains the first letter of each essential amino acid, the mnemonic PVT TIM HaLL ("private Tim Hall") is a popular method for remembering these amino acids. In terms of nutrition, a single complete protein provides all nine essential amino acids. A complete protein is one that contains all of the essential amino acids. Except for soy, complete proteins are typically derived from animal-based sources of nutrition. The essential amino acids can also be obtained from incomplete proteins, which are typically found in plant-based foods. The term "limiting amino acid" refers to the essential amino acid that is present in the least amount in a food protein in comparison to a reference food protein, such as egg whites. The term "limiting amino acid" can also refer to an essential amino acid that does not meet human minimum requirements. Amino acids are the fundamental building blocks of proteins and the nitrogenous backbones of compounds such as neurotransmitters and hormones. An amino acid is an organic compound in chemistry that contains both an amino (-NH2) and a carboxylic acid (-COOH) functional group, thus the name amino acid.(Boyle, 2008)(Pollock, 2007)(Stollar & Smith, 2020)

3.4.4.1.1 function of proteins

The proteins in a cell determine its health and function. Proteins are in charge of nearly every aspect of cellular life, including cell shape and inner organization, product production and waste cleanup, and routine maintenance. Proteins, like other cell components, receive external signals and mobilize intracellular responses. They are the cell's workhorse macromolecules, as diverse as the functions they serve. Proteins can be large or small, mostly hydrophilic or mostly hydrophobic, exist alone or as part of a multi-unit structure, change shape frequently or remain almost immobile. All of these distinctions stem from the distinct

amino acid sequences that comprise proteins. Fully folded proteins also have distinct surface characteristics that influence how they interact with other molecules. Proteins' conformation can change in subtle or dramatic ways when they bind with other molecules. Protein functions, not surprisingly, are as diverse as protein structures. Structural proteins, for example, maintain cell shape, similar to a skeleton, and they compose structural elements in connective tissues such as cartilage and bone in vertebrates. Enzymes are a type of protein that catalyzes the biochemical reactions that occur in cells. Other proteins, on the other hand, act as sensors, changing shape and activity in response to metabolic signals or messages from outside the cell. Cells also secrete a variety of proteins that either become part of the extracellular matrix or play a role in intercellular communication. After translation and folding are complete, proteins are sometimes altered. In such cases, transferase enzymes add small modifier groups to the protein, such as phosphates or carboxyl groups. These modifications frequently alter protein conformation and function as molecular switches that turn a protein's activity on or off. Many post-translational modifications are reversible, though the reverse reactions are catalyzed by different enzymes. Phosphate groups are added to proteins by enzymes called kinases, but they must be removed by enzymes called phosphatases. (Whitford et al., 2013) (Braithwaite et al., 2012)

To catalyze metabolic reactions, cells rely on thousands of different enzymes. Enzymes are proteins that increase the likelihood of a biochemical reaction occurring by lowering the activation energy of the reaction, allowing these reactions to occur thousands or even millions of times faster than they would without a catalyst. Enzymes have a high affinity for their substrates. They bind these substrates at complementary areas on their surfaces, resulting in a snug fit that many scientists liken to a lock and key. Enzymes function by binding one or more substrates, bringing them together to allow a reaction to occur, and then releasing them once the reaction is complete. When enzymes bind to substrates, they undergo a conformational shift that orients or strains the substrates, making them more reactive. An enzyme's name usually refers to the type of biochemical reaction that it catalyzes. Proteases, for example, degrade proteins while dehydrogenases oxidize a substrate by removing hydrogen atoms. The "-ase" suffix identifies a protein as an enzyme in general, whereas the first part of an enzyme's name refers to the reaction that it catalyzes. The proteins in the plasma membrane typically assist the cell in interacting with its surroundings. Plasma membrane proteins, for example, ferry nutrients across the plasma membrane, receive chemical signals from outside the cell, translate chemical signals into intracellular action, and sometimes anchor the cell in a specific location. The overall surfaces of membrane proteins

are mosaics, with patches of hydrophobic amino acids where the proteins come into contact with lipids in the membrane bilayer and patches of hydrophilic amino acids where the proteins extend into the water-based cytoplasm. Many proteins can move across the plasma membrane via a process known as membrane diffusion. The fluid mosaic model of the cell membrane refers to the concept of membrane-bound proteins that can travel within the membrane. Membrane proteins that extend beyond the lipid bilayer into the extracellular environment are also hydrophilic and frequently modified by sugar molecules. Other proteins are associated with but not inserted into the membrane. They are sometimes anchored to membrane lipids or bound to other membrane proteins. (Alberts et al., 2002)(Alberts et al., 2017)

3.4.5 Water in Foods

Water is a major component of most foods and has a significant impact on their physical and chemical properties. However, it is not just the amount of water that is important; it is also the availability of water to interact and react with food components. Measuring the equilibrium relative humidity is a simple way to estimate water availability. Water activity (Aw) is then defined as the ratio of this vapour pressure to pure water at the same temperature and pressure. The relationship between water content and Aw in foods and their components is not simple, but it frequently takes the form. It is worth noting that these adsorption and desorption isotherms frequently exhibit hysteresis, indicating the first issue with the measurement. Although Aw is an equilibrium property, hysteresis implies that systems are not always in equilibrium. Adsorption curves have also been mathematically modelled using a series of water layers. The 'binding' of water to the food substrate is often used to identify the first monolayer. Such models allow for numerical comparisons of food components and whole structures, but it should be noted that they may not accurately represent the position and dynamics of water molecules. Nonetheless, Aw and absorption curves have been used successfully in monitoring product quality, particularly microbiological stability. The concentration of the substrate and the proximity of the reactants. It should be noted that lowering Aw can stop biological deterioration, which is recognized as a primary barrier to microbial growth. This is most likely due to the fact that the components of foods that are susceptible to spoilage have a high moisture content, where hysteresis is minimal, and the Aw measurement correlates with the amount of water available for microbial growth. As a result, while we now understand that Aw is not the determining

property of water's role in foods, it remains a useful diagnostic for safety and stability.(Troller et al., 2012)(Mathlouthi, 2001)(Passone et al., 2007)

3.4.6 Vitamins

Vitamins are a diverse set of chemical molecules that are typically incapable of being manufactured by the human body yet are required for the proper maintenance of its regular activities. Under normal circumstances, we may receive the various vitamins via food and proper nutrition, but minimum nutritional needs are frequently not satisfied, necessitating the use of supplements.

Vitamins are necessary for metabolism, as well as for development and optimal bodily function. Only vitamin D is created by the body; all other vitamins must be received through diet. Many disorders are caused by a vitamin deficiency, such as scurvy (vitamin C) or beriberi (vitamin D). (Zempleni et al., 2013)

Vitamins are categorized into two types based on their solubility: water-soluble vitamins and fat-soluble vitamins. The first group consists of B vitamins and vitamin C, whereas the second group consists of vitamins A, D, E, and K. Vitamin stability and bioaccessibility in food systems are frequently jeopardized by variables such as poor permeability and/or solubility inside the gut, lack of stability (temperature and oxygen), and food processing technologies, as well as elements in the gastrointestinal tract (pH, enzymes, presence of other nutrients). As a result, it's worth learning about new technologies that can increase the stability and bioaccessibility of water- and fat-soluble vitamins.(Gironés-Vilaplana et al., 2017)

β-Carotene is an orange pigment found in fruits and vegetables that belongs to the carotenoids class of compounds. -carotene is the primary dietary source of provitamin A among the carotenoids. Broccoli, carrots, kale, pepper, red, and sweet peppers are dietary sources. Pumpkin, spinach, winter squash, lettuce, cantaloupe, and melon.(Rasmussen et al., 2013)(Halver, 2003)

3.4.6.1.1 Ascorbic acid (vitamin C)

Ascorbic acid (vitamin C) is a critical redox cofactor in plant and animal systems. Although most animals produce enough ascorbic acid, ascorbic acid is a genuine vitamin for others due to an inability to carry out adequate synthesis or biosynthesis. This is true for humans and other higher primates, a small number of other mammals (such as guinea pigs and bats), as well as several bird and fish species. Because of abnormalities in ascorbic-acid-

specific enzymatic steps and processes, a deficit produces the illness scurvy in humans, which results in a variety of pathologic symptoms. (Sanseverino, 2005) (Johnston et al., 2007)

Vitamin C aids in the treatment of infections and wound healing, and it is a powerful antioxidant capable of neutralizing free radicals. It is required for the production of collagen, a fibrous protein found in connective tissue that is woven throughout the body's many systems, including the neurological, immunological, bone, cartilage, blood, and others. The vitamin aids in the production of many hormones and chemical messengers that are employed in the brain and nerves. Ascorbic acid is an essential cofactor for the enzyme prolyl hydroxylase, and a deficiency of this vitamin results in the accumulation of abnormal collagen. The redox capacity of ascorbic acid forms the basis for its function as an enzyme cofactor, but this ability also has established vitamin C as potentially an important water-soluble antioxidant. Chemically, ascorbic acid can react with superoxide, hydroxyl and peroxyl radicals, hydrogen peroxide, hypochlorite, and singlet oxygen.(Steinberg & Rucker, 2013) Foods high in vitamin C include blackberries, blueberries, broccoli, Brussels sprouts, cabbage, grapefruit, lemon, lime, tomato, orange, and cauliflower. Fruits and vegetables provide more than 90% of the vitamin C in the human diet. The richest sources of vitamin C are fresh fruits and vegetables, but the amount varies greatly between species and cultivars. Vitamin content in horticultural crops is also strongly influenced by a variety of climatic environments, preharvest and postharvest factors, including preharvest climatic conditions, maturity, harvesting methods, and postharvest handling procedures. The fruit of acerola, also known as Barbados cherry or west indian cherry, has the highest concentration of ascorbic acid, accumulating 17-46 g/kg edible portion. Australian fruit known as Kakadu plum or billygoat plum has a comparable vitamin C content, 23-32 g/kg edible portioning. Much more important are sources with a lower average vitamin level, such as potatoes, which are known to be the most important source of vitamin C. Fruits from the subtropics. Subtropical fruits, particularly oranges, lemons, and limes, provide a significant portion of the vitamin C requirements during the winter and spring months.

vitamin c content of vegetables and fruits (mg/L): Apples 15-50 mg/L,Pears 20-40 mg/L Plums 24-45 mg/L, Peaches 70-100 mg/L, Oranges 300-600 mg/L, Lemons 300-640 mg/L, Bananas 100-350 mg/L, Kiwi 700-1630 mg/L, Melons 130-590 mg/L, Mango 100-350 mg/L, Grapes 20-50 mg/L.(Velisek, et al., 2020)

3.4.6.1.2 Vitamin B Group

The B vitamins are essential cofactors in enzyme processes. They are water-soluble, and the excess is excreted in the urine. Thiamine (vitamin B1), riboflavin (vitamin B2), niacin (nicotinic acid, nicotinic acid amide, vitamin B3), pantothenic acid (vitamin B5), vitamin B6 (pyridoxine, pyridoxal, pyridoxamine), biotin (vitamin B7), folic acid (pteroylglutamic acid, vitamin B9), and cobalamin are the most (vitaminB12). Elevated B vitamin levels are required in conjunction with increased physical activity, as B vitamins are involved in the breakdown of carbohydrates and lipids for energy generation. As a result, their relevance in athletes is heightened, and they are critical to their performance levels. It should also be mentioned that certain B vitamins are required to assist in the formation of haemoglobin in red blood cells, which is a primary factor of oxygen supply to the muscles during aerobic endurance activity. Choline is a B vitamin that may help with memory, learning, and mood. Other vitamins and minerals may aid in the treatment of depression symptoms. Wheat germ and eggs contain choline. Folate-rich foods include spinach, turnip greens, kale, citrus, dried beans or peas, asparagus, and tomatoes. Omega-3 foods include wild salmon, tuna, flaxseed oil, canola oil, and olive oil. (Baj & Sieniawska, 2017)

3.4.6.1.3 Vitamin E

Vitamin E is the body's primary fat-soluble antioxidant (Rafeeq et al., 2020). It is found in biological membranes, where it shields the polyunsaturated fatty acids (PUFA) of membrane phospholipids against free radical oxidation. Tocopherols and tocotrienols are structurally similar, fat-soluble, plant-derived natural compounds that make up vitamin E. Vitamin E is currently thought to reduce free radical-chain peroxidation of polyunsaturated lipids in membranes and lipoproteins. For mammals, the most physiologically active form is α-tocopherol, which is the predominant form of vitamin E present in blood and tissues. Foods high in vitamin E include sunflower oil, corn oil, peanuts, and soybean oil, and some others.(Sevanian & Ursini, 2000)(Rigotti, 2007)

3.4.7 Minerals

minerals play an integral part in many of the human body’s principal functions. There are a variety of important minerals including macronutrients, micronutrient minerals, and trace elements that help prevent diabetes, obesity, high blood pressure, and other

cardiovascular diseases. Minerals are vital nutrients that power important cellular and physiological activities. Each mineral is absorbed in the stomach by distinct, complicated processes that may include a cascade of receptors and binding proteins. Foods can both offer minerals and include components that influence mineral bioavailability in the digestive system. (Drago, 2017)(Maathuis, 2009)

3.4.7.1.1 Iron (Fe)

Iron is a micronutrient that is a component of haemoglobin, which is necessary for the transfer of oxygen and CO2 in the blood. It is also a component of tissue enzymes such as cytochromes (essential for energy synthesis) and immune system enzymes. The most frequent nutritional disorder in the world is iron deficiency. Deficiency occurs when the quantity of iron absorbed is insufficient to satisfy the needs. This is more likely when greater levels are required (e.g., during pregnancy, growth, menstrual loss, parasite infections) or when dietary ingredients affect the absorption, or the quantity given by the diet is inadequate. Micronutrient nutritional deficits in underdeveloped nations are caused by the decreased dietary quality of ingested iron. Indeed, impoverished populations consume more grains and tubers, limiting their intake of more expensive animal items, fruits, and vegetables. Inhibitory factors, which are typically found in diets high in vegetables and low in heme iron, are to blame for the high prevalence of iron deficiency anaemia. (Levi & Rovida, 2009) Haem is the most common form of iron found in animal tissue (particularly myoglobulin and haemoglobulin). Iron is bound in conalbumin in egg white. and phosphoprotein phostvin in the yolk Iron is bound in a variety of compounds in plants, particularly phitic acids, aliphatic hydroxilcarboxilic acids, aminocarboxilic acids, thiols, phenolic substances, nucleotides, peptides, and proteins. vegetables and iron-rich foods 47-68 mg/kg peas Beans: 59-82 mg/kg; soybeans: 50-110 mg/kg; spinach: 10-40 mg/kg; potatoes: 3.0-8.4 mg/kg; apples: 2.3-4.8 mg/kg; bananas: 3.2-5.5 mg/kg; strawberries: 3.6-9.6 mg/kg; oranges: 1.3-5.0 mg/kg. (Velisek, et al., 2020)

3.4.7.1.2 Calcium (Ca)

In the human body, calcium is the most abundant mineral. More than 99 per cent of it is present in bones, where it contributes to their structure and strength. Another very tiny part is

involved in controlling key activities such as muscle contraction, nerve impulse transmission, enzyme functioning, and cell membrane maintenance. Its importance in metabolic control cannot be overstated. In terms of both amount and bioavailability, dairy products are a great supply of this mineral. Other foods with lower quantities of calcium that are less bioavailable include soy products, beans, green vegetables, sardines, mackerel, dried fruits, and seaweed. iCa2+ is the biochemically active species, accounting for approximately 45 per cent of total serum calcium. Because albumin binds more than 80% of protein-bound calcium, the total calcium concentration in serum decreases in hypoalbuminemia individuals. (Darwish et al., 2009)(Gironés-Vilaplana et al., 2017) calcium rich vegetables and fruits are: 440-780 mg/kg, beans: 300-1800 mg/kg, soybeans: 1300-1800 mg/kg, cauliflower: 180-310 mg/kg, spinach: 700-1250 mg/kg, lettuce: 400-800 mg/kg, carrot:240-480 mg/kg, onion: 200-440 mg/kg. (Velisek, et al., 2020)

3.4.8 Antioxidants

The antioxidant content of vegetables and fruits may contribute to their protective effects. These antioxidants may aid in the relief of oxidative stress, for example. preventing free radicals from causing harm to biomolecules including proteins, DNA, and lipids. The complex variety of phytochemicals found in vegetables and fruit may give better protection than a single phytochemical thanks to additive and synergistic effects. one of the reasons why a diet rich in fruits and vegetables of various colors is suggested. (Azadeh et al., 2020)

These findings emphasize the need of measuring antioxidants in fruits and vegetables in their whole. Data on the antioxidant content of a wide range of vegetables and fruits would be valuable for epidemiological studies and dietary guidance. Antioxidants are usually used to enhance the oxidative stability of foods that are prone to lipid oxidation. Antioxidant techniques are based on the physical and chemical composition of food items since, depending on their antioxidant capabilities, one antioxidant may be effective in one product but not in another.(Stanner & Weichselbaum, 2013)(Singh Makhaik et al., 2021)

3.5 Role of Vegetables in Human Nutrition and Disease

Vegetables are annual or perennial horticultural crops that have various sections (roots, stalks, flowers, fruits, leaves, and so on) that can be consumed whole or in part, cooked or uncooked. Vegetables are vital for human nutrition because they include bioactive

nutritional molecules such as dietary fibre, vitamins, and minerals, as well as non-nutritive phytochemicals (phenolic compounds, flavonoids, bioactive peptides, etc.). These nutritional and non-nutrient molecules lower the risk of chronic illnesses such as heart disease, diabetes, certain cancers, and obesity. With a rising interest in the effect of foods on keeping healthy and preserving health, people have begun to adjust their eating habits in recent years. "Western" diets are distinguished by an increase in calories, sugar, saturated fats, and animal protein, as well as a decrease in vegetable and fruit consumption. When this diet is paired with a lack of physical exercise, the occurrence and frequency of illnesses such as obesity, diabetes, and cardiovascular pathologies rise. In healthy diets, eating plant-based foods such as fruits and vegetables, cereals, legumes, and nuts, replacing butter with healthy oils such as olive oil and canola oil, using herbs and spices to add flavor instead of salt, limiting red meat to several times per month, and eating fish and poultry at least twice a week are all recommended (Mediterranean diet model). Epidemiological research and clinical trials suggest that the Mediterranean diet is related to a variety of good health outcomes, including a lower risk of different chronic diseases, a lower overall mortality rate, and an enhanced chance of healthy ageing. The high consumption of vegetables, and therefore fibre, vitamins, minerals, flavonoids, phytoestrogens, sulfur compounds, phenolic compounds such as monoterpenes, and bioactive peptides, is one of the most essential elements of these diets. (Azadeh et al., 2020)(Ülger et al., 2018)

3.5.1 The efect of vegetables on some disease

Diabetes mellitus (DM), obesity, and metabolic syndrome (MS) have all become more prevalent in recent years, paralleling an increase in bad eating habits and unhealthy lifestyle choices. The regulation of eating habits is one of the most fundamental parts of illness control and management in people with these health issues. It is critical in medical nutrition treatment for these persons to satisfy their energy and nutritional demands, as well as incorporate foods with functional activities against the consequences of these illnesses in their diet. Secondary substances contained in vegetables, known as phytochemical compounds (carotenoids, alkaloids, terpenoids, and phenolics), are suggested to be protective against certain illnesses.

Onions and garlic are among the vegetables considered to be protective against diabetes, obesity, and multiple sclerosis due to their volatile oils, organosulfur compounds, and flavonoids(Belemkar et al., 2013). These vegetables' organosulfur compounds, such as S-methyl cysteine, and flavonoids, such as quercetin, have a functional impact by modulating

the activities of certain glucose metabolism enzymes, enhancing insulin secretion and sensitivity, and raising NADP+ and NADPH activities. Furthermore, these veggies block the enzymes -glucosidase and α-amylase, preventing D-glucose production from oligosaccharides and disaccharides and delaying glucose absorption from the intestines. Onion and garlic are particularly protective against dyslipidemia and oxidative stress, which are common in diabetes and multiple sclerosis.

Scientists discovered that obese Type 2 diabetes patients who used garlic tablets in addition to metformin had significantly higher fasting blood glucose (FBG), postprandial blood glucose, total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL), C-reactive protein (CRP), and adenosine deaminase levels than patients who only used metformin (Kumar et al., 2013). Garlic pills for 12 weeks effectively lowered TC and LDL levels while increasing high-density lipoprotein cholesterol (HDL) levels in dyslipidemic people with Type 2 DM. Despite comparable studies indicating that garlic has a good effect on blood glucose levels and plasma lipid profile in the presence of diabetes, garlic was also shown to improve antioxidant enzyme activities in diabetes and lower bioactive aldehyde levels. Garlic eating was reported to boost adiponectin levels in MS patients. Garlic eating in MS patients is likely to be protective against cardiovascular illnesses because of adiponectin's anti-atherogenic and antiatherosclerotic properties (CVDs). Furthermore, garlic has been shown to improve insulin resistance in rats with MS caused by high fructose content meals. (Eidi et al., 2006)

Garlic added animal feed lowered TG and TC levels, as well as body weight and epididymal fat formation in obese rats generated by high-fat diets. Similarly, garlic decreased visceral and epididymal fat formation while decreasing atherogenic index and cardiac risk factors in obese rats fed a high-fat diet(Lee et al., 2012).

It was discovered that adding onion powder to animal feed in diabetic rats caused by aloxone or streptozotocin had a hypoglycemic effect. In research comparing the efficiency of glibenclamide, an oral antidiabetic medicine, with onion application at various dosages in DM rats, 300 mg/kg onion extract treatment lowered fasting glucose levels by 75.4 per cent, whereas 2.5 mg/kg glibenclamide reduced fasting glucose levels by 76.4 per cent. (Ozougwu et al., 2009)

Onion eating in the presence of DM raised the levels of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), according to studies. In these investigations, onion was found to lower bioactive aldehyde

levels such as malondialdehyde (MDA), which is produced by the breakdown of lipid hydroperoxides. (Ogunmodede et al., 2011)

Onion has been shown to lower insulin resistance and increase FBG levels in MS Zuckertype mice. An onion extract was demonstrated to lower weight growth, epididymal fat buildup, and blood TC levels in obese BALB/c mice fed a high-fat diet. A daily onion consumption of 100–120 g was found to dramatically lower TC and LDL cholesterol levels in obese individuals with the polycystic ovarian syndrome.(Ebrahimi-Mamaghani et al., 2014)

3.5.2 Efects on cardiovascular disease

CVDs are the leading cause of death and disease in the world. According to the Global Burden of Disease survey, CVDs were responsible for 29.6 per cent of all deaths worldwide. (Lozano et al., 2012)The major cause of these deaths is the rise in bad eating habits and eating environments. its. The majority of the risk factors linked with CVDs are reversible, and non-pharmacologic treatments such as good eating habits and lifestyle adjustments may help manage the disease's risk factors. Vegetable consumption, which is an essential element of a balanced diet, has been found to lower CVD-related mortality rates and improve risk factors.(Roth et al., 2015) Vegetables are heart-healthy because they are low in saturated fat, trans fat, and cholesterol and high in bioactive compounds like flavonoids, phytoestrogens (lignans, coumestans, isoflavones, resveratrol, and lycopene), organosulfur compounds, soluble dietary fibres (-glucan, pectin, and psyllium), isothiocyanates, monoterpenes, and (sitostanol, stigmasterol, and campesterol).(Kris-Etherton et al., 2002)

According to epidemiological research, there is an inverse link between garlic intake and the development of CVD. Garlic and garlic components have been shown in studies to have cholesterol and lipid-lowering effects by inhibiting key enzymes involved in cholesterol and fatty acid synthesis (monooxygenase and HMG-CoA reductase)(Yeh & Liu, 2001), anti-platelet effects by inhibiting cyclooxygenase enzyme activity, and fibrinolytic effects by inhibiting lipid peroxidation and hemolysis in oxidized erythrocytes. Onion and garlic were also shown to reduce blood pressure by increasing intracellular nitric oxide and hydrogen sulfide production and blocking angiotensin-converting enzyme activity. Garlic was also demonstrated to lower the levels of reactive oxygen species (ROS), which are suspected to play a role in the pathophysiology of CVD, as well as boost antioxidant capacity.

Although epidemiological research on the association between onion intake and CVD risk and CVD-related mortality rates is sparse, a study done in Finland discovered that CVD-

induced mortality was lower in persons who consumed more onions than those who consumed less. (Knekt et al., 1996)

Onion, like garlic, enhances cardiovascular health by containing sulfurous chemicals, particularly flavonoids such as quercetin. S-alk(en)yl-L-cysteine sulfoxides are transformed into thiosulinates and copaenes by the enzyme alliinase when an onion is chopped, and these chemicals suppress platelet aggregation(Briggs et al., 2001). Because platelet aggregation is a major risk factor for the development of coronary thrombosis and atherosclerosis, onion intake may be advantageous in people who are predisposed to CVD. Furthermore, it has been found that eating onions decreases CVD risk in hypercholesterolemic rats by lowering the heightened inflammatory indicators associated with a high cholesterol diet and boosting the levels of antioxidant enzymes. Onion also reduces risk factors by addressing dyslipidemia, which is observed in various chronic conditions such as diabetes.(Akash et al., 2014) (Ülger et al., 2018)

4 Material and methods

The research was carried out at the food sciences department of the Czech University of Life Sciences' Prague campus in Prague, Czech Republic. The vegetables and fruits used in the study were acquired from several supermarkets in Prague, namely Albert, BILLA, and Kaufland.

Aqueous extraction will be used to process samples from 35 common vegetable and fruit species from various families. On a Bruker 500MHz spectrometer, ^{1}H and 2D NMR spectra will be acquired, Fourier-transformed, and analyzed using standard methods in NMR spectroscopy, NMR spectral elucidation, and Chenomx profiling.

4.1 Sample collection

Vegetables and fruits were harvested for the study from September 2021 to February 2022. The vegetables and fruits were classified by country of origin. For each research material, the following information was obtained: release date and expiration date, as well as manufacturing territory.

The colour, fragrance, taste, form, and physical qualities of the veggies and fruits were all inspected and rated on the spot. Despite their uneven physical conditions, they were chosen at random to establish the authenticity of the product quality and to assess the quality of the products we eat on a regular basis. Thus, the storage conditions did not have a substantial influence on the product, an analytical sample was prepared no later than two days after acquisition.

4.2 Sorting samples based on their origin

Veggies and Fruits	Date of collection of study material	Origin
Fennel	23.11.2021	Spain
Pumpkin	09.11.2021	Czech Republic
lettuce	11.11.2021	Spain
Pineapple	11.11.2021	Costa Rica
Cauliflower	19.11.2021	Czech Republic
Tomato	23.11.2021	Spain
Phisalys	23.11.2021	Italy
Celery	09.11.2021	Czech Republic
Pomegranate	11.08.2021	Czech Republic
Kiwi	19.10.2021	New Zealand
Common cornsalad	19.11.2021	Czech Republic
Beetroot	08.12.2021	Czech Republic
Courgete	11.11.2021	Spain
Butterhead lettuce	08.12.2021	Czech Republic
Leek	09.11.2021	Czech Republic
Mango	11.12.2021	Brazil
Grapepfruit	21.10.2021	Israel
Oroblanco	08.11.2021	Israel
Garlic	10.11.2021	Spain
Persimon kaki	11.11.2021	Bulgaria

Radish	08.11.2021	Italy
Apple	18.10.2021	Czech Republic
Grape	19.10.2021	Italy
Kohlrabi	18.11.2021	Italy
Raspberry	18.10.2021	Czech Republic
Banna	24.01.2022	Cameroon
Pear	13.10.2021	Czech Republic
Grapepfruit 2	20.10.2021	Israel
Lemon	18.10.2021	Turkey
Bluberry	24.01.2022	Czech Republic
Lemon 2	24.01.2022	Turkey
Cucumber	10.11.2021	Czech Republic
Bell pepper	19.11.2021	Czech Republic
Lime	19.10.2021	Mexico
Parsley	18.11.2021	Czech Republic

4.3 Sample grinding and extraction for ^{1}H NMR analysis

Under running water, each vegetable and fruit was thoroughly washed. and, if required, was technically prepared: peeling, cleaning from unusable parts of vegetables and fruits.
after cleaning of materials, they were ground by a special grinder (Retsch Type GM 200) for about 3 minutes, if needed till 6-7 minutes. They were measured on an analytical balance and obtained a fixed weight for equal concentration with water because we used the same amount of water in the grinder along with the sample. Each prepared sample was placed in a 15ml centrifuge tube for centrifugation. samples were Centrified by centrifuge apart.

A centrifuge is a device that separates different components of a fluid using centrifugal force. This is accomplished by rapidly spinning the fluid within a container, separating fluids of different densities (e.g., cream from milk) or liquids from solids. For best results, each centrifuged sample was centrifuged a second time for 10 minutes if necessary. Finally, all centrifuged samples are stored in a 2 ml Eppendorf tube for refrigerator and, if necessary, they

are again centrifuged in a 2 ml Eppendorf tube with a proper centrifuge, and we took a sample from this tube. were used Pipette for transportation in a new 2 ml Eppendorf tube.
finally, we stored samples at about -40 °C for 1H MRR analysis.

4.3.1 Sample Preparation For ^{1}H NMR analysis

1)the samples were transfered in a 2 ml Eppendorf tube and vortexed for about 15 seconds.
(A vortex mixer is a simple device that is frequently used in laboratories to mix small vials of liquid. It consists of an electric motor with a vertically oriented drive shaft attached to a cupped rubber piece mounted slightly off-centre. The rubber piece oscillates rapidly in a circular motion as the motor runs. When a test tube or other suitable container is pressed into the rubber cup (or touched to its edge), motion is transmitted to the liquid inside, resulting in the formation of a vortex)
2) all samples were transferred into a new Eppendorf tube and centrifuged for 5 minutes max speed of about 16. 3) 540 µL D_2O and 60µL of 1,5 M phosphate buffer (K_2HPO_4 and NaH_2PO_4, pH 4 with 5 nM TSP and 0,2NaN_3 in D_2O) were added to the Eppendorf tube. 4) Used again vortexer again for 15 seconds. 5) 600 ml of the sample solution was transferred into the NMR tube after it had been mixed.

4.3.2 Measurement, data processing, and analysis of ^{1}H NMR.

All spectra were recorded using a Bruker III Avance spectrometer outfitted with a board band fluorine observation (BBFO) SmartProbeTM with z-axis gradients (Bruker bioSpin GmbH, Rheinstetten, Germany) and set to a proton NMR frequency of 500.23 MHz. The temperature was set at 298 k (25°C). Under the same conditions, ^{1} H NMR spectra were acquired and processed. To suppress the residual water signal at 4,704 ppm, the Bruker pulse sequence (noesypr1d) was used. 128 scans and dummy scans were collected as 64 k data points for each sample using a spectral bandwidth of 8 k Hz, receiver gain of approximately 18, relaxation delay of 1 s, the acquisition time of 4.00 s, and mixing time of 0.01 s. The overall acquisition time was 11 minutes. Tuning, lock, gain 90°C pulse calibration, and shimming for each sample were done by an automated routine (atma, lock, rga, pulsecal and topshim). Prior to Fourier transformation, 0.3 Hz line broadening was applied

4.5 Non-target profiling, target profiling, and spectral processing

The spectra were corrected with basic line correction and phase correction using the Topspin 3,5p17 software (Bruker., Germany). Data was entered into the MestReNova software (Mestrelab Research, Spain), water was removed, and a single spectrum unit of 0.01 compound was used for identification by the Chenomx library manager and Chenomx profiling program. Several compounds were used for identification. By the Chenomx library manager and the Chamonx profiling program.

4.6 Statistical Analisys

To conduct statistical analysis, data were entered into a statistical program called MetaboAnlyst (Wishart Research group at the University of Alberta, Canada. www.metaboanalyst.ca). The data were auto-scaled and normalized. scaled and normalized. For target profiling data, principal component analysis (PCA) and the heatmap was created using hierarchical clustering (HC). The Pearson distance was used to measure the similarity between group averages in this technique, while the average linkage method was used for cluster aggregation.

5 Result

5.1 Table.1. Concentration of selected analytes in the samples

mg/dL						
Vegetables and Fruits	Fructose	Glucose	Sucrose	Mono+disaccahrides	Citrate	Malate
Parsley	573.0	469.1	3645.0	4687.1	0.0	0.0
Fennel	2717.6	2178.7	493.0	5389.3	0.0	0.0
Pumpkin	2274.1	2210.3	0.0	4484.4	0.0	0.0
Lettuce	817.7	543.7	0.0	1361.4	0.0	0.0
Pineapple	1029.6	968.4	4512.0	6510.0	0.0	0.0
Cauliflower	2012.7	2870.4	500.1	5383.3	0.0	0.0
Tomato	1105.3	633.7	0.0	1739.0	264.1	0.0
Physalis	3619.6	2709.0	4683.7	11012.3	1895.1	0.0
Celery	1908.5	66.2	935.6	2910.2	0.0	0.0
Pomegranate	7354.9	5840.3	0.0	13195.2	2453.4	0.0
Kiwi	2016.5	1586.1	26.9	3629.5	298.2	0.0
Common cornsalad	63.8	134.1	51.1	248.9	0.0	0.0
Beetroot	26.3	660.1	4716.9	5403.3	0.0	0.0
Courgette	1982.7	1301.9	0.0	3284.6	0.0	0.0
Butterhead lettuce	1159.7	1137.6	0.0	2297.4	0.0	0.0
Leek	3384.1	33.7	2765.0	6182.8	0.0	0.0
Mango	13323.8	5861.2	19692.2	38877.3	1418.0	0.0
Grapefruit	555.9	567.5	1223.2	2346.6	441.9	0.0
Oroblanco	9683.2	7584.6	21818.6	39086.4	2664.3	0.0
Garlic	19184.1	7416.3	15256.5	41856.9	0.0	0.0
Persimon Kaki	1037.7	1362.2	1067.3	3467.3	0.0	0.0
Radish	1281.2	1041.5	0.0	2322.6	0.0	0.0

Apple	3282.3	1892.6	8747.8	13922.7	0.0	1084.4
Grape	12680.4	10646.9	0.0	23327.2	0.0	0.0
Kohlrabi	872.5	1418.5	283.1	2574.0	0.0	0.0
Raspberry	7647.4	4920.4	0.0	12567.8	6561.6	0.0
Banana	401.4	308.9	1339.6	2049.8	0.0	0.0
Pear	9085.3	1697.6	2721.8	13504.7	0.0	0.0
Grapefruit 2	4185.5	3521.2	9210.2	16916.9	1045.0	0.0
Lemon	4403.6	3638.1	9273.8	17315.6	2078.0	0.0
Bluberry	7365.4	5285.2	0.0	12650.6	1076.8	0.0
Lemon 2	3041.0	2677.9	599.4	6318.3	14863.5	0.0
Cucumber	977.9	690.5	0.0	1668.5	0.0	0.0
Bell pepper	4168.9	2072.4	0.0	6241.3	0.0	0.0
Lime	1491.3	1350.2	528.4	3369.9	13566.5	0.0

5.2 Principal component analysis

The fundamental bilinear factor model is the unsupervised method known as principal component analysis (PCA). PCA decomposes the data into score vectors and loading vectors, which when outer products of and added together, recreate the original data. Loading vectors are associated with each class, correlated with the original variables, and oriented toward the direction of maximum variance of variables in order to highlight possible differences or similarities among samples. The data matrix of 35 samples classified by type was subjected to PCA analysis. The PCA score plot revealed a clear separation between the fruit and vegetable clusters, with the first two principal components accounting for 71.3 per cent and 11.4 per cent of the variation, respectively (Fig. 1). there can be observed closeness Between A and B classes. Garlic and mango seemed to have the highest fructose concentrations of vegetables and fruits, at 191.841 g/L and 133.238 g/L, respectively. Beetroot and banana seemed to have the lowest fructose levels, at 0.26,3 g/L and 4.014 g/L, respectively. According to NMR, the

highest glucose concentrations of vegetables and fruits were found in garlic and grape, which appeared at 74.163 g/L and 106.469 g/L, respectively. The lowest glucose levels were found in leek and banana, which appeared at 0.337 g/L and 3.089 g/L, respectively. A high rate of sucrose discovered was from vegetables in garlic about 152.565 g/L and in fruits, Oroblanco was the highest rate at about sucrose concentration of 218.186 g/L. A high rate of sucrose discovered was from vegetables in garlic about 152.565 g/L and in fruits, Oroblanco was the highest rate at about sucrose concentration of 218.186 g/L. The highest level of mono+disaccharides was found in garlic among vegetables and was approximately 414.569 g/L, while the lowest level was found in common cornsalad and was 2.489 g/l. The highest concentration of mono+disacarides in fruits was found in oroblano, which had a concentration of 390.864 g/l, and the lowest concentration was found in banana, which had a concentration of 20.498 g/l.

Citric acid concentrations in some vegetables and fruits ranged from 2.641 g/l to 148.635 g/l

Figure.1. PCA analysis, No normalisation, paretto scaling applied. Component loadings are showen in the biplot on the right.

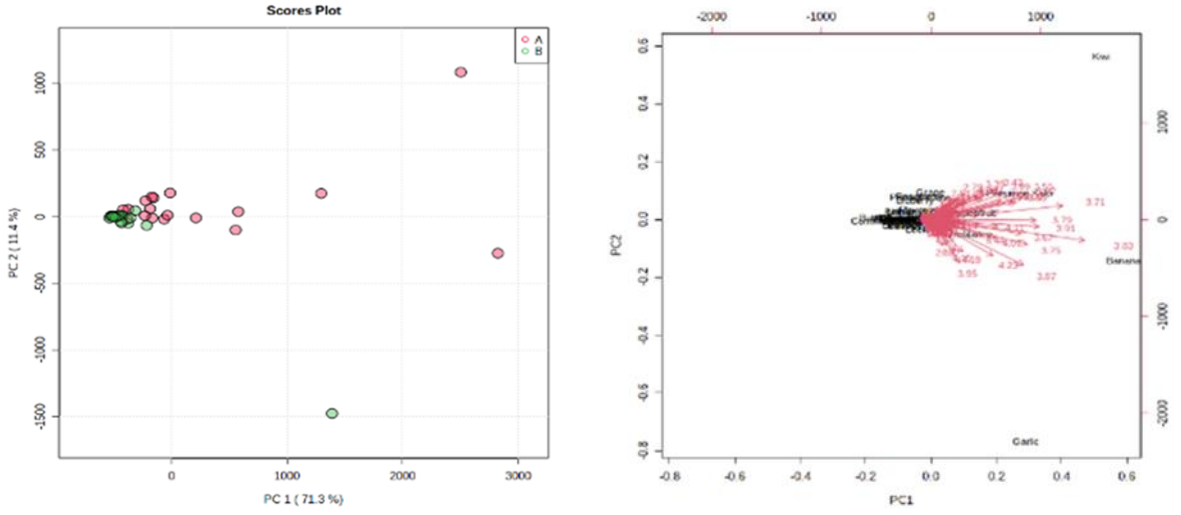
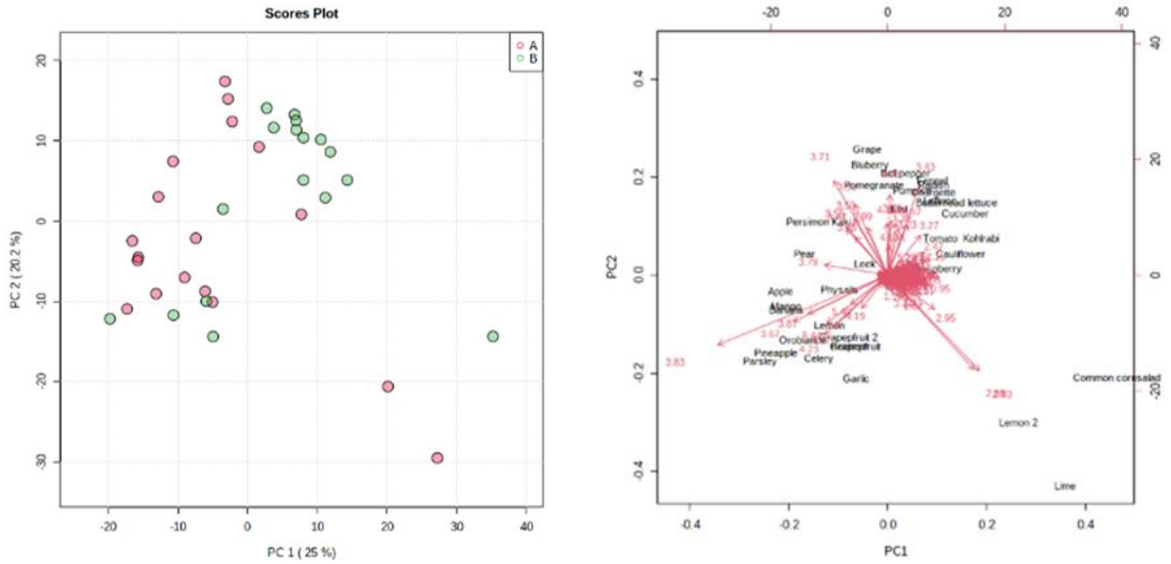


Figure.2. Normalisation by sum, paretto scaling PCA analysis. Loadings are shown biplot on the right



The PCA score plot revealed a distinct separation between the fruit and vegetable clusters, with the first two principal components accounting for 20% and 25% of the variation, respectively (Fig.2). it clearly demonstrates that the loading plot revealed high levels of bins containing signals from lemon 2 and lime, distinguishing them from other vegetables and fruit samples. This could be due to the high number of citric acids in the lemon 2 and lime samples when compared to other samples.

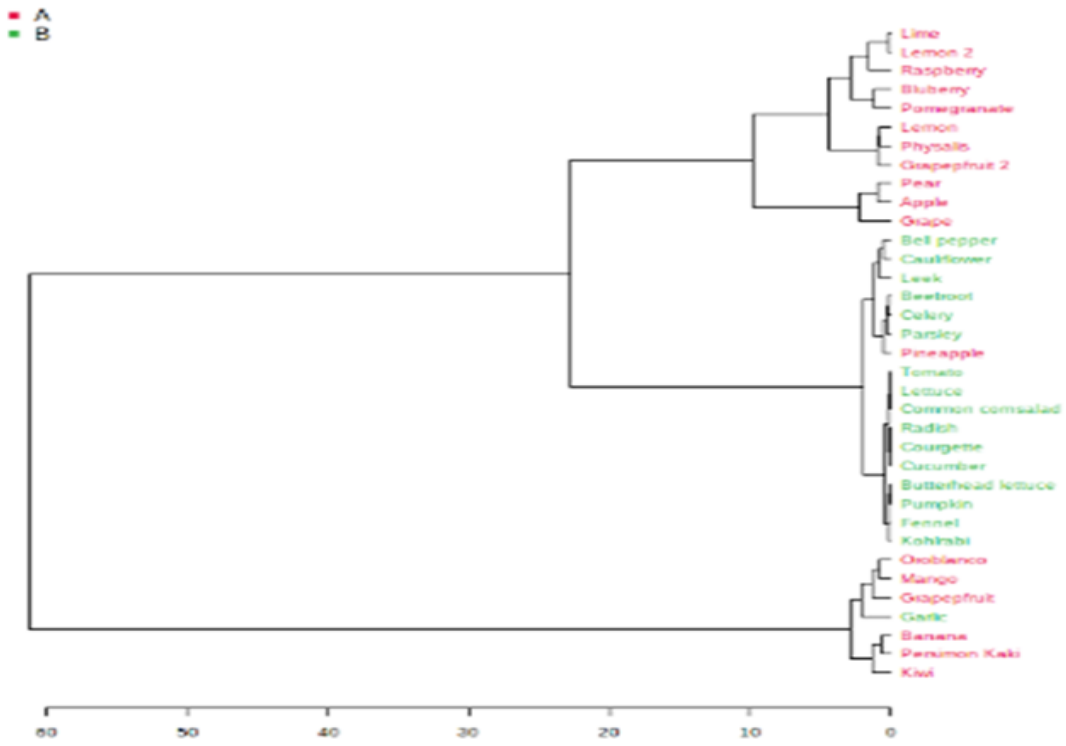
5.3 Cluster analysis

When the data being analyzed has some sort of response associated with each sample, this can often be used to create models for prediction of the response, exploring the mechanisms of the model, identifying important variables (e.g., biomarkers), or for other purposes. There are two kinds of responses: continuous (regression) and discrete (classification). There are also ordinal responses and related types. in our case was done Hierarchial clustering.

In hierarchical cluster analysis, samples are grouped based on similarities, without regard for class membership information. The results of HCA are shown as a dendrogram (Fig. 3), with three well-defined clusters visible. Samples will be grouped into clusters based on their proximity or similarity. Cluster analysis (CA) requires less information (only distances) than PCA. It's fascinating to see what kind of classification can be made solely on the basis of distances. Hierarchical clustering derived from ^{1}H NMR spectra of fruits sample (A) and wine grape varieties (B). Fruits (Lime, Pineapple, Pomegranate, Kiwi, Mango, Grapefruit, Oroblanco, Persimmon, Kaki, Apple, Grape, Raspberry, Banana, Pear, Grapefruit 2, Lemon2, Blueberry, Lemon)

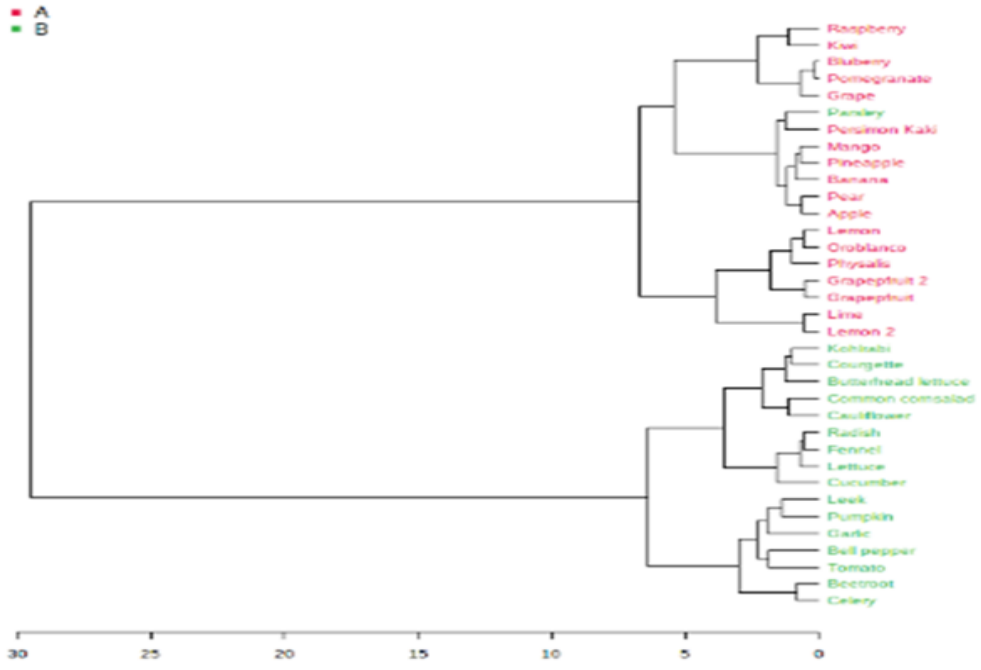
Vegetable (Parsley, fennel, Pumpkin, Lettuce, Cauliflower, Tomato, Physalis, Celery, Common cornsalad, Beetroot, Courgette, Butterhead lettuce, Leek, Garlic, Radish, Kohlrabi, Cucumber, Bell pepper).

Figure.3. Sum normalisation, paretto scaling Hierarchical clustering



We can see distinct group of samples is discernible, consisting of Lime, Lemon, and Raspberry. These fruits are associated with high levels of citrate concentration, and they are linked to the above-mentioned chemical. Citrate levels in Lime samples are 135,665 g/L, while Lemon 2 samples are 148,635 g/L. In addition, we can see mango and grapefruit in the third cluster. because they are related to each other's high citric acid content Citric acid concentrations in g/L are respectively (14,180 and 44,19) in mango and grapefruit samples

Figure.4. Sum normalisation, paretto scaling Hierarchical clustering



Derived from the figure.4. it is reasonable to assume that Clustering techniques group subjects in such a way that subjects in the same group are more similar to one another than subjects in other groups The more similarity there is within a group and the greater the difference between groups, the more distinct the clustering is. The distinction between fruits and vegetables in the figure is clear.

5.4 Hierarchical clustering of vegetable and fruit varieties

A heatmap is a data visualization tool that uses colour to represent a set of parameters and a score on multiple objectives. It is a two-dimensional array with groups and bins as dimensions, and groups are clustered based on similarity. Each row in the heatmap represents a bin ranked by ANOVA, and each column represents a category group average ordered by HC. HC is an unsupervised method that recognizes and distributes data groupings based on their affinity in progressive dissimilarity clusters. These clusters are represented as a dendrogram, with the assumption that closer objects in a space defined by variables have greater property similarity. HC was used to validate the classification of types based on their mutual dissimilarities.

Figure.5. Hierarchical clustering of vegetable and fruit varieties

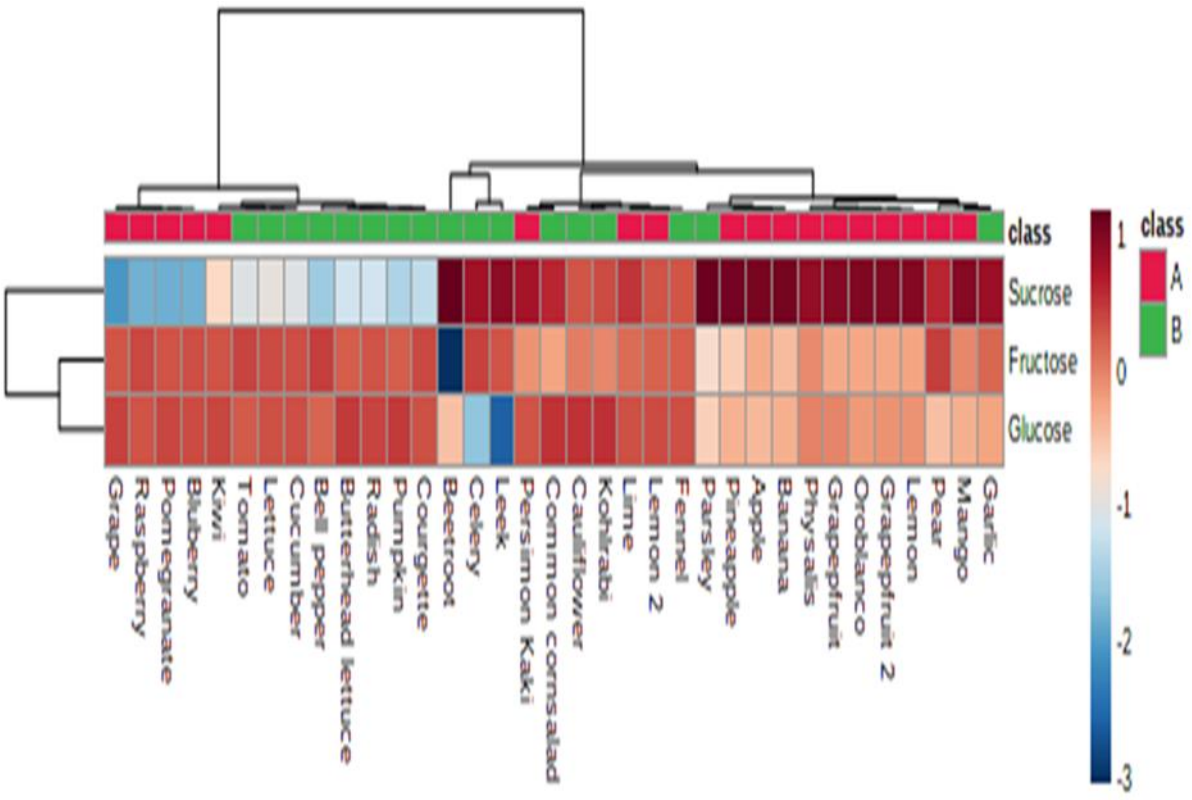


Figure.5. depicts the resulting heatmap, which includes the dendrogram and the main descriptive values. The grouping of samples on types revealed two distinct clades, which corresponded to the results of PCA. The first clade(red) contained fruits, and the second included vegetables. heatmap showed clustering of fruit and vegetable varieties that were represented by more than 30 samples in our research set. Samples of vegetables and fruits were divided into two distinct clades based on sum-normalised carbohydrate concentration. The intensity of the colour indicates the concentration of carbohydrates in the sample. For example, we can compare beetroot Sucrose concentration with beetroot fructose concentration, and the colour intensity is noticeably different. The heatmap colour reflects information about the carbohydrate's concentration of Leek and celery, allowing us to distinguish between them. Their glucose level is markedly different from their fructose and sucrose levels, and their glucose level is comparable to that of most fruits. Their glucose level is also noticeably different from vegetable glucose levels, as evidenced by our heatmap

6 Discussion

The study discovered that carbohydrates are the most important food component to investigate and differentiate fruits and vegetables within their own species or between species and to create classification between vegetables and fruits based on carbohydrates and organic acids. Carbohydrate signals in ^{1}H NMR can reveal food authenticity and adulteration, providing infallible information about concrete food products and their quality.

As we The HCA grouped samples in clusters, based on their similarities calculated from distances between samples, using all variables simultaneously. This approach proved to cluster correctly biological replicates from the 35 species of fruits, and vegetables and provided an overview (dendrogram) of their similarities. As shown in (Fig. 3) lemon is closely related to lime, followed by a raspberry. This result reflected the relationships among these plants in the phylogenetic tree where they are genetically related to each other in the same order as in the HCA dendrogram (Fig.4). Thus, it is remarkable how they are clustered. Plotting score vectors against each other, often with colour codes, can assist in elucidating relationships between observations and how these are connected and grouped, as in our study.

As we know, the unsupervised method known as principal component analysis employs the fundamental bilinear factor model.PCA breaks down the data into score vectors and loading vectors, which when outer products of and added together recreate the original data. The loading directions are chosen to maximize the variation spanned by each vector, with the first orthogonal component containing the most variation and subsequent orthogonal components containing decreasing amounts of variation. The most interesting phenomena are typically found in the first few components, while the majority of components are regarded as uninteresting or noisy. (Liland, 2011)

Loading vectors plotted as spectra can show where the components found their variation and thus which metabolites have affected the relationships in the score vectors, as seen in PCA analysis above. Plotting loading vectors against one another reveals information about the relationship between variables. In biplots, the combination of loading and score plots will frequently reveal which observations are linked to which variables. This is a particularly intriguing process for differentiation. The orthogonality between components imposes a rigid structure that is well suited for extracting as much information as possible from the samples but does not separate natural phenomena well in the components because most naturally occurring phenomena are not orthogonal. Both the alternating least squares variant of PCA

without orthogonalization and independent component analysis outperform in this regard but at the expense of less compression in the first components.(Liland, 2011)

As I previously stated, NMR has revealed Carbohydrates during our research, and there is a general statement from a scientific paper where we can draw parallels. Carbohydrates are compounds that primarily consist of carbon, hydrogen, and oxygen molecules, but can also contain nitrogen and phosphorus. They are the most abundant and widely distributed compounds in horticultural commodities. Their content in fruits and vegetables ranges from less than 1.0 per cent to more than 60%. They are typically estimated to be between 50 and 80 per cent of the dry weight of vegetables and fruits. In general, carbohydrates in leafy and stem vegetables range from 2% to 9% of fresh weight, while carbohydrates in root vegetables and tubers range from 15% to 25% of fresh weight and in citrus fruits range from 10% to 12% of fresh weight. Carbohydrates play an important role in horticultural commodities because they contribute to the texture, flavour, colour, and nutritional value of the products. Plant cells require celluloses, hemicelluloses, and pectins to function properly. Starch is a polysaccharide that is stored in unripe fruits and vegetables and is converted into simple carbohydrates during ripening. Sugars help to make fruits and vegetables sweeter. The flavour of vegetables and fruits is determined by the ratio of sugars, organic acids, and other compounds such as phenolic compounds.(Song et al., 2020)

Citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid) is a weak tricarboxylic acid found in citrus fruits. It exists primarily as the trivalent anion at physiological blood pH and, to a lesser extent, in urine. Citric acid is a common food additive used to add acidity and a sour taste to foods and beverages. Citrate salts of various metals are used to deliver minerals in biologically available forms; dietary supplements and medications are examples. Citric acid is most concentrated in lemons and limes, accounting for up to 8% of the dry fruit weight. Endogenous metabolism in the mitochondria via ATP production in the citric acid cycle is a major source of citric acid in vivo. (Penniston et al., 2007)

Honey is a supersaturated natural sugar solution in which the monosaccharides fructose and glucose are the most abundant (typically accounting for 80–85 percent of the honey's solids). The most common methods of adulteration of honey are the addition of sucrose, which cannot exceed 1% of its dried mass, or overfeeding bees with sugar and other types of sucrose. Other methods of adulteration encountered in the honey industry include the addition of fructose or industrial glucose, thereby changing the fructose/glucose ratio, which

in pure honey must be 1–1.2. When the ratio deviates from this value, it is assumed that the honey has been tainted. (Puscas et al., 2013)

According to the sugar role in honey, we can state that macronutrients such as carbohydrates and their monosaccharides: sucrose, fructose, and glucose, as well as their quantification and rapid analysis, are critical for detecting adulteration and determining quality.

According to our findings, apples have a high concentration of malate, implying that the quality of apples is determined by this. Malate concentration influences apple sensory quality, as evidenced by a plethora of research findings.

(C. Li et al., 2020)Acidity is a major contributor to the overall taste and flavor of apple (*Malus domestica*) fruit. Organic acids are collectively responsible for acidity, but malic acid accounts for more than 90% of total acid and is largely responsible for apple fruit acidity. The majority of the malic acid in apple fruit is found in the vacuole of the parenchyma cells, and its concentration follows a developmental pattern that peaks 4 to 6 weeks after bloom and then gradually declines until fruit harvest. While malate synthesis and degradation can have an impact on fruit malate levels, apple fruit acidity appears to be primarily determined by intracellular malate transport between the cytosol and the vacuole. Malate is a significant metabolite in glycolysis and the Krebs cycle, but its accumulation in the vacuole also influences the acidity of many fleshy fruits.

As we know malic acid has two stereoisomeric forms (L- and D-enantiomers), but only the L-isomer occurs naturally. L-isomers are abundant in apples and can be used to detect adulteration.

(Elkins,et al., 1994)In pure apple juice, L-malic acid is the dominant acid, and no D-malic acid should be present. Synthetic malic acid contains 50% D-malic acid, is cheap, and can be used to make fake apple juice. L-Malic/total malic ratios of 0.9 or less indicate a tainted sample. A collaborative study involving fourteen laboratories was conducted to determine the L-malic/total malic acid ratio in apple juice. Each laboratory received ten samples of apple juice. The authenticity of the samples ranged from 0% to 100%. In all cases, the coefficients of variation were acceptable, i.e., less than 5%.

To assess the possibility of authentication coupled with chemometrics, the sugar and organic acid profiles of various fruit juices (including apple, pear, peach, grape, sweet cherry, strawberry, and blueberry with various varieties) were compared.in grape and blueberry glucose and fructose were the predominant sugars.(J. Li et al., 2020) The same results are

shown in our research table, where the predominant sugars in grapes and blueberries are glucose and fructose.

Our tomato hypothesis was proven correct. The most abundant sugars in the tomatoes sample that we measured were fructose and glucose, which were 11.053 g/L and 6.337 g/L, respectively. The same expectations and outcomes can be found in other research papers.

Sugar and acid content are important factors in tomato fruit flavor, and high but balanced levels of sugars and organic acids are desired. Sugar and acid content are both important characteristics. Citric acid is the dominant organic acid at all stages, but unripe green tomatoes may contain significant amounts of malic acid, whereas its content in ripe fruits is relatively low. The fruits of the cultivated tomato (*Lycopersicon esculentum*) are primarily composed of glucose and fructose, with only trace amounts of sucrose. However, the reported values for citric acid were very low, 0.36-0.55 g/l, which was also the case in our study. (Agius et al., 2018)

Blueberries have a cellulose content of 3.5 percent and a soluble pectin content of 0.7 percent. Total sugars account for more than 10% of the berry's fresh weight. The primary reducing sugars in blueberries are glucose and fructose, Ripe blueberries have an acid content ranging from 1% to 2%, with citric acid constituting the primary organic acid (1.2%) in blueberries. Blueberries are high in the amino acid arginine. Blueberries have a vitamin C content of 22.1 mg per 100 g FW. (Padmanabhan et al., 2016) from our results predominant sgar are glucose and fructose.

As we all know, NMR spectroscopy can be used to determine the absolute configuration of stereoisomers. As a result, we can say that it is a one-of-a-kind technique for adulteration that we measure in fruits and vegetable carbohydrates and organic acids, which, as previously stated, could be critical for revealing adulteration.

Fruit juices are tainted by the addition of water, sugar, acid, synthetic flavour and aromatic substances, dyes, and peel extracts. Furthermore, expensive juices can be contaminated with inexpensive juices. Juice adulteration allows producers to reduce raw material costs while increasing economic profit. Citric, D-isocitric, and L-malic are the basic organic acids found in fruit juices. Because the contents of these organic acids, as well as the citric acid: D-isocitric acid ratios, differ between juices, they can serve as indicators of their authenticity. These parameters are compared to standard values, such as those found in the Code of Practice [1996,] (J. Li et al., 2020). The Code of Practice was developed by the Association of the Industry of Juices and Nectars from Fruits and Vegetables of the European

Union (AIJN), a non-profit organization dedicated to standardizing quality rules and determining the authenticity of fruit juices. Deviations from accepted norms indicate the presence of undeclared juice addition.

Quantitative Assessment of Citric Acid in Lemon Juice, Lime Juice, and Commercially-Available Fruit Juice Products, a research paper published data on citrate concentration in lemons, limes, and oranges. The concentrations were computed (Grams per Liter). Citric acid concentrations in lemon juice were 48g/L, lime juice 45.8, grapefruit 25.00 g/L, and orange juice 16.9g/L, in that order.(Penniston et al., 2008)

The University of Chicago Press Journals showed us a research paper from about citric acid concentration in lemon, which determined citric acid concentrations in 10 different varieties of lemon at different ripening times. (mg/ml) was used to calculate concentration. results are shown according to varieties, concentration and date. Lisbon 54.54 mg/ml 11/30, Eureka 48,15 mg/ml 12/6, Eureka 58,99 mg/ml 12/12, Lisbon 61,59 mg/ml 12/17, Lisbon 58,07 mg/ml 12/26, Eureka 53,01 mg/ml 1/10, Eureka 51,83 mg/ml 1/16, Lisbon 56,68mg/ml 1/18, Lisbon 58,46 mg/ml 1/22, Lisbon 55,15 mg/ml 1/27.(Sinclair, et al., 1945)

Main Organic Acid Distribution of Authentic Citrus Juices in Turkey, a scientific paper published information about Citric acids concentration in citrus. All citrus varieties were sourced from the Mediterranean region, which is Turkey's main citrus cultivation area. Four lemon varieties, four grapefruit varieties, and four orange varieties were studied. In lemon varieties Kütdiken’s citric acids concentration was 60.32 g/l, in Karalimon 48,54 g/l, 51,86 g/l citric acids were in Interdonato and in lamas was 59,72 g/l citric acids.

Of 4 types of Grapefruits varieties, the highest citric acid concentration was determined in the red Blush grapefruit variety respectively 24,25 g/l, and the lowest concentration was discovered as called name Star Ruby 16,96 g/l citric acid.

The highest citric acid concentration was discovered in the authentic variety respectively 15,65 g/l, and the lowest concentration was discovered as called Washington 11,10 g/l citric acid.

lemons, grapefruits and oranges' average citric acids concentrations were respectively 55.11 g/l, 19.61 g/l, 13.28 g/l. (Karadeniz, et al., 2004)

The organic acids of grapefruit juices scientific paper published information about citric acids concentration in grapefruit. grapefruits were sorted according to location and sampling date. scientists analysed 14 samples according to 5 different locations. the highest citric acid concentration was from (Indio) called the cultivation area, concentration was 22.84 mg/ml. the lowest concentration was 6th sample from the riverside and its concentration was 17.54

mg/ml. also, grapefruit from Hemet showed an average concentration of citric acids and it was 17,72 mg/ml. and grapefruits from Sunnymead and West riverside showed citric acids concentration accordingly 18.48 mg/ml and 18.22 mg/ml.(W. B. Sinclair & Eny, 1946) The concentrations of fruits in our results are similar to those found in all of the above-mentioned scientific papers.

Between Onion and Garlic, a scientific paper revealed fructose glucose concentration between garlic and onion

the concentration of fructose in garlic was 89.2 mg/g and in onion, fructose concentration was about 67.2 mg/g. glucose concentration in garlic and onion was respectively 10.8 and 32.8 mg/g. (Ohsumi & Hayashi, 1994)

Profiles of Sugar and Organic Acid of Fruit Juices: A Comparative Study and Implication for Authentication, called the research paper revealed sucrose glucose-fructose concentration in fruits. especially in apple, pear, strawberry, blueberry and grape species was determined carbohydrates. especially in apple, pear, strawberry, blueberry and grape species was determined carbohydrates. The concentration was measured in g/L. The concentrations of sucrose, glucose and fructose in the apple were 25.56 g/L, 24.27 g/L, and 70.31 g/L, respectively. Pear's sucrose concentration was 33.66 g/L. Fructose and glucose concentrations were 40.12 g/L and 17.79 g/L, respectively(J. Li et al., 2020). According to our findings, the above-mentioned carbohydrates concentrations were 27.218 g/l, 90.853, and 16.976 g/l. We have some differences with fructose, which could be due to transportation from the manufacturer country and ripening period.

The same study discovered monosaccharide concentrations in grapes, and we can draw a parallel to our findings. Sucrose concentration was 0.55 g/l, while glucose and fructose concentrations were approximately 90.59 g/l and 95.38 g/l, respectively. According to our findings, the concentrations of sucrose, glucose, and fructose were 0.00 g/L, 106.469 g/l, and 126.804 g/l, respectively. We can say that the grape quality and monosaccharide content of our grapes were satisfactory.

According to our research, the concentrations of sucrose, glucose, and fructose in blueberry were 0.00 g/L, 52.852 g/L, and 74,654 g/L, respectively. Almost the same result can be found in the previously mentioned research paper, where the sugar carbohydrates concentrations of the above-mentioned fruits were sucrose 0.42 g/l, glucose 56.21 g/l, and fructose 59.90 g/l.

In temperate zones, the apple (*Malus domestica Borkh*.) is a major fruit crop. Dessert apples have become increasingly popular due to market preferences such as good taste, high nutritional properties, storability, and convenience. Malic acid, which accounts for up to 90% of total organic acids in cultivated apples, is a major determinant of fruit acidity.(Ma et al., 2018)

Our results showed a malic acid concentration of approximately 10.844 g/l, which is quite satisfactory and comparable to other scientific papers that showed a malic acid concentration of 4,7404 g/l in apple(J. Li et al., 2020)

Determination of Predominant Organic Acid Components in Malus Species: Correlation with Apple Domestication-scintific paper, showed us the variation of fruit organic acid content in the apple germ plasm by examining the organic acid components of mature fruits from 101 apple accessions, including 53 apple cultivars and 58 wild relatives. where was analised especially malic acids in apple. from 101 apple varieties malic acids ratio was fluctuated from 2,58 to 29.27 mg/g. According to the results of our study paper, the malic acid content of apples was 10.84 g/l. The same results are presented in the above-mentioned scientific paper about apple samples, where the concentration was estimated to be mg/g, which is equal to g/l. The concentration of malic acid in nine apple samples was found to be very close to our results. Malic acid concentrations in samples 9, 14, 16, 21, 22, 34, 42, 61, and 68 range from 10.03 mg/g to 10.80 mg/g. which is exactly the same as our outcome. (Ma et al., 2018)

According to our findings, the sugar content of grapes was 12,680 g/100g for fructose and 10,646 g/100g for glucose. Almost identical results were revealed in the scientific paper Sugars, organic acids, and phenolic compounds of ancient grape cultivars (*Vitis vinifera L.*) from the Igdir province of Eastern Turkey. there were estimated about 9 gape cultivars and their fructose glucose concentration. The second cultivar, Beyaz Kismis, had the highest fructose concentration, which was approximately 15.55 g/100g. The lowest concentration was found in the eighth cultivar, Miskali, with a fructose concentration of 8.03 g/100g. The glucose concentration ranged from 9.51 g/100g in the Miskali cultivar to 15.21 g/100g in the Hacabas cultivar.

There may be minor differences between our results and those of other scientific papers; this difference may be due to the transportation of vegetables and fruits, as well as the ripening period. As the fruit matures, the accumulated starch is hydrolyzed into sugars (glucose, fructose, or sugars), which is a typical event for fruit ripening. The action of invertase is likely to be involved in the further breakdown of sucrose into glucose and

fructose. In vegetables such as potatoes and peas, on the other hand, the higher sucrose content, which remains high at the fresh immature stage, converts to starch as maturity approaches. Ripening is the integration of processes that occur in the later stages of a fruit's maturation and into the early stages of senescence. These processes soften the fruit, change its color, and increase its sugar content in order to make it suitable for human consumption. Maturation refers to the processes that result in fruit ripening. Fruit that is less mature and not ripe (that is, green fruit such as bananas and apples) is less acceptable for consumption than fruit that is more mature and fully ripe. (Toivonen et al., 2007)

This could be a reasonable explanation for minor differences between research papers. When we mentioned food authentification by NMR above, we highlighted authentification according to food geographical territories, and each geographic territory had a specific influence on the characteristics of food, such as high or low chemical concentrations, species differentiation by pH, and so on. When it comes to discovering similarities between researcher papers, this could be a critical factor. When one species of fruit or vegetable is examined, differences will be found so their geographical location could be the reason.

Specific findings can be applied to my future studies such as clinical nutrition, in which the carbohydrate content of vegetables and fruits will be considered in order to eliminate type 2 diabetes.

7 Conclusion

We investigated the capability of ^{1}H NMR spectroscopy for quality control of vegetables and fruits in this study. ^{1}H NMR spectroscopy, in conjunction with advanced data analysis and chemometrics, was effective in the classification of fruits and vegetables, not only in distinguishing them from one another, but also in differentiating them by specific chemicals that are specific to concrete fruits or vegetables.

Thanks to a large dataset of 35 vegetables and fruits spectra, we were able to understand the significance of sugar and organic acid in the species. Sugar is a macronutrient found in fruits and vegetables that is unique to each species and common; however, one of the most distinguishing features of each species, as measured by ^{1}H NMR.

The presence of sugar and organic acids was a determining factor in the rapid classification of vegetables and fruits. The classification of species based on their sugar content is necessary for the prevention of some diseases associated with excessive sugar consumption in daily diets.

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Figure.3. Sum normalisation, paretto scaling Hierarchical clustering

Figure.4. Sum normalisation, paretto scaling Hierarchical clustering