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Pedagogical Handout

FUNCTIONAL FOOD AND NEW PRODUCTS

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Designed to Students of Food Sciences Field

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Forword

Functional foods are sorts of foods that provide health benefits beyond their "core nutrients", i.e. micronutrients and macronutrients, such as vitamins, minerals, carbohydrates, lipids, and proteins. Functional food must be considered to be a part of a healthy diet to exercise its potential benefit.

New food products include genetically modified foods, fourth-line food products, new meat products, Sous vide cooking, Low-fat products, and extruded products. These manufactured sorts of food are available on all supermarket shelves and have been widely consumed over the last decade.

The objective of this handout is to present the functional foods module by detailing six chapters: General information on functional foods, probiotics and prebiotics, dietary fibers, polyphenols, alkaloids and terpenes, and new food products.

Firstly, and for each bioactive substance, we have described the ingredients' definition, history, and effect on health and regulations.

Secondly, we proposed practical work including five protocols on the analysis of certain types of functional molecules in foods as well as the evaluation of their antioxidant activity. This type of practical work was carried out for Master 2 students: Biochemistry of Nutrition, Nutrition and Pathology.

List of abbreviations

AACC: American Association of Cereal Chemists

AOAC: Association of Official Agricultural Chemists

CAGR: Compound Annual Growth Rate

CKD: Chronic Kidney Disease

CoA: Coenzyme A

DA: Dalton

DAP: Dimethyl Allyl Phosphate

DMAPP: DiMethylAllyl diphosphate

DOXP: DeOxy Xylulose Phosphate

EOs : Essential Oils

EU : European Union

FDA: Food and Drug Administration

FOS: Fructo-Oligo-Saccharides

FOSHU: Food for Specified Health Use

FPP : Farnesyl diphosphate

GDP : Geranyl DiPhosphate

GGDP: GeranylGeranyl DiPhosphate

GI: Gastro-Intestinal

GM: genetically modified

GOS: Galacto-Oligo-Saccharides

GPP: geranyl diphosphate

IBS: Irritable Bowel Syndrome

IPP : IsoPentenyl Pyrophosphate

IxS: Indoxylsulfate

MECP: Methyl Erythritol Cyc lo Pyrophosphate

MVA : Mevalonic Acid

NMR: Nuclear Magnetic Resonance

pCS: p-CreSylsulfate

POS: Pectic-Oligo-Saccharides

ROS: Reactive Oxygen Species

RS: Resistant Starch

SCFA: Short-Chain Fatty Acids

SGLT: Sodium/GLucose coTransporter

TPSs: Terpene synthases

XO: Xanthine Oxidase

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Introduction

Introduction

Over the past two decades, there has been a significant increase in the knowledge regarding the impact of diet on health and well-being. This has led to the creation of new and healthier foods that can help reduce the risk of chronic diseases. The modified traditional foods, called functional foods, are designed to have health benefits compared to their non-modified counterparts (Kaur et Das, 2011).

This educational part is particularly aimed at Master students of biological sciences, and food sciences, and food sciences, allowing them to increase their knowledge of functional food sciences. It is a tool that allows you to learn about the different substances and functional ingredients that exist either in the usual foods that we consume every day or in new food products manufactured to have benefits for human health.

This paper describes all types of functional molecules and new food products. Three main types of functional ingredients prove their effectiveness in functional foods (pro/prebiotics, dietary fiber, and polyphenols). Regarding the new products that are currently the most demanded by the consumer, we can cite six categories of food: genetically modified functional foods, 4th range products, products obtained by cooking-extrusion, vacuum-packed products, new meat products, and low-fat products (in sugar). we began our work with chapter 1 which brings general information on functional foods. Pro/prebiotics are discussed in Chapter 2. Dietary fibers are presented in Chapter 3. Chapter 4 describes polyphenols. Alkaloids and terpenes are evoked in Chapter 5. In the last chapter (Chapter 6), we described all types of new food products.

In the second part of this handout, we proposed a practicum on the extraction of a functional molecule called lycopene as well as its dosage by the spectrophotometric method. Other practicum highlights three types of extraction of bioactive molecules from plants such as decoction, maceration, and infusion. The 4th practicum concerns the phytochemical screening of certain bioactive compounds and the last practicum on the determination of phenolic compounds and the evaluation of the antioxidant activity of certain

functional molecules. This practicum constitutes a type of teaching based on practical learning with the carrying out of experiments making it possible to

verify and supplement the knowledge provided in the theoretical courses of the functional foods and new products unit.

Chapter I

Basic Facts and Emerging Concepts on Functional Foods

I Functional food concept

Functional food is not and cannot be a defined or characterized entity. Indeed, a wide variety of food products are or will be, in the future, cataloged as such. These foods contain or will contain a multitude of components classified or not as nutrients, recognized as capable of influencing a wide variety of functions involved in the state of well-being and health or in reducing the risk of disease. Therefore, there will probably not be a simple and universal definition of functional food. It is therefore first and foremost a concept which, if it is centered on "the functions to be modulated" more than on "the products to be developed" has every chance of becoming universal and not easily influenced by local eating habits or cultural traditions ([Roberfroid et al., 2008](#)).

I.1 Japanese definition

Japan was the birthplace of the term functional food and it was at the forefront of their development. Functional foods are replaced by the term FOSHU: (Food for Specified Health Use)

- Foods that are known to have a specific health effect attributable to some of their constituents.
- Foods from which allergens have been extracted.
- Foods for which the effects attributable to the addition or extraction of components have been scientifically assessed and for which claims of health benefits associated with their consumption are authorized.
- Foods that pose no health or hygiene risk.

I.2 Other concepts

Since 1990, other terms inspired by the Japanese approach have emerged. These terms include more exotic words and expressions such as: Nutraceutical, Designer food, F(ph)armafood, Medifood, Viitafood, to which more traditional terms must be added such as food supplement, dietary food, fortified enriched food. These terms and expressions refer to Food substances

that provide medical and/or health benefits including the prevention and treatment of disease (Roberfroid, 2002).

I.2.1 Definition of nutraceutical

A product derived from food and sold in a powder, pill, or other pharmaceutical form that is not usually associated with food and for which physiological benefits or a role in the protection against chronic diseases has been demonstrated (Tur et Bibiloni, 2016).

I.2.2 Definition of the allegation

Allegation means "any message, statement or representation (regardless of the means or form of its transmission) which states, implies or suggests that a foodstuff has specific characteristics, properties or effects linked in particular to its nature, its composition, nutritional value, method of production and processing or any other quality" (Baelde, 2000).

II History of functional foods

The term "Functional Food" was born in the mid-1980s in Japan. In 1984, a large-scale national project, led by a research team from the University of Tokyo and other prominent food researchers, was started under the auspices of the Japanese Ministry of Education, Science and Culture. The project studied "Foods for Health".

In 1910, the eminent biochemist Umetaro Su Zuki (University of Tokyo) studied rice bran and discovered an anti-beriberian principle named Oryzanine, later renamed thiamine (vitamin B₁). With this discovery, the project moved from humble beginnings to chart a unique development path through special insights into physiologically functional non-nutrients from common foods (Table 1). The research team first proposed three categories of food functionality (Figure 1) and then focused their study on the "tertiary" function evoked by non-nutritive components. Then, political actions were followed for new legislation.

In 1991, Japan's Ministry of Health and Welfare launched the world's first policy to legally allow the marketing of selected functional foods, called "foods for specific health purposes" (FOSHU). Each of FOSHU products can guarantee some degree of health benefit. These scientific and political

activities were reported in Nature Journal under the title " Japan explores the boundary between food and medicine, " reminding us of the old saying. "Medicine and food are isogonic."

In 1991 and the United States, Dr. Jeffrey Bland founded the Institute of Functional Medicine. More than 100,000 licensed healthcare professionals have participated in training programs offered by the institute over the past 26



Figure 1: Three categories of proposed dietary functions (Vattem and Maitin, 2015).

years (Bland, 2018).

Table 1: Common non-nutrient components with possible functions for reducing disease risk (Vattem and Maitin, 2015).

Component	Origin	Disease
Carotenoids		
Carotene	Lycopene (tomato)	Oxidative stress
Xanthophyll	Lutein (vegetal)	Oxidative stress
Flavonoid		
Flavone	Quercetin (vegetal)	Oxidative stress
Flavonol	Nobiletin (citrus)	Oxidative stress
Isoflavone	Daidzein (soja)	Osteoporosis

Flavanone	Naringin (grapefruit)	Diabetes
Catechin	Epigallocatechin Gallate 3- or 4-methyl ester (tea)	Allergies
Anthocyanin	Cyanidin (Red bean)	Oxidative stress
Simple polyphenol	Chlorogenic acid (coffee)	Cancer
Phenylpropanoid	Curcumin (Curcuma)	Cancer
Isoprenoid	Ubiquinone (ubiquitous)	Oxidative stress
Triterpenoid	Soyasaponin (soy)	Oxidative stress
Phytosterol	B-Sitosterol (soy)	Hypercholesterolemia
Chromanol derivative	Tocotrienol (soy)	Oxidative stress
Isothiocyanate	Sulforaphane (broccoli)	Detoxification
Sulfoxide	Alliin (garlic)	Coagulation
Vanilloid	Capsiate (pepper)	Obesity
Alkaloid	Caffeine (coffee)	Obesity
Lignan	Sesamin (sesame)	Hangover
Organic acid	Acetic Acid (vinegar)	Hypertension
amino acid	γ -Aminobutyric acid (rice)	Hypertension
	B-Conglycinine (soy)	Obesity
	Val-Pro-Pro (rotten milk)	Hypercholesterolemia
Protein	3-Diacylglycerol (cooking oil)	Obesity
Oligopeptide	Alginic acid (algae)	Constipation
Lipid	Manno-oligosaccharide (coffee)	Diabetes
Polysaccharide	Bifidobacterium lactis (yogurt)	
Prebiotic	Neoculine (Curculigo latifolia)	
Probiotic	Val-Pro-Pro (rotten milk)	

Sweeteners		
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As of March 2012, 990 FOSHU products in 13 categories were approved in Japan. Ameal-S, a sour milk product containing milk protein-derived antihypertensive peptides (Val Pro-Pro and Ile-Pro-Pro), is an example of an approved FOSHU product ([Arihara et Ohata, 2011](#)).

Most FOSHU products utilize functional ingredients to help in the maintenance of a healthy human body. Such food ingredients are listed in [Table 2](#).

Table 2: Representative functional ingredients used for FOSHU products ([Arihara et Ohata, 2011](#)).

Ingredients	Targets
Dietary fibers	Intestinal disorder Cholesterol level Blood sugar level
Lactic acid bacteria	Intestinal disorder
Oligosaccharides	Intestinal disorder
Soy proteins	Cholesterol level
Sugar alcohols	Dental caries
Peptides	Mineral absorption Blood pressure level Cholesterol level Triacylglycerol level
Calcium/iron	Mineral level
Polyphenols	Dental caries Blood sugar level
Glycocides	Blood pressure level
Sterol esters	Cholesterol level
4-Aminobutanoic acid	Blood pressure level



III Origin of functional food

Depending on their origin, functional foods can be classified into two main groups: Functional foods of plant origin and animal origin:

III.1 Functional foods of plant origin

Two examples can be cited; oats (beta-glucan) in the reduction of cardiovascular diseases, and soy (isoflavones) in the reduction of cardiovascular diseases and cancer.

III.1 Functional foods of animal origin

We can give the example of fish (Omega 3) used in the regulation of blood pressure ([Nayak et al., 2021](#)).

VI Physiological action of functional foods

Functional foods are essential to support various physiological and pathophysiological processes. They impact biological responses in the body, leading to significant health benefits in different areas of human physiology. Figure 2 shows the importance of functional foods in the current physiological areas of interest, including oxidative stress and cancer prevention gastrointestinal health, cardiovascular health, regulation of basic metabolism processes, physiology of cognitive and mental performance, physical performance, neurodegenerative diseases, and cardiometabolic syndrome, growth and development in children ([Aguilar et al., 2019](#)).

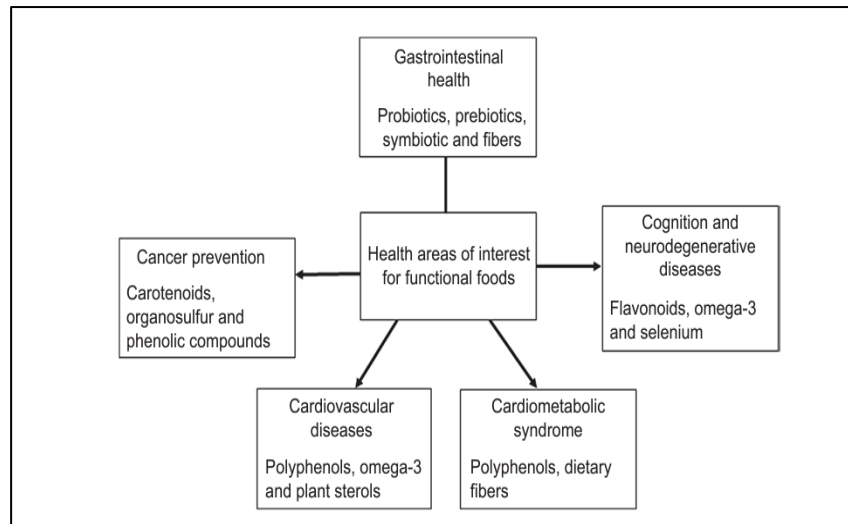


Figure 2: Health areas of interest for functional foods (Aguilar et al., 2019).

V Functional Food Development Strategy

V.1 Objectives of the strategy

The development of functional foods aims to identify and understand (at least partially) the beneficial interactions between supposedly functional food and a target function.

V.2 Expected result

- Demonstration of a functional effect and formulation of hypotheses to be tested in nutritional studies in humans.
- Evaluate the safety of ingesting the amount of food necessary to achieve the desired effect; plan intervention studies in humans to demonstrate the effect of the functional food on the target function (direct demonstration and use of valid markers).
- Monitor the long-term consequences of interactions between the functional food and/or modulated target functions.

VI Functional food consumption worldwide

The largest functional food markets are in Japan, Korea, the United States, and Europe (Figure 3). In the European Union, Spain, Finland, Cyprus, the Netherlands, and Sweden exhibit high consumption of functional foods. The market size and success of functional foods depend on the

acceptance and attitude of consumers towards them. It has been observed that Americans tend to be more accepting of and receptive to functional foods, while Europeans are more critical and inquisitive about them. Specifically, Danish consumers have been found to hold more negative attitudes towards functional foods compared to their American and Finnish counterparts. Compared to Americans and Canadians, French consumers seem less aware of functional foods. Finnish consumers, on the other hand, have the most positive attitudes toward functional foods (Tur et Bibiloni, 2016).

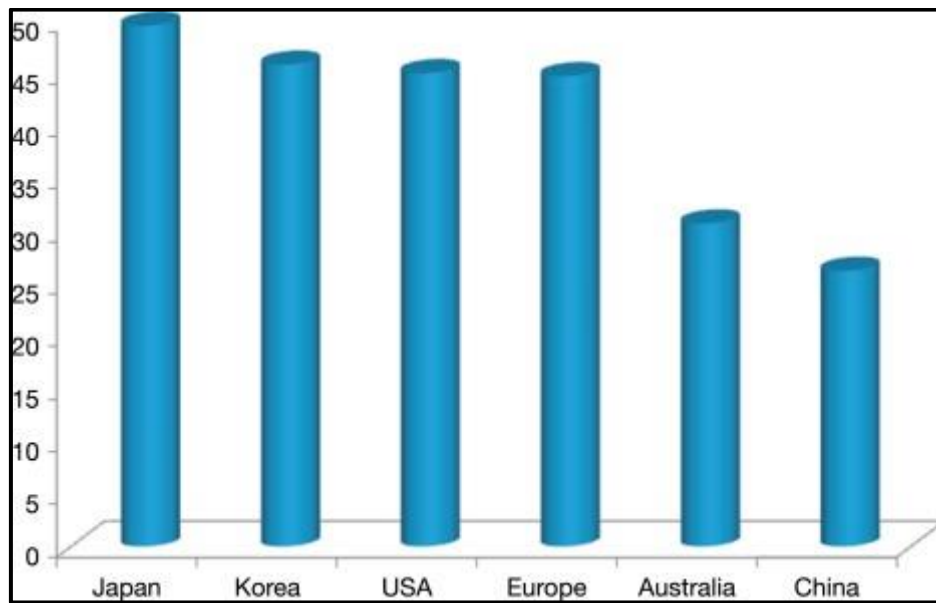


Figure 3: Proportion of functional food consumers worldwide (Tur et Bibiloni, 2016).

VII Functional food consumption depending on age and gender

The consumption of functional food varies according to the age and gender of consumers. Functional foods, such as low-fat/skimmed milk, fruit juice, cereals, and probiotics, are consumed by people of all ages; While cholesterol-lowering products and soy products are popular, they are primarily consumed by adults and seniors. Functional foods may appeal more to females overall, but there are still products that can interest either gender. Research has shown that education is a key factor in promoting healthy eating habits, and it has been noted that functional food users tend to have higher levels of education compared to non-users. Consumption of cholesterol-lowering products increases with diet-related issues like high cholesterol, high blood pressure, and diabetes. People with diet-related issues consumed more cholesterol-lowering products than those without such issues; those with cardiovascular diseases used them more frequently. Other main interests include blood sugar control and weight reduction (Tur et Bibiloni, 2016).

VIII Functional food regulation

Most countries have not yet established well-defined regulations for functional foods. Legislators in Japan, the USA, and Europe have distinct approaches to functional foods. In the USA, functional foods fall under the same regulatory framework as traditional foods and dietary supplements. The regulatory scheme of the FDA does not acknowledge functional foods as a separate category for regulation purposes. While developing a functional food that complies with EU legislation is complex (Tur et Bibiloni, 2016).

Chapter II

Probiotics and Prebiotic

I Probiotics

I.1 Definition of probiotics

In 2001, the Food and Agriculture Organization of the United Nations and the World Health Organization jointly established guidelines for the use of the term "probiotic" in foods and formulated its definition "live microorganisms which, when given in adequate amounts, produce a health benefit to the host" (Rambaud *et al.*, 2004).

I.2 History

The term probiotic was coined in 1953 by German scientist Werner Kollath. "active substances that are essential for a healthy development of life."

In 1965, Lilly and Stillwell used the term "probiotics" to refer to substances secreted by one organism that stimulate the growth of another. Fuller later defined probiotics more specifically in 1992 as "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance." (Ebner *et al.*, 2014).

The history of probiotics began in the early 1900s with Elie Metchnikoff's studies at the Pasteur Institute in Paris. Louis Pasteur identified the microorganisms responsible for fermentation, while Metchnikoff investigated their impact on human health. The enhanced longevity of Bulgarian rural people is attributed to their regular consumption of fermented dairy products, such as yogurt. This is linked to the discovery of the Bulgarian bacillus by a 27-year-old physician named Stamen Grigorov. It is further suggested that lactobacilli can counteract the putrefactive effects of gastrointestinal metabolism that contribute to illness and aging. Hippocrates stated 2000 years ago that poor digestion is the root of all evil. Metchnikoff claimed that toxins causing aging originate from bacterial putrefaction in the large intestine and are released into circulation. He referred to these microbes as putrefying bacteria, which are now recognized as proteolytic clostridia (Ebner *et al.*, 2014).

In 2013, experts published a consensus document defining probiotics as live microorganisms that confer a health benefit to the host when administered in

sufficient amounts. Modern technology has allowed for the selection of specific strains of bacteria that produce fermented milk with excellent nutritional and organoleptic properties, surpassing those of other strains. As per a consensus document, yogurt and other fermented milk products can be regarded as the first functional foods (Ebner et al., 2014).

I.3 Major types of probiotics

The Table below presents the main types of probiotics used. Each strain is different and will cause several quite different physiological effects. Some strains secrete anti-inflammatory interleukins, others will have a role in pain. A third will secrete a group B vitamin (Markowiak et al., 2017).

Table 3: Major types of probiotics and their effects (Markowiak et al., 2017).

Strain	Effects
<i>Lactobacillus rhamnosus</i>	Prevention of prebiotic-related diarrhea
<i>Bifidobacterium longum</i> , <i>Lactobacillus helveticus</i> , <i>Lactobacillus lactis</i> , <i>Streptococcus thermophiles</i>	Prevention of irritable bowel syndrome with constipation
<i>Lactobacillus gasseri</i>	strengthening the gut barrier
<i>Lactobacillus plantarum</i>	prevention of vulvovaginal candidiasis

2017).

I.4 Mechanism of action of probiotics

The potential mechanisms by which probiotic microorganisms could influence the gut microbiota are illustrated in Figure 4.

I.4.1 Competition for nutrients

Probiotics compete with pathogenic bacteria by reducing the amount of nutrients accessible to pathogens. Consumption of probiotic cultures can modulate

the microbiota or modify its metabolic properties by competition for nutritional substrates (O'Toole et Cooney, 2008).

I.4.2 Fermentation of undigested food components: Bioconversion

Two types of fermentation can be cited; saccharolytic fermentation of oligosaccharides which allows the production of butyrate used as an energy source for epithelial cells, and proteolytic fermentation which allows the degradation of proteins and facilitates their digestion (O'Toole et Cooney, 2008).

I.4.3 Production of useful substances

Research on probiotics has shown that their presence in the large intestine is manifested by the production of essential nutrients like vitamin K (Quigley et Quera, 2006), and short-chain fatty acids (Ohashi et Ushida, 2009).

I.4.4 Direct antagonism

Probiotic bacteria probably impact gut microbiota through direct antagonism. It has been shown in several recent studies that they can modulate the number of certain model organisms in experimental systems. For example, the probiotic strains of *L. salivarius* inhibit the growth of *H. pylori* in vitro by mechanisms involving lactic acid secretion. This acid lowers the pH of the intestine which inhibits the growth of pathogens (Ryan et al., 2008). The natural competition between commensal bacteria and opportunistic pathogens can therefore be mediated by mechanisms such as the production of bacteriocins, which can be exploited to use probiotics to modulate the microbiota.

I.4.5 Competition for the bindings

Administering probiotics prevents pathogens from easily attaching and causing infections. This mechanism is called competitive exclusion or resistance to colonization (Vieira et al., 2008).

I.4.6 Tight Junction Reinforcement

Commensal bacteria and probiotics can enhance tight junction barrier function and decrease intestinal permeability (Alvarez et al., 2016). This reinforcement is manifested by the production of a glycoprotein called mucin 2 secreted by epithelial cells (Chang et al., 2020).

I.4.7 Immune system regulation

Probiotics promote the differentiation of B cells into plasma cells which produce type A immunoglobulins called secretory IgA (sIgA). These have anti-inflammatory power, they act with pathogens and prevent their access to the lamina propria (Carasi *et al.*, 2017).

I.4.8 Immune System Modulation

Probiotics influence the functioning of dendritic cells, which detect possible dangers inside the intestine and trigger a T-cell reaction (Meijerink et Wells, 2010).

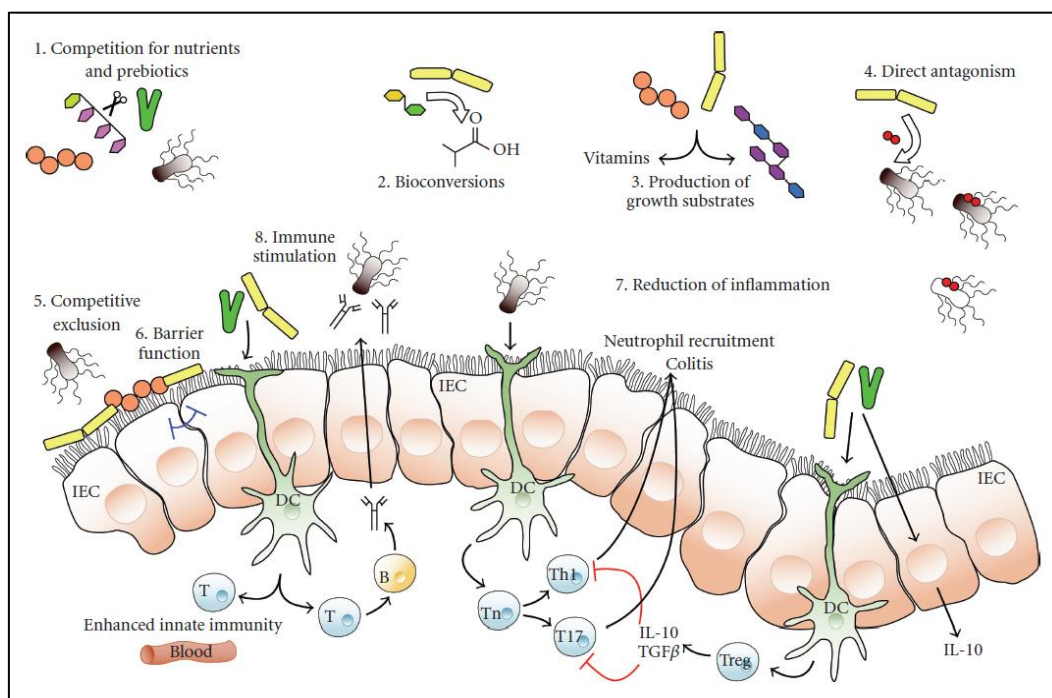


Figure 4: Mechanisms of action of probiotics (O'Toole and Cooney, 2008).

I.5 Clinical application of probiotics

Current insights into the clinical applications for the various probiotics in the field of gastroenterology are summarized below:

I.5.1 Application in Clinical Gastrology

I.5.1.1 Prevention of intestinal diseases

I.5.1.1.1 Prevention of chronic inflammatory bowel diseases

a- Pouchitis

There is good evidence that certain probiotics are useful in the prevention of pouchitis and in the prevention of subsequent relapses after induction of remission by antibiotics. Probiotics may be recommended in patients with mild pouchitis or as maintenance therapy in patients in remission ([Zubillaga et al., 2001](#)).

b- Ulcerative colitis

Some probiotics are safe and as effective as conventional treatments in inducing higher response and remission rates in mild and moderate ulcerative colitis in adults and children ([Zubillaga et al., 2001](#)).

c- Crohn's disease

Studies conducted in the context of Crohn's disease have concluded that there is no evidence in favor of the beneficial effect of probiotics in the maintenance of remission of Crohn's disease ([Zubillaga et al., 2001](#)).

I.5.1.1.2 Irritable Bowel Syndrome (IBS)

A reduction in intestinal bloating and flatulence as a result of probiotic treatment is a consistent observation in published studies; a few strains can additionally relieve pain and provide overall relief. There is literature suggesting that some probiotics can improve symptoms and quality of life in patients with functional abdominal pain ([Zubillaga et al., 2001](#)).

I.5.1.1.3 Prevention of colorectal cancer

Although diet is believed to contribute to the development of colorectal cancer and probiotics have been shown to improve biomarkers associated with such cancer, there is very little evidence from human studies that show some benefit in preventing colorectal cancer ([Zubillaga et al., 2001](#)).

I.5.2 Treatment and prevention of diarrhea

I.5.2.1 Treatment of acute diarrhea

Different strains of probiotics have proven their usefulness in reducing the severity and duration of acute infectious diarrhea in children. Oral administration of probiotics reduces the duration of acute diarrheal illness in children by approximately one day. Several meta-analyses of controlled clinical trials testing other strains of probiotics have been published and show consistent results suggesting the safety and efficacy of probiotics. The mechanisms of action may, however, be strain-related ([Zubillaga et al., 2001](#)).

I.5.2.2 Prevention of acute diarrhea

In the prevention of diarrhea in children and adults, there is evidence that certain probiotics are effective in a few specific cases ([Zubillaga et al., 2001](#)).

I.5.2.3 Prevention of antibiotic-associated diarrhea

In the prevention of antibiotic-associated diarrhea, there is strong evidence in favor of the efficacy of probiotics in adults and children under antibiotic treatment ([Zubillaga et al., 2001](#)).

I.5.2.4 Prevention of *Clostridium difficile*-associated diarrhea

In 2016, a meta-analysis showed that probiotics may decrease the risk of developing *C. difficile* diarrhea in patients on antibiotic treatment, but the authors say further studies are needed to determine the optimal dosage and strain ([Zubillaga et al., 2001](#)).

I.5.2.5 Prevention of radiation-induced diarrhea

The gut microbiota likely plays an important role in radiation-induced diarrhea by enhancing gut barrier function, enhancing innate immunity, and stimulating gut repair mechanisms. A meta-analysis in 2013 concluded that probiotics may be beneficial in the prevention and possibly also in the treatment of radiation-induced diarrhea.

I.5.3 Eradication of *Helicobacter pylori*

The Consensus Maastricht V/Florence report on the treatment of *H. pylori* infection concluded that probiotics and prebiotics show promise in reducing side

effects during treatment for *H. pylori*. A meta-analysis of randomized studies in 2014 suggested that the addition of certain probiotics to anti-*H. pylori* may also be effective in increasing the eradication rate and may be useful in patients in whom eradication has failed. There is no evidence to support the concept that a probiotic alone, without any concomitant antibiotic treatment, could be effective (Zubillaga et al., 2001).

I.5.4 Prevention and treatment of hepatic encephalopathy

Prebiotics like lactulose are commonly used in the prevention and treatment of hepatic encephalopathy. There is some evidence that a particular probiotic can treat minimal hepatic encephalopathy (Zubillaga et al., 2001).

I.5.5 Immune response

There is evidence to suggest that several strains of probiotics and the prebiotic oligofructose help enhance immune response. Evidence suggesting an increased immune response has been obtained in studies aimed at preventing acute infectious disease (hospital-associated diarrhea in children, common cold episodes) and in other studies that have tested the antibody response to vaccines (Zubillaga et al., 2001).

II Prebiotics

II.1 Definition of prebiotics

A Prebiotic has been defined as an “indigestible food ingredient that selectively stimulates in the colon the multiplication or activity of one or a limited number of bacterial groups likely to ameliorate the host physiology” ([Roberfroid, 1995](#)).

A prebiotic is an indigestible food ingredient that provides health benefits by selectively stimulating the growth and/or metabolic activity of one or a limited number of gut bacteria ([Brownawell et al., 2012](#)).

II.2 History

The term "prebiotic" was introduced in a publication in the 1950s. Researchers described the presence of a so-called ‘Bifidus factor’ in human milk, a component that enriched for bifidobacteria in infants. Later, this factor was identified and shown to consist of complex oligosaccharides and glycans. Oligosaccharides were also detected in bovine milk and milk products, but their physiological role was unclear. Remarkably, there were even reports of human subjects consuming massive doses of lactose to enrich beneficial lactic acid bacteria in the gut. Fructans and other fermentable fibers likely comprised an important part of the diet of humans tens of thousands of years ago ([Hutkin et al., 2016](#)).

In 1995, Glenn Gibson and Marcel Roberfroid introduced the prebiotic concept. They defined a prebiotic as “a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health”. Although this original definition has been revised multiple times, the main features have mostly been retained ([Hutkin et al., 2016](#)).

Even by the early 2000s, the development of next-generation, rationally selected prebiotics was proposed. Thus, resistant starch (RS), pectin and other fiber components, and milk oligosaccharides are now proposed as having prebiotic potential ([Hutkin et al., 2016](#)).

II.3 Types of Prebiotics

II.3.1 Fructans

Fructans are fructose polymers of four to several hundred residues that possess a sucrose core. Fructans are found widely in plants and appear to act as reserve carbohydrates, and also improve cold and drought tolerance. Some bacteria and fungi can produce fructans (Davani-Davari *et al.*, 2019).

There are three main types of fructans: inulins, levans (or phleins), and gramminans. Linear inulin consists of β -1,2-linked fructose residues attached to a sucrose core and is found in members of the Asterales family (e.g. chicory). Inulin neo-series possesses two β -1,2-linked fructose chains attached to the sucrose core and is found in members of the Liliaceae family (e.g. onions). Levan fructans are found in grasses and consist of a β -2,6-linked fructose chain attached to sucrose. Mixed fructans consisting of β -2,6-linked fructose residues with β -1,2-branches are termed gramminans and are also found in grasses (Figure 5) (Stick *et al.*, 2009).

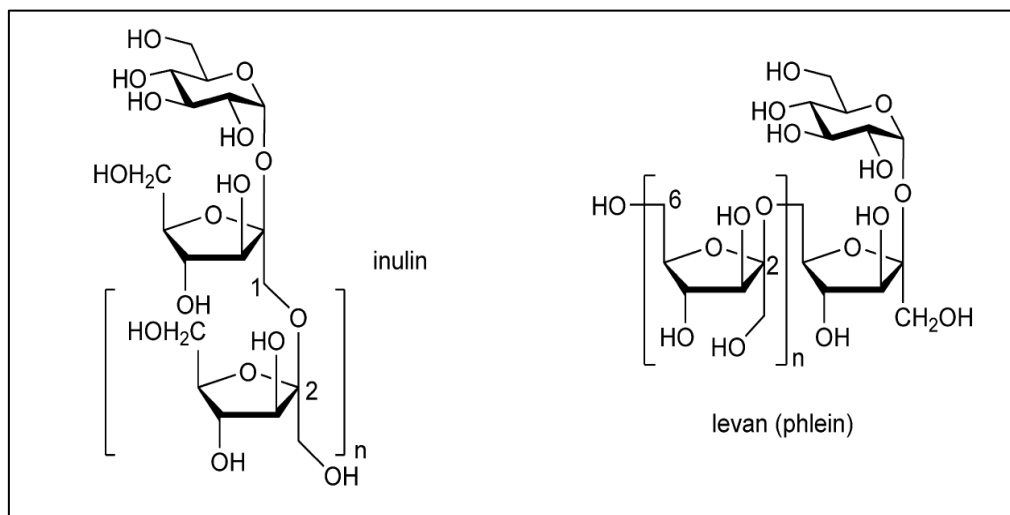


Figure 5: Main types of fructans (Stick *et al.*, 2009).

Fructans are used widely in the food industry. Chicory inulin is the main fructan food additive and forms an emulsion with water that possesses a similar texture to fat and is used in yogurts and ice cream as a fat substitute. Fructans are classified as soluble fibers and are available as a food supplement. Fructans are touted to have the capability of promoting the growth of beneficial bacteria within the gastrointestinal tract and may provide relief from inflammatory bowel disease. 1-Kestose (isoketose) and nystose are inulin oligosaccharides comprised of one or two

b-1,2-linked fructose residues, respectively, attached to sucrose. They are obtained from sucrose using a fructosyltransferase and are marketed as low-calorie, non-cariogenic sweeteners. Fructan biosynthesis takes place in the vacuole of the plant cell and uses 1-kestose as a primer. Unusually, for a polysaccharide biosynthesis pathway, fructan biosynthesis uses sucrose as a fructosyl donor, rather than a sugar-phosphate donor (Stick *et al.*, 2009).

II.3.2 Galacto-Oligosaccharides (GOS)

GOS was recently defined as “a mixture of those substances produced from lactose, comprising between 2 and 8 saccharide units, with one of these units being a terminal glucose and the remaining saccharide units being galactose and disaccharides comprising 2 units of galactose”. The global market size of GOS was recently estimated to be about 20,000 tons with a compound annual growth rate (CAGR) of 10% to 20% (Torres *et al.*, 2010).

II.3.3 Starch and Glucose-Derived Oligosaccharides

There is a kind of starch that is resistant to upper gut digestion known as resistant starch (RS). RS can promote health by producing a high level of butyrate; so it has been suggested to be classified as a prebiotic. Various groups of Firmicutes show the highest incorporation with a high amount of RS. An *in vitro* study demonstrated that RS could also be degraded by *Ruminococcus bromii*, and *Bifidobacterium adolescentis*, and also to a lesser extent by *Eubacterium rectale* and *Bacteroides thetaiotaomicron*. However, in the mixed bacterial and fecal incubations, RS degradation is impossible in the absence of *R. bromii*. Polydextrose is a glucose-derived oligosaccharide. It consists of glucan with a lot of branches and glycosidic linkages. There is some evidence that it can stimulate *Bifidobacteria*, but it has not been confirmed yet (Davani-Davari, 2019). There are four types of resistant starch (Table 4).

II.3.3.1 Resistant starch

There are different types of resistant starch (RS). This is analogous to dietary fiber, for which there are many different types and sources. Fibers exhibit a broad range of physiological effects and so do RSs. Four classes of RS have been described (Table 4). Three are found in nature or are formed as part of normal cooking

procedures while the fourth class can be produced using chemical modification methods that are approved for use in foods. Commercial sources of RS-rich ingredients are available for increasing the RS and/or dietary fiber content of foods and these will be described later. RS ingredients from all four classes of RS are available commercially.

Table 4: Classification of resistant starches (Champ, 2004).

Type	Main characteristic	Examples of RS or RS sources
RS 1	Physically inaccessible starch	Grain legumes (beans, lentils)
RS 2	Resistant starch granules	Unripe banana, raw potatoes
RS 3	Retrograded starch	Cooked and cooled potatoes, stale bread, Novelose 330
RS 4	Chemically modified starch	Cross-bonded starches (with high degree of reticulation to common chemically modified starches)

II.3.4 Other Oligosaccharides

Some oligosaccharides are originated from a polysaccharide known as pectin. This type of oligosaccharide is called pectic-oligosaccharide (POS). They are based on the extension of galacturonic acid (homogalacturonan) or rhamnose (rhamnogalacturonan I). The carboxyl groups may be substituted with methyl esterification, and the structure can be acetylated at C2 or C3. Various types of sugars (e.g., arabinose, galactose, and xylose) or ferulic acid are linked to the side chains. Their structures vary significantly depending on the sources of POSs (Davani-Davari, 2019).

II.3.5 Non-Carbohydrate Oligosaccharides

Although carbohydrates are more likely to meet the criteria of prebiotics definition, some compounds are not classified as carbohydrates but are recommended to be classified as prebiotics, such as cocoa-derived flavanols. In vivo and in vitro experiments demonstrate that flavanols can stimulate lactic acid bacteria (Davani-Davari, 2019).

II.4 Production of Prebiotics

Prebiotics play an important role in human health. They naturally exist in different dietary food products, including asparagus, sugar beet, garlic, chicory, onion, Jerusalem artichoke, wheat, honey, banana, barley, tomato, rye, soybean, human's and cow's milk, peas, beans, etc., and recently, seaweeds and microalgae. Because of their low concentration in foods, they are manufactured on industrial large scales. Some of the prebiotics are produced by using lactose, sucrose, and starch as raw material. Since most prebiotics are classified as GOS and FOS on an industrial scale (Figure 6), there are many relevant studies on their production (Davani-Davari, 2019).

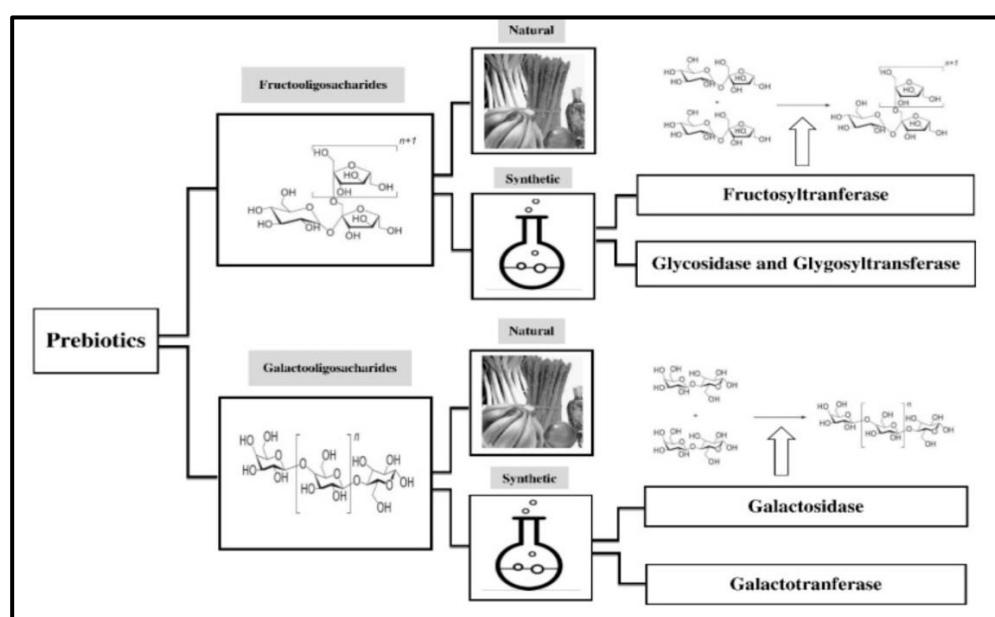


Figure 6: Sources and production of major prebiotics (Davani-Davari, 2019).

II.5 Food rich in prebiotics

Foods rich in prebiotics are grouped into several families. The [Table](#) below presents the different categories of foods containing prebiotics:

Table 5: Food rich in prebiotics (Charalampopoulos et Rastall, 2012).

Food rich in prebiotics		
Vegetables	Garlic+++ Artichoke+++ Asparagus++ Beet+ Broccoli+ Chestnut Chicory+++ Cabbage+ Shallot	Endive+ Fennel Green bean Onion++ Parsnip++ Dandelion++ Leek++ Tomato
Legumes	Lentil Flageolet Chickpea+	Black bean Red bean+
Fruits	Pineapple+ Banana + Quince red fruits (raspberries, strawberries, blueberries, blackberries) Dried fruits (grapes, apricots, prunes, figs, dates, persimmon)	Mango Nectarine+ Grapefruit+ Fishing+ Pear Apple
Seeds	Oats (gluten) Whole wheat++ (gluten) Flax	Barley (gluten) Rye++ (gluten) Chicory++
Roots	Parsnips	Jerusalem artichoke++
Oilseeds	Almonds Pistachio Nuts	

II.6 Food Applications of Prebiotics

Both important technological characteristics and interesting nutritional properties have been shown by Prebiotics. Some of them are found in fruits and vegetables and some can be processed industrially from renewable materials. In foods, they affect considerably by improving organoleptic characteristics, enhancing both taste and mouth feel. As functional food ingredients prebiotics must be chemically stable to processing treatments of food, such as high temperature, low pH, and Maillard reaction conditions. Therefore, if the prebiotic was degraded to its component mono- and disaccharides or chemically altered, a prebiotic would no longer provide selective stimulation of beneficial microorganisms and it was unavailable for bacterial metabolism. A study ascertained the effect on the prebiotic activity by processing conditions of commercial prebiotics using a prebiotic activity assay. The results showed that a significant decrease in prebiotic activity was only seen when heating at low pH, with one of the fructo-oligosaccharides (FOS) products being the least stable. Little change in activity was caused by other conditions. These results help us in selecting of prebiotics as functional food ingredients and predicting the extent to which processing affects prebiotic activity ([Franck, 2008](#)).

Presently known prebiotics and prebiotic candidates are non-digestible oligosaccharides. They are acquired either by extraction from plants (e.g., chicory inulin), enzymatic hydrolysis (e.g., oligo-fructose from inulin), or by synthesis (by trans-glycosylation reactions) from mono or disaccharides such as sucrose (fructooligosaccharides) or lactose (trans-galactosylated oligosaccharides or galactooligosaccharides). Among these prebiotics, inulin and oligosaccharides have been the most studied prebiotics and recognized as dietary fibers in most countries. Prebiotics can be used to offer a double benefit such as an improved organoleptic quality and a better-balanced nutritional composition. As fiber ingredients the use of inulin and nondigestible oligosaccharides is simple and often leads to enhance taste and texture. Specific colonic bacteria, such as bifidobacteria and lactobacilli species readily ferment these particular forms of dietary fibre and by raising their cell population with the parallel production of short-chain fatty acids. These acids, mainly butyrate, acetate, and propionate provide acidification of the bowel as well as metabolic energy for the host. Lactose occurs wholly in the milk of mammals and one might observe from a teleological point of view that lactose ingestion provides specific

health benefits beyond just being a source of energy to the suckling animal. It is used in different foods such as baby foods, cakes, biscuits, chocolates, sugar confectionary, and soups and sauces and the global demand has grown considerably over the previous 10 years to amount to approximately 500,000 t per annum. Currently, more and more prebiotics are used in different as an ingredient that helps in the growth of health-promoting colon microorganisms particularly probiotics, and offer additional health benefits (Bamigbade *et al.*, 2022). Food applications are illustrated in Table 6.

Table 6: Food Applications of Prebiotics (Franck, 2008).

Application	Functionality
Dairy products (yogurts, cheeses, desserts, drinks)	Fat or sugar replacement, body and mouthfeel, foam stabilization, fiber, and prebiotic
Frozen desserts	Fat or sugar replacement, texture, and mouthfeel, melting behavior
Fruit preparations	Sugar replacement, synergy with intense sweeteners, body, and mouthfeel, fiber, and prebiotic
Breakfast cereals and extruded snacks	Sugar replacement, crispiness and expansion, fibre, and prebiotic
Baked goods and breads	Sugar replacement, moisture retention, fibre, and prebiotic
Fillings	Fat or sugar replacement, texture, and Mouthfeel
Tablets and confectionery	Sugar replacement, fibre, and prebiotic
Chocolate	Sugar replacement, heat resistance and fibre
Dietetic products and meal replacers	Fat or sugar replacement, synergy with intense sweeteners, body and mouthfeel, fiber, and prebiotic
Table spreads and butter products	Fat replacement, texture and spreadability, stability, fiber, and prebiotic

Salad dressings	Fat replacement, mouthfeel , and body
Meat products	Fat replacement, texture and stability, and fiber

II.7 Mechanism of action of prebiotics

In recent decades, several studies have underlined the health benefits of prebiotics, including effects on the gastrointestinal tract (i.e., the prevention of pathogen damage or immune system modulation, the improvement of gut barrier function, a reduction in the pathogenic bacteria population, the production of short-chain fatty acids, SCFAs), on the cardiovascular system (i.e., a reduction in blood lipid levels or effects on insulin resistance), on mental health (i.e., metabolites that influence brain function, energy and cognition) and on bone (i.e., mineral bioavailability), etc. Regarding the potential effects on the GI tract, non-digestible carbohydrates, such as prebiotics, can deeply modulate the composition and activity of intestinal microbiota ([Figure 7](#)). Generally, in the human intestine, the enzymes that hydrolyze the polymer bonds of these prebiotics are lacking, so they can resist small-intestinal digestion and reach the colon intact, where they undergo fermentation by beneficial bacteria, such as *Lactobacilli* and *Bifidobacteria*. As gut microbiota have been involved in the pathogenesis of several GI disorders, there is an increasing interest in dietary strategies to modulate microbiota. For this reason, research has focused on the use of prebiotics, since many of these polysaccharides can be metabolized by intestinal microbiota, leading to the production of SCFAs (including acetate, butyrate, and propionate). The action of prebiotics on microbial diversity in the colon is still under debate. Indeed, the few human studies that have been conducted provided contradictory observations regarding SCFA levels. An interesting study by Liu et al., carried out using healthy volunteers, found a significant decrease in butyrate producers' levels and an increase in *Bifidobacterium* levels, after the administration of FOS and GOS, which can be caused by high levels of lactic acid, responsible for creating an environment inhospitable for butyrate producers. A limitation of this study is that this intervention was conducted for 14 days, and longer intervention studies may be fundamental to better exploring the effects of prebiotic administration ([Markowiak et al., 2017](#)). A specific advantage of prebiotics is the growth of target microorganisms that, in turn, compete with species that are injurious to energy sources and exclude them by protecting or promoting the

production of beneficial fermentation substances, such as SCFAs, which have immunomodulatory properties, influencing toll-like receptor-4 signaling and the production of pro-inflammatory cytokines. Among many potential prebiotics assessed, only a few substrates, i.e., inulin, FOS, and GOS, have been validated using human studies. Nevertheless, fructans are known to be the main substrate of healthy microbes, GOS and lactulose, which seems to determine a major growth of *Lactobacilli* and *Bifidobacteria*, compared to inulin. Studies have shown that gastric acid secretion can promote the oxidation of lipids and other food substances. According to their studies, dietary antioxidants (including inulin) may play a role in preventing lipid peroxidation in the stomach. In general, dietary supplementation of inulin or oligo-fructose contributes to protection from oxidative stress, consequently preventing inflammatory reactions associated with oxidative stress ([Markowiak et al., 2017](#)).

Moreover, it is interesting to note that the process called “cross-feeding”, in which the metabolism of dietary fibers, mostly prebiotics, from some microbes indirectly stimulates the growth of other ones, and the products of fermentation become, in turn, a substrate for the growth of other bacteria.

For example, the products of fermentation of *Bifidobacteria* and *Lactobacilli*, the main utilizers of fructans, are lactate and acetate, which can be used as an energy source from other bacteria, including *Eubacterium*, *Roseburia*, and *Faecalibacterium*, that, in turn, produce butyrate. Thus, it has been shown that the dietary consumption of fructans is related to an increase in butyrate levels, even though the primary increases in bacteria, following the consumption of fructans, do not directly produce butyrate.

Briefly, the mechanisms of action known to date of fructans, GOS, and Lactulose are reported in this section, with a final focus on new interesting molecules, that are showing prebiotic effects ([Markowiak et al., 2017](#)).

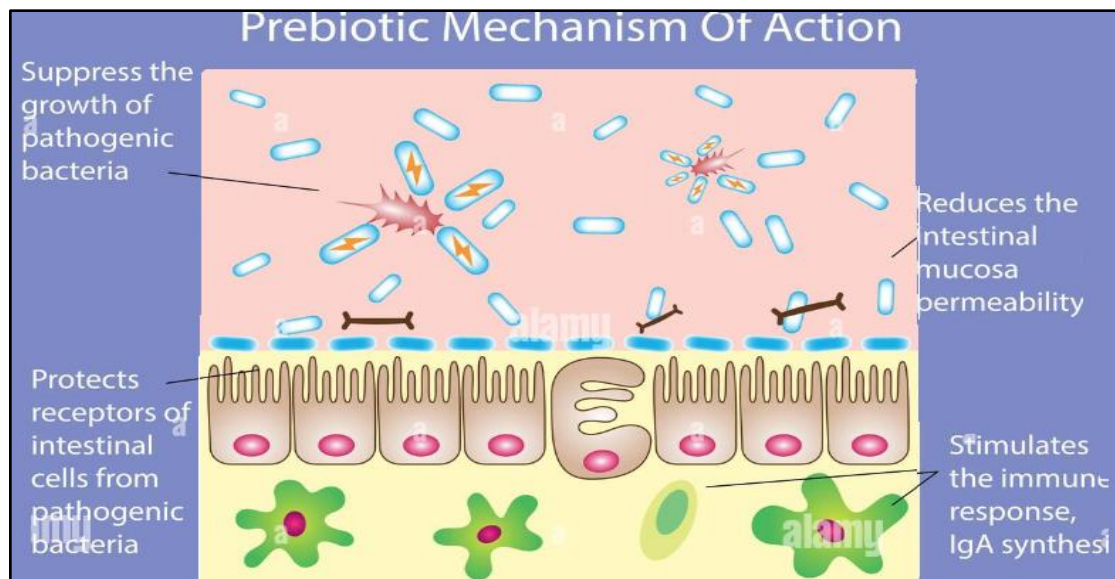


Figure 7: Mechanism of action of prebiotics (Markowiak *et al.*, 2017).

II.8 Safe use in food

Inulin, oligofructose, and galactooligosaccharides have been evaluated by the health authorities in most countries and have been confirmed as “safe”. Studies conducted to evaluate potential toxic effects in animals and humans revealed no adverse effects. The only side effects noted were spasmodic bloating, flatulence, and soft stools following ingestion of large quantities. These effects are comparable to those observed with all soluble dietary fibers. In practice, the user levels of prebiotics (typically 2–4g/serving) are far below the amounts at which intestinal discomfort occurs. The caloric value of non-digestible oligosaccharides has been estimated between 1 and 2 kcal/g (Franck, 2008).

III Pro/prebiotics security

Pre/probiotics are currently sending back a positive image of their benefits by being present in certain foods (especially dairy products), in food supplements, or even in drugs. Their conditions of placing on the market are defined according to their application: drug or food. They are therefore qualified as “health foods”, a border between traditional foods and medicines as shown in the Figure below which illustrates the place of pre/probiotic products as medicines or health foods (Cunningham *et al.*, 2021).

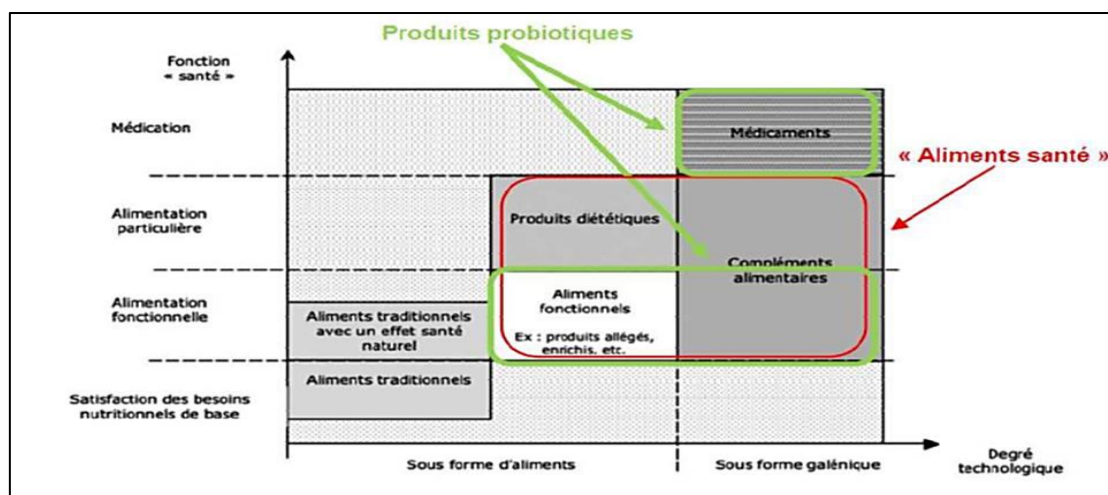


Figure 8: Current positioning of pro/prebiotics (Cunningham et al., 2021).

Chapter III

Dietary Fibers

I Definition

The most accepted definition would be proposed by AACC in 2001: “Dietary fiber is the remnants of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin, and associated plant substances. Dietary fiber exhibits one or more of either laxation (fecal bulking and softening; increased frequency; and/or regularity), blood cholesterol attenuation, and/or blood glucose attenuation” (Cui et Roberts, 2009).

II History

Since the early 1950s, various definitions of dietary fiber have been proposed by different countries and organizations.

In 1953, Hipsley defined dietary fiber as a term for nondigestible constituents that make up the plant cell wall, encompassing the “unavailable carbohydrate” that had been described much earlier by McCance and Lawrence. This definition was expanded by Trowell (1972) based on: (1) some hypotheses relating dietary fiber to health (“dietary fiber hypothesis”) including prevention of diverticular disease and colon cancer; (2) a concern for the adverse effects from consuming diets high in refined carbohydrates, termed The Saccharine Disease; and (3) the need to replace the term “crude fiber”. Based on the above concerns, dietary fiber was defined as “the skeletal remains of plant cells that are resistant to digestion (hydrolysis) by enzymes of man”.

In 1976, Trowell and colleagues recognized the inadequacy of the 1972 definition because it was not known at the time of the first definition that components of the plant cell other than the cell wall, including mucilages, storage polysaccharides, and algal polysaccharides, were not hydrolyzed by the alimentary enzymes. Therefore, dietary fiber was redefined.

In 1984, New Zealand Food Regulations defined dietary fiber as the “edible plant material not hydrolysed by the endogenous enzymes of the human digestive tract”; it was to be measured by the first method of analysis accepted by AOAC

(Institute of Medicine (US) Panel on the Definition of Dietary Fiber and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2001).

In 1987, the U.S. Food and Drug Administration (FDA) adopted AOAC method 985.29 for regulatory purposes to identify dietary fiber as a mixture of nonstarch polysaccharides, lignin, and some resistant starch. Related methods that isolated the same components as AOAC method 985.29 were developed independently and accepted by AOAC in subsequent years. These methods are also accepted by FDA.

In 1988, Health Canada published guidelines for novel fiber sources and food products containing them that can be labeled as a source of fiber in addition to those included in their 1985 definition. The rationale for these guidelines was that there were safety issues unique to novel sources of fiber, and if a product was represented as containing fiber, it should have the beneficial physiological effects associated with dietary fiber that the public expects. The guidelines indicate that both the safety and efficacy of the fiber source have to be established for the product to be identified as a source of dietary fiber in Canada, and this has to be done through experiments using human subjects. Three measures of efficacy were identified: (1) laxation, (2) normalization of blood lipid levels, and (3) attenuation of blood glucose responses. Detailed guidelines were later produced for the clinical studies required to assess laxation effects, as this was the physiological function most often used by the industry when seeking approval for a novel fiber source.

In 1995, a definition for dietary fiber appeared in the Codex Alimentarius Guidelines on Nutrition Labelling. The Codex allows the analytical methods AOAC 985.29 and AOAC 991.43 for measurement of dietary fiber in special foods and infant formula. There have been recent attempts to revise the Codex definition; however, there has not been a consensus on the inclusion of animals and other chemically characterized substances (Institute of Medicine (US) Panel on the Definition of Dietary Fiber and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2001).

Several countries in Europe published definitions for dietary fiber in the late 1980s and early 1990s, including Germany, Belgium, and Italy. For labeling purposes, Denmark, Finland, Norway, and Sweden have defined dietary fiber as edible material that cannot be degraded by human endogenous enzymes, as measured by AOAC

method 985.29. The issue regarding inclusion or exclusion of inulin and fructo-oligosaccharides has been handled somewhat differently by these countries in the absence of European Union regulation. In Denmark and Norway, fructans have been allowed to be included as dietary fiber on the food labels since 1995 and 1998, respectively (i.e., before the approval of AOAC method 997.08). Sweden made a similar decision in 1999, specifying AOAC method 997.08. In 1998, the Food Administration of Finland recommended that inulin and oligo-fructose be labeled separately and not be included as dietary fiber. In 2001, however, AOAC method 997.08 was added to 985.29 for analysis of dietary fiber, implying that inulin and oligo-fructose can now be labeled as dietary fiber in the four Nordic countries ([Institute of Medicine \(US\) Panel on the Definition of Dietary Fiber and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 2001](#)).

III Classification

Dietary fiber can be classified in many different ways such as structure and solubility. In terms of structure, polysaccharides are categorized into linear or nonlinear molecules. Based on solubility, they can be divided into soluble or insoluble dietary fiber. Insoluble dietary fiber consists mainly of cell wall components (e.g., cellulose, lignin, hemicellulose), while soluble dietary fiber consists of noncellulosic polysaccharides (e.g., pectin, gums, mucilage) ([Dai et Chau, 2017](#)).

The classification of dietary fiber components based on water solubility and fermentability is presented in [Table 7](#).

Table 7: Classification of dietary fiber components based on water solubility/fermentability ([Dhingra et al., 2012](#)).

Characteristic	Fiber component	Description	Main food sources
Water insoluble/Less fermented	Cellulose	Main structural component of plant cell wall. Insoluble in	Plants (vegetables, sugar beet, various brans)

		concentrated alkali, soluble in concentrated acid.	
	Hemicellulose	Cell wall polysaccharides, which contain backbone of β -1,4 glucosidic linkages. Soluble in dilute alkali.	Cereal grains
	Lignin	Non-carbohydrate cell wall component. Complex cross-linked phenyl propane polymer. Resists bacterial degradation.	Woody plants
Water soluble/Well fermented	Pectin	Components of primary cell wall with D-galacturonic acid as principal components. Generally water soluble and gel forming	Fruits, vegetables, legumes, sugar beet, potato
	Gums	Secreted at site of plant injury by	Leguminous seed plants (guar, locust

		specialized secretory cells. Food and pharmaceutical use.	bean), seaweed extracts (carrageenan, alginates), microbial gums (xanthan, gellan)
	Mucilages	Synthesized by plant, prevent desiccation of seed endosperm. Food industry use, hydrophilic, stabilizer.	Plant extracts (gum acacia, gum karaya, gum tragacanth)

III Dietary fiber content in various foods

Dietary fiber is naturally present in cereals, vegetables, fruits and nuts. The amount and composition of fiber differ from food to food. A fiber-rich diet is lower in energy density, often has a lower fat content, is larger in volume and is richer in micronutrients. This larger mass of food takes longer to eat and its presence in the stomach may bring a feeling of satiety sooner, although this feeling of fullness is short term. It is suggested that healthy adults should eat between 20 and 35 g of dietary fiber each day. Several non-starch foods provide up to 20–35 g of fiber/100 g dry weight and other those containing starch provide about 10 g/100 g of dry weight and the content of fiber of fruits and vegetables is 1.5–2.5 g/100 g of dry weight. Other studies reported, cereals to be one of the main sources of dietary fiber, contributing to about 50% of the fiber intake in western countries, 30–40% dietary fiber may come from vegetables, about 16% from fruits and the remaining 3% from other minor sources. Dietary fiber content of various food sources is presented in [Table 8 \(Dhingra et al., 2012\)](#).

Table 8: Dietary fiber content of various food sources ([Dhingra et al., 2012](#)).

Source	Dietary fiber (g/100 g edible portion)		
	Total	Insoluble	Soluble
Grains			
Barley	17.3	—	—
Corn	13.4	—	—
Oats	10.3	6.5	3.8
Rice (dry)	1.3	1.0	0.3
Rice (cooked)	0.7	0.7	0.0
Wheat (whole grain)	12.6	10.2	2.3
Wheat germ	14.0	12.9	1.1
Legumes and pulses			
Green beans	1.90	1.40	0.50
Soy	15.0	—	—
Peas, green frozen	3.5	3.2	0.3
Kidney beans, canned	6.3	4.7	1.6
Lentils, raw	11.4	10.3	1.1
Vegetables			
Potato, no skin	1.30	1.0	0.30
Bitter gourd	16.6	13.5	3.1
Beetroot	7.8	5.4	2.4
Fenugreek leaves	4.9	4.2	0.7
Ladyfinger	4.3	3.0	1.3

Spinach, raw	2.6	2.1	0.5
Turnips	2.0	1.5	0.5
Tomato, raw	1.2	0.8	0.4
Green onions, raw	2.2	2.2	0.0
Eggplant	6.6	5.3	1.3
Cucumbers, peeled	0.6	0.5	0.1
Cauliflower, raw	1.8	1.1	0.7
Celery, raw	1.5	1.0	0.5
Carrot, raw	2.5	2.30	0.20
Broccoli, raw	3.29	3.00	0.29
Fruits			
Apple, unpeeled	2.0	1.8	0.2
Kiwi	3.39	2.61	0.80
Mango	1.80	1.06	0.74
Pineapple	1.20	1.10	0.10
Pomegranate	0.60	0.49	0.11
Watermelon	0.50	0.30	0.20
Grapes	1.2	0.7	0.5
Oranges	1.8	0.7	1.1
Plums	1.6	0.7	0.9
Strawberry	2.2	1.3	0.9
Bananas	1.7	1.2	0.5
Peach	1.9	1.0	0.9
Pear	3.0	2.0	1.0

Nuts and seeds			
Almonds	11.20	10.10	1.10
Coconut, raw	9.0	8.5	0.5
Peanut, dry roasted	8.0	7.5	0.5
Cashew, oil roasted	6.0	—	—
Seasame seed	7.79	5.89	1.90
Flaxseed	22.33	10.15	12.18

IV Dietary fiber fate

The food chain of degradation of dietary fiber is shown in [Figure 9](#). The bacterial communities: Polysaccharide degraders, Oligo/monosaccharide degraders, or utilizers correspond to functional communities of microorganisms. These bring together several species of bacteria capable of ensuring the function of fiber degradation (fibrolytic), fermentation of oses (glycolytic), use of succinate, lactate (succinates lactates users) or formate or use of gaseous hydrogen (H₂) (hydrogenotrophs) ([Scott et al., 2008](#)).

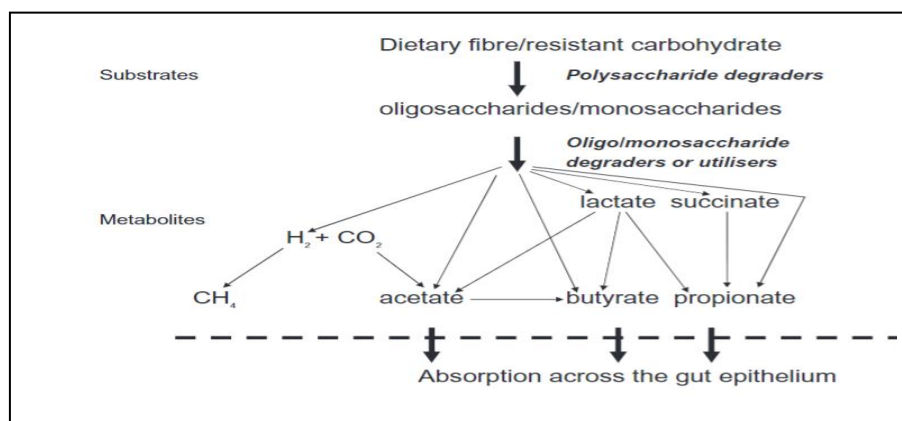


Figure 9: Diagram illustrating the sequential degradation of dietary substrates ([Scott et al., 2008](#)).

IV Benefits of dietary fiber

IV.1 Gastrointestinal health and constipation

The benefits of dietary fiber in the maintenance of GI health and prevention of constipation are well established. Dietary fiber (i) increase the retention of water in the colon which results in softer stools, (ii) increase fecal bacterial mass and nitrogen excretion, (iii) maintain a healthy colonic microbiota ecosystem by promoting the growth of beneficial bacteria such as bifidobacterial and lactobacilli, and (iv) increase the production of short chain fatty acids (SCFAs). SCFAs, such as acetate, butyrate, and propionate, are released from saccharolytic bacteria when the dietary fiber is fermented and have anti-inflammatory and immunomodulatory effects, enhancing the integrity of the gut barrier. Studies using dietary fiber blends demonstrated an improvement in constipation, an increase in daily bowel movements, and an improved stool consistency ([Snauwaert et al., 2023](#)).

IV.2 Uremic toxin production, inflammation, and oxidative stress

A high fiber intake has been linked to a lower production of gut-derived uremic toxins. Gut-derived uremic toxins are organic metabolites produced by the gut microbiota which accumulate in patients with progressive decline in kidney function due to decreased renal excretion and/or increased generation of these metabolites. The two most widely studied gut-derived uremic toxins are indoxylsulfate (IxS) and p-cresylsulfate (pCS), and their pro-inflammatory and pro-oxidative properties are well established in experimental studies. By inducing the production of reactive oxygen species, these gut-derived metabolites activate the nuclear factor-kappa B pathway leading to oxidative stress and the production of pro-inflammatory cytokines. IxS and pCS are mainly produced by proteolytic bacteria. The proteolytic and saccharolytic fermentation characteristics of the microbiome are related to nutrient availability, i.e. the balance between respectively carbohydrate (such as fiber) and undigested protein. Therefore, a higher intake of dietary fiber shifts a dominantly proteolytic microbial metabolism to a saccharolytic one, characterized by the incorporation of amino acids to bacterial growth and the use of dietary fiber as a source of energy, rather than being metabolised in precursors of uremic solutes. Besides a decrease in gut-derived uremic toxin production, a high dietary fiber intake has been shown to decrease inflammation and oxidative stress by improving the

integrity of the gut barrier through SCFA production with subsequent decrease in bacterial translocation ([Snauwaert et al., 2023](#)).

IV.3 Cardiovascular diseases, risk factors, and all-cause mortality

It is hypothesized that dietary fiber may protect against cardiovascular morbidity and mortality by attenuating risk factors such as metabolic syndrome, diabetes mellitus, hyperlipidemia, and obesity. This protection might be achieved through decreasing gut-derived uremic toxin production, decreasing inflammation and oxidative stress, and stimulating SCFA production and translocation into the blood which in turn results in improved insulin sensitivity and a more alkaline environment. A meta-analysis of seventeen cohort studies published in 2013 by Threapleton et al. confirmed the association between low dietary fiber consumption and increased risk of cardiovascular disease in healthy adults. Similarly, a meta-analysis of seven prospective cohort studies observed an 11% reduction in mortality risk for each 10 g/day increments of dietary fiber consumed ([Snauwaert et al., 2023](#)).

IV.4 Progression of kidney function

An intriguing hypothesis is that an optimal intake of dietary fiber may protect against Chronic Kidney Disease (CKD) progression. The latter has been addressed in two trials in adults with diabetes: dietary fiber supplementation (10 g of a soluble fiber supplement added to the subject's usual diet) did not have any beneficial effects on changes in estimated glomerular filtration rate or microalbuminuria. A vegetarian diet rich in fiber was associated with a reduction in albuminuria in adults with types 1 and 2 diabetes, perhaps as a consequence of better glycemic control. In another meta-analysis based on 14 trials on 143 patients with CKD. Some studies demonstrated that dietary fiber supplementation significantly reduced serum urea and creatinine levels compared with placebo. Further studies are needed to better explore the possible nephron-protective role of fiber in CKD, particularly in non-diabetic patients and children ([Snauwaert et al., 2023](#)).

V Methods of analysis of dietary fiber

V.1 Enzymic-gravimetric methods

It involves enzymic treatments for starch and protein removal, precipitation of soluble fiber components by aqueous ethanol, isolation and weighing of the dietary fiber residue, and correction for protein and ash in the residue ([Dhingra et al., 2012](#)).

V.2 Enzymic-chemical methods

This method involves enzymic removal of starch, and precipitation with 80% (v/v) ethanol to separate the soluble dietary fiber polysaccharides from low-molecular-weight sugars and starch hydrolysis products ([Dhingra et al., 2012](#)).

VI Nutritional claims

VI. 1 Source of fiber

A claim that a food is a source of fiber, or any other claim likely to have the same meaning for the consumer, may only be made if the product contains at least 3 g of fiber per 100 g or at least 1.5 g of fiber per 100 kcal ([Gębski et al., 2019](#)).

VII. Rich in fiber

A claim that a food is rich in fiber, or any other claim likely to have the same meaning for the consumer, may only be made if the product contains at least 6 g of fiber per 100 g or at least 3 g of fiber per 100 kcal ([Gębski et al., 2019](#)).

Chapter IV

Polyphenols

I Definition

The term polyphenol or phenolic compounds replaces the old term vegetable tannin: the presence of one or more benzene rings (Benzenes), bearing one or more hydroxyl (OH) functions (Badria *et al.*, 2022).

II Historical background of polyphenols

Polyphenols have a rich history and were originally known as "vegetable tannins". These natural products were used in various plant extracts to convert animal skins into leather. These compounds derived from plants were later referred to as polyphenols (Belščak-Cvitanović *et al.*, 2018).

Since the compounds found in tanning plant extracts were in high demand in the leather industry, significant efforts were made from the early 20th century onwards to study the chemistry of these extracts. The aim was to better understand the polyphenolic constituents that make up their structure. Research on plant polyphenols shifted gears after 1945, as the discovery of paper chromatography and more and more advanced analytical techniques made it possible to separate innumerable individual constituents. In 1957, an industrial chemist, Theodore White, pointed out that the term "tannin" should strictly refer to plant polyphenolic materials having molecular masses between 500 and 3000Da and a sufficiently large number of phenolic groups to be capable of forming hydrogen-bonded cross-linked structures with collagen molecules (the act of tanning). The Plant Phenolic Group was founded in 1957 by E. C. Bate-Smith and Tony Swain due to the surge in polyphenols research. In 1962, Swain and Bate-Smith proposed a definition of plant polyphenols as "water-soluble phenolic compounds with molecular weights ranging from 500 to 3000 Da. In addition to giving the usual phenolic reactions, they possess unique properties such as the ability to precipitate proteins like alkaloids and gelatin from solutions" (Belščak-Cvitanović *et al.*, 2018).

Edwin Haslam later refined the definition at the molecular level, expanding on the work of Bate-Smith, Swain, and White. According to his revised definition, the term "polyphenols" should be used to describe water-soluble plant phenolic compounds with molecular masses ranging from 500 to 3000-4000 Da. These compounds should have between 12 to 16 phenolic hydroxy groups on 5 to 7 aromatic

rings per 1000 Da of relative molecular mass. The original classification of plant phenolics as “polyphenols” or not was. The original classification of plant phenolics as “polyphenols” or not was based primarily on their ability to complex with other biomolecules by White *et al.* based primarily on their ability to complex with other biomolecules by White *et al.* The initial definition of polyphenolic substances was not precise enough, as it only classified them into three groups of natural products containing polyhydroxyphenyl, according to certain restrictions. To improve the accuracy of the definition and taking into account chemical considerations, researchers proposed a new definition of polyphenols: "Polyphenols" should refer specifically to plant secondary metabolites derived from the shikimate-derived phenylpropanoid and/or the polyketide pathway, possessing more than one phenolic ring and having no nitrogen-based functional group in their most basic structural expression (Belščak-Cvitanović *et al.*, 2018).

III Pathways of biosynthesis of polyphenols

Despite the exceptional diversity of phenolic compound structures, there are only two main pathways of their biosynthesis: shikimic and acetate-malonate. In higher plants, the shikimic pathway occupies one of the central places in cellular metabolism (Babenko *et al.*, 2019).

III.1 Shikimic acid pathway

The shikimate biosynthesis pathway provides precursors for aromatic molecules in bacteria, fungi, apicomplexan, and plants, but not in animals. This pathway is a crucial connection between primary and secondary metabolism in higher plants. In microorganisms, the shikimate pathway produces the essential aromatic amino acids L-phenylalanine, L-tyrosine, and L-tryptophan, which serve as the building blocks for protein biosynthesis (Figure). But in plants, these aromatic amino acids are not only crucial components of protein biosynthesis; they also serve as precursors for diverse secondary metabolites that are important for plant growth. These secondary metabolites are called phenolic compounds and are synthesized when needed by the plant. The aromatic phenolic compounds synthesized from L-Phe and L-Tyr are cinnamic acids and esters, coumarins, phenylpropenes, chromones (C6-C3), stilbenes, anthraquinones (C6-C2-C6), chalcones, flavonoids, isoflavonoids, neoflavonoids (C6-C3-C6), and their dimers and trimers, respectively (C6-C3-C6)_{2,3},

lignans, neolignans (C6-C3)₂, lignans (C6-C3)_n, aromatic polyketides, or diphenylheptanoids (C6-C7-C6). L-Trp is a precursor of alkaloids in the secondary metabolism ([Santos-Sánchez et al., 2018](#)).

III.2 Acetate-malonate pathway

For the synthesis of polyphenol chemicals, the acetate-malonate route (Figure) is common of fungus, lichens, and microorganisms. Due to its inclusion, blooming plants create flavonoids, of which the phenylpropanoid ring is produced through the acetate-malonate pathway. Starting with coumarin-CoA and three malonyl-CoA molecules, their condensation yields a chalcone. The reaction is catalyzed by chalcone synthase, which is the key enzyme in the biosynthesis pathway of flavonoids and polyphenols. The formed tetraoxychalcone is converted to flavanone naringenin, which then serves as the precursor for almost all other classes of flavonoids, except for chalcones and dihydrochalcones ([Figure 10](#)). Flavonoids, derivatives of 3-phenylchroman, give rise to numerous isoflavonoids (derivatives of 2-phenylchroman). Accumulation of intermediates and end-products of the flavonoid pathway occurs at their site of synthesis ([Babenko et al., 2019](#)).

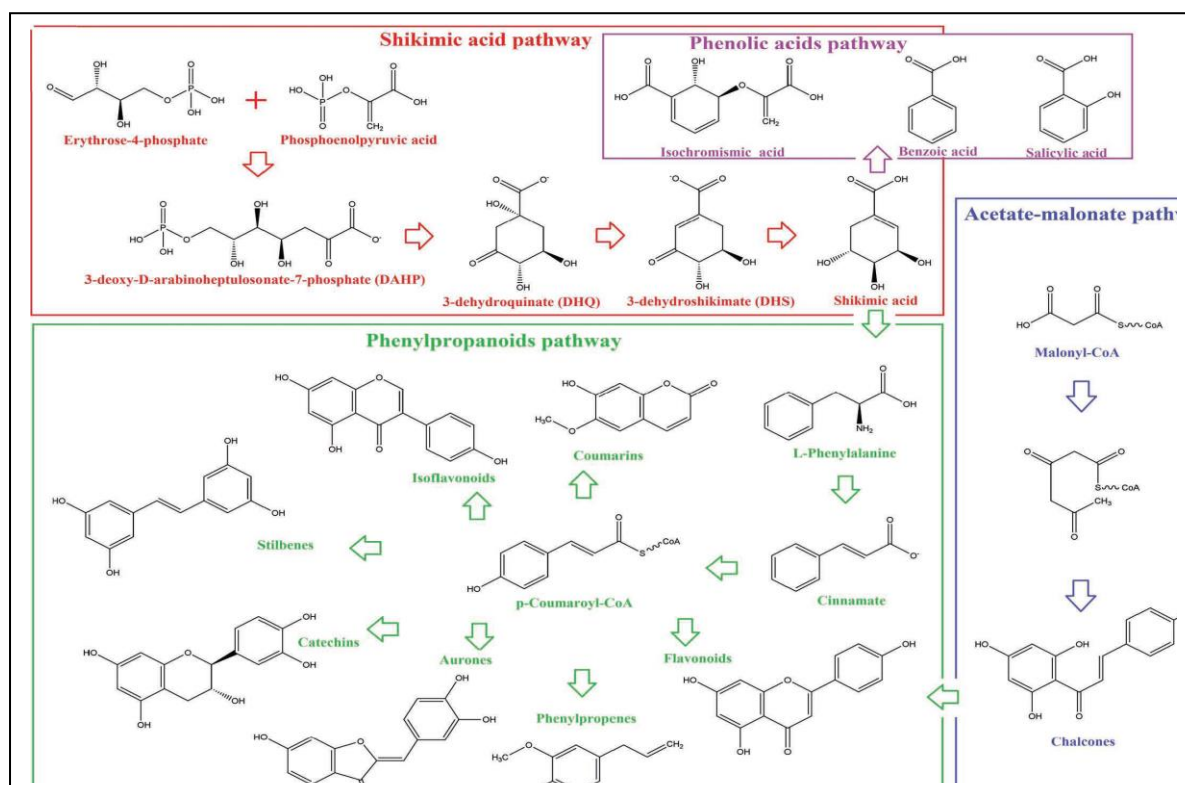


Figure 10: Shikimic acid pathway ([Babenko et al., 2019](#)).

IV Classification of polyphenols

Phenolic compounds known as polyphenols have been categorized based on their biological function, natural distribution, chemical structure, and origin. With regards to their occurrence in nature, they can be classified into three categories: compounds that are found in low quantities (such as simple phenols, pyrocatechol, hydroquinone, resorcinol, aldehydes that are obtained from benzoic acids and are present in essential oils, such as vanillin), compounds that are widely distributed (which include flavonoids and their derivatives, coumarins, and phenolic acids such as benzoic and cinnamic acid and their derivatives), and polymers (tannins and lignins) ([Figure 11](#)) ([Vuolo et al., 2019](#)).

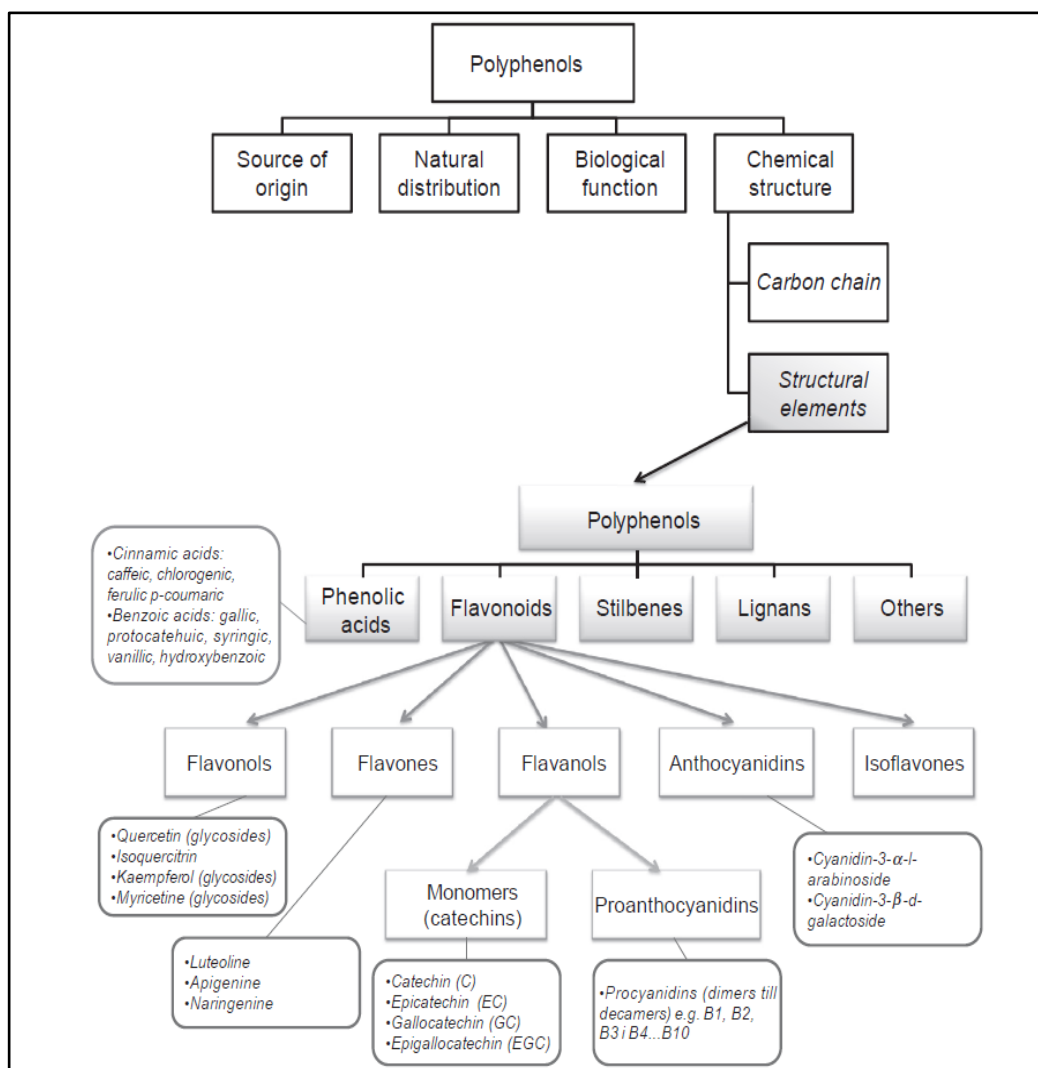


Figure 11: Different classifications of plant polyphenols and polyphenolic classes based on the number of phenol rings and their structural elements (Vuolo et al., 2019).

Based on the basic carbon structure, there is a wide range of phenolic compounds that occur in nature. Currently, more than 8000 phenolic compound structures are known (Pandey et al., 2009). They can be categorized into several classes as shown in Table 9.

Table 9: Phenolic compounds in plants (Sun et al., 2023).

Class	Structure
Simple phenolics, benzoquinones	C ₆
Hydroxybenzoic acids	C ₆ –C ₁
Hydroxycinnamic acids, phenylpropanoids	C ₆ –C ₃
Acetophenones, phenylacetic acids	C ₆ –C ₂
Xanthones	C ₆ –C ₁ –C ₆
Stilbenes, anthraquinones	C ₆ –C ₂ –C ₆
Flavonoids, isoflavonoids	C ₆ –C ₃ –C ₆
Lignans, neolignans	(C ₆ –C ₃) ₂
Lignins	(C ₆ –C ₃) _n
Condensed tannins (proanthocyanidins or flavolans)	(C ₆ –C ₃ –C ₆) _n

V Bioavailability of polyphenols

The main data on the bioavailability of dietary polyphenols are summarized as follows:

V.1 Absorption within the stomach

Only anthocyanins and some hydroxycinnamic acids in bound form such as chlorogenic acid (phenol acid) can be absorbed directly from the stomach (Pandy et al., 2009).

V.2 Absorption from the small intestine

Polyphenol aglycones (e.g. flavanols) and O- β -D-glucosides (complex phenolic compound) can be absorbed in the small intestine, the first ones by passive diffusion, the latter by two mechanisms: Direct absorption of glucosides via the sodium-dependent glucose transporter (glucose and galactose transporters found in the intestinal mucosa of the small intestine (SGLT1) and the proximal tubules of the nephrons (SGLT2 and SGLT1) for renal glucose reabsorption. It is followed by the hydrolysis of glucosides in the cytosol by a cytosolic β -glucosidase (Figure 12) (Scheepens et al., 2010).

Another mechanism involving lactase phloridzin hydrolase, an intestinal brush border glucosidase that catalyzes the extracellular hydrolysis of glucosides, has also been demonstrated. It is followed by the absorption of the aglycone thus released by passive diffusion.

V.3 Absorption from the colon

Polyphenols not absorbed in the stomach and small intestine reach the colon, then are catabolized by the colonic microflora, having various enzymatic activities, before being absorbed. In particular, rutin (flavonoid), a common dietary flavonol (quercetin (3-O- β -D-(L-rhamnosyl- α -1,6-D-glucoside)), must be hydrolyzed by enzymes bacteria before being absorbed in the colon and reaching the blood circulation (in the form of quercetin conjugates) (Figure 12) (Scheepens et al., 2010).

V.4 Conjugation at the liver level

The absorbed polyphenol fraction undergoes conjugation in enterocytes and hepatocytes. Thus the circulating forms are typically glucuronides, sulfates (SO_4^{2-}), and sulfoglucuronides. In addition, catechol groups, important for antioxidant activity, are partially methylated by catechol O-methyltransferase in the live (Figure 12) (Scheepens *et al.*, 2010).

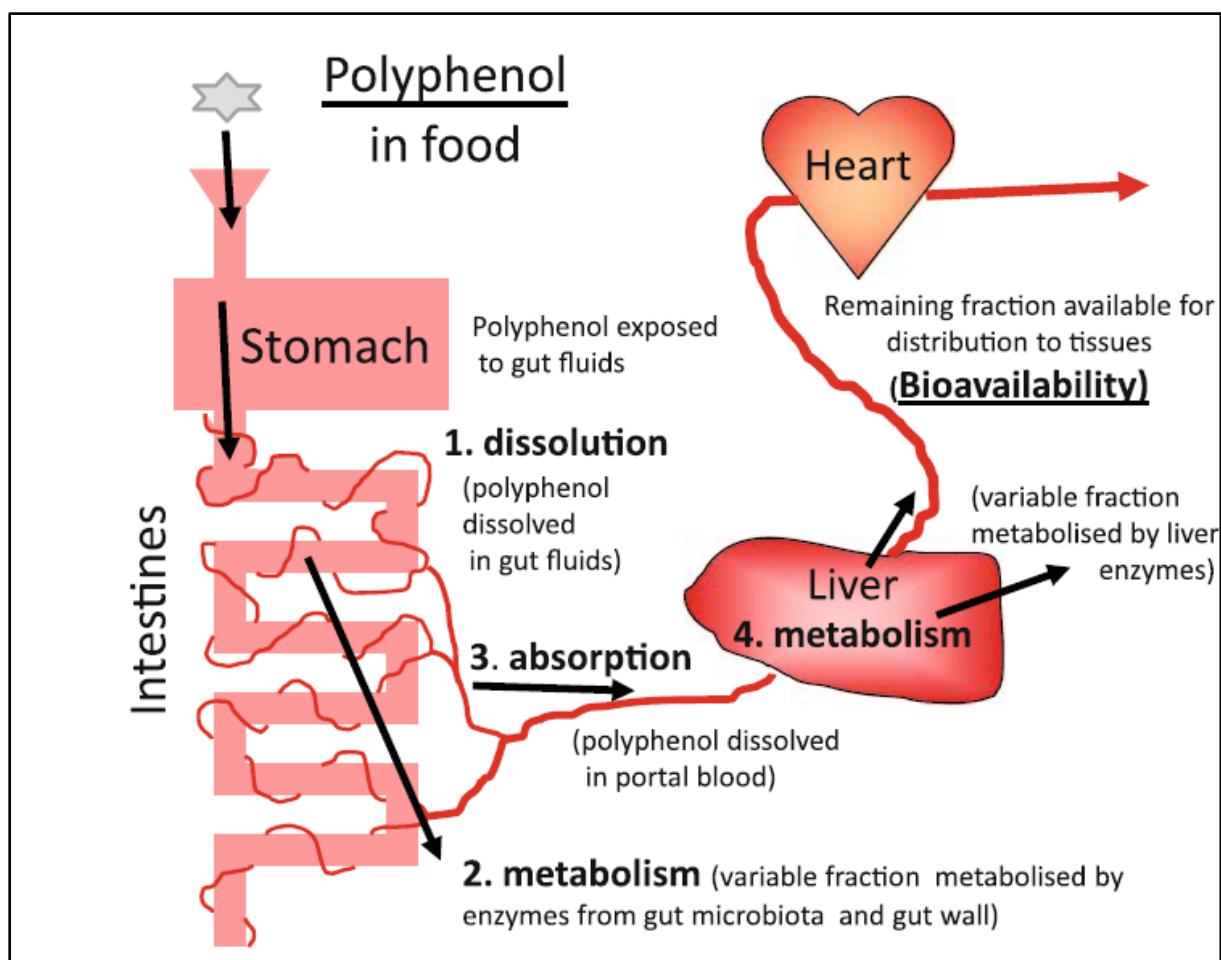


Figure 12: Schematic representation of the major processes governing polyphenol bioavailability (Scheepens *et al.*, 2010).

VI Mechanism of action of polyphenols

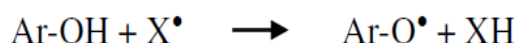
Phenolic compounds exert antioxidant activity via several mechanisms:

1. Direct trapping of ROS (reactive oxygen species)
2. Inhibition of ROS-generating enzymes
3. Chelation of transition metal ions, responsible for the production of ROS

4. Induction of the biosynthesis of antioxidant enzymes (Kruk et al., 2022).

VI.1 Trapping of free radicals

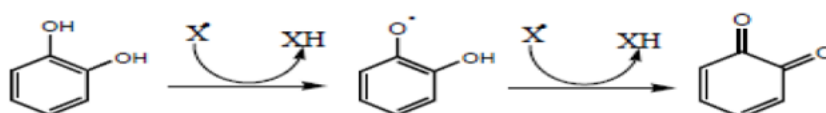
Polyphenols (Ar-OH) are capable of rapidly reducing oxidizing free radicals such as superoxide ($\text{O}_2^\bullet$), peroxy ($\text{ROO}^\bullet$), alkoxy ($\text{RO}^\bullet$) and hydroxyl ($\text{OH}^\bullet$) by transfer of hydrogen:



$\text{X}^\bullet$: Represents one of the EROs mentioned above;

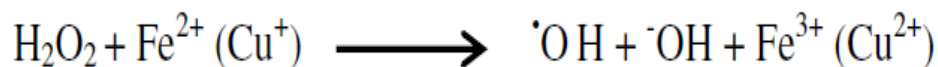
$\text{Ar-O}^\bullet$: aryloxy radical which, if it is a catechol nucleus, can react with another radical to form a more stable o-quinone

The reaction below shows the trapping of ROS ($\text{X}^\bullet$) by a catechol nucleus (Kruk et al., 2022).



VI.2 Chelation of metal ions

The inhibition of the formation of free radicals by the chelation of transition metals such as iron (Fe^{2+}) and copper (Cu^+) responsible for the production of the $\text{OH}^\bullet$ radical by the reduction of H_2O_2 during the Fenton reaction:



Polyphenols abundant in food, notably flavonoids, sequester these metal ions at different sites (Figure 13).

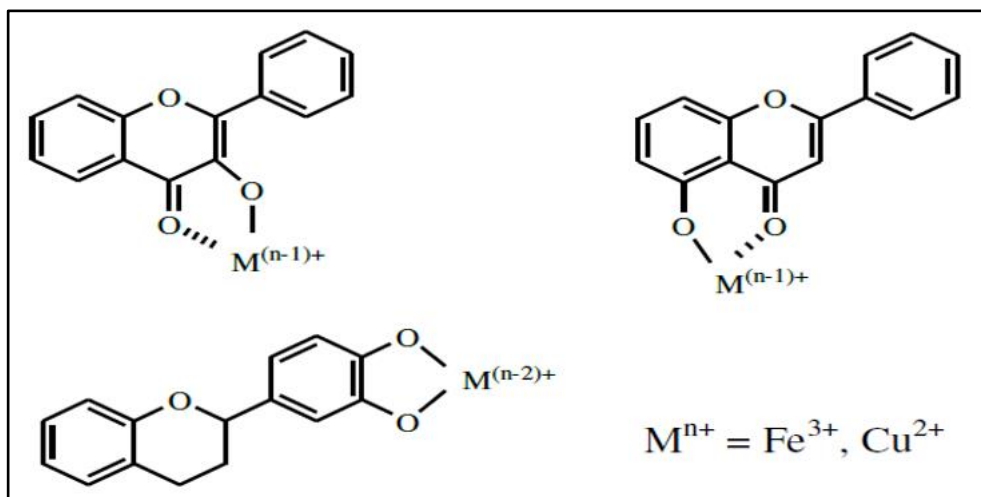


Figure 13: Sites of chelation of metal ions by flavonoids (Kasprzak et al., 2015).

VI.3 Inhibition of enzymes

The xanthine oxidase (XO), liver enzyme involved in gout disease, and which catalyzes a purine catabolism reaction, by transforming hypoxanthine into xanthine and xanthine into uric acid (Figure 14) (Masuoka et al., 2018).

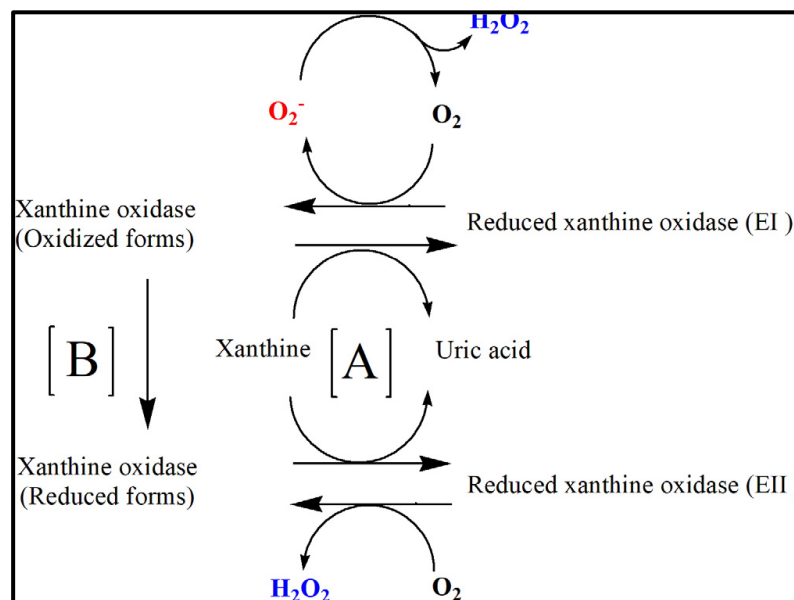


Figure 14: Proposed mechanism of the O_2^- or hydrogen peroxide generation catalyzed by xanthine oxidase. [A]: Uric acid formation inhibitors, [B]: reducing agents of the enzyme (molecule) (Masuoka et al., 2018).

VII Polyphenol compounds of common foods

About 10,000 phenolic compounds of plant origin have been identified, ranging from simple molecules to highly polymerized compounds. [Table 10](#) represents their occurrence in common foods ([Rossi et al., 2008](#)).

Table 10: The most studied polyphenols and their food source ([Rossi et al., 2008](#)).

Polyphenols		
Class	Compound	Natural sources
Non flavonoids	Curcumin	Turmeric
	Resveratrol	Grapes
Anthocyanins	Cyanidin	Berries
	Petunidin	Strawberries, tea
Flavones	Apigenin	Apple skins
	Kaempferol	Broccoli
	Myricetin	Fruits peels
	Quercetin	Lettuce, olives and onions
Flavanones	Fisetin	Citrus fruit
	Hesperitin	Citrus peel
Catechins	Catechin	Red wine
	Epigallocatechin-3-gallate	Tea

Chapter V

Alkaloids and

Terpenes

I Alkaloids

I.1 Definition

Plant alkaloids, one of the largest groups of natural products, are a highly diverse group of chemical compounds. The group of alkaloids consists of structurally diverse and biologically unrelated molecules, many of which have potent pharmacological effects. Plant alkaloids include well-known drugs such as narcotic analgesics morphine and codeine, apomorphine (a derivative of morphine) used in Parkinson's disease, muscle relaxant papaverine, and antimicrobial agents sanguinarine and berberine. It is worth noting that many plant compounds have been used in the development of powerful anti-cancer drugs (Bribi, 2018).

I.2 Historical background of alkaloids

The term alkaloid was coined by W. Meisner in the early 19th century to describe natural substances that react like bases, that is, like alkalis (from the Arabic word alkali, meaning soda, and the Greek word eidos, meaning appearance). Alkaloids are nitrogen-containing natural metabolites that lack a precise definition, making it challenging to distinguish them from other similar compounds (Bribi, 2018).

Plants containing alkaloids have been used for medicinal purposes for at least 3000 years. However, it wasn't until the 19th century that researchers were able to isolate and characterize the compounds responsible for their activity. Recently, several challenging chemical problems were solved and their structures determined. The percolation process introduced in the nineteenth century coincided with the first isolations of alkaloids from drugs containing them.

Although alkaloids were discovered during the early 19th century, it wasn't until then that therapeutically active substances were isolated. Opium, which has been used for centuries for its analgesic and narcotic properties, was the first crude drug to be chemically investigated. In 1803, Derosne extracted a mixture of narcotine and morphine from opium and was the first to isolate a vegetable alkali (Schiff, 2002).

In 1806, Serturner identified the alkaline nature of the somniferous principle in opium and later named it morphine. Shortly afterward, between 1817 and 1820,

two French pharmacists, Pelletier and Caventou, discovered an impressive series of active compounds: caffeine, emetine from ipecac, strychnine from nuxvomica, quinine and cinchonine from cinchona bark, followed a little later by coniine. Today, advanced NMR techniques and X-ray diffraction spectrometry allow for the elucidation of complex structures. Due to their significance, numerous attempts have been made to produce alkaloids in plant tissue culture. Approximately 30 alkaloids constitute the majority of commercial interest, particularly as medicines, flavorings, or poisons (Bribi, 2018).

I.3 Classification of alkaloids

One of the most fascinating and significant subjects in bioorganic chemistry is the chemistry of alkaloids. The term "alkaloid" was first used to describe nitrogen-containing compounds of plant origin that had a notably basic nature, but now it has much broader applications. Alkaloids are a diverse group of naturally occurring organic compounds that contain nitrogen and are produced by plants, microorganisms, marine organisms, and fungi. Compared to other natural compounds, alkaloids have an almost unlimited range of structural frameworks, and their molecules contain an N atom, making them highly variable. Alkaloids are classified based on distinct characteristics like natural sources or chemical nature. However, the most accurate and widely accepted classification is based on their principal C-N skeleton structure. According to this classification, alkaloids can be grouped into various categories (Figure 15) that include pyrrolidine, pyridine, quinoline, isoquinoline, indole, quinazoline, steroidal, diterpenoid, and other alkaloids. Each of these groups is subdivided into several subgroups depending on the structural features of its representatives.

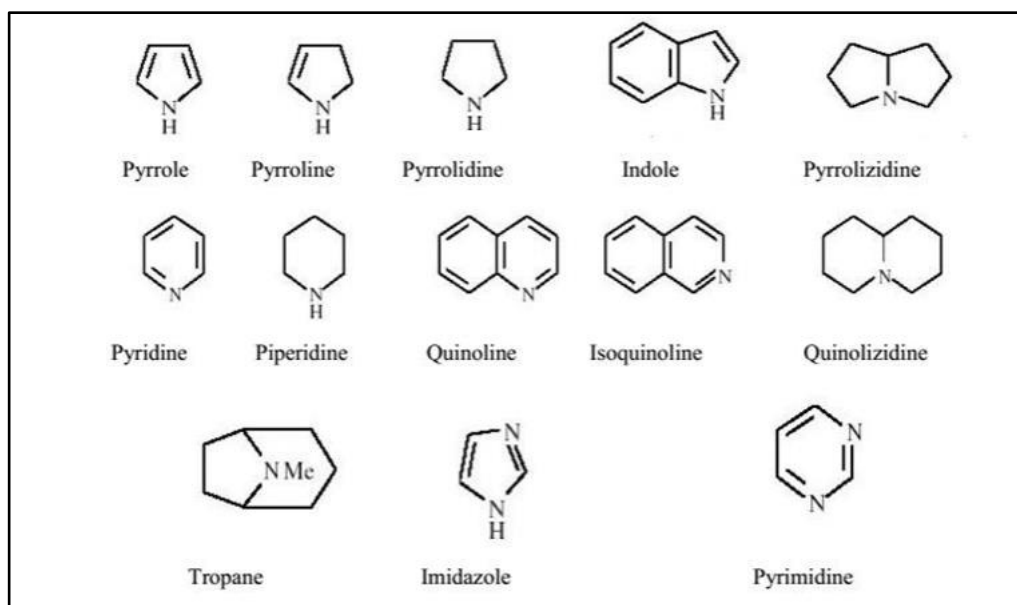


Figure 15: Heterocyclic structure of the skeleton constituting the group of alkaloids (Bribi, 2018).

I.4 Pathways of biosynthesis of alkaloids

The initial stages of an alkaloid pathway are essential, as they serve as the entrance to a unique chemical territory. These stages are essential metabolically, as they redirect flow from primary to specialized metabolism. They are also significant chemically and enzymatically, as they involve the creation of a new molecular structure. Moreover, they have a fundamental role in the development of these pathways (Figure 16).

The initial stages of complex alkaloid biosynthesis usually involve four steps as follows:

1. Collection of an amine precursor.
2. Collection of an aldehyde precursor.
3. Creation of an iminium cation.
4. A Mannich-like reaction.

This final step is often referred to as the "scaffold-forming," signature, or first-commitment step in a pathway.

I.4.1 Amine accumulation

Plants use amino acid metabolism to produce nitrogen in complex alkaloids. However, most alkaloids don't use amino acids directly but rather rely on primary amine derivatives. Green plants contain primary amines that play specialized roles in their development, reproduction, and response to stress. These amines accumulate in concentrations beyond what is typical to enable flux to be directed into alkaloid metabolism without disrupting existing pathways. Such accumulation often requires changes to metabolism, which are brought about by gene duplication. Recently, the contribution of primary metabolism to plant chemical diversity has been reviewed.

For essential or abundant amines, such as putrescine or tyramine, accumulation may result from higher expression or duplication of a pre-existing gene without modification to the enzyme's substrate scope. Accumulation of amines that are typically at low concentrations or absent, such as cadaverine or homospermidine, may require the evolution of new enzyme activity through modification of substrate scope. Other possible routes to the accumulation of amines include boosting the concentration of amino acid precursors or reducing degradative catabolic pathways. The amines contributing to alkaloid biosynthesis can be split into two categories: polyamines, derived from lysine, arginine, and ornithine; and aromatic amines, derived from tryptophan and tyrosine ([Figure 16](#)) ([Lichman, 2021](#)).

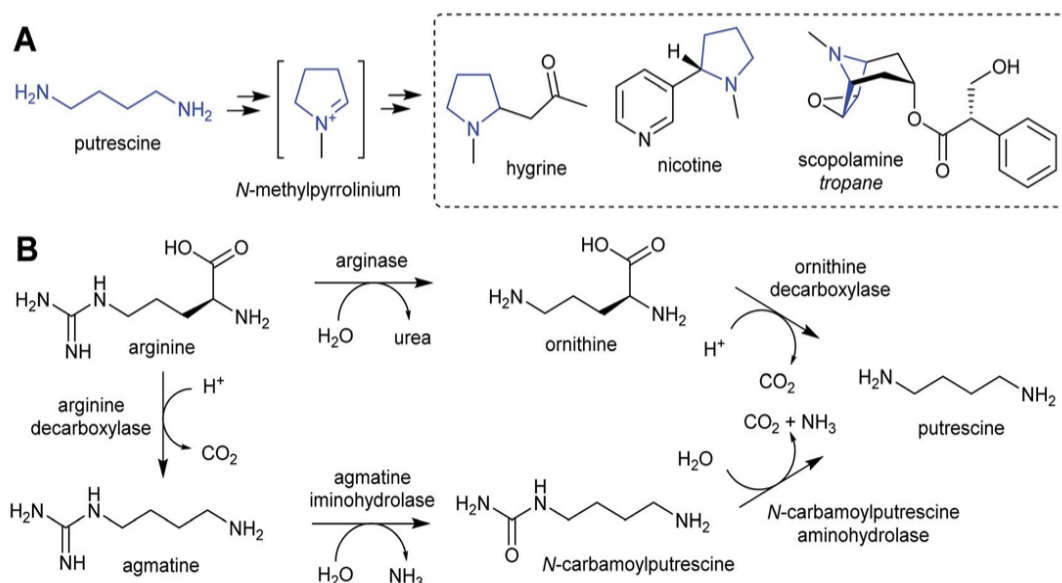


Figure 16: Putrescine-derived alkaloids. (A) Putrescine-derived alkaloids (moiety originating from putrescine highlighted in blue). (B) Biosynthesis of putrescine from ornithine or arginine (Lichman, 2021).

I.4.2 Aldehyde accumulation

Complex alkaloid biosynthesis typically requires an aldehyde precursor. The aldehyde must accumulate to a high concentration and near the amine precursor to enable the next stage of general alkaloid biosynthesis, the formation of a reactive iminium intermediate. For polyamine-derived alkaloids, this aldehyde is formed through oxidative deamination of a terminal amine on the polyamine precursor, forming an amino aldehyde. In aromatic amine-derived alkaloids, the aldehyde moiety is present on a distinct molecule, typically as a secondary metabolite (Lichman, 2021).

I.4.3 Iminium Formation

A primary amine can condense reversibly with an aldehyde to form an electrophilic iminium cation, also known as a Schiff base (Scheme). In water, the equilibrium is typically on the side of the reactants, and the concentration of iminium ions is low. The position of the equilibrium can shift towards the products if the iminium is cyclic or conjugated. Surprisingly, the ratio of neutral imine to charged iminium in solution is largely dependent on the pK_aH of the amine precursor ($\sim 9\text{--}10$), rather than the pK_aH of the iminium itself (~ 7), meaning that at physiologically relevant pHs the reactive iminium will be more abundant than the imine. Iminiums are highly

electrophilic and form rapidly, so even when they not at high concentrations, the product of their reaction with a nucleophile can accumulate, provided the reaction is irreversible. This reaction of an iminium with a nucleophile is described here as a Mannich-like reaction, which quenches the reactivity of both the iminium and nucleophile ([Lichman, 2021](#)).

II Terpenes

II.1 Introduction and Historical Background

The history of terpenes dates back to various civilizations. The essential oils were used in ancient Egypt for various ceremonies. Camphor was introduced by Arabs around the 11th century. The process of distillation of oils from rosemary and sage was described by Arnaud de Villanosa (1st century). Analyses of oils obtained from plants started by JJ Houston in 1818. Dumas proposed the name “Terpene” derived from turpentine. In 1887, Wallah O proposed that the isoprenoid unit (5C) is present always in terpenes. The structure of beta carotene from carrot was isolated by Wackenrodter and its correct molecular formula was determined by Will Statter (Kandi et al., 2015).

II.2 Molecular Structure of Terpenes

Terpenes are polymers of five-carbon hydrocarbon isoprene and are miscellaneous lipids found in all living organisms and natural products. The isoprene (5C) unit is the nature’s favorite building block. Terpenes have many isoprene units attached in a regular head-to-tail fashion. The side chains in vitamins A, E, K, beta carotene, and squalene are examples of terpenes (Figure 17).

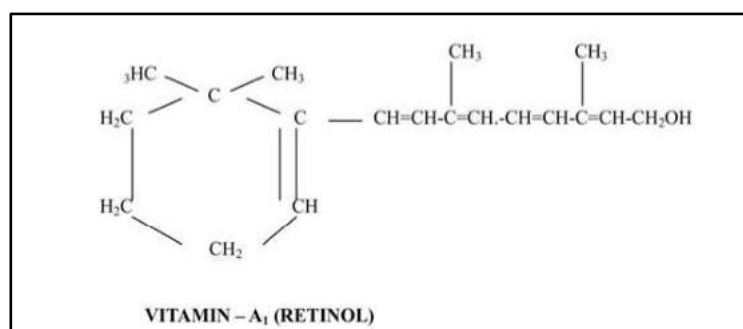


Figure 17: Molecular structure of terpenes as observed as a side chain in Vitamin A (Kandi et al., 2015).

Natural rubber is polyterpene containing hundreds of isoprenes arranged in a regular linear order. The terpenes of natural product emit characteristic fragrance. The products derived from terpenes in living organism makes it special as it leads to formation of squalene, cholesterol, sidechains of fat soluble vitamins like A, E, K and ubiquinone (a component of Electron Transport Chain). By definition terpenes are volatile, unsaturated aromatic hydrocarbons found in all living organisms and

essential oils of plants. Termites swallow tail butterflies, conifers, citrus plants, eucalyptus tree etc. The molecular Structure of terpenes includes polymers of isoprenoid units (5C) (Figure 18). The number of carbons present in terpenes is multiples of 5. The isoprenoid units are arranged in head to tail fashion to form terpenes. Terpenoids are also terpenes but contain an oxygen atom. Terpenes are released by trees more actively in warmer weather and acts as a natural form of cloud seeding. The clouds reflect sunlight, allowing the forest to regulate its temperature (Kandi et al., 2015).

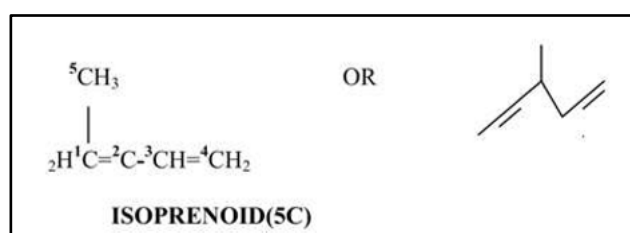


Figure 18: The molecular structure of terpenes as observed in polymers of isoprenoid units (5C) (Kandi et al., 2015).

II.3 Classification of terpenes

Terpenes are classified based on the attached isoprenoid unit as Monoterpenes (2 isoprenoid units and 10 carbon atoms), Sesquiterpenes (3 isoprenoid units and 15 carbon atoms), Diterpenes (4 isoprenoid units and 20 carbon atoms), Sesterterpenes (5 isoprenoid units and 25 carbon atoms), Triterpenes (6 isoprenoid units and 30 carbon atoms), Tetraterpenes (8 isoprenoid units and 40 carbon atoms) and Polyterpenes (many isoprenoid units). There are two metabolic pathways for biosynthesis synthesis of terpenes, including the Mevalonic acid pathway and Methyl erythritol Phosphate / Deoxy Xylulose Phosphate pathway. The Mevalonic acid pathway is similar to cholesterol synthesis via the enzyme HMG-CoASH reductase. These reactions take place in the cytosol (Figure 19) (Kandi et al., 2015).

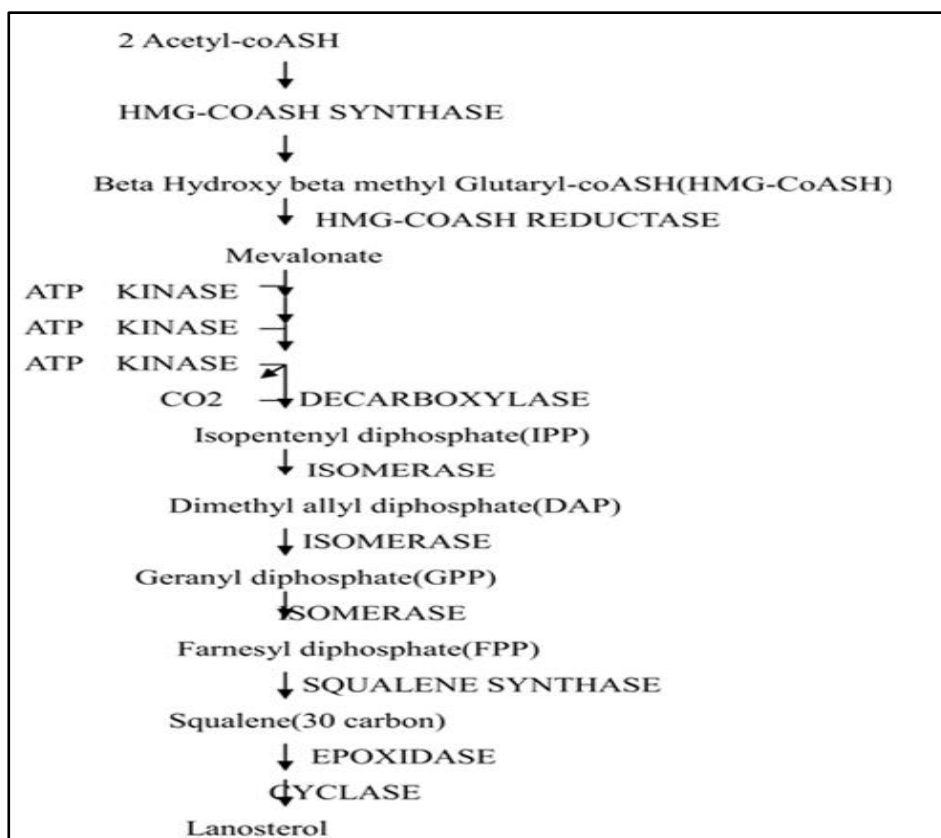


Figure 19: The Mevalonic acid pathway that occurs in the cytosol of living cells (Kandi et al., 2015).

In this pathway, the formation of isopentenyl diphosphate, a 5 carbon compound is the initial stage of terpene formation. The squalene that is formed in this pathway, a 30 carbon cyclic compound is an unsaturated hydrocarbon present in shark and mammalian liver and human sebum. The squalene leads to synthesis of cholesterol, carotenoids, ubiquinone. The Methyl erythritol phosphate or Deoxy xylulose phosphate pathway (DOXP) is also called non-mevalonic acid pathway which occurs in plastids of plants, apicomplexa group of protozoa and many bacteria (Figure 20). After the formation of IPP and DAP the pathway is similar in both. Synthesis of all higher terpenes/terpenoids occurs via formation of GPP and FPP (Kandi et al., 2015).

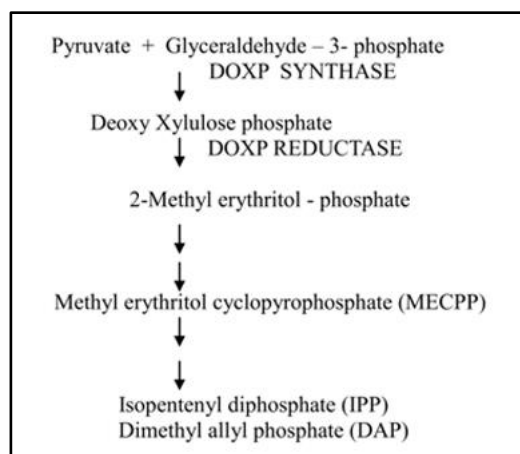


Figure 20: The Methyl erythritol phosphate or non-mevalonic acid pathway (Kandi et al., 2015).

II.4 Biosynthesis of Plant Terpenoids

II.4.1 Biosynthesis of IPP and DMAPP

Terpenoids are derived from the universal C₅ precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP), which in higher plants are generated from two independent pathways located in separate intracellular compartments. In cytosol, IPP is derived from the long-known mevalonic acid (MVA) pathway that starts with the condensation of acetyl-CoA. In plastids, IPP is formed from pyruvate and glyceraldehydes 3phosphate. This MVA-independent pathway, also called MEP pathway after the key intermediate methylerythritol phosphate (MEP), was discovered only in the last 10 years. Initial research indicated that the cytosolic IPP serves as a precursor of farnesyl diphosphate (FDP) for sesquiterpenes and triterpenes, whereas the plastidial IPP provides the precursors for geranyl diphosphate (GDP) and GGDP for mono-, di-, and tetra-terpenes. However, cross-talk between these two IPP biosynthetic pathways is prevalent, particularly in the direction from plastids to cytosol. The cross-talk between the MVA and MEP pathways in tobacco BY-2 suspension cells has been demonstrated; although the exact intermediates were not identified, the authors provided evidence that under certain conditions, exchange of metabolites of the cytosolic and the plastidial isoprenoid pathways across the plastid envelope occurred and the transport might be bi-directional. Interestingly, a recent report showed that in snapdragon flowers the plastid-localized MEP pathway provided IPP precursors

for both plastidial monoterpene and cytosolic sesquiterpene biosynthesis (Cheng et al., 2007).

II.4.2 Formation of GPP, FDP and GGDP

In the second phase of terpene biosynthesis, IPP and DMAPP are used by prenyltransferases in head-to-tail condensation reactions to produce GDP, FDP and GGDP, the immediate precursors of monoterpenes, sesquiterpenes and diterpenes, respectively. These reactions are catalyzed by short-chain prenyltransferases, including GDP synthase, FDP synthase, and GGDP synthase. GDP synthase catalyzes the condensation reaction of IPP and DMAPP to form GDP. FDP synthase sequentially adds two molecules of IPP to DMAPP to form the C₁₅ diphosphate precursor of sesquiterpenes and triterpenes, and GGDP synthase adds three molecules of IPP to DMAPP to form the C₂₀ diphosphate precursor of diterpenes and tetraterpenes. GDP synthase contains two heteromeric subunits, both of which are required for prenyltransferase activity. FDP synthases and GGDP synthases are functional homodimers. The large subunit of peppermint (*Mentha piperita*) GDP synthase shows a high level of amino acid sequence identity (56%–75%) with plant GGDP synthases, whereas the sequence of the smaller subunit is divergent from the sequence of GGDP synthases, with 24%–29% sequence identity only. Genes encoding FDP synthases and GGDP synthases have been isolated from many plant species. They play a central role in both primary and secondary metabolisms of terpenoids. On the contrary, GDP synthases have been characterized from a small number of plant species only, mostly from tissues that produce abundant quantities of monoterpenes. These enzymes function at the branch point of terpenoid metabolism, thus playing a regulatory role in controlling IPP flux into different families of terpenoids (Figure 21) (Cheng et al., 2007).

II.4.3 Plant terpene synthases

In the third stage of terpene biosynthesis, the allylic prenyldiphosphates of GDP, FDP and GGDP are used by Terpene synthases (TPSs) to form monoterpenes, sesquiterpenes, and diterpenes, respectively. Investigation of TPSs has been an active area of plant secondary metabolism research and many genes have been isolated from various gymnosperm and angiosperm species, including, for a few examples, *Picea abies*, *Taxus brevifolia*, *Taxus media*, *Arabidopsis thaliana*, *Artemisia annua*, *Cucumis sativus*, *Nicotiana attenuata*, and *Mentha piperita*. Recently, several

sesquiterpene synthases from maize (*Zea mays*) have been cloned and characterized. Rice has served as a model plant of monocotyledons due to the availability of its genome sequence and the readiness of genetic transformation. (Cheng et al., 2007).

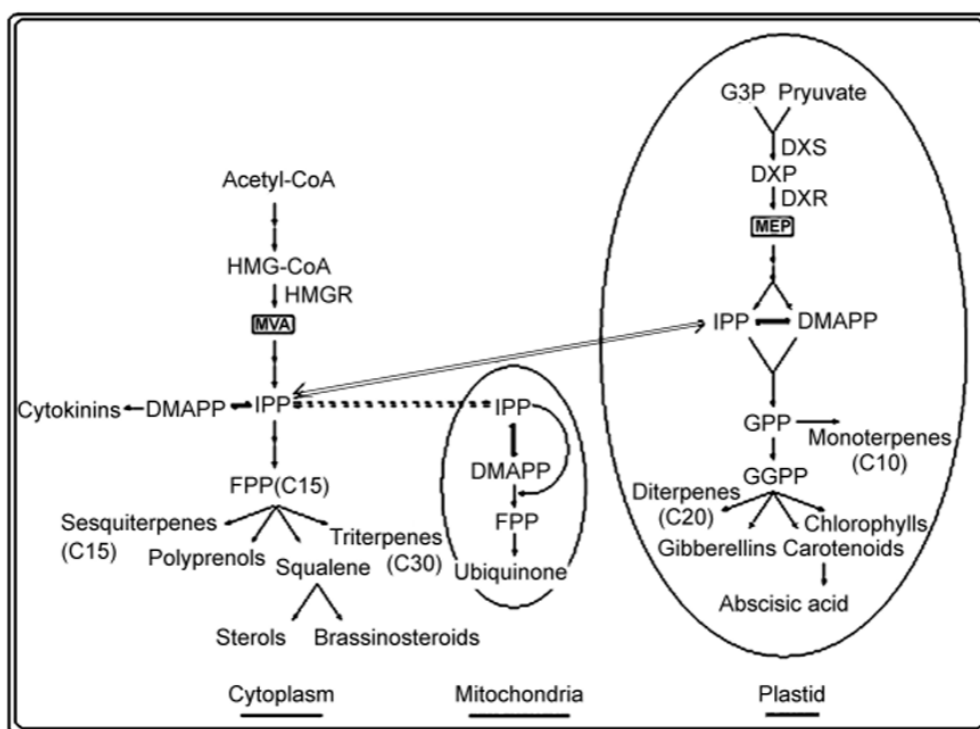


Figure 21: The biosynthesis pathway of isoprenoids in plant cells (Cheng et al., 2007).

II.5 Role of terpenes and terpenoids on human health

In recent decades, numerous studies have reported that terpenes and terpenoids are essential in supporting human health. These bioactive compounds consisting of several isoprene units is the largest class of organic compounds produced in the essential oils (Eos) of various plants. It has a significant role to treat various types of diseases, in many studies in vitro and in vivo using as anticancer agents, antimicrobial, anti-inflammatory, antioxidants, antiallergic, neuroprotective, anti-aggregator, anti-coagulation, sedative and analgesic through the activity of monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes and glycoside compounds. The content of this compound can be found in several nutritional and health products of humans because it is a source of vitamins A, E, K, and coenzyme Q10. Even carotenoid and tocopherol compounds in this group are essential sources of vitamins, mostly in animals, including humans. The application

of terpenes compounds in everyday human life and health is widely used in pharmaceutical, nutraceutical, food and beverage products, cosmetics, perfumes, synthetic chemicals, aroma and flavor additives, rubber products, and the biofuel industry (Figure 22) (Masyita *et al.*, 2022).

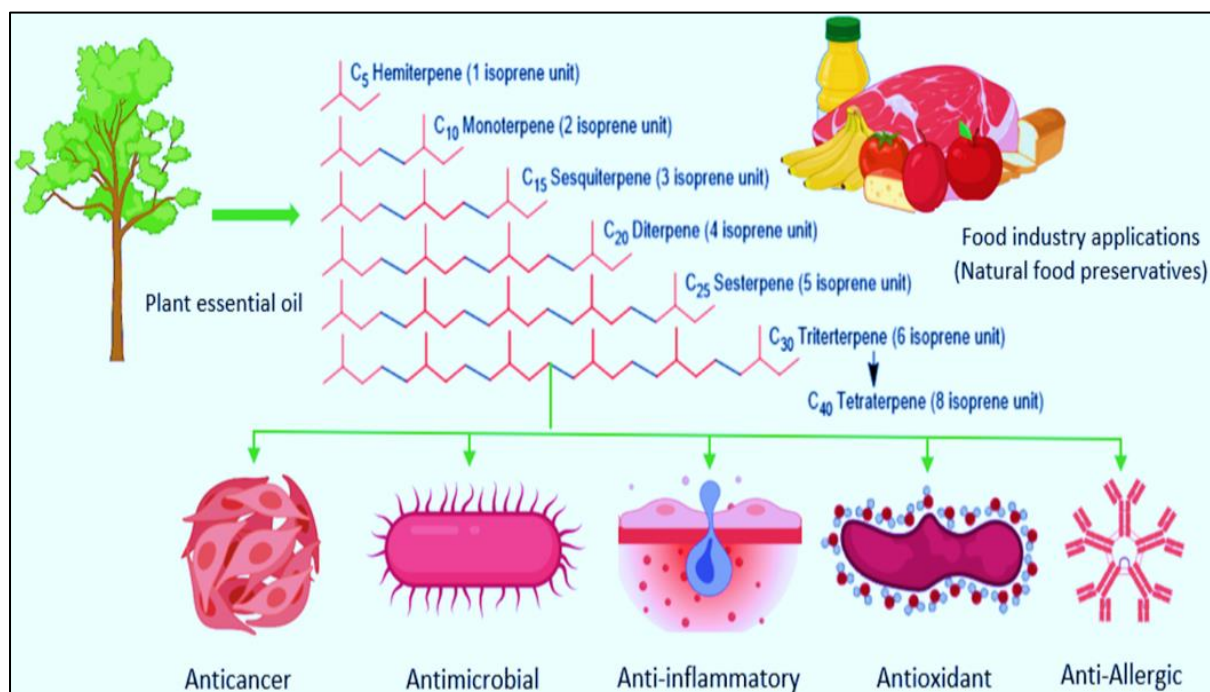


Figure 22: The potential benefits of terpenes and terpenoids found in essential oils (Masyita *et al.*, 2022).

Chapter VI

New Food Products

I Definition and historical background

The last two decades have seen considerable research and development efforts dedicated to new food technologies. The “FlavrSavr” tomato (the first genetically modified (GM) food developed in 1994) and the birth of Dolly the sheep (the first mammal to be cloned from an adult somatic cell developed in 1996) are some of the well-known examples that gave rise to much debate and controversy in the media when they were first developed. Nowadays, consumers are exposed to several applications of emerging technologies such as GM maize in Spain and irradiated poultry meat in the Netherlands. However, even if a variety of food ingredients and food contact materials derived from new food technologies are already available in some European countries, the application is at an early stage and is widely expected to grow. One of the reasons for such interest in new food technologies is the anticipated range of benefits they can bring to the consumer and the food sector. The reported advantages include safer, healthier more nutritious foods using less energy, water and chemicals and producing less waste. However, the toxicological nature of hazard, likelihood of exposure and risk to consumers from some new food technologies are largely unknown.

II Types of new food products

II.1 Genetically modified functional foods

GM functional foods and non-GM functional foods have the same objective, that is, to provide demonstrated physiological benefits and reduce the risk of various diseases such as cancer, cardiovascular disease and diabetes. For example. Tomatoes with a high content of lycopene, a powerful antioxidant, spinach rich in iron and folic acid, two compounds that can prevent heart disease, are examples of functional foods, i.e. which therefore go to the beyond basic nutritional functions.

II.1.1 Research in the field of genetically modified functional foods

The axes of research in the field of genetic exploitation in functional foods are progressing over time, leading to the birth of functional foods with health benefits (Table 12) (Singh *et al.*, 2014).

Table 11: Genetically modified functional foods (Singh et al., 2014).

Breeding	Genetic modification	Advantages
Potato	Modifying the potato so that it produces more starch	By producing more starch, the potato absorbs less fat when left in oil during cooking
Tomato	Tomatoes are genetically modified to contain four times the normal amount of lycopene (a carotenoid). The biosynthesis of this compound using enzymes is now well known. All these enzymes have been cloned and we are now trying to make them accumulate in the tomato.	carotenoids are powerful antioxidants. they can reduce the risk of coronary heart disease and certain types of cancer.
Golden rice	introduction of the daffodil gene (phytoene desaturase). Another from a bacteria (phytoene desaturase of bacterial origin) and another which is a resistance gene to hygromycin (antibiotic used to select transgenic plants) in rice so that it produces beta-carotene, the precursor of vitamin A.	This rice could help prevent vitamin A deficiencies which can cause certain health problems such as diarrhea, measles, as well as eye diseases.

Rice	incorporation of iron-bearing soy protein in rice seeds so that it produces about three times more of this vitamin than conventional rice. research is also being done to increase other plants' iron content from certain pea components.	iron prevents anemia and certain learning disorders in children. moreover, it allows to have a greater resistance to infections.
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II.2 New technologies and forms of consumption of functional foods

II.2.1 Fourth-range products

The fourth range includes fresh, raw, washed, peeled and cut vegetables and fruits. (classic and mixed salads, raw vegetables, fresh herbs, vegetables, and fruits). The products are packaged in ambient air or modified atmosphere or even under vacuum, in bags or trays. Their shelf life (DLC) varies from 4 to 10 days depending on the product ([Martínez et al., 2022](#)).

II.2.2 Products obtained by cooking-extrusion

Extrusion is a thermomechanical manufacturing process by which a compressed material is forced to pass through a die having the section of the part to obtain. An extrudate is continuously formed, a long product (tube, pipe, profile, textile fiber) and/or flat product (plate, sheet, film). Production rates are high ([Figure 23](#)).

Extrusion has become an important processing technique in an increasing variety of food processes. The use of extrusion cooking has many distinct advantages:

- Versatility: a wide variety of food can be produced on the same basic extrusion

system.

- High productivity: an extruder has greater production capability than other cooking/forming systems.

- Low cost: labor and floor space requirements per unit of production are smaller

than for other cooking/forming systems.

- Product shapes: extruders can produce shapes not easily obtained using other

production methods.

- High product quality: the high temperature and short time process minimizes

nutrient degradation while destroying most microorganisms or other pests.

- Production of new foods: extruders can modify vegetable proteins, starches,

and other food materials to produce new food products. No effluents: no process effluents or hazardous materials are produced ([Harper, 1979](#)).

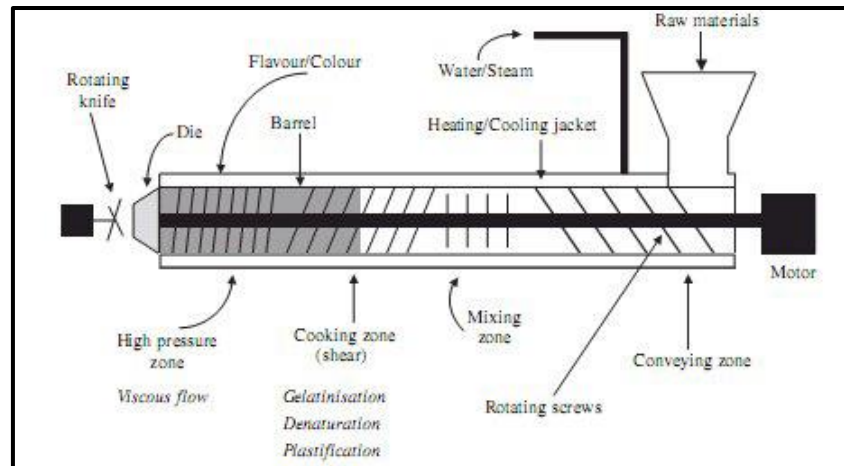


Figure 23: Schematic representation of food extrusion cooking process program (Oonsivilai, Oonsivilai, 2008).

II.2.3 Sous vide cooking

Sous vide or vacuum cooking refers to a cooking method of placing food in a vacuum bag and cooking under strictly controlled temperature and time conditions. SV differs from traditional cooking in various aspects. First, the ingredients are sealed in a vacuum bag and cooked at low temperatures. Such pasteurization conditions can avoid the risk of bacterial contamination while inhibiting the growth of anaerobic bacteria in food during storage. Thus, the cooked food can be stored for a more extended period and can rapidly cool down after cooking. Second, the heating temperature and time can be precisely controlled. Vacuum-sealed packaging can effectively transfer heat while preventing oxidation and loss of volatile substances and moisture, resulting in a better flavor of foods (Baldwin, 2012; Cui Z, 2021).

II.2.4 New meat products

The recent alert launched by the WHO associating the consumption of meat and meat products with the incidence of cancer has increased consumer concern regarding the composition of these products.

To obtain healthier meats and meat products, different strategies are put in place:

- Modification of fat composition: by changing the animal's diet and/or production system.
- Reducing the amount of salt and sodium in processed meatproducts

- Reducing of nitrite or replacement in meat products

Food safety monitoring is particularly important because both of these last acts as antimicrobials to varying degrees ([Weiss et al., 2010](#)).

II.2.5 Low-fat products (in sugar)

II.2.5.1 Definition of light product

The legislation defines a low-fat product as a product whose content of a nutrient (sugar, fat) or calories is reduced by at least 25% compared to the reference product ([Reedy, 1977](#)).

II.2.5.2 Definition of low sugar product

The names are varied: “low in sugar”, “light” or “with reduced sugar content”. they are either by reducing added sugar or by using sweeteners (aspartame, polyols) with powerful sweetening power ([Dodevska, 2022](#)).

To emphasize a certain ingredient that the food contains more or less of than is habitual, nutrition statements contained on the list of approved nutrition statements can be used:

II.2.5.2.1 Low Sugar

Statement claiming that a certain food has a low quantity of sugar and any statement that is likely to have the same meaning for the consumer, can only be placed on a product if the said product contains no more than 5 g of sugar per 100 g of solid product, or 2.5 g of sugar per 100 ml of liquid product ([Dodevska, 2022](#)).

II.2.5.2.2 No Sugar

A statement that a food contains no sugar and any statement likely to have the same meaning for the consumer can only be placed on a product if the product contains no more than 0.5 g of sugar per 100 g or 100 ml ([Dodevska, 2022](#)).

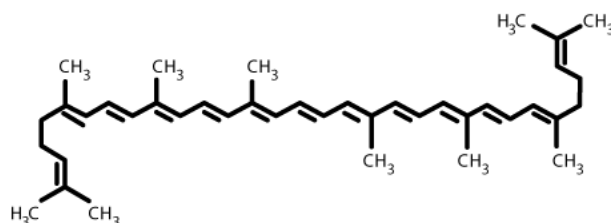
II.2.5.2.3 No Sugar added

Statement that a food contains no added sugar and any statement likely to have the same meaning for the consumer may only be placed on a product if the product contains no added monosaccharides or disaccharides, or any other good used for its sweetening properties. If sugars are naturally present in such food, the label must also display the following statement: “Contains naturally occurring sugars” (Dodevska, 2022).

Practicum

Practicum 1: Extraction of lycopene from tomato juice

Definition of lycopene: This is a molecule composed of a long chain of carbon and hydrogen atoms. It belongs to the large family of carotenoids (like the carotene found in carrots). It is mainly found in red fruits and vegetables, particularly tomatoes (Periago *et al.*, 2004).



Protocol

1- Materiel and reagents:

- Hexane
- Acetone
- Ethanol
- Separation bulb
- 125 ml balloon
- Magnetic stirrer+bar
- 2 beakers of 100ml
- Filter paper
- Oven

2- Extraction

- In a 125ml flask, add 4 grams of previously filtered tomato juice and cover with aluminum foil.
- Add a mixture of 100ml of hexane-acetone-ethanol (v:v:v) (50:25:25)
- Close the flask and shake for 10min
- Add 15ml of water and shake for 5min
- Place the mixture in a separatory funnel and leave to settle for 30 min.
- Collect the aqueous phase and the organic phase in two different beakers.
- Making the observation
- Dry the sample in the oven until dehydrated lycopene is obtained (use for the next lab course).

4- Personal work

- What is the principle of lycopene extraction?
- What is the utility of using organic solvents in this experiment?
- In which phase is lycopene recovered and why?

5- Response

- The principle is based on the migration of lycopene from the aqueous phase to the oily phase
- Pure allow better extraction of lycopene which is soluble in organic solvents.
- the phase that the lycopene recovered oily phase.

Practicum 2: Lycopene determination by spectrophotometry (Davis et al., 2003)

Protocol (Davis et al., 2003)

1- Spectrophotometer calibration

From a standard lycopene solution at 1g/L, make a range of 5 tubes containing 0.0021 to 0.009 mg of albumin per tube.

Complete and carry out the calibration range according to the following table:

C (mg/ml)	0	0,0021	0,005	0,007	0,009	S1	S2
OD	0	0,556	1,349	1,998	2,5	?	?

OD: optical density, S1: Sample 1, S2: Sample 2

2- Working method

- Add 2 ml of n-hexane to the lycopene extract (tomato juice extract S1, tomato paste extract S2).
- Mix for 3min.
- Read the absorbance at 472 nm against the control tube of the range: tube 0.
- After developing the standard range $OD = f$ (quantities of lycopene in mg), complete the table above.

3- Experimental results

- Trace the calibration curve: $A = f$ (amount of lycopene/tube) on millimeter paper and using modeling software.
- Calculate the average lycopene composition of tomato juice (in g/L) then deduce the lycopene content per 100g of tomato
- Interpret the results.

4- Data:

Lycopene composition of tomatoes

Tomato juice: 9.3 ml (mg)%

Tomato paste: 29.3 ml (mg)%

Practicum 3: Extraction of functional ingredients from plants

(Use of filtration, centrifugation, and rotary evaporation)

Definitions of some processes for extracting functional ingredients from plants

Infusion:

Boiling water is poured over the plant's finely chopped leaves or flowers so that they release all the aroma and their active ingredients. Leave to infuse for ten minutes (this is the principle of tea preparation) ([Martins N, et al., 2015](#)).

Decoction:

The plant is put in cold water. Bring to a boil for a few minutes. This method of transformation does not allow the extraction of as many active ingredients as the infusion, but it is suitable for roots and bark for which extraction is difficult (principle of preparing tisane) ([Martins N, et al., 2015](#)).

Maceration:

To perform this extraction, we adopted the protocol of [Casassa et al., 2014](#). The action of allowing a substance to remain cold in an organic solvent to extract its soluble constituents.

Protocol:

1- Preparation of raw noble laurel extract by maceration

- Using a mortar, pound 5 g of the plant. Put the powder obtained in an Erlenmeyer flask containing 50 ml of absolute ethanol, add a magnetic bar in the Erlenmeyer flask, and stir using a magnetic stirrer overnight at room temperature, the extract is then filtered and then evaporated under reduced dry pressure ($T^{\circ} = 45^{\circ}\text{C}$) by a rotary evaporator. The dry residue is taken up in a few milliliters of methanol and stored at $+4^{\circ}\text{C}$.
- Determine the dry extract yield by calculating the following ratio:

$$\text{Yield (Y) \%} = [W_1 - W_2 / W_3] \times 100$$

P1: Weight of the balloon after evaporation;

P2: Weight of the flask before evaporation (empty flask);

P3: Weight of the starting dry plant material.

- Determine the concentration of your extract after recovery in methanol.

2- Preparation of mint infusion

- Weigh 2g of mint leaves in a beaker, pour 20ml of boiling distilled water over the leaves, and leave to infuse for 15 minutes, filter, and store the extract (filtrate) at 4°C ([Martins N, et al., 2015](#)).

3- Preparation of cinnamon decoction:

- In a flask (or heat-resistant beaker), weigh 2 g of cinnamon bark, add 20ml of distilled water, and bring everything to a boil for approximately 15 to 20 minutes. Leave to cool and filter to remove large particles of bark, then centrifuge the filtrate to eliminate all traces of cinnamon powder, separate the supernatant (extract) and store it at 4°C ([Martins N, et al., 2015](#)).

Practicum 4: Phytochemical screening of functional compounds

1- Alkaloids:

- The tests are carried out by precipitation reactions with Dragendorff's reagent. We put 0.2 ml of each methanolic extract in a test tube and added 5ml 1% HCL. After cooling, this mixture was put in a water bath and 5 drops of Dragendroff reagents were added. A positive test is revealed respectively by the appearance of a white or brown precipitate (Sreevidya, 2003).

2- Flavonoids

- Flavonoids were investigated by the cyanidin reaction. Put 3ml of each methanolic extract in a test tube then add a few drops of concentrated HCL and a few twists of magnesium. The presence of flavonoids is highlighted by the coloring: pink, orange, or red (Muthukumaran et al., 2016).

3- Terpenoids

- Place 5 ml of each extract in a test tube then add 2 ml of chloroform and 3 ml of concentrated H₂SO₄. The presence of Terpenoids is revealed by the appearance of two phases and a brown color at the interphase (Jiang et al., 2016).

4- Steroids

- Add 5 ml of extract at 5 ml of acetic anhydrous, and more than 20 drops of concentrated H₂SO₄. With this reaction, steroids give a purple color turning green (Gonzalo-Lumbreras et al., 2001).

5- Tannins

- The presence of tannins is demonstrated by adding to 1 ml of each extract, 1 ml of water, and 1 to 2 drops of FeCl₃ solution diluted to 1% (Muthukumaran et al., 2016).

1- The appearance of a dark green or blue-green color indicates the presence of tannins.

2- The appearance of a dark green color indicates the presence of catechic tannins.

3- The appearance of a blue-green color indicates the presence of gallic tannins

6- Saponosides

- Put 2 ml of each extract in a test tube, add 2 ml of distilled water, and shake vigorously for 30 seconds horizontally. The presence of Saponosides is revealed by the appearance of foam (N'Guessan *et al.*, 2009).

7- Quinones

- Put 5 ml of extract in a test tube then add a few drops of 1% NaOH, the appearance of a color that turns yellow, red, or purple indicates the presence of quinones (Kasouni *et al.*, 2021).

8- Anthraquinones

- Anthraquinones were identified by the following procedure: Put 10 ml of extract in a test tube, add 5 ml of 10% NH₄OH then shake vigorously. The appearance of purple color indicates a positive test (Oloyede, 2005).

Practicum 5: Determination of phenolic compounds

1- Polyphenol determination

- The total polyphenol content was estimated according to the colorimetric method based on Folin Ciocalteu reagent. The reagent consists of phosphotungstic acid $\text{H}_3\text{PW}_{12}\text{O}_{40}$ and phosphomolybdic acid $\text{H}_3\text{PMo}_{12}\text{O}_4$, which are reduced during the oxidation of phenols to blue oxides of tungsten (W_8O_{23}) and molybdenum (Mo_8O_3), which helps us to measure phenols in the visible at a wavelength of around 765 nm (Rossi et Singleton, 1966).
- For this purpose, methanol solutions were prepared from each extract starting from 1 mg/ml. This solution was also used for the determination of flavonoids. From each solution, 200 μl of extract is added to 1 ml of Folin reagent diluted 10 times and 0.8 ml 7.5% sodium carbonate. Total polyphenols are determined after 30 min of incubation at room temperature by measuring the absorbance at 765 nm (UV-visible Spectrophotometer).
- A calibration curve was carried out in parallel under the same operating conditions using gallic acid at different concentrations (0 à 1000 $\mu\text{g}/\text{ml}$).

2- Flavonoid determination

- The determination of flavonoids was carried out by a method based on the formation of a very stable complex, between aluminum chloride and the oxygen atoms present on carbons 4 and 5 of the flavonoids.
- In a test tube, 500 μl of each extract were mixed with 1500 μl of distilled water then 150 μl of 5% sodium nitrite were added. The mixture is incubated for 5 min in the dark and at room temperature. After incubation, a volume of 153 μl of 10% AlCl_3 was added. The mixture was reincubated under the conditions previously mentioned for 6 min. Finally, 533 μl 4% NaOH is added to the mixture. The absorbance is read immediately at 510 nm.
- On the other hand, the calibration curve is produced by catechin, used as a standard, at different concentrations. Results are expressed as mg equivalent of catechin per gram of sample (Zhishen et al., 1999).

3- Condensed tannin determination

- The quantities of condensed tannins are estimated using the vanillin method in an acid medium ([Julkunen-Titto, 1985](#)).
- A volume of 50 μl of each extract is added to 1500 μl of the vanillin/methanol solution (4%, m/v) and then mixed using a vortex. Then, 750 μl of concentrated hydrochloric acid (HCl) is added and left to react at room temperature for 20 min. The absorbance at 550 nm is measured against a blank. The concentration of tannins is estimated in milligram (mg) catechin equivalents per gram (g) of dry matter weight (EC)/g) from the calibration curve.

Practicum 6: Evaluation of antioxidant activity

- The antioxidant activity was evaluated according to the free radical scavenging activity of picrylhydrazyl 2,2-diphenyl-1-(DPPH) (Blois, 1958).
- The DPPH test makes it possible to measure the anti-radical power, it measures the capacity of an antioxidant to reduce the chemical radical DPPH (2,2-diphenyl-1-picrylhydrazyl) by transfer of hydrogen. DPPH, initially purple, transforms into DPPH-H, pale yellow.

The discoloration of the solution is proportional to the concentration and reducing potential of the samples.

- To evaluate the antioxidant activity, we put 3 mg of each total extract of roots and leaves diluted in 1 ml of methanol. Then each methanolic solution is diluted to decreasing concentrations.
- A volume of 10 µl of different concentrations in mg/ml of each extract is added to 1990 µl of the freshly prepared methanolic solution of DPPH (0.025g/l).
- The absorbance is measured at 515 nm after 30 min of incubation at room temperature. The percentages of inhibition of the DPPH radical are calculated from the following formula:

$$\text{Inhibition percentage } I (\%) = \frac{(\text{control ABS} - \text{Test ABS}) * 100}{\text{control ABS}}$$

I%: Inhibition percentage

Control Abs: Absorbance of control

Test Abs: Absorbance of the test performed

- The minimum inhibition concentration of 50% of DPPH (IC₅₀) is calculated graphically from the linear trend curves and then used for the comparison of the antioxidant activity of the different samples.

Conclusion

Conclusion

In this handout, we have detailed the lectures of the “Functional food and new products” module designed to Second Year Master students, specialties: Applied Biochemistry, Biochemistry of Nutrition and Nutrition and Pathology.

Functional ingredients prove their effectiveness in the medical and agri-food fields because they can be used as food supplements and medicines at the same time.

At the end of this research in the field of functional foods, it is important to conclude that any food is "functional" in the sense that its nutrients which compose it and its effect will participate in an effect on the body and therefore fulfill a function. It is therefore towards a new conception of the functionality of foods that we must move for the years to come, a conception which must be based on a new paradigm more following the functioning of reality, which is today described as a holistic approach.

The functional impact of diet on health cannot therefore be reduced to the sole provision of energy and nutrients. Food therefore includes physiological (including genetic factors), psychological, socio-economic, cultural, pleasure, and also environmental and animal welfare dimensions.

Diet is not a sufficient factor to modulate and guarantee health. We must broaden our level of observation, particularly to the lifestyle. An observational study published in 2009 clearly illustrates this observation: the authors studied the impact of four lifestyle factors (not smoking, body mass index less than 30, getting 3.5 hours or more of physical activity per week, and adhering to a healthy diet rich in fruits, vegetables, whole meal bread and low meat) on the risk of developing cardiovascular diseases (myocardial infarction and stroke), cancers or type 2 diabetes in 23,153 German adults aged 35 to 65 from the EPIC cohort (European Prospective Investigation Into Cancer and Nutrition) ([Ford et al., 2009](#)). Participants meeting all four criteria for a healthy lifestyle have a 78% reduced risk of developing chronic diseases, and this reduction becomes less significant as the number of criteria decreases (e.g., 21% reduction for the only criterion “healthy diet” and reduction of 24% for the sole criterion “physical activity”).

This study clearly shows that taking physical activity into account is an important criterion to consider, and therefore that a holistic approach to diet is necessary. Indeed, participants adhering to both the “healthy diet” and “physical activity” criteria have a reduced risk of 66%, i.e. 45% more than when only taking diet into account, and 42% more than 'by only taking into account physical activity. So, adhering to four simple healthy lifestyle factors can have a strong impact on the prevention of chronic diseases. We must broaden the observation beyond functional food which is a tool of functional nutrition.

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