Hort4EUGreen

Strategic Partnership No. 2020-1-RO01-KA203-080398

NUTRITIONAL QUALITY OF HORTICULTURAL PRODUCTS

Food nutritional quality

Volume 3

University
of Agronomic Sciences
and Veterinary Medicine
of Bucharest

ЛЕСОТЕХНИЧЕСКИ
УНИВЕРСИТЕТ

MATE

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CONTRIBUTORS

UNIVERSITY
OF AGRONOMIC SCIENCES
AND VETERINARY MEDICINE
OF BUCHAREST

USAMV
Liliana BĂDULESCU
Monica BADEA
Violeta Alexandra ION
Oana - Crina BUJOR
Andreea BARBU
Aurora DOBRIN
Carmen CONSTANTIN
Ioana CĂTUNEANU
Lavinia ILIESCU

Editing:
Oana VENAT
Alexandra CORNEA

University of Forestry
Natalia KOLEVA

ЛЕСОТЕХНИЧЕСКИ
УНИВЕРСИТЕТ

ZMMAE
Okray OREL

UNI-MATE
Szandra KLÁTYIK

MATE
MAGYAR AGRÁR- ÉS
ÉLETTUDOMÁNYI EGYETEM

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"Enhancing practical skills of horticulture specialists to better address the demands of the European Green Deal"

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Module No. 3

Food nutrition quality

Introduction

The nutritional quality, as an extended definition, refers at the value that one product has for the consumer's physical health, growth, development, reproduction and psychological or emotional well-being. This value can be conferred by the substance itself, considering all the mineral and organic components of foods (all ingredients, compounds either beneficial or harmful) and the feelings, the state of well-being/indisposition that foods can cause in consumers (Köpke, 2005). Otherwise said, the nutritive quality is an indicator of the contribution of a food to the nutrient content of the diet. The food value depends on the quantity of a food which is digested and absorbed and the amounts of the essential nutrients (protein, fat, carbohydrate, minerals, vitamins) which is contained by that ingested amount of food. Food quality has a great importance in our diet, and most important in the diets associated with chronic diseases. Nutrients are not consumed in isolation, but as components of whole foods and in an infinite number of combinations.

Foods contain a myriad of chemical substances, of which some play a major role, some a secondary role, while some either serves no role in human metabolism or their role has not yet been elucidated. In order to develop individual diet recommendations, that are nutritionally adequate for a better human health and well-being, there is a continue need for learning and understanding the nutrient composition of food products. Continuous research is being carried out on food with high nutritional value, that needs to be valorized accordingly and the last decades were full of new superfood placed into the market. There is a continuous necessity and an opportunity for teaching staff and master students to practically learn the analytical methods used for the identification and quantification of nutrients and the bioactive compounds with increased nutritional value. These aspects are largely presented on the first module, entitled “Current trends and importance in food chain”.

For being in direct relation with people safety and health, the nutritional quality aspects of horticultural products have been included in the EU regulations and international or national standards. To apply the quality standards for vegetables and fruits, different criteria, specific to each product are used, and the normative acts differentiate the quality of fruits and vegetables by three categories: extra, quality I and quality II. The international organizations responsible for food safety and quality are: Food and Agriculture Organization (FAO), World Health Organization (WHO), European Food Safety Authority (EFSA) and national bodies.

For countries that don't have the resources or infrastructure to develop their own safety evaluations and regulations, the Joint FAO/WHO Food Standards Programme, known as the Codex Alimentarius, was developed. These aspects are largely presented on the first module, entitled "Regulations on nutritional quality of horticultural products".

Horticultural products have their quality traits in direct relation with soil and climate variations, with cultivars/varieties/species, with the applied cultivation technology or specific agricultural practices, etc. (Köpke, 2005) and the values of each component can be affected by the soil and growing conditions, handling and storage, and processing. Recently, it has been claimed on social media, in an intense debate, that our food is far less nutritious than it used to be (McGrath & Shewry, 2020) and that the vanishing nutrients it's just a hazard of climate change (Suglia, 2018).

Sellers of vitamins also claim our food doesn't contain all the nutrients it once did. This might be due to soil depletion, cold storage, food ripening off the vine, and global transport of out-of-season foods as the foods contain the greatest amount of nutrients when they are eaten right after the harvest.

Summary

In this module, aspects related to nutritional quality, including the classification of compounds with nutritional value in fruits and vegetables (carbohydrates, protides, lipids, antioxidants, minerals, and the analytical techniques used for identification and quantification of these nutritional compounds, as general physico-chemical analysis, spectrophotometry (total phenolic content, total flavonoid content, total carotene and lycopene content, chromatography and spectrometry, atomic absorption spectrophotometry, inductively coupled plasma, mass spectrometry are presented.

Food nutritional quality is a crucial aspect of nutrition science. It's directly related to the field of scientific research and refers to the overall nutrient content of a food item and its ability to provide essential nutrients while minimizing harmful substances.

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Learning outcome descriptors

By the end of the module, the trainees should be able to prove they acquired both general and transferable skills and knowledge, understanding and professional skills.

General and transferable skills

1	Plan a research task.
2	Work independently or with a minimal guidance where appropriate.
3	Work in team with minimal guidance where appropriate.
4	Show good written and oral communication skills.
5	Demonstrate computer literacy
6	Perform online (computer) search to develop information technology skills in order to retrieve information from a variety of sources.

Knowledge, understanding and professional skills

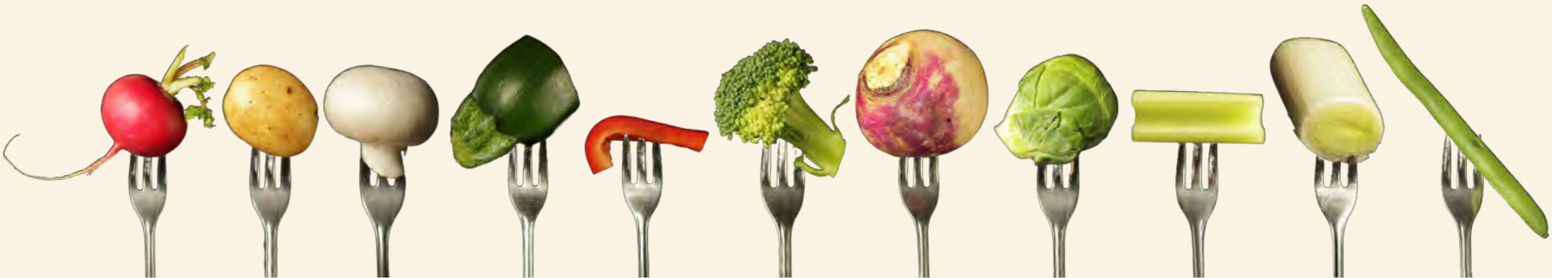
Specify the main factors that should be taken into consideration before
Plan the different steps of the acquiring knowledge.

Unit 3.1: Classification of compounds with nutritional value in fruits and vegetables

Monica Badea, Liliana Bădulescu, Aurora Dobrin, Andreea Barbu, Ioana Cătuneanu, Lavinia Iliescu

Horticultural products are important sources of substances for human nutrition due to their rich content in vitamins, mineral salts, plastics (carbohydrates, lipids and proteins) and secondary substances (phenols, alkaloids, organic acids, glycosides).

Fruits and vegetables, due to the variation of their chemical composition, play an important role in nutrition and also in preventing and combating many diseases. Plants are the only organism that can use inorganic substance in the environment to synthesize organic compounds.



3.1.1 Carbohydrates

Carbohydrates are organic compounds, the primary result in the process of photosynthesis.

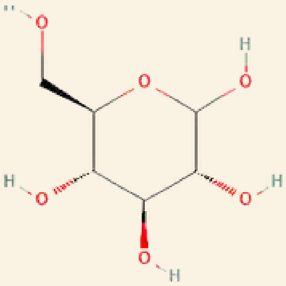
Lipids and proteins are produced out of these substances, resulting from the primary metabolism of plants. Intermediate or final substances, resulting from the metabolism of primary substances (carbohydrates, proteins, lipids), are referred to as secondary substances, are formed in the biochemical cycles that make up secondary metabolism. These substances are organic acids, alkaloids, flavones, terpenes, glycosides, etc.

It is the category most present in fruit and vegetables (representing 50-80% of the total dry weight). Carbohydrates play a role in storing energy reserves and forming an important part of the structural frame of cells.

"Let thy food be thy medicine. Which means our food should be our medicine and vice versa"
Hippocrates

The simple ones are the result of photosynthesis. Glucose and fructose are the predominant forms of sugars especially in fruit. Glucose, fructose, and sucrose are the sugars that make fruit and vegetables taste sweet.

D-GLUCOSE



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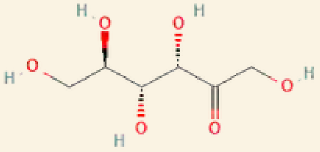
D-(-)-FRUCTOSE



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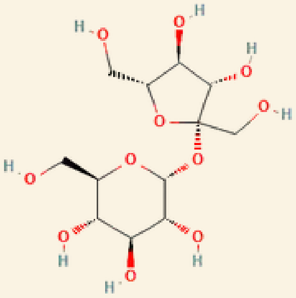
SUCROSE



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The amount of glucose, fructose and sucrose varies depending on the fruit and the degree of ripeness of the fruit. There are also fruits that contain more glucose and fructose (apples, strawberries, grapes, pears) and less sucrose. Vegetables such as onions, carrots, peas and sweet potatoes have a higher sucrose content. One category of carbohydrates is starch, which is present in large quantities in starchy plants (potato, beans).

Total carbohydrate content of the main species of vegetables and fruits

SPECIES	AVERAGE 100%	LIMIT 100%	SPECIES	AVERAGE 100%	LIMIT 100%
Pepper	3,0	1,5-6,6	Cranberries	9,2	6,2-11,9
Potatoes	1,2	0,4-3,4	Bananas	18,0	11,4-27,9
Cucumbers	1,9	1,2-3,4	Apricots	10,1	9,6-13,8
Onion	8,4	4,7-10,2	Chestnuts	28,0	26,0-29,0
Cauliflower	2,5	1,7-4,8	Cherries	11,8	6,4-15,3
Carrot	6,9	5,8-8,2	Quince	10,1	6,5-12,9
Leek	6,3	4,5-9,8	Lemon	2,2	0,9-3,6
Spinach	3,1	2,4-3,7	Appels	11,6	6,0-16,7
Asparagus	2,.3	2,0-3,2	Nuts	12,5	7,8-16,2
Tomato	3,8	1,8-4,3	Pears	11,8	6,5-14,9
Celery	3,6	0,9-4,6	Peaches	10,5	6,3-12,4
Garlic	25,0	20,6-30,9	Plums	12,3	7,2-14,9
White cabbage	4,5	2,9-5,8	Cherry	10,2	6,0-14,0
Eggplants	2,5	0,7-5,4	Raspberry	4,5	3,0-9,3

Source: Souci et al., 1975, Gherghi et al., 1979 cited by Gherghi et al., 2001

Classification of carbohydrates

Monoglycosides, oligoglycosides and polyglycosides have been identified in fruits and vegetables.

Monoglycides are substances containing in the molecule a carbonyl group and several hydroxyl groups, with a linear chain, very rarely branched. Depending on the number of carbon atoms, monoglycosides are divided into: trioses, tetrodes, pentodes, hexoses and heptodes.

Triazines contain three carbon atoms, are found as phosphate esters forming intermediates of carbohydrate metabolism such as dihydroxyacetone and D-aldehyde glycerol.

Tetroses contain four carbon atoms, e.g. D-erythrose which is involved as a phosphate ester in the photosynthesis process.

Pentoses are widespread in nature, occurring in fairly small amounts in nature, larger amounts are found in the formation of polyglycosides, phosphoric acid esters and glycosides. It is also a component of enzymes, vitamins and nucleotides. The main pentoses are: D-xylose, D-ribose, D-xylose, d-Xylulose and D-ribulose. Ribulose, xylulose and ribose play an important role in carbohydrate transformation processes.

Hexoses are found in the free state but also as oligoglycosides, polyglycosides, esters, glycosides and derivatives. The most common hexoses are: D-glucose, D-galactose, D-mannose, D-fructose and L-mannose.

Heptoses are most often found in the form of the diphosphate ester of D-sedoheptulose, intervening in the process of photosynthesis. Found in vegetables and fruit. There are also carbohydrates with 8 and 9 carbon atoms, such as octaves and nonoses. They are found in very small quantities in plant products.

Oligoglycans

The most common are oligoglycans made up of hexoses, which are common in vegetables and fruit. Representative for this group is a non-reducing diglucose called **sucrose** (C12H22O11) composed of D-glucose and D-fructose.

Maltose is formed by the condensation of two glucose molecules and is a reducing diglucose. It is found in higher amounts in **bananas and grapes.**

Other oligoglycans include rutinose (fruit), gencybiosis (fruit), hesperidin, **amygdalin**, **rutin**, **cellobiose** (fruit and vegetables), **raffinose** (plums, grapes), **stachyose** (beans, lentils, grapes).

Polyglucose

Homopolyglucoses -Arabane-highlighted pentosans enter the cell wall structure of fruits and vegetables, creating the link between hemicellulose, cellulose and pectin molecules. From fructose molecules, linked b--(2,1)-glycosidically, fructans are formed, from glucose, glucans are synthesized, from galactose, galactans, and from mannose, mannans.

Hexozans, which **include fructans, mannans, glucans and galactans**, are commonly found in horticultural products and are part of the cell wall structure.

Inulin

is the main reserve carbohydrate in Allium cepa (onion) bulbs, it is also found in Arctium lappa (burdock), Cynara scolymus (artichoke), Inula helenium (big grass), and Echinacea spp. (echinacea). Inulin has immunostimulatory activity, in case of hypoglycaemia it stabilises blood sugars and has a diuretic action.

Galactans

have D-galactose and L-galactose in their structure.

Mannans

are made up of units formed from β-mannose, they enter into the composition of cell walls.

Fructans

consist of D-fructose, also known as fructooligosaccharides. They are found in asparagus, leeks, garlic, artichokes, endives. The main fructans are inulin and asparagose from asparagus.

Glucans

are formed from D-glucose molecules, the main glucans are **starch** and **cellulose**. Glucans, i.e. b-(1-3)-glucans, are polyglucans with immunostimulatory, antitumor, bactericidal, anti-inflammatory effects. Such bioactive polyglucoses are found in *Aloe spp*.(aloe), in the roots of *Glycyrrhiza glabra* (liquorice) and the fruits of *Zizyphus jujuba* (Dobrogean olive tree).

Starch is the main form of carbohydrate storage in plants, made up of amylose and amylopectin. An important source of carbohydrates for human health is resistant starch. The richest sources of resistant starch are legumes (35%) (Marlett and Longacre, 1996).

Amylose is in the form of a chain, made up of 1,4-a-glycosidically linked D-glucose molecules, while amylopectin has a chain similar to amylose, to which shorter chains of glucose are linked by 1,6-a-glycosidic bonds. The amylose and amylopectin chains are deposited in concentric layers forming starch granules.

Starch accumulates as a reserve substance in roots, rhizomes, tubers, fruits and seeds. During the vegetative stage, starch is enzymatically hydrolysed to provide α-D-glucose used in respiration and growth processes.

Starch is the main reserve substance in potato tubers and cereal kernels (Burzo, 2015). Thus, the starch content varies between species within the following limits:

- 70 - 80 % in barley seed
- 64.7 - 66.0 % in maize seed
- 63.7 - 67.9 % in wheat seeds
- 49.8 - 63.0 % in oat grains
- 13.2 - 23.0 % in potato tubers

Cellulose is part of the structure of cell walls and is made up of D-glucose molecules linked by 1,4-β-glycosidic bonds. The cell wall in fruit and vegetables accounts for 1 to 2% of the weight, 33% of which is cellulose. During ripening of fruits and vegetables the cell wall undergoes changes resulting in a change in cellulose content (Brummell, 2006). It is often accompanied by hemicelluloses and pectic substances.

Heteropolyglucoses

Pectic substances

Pectins are substances that form the middle lamella, representing the binder between cells and forming the cell walls. These polyglucoses are synthesised on the outside of cell walls (intercellular) from polygalacturonic acids produced by the Golgi complex. They consist of α-galacturonic acid molecules linked by 1,4-α-glycosidic bonds, to which other polyglucoses are added, such as: D-xylans, L-arabans and D-galactans. They are found in greater quantities in the fruits of *Cydonia oblonga* (quince) and *Malus domestica* (apples).

Hemicelluloses enter into the structure of cell walls along with cellulose and pectins. Hemicelluloses are mostly made up of xylans, which are joined by mannans, arabans, galactans and pectins.

Mucilages are made up of pentoses, hexoses and polygalacturonic acids, which swell in contact with water. (Burzo, 2015). They are found in various plant organs: roots, stem bark, leaves and seeds.

Mucilages have an emollient, laxative and antidiarrhoeal effect, inhibit fermentative processes and the growth of bacteria. They can bind toxins in the digestive tract and because they are less digestible, they are eliminated from the system along with the bolus that forms.

Glucose, fructose and sucrose are the main energy substances in sucrophilic plants, their content varying within the following limits:

- glucose between 1.16 % in peaches and 7.28 % in grapes;
- fructose ranges from 0.87% in apricots to 7.33% in grapes;
- sucrose ranges from 0.22% in cherries to 10.6% in bananas.

Carbohydrates are the primary products of primary metabolism, from which all other plant substances are biosynthesised. Together with lipids, they are the main energy substances in plants, with the metabolism of one molecule of hexose resulting in 1240 kJ biochemical energy and 1600 kJ caloric energy.

Carbohydrates are important not only in terms of their energy value, but also because of their content of cellulose, hemicellulose, pectins and lignin, which make up dietary fibre.

They have a high capacity to retain water and some toxic substances (uronic acids), which are excreted with faeces. It is believed that because of this role, dietary fibre helps prevent colon cancer.

For more information regarding main chemical components of fruits and vegetables, please visit:

Link here:

Link here:

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3.1.2 Protides

Protides are widespread in all plant organisms, being basic substances of living matter, entering into the composition of cell membranes, enzymes and nucleic acids, having structural, functional and catalytic roles.

Protides are made up of carbon, hydrogen, oxygen and nitrogen and are called quaternary substances, nitrogen being the characteristic element that distinguishes them from lipids and carbohydrates, organic substances called ternary.

Protides content varies by species, variety, organ and also by tissue within the same organ.

Peptides, depending on their structure, comprise three groups:

- amino acids;
- peptides;
- proteids.

Amino acids are organic substances that have amino (-NH2) and carboxyl (-COOH) groups in their molecule. They are found in the composition of peptides and proteids or in the free state.

Peptides are formed by the condensation of 2-4 amino acids joined together by peptide bonds. Peptides are formed by the bonding of free amino acids, or following the hydrolysis of proteins. Of the peptide category, **glutathione** ($C_{10}H_{17}N_3O_6S$) is a tripeptide that is present in almost all living tissues, most abundantly in the wheat embryo.

Glutathione plays an essential role in the oxidoreduction processes due to the -SH group. It is also involved in the maintenance of redox potential, it is part of the composition of enzymes such as glutathione reductase and glyoxalase. It is found in higher amounts in: cauliflower, cabbage, avocado, asparagus, broccoli and spinach.

Proteids are macromolecular substances made up of amino acids linked by peptide bonds. Proteids are classified according to their chemical composition into **heteroproteids** and **haloproteids**.

Haloproteids (proteins) are made up of a large number of amino acids that are linked by peptide bonds. They play a particularly important role in living organisms, performing several functions: energetic, plastic, enzymatic, immunological, etc. From the protein category, **albumins** and **globulins** have been frequently found in horticultural products.

Albumins are low molecular weight proteins present in all plant organs, with legumes (soya, peas, lentils), lucin (castor) and soya being the most common.

Globulins are high molecular weight proteins that are widespread in the plant kingdom. The most common of these are: legumin (peas, beans, lentils), phasoline (beans), tuberine (potato tubers), glycinine (soya), coriline (alum seeds), viciline (pea).

Protein content varies by species, for example, 20% in peas and 37% in soya beans. Proteins are used in nutrition not only in terms of their energy value but also in terms of their content of essential amino acids such as leucine, phenylalanine, arginine, isoleucine, lysine, methionine, tyrosine, threonine, tryptophan and valine.

The average protein content of some vegetables and fruits

SPECIES	PROTEINS %	SPECIES	PROTEINS %
Pepper	1,2	Cranberries	6,0
Potatoes	2,1	Peanuts	13,4
Cucumbers	0,6	Bananas	1,1
Onion	1,2	Chestnuts	7,1
Cauliflower	2,5	Apples	0,3
Green beans	2,3	Almond	18,3
Green pea	6,5	Nuts	16,4
Carrot	1,1	Peras	0,5
Tomato	1,1	Peaches	0,8
Celery	1,6	Plums	0,7
Garlic	6,5	Figs	1,3
Eggplants	1,4	Raspberry	1,2

Source: Souci et al., (1975), Gherghi et al. (1979) cited by Gherghi et al., 2001.

The daily requirement of essential amino acids for a person weighing about 70 kilograms is: 1.400 mg isoleucine, 2,730 mg leucine, 2,100 mg lysine, 1,050 mg methionine + cysteine, 1,750 mg phenylalanine + tyrosine, 1,050 mg threonine, 280 mg tryptophan and 1,820 mg valine.

Heteroproteids are macromolecular substances, consisting of amino acids and some prosthetic groups (substances of a non-protein nature). The most common heteroproteids found in horticultural products are lipoproteids, nucleoproteids and chromoproteids.

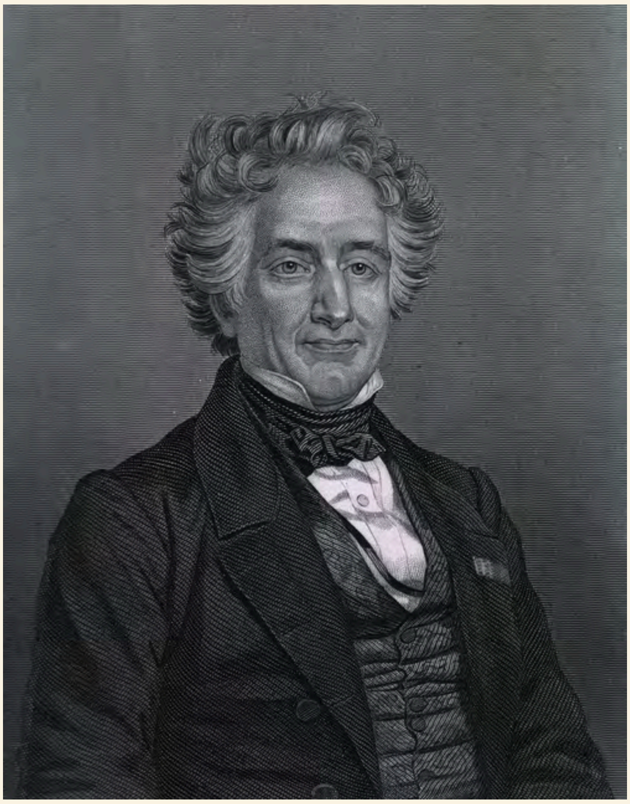
Lipoproteids play a plastic role, they are part of cellular and intracellular membranes. They have been found in carrots, tomatoes, apples and soybeans.

Nucleoproteids play a very important role in cell division processes, in the transmission of hereditary traits and in protein production.

Chromoproteids present as a prosthetic group compounds with porphyrin nucleus participating in the synthesis of carbohydrates, in oxidation-reduction processes, in horticultural products (vegetables and fruits) having also enzymatic functions. **Chloroplastin** or **chloroglobin**, a chlorophyll-holoprotein complex, belongs to the category of chromoproteids. It plays a very important role in the process of photosynthesis, absorbing and transmitting light energy used in the photochemical decomposition of water. Chloroplastin (chloroglobin) is found in all green plant organs containing the pigment chlorophyll.

3.1.3 Lipids

Lipids are a class of organic substances made up of fatty acids and their derivatives esterified with different alcohols. M.E. Chevreul (1811- 1823) was the one who discovered lipids in plants and noted that in the molecular structure there are fatty acids and alcohols, or aminoalcohols. They are water insoluble (hydrophobic) and soluble in organic solvents (e.g. petroleum ether, benzene, chloroform, etc.).



Michel-Eugène Chevreul, c. 1860.

Some lipids combine with proteins to form lipoprotein complexes that are involved in the process of respiration. Depending on their structure, lipids in the plant kingdom are divided into:

- Simple lipids;
- Complex lipids.

The main alcohols and aminoalcohols that enter into the lipid structure of horticultural products are: **arachic alcohol**, **ceryl alcohol**, **myricyl alcohol**, **ceryl alcohol**, **glycerol**, **choline**, **cetyl alcohol**, **stearic alcohol**.

The most important phytosterols present in fruits and vegetables are: campesterol (peas, bananas, chestnuts, almonds, walnuts peaches, grapes etc), β-sitosterol (cucumber, onion, cauliflower, asparagus, apricots, strawberries, chestnuts, apples, almonds, walnuts, figs, peaches etc), stigmasterol (cauliflower, peas, lettuce, asparagus, almonds, peaches, figs, aubergines etc), β- and γ-stanol have been identified in peas.

SPECIES	**g/100g**	**SPECIES**	**g/100g**
Parsley	0,48	Apricots	0,13
Salad	0,22	Plums	0,17
Celery	0,33	Almond	54,10
Garlic	0,12	Grapes	0,28
Eggplants	0,18	Nuts	62,50
Cucumbers	0,20	Carrot	0,20
Peanuts	61,60	Onion	0,25
Pears	0,29		

Lipid content in some species of vegetables and fruits (Gherghi et al.2001).

Glycerides are esters of glycerol with fatty acids, they are widespread in horticultural products, participating in the regulation of membrane permeability (vegetable pulp) but also as a nutrient for the embryo (fruit seeds - peaches, cherries, plums, grapes and tomatoes).

Cerides are esters of fatty acids with long chains of monohydric alcohols. Cerides are substances that are part of plant waxes present on the surface of some vegetables and fruits. Plant waxes also contain fatty acids, alcohols, aldehydes and hydrocarbons in addition to cerides and ketones. Plant waxes are found on the surface of vegetables and fruit, reducing high water loss through transpiration, and have a protective role against pathogens.

Sterides are natural esters of fatty acids (commonly found - palmitic, oleic, stearic and linoleic acid) with sterols (polycyclic alcohols). These simple lipids are found in smaller quantities and mostly occur alongside glycerides.

Simple lipids - are made up of C, H and O are esters consisting of an organic acid and alcohol.

Complex lipids are substances that contain, along with alcohols and fatty acids, substances such as aminoalcohols, phosphoric acid, amino acids and sometimes carbohydrates or inositol.

Phosphatides are esters of glycerol with fatty acids and phosphoric acid, which is esterified with an amino alcohol, amino acid or inositol. Phosphatides enter into the lipoprotein membrane structure of cell organelles playing a role in regulating the permeability of cell membranes and also play a role in the transport of carbohydrates, proteins in the plant. Phosphatides are found in apples, cabbage and tomatoes in concentrations between 0.1 and 0.6%.

The most representative phosphatides are: **glycerophosphates** (spinach, tomatoes, kale), **lectins** (apples, beetroot, potatoes), **cephalins** (green tomatoes), **serine phosphatides** (in larger quantities in apple flesh), **inositolphosphatides** (apples, peas, pears).

Phosphatides are found mainly in the lipoprotein membrane of cell organelles and in the spheroids of the cytoplasm of fruits and vegetables, where the amount varies depending on the species.

Glycolipids are lipids that have nitrogen in their molecule and are considered amides from a chemical point of view, being part of the lipoprotein membrane structure of cell organelles in fruits and vegetables. A higher amount of lipids was identified in hazelnuts, almonds, walnuts, the content varying according to species and variety.

From a physiological point of view, lipids have several important functions: they are reserve substances for oil plants (triglycerides), they are structural components of plasma membranes (phospholipids), they constitute the protective coating for external tissues (waxes), they are involved in cell recognition (glycolipids) and some sterols may have a hormonal role, stimulating growth and development processes.

Lipids are the main energy substance for oilseed plants. During germination of the seeds of these plants, lipids are also used for the biosynthesis of the carbohydrates necessary for this process to take place, via gluconeogenesis.

Lipids are reserve substances for oil plants (triglycerides), they are part of the structure of plasma membranes (phospholipids), they are protective coatings for external tissues (waxes), they are involved in cell recognition (glycolipids) and some sterols may have a hormonal role, stimulating growth and development processes. For oilseed plants, lipids are the main energy substance during seed germination and are also used for carbohydrate biosynthesis.

For more information for lipids, please visit:

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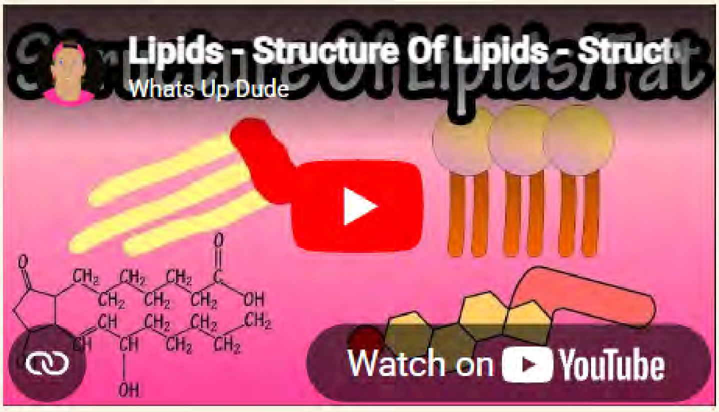

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3.1.4 Vitamins

The term vitamin comes from the words “vital amine” a number of 14 vitamins are known: vitamin A (retinol), B vitamins (thiamine), riboflavin, niacin, pantothenic acid, pyridoxine, folic acid, choline, biotin, cyanocobalamin, vitamin C, vitamin D, vitamin E, vitamin K. Vitamins A, D, E and K are fat-soluble, vitamins B and C are water-soluble.

Vitamins are necessary for normal development and cannot be synthesised by the body in sufficient quantities, requiring supplementation through diet. Vitamins in fruit and vegetables play an important role in human nutrition as catalysts for many vital body functions. Lack of vitamins in the body, or insufficient amounts, causes metabolic changes that are revealed by avitaminosis.

The vitamin content differs between species (Salunche et al., 1991). Differences were also observed in the vitamin content of fruit and vegetables depending on the variety, growing environment and orchard conditions. (Rodrigues-Amaya, 2001; Lee and Kader, 2000.

Vitamin A

Fat-soluble causes the orange, yellow and red color of fruit and vegetables. Influences cell division, visual function, bone development and reproduction. **Carotenoids** in plants have functions related to the interception of radiation, particularly in the blue-green region of the spectrum, which can be transferred to photosynthetic centers (Kopsell and Kopsell, 2006).

Fruits and vegetables provide about 30% of vitamin A through the diet (Hiza and Bente, 2007). It has been found that vitamin A is accumulated in greater quantities in the skin of tomatoes and peppers (Rodrigues - Amaya, 2001).

Vitamin A is found in: carrots, spinach, lettuce, oranges, peppers, tomatoes, pumpkin, melon, etc. Beta-carotene is the most studied carotenoid and is found in large amounts in carrots. Carotene present in vegetables does not change during processing. Carotene absorption is achieved if some fat (about 15%) is included in the diet.

The way it is prepared influences carotene absorption. Thus carrots eaten in pureed form have better carotene absorption than shredded carrots. Vitamin A deficiency can lead to severe anemia, decreased visual acuity, slowed growth in children, dry skin, etc.

Total beta-carotene content in some vegetables and fruits

SPECIES	**mg/100g fresh product**	**SPECIES**	**mg/100g fresh product**
Pepper	25,0-35,0	Cranberries	02,-0,3
broccilli	0,78-5,02	Bananas	0,38-8,3
Cucumbers	014-0,23	Apricots	1,52-19,57
Onion	0,39	Strawberries	0,03-0,1
Cauliflower	0,01-00,8	Cherries	0,01
Carrot	3,5-18,2	Oranges	0,02-3,5
Spinach	3,9-9,9	Lemon	0,4-0,8
Aspaargus	0,49	Appels	0,02-0,8
Tomato	0,19-12,0	Pears	0,01-0,2
Celery	0,15-0,57	Peaches	0,08-2,00
Cabbage	0,01-0,13	Plums	0,11

Source: Dobreanu, (1977, 1979), O’Neill et al. (2001), Tanumihardjo (2013), Arscott (2013) cited by Burzo, 2018

B-complex vitamins

Thiamine (vitamin B1)

Vitamin B1 is also called aneurine, having beneficial action on the nervous, digestive cardiovascular and muscular system. Has a coenzyme role in decarboxylation reactions in carbohydrate and protidic metabolism. Leguminous plants contain a large amount of thiamine, which has a stable structure at high temperatures, especially if the solution is slightly acidic. Losses during the processing of vegetables and fruit range from 25-40%. Thiamin is found in peas, walnuts, peanuts, dried beans, cauliflower and potatoes. A higher amount is also found in brewer's yeast. Deficiency in vitamin B1 can lead to the disease called beriberi.

Riboflavin (vitamin B2)

Vitamin B2 is a water-soluble vitamin with action in metabolic processes. It is found in plants in the free state or as coenzymes. It is found in almonds, parsley, mushrooms, peanuts, broccoli, spinach, asparagus.

Riboflavin is involved in oxido-reduction reactions, it is a coenzyme in some reactions of carbohydrate metabolism contributes to maintaining healthy nails and hair and skin. Riboflavin deficiency causes fatigue, impaired vision, hair loss, lowers the body's resistance to infection, skin damage, etc.

Source: https://newsmed.ro/vitamina-b2-rol-deficit-si-supradozaj

Niacin (vitamin B3, PP, nicotinamide)

Vitamin B3 is a relatively stable vitamin that can be synthesized in the body from tryptophan. The term "niacin" is non-technical and is used because there are several different chemical forms of vitamin B3. These forms include nicotinic acid and nicotinamide, which is also called niacinamide. This vitamin plays a role in the metabolism of carbohydrates, proteins and lipids, stimulation of gastric secretion, vasodilating properties, etc. Recent studies have shown that niacinamide may have anti-aging effects. Almonds contain a high amount of niacin, as also leguminous plants such as green peas, asparagus, etc.). It is also found in dried barm, mushrooms. Deficiency in this vitamin can lead to a disease called pellagra.

Source: https://www.revistagalenus.ro/arhiva/revista-galenus-2008/vitamina-pp-2/

Pantothenic acid (vitamin B5)

Vitamin B5 is a water-soluble vitamin found in almost all foods in varying concentrations. Higher amounts of vit. B5 is found in broccoli, sweet potato, peas, soya and nuts. Pantothenic acid helps the normal functioning of the digestive system, lowering cholesterol levels. Symptoms of vitamin B5 deficiency are sleep disturbances, fatigue, paresthesia of the limbs, headaches.

Pyridoxine (vitamin B6)

Vitamin B6 is also called **adenine**, is a cofactor in many reactions, playing an important role in protidic, carbohydrate and lipid metabolism. Pyridoxine participates in biochemical processes as a coenzyme, intervenes in the decarboxylation and dehydrogenation of amino acids, in the proper functioning of the nervous system, immune system, biosynthesis of neurotransmitters, etc. Vitamin B6 deficiency leads to a number of disorders with dermatological (dermatitis around the eyes), nervous (dizziness, vomiting, etc.), weight loss, anaemia. Important sources of vitamin B6 are: beans, cabbage, potatoes, spinach, prunes, bananas, avocados, nuts.

Biotin (vitamin B7)

Vitamin B7 is also known as Bios 11 b, coenzyme R or vitamin H and is an indispensable dietary factor. Biotin is involved in lipid, protein and carbohydrate metabolism, functions as an enzyme cofactor, is involved in numerous biochemical reactions. It can also be synthesised by bacterial flora in the large intestine. It is a relatively stable vitamin during food processing, deficiency leads to insomnia, depression and muscle pain, hair loss, dermatitis, conjunctivitis. It is found in fruits and vegetables such as avocado, broccoli, beans, cauliflower, nuts, peas, potatoes, spinach, bananas

The *Dietary Guidelines for Americans* describes a healthy dietary pattern as one that: a) includes a variety of vegetables; fruits; grains (at least half whole grains), fat-free and low-fat milk, yogurt, and cheese and oils. b) includes a variety of protein foods such as lean meats; poultry; eggs; seafood; beans, peas, and lentils; nuts and seeds; and soy products.

Source: https://ods.od.nih.gov/factsheets/Biotin-HealthProfessional/

Folic acid (vitamin B9)

Vitamin B9, also called **pteroylglutamic acid**, plays a role in cell reproduction and division. Folic acid biosynthesis involves the participation of purines or purinnucleotides used in the enzymatic reaction chain (Brenner and Lenthardt, 1961). Involved in the metabolism of purines, riboflavin and flavin, folic acid prevents depression, reduces homocysteine levels, supports the immune system and nervous system. It is found in appreciable amounts in spinach, bean seeds, asparagus, red beetroot, white cabbage, cauliflower, nuts and peanuts. Folic acid deficiency leads to anemia, depression, memory disorders, allergies, etc.

The *Dietary Guidelines for Americans* describes a healthy dietary pattern as one that: a) includes a variety of vegetables; fruits; grains (at least half whole grains), fat-free and low-fat milk, yogurt, and cheese and oils. b) includes a variety of protein foods such as lean meats; poultry; eggs; seafood; beans, peas, and lentils; nuts and seeds; and soy products.

Source: https://ods.od.nih.gov/factsheets/Biotin-HealthProfessional/

Cycobalamin (B12, cobalamin)

Vitamin B12 has a complex structure required for erythrocyte maturation and in DNA synthesis. It also helps maintain the myelin sheath. Not found in fruits and vegetables.

Ascorbic acid (vitamin C)

Vitamin C is water-soluble and cannot be synthesized by the human body. Biosynthesis takes place in plant tissues through a sequence of photochemical reactions, starting from D-glucose or D-galactose. This process takes place in mitochondria and partly in microsomal fractions. Ascorbic acid has a lot of benefits for the human body, high intake of Vitamin C is associated with fewer diseases (Carr and Frei. 1999). In its presence there is a better absorption of some elements (iron for example).

The main sources of vitamin C are fresh fruit and vegetables and their juices. About 90% of the body's vitamin C requirements come from fruit and vegetables (Hiza and Bente, 2007). Fruits and vegetables rich in vitamin C include: strawberries, blackcurrants, kiwis, currants, citrus fruits, broccoli, chilli peppers, parsley, cauliflower, spinach, etc.

The vitamin C content of fruits and vegetables varies according to variety, environmental factors and storage and processing conditions. **Storing** fruit and vegetables at **high temperatures** causes ascorbic acid levels to drop, it is very sensitive to oxidation.

Also, **thermal processing** loses about 40-80% of vitamin C. Steaming reduces vitamin C loss. **Long-term freezing** (12 months) can lead to a significant loss of vitamin C, 33-50%, (Ancos et al., 2000).

Ascorbic acid is an enzyme cofactor, contributes to the breakdown of free radicals and participates in oxido-reduction reactions in the body. Lack of ascorbic acid in the body leads to a disease called scurvy.

Ascorbic acid content of vegetables and fruits

SPECIES	mg/100g	SPECIES	mg/100g
Pepper	139-190	Gooseberry	1,0
Broccoli	41-146	Bananas	0,45
Cucumbers	5-11	Apricots	0,50
Onion	6-11	Strawberries	0,22
Cauliflower	17,2-81,0	Cherries	0,27
Carrot	3,9	Peanuts	28,0
Cabbage	18,8-46	Chestnuts	7,50
Garlic	14	Appels	0,75
Eggplants	5	Orange	0,24
Spinach	28-52	Lemon	0,80
Salad	4-13	Blackberries	9,70
Tomato	16-24	Plums	0,80
Parsley	35	Peanuts	28,0

Source: Soci et al. (1972), Chu et al., (2002) cited by Burzo, 2018.

Vitamin E (tocopherols and tocotrienols) can come in 8 different forms (4 tocopherols and 4 tocotrienols). All isomers consist of aromatic rings containing a hydroxyl group. The different isomers are called alpha, beta, gamma and delta , their number being given by the position of the methyl group in the ring.

The most active is alpha-tocopherol. It is found in higher amounts in oily seeds, avocados, almonds and less in leafy vegetables. Tocopherols have antioxidant properties, inhibiting the biodegradation of vitamin A and membrane fatty acids by free radicals.

Vitamin E is called the fertility vitamin and also helps reduce the risk of coronary heart disease in men. It is fat soluble, heat resistant (up to 250°C) and extremely sensitive to oxygen and light. Foods rich in tocopherols include: olive oil, sunflower oil, lentils, corn germ oil, blackberries, walnuts, peanuts, almonds, dandelion leaves, flax seeds, dark leafy greens (more accumulates in the leaves), etc. Vitamin E deficiency can cause infertility, menstrual problems, neuromuscular disorders, damage to red blood cells and nerves, etc.

Vitamin D

Vitamin D is a fat-soluble and the main forms are ergocalciferol and cholecalciferol. Vitamin D helps bone growth and remodeling by binding calcium, maintains muscle function and strengthens the immune system, contributes to a well-functioning thyroid, parathyroid and pituitary glands. Vitamin D deficiency leads to rickets in children and osteomalacia in adults. In fruit and vegetables it is present in very small amounts. The most important source of vitamin D is sunlight.

People can develop vitamin D deficiency when usual intakes are lower over time than recommended levels, exposure to sunlight is limited, the kidneys cannot convert 25(OH)D to its active form, or absorption of vitamin D from the digestive tract is inadequate. Diets low in vitamin D are more common in people who have milk allergy or lactose intolerance and those who consume an ovo-vegetarian or vegan diet.

Source: Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press, 2010.

Vitamin K

Vitamin K plays an essential role in the blood clotting process. Vitamin K also has other benefits such as reducing blood pressure, improving memory, maintaining bone health, etc. It is found in high amounts in green leafy vegetables such as spinach and lettuce and in cruciferous vegetables, but can also be found in green beans, kiwi fruit, soybean oil, avocados, cashews, peanuts, walnuts, grapes.

For more details, please visit:

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3.1.5 Antioxidants

Antioxidants in fruit and vegetables:

1. Vitamin C (ascorbic acid) is the most important antioxidant substance for neutralising the harmful effects of free radicals. Contributes to the regeneration of vitamin E, glutathione and antioxidant pigments (xanthophylls, carotenes). Antioxidant activity is achieved by the release of an electron to free radicals which oxidise simultaneously with the transition from the reduced form (L-ascorbate) to the L-dehydroascorbate form. (Burzo, 2018).

2. Carotenoids have powerful antioxidant properties. The group of carotenoid pigments includes about 700 compounds located in the chromoplasts, in the free state, as carotenoid glycosides, dissolved in lipids, crystallized, as carotenoid-protein compounds. Of these 50 are commonly found in the human diet (Fiedor and Burda, 2014) such as α-carotene, β-carotene, lycopene, phytofluene, lutein, zeaxanthin. Carotenoid pigments are present in almost all plants where photosynthesis takes place, especially in vegetables and fruits.

Tocopherols and tocotrienols

α-tocopherol has a very active hydroxyl group, with the highest efficiency in the decomposition of free radicals. It also has an important role in the protection of skin and membrane phospholipids and as an antioxidant for free radicals generated by ultraviolet radiation (Duval and Poșeman, 1995).

It inhibits singlet oxygen-induced oxidations and can react with radicals and non-radicals: hydroxyl, peroxyl, nitrogen dioxide and superoxide. Tocopherols are found in low amounts in vegetables and in higher amounts in peanuts (20.20 mg/100g), walnuts (24.70 mg/100g) and hazelnuts (28.00 mg/100g).

Phenolic compounds

include compounds derived from aromatic amino acids (tyrosine and phenylalanine), provide UV protection. Phenolic compounds are responsible for the bitter taste of some fruits and vegetables. From the category of these substances, phenolic acids, phenolic diterpenes, flavonoids, coumarins, stilbenes and volatile oils have been identified in plants (Burzo, 2015).

Total phenol content in some fruits

SPECIES	mg/100mg gallic acid equivalent	SPECIES	mg/100mg gallic acid equivalent
Cranberries	124-531	Pears	218-220
Bananas	231	Melon	124
Apricots	50,6-133,0	Watermelon	59
Strawberries	42,0-368,0	Peaches	40,7-163
Citrus	126,0-127,8	Plums	320-478
Blackblueberry	660-820	Grapes	121-145
Apples	48,0-262	Black grapes	175-185
Nectarines	107	Cherry	256-339

Source: Wu et al. (2004), Dragovic-Uzleac et al. (2009) cited by Burzo, 2018.

Total phenol content in some vegetables

SPECIES	mg/100g	SPECIES	mg/100g
Pepper	44,6-156,1	Leek	41,6-88,2
Artichoke	330,4	Spinach	72,0-89,4
Broccoli	109-198	Asparagus	64,0
Cucumbers	18,9	Tomato	30,0-32,3
Onion	23,6-88,0	Celery	13,5
Cauliflower	62,3	Garlic	81,2
Carrot	14,6	Cabbage	52,5-105,2
Pepper	44,6-156,1	Eggplants	57,4-64,8

Source: Ninfali et al. (2005), cited by Burzo, 2018

As phenols can rapidly donate hydrogen atoms from OH groups they are considered the best antioxidants (Foti, 2007).

3. **Phenolic acids** have a phenolic radical and a carboxyl group with the structure C6C1. Among the phenolic acids with antioxidant activity we can note: ferulic acid, ellagic acid, vanillic acid, gallic acid, p-coumaric acid, protocatechuic acid, etc. Gallic acid is found in strawberries, mango, soy and has antioxidant and anticarcinogenic properties (Burzo, 2018). Ferulic acid accounts for 90% of the total phenolic acids present in cereals (Manach et al., 2004). Their antioxidant activity depends on their structure and the hydroxyl bonds present in the molecule.

Flavonoids are phenolic compounds that have two aromatic rings in their structure. They are divided into several classes:

- **Flavones and flavanols** are present in high amounts in onions, blueberries, celery
- **Flavanones and flavanols are** present in large quantities in the genus Citrus
- **Isoflavones** are phytoestrogens present in legumes (soy for example)
- **Proanthocyanidins** are commonly found in grapes.
- **Anthocyanidin**s pigments that give fruits and vegetables their red or purple colour.

A high content of flavonoids was found in broccoli (60.1 mg/100g), artichokes 285.2 mg/100g, red beetroot (92.8 mg/100g), chicory (89.10 mg/100g) parsley (52.2 mg/100g) cabbage (45.7 mg/100g).

Content of flavonoids and flavonoids in some vegetable species

SPECIES	**Flavonoids mg/100g**	**Flavonols mg/100g**	**SPECIES**	**Flavonoids mg/100g**	**Flavonols mg/100g**
Pepper	7,0-9,9	0,56-0,91	Leek	10,1	1,01
Artichoke	285,2	0,88	Spinach	32,5	1,34
Broccoli	60,1	0,64	Asparagus	24,6	0,77
Cucumbers	4,7	0,41	Tomato	6,1-7,0	0,15-0,48
Onion	3,6-6,4	0,21-0,28	Celery	6,1	0,51
Chicory	89,10	1,13	Garlic	12,4	1,69
Cauliflower	32,0	0,72	Cabbage	45,7	0,66
Carrot	12,8	0,53	Eggplants	25,7-28,4	0,35-0,73

Source: USDA, 2010 quoted by Burzo, 2018

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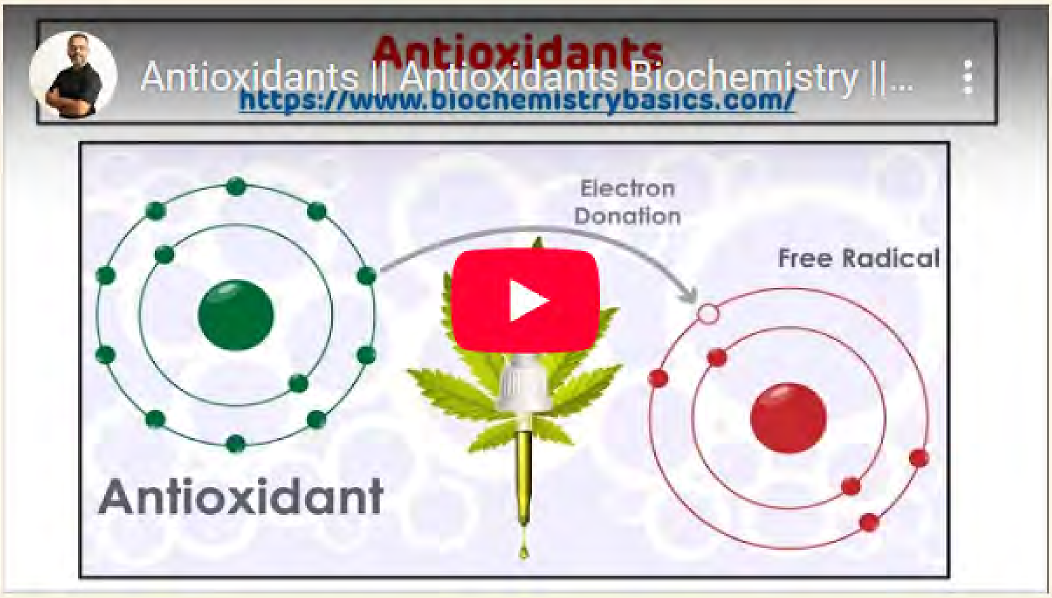

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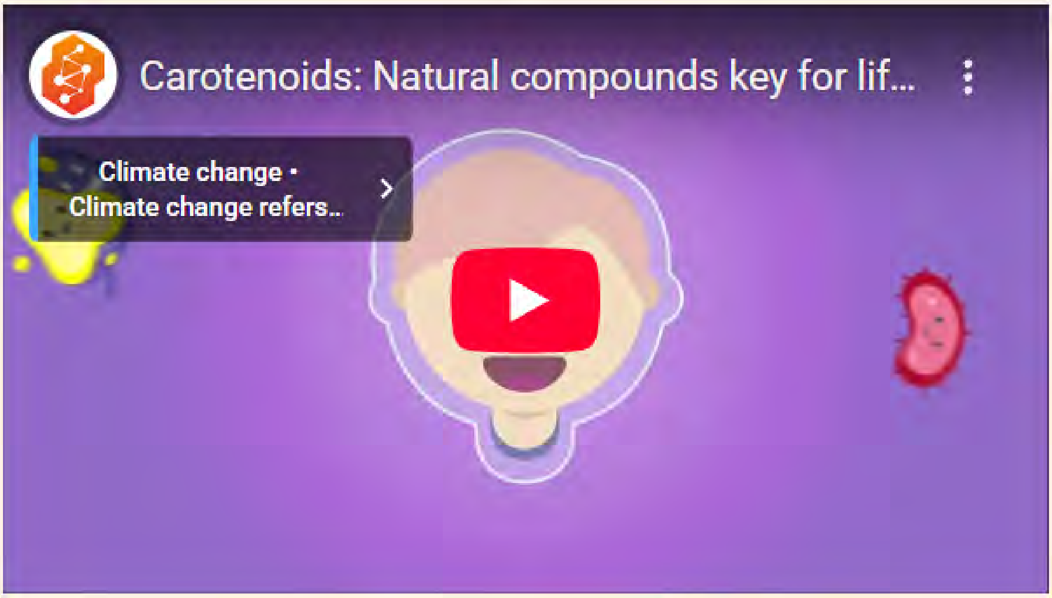

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3.1.6 Minerals

Mineral sources from fruit and vegetables

Plants, like all other living things, need food for their growth and development. Plants require 16 essential elements. Carbon, hydrogen, and oxygen are derived from the atmosphere and soil water. The remaining 13 essential elements (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine) are supplied either from soil minerals and soil organic matter or by organic or inorganic fertilizers.

More than sixty elements of the 105 discovered so far are found in different plants. Some plant species accumulate selenium, some others gold, while some plants growing near nuclear test sites take up radioactive strontium. There are techniques that are able to detect the minerals even at a very low concentration (10-8 g/ mL). The mineral content of plants is given by the ash value.

According to the concentration of each nutrient, they are classified into **macronutrients** and **micronutrients**.
The macronutrient category includes: calcium, magnesium, potassium, phosphorus, nitrogen. Their concentrations in plants range from 1000-15000 µg per gram dry mass.

Micronutrients present in plants have lower concentrations from 100-10000 and include: copper, iron, manganese, sodium, chlorine, selenium, sulphur.

Vegetables are a richer source of minerals than fruit. The effects of minerals on the human body are direct and indirect. It has been found that the excess or deficiency of a mineral is strictly related to the presence or absence of other nutrients, so a balanced diet containing both macro and micronutrients is needed to maintain optimal health.

Efforts are currently being made to apply a post-harvest strategy to improve the mineral content of fruit and vegetables using fortification methods.

For more information please visit:

Link here

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Hort4EUGreen

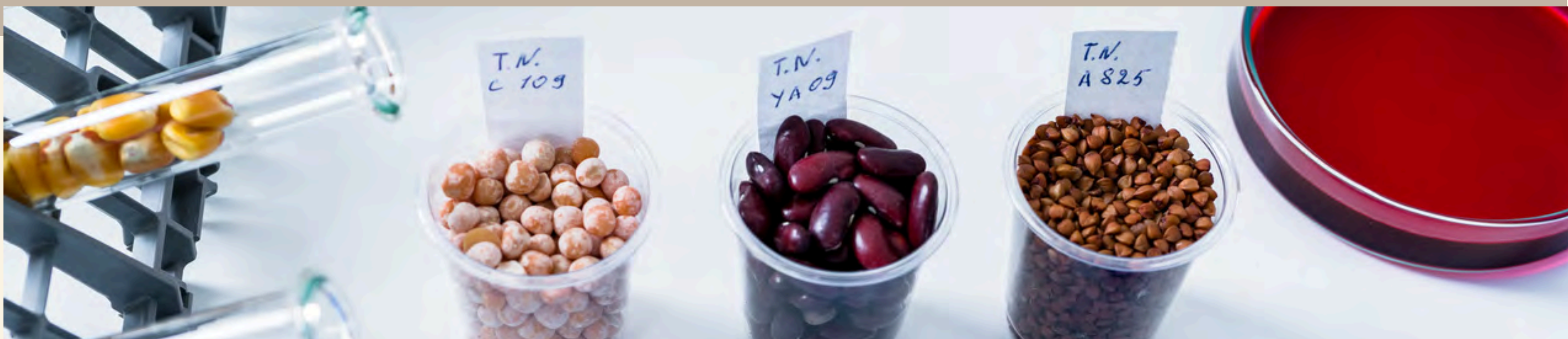
Unit 3.2 Analytical techniques used for identification and quantification of nutritional compounds

Carmen Constantin, Aurora Dobrin,
Andreea Barbu, Ioana Cătuneanu, Iliescu Lavinia,
Oana-Crina Bujor, Violeta Alexandra Ion,
Liliana Bădulescu

Source here

The science of food and nutritional analysis has come a long way, evolving from its humble beginnings in the nineteenth century to a multidisciplinary field that plays a central role in ensuring food quality, safety, and nutritional value. As consumers continue to emphasize the importance of understanding what they eat, the development and application of advanced analytical techniques remain integral to this ongoing journey of discovery and assurance in the realm of food science.

(Source: https://www.sciencedirect.com/science/article/pii/B0123693977001758)



3.2.1 General physico-chemical analysis

The main physico-chemical characteristics to be analysed for fruit and vegetables are: **texture**, **firmness**, the **specific mass**, the **specific heat**, the **degree of maturation.**

TEXTURE

The texture of horticultural products comes from the development and specialization of embryonic (primary) tissues as definitive tissues. The epidermis is a primary defense tissue, consisting of a layer of compact cells, which protects the leaves and fruits on the outside. In lettuce and cabbage, the cells are polyhedral, with chlorophyll. Apples, cherries and strawberries have anthocyanin pigments in the epidermis, carrots contain carotene, lycopene tomatoes, and cauliflower has no pigments in white varieties. The epidermis of apples or cabbage is covered by the cuticle, a thin layer of wax, which can be continuous, or in the form of granules, scales. Plums and grapes have plum-covered epidermis. Peaches and gooseberries have hairy epidermis. The leafy vegetables have stomata in the epidermis, and the fruits and stems, the lentils, which allow the exchange of gases and vapors with the outside.

> "The sampling procedure depends on type of analyzed food materials. Sometimes sampling procedure is presented by authorities in general regulations and food policies. In this case, type of sampling method is presented in detail. It is said that approx. 66% of errors in analysis comes from wrong sampling".
>
> Source: Deleanu, C., Nuclear Magnetic Resonance spectroscopy applications/Food in " Encyclopedia of Analytical Science (Second Edition), Elsevier, 2005, 303-315.

The rhizoderm (root epidermis) also has lentils. The suberum is a secondary defense tissue that consists of several layers of cells, with thickened walls (by suberification), which protect the outside of the potato tubers. Ghimbăşan Rodica et al., (1979), studied the formation of the suber in correlation with the resistance to mechanical damage of the potato. The thickness of the suber and the number of layers of cells differ depending on the variety and the area analyzed on the tuber. In the early stages, the suberification is uneven (the suberum is thicker at the base), but the differences disappear completely at maturity. In many species the suberification of the lentils causes their partial or total closure. The rhytidoma protects the outside of the neck.

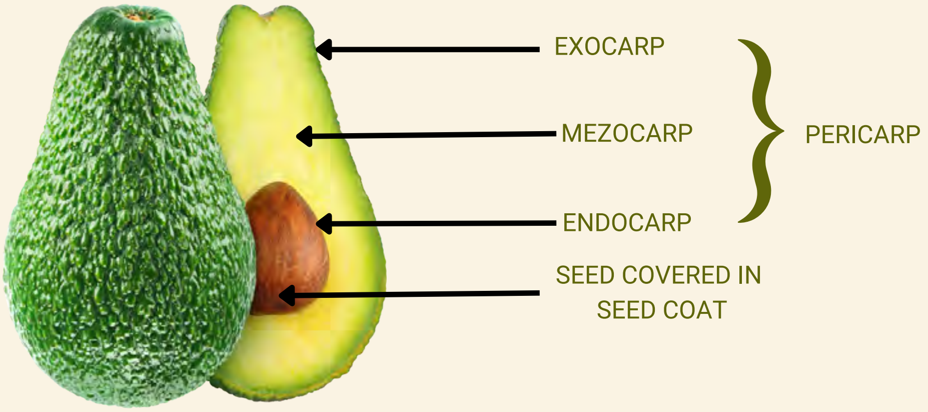
The affected products may form scar tissue under certain conditions. Potato tubers or some roots develop layers of suberified cells under the damaged cells with the role of defense and protection. After Burton, W.G. (1982), these tissues are formed under the action of natural bioactive substances (gibberellin and ethylene), which promote the formation of membranes and the synthesis of the necessary enzymes. The rate of suberification depends on the temperature, and its low values inhibit this process.

In some affected fruits, lignified areas appear (Burzo, I., 1986). Trophic (fundamental) tissues are characteristic of the edible part of fruits or vegetables. The cells have thin walls, with large intercellular spaces 27 (12-29% for roots and fruits, higher values for leaves). Salad, spinach and cabbage have assimilating parenchymal tissues, with a role in photosynthesis. The storage parenchyma tissues play a role in the accumulation of reserve substances: the medullary parenchyma in potatoes and the woody parenchyma in radishes store starch. The Liberian parenchyma of carrots stores starch, sucrose and carotene.

The cortical parenchyma of the gules stores starch and sucrose. The fundamental parenchyma of beans, hazelnuts and walnuts stores lipids, proteins and starch. The sclerenchyma is a supporting (mechanical) tissue made up of tightly bound cells with thickened walls by lignification. It is found in the form of elongated fibers in the hazelnut epicarp. In the form of sclerenchyma sclerosis, it forms the shell of drupaceous seeds, or the shell (mesocarp) of walnuts. Pear and quince sclereids have no supporting role. Secretory tissues that accumulate products of metabolism are found, for example in garlic or onions, made up of cells with thin walls, cellulose, which accumulate oxalates. Lettuce, elongated cells that secrete latex, are present in lettuce (*Lactuca spp*).

Glandular cells form in the citrus a spherical or oval group that delimits a space into which the secreted volatile (essential) oils flow. In *Umbelliferae* there are secretory channels that also contain volatile oils (dill, celery, larch, etc.). The fruit tissues are stratified into three areas: epicarp, mesocarp and endocarp. The epicarp exists as a thin layer of drupaceous or grape (covered with prunes or hairs, in some cases), is a thicker shell (melons, citrus), or a lignified shell (hazelnuts). The mesocarp or exocarp (outer shell) is thin in citrus, fleshy in apples and lignified in nuts. The endocarp (central tissue) is lignified in pips, parchments (seminal lodges) in seeds, or fleshy in citrus. The structure of a product is defined by the size or type of the component cells and their placement in the tissues. The texture of the same product appears and consists of the way of joining and associating the different tissues that make up the mass of plant organs. The degree of compactness or looseness of fruits or vegetables is determined by the shape, size or joint of the cells in the tissues and the complexity of the structure of the tissues in the organs.

AVOCADO
FRUIT
STRUCTURE



This complex combination of factors determines whether a vegetable product is loose (leafy vegetables, cabbage, apples) or compact (potatoes). Intercellular spaces, vacuolar spaces and the nature of tissues differ from product to product, evolving according to the degree of maturity, enzymatic activity or the degree of turgidity. An apple fruit in the early stages of maturity differs greatly in this respect from the same fruit or others at physiological maturity. The degree of firmness (after A. Gherghi) or the structotextural firmness (after I.F. Radu) results from the interdependence between texture and structure. When measured, it is associated with puncture resistance, specifically expressed.

Products with thin cell membranes, with less modified epidermal cells (in the case of cherries, sour cherries, plums, grapes, blueberries, currants, tomatoes, cucumbers), have a weak firmness at a time after harvest, but maintain their elasticity. Leafy vegetables, bean or pea pods, early potatoes, peppers, asparagus, evolve in a shorter or longer time to a state of wilting, more superficial (reversible) or deeper (irreversible). Lettuce varieties in protected crops have leaves with a much softer and more sensitive conformation than varieties in field crops, which have leaves with a semi-fine or coarse conformation. Asparagus etiolated shoots or some overly perishable leafy vegetables are prized for their tenderness at harvest.

The more perishable roots (root parsley, parsnip, root celery) have large cells with very thin cell walls and large intercellular spaces. Due to the large number of lentils on the surface of the roots, although they have a thicker rhizoderm, they are easily permeable to gases and easily lose water.

Dehydration reduces the resistance to attack by microorganisms and disorders occur in the respiration process, and the storage capacity is affected. The carrot actually makes the transition from one group to another, having a very thin rhizoderm, practically being difficult to establish the boundary between the cuticle and the parenchyma. Winter radishes and beets have a higher storage capacity than carrots due to their specific firmness.

The texture of potato tubers is characterized by Burtea, O. et al., (1980) by: structure (granularity, perceived during consumption); firmness (crushing, disintegration on the surface after boiling); consistency (flouriness, degree of crushing, by crushing after boiling). There are 4 types of potato varieties: A-for salad, B-for food, C-for puree, D-for industry.

FIRMNESS

The firmness is a guarantee of maintaining quality during handling, transport or marketing. Tomatoes, peaches, apricots, melons and other products that evolve after harvest are not of quality without the firmness required by the beneficiaries. The firmness of the fruit is determined by the enzymatic transformation of the insoluble protopectin in the tissues, which has a high degree of polymerization and numerous bonds between the molecular chains. Tissue softening is due to a whole series of hydrolysis and depolymerization reactions, characteristic depending on the species or variety. There are in-depth studies on the relationship of this evolution with the time of harvest, storage temperature, atmospheric composition, the presence of ethylene, the role of Ca2 + in tissues, etc. The firmness of some vegetable species is caused, in the last resort, also by the chemical composition: cellulose in the tissues, their lignification or suberification, and in potatoes the content in starch (amylose) and soluble pectins. In dynamics, other specific evolutions appear. During maturation, some species experience an increase in intracellular space, which contributes to increased loosening of tissues. The ratio between turgidity and elasticity, regardless of the species, also influences the firmness, determining the way of capitalization depending on the persistence of tissue hydration.

Knowing the firmness of some horticultural products is important for determining the time or manner of harvesting, packaging and transport, as well as the shelf life. The empirical assessment, by simple pressing, has been replaced in laboratories with various instruments or apparatus, which allow a precise determination. When we have to characterize a newly created variety or when the issue of export is raised, these devices prove to be very useful for the correct determination, in conditions of repeatability, of the degree of maturation at a given moment. However, there are two constructive variants: the portable version for fruits and the laboratory version, with stand and automatic operation. The texturometers have a pneumatic chamber, equipped with a piston. A rack and crank system allows increasing pressure to be exerted by means of a piston, finished at the bottom with several punches, 30 which come into contact with the product. A hydraulic pressure gauge signals the maximum breakthrough pressure. The Christel texturometer is a better-known device. Maturometers have a construction principle similar to texturometers.

For more informations, please visit:

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3.2.2 Physical properties of horticultural products

THE SPECIFIC MASS

The specific mass of a horticultural product is defined as the ratio of its mass to its volume, or the mass of a unit volume of that product (g/cm3). The specific mass is determined for each product by the specific mass of the components: air = 0.0012; water = 1.0; cellulose = 1,265; glucose = 1.56; fructose = 1,669; citric acid = 1,542; malic acid = 1,601; tartaric acid = 1,759 and others. Products with large gaps have a minimum specific mass: peppers (0.53) and apples (0.650). Compact products rich in compounds with high specific mass are characterized by high values: blackberries (1.2545), peaches (1.1875), potatoes (1.128). We specify that these are average values.

Horticultural products can be classified into three major groups, from this point of view:

- with a small specific meal: peppers, lettuce, mushrooms, okra, cabbage, eggplant, apples;
- with a medium specific mass, but lighter than water are: peas, melons, onions, parsley, quinces, strawberries;
- heavier than water (density greater than 1): potatoes, pod beans, carrots, cherries, sour cherries, blackberries, raspberries, currants, plums, grapes, pears.

The specific mass of small fruits is higher than those of large ones, being more compact, more resistant to handling and transport, better keeping their turgidity until the end of storage. The volumetric mass of a horticultural product represents the mass (kg) of the products occupying the volume of 1 m3 (kg/m3). It is a property whose knowledge is very important for those who pack or store the products. The oscillation limits are between vegetables between 120 kg/m3 (spinach) and 1000 kg/m3 (tomatoes), and for fruits between 400 kg/m3 (apples) and 620 kg/m3 (cherries). Voluminous products with a low specific mass also have a small volumetric mass.

THE SPECIFIC HEAT

The specific heat is the amount of heat required, at constant volume, to raise the temperature of one unit mass (kg) of product by one degree Celsius. It is a thermophysical constant of great importance in the storage process, expressing itself (correctly) in J/Kg°K. Other units: Kcal/kg°C, or Kwh/kg°C. The specific heat of horticultural products is equal to the weighted average specific heat of the constituent substances. Glucose and fructose have 1.47 Kj/kg°K, starch = 1.38; proteins = 1.55; lipids = 1.76, and water 4.1868 kj/kg°K.

There are also estimates of the calculation of specific heat, starting from the dry matter (SU) and moisture (U) content of a product.

$$C = (U + 0.3\ SU)\ Kcal/kg°C$$
or
$$C = (U + 0.3\ SU) \times 4.1868\ Kj/kg°K$$

Specific heat expressed in kj/kgK: a) for vegetables varies between 3.14 (garlic) and 4.07 (greenhouse cucumbers; b) for fruit, between 1.6 (nuts) and 3.85/4.02 (strawberries).

The characteristic values of each product are found in the tables, used in the calculation of the required cold (Qrp). Qrp = T G · c · dt G = mass of the product in kg; dt = temperature difference (t2-t1); T = cooling time, (h); (1 kj/kgK = 0.38885 kcal/kgK).

THERMAL CONDUCTIVITY (Λ)

Thermal conductivity (λ) is a thermophysical constant characteristic of each product, used in various calculations of design or operation of warehouses, in refrigeration or freezing. Heat conduction and heat exchange are quite difficult for horticultural products, which are poorly conductive to heat.

However, they heat up during the day and cool down at night, resisting the reception or release of heat, conditioned by their water and air content. The units of measurement are: J/m h 0K = W/m 0K or Kcal/m h °C.

In vegetables, λ ranges from a maximum of 0.99-1.86 W/m0K (cabbage) to a minimum of 0.5-0.62 W/m°K (potatoes, carrots, beets, squash). In fruit, λ is between 0.54-1.35 W/m°K (0.67 strawberries) and 0.24-0.33 W/m K (plums, gooseberries).

Thermal conductivity is influenced by the texture, structure and moisture content of tissues. Water-rich products have a high λ. The looser a product is, or the larger the gap space, the lower its thermal conductivity. Fruits with thin epicarp (peel) have lower values of this constant than those with thick peel, and peeled ones have 2 times lower values than those with peel.

Freezing temperature (in technical terms, freezing point) is the thermal threshold (t°C) at which the free water from horticultural products passes into a solid state. The water in the cells contains dissolved mineral and organic substances, so this thermal value is always below 0°C. This is obvious if we notice that the salad freezes at -0.3°C (higher temperature, low dry matter content), and the nuts at -6.7°C, -6.9°C (inverse relationship).

Horticultural products are grouped into five classes of differentiated **frost resistance**:

- particularly sensitive to frost: Witloof endives, cucumbers, melons, spinach, lettuce, tomatoes, cabbage, eggplant and fruit currants, being affected at relatively low negative temperatures (-0.3°C and -1.0°C);
- most sensitive to low temperatures (-1.0°C/-2.0°C) are most products: peppers, potatoes, onions, mushrooms, cauliflower, cauliflower, garden peas, carrots, parsley, parsnips, asparagus, celery,strawberries, blackberries, peaches, raspberries;
- sensitive to low temperatures (-2.0°C/-3.0°C): radishes, cherries, apples, pears, cherries, plums, early grape varieties;
- slightly sensitive to low temperatures (-3.0°C/-4.0°C): garlic, most grapes, some varieties of plums or sweeter cherries;
- relatively resistant to low temperatures (-4.0°C/-7.0°C): nuts, almonds, chestnuts, hazelnuts, grape varieties richer in sugars.

The refrigerated storage temperature must be set outside the freezing temperature, and the harvesting period will take into account the danger of frost.

The factors influencing the freezing temperature are: the degree of maturation, the belonging to a certain species or a certain variety, as well as the duration of exposure to low temperatures.

THE DEGREE OF MATURATION

The degree of maturation, correlated with the **soluble dry matter content**, is proportional to the freezing temperature, in a certain species and in a certain variety. The higher the soluble dry matter content, the higher the freezing temperature (in absolute terms). The variety factor acts identically. In grapes, the freezing temperature is lower in the early Cardinal variety (-2.6°C) and much higher in absolute terms in the Muscat Hamburg variety (-4.6°C). In apples, the award-winning Jonathan or Wagener varieties are much more sensitive to low temperatures than Red Delicious, Golden Delicious and others, which are richer in carbohydrates.

The species factor differentiates the species from each other, even at the same soluble dry matter content. Plums freeze easier than cherries, even at significantly higher carbohydrate content.

The duration of exposure to low temperatures is a very important factor. Short-term exposure causes extracellular frost, more or less reversible. Prolonged exposure causes frost, both extracellular and especially intracellular, and therefore irreversible, because ice needles irreparably damage the cell. The lightly frozen salad (-0.3°C/-0.5°C) withstands temperatures of up to -2°C in the short term (frost, frost, frost). Although the leaves freeze, after thawing they largely return to their original appearance, except for small portions that have a wrinkled surface. At -4°C, the destruction of the salad is irreversible, especially when kept for more than 24 hours, and the head obviously loses its commercial appearance. Potatoes have a freezing point between -0.8°C and -1.5°C. After 8 hours of exposure to temperatures of -4°C/-5°C, the tubers still do not show external symptoms of impairment, and internal symptoms appear in a small number (8%). After 24 hours at the same temperature, an exudate appears on the potatoes, and the tubers affected by the low temperatures exceed one third. After 3 days of exposure, the depreciated tubers reach 75-80%. Freezing at -8°C irreparably destroys the potatoes, especially if they occur slowly. Cell walls are broken, water is lost. Through slow thawing, the consistency of the tubers remains floury. Rapid thawing causes irreparable destruction, the potatoes turn black and turn into a damp mass.

The intercellular spaces are small, the frost is intracellular. Onions freeze between -1°C and -1.7°C. At temperatures down to -8°C there are no signs of degradation, especially if thawing occurs slowly. Carrots are also more resistant to low temperatures. Although the freezing point is between -1.4°C and -2.2°C, at negative temperatures down to -8°C they also show no signs of degradation. Due to the larger intercellular spaces, the frost affects the inside of the cells less, but after thawing, some radial cavities are formed inside. After Niculiţă, P. et al. (1986), as a result of the lowering of the temperature, the first phase of the transition of free water from the extracellular spaces takes place in the first phase. The water in the cells, cooled, with a higher vapor pressure than the ice formed on the outside, migrates through the cell walls, causing the growth of the crystals already formed. The process continues as long as the intracellular water remains liquid and the vapor pressure remains higher than that of the outer ice. The slower this process, the more intense the transport of intracellular water to the outside of the cells takes place, at temperatures well below freezing.

3.2.1.2 Other physical properties (bioelectric, mechanical, shape, size)

THE BIOELECTRICAL PROPERTIES, ELECTRICAL CONDUCTIVITY AND ELECTRICAL IMPEDANCE

The bioelectrical properties, electrical conductivity and electrical impedance of the tissues of horticultural products are due to the ions of mineral salts or acid radicals in the cells, which fulfill the role of an electrolyte. Crossing the membranes, they are oriented according to the electric charge, towards the two electrodes between which there is a potential difference. Determinations are made with different types of conductometers. The essential condition is to perform them at a constant temperature. The units of measurement used for conductivity are **μ S** (microsiemens) or **mV** (millivolts), and for electrical impedance ohms. Factors that influence membrane permeability also influence the electrical conductivity of tissues. Values increase in ripe fruits and those that tend to come out of vegetative rest. Freezing, cryptogamic diseases, shocks or pressures also cause these values to increase as a result of damage to cellular integrity. The practical importance of such determinations is obvious, but they are performed only in special cases, in research or expertise laboratories for the operative detection of hidden defects.

PUNCTURE RESISTANCE

Puncture resistance is the physical expression of the structo-textural firmness measured in N/cm2 or kgf cm2. The shape and size of the products, studied in the description of the varieties; have a useful application to fruit, namely the appreciation of the degree of ripening of apples based on stage T. Stage T corresponds to the moment when the peduncle forms a T with the base of the young fruit (null cavity). The graphic method allows the establishment of the day (calendar date) in which the fruit has reached this stage, by subsequent measurements of the depth of the peduncular cavity and their transposition on graph paper. Where the hypotenuse of the formed right triangle intersects the horizontal axis (large chain) is the stage T marked with 0 mm. Knowing the number of days between the two measurements (2 mm-4 mm), one can approximate to the left the date when stage T appeared.

WATER AND DRY MATTER CONTENT OF HORTICULTURAL PRODUCTS.

Both water and dry matter are the result of a gradual accumulation, each product having a specific composition.

THE DRY MATTER

The dry matter in horticultural products contains organic substances (over 95%), but also a small amount of minerals. Total dry matter (%) obtained by evaporation of total moisture, in laboratory conditions, in an oven or by distillation - entrainment. The soluble dry matter, expressed as a percentage by mass, is determined refractometrically and is equivalent to a solution of sucrose, which has the same refractive index as the extract or juice obtained for analysis, from a particular product.

THE WATER CONTENT

The water content of horticultural products is the environment for biochemical reactions, participates in the circulation of solubilized substances, contributes to regulating plant temperature, maintaining turgidity, the proper development of growth and development. Water-rich products have high specific heat and more intense metabolic activity, being much more perishable. The forms in which water is found in products, free water and bound water are retained in a special way in tissues, consequently having different functions or properties.

FREE WATER

Free water is found in vacuoles, containing dissolved various substances (carbohydrates, salts, organic acids, etc.). It is retained mechanically or by capillarity. By pressing, centrifugation or evaporation, it can be separated or removed relatively easily.

BOUND WATER

Bound water is represented by four inactive forms: colloidal water of swelling, colloidal water of absorption, water of crystallization and water of constitution (L. Rey, cit. By I.F. Radu, 1985).

BOUND WATER

Colloidal water freezes at much lower temperatures than free water, it can only be partially extracted by fragmenting the product and keeping it at 105°C for 8-12 hours. The colloidal swelling water is osmotically bound to the colloidal particles.

THE COLLOIDAL ADSORPTION WATER

The colloidal adsorption water (hydration water) is molecularly retained on the surface of the colloidal particles and can be partially removed by cryodehydration.

CRYSTALLIZATION WATER

Crystallization water is required for some constituents of horticultural products to crystallize in a particular system.

THE CONSTITUENT WATER

The constituent water represents the amount of hydrogen and oxygen in a ratio of 2:1 in the constituent molecules of the products. The latter two forms may be separated or extracted only by methods which cause destruction of the structure or constituents of the product. In addition to the forms of water inside the products, the external humidity (water) must also be mentioned. Regardless of the internal content, it is an important indicator of the appreciation of storage capacity (eg onions).

TOTAL MOISTURE

Total moisture is the sum of free and bound water (especially colloidal swelling water), which can be removed without compromising the value of the product. Together with the total dry matter, their amount is 100%. The total moisture content varies widely, depending on the nature of the product and its degree of maturation.

For vegetables, the maximum content is 90-97% (cucumbers) and the minimum values are 72-78% (garden peas).

THE CONSTITUENT WATER

In fruit, strawberries are 84-93%, while nuts are only 6-8%. In the same product, the total moisture content differs depending on the fabric. In apples, the pulp contains the largest amount of water (86-87%), and the seeds only 45%. In parsley, the leaves contain 80% water, the roots 85%, and the seeds 9%.

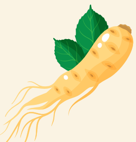
Horticultural products in our country can be grouped, depending on their **content in total humidity**, in **5 groups**.

- The first group includes nuts, which have less than 10% total moisture and chestnuts (**47-53%**).
- The second group (**70-80%**) includes potatoes, green peas, parsnips, grapes, plums.
- The third group (**80-85%**) contains onions, parsley, melons, zucchini, leeks and most fruits.
- The fourth group (**85-90%**) consists of peppers, cauliflower, green beans, kale, carrots, radishes, beets, celery, cabbage, strawberries and peaches.
- In the fifth group, the products have the maximum total moisture content (**over 90%**), including only vegetables: cucumbers, mushrooms, melons, lettuce, spinach, asparagus, tomatoes and eggplant.

Viewed in dynamics, the percentage of total water decreases during maturation, due to the accumulation of dry matter.

After harvesting, the moisture content of the products begins to decrease. Moisture loss occurs not only through perspiration but also through evaporation, depending on the structure and texture of the product. Perishable products, with a high percentage of water, without protective suberified tissues, register high moisture losses in only 5-6 days after harvest, even at lower temperatures.

Leafy vegetables, the fruits of fruit bushes, are specifically valued, being very sensitive.



Products such as roots, potatoes, apples lose 6-8% of their total moisture in 6 months of storage, depending on the species, variety or storage technology.

Nuts can absorb moisture from the atmosphere if they have been excessively dehydrated (less than 6%) or when the relative humidity in the store exceeds 70-75%.

The soluble dry matter consists of soluble carbohydrates and soluble non-carbohydrate substances which together influence the refractive index of the aqueous extract, obtained under laboratory conditions from a particular horticultural product.

THE SOLUBLE DRY

The soluble dry matter is determined refractometrically and is expressed in **g% S.U.S**. (or refractometric degrees). The identification between the soluble dry matter and the carbohydrate content is incorrect. However, there are calculation formulas and equivalence tables, which differ depending on the species, but the results (estimates, approximations) are often higher than the actual values. The most commonly used equivalence tables were for grapes and apples. In the sugar industry, Brix grades are used to express the same values, and in many specialized technical works in the West, except Germany, Brix grades are used, in the sense of U.S. grams%.

THE NON-FERMENTABLE SOLUBLE SUBSTANCES

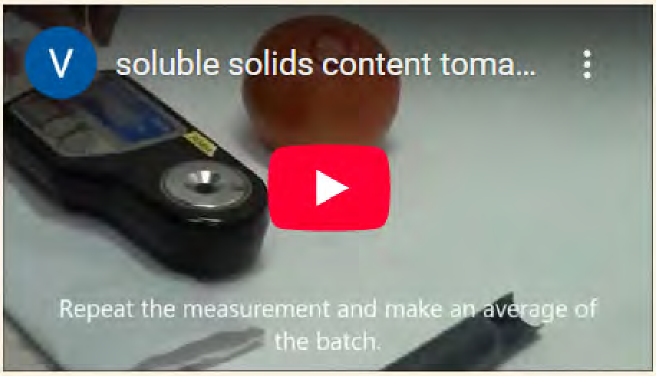
The non-fermentable soluble substances (in technical language "non-sugar") in fruit musts are numerous. In kernels, sorbitol and organic acids are the main representatives. In a filtered cherry must (concrete case), having 14.3% soluble dry matter, the non-fermentable extract was 65 g/l: 40 g/l sorbitol, 10 g/l organic acids, 4 g/l glycerin, 3 g/l protein, 3 g/l galacturonic acid, 3 g/l tannins. The total organic non-fermentable substances is 63 g/l to which are added 2 g/l soluble mineral substances.

THE TITRATABLE ACIDITY

The titratable acidity of the horticultural products is determined by the direct neutralization (titration) of the extract obtained volumetrically from a certain product, with sodium hydroxide (n/10). The titratable acidity is caused by the presence of dissociated free acids, acid salts as well as other compounds with acid reaction (phenols, phosphoric acid and even ascorbic acid to a much lesser extent). It is expressed in grams or milliequivalents of the acid predominant in the product.

1. Malic acid for apples, pears, apricots, cherries, quinces, peaches, plums, cherries, broccoli, carrots, potatoes, resale;
2. Citric acid for strawberries, currants, blueberries, apples, raspberries, beans, tomatoes;
3. Tartaric acid for grapes, etc.

For more information, please visit:



Repeat the measurement and make an average of the batch.

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3.2.2 Spectrophotometry (UV-VIS, FT-IR)

Ultraviolet and visible light range (UV/VIS) is widely applied in research, production and quality control for the classification and study of substances. Many molecules contain chromophores which will absorb specific wavelengths of ultra violet or visible light. In other words, UV/VIS spectroscopy is based on the absorption of light by a sample. Also, the technique is useful in the identification of pure drug compounds, in forensic (NicDaéid, 2019).

Depending of the amount of light absorbed by the sample, important information can be obtained like purity of the sample in spectra and their quantification depending on the amount of sample. Due to simplicity and reliability (Leder and Porcu, 2018), this technique has already been used in many research areas. According to Haque (2021), specific applications of UV–VIS are related to various different food matrices (e.g., meat, milk, coffee, wine, vegetables, fruits, drinks, and olive oil) with respect to food composition, authentication, adulteration, and quality).

UV-vis spectroscopy is one of the most popular analytical techniques because it is an inexpensive, simple, flexible, non-destructive, analytical method appropriate for a wide class of organic compounds and some inorganic species.
UV-vis spectrophotometers measure the absorbance or transmittance of light passing through a medium as a function of the wavelength
(Rocha et al., 2018).

The UV/VIS spectrophotometer measures the intensity of light passing through a sample solution in a cuvette, and compares it to the intensity of the light before it passes through the sample. The main components of a UV/VIS spectrophotometer are a light source, a sample holder, a dispersive device to separate the different wavelengths of the light and a suitable detector (figure 1).

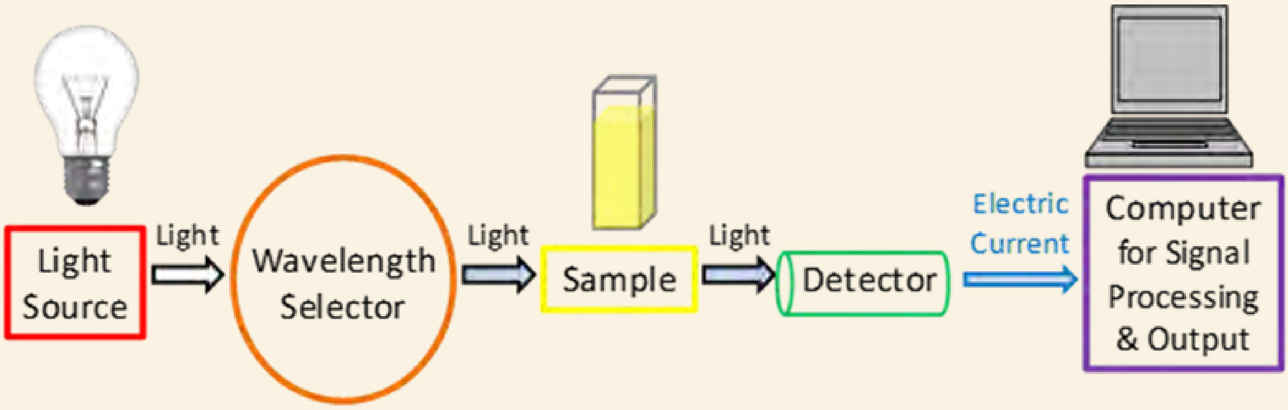

Figure 1. A simplified schematic of the main components in a UV-Vis spectrophotometer
Source: https://www.technologynetworks.com/

Light has a certain amount of energy which is inversely proportional to its wavelength. Humans are able to see a spectrum of visible light, from approximately 380 nm, which we see as violet, to 780 nm, which we see as red. UV light has wavelengths shorter than that of visible light to approximately 100 nm. Therefore, light can be described by its wavelength, which can be useful in UV-VIS spectroscopy to analyze or identify different substances by locating the specific wavelengths corresponding to maximum absorbance.

UV-Vis can be used in a qualitative manner, to identify functional groups or confirm the identity of a compound by matching the absorbance spectrum.

It can also be used in a quantitative manner, as concentration of the analyte is related to the absorbance using Beer's Law ($A=\varepsilon bC$ where ε is the molar attenuation coefficient, b is path length, and C is concentration). The UV extends from 100–400 nm and the visible spectrum from 400–700 nm (www.jove.com).

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3.2.2.1 Total phenolic content

Plants are a natural source of bioactive compounds like secondary metabolites and antioxidants. One of the most abundant secondary metabolites in plants are phenolic compounds.

Playing a key role in pigmentation, growth and reproduction of the plant, attractants for pollinators, together with resistance to pathogens and predators (Noreen ae al., 2017), phenolic compounds gained interest because of their perceived health-beneficial effects (Blainski et al., 2013). Regarding their impact on human health, they have been reported to exhibit anti-carcinogenic, anti-atherogenic, antiulcer, anti-thrombotic, anti-inflammatory, immunomodulating, antimicrobial, vasodilatory and analgesic effects. The beneficial effects on human health may be due to their free radical scavenger properties, blocking the reactive oxygen species. According to Blainski et al. (2013), phenolics include simple phenols, phenolic acids (benzoic and cinnamic acid derivatives), coumarins, flavonoids, stilbenes, hydrolyzable and condensed tannins, lignans, and lignins, being the most widely occurring secondary metabolites in the plant kingdom.

They occur in a variety of fruits, vegetables, nuts, seeds, flowers, bark, beverages, and even some manufactured food, as a natural ingredient. Therefore, their quantification is a common practice for selecting genotypes, maturity stages, storage and processing conditions (Sanchez-Rangel et al., 2013).

The **Folin-Ciocalteu assay** is the most commonly used procedure to determine TPC of food extracts. The Folin-Ciocalteu is a colorimetric method is an electron transfer-based assay the between Folin-Ciocalteu reagent and phenolic content, and gives reducing capacity which is expressed as phenolic content (Noreen H. et al, 2017). Total phenolic content of plant extracts and their yield depends on the extraction procedure. The determination of the total phenolic content was performed according to a spectrophotometric method adapted according to Folin – Ciocâlteu (Singleton V.L. et al., 1999).

Reagents:

- 20% Na2CO3 solution;
- Folin-Ciocâlteu reagent.

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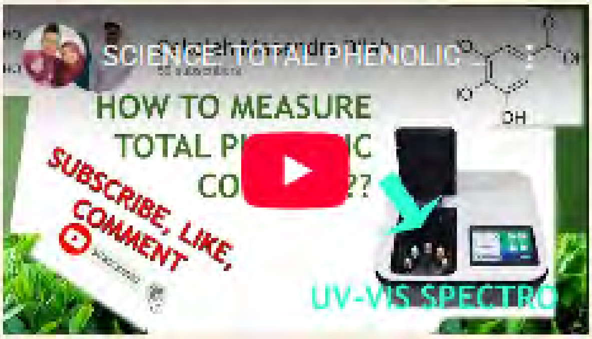
For more information, please visit:



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3.2.2.2 Total flavonoid content

Generally, the phytochemicals are divided into two major categories, firstly primary metabolites (carbohydrates, lipids, proteins), and secondly, secondary metabolites (alkaloids, terpenoids, and phenolic compounds). The primary metabolites are responsible for the growth and development of plants. The secondary metabolites play an important role in defense mechanisms against the environmental pollutants (Patle et al., 2020). Among these **flavonoids** are considered to be a very important class of biomolecules having a significant medicinal property for the human being, protecting the body from damage caused by oxidizing agents.

Flavonoids are one of the largest (> 6000 identified) and most distributed groups of secondary plant metabolites and are found in practically all photosynthesizing plants. Flavonoids are polyphenolic compounds showing a common basic C6-C3-C6 skeleton structure consisting of a heterocyclic pyran or pyrone ring flanked by a benzene ring on each side (Guang et al., 2013). Most flavonoids are present in plants as esters, glycosides, or polymers. The chemical structure of flavonoids determines the absorption range (Sánchez-Gavilán et al., 2021).

The basic structure of flavonoids was presented. Flavonoids with 3',4'-dihydroxy-substituted structure can show special color by reacting with the system of NaNO2–Al(NO3)3–NaOH. The color reaction of flavonoids and chromogenic system is presented. The method is based on the reaction of aluminum ion with flavonoid at alkaline medium forming red chelates. By measuring the absorption of such red chelates, it is possible to determine the flavonoids. (Zu et al., 2010).

The determination of the total flavonoid content may be performed according to a spectrophotometric method adapted after Zhishen et al. (1999).

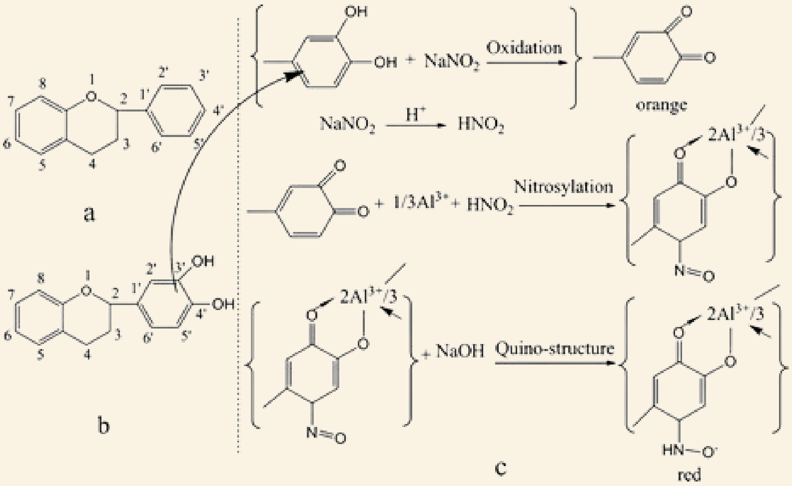
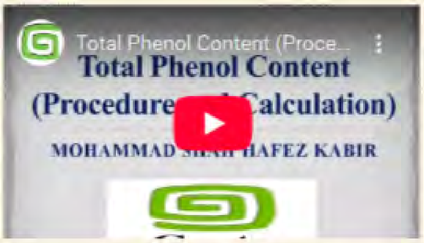


Figure 2. The color reaction of flavonoids and chromogenic system

For more information related to UV/VIS analysis and principle, please visit:



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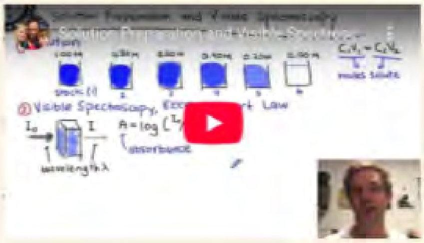

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3.2.2.3 Total monomeric anthocyanin pigment content

Anthocyanins are naturally occurring pigments in all plant tissues throughout the plant kingdom, being present widespread in nature and responsible for red, purple, or blue colors in different organs of plant such as stem, leaf, flower, fruit, root, and tubers.

The anthocyanins are all based chemically on a single aromatic structure, and all are derived from this compound by the addition or subtraction of hydroxyl groups and by the number of sugars and their position on the aglycon. Because of their specific chemical structure, they are described by an electron deficiency, and make extremely reactive towards ROS (reactive oxygen species)(Pervaiz T, et al., 2017; Hurst , 2002).

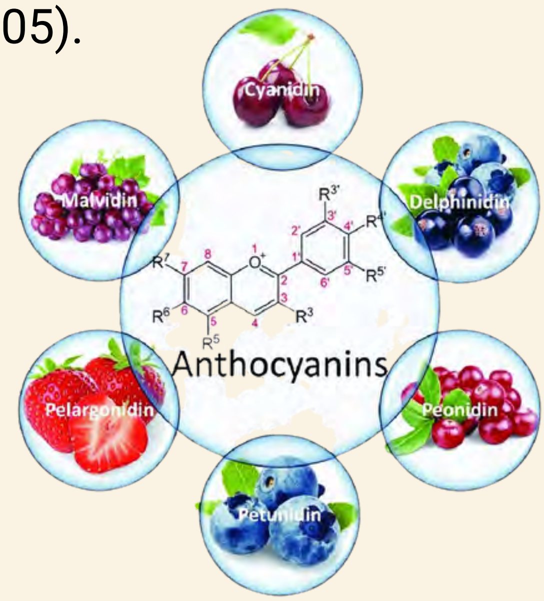
The center of the anthocyanidin, the flavylium, has the specifical flavonoid structure, surrounded by one fused aromatic ring (A ring) one heterocyclic benzopyran ring (C ring), and one phenyl component (Pervaiz T, et al., 2017).

The majority of the anthocyanins occur as monoglycosides and diglycosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin (Pervaiz T, et al., 2017; Lee et al., 2005).

A spectrophotometric method used for the determination of total monomeric anthocyanin content, based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5 it is the pH differential method.

Monomeric anthocyanin pigments reversibly change color with a change in pH; The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration. Results are expressed on a cyanidin-3-glucoside basis. Degraded anthocyanins in the polymeric form are resistant to color change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0 (Giusti et al., 2001, AOAC. 2005).

Absorbance should be measured at the λmax of the pigment solution, and the pigment content should be calculated by using the molecular weight (MW) and molar extinction coefficient of the major anthocyanin in the matrix (Lee et al., 2005).



Source: Cosme, F., Gonçalves, B., Bacelar, E., Inês, A., Jordão, A , Vilela, A. (2017). Genotype, environment and management practices on red/ dark-colored fruits phenolic composition and its impact on sensory attributes and potential health benefits. 10.5772/66881.

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3.2.2.4 Total carotene and lycopene content

The carotenoid composition of foods is influenced by factors such as cultivar or variety, part of the plant consumed, stage of maturity, climate or geographic site of production, handling during harvesting and postharvest, processing and storage (Dias et all.,2021; Meléndez-Martínez et al., 2021). In order to identification of carotenoids, the ultraviolet and visible spectrum can be consider the first diagnostic tool.

The unsaturated **acyclic carotenoid lycopene**, have 11 conjugated double bonds, the color it is red and absorbs at the following wavelengths: 444, 470, and 502 nm in petroleum ether. For a carotenoid to have a perceptible color it has to have at least 7 conjugated double bonds are needed (carotene color is light yellow). ζ-Carotene being acyclic, its spectrum has three well-defined peaks and wavelengths at: 378, 400, and 425 nm in hexane/petroleum ether, wavelengths much lower than those of lycopene (Rodriguez-Amaya & Kimura, 2004)

Phytoene (3 conjugated double bonds) and **phytofluene** (5 conjugated double bonds), are colorless and absorb maximally at 276, 286, and 297 nm and at 331, 348, and 367 nm, respectively. They precede ζ-carotene in the desaturation biosynthetic pathway.

Neurosporene has the structure intermediate between ζ carotene and lycopene (9 conjugated double bonds), exhibits maximum absorption at 414, 439, and 467 nm, in petroleum ether (Rodriguez-Amaya & Kimura, 2004, Nollet et al., 2012)

β-carotene, has the same number of conjugated double bonds as lycopene, its color is yellow orange and has λmax at 450 and 477 nm and 425 nm in hexane and petroleum ether (Figure3).
Phytoene (3 conjugated double bonds) and **phytofluene** (5 conjugated double bonds), are colorless and absorb maximally at 276, 286, and 297 nm and at 331, 348, and 367 nm, respectively. They precede ζ-carotene in the desaturation biosynthetic pathway.

Figure 3. β-carotene- ultraviolet and visible light absorption spectra in hexane and ethanol

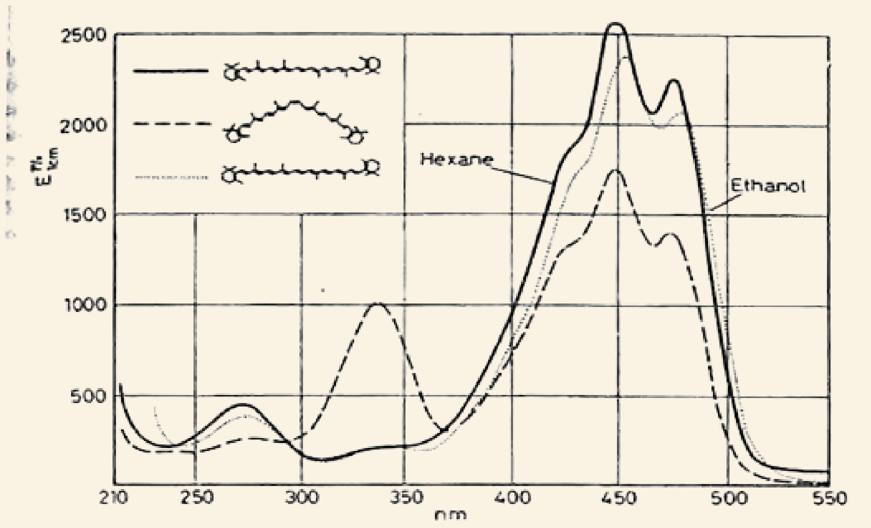

Figure 11. Ultraviolet and visible light absorption spectra of β-carotene (——— in hexane, · · · in ethanol) and 15,15′-*cis*-β-carotene (— — — —) (in hexane).

Source: http://lipidbank.jp/cgi-bin/detail.cgi?id=VCA0001

α- carotene is light yellow and its absorption spectrum have λmax at : 422, 445, and 473 nm.

The spectra of lutein, zeinoxanthin, and α-cryptoxanthin resemble that of α-carotene, and those of β-cryptoxanthin and zeaxanthin are identical to that of β-carotene (Rodriguez-Amaya, 2015).

Special precautions in carotenoid analysis

The instability of carotenoids during processes of extraction, handling, and elimination of organic solvents makes the preparation of a sample for analysis an extremely delicate task. Exposure to light, especially direct sunlight or UV light, induces trans-cis photoisomerization and photodestruction of carotenoids. Thus, carotenoid work must be done under subdued light. Open columns and vessels containing carotenoids should be wrapped with aluminium foil (Meléndez-Martínez et al., 2021, Rodriguez-Amaya, 2015).

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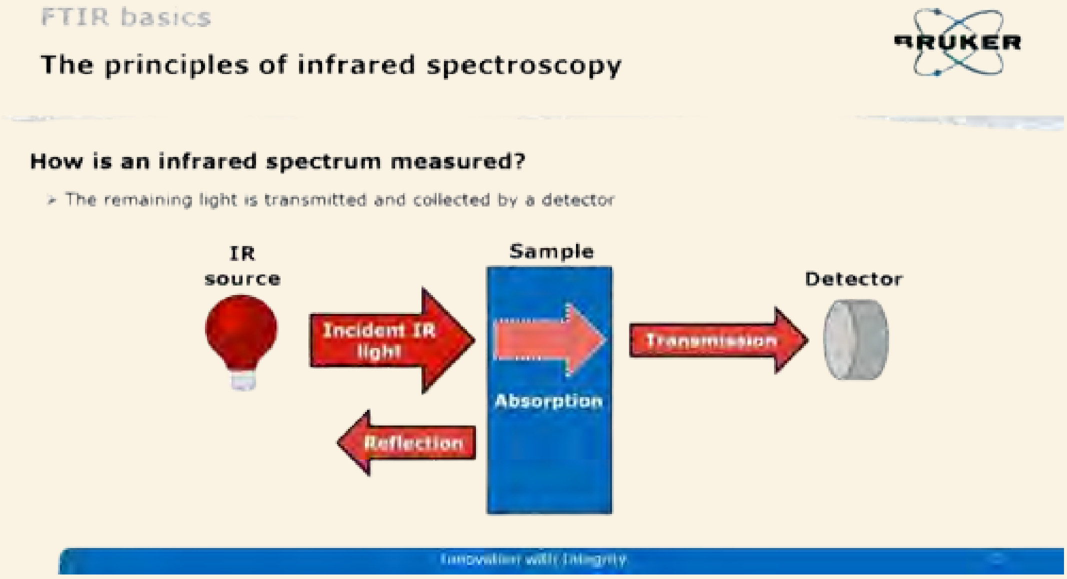
3.2.2.5

FT-IR

The principle of the Infrared spectroscopy (IR) is that most molecules absorb light in the infrared region of the electromagnetic spectrum, converting it to molecular vibration. Infrared spectrum is molecular vibrational spectrum. The absorption is characteristic of the nature of the chemical bonds present in the sample (bruker.com).

Figure 4. The principles of IR



FTIR basics

The principles of infrared spectroscopy

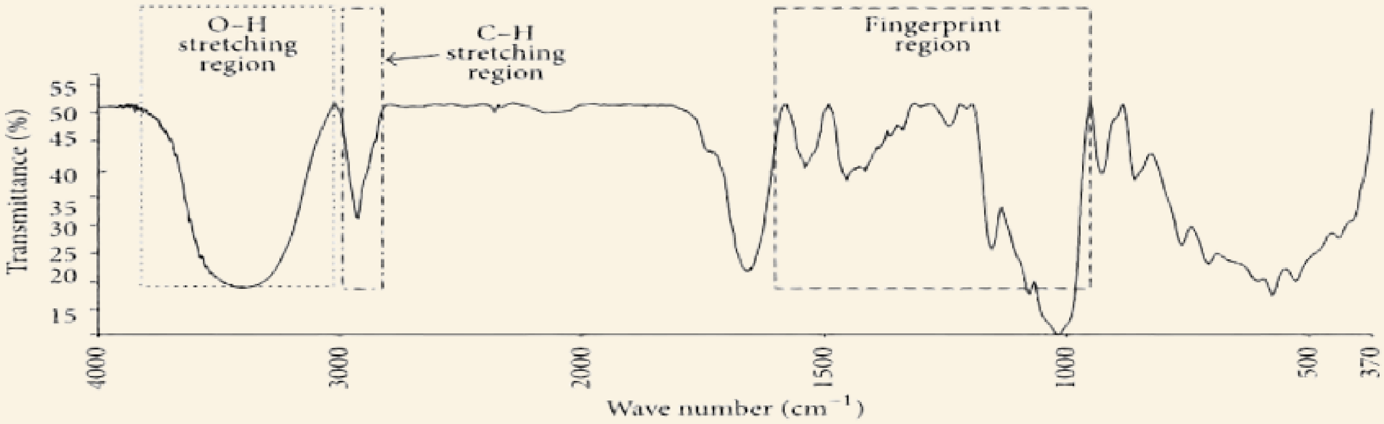
How is an infrared spectrum measured?

- The remaining light is transmitted and collected by a detector

Source: https://www.bruker.com/en/products-and-solutions/infrared-and-raman/ft-ir-routine-spectrometer/what-is-ft-ir-spectroscopy.html

The specific absorption range for infrared absorption spectroscopy is 600 – 4000 cm-1 because the absorption radiation of most organic compounds and inorganic ions is within this region (chem.libretexts.org). The result is an IR spectrum that serves as a characteristic "molecular fingerprint" that can be used to identify organic and inorganic samples (bruker.com).

Figure 5. Spectrum of spelt flour type



Source: Sujka K.et al.,2017

FT-IR instruments collect data from all wavenumbers in one pass, and then translate the resulting interference pattern into an IR spectrum. The digital processing capabilities of FT-IR instruments enabled new chemometric techniques for qualitative and quantitative analysis.

Because all of the frequencies are measured simultaneously, most measurements by FT-IR are made in a matter of seconds.
FT-IR systems are very flexible, extremely accurate and reproducible, designed for routine applications, in quality control, quality assurance application and method development being also a very important tool in food fraud and adulteration detection (thermonicolet.com, MPA User Manual, 2011).

FT-MIR and FT-NIR can analyze multiple parameters simultaneously. The IR methods are safer and more environmentally friendly than wet-chemistry or chromatographic methods because no hazardous chemicals or solvents are used. In most cases, no sample preparation is required (INFORM 2016).

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MPA User Manual (2011), Bruker Optik.
https://foodsafetytech.com/feature_article/food-fraud-and-adulteration-detection-using-ftir-spectroscopy/

3.2.3 Chromatography (GC, LC)

Violeta - Alexandra Ion,
Oana - Crina Bujor

Chromatography is widely used separation technique in quality assessment of horticultural products for the qualitative and quantitative analysis of components in both raw and processed products. Specifically, chromatography is a physical separation method in which the components of a mixture are partitioned between two phases.

One of the phases called the stationary phase is immobile being in the form of a porous bed, bulk liquid, layer or film, whereas the other phase, named mobile phase (eluent), is a fluid that percolates through or over the stationary phase. The separation results from repeated sorption/desorption process during the movement of the sample components along the stationary phase in the general direction of mobile phase (Poole, 2000).

The figure 1 shows the schematic representation of separation of compounds from a sample. The different components in the sample mixture are transported through the column by the flow of the mobile phase and reach zones where new equilibria are formed again and again (Meyer, 2006).

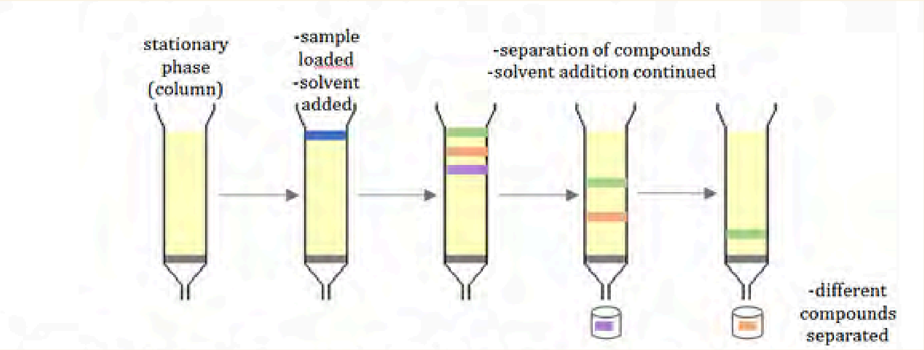


Figure 1 - Schematic representation of a column chromatographic separation

Given the large difference in the properties of the molecules, the separation can easily be made. The compounds are separated as a function of **time (or volume of mobile phase)**. Immediately after leaving the column, the compounds are identified by a physico-chemical analyzer, called a **detector**, capable to giving a signal proportional to the mass or the concentration of the component solution in the mobile phase (Naşcu and Jäntschi L., 2006).

The graphic representation of the detector signal as a function of time is called a **chromatogram (figure 2)**. The y-axis is the response of the detector and the x-axis is the time or the volume of mobile phase.

The eluted compounds are recorded as **Gaussian (bell-shaped) curves** and the signals obtained are called **peaks** (figure 2). The position of each peak in the chromatogram gives us information's about the identity of the compound and the area under the peak is a function of the concentration or amount of each compound (Poole, 2000).

The information obtained from a chromatogram contain both qualitative and quantitative data on separation efficiency (Meyer, 2010) as presented in the Figure 2:

- the retention time of a component is always constant under identical chromatographic conditions;
- **w** is the peak width at the baseline;
- ***t_0*** is the **dead time** or retention time of an unretained solute, i.e. the time required by the mobile phase to pass through the column;
- ***t_R*** is **the retention time and represent** the period between the injection of the sample and recording of the peak maximum; two compounds can be separated if they have different retention times;
- both **the area and height of a peak** are proportional to **the amount of a compound;**
- the column dimensions, type of stationary phase, mobile phase composition and flow velocity, sample size and temperature provide the **chromatographic conditions**.

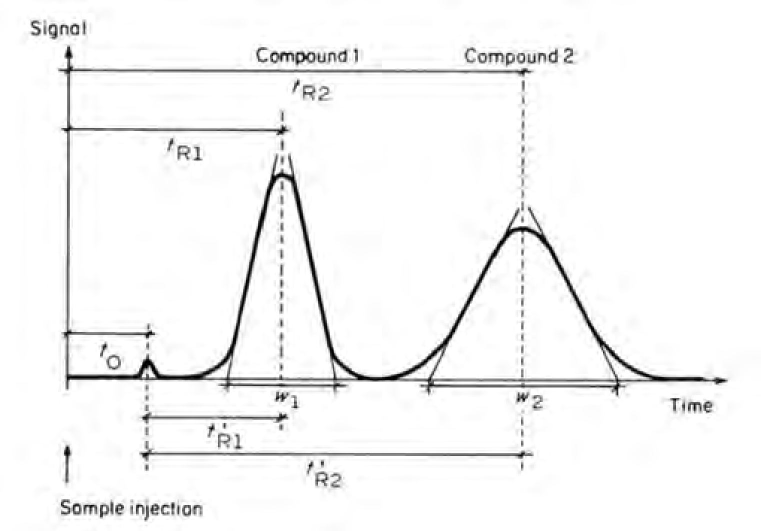


Figure 2. The characteristic features of chromatogram (Meyer V. R., 2006, Pitfalls and Errors of HPLC in Pictures, 2nd Ed.)

Types of chromatography

Why do different compounds have different affinities towards the stationary and mobile phases? “Polarity” of the compounds dictates their affinities towards the stationary and mobile phases.

Based on the polarity of the two phases, there are know the **normal-phase chromatography** which is performed on a stationary phase that is polar (hydrophilic) and use a non-polar mobile phase (hydrophobic). On the other side, a particular form of bonded-phase chromatography is **reversed-phase chromatography (RPC)**, in which the mobile phase is more polar than the stationary phase (for most practical applications the mobile phase is an aqueous solution) being the most popular form of liquid chromatography because of its applicability to polar compounds (Poole, 2000).

For example, we supposed to have a mixture of two molecules A and B, where ‘A’ is a protein and ‘B’ is a lipid; the column is packed with silica, which is polar in nature; our mobile phase is hexane, which is non-polar in nature. ‘A’, being polar in nature, will adsorb on to the polar stationary phase (silica). ‘B’ being non-polar in nature, will readily dissolve in the non-polar mobile phase (hexane) without adhering to silica, and will thus elute out of the column with hexane. Once B is eluted out, the mobile phase will be changed to something polar like acetonitrile.

Types of chromatography

By doing so we will now force A to detach from the silica and dissolve in the polar solvent, acetonitrile, and get eluted out of the column with acetonitrile.

According to the type of mobile phase, many types of chromatography are distinguished:

- high-performance liquid chromatography (HPLC), gas chromatography (GC);
- ion-exchange chromatography (IEC or IC);
- affinity chromatography (AC);
- supercritical fluid chromatography (SFC).

All these methods employ the same principles of chromatography.

High-performance liquid chromatography (HPLC)

High-performance liquid chromatography is a modern separation technique of liquid chromatography in which the mobile phase is a liquid. HPLC also refers to high-pressure liquid chromatography considering that the stationary phase requires very small particles and a high pressure is necessary for forcing the mobile phase through the column (Meyer, 2010).

The classical HPLC systems use pumps that can deliver the volumetric flow rate between 0.1 mL/min to 10 mL/min fluid and generate up to 6000 psi (about 400 bar; 1 bar ¼ 14.5,037,738 psi) (Moldoveanu & David, 2017).

The modern instruments, know it as UPLC or U-HPLC, can work up to 8500 psi (about 600 bar) or higher up to 18000 psi (about 1200 bar for up to 5 mL/min).

The elements of modern HPLC and UPLC are shown in **Figure 3.**

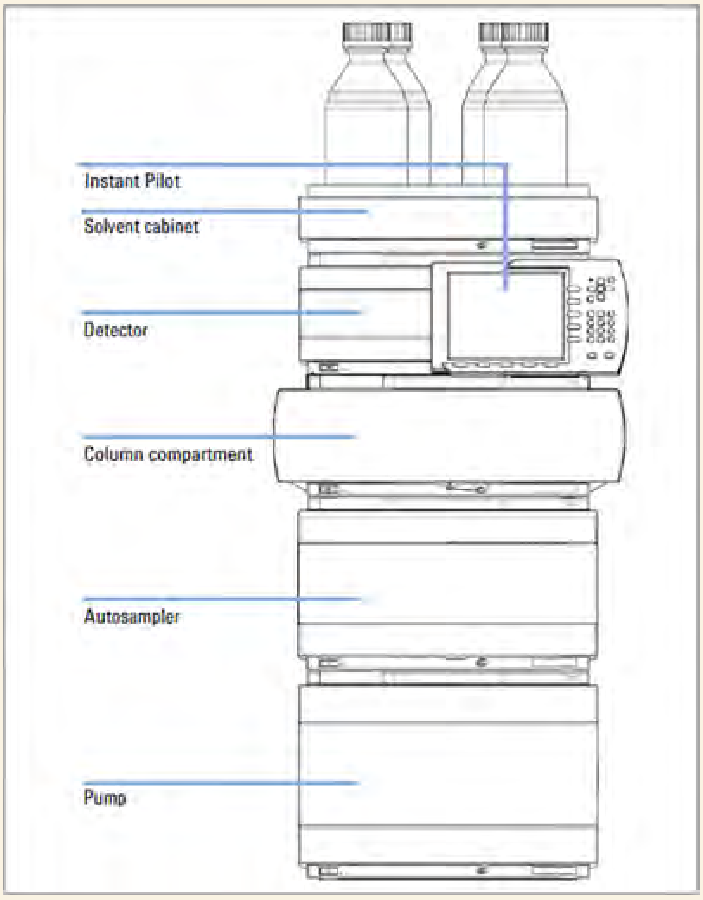


Figure 3 -1 Configuration for HPLC 1290 Infinity with binary pump (Agilent 1200 Infinity Series DAD User Manual)

Figure 3 -2 Waters ACQUITY UPLC chromatograph (Waters, Milford, MA) equipped with UV-PDA and FLR detectors

Practical principles of HPLC

An HPLC instrument includes the following parts:

1.solvent supply system (solvent reservoir, transfer line with frit)

An HPLC instrument includes the following parts:

1.solvent supply system (solvent reservoir, transfer line with frit)
2. high-pressure pumping system
3. injector which included in the autosampler
4. thermostatted column holder
5. chromatographic column (possibly with a guard column or precolumn)
6. one or more detectors
7. computer with dedicated programs which control the instrument and do the data acquisition and processing.

Columns for HPLC

The stationary phase is contained inside a column that is designed for performing the separation of the sample compounds.

Figure 4. Columns for HPLC, from Agilent manufacturer, of different sizes (https://www.agilent.com/)

Generally, the HPLC columns are made of 316 grade stainless steel, which is austenitic chromium–nickel–molybdenum steel, resistant to the usual HPLC pressure and also relatively inert to chemical corrosion (chloride ions and lithium ions at low pH being important exceptions) (Meyer, 2010).

In HPLC, internal diameters for the analytical columns are between 2 and 10 mm, and length between 50 and 250 mm (other dimensions are also possible) whereas, in UPLC the utilized columns are typically narrow (e.g., 2 mm i.d.) and short (e.g., 50 mm) (Moldoveanu & David, 2017). A HPLC separation of carotenoids on a 250 x 4.6 mm i.d. YMC C30 column, at 444 nm is shows in Figure 5.

To enhance column lifetime, guard columns should be used. Guard columns are very short with length between 0.5–3.0 cm, are packed with the same stationary phase (or equivalent) as that used in the analytical column and must be used only for short periods and should be replaced after a limited number of injections (e.g. in the order of 50–200) (Robards and Ryan, 2022).

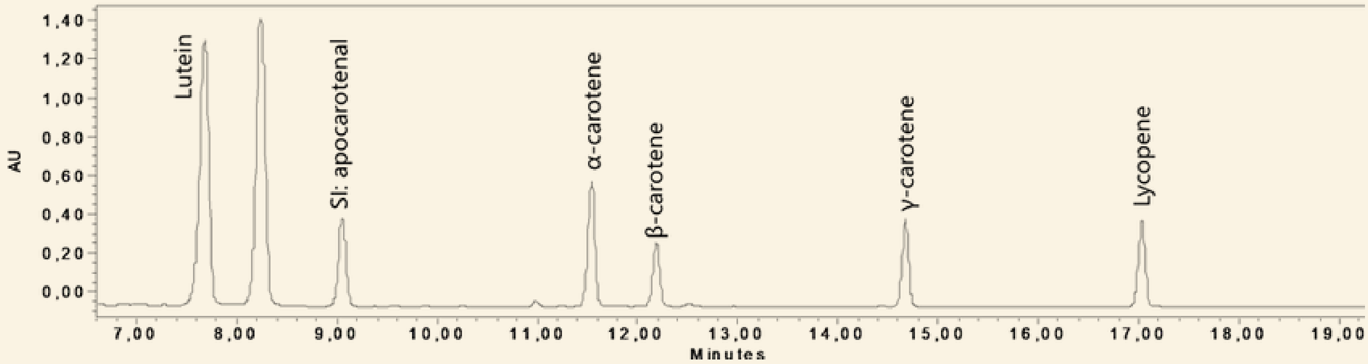


Figure 5. Cromatographic profile of standard carotenoids

The pump for HPLC is considered one of the most important components because it allows a constant flow of the eluent through the entire system: injector, column, detector, greatly increasing the separation speed (Naşcu and Jäntschi, 2006).

Regarding the types of detectors used in HPLC and UPLC, the more common re the following (Moldoveanu & David, 2017; Meyer, 2010):

1. UV-Vis spectrophotometers

- can have a fixed wavelength, a variable wavelength capability with unique wavelength detection, or variable wavelength capability with multiple wavelength detection such as the diode array detector (DAD);
- measure the absorbance Aʎ of the eluent, which is related to instantaneous concentration of the analyte;

2. Fluorescence detectors (FLDs)

- is used for the measurement of analytes that display fluorescence or of which fluorescing derivatives can be obtained are picked up with high sensitivity (may be up to 1000 times greater than with UV detection);

3. Chemiluminescence (CL) detectors

- are used when the analyte may be involved in emission of light as a result of a chemical reaction.

4. Refractive index detectors (RI or RID)

- record all eluting zones which have a refractive index different to that of the pure mobile phase and the signal is the more intense the greater is the difference between the refractive indices of the sample and eluent;

- are about 1000 times less sensitive than UV detectors (detection limit ca. 5x10-7 g of sample per millilitre of eluate under the most favourable conditions, in contrast to 5x10-10 g/ml with UV detectors);
- is sensitive to temperature changes, and a constant temperature must be maintained during measurements.

5. Mass spectrometric detectors combined with HPLC are one of the best tools for chemical analysis:

- responds to the instantaneous amount of solute passing through the detector;
- LC-MS and LC-MS/MS can provide very good sensitivity and selectivity compared to other detection techniques having the capability to measure ng/mL levels of compounds in the sample, to differentiate between molecules with different mass and fragmentation patterns, as well as the potential identification capability make LC-MS and LC-MS/MS invaluable techniques;

6. Electrochemical detectors

- different types are amperometric, coulometric, potentiometric and conductometric detectors;
- are useful in particular for ion chromatography;

7. Evaporative light-scattering detector (ELSD):

- is an instrument for the nonselective detection of nonvolatile analytes;

- the column eluate is nebulized in a stream of inert gas, the liquid droplets are then evaporated, thus producing solid particles which are passed through a laser, LED, or polychromatic light beam.

HPLC with different detection systems, such as diode array detector (DAD), mass or tandem mass spectrometry has become a dominating analytical tool for the separation and determination of polyphenols in fruits and vegetables (Ignat et al., 2011).

Gas chromatography (GC)

Like HPLC, the gas chromatography technique is the chromatographic analysis methods widely applied in chemical analyses in which the mobile phase is a gas. It is the technique used for the separation and analysis of volatile compounds but has been used for analysis of liquids, gases, and volatile solids. An example of essential oil from basil leaves (fresh and dried) identified by GC is presented in Figure 6.

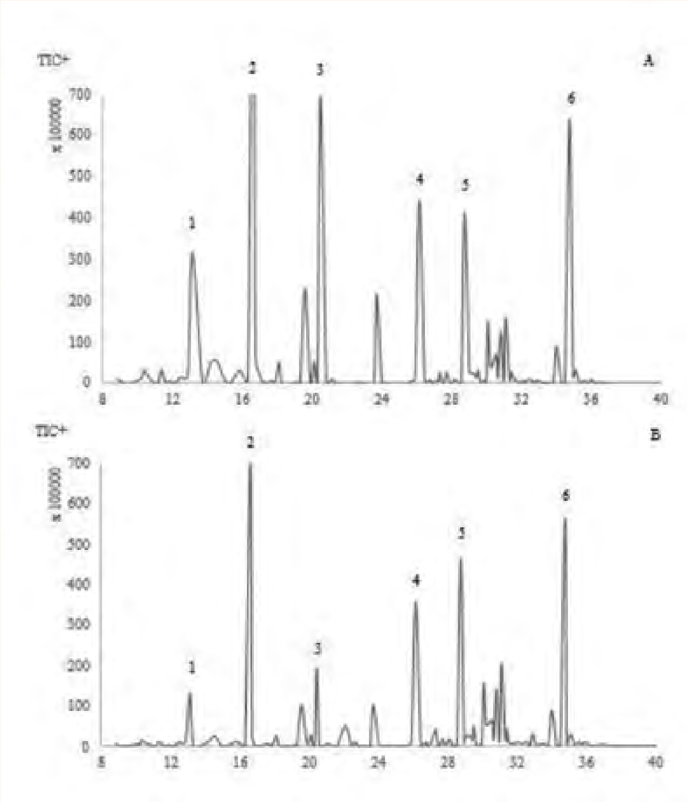
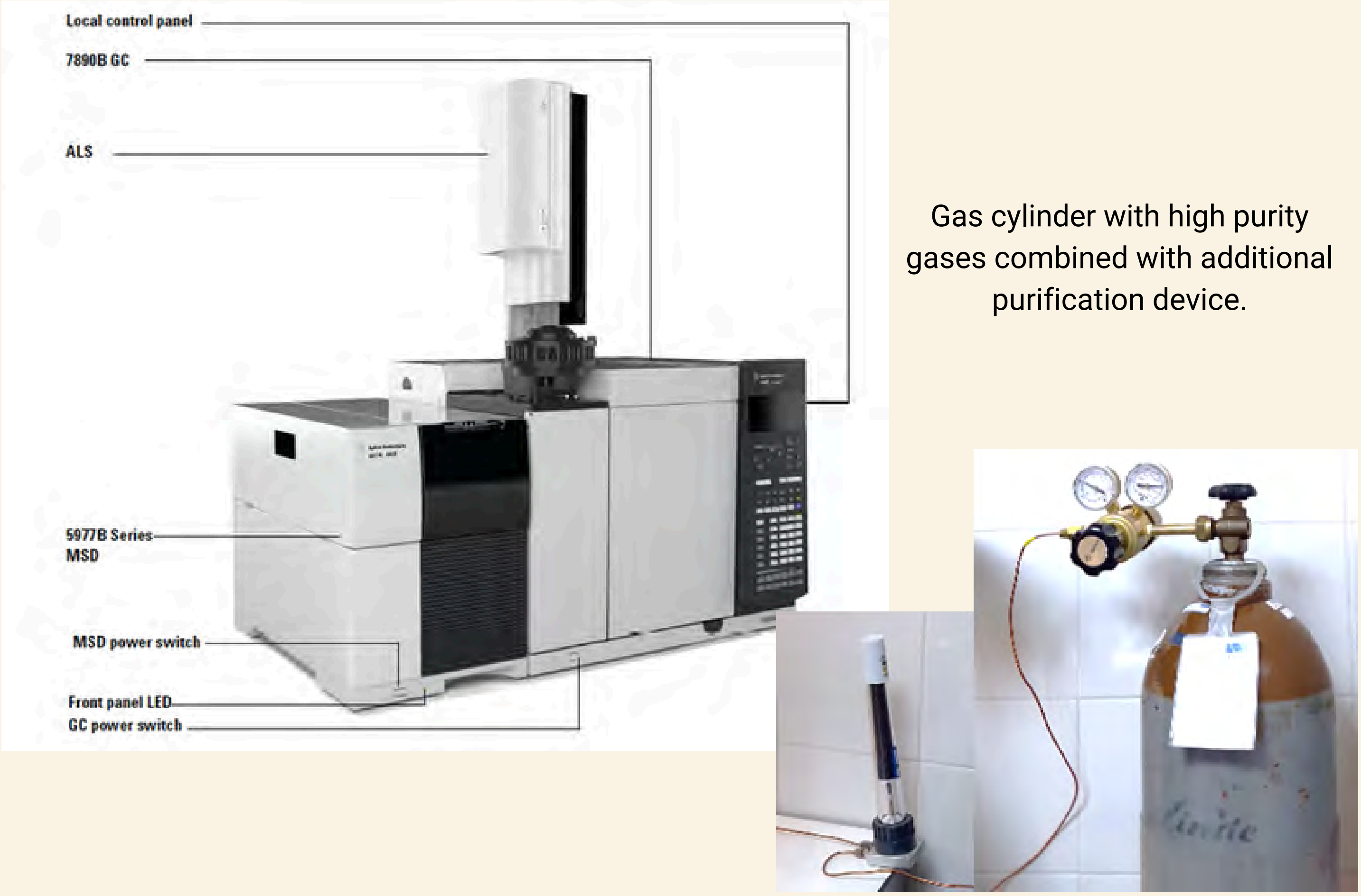


Figure 6 . Essential oil chromatographic profile of fresh basil leaves (A) and freeze-dried basil leaves (B): 1) 1,8 Cineole; 2) Linalool; 3) Methyl chavicol; 4) Eugenol; 5) α-Bergamotene; 6) α-epi-Cadinol (Ion et al., 2020).

The substances to be analyzed are introduced into the separation column, at a vaporization temperature, which can sometimes be higher than the decomposition temperature of the substances to be analyzed. The column temperature must be kept above the "dew point" of the sample, but not above its boiling point (McNair and Miller, 1997).

The basic parts of a simple gas chromatograph re: carrier gas, flow controller, injector, column, detector, and data system, as shows in the Figure 7.



Gas cylinder with high purity gases combined with additional purification device.

Figure 7. 5977B Series GC/MSD system, shown with Agilent 7890B GC and Automatic Liquid Sampler(ALS) 80 (Agilent 5975/5977 Series MSD Operation Manual)

Two types of columns are used in GC, the first ones re metal tubes packed with inert supports on which stationary liquids were coated (a, Figure 8) and the second ones are made of fused silica and are open tubes (OT) (b, Figure 8) with capillary dimensions with the stationary liquid phase is coated on the inside surface of the capillary wall.

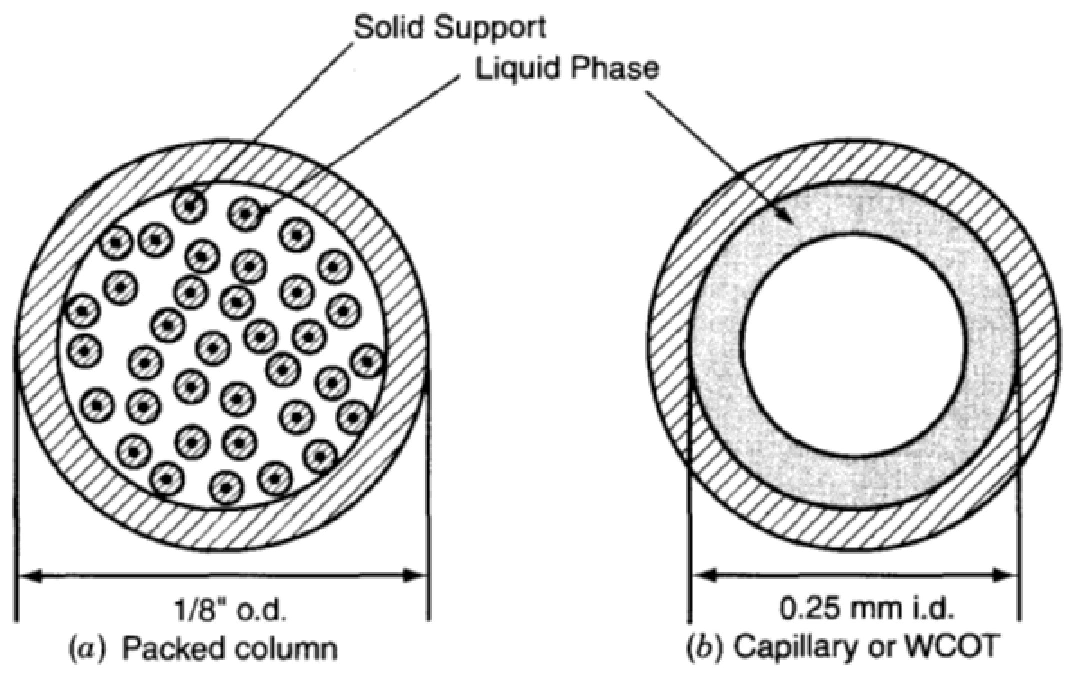


Figure 8. Types of GC column: (a) packed column and (b) open tubular column (McNair and Miller, 1997).

Characteristics of the GC elution process is represented by retention data such as the retention volumes, the retention temperatures or the retention times (tR) -characteristic for each compound under specific analytical conditions. Must be taken in consideration that the retention time is not unique to a compound, and two or more substances can share the same retention time, being generally influenced by several operating parameters (e.g. carrier gas type and flow, temperature, column dimensions and stationary phase polarity and thickness affect retention time) (Bizzo et al., 2023)

Name of detector	Selective*
1. Flame Ionization Detector (FID)	No
2. Thermal Conductivity Detector (TCD) (Katharometer)	No
3. Electron Capture Detector (ECD) X	X
4. Nitrogen/Phosphorous Detector (NPD); Alkali Flame Ionization Detector (AFID); Thermionic Ionization Detector (TID)	N, P, X
5. Photoionization Detector (PID); Discharge, Ionization Detector (DID)	aromatics
6. Helium Ionization Detector (HID) No	No
Emission type detectors	
7. Flame Photometric Detector (FPD)	S, P
8. Plasma Atomic Emission (AED)	Metals, X, C, 0
Electrochemical detectors	
9. Hall Electrolytic Conductivity (HECD)	S, N, X
Other types of detector	
10. Chemiluminescent	S
11. Gas Density Detector (GADE)	No
12. Radioactivity Detector	3H, 14C
13. Mass Spectrometer (MS or MSD)	Yes
14. Fourier Transform Infrared (FTIR)	Yes
*X = Halogen	

Table 1. Common available detectors (from McNair and Miller, 1997)

Beside these, the limitation of GC is analytical derivatization, where analytes are derivatized prior to injection for analysis which is a time-consuming extra step in the sample preparation procedure. (Atapattu and Temerdashev, 2023). Another limitation of offline analytical derivatization is that certain reagents, e.g., silylation-type reactions, are accompanied by side reactions due to exposure to moisture in samples, often resulting in extra peaks in the chromatograms and reducing yields and lowering the sensitivity of the analysis.

Further, the classification of the detectors, universal, selective, or specific, is presented in Table 1 (Poole, 2000; McNair and Miller, 1997).

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* https://www.khanacademy.org/science/

Agilent 5975/5977 Series MSD Operation Manual

3.2.4 Spectrometry (AAS, ICP)

3.2.4.1 Atomic Absorption Spectrophotometry

Atomic Absorption Spectrophotometry (AAS) is a sensitive method of analysis that provides a high degree of accuracy, and it is used to determine how much of certain elements are in a sample.

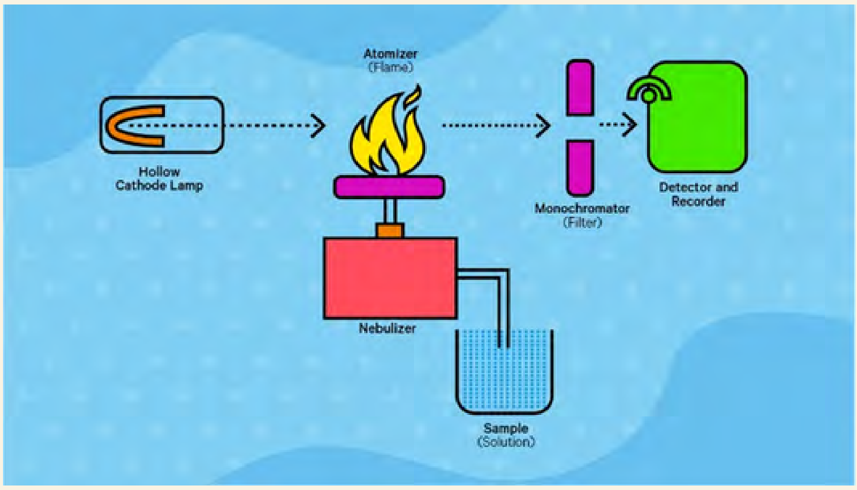
The basic principles it is that atoms or ions can absorb light at a specific, unique wavelength. The energy (light) is absorbed by the atom, when the specific wavelength of light is provided (scimed.co.uk).

The amount of light absorbed at the specific wavelength is directly proportional to the concentration of the absorbing ions or atoms. When the atom is exposed to its own unique wavelength, it can absorb the energy (photons) and electrons move from a ground state to excited states (technologynetworks.com). The changes in these wavelengths of light, before and after absorption, will appear as peaks of energy absorption in a readout (agilent.com).

The main components of an atomic absorption spectrometer are: the light source, the atomization system, the monochromator and the detection system (Figure 6). The samples are aspirated by a pneumatic nebulizer and sprayed into the mixing chamber. In the mixing chamber, the sample aerosol is mixed with acetylene and oxidant before it emerges from the burner slot. The flame is either 5 or 10 cm long and a few millimetres wide, depending on the burner used (scimed.co.uk).

The mirrors direct the light beams from the cathode and D2 lamps, and the beam selector splits the beam into component wavelengths. The photon detector counts light in photons. For reducing the background interference a monochromator is placed between the sample and the detector.

Figure 6. Schematic diagram of an atomic absorption spectrometer



Source: https://www.technologynetworks.com/analysis/articles/atomic-absorption-spectroscopy-principles-and-applications-356829

For more information, please visit:

Discover the Hidden World of AAS: Metal ...

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3.2.4.2 ICP (Inductively Coupled Plasma)

In any society food and nutrition are very important with significant economic, social and environmental impact. Food quality and its safety are the major issues of the current industry. The continuous increasing demand of food safety has stimulated research regarding the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins. There are several metals essential for life, others with different biological functions, and some others may cause diseases. The elements causing toxicity are accumulated in the body through the food chain, water and air (Voica et al., 2012).

To ensure food safety and safeguard human health, the characterization of the elemental composition of a wide range of food types is required. Since the concentration ranges for different elements vary significantly across different foods, various techniques have been used for sample characterization.

Thus, inductively coupled plasma mass spectrometry (ICP-MS) has become one of the most popular analytical techniques for elemental analysis in all types of samples including food and beverages (Garcia et al., 2015).

This is due to the analytical characteristics: very low detection limits (ppb), multi-element capabilities, the possibility of measuring isotope ratios and its easy coupling with other chromatographic techniques. According to Aceto et al., (2016), ICP-MS is one of the most used techniques in food classification starting from the most simple production chains (fruit and vegetable products) to the most complex ones (meat and fishes).

Regarding the ICP – MS structure, the following components are included: the sample introduction system, inductively coupled plasma (ICP), interface, ion optics, mass analyzer and detector. The samples are brought to a liquid state by mineralization/digestion. Liquid samples are first nebulized in the sample introduction system, creating a fine aerosol that is subsequently transferred to the argon plasma. The high-temperature plasma atomises and ionises the sample, generating ions which are then extracted through the interface region and into a set of electrostatic lenses called the ion optics. The ion optics focuses and guides the ion beam into the quadrupole mass analyser. The mass analyser separates ions according to their mass-charge ratio (m/z), and these ions are measured at the detector (Wilschefski and Baxter, 2019). The results are expressed as ppb (µg/l) and depending on the nature of the sample can be expressed in mg/ l, mg / kg, g/kg, g/100g.

A photomultiplier at the exit of the monochromator measures, synchronously with the clocking of the light sources, the intensity of the impinging radiation. The detector measures the intensity of the beam of light and converts it to absorption data (Operating Manual, ZEEnit 700 P, 2019).

The software of the equipment enables precise instrument control, and acquiring, manipulating and interpreting the data that the process generates.

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3.2.5 Mass spectrometry (MS)AAS, ICP)

Mass spectrometry (MS) is an investigative technique with broad applications in analytical chemistry, such as: determination of atomic and molecular masses; isotopic composition of elements; or identification of organic compounds.

The first commercialised MS tools appeared after 1950, being used in the oil processing industry. After the 1960s, mass spectrometry became the most important detection technique in both gas chromatography and liquid chromatography.

According to Jocelyn Paré et al., (1997), mass spectrometry has three main functions — namely, (1) molecule ionization, (2) the separation of ionized molecules according to their mass-to-charge-ratio, and (3) the determination of the respective abundance of each ion produced. The key principle underlying the electron impact ionization (EI) process is that the substances are ionized under vacuum conditions, the number of ion-molecule collisions is very small, and the fragmentation patterns are almost exclusively the result of unimolecular dissociation reactions.

Mass spectrometry is an analytic technique by which chemical substances are identified by the sorting of gaseous ions in electric and magnetic fields according to their mass-to-charge ratios. The instruments used in such studies are called mass spectrometers and mass spectrographs, and they operate on the principle that moving ions may be deflected by electric fields.

Mass spectrometry (MS) is regarded as an instrumental technique for separation of electrically charged species in the gas phase (Urban, 2016). The major components of the MS include inlet system, ion source, mass analyzer, detector, signal processor, and vacuum system. The inlet system allows introduction of the analyte into the MS without disrupting the high vacuum existing within the MS. The reservoir inlet permits volatile and thermally stable liquids and gases to be bled into the ion source from a vessel of higher pressure. A heated direct insertion probe is used to insert less volatile solid and liquid analytes directly into the ionization chamber for vaporization. The ion source is the heart of the MS, where the analyte is converted into energized ions by bombardment with a high-energy electron beam, by interaction with other ions, by bombardment with a beam of high energy atoms, by bombardment with a beam of high energy laser light, or by the production of gas phase ions from ions in solution. The generated ions are drawn into the mass analyzer by an electrical field. The molecular weight of the analyte is inferred from the mass of the molecular ion, and the structure and identity of the analyte is inferred from the pattern of fragment ions (Williams et al., 1997). Electric signals, produced by the ion detector, are subsequently processed to produce mass spectra (Urban, 2016).

For more informations, please visit:

Link here

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Unit 3.3 Food fraud - investigation ways

Violeta - Alexandra Ion, Natalia Koleva, Szandra Klatyik, Okray Orel

Inside EU, there is Knowledge Centre for Food Fraud and Quality (KC-FFQ), that produces and makes sense of scientific information to protect the authenticity and quality of food in the EU.

What is the difference between food fraud and food adulteration?

Food fraud deceives the consumers by providing them with lower quality foodstuff, against their knowledge and will. Economically-motivated adulteration deprives the consumers of the quality products they intend to purchase. It can also have serious implications on food safety and the health of consumers.

Definition of Food Fraud

- CEN Workshop Agreement 86 Food fraud: intentionally causing a mismatch between food product claims and food product characteristics.
- CX/FICS 18/24/7 Food fraud: any deliberate action of businesses or individuals to deceive others in regards to the integrity of food to gain undue advantage. Types of food fraud include but not limited to: adulteration, substitution, dilution, tampering, simulation, counterfeiting, and misrepresentation.

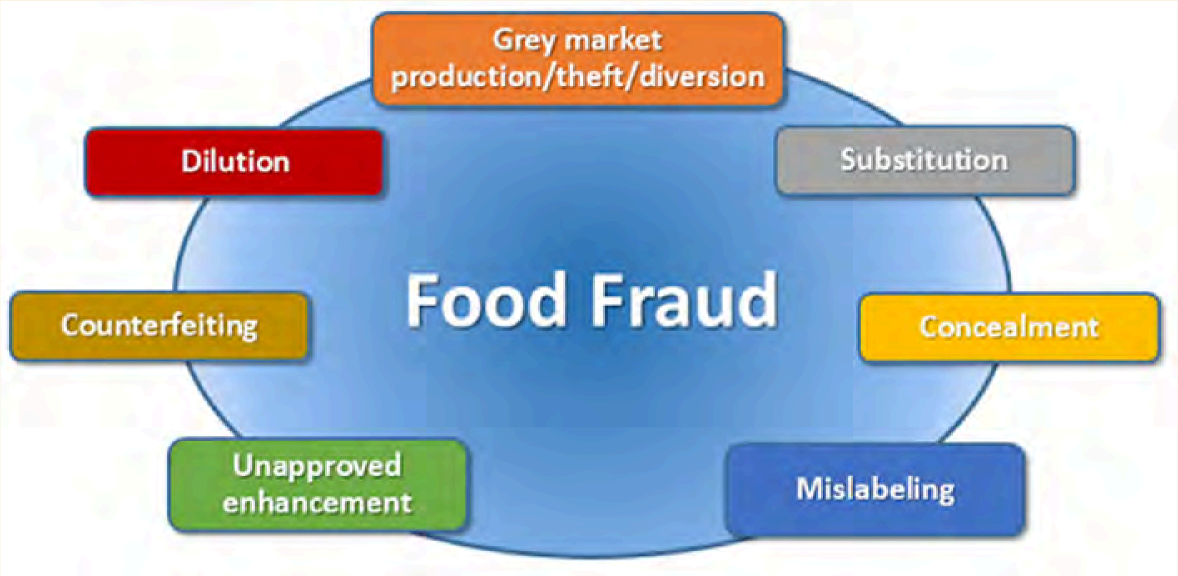
OCR Regulation (EU) 2017/625, Article 9, General rules on official controls:

1. Competent authorities shall perform official controls on all operators regularly, on a risk basis and with appropriate frequency, taking account of:

> [....] any information indicating the likelihood that consumers might be misled, in particular as to the nature, identity, properties, composition, quantity, durability, country of origin or place of provenance, method of manufacture or production of food.

2. Competent authorities shall perform official controls regularly, with appropriate frequencies determined on a risk basis, to identify possible intentional violations of the rules [....] perpetrated through fraudulent or deceptive practices, and taking into account information regarding such violations shared through the mechanisms of administrative assistance

Fraud in the context of food means that the description of the origin of food, its composition and how it has been obtained and/or prepared, shall be truthful, i.e. nothing of lesser economic value must be added, or removed if it is of higher economic value.



Food safety is generally controlled with official methods by determining physicochemical or biological parameters in accordance with established specifications and tolerances.

Top most common food frauds

According to the scientists, these foods are the most common sources of food fraud: olive oil, milk, honey, saffron, orange juice, apple juice, grape wine, vanilla extract and fish. Different international organisations are working in the development of the trustable methods for food analysis and determination fo the frauds. For some of the food there are already developedseveral methods of analysis, as follow:

- Methods of analysis of Honey (PDF) (International Honey Commission (IHC))
- Methods of analysis of Wine (International Organisation of Vine and Wine (OIV))
- Methods of analysis of Olive Oil (International Olive Council (IOC))

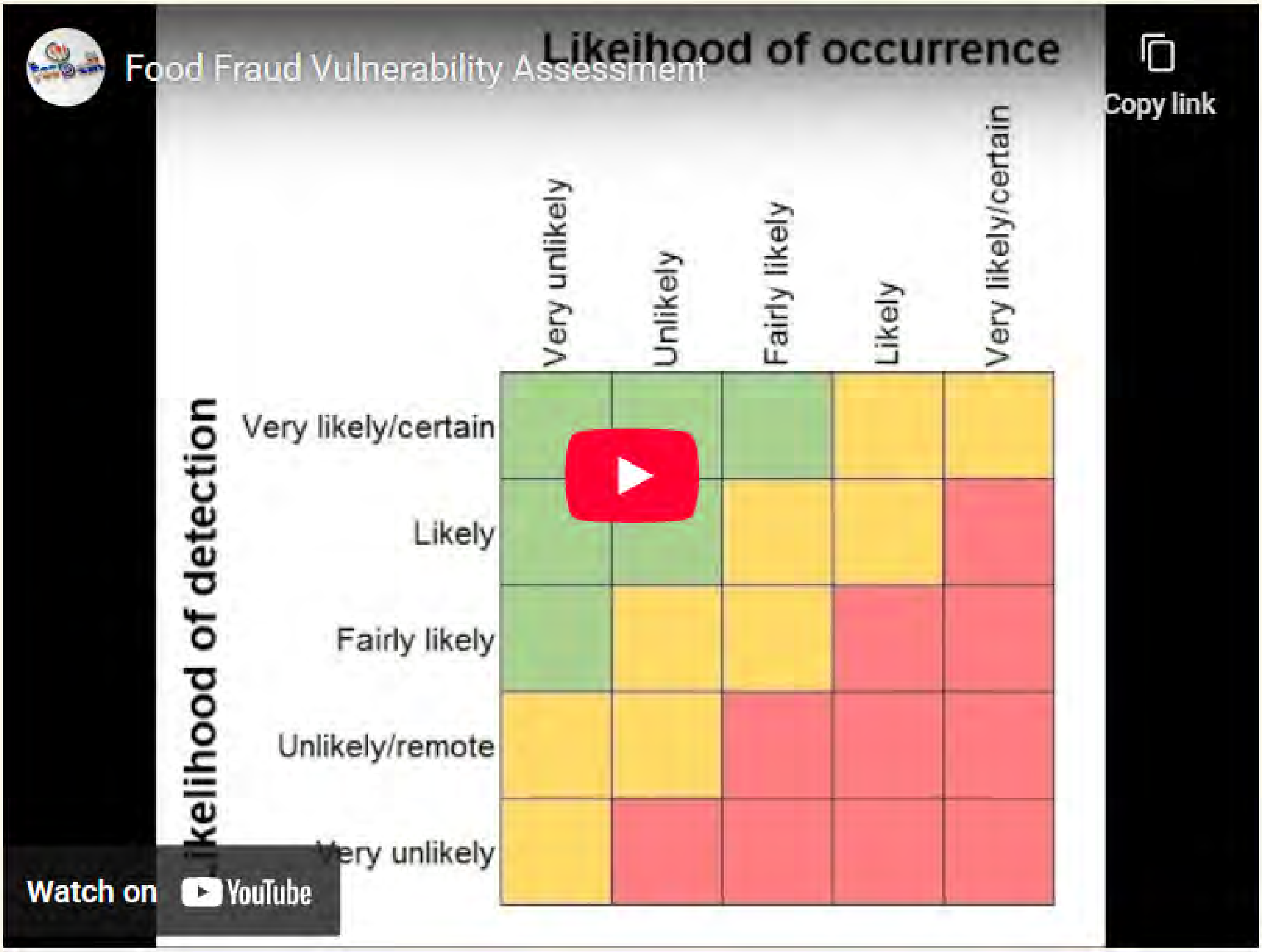
Another thypes of fraud related to fraud are those related to authentication.

Analytical tests for authentication purposes such as recognition of the detection of adulteration or geographic origin are more complex. Specifications for agri-food products with geographic indication are usually based on subjective characteristics such as organoleptic properties. Physicochemical parameters do not allow differentiation from similar products and are generally not characteristic of the geographic origin.

Therefore, adulterations or imitation products are offered to consumers and compete in an unfair manner with the genuine product.

For more information, please visit:

https://knowledge4policy.ec.europa.eu/food-fraud-quality/topic/food-fraud_en#faq



Food Fraud Vulnerability Assessment

Likeihood of occurrence

ikelihood of detection

	Very unlikely	Unlikely	Fairly likely	Likely	Very likely/certain
Very likely/certain	🟩	🟩	🟩	🟨	🟨
Likely	🟩	🟩	🟨	🟨	🟥
Fairly likely	🟩	🟨	🟨	🟥	🟥
Unlikely/remote	🟨	🟨	🟥	🟥	🟥
Very unlikely	🟨	🟥	🟥	🟥	🟥

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