People's and Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
University A. MIRA - Bejaia

Faculty of Natural and Life Sciences
Department of Food Sciences

Food Biochemistry
Part I

Accomplished by
Mme MADDI Née SOUFI Ouahiba

University year 2023/2024

Preamble

Knowledge in food biochemistry has grown exponentially and is disseminated. The two areas of food biochemistry and food processing are becoming closely interrelated. Fundamental knowledge in food biochemistry is crucial to enable food technologists and food processing engineers to rationalize and develop more effective strategies to produce and preserve food in safe and stable forms. The present document which includes the first part designed to fill the gap by assembling information regarding the basic principles of food science and the various reactions and modifications involved in technological processes; three separated chapters are presented:

- *Impact of treatments on the properties of food Constituents*
- *Scission reactions*
- *Oxidation reactions*

The contents of this handbook have been enriched by knowledge contributed by other authors. The aim of this document is to give a synthetic presentation of the chemical and biochemical aspects of food science, as well as the technological aspects of the unit operations involved in transforming raw materials into food products, the various modifications that can be made to food ingredients, and the quality control of these products through the description of a number of analytical methods. Furthermore, the chapters presented in this support cover the various themes of major importance in food science: proteins, lipids and carbohydrates. This book is intended for the various academic license affiliated with the food science speciality, in particular those of quality control and analysis, agri-food technology, as well as master's degrees in product quality and food safety, and food preservation.

Finally, i would like to express my gratitude to all those who have contributed directly or indirectly to perform this document through their sound advice and critical rereading. I would also like to pay homage to Pr Louaileche H., who once taught me the basics of food science.

List of tables

List of figures

Contents

Introduction

Food processing is usually a required set of steps applied to food before its consumption. The main reasons for food p1rocessing include: imparting a desirable modification in the food composition, maintaining the food quality, sustain the availability of products at various times and places (products provision in out of season), food diversity (creating diverse food products to different consumers), increasing shelf life, and preparation of ready-to-eat products. Changes occurring during processing can be beneficial, such as the inactivation of microorganisms and destruction of toxins, increasing the bioavailability of some nutrients, development of desirable flavor and texture attributes, and extending shelf life, or it may be detrimental, such as the destructive effect of heat on nutritional value of food (e.g., loss of vitaminsand bioactive compounds) and the formation of harmful components (e.g., acrylamide, trans fatty acids), Moreover, a loss of amino acids can occur, depending on the severity of the treatment.

The most usual processes in food industries include heating, cooking, baking, freezing, milling, canning, fermentation, drying, salting (pickling, curing, or brining), extrusion, and smoking. Among these processes, thermal processing (e.g., cooking, roasting, grilling, frying, boiling, pasteurizing, and sterilizing) are considered to be the most efficient in destroying pathogens, but they also have the most drastic effect food on composition, characteristics, and properties.

Chapter I: Impact of treatments on the properties of food constituents

I.1. Introduction

One of the aims of a technological process is to apply unit operations in order to transforming raw materials into finished products. These stages involve favorable or unfavorable changes to the food (hygienic quality, organoleptic, physico-chemical and nutritional). The types of treatment include:

- **Physical treatments:** the use of temperature (cooking, pasteurization, sterilization...), radiation (UV), oxygen...
- **Chemical treatments:** alkaline, acid (dairy products). In addition to many transformations can lead to a reduction in the value of the product. or produce toxic degradation products. Among reactions

a. Modification of carbohydrates: starches are not easily digestible. Their digestibility is increased by cooking, which causes gelatinization, facilitating degradation in the intestine.

b. Modification of proteins: freezing has no effect on its own. Some diffusion lossescan be observed during thawing.

c. Modification of lipids: during culinary preparation or processing, the fats can undergo a number of transformations that affect their value nutritional and organoleptic quality. Fats oxidize depending on the degree of oxidation. unsaturation of fatty acids. Rancidity in air or in the presence of oxygen is favored by light and heat

I.2. Functional properties of food components

Food is a heterogeneous system made up of several phases with different characteristics, differences in density. The functional properties of the components (protein, polysaccharide, and lipid) are the expression of their characteristics including physicochemical properties. The different functionalities are the source of interactions molecular properties. There are three types of properties:

- **Hydration properties:** due to the interactions between the constituent and water: absorption, swelling, viscosity, thickening properties....
- **Textural properties**: due to molecule-molecule interactions
- **Surface (interfacial) properties**: the water/oil interfaces of emulsions or water/gas (foams); these interfaces are stabilized by molecules amphiphilic.

Among food constituent, proteins show a large number of functions and functionalproperties and some of the most important are shown on table 1. Protein functionality is evident by its interaction with other components within the food or chemical system. These interactions may involve solvent molecules, solute molecules, other protein molecules or substances that are dispersed in the solvent such as oil or air.

Table 1. Typical functional properties performed by proteins in food systems

Functional Property	Mode of action	Food system
Solubility	Protein solvation, pH dependent	Beverages
Water absorption and binding	Hydrogen-bonding of HOH, entrapment of HOH (no drip)	Meats, sausages, breads, cakes
Viscosity	Thickening, HOH binding	Soups, gravies
Gelation	Protein matrix formation and setting	Meats, curds, cheese
Cohesion-adhesion	Protein acts as adhesive material	Meats, sausages, baked goods, pasta products
Elasticity	Hydrophobic bonding in gluten, disulfide links in gels (deformable)	Meats, bakery
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna, soup, cakes
Fat adsorption	Binding of free fat	Meats, sausages, donuts
Flavor binding	Adsorption, entrapment, release	Simulated meats, bakery, etc.
Foaming	Forms stable films to entrap gas	Whipped toppings, chiffon desserts, angel cakes

I.2.1. Hydration properties

When bread or sausages are made, the proteins are endowed with the following properties hydration properties that enable them to absorb and retain water thanks tothe hydrogen bonds with surrounding water molecules. In addition to proteins polysaccharides have strong thickening properties. e.g. starch which is often used asa thickening agent to increase the viscosity of sausages and soups. The main hydration steps are shown in figure 1.

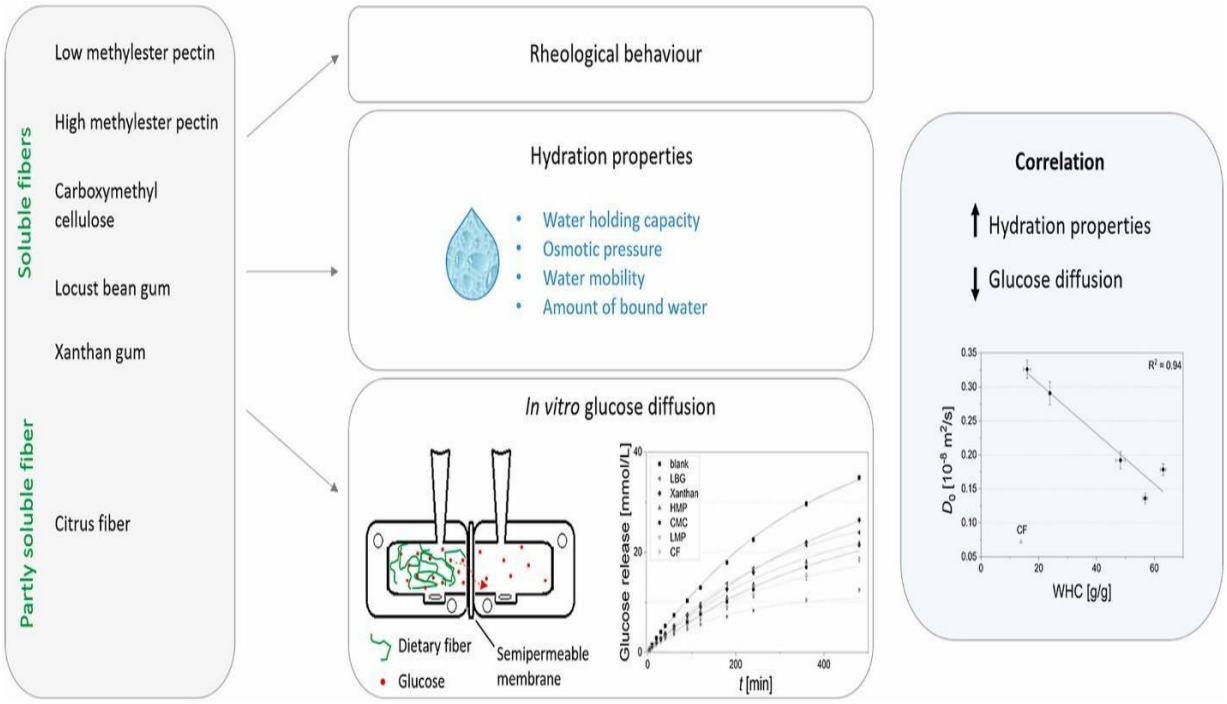


Figure 1. Hydration properties.

Most raw materials containing cereal fibers are ground for better acceptance of the final product and this process can affect hydration properties. Swelling and water- binding capacity of pea hull fibers are decreased by grinding, whereas the water- holding capacity was slightly increased. The kinetics of water-uptake was also different, and the ground product hydrated instantaneously in contrast to the unground product, which reached equilibrium only after 30 minutes. This was related to the differences in surface area.

Heat-treatment can also change hydration properties. For example, boiling increasedthe water-binding capacity slightly in wheat bran and apple fiber products, whereas autoclaving, steam-cooking and roasting had no significant effects. The kinetics of water uptake, however, was different for steam-cooking and roasting. Thus, both products exposed to steam-cooking had a very rapid water-uptake, whereas the roasted sample had a slow uptake. For example, extrusion-cooking of pea-hulls, sugar-beet fibers,wheat bran and lemon fibers had only slight effects on the water-binding capacity.

I.2.2. Gelling properties

This is an interesting property used in the development of products. by creating a solidstructure; the properties of the most common pectin's are also very interesting from a food technology point of view is their ability to form gels (in case of jams) (figure 2). With the exception of gelatin, these are all polysaccharides made up of hexose, pentose or uronic acid, which are commonly added to biscuits and soups, desserts....

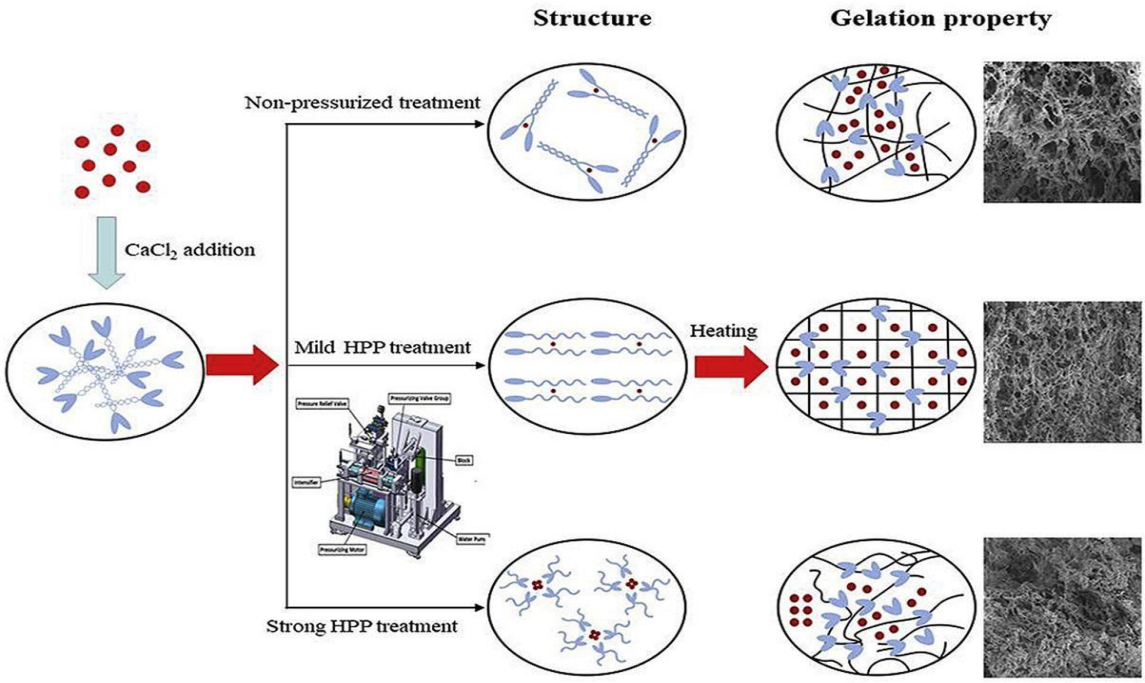


Figure 2. Gel formation.

I.2.3. Foaming properties

A foam is a dispersion of a gas bubble in a liquid phase. Among dietary components, proteins play an important role in the formation of proteins: foams; desserts (whipped cream, egg whites, etc.). The main processes in foams that can be controlled efficiently using appropriate surfactants, polymers, particles and their mixtures are: (a) Foaminess — foam volume and bubble size; (b) Foam stability to liquid drainage, bubble Ostwald ripening and bubble coalescence; and (c) Foam rheological properties and bubble size in sheared foams (figure 3).

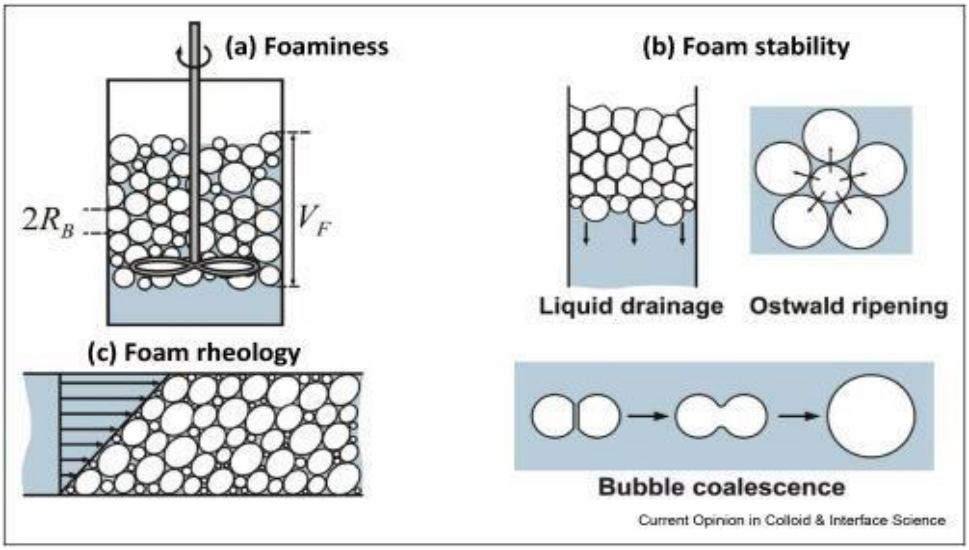


Figure 3. Foaming properties.

I.2.4. Emulsifying property

Emulsions are dispersing systems of one or more immiscible liquids. They are stabilized by emulsifiers — compounds which form interface films and thus prevent the disperse phases from flowing together. Due to their amphipathic nature, proteins can stabilize o/w emulsions such as milk. This property is used at a large scale inthe production of food preparations.

The adsorption of a protein at the interface of an oil droplet is thermodynamically favored because the hydrophobic amino acid residues can escape the hydrogen bridge network of the surrounding water molecules. In addition, contact of the protein with the oil droplet results in the

displacement of water moleculesfrom the hydrophobic regions of the oil-water boundary layer. Therefore, the suitability of a protein as an emulsifier depends on the rate at which it diffuses into the interface and on the deformability of its conformation under the influence of interfacial tension (surface denaturation).

The diffusion rate depends on the temperature and the molecular weight, which in turncan be influenced by the pH and the ionic strength. The adsorbance ability depends on the exposure of hydrophilic and hydrophobic groups and thus on the amino acid profile, as well as on the pH, the ion strength and the temperature.

The confirmative stability depends in the amino acid composition, the molecular weight and the intra-molecular disulfide bonds. Therefore, a protein with ideal qualities as an emulsifier for an oil-in-water emulsion would have a relatively lowmolecular weight, a balanced amino acid composition in terms of charged, polar and non-polar residues, good water solubility, well-developed surface hydrophobicity, and a relatively stable conformation. The β-casein molecule meets these requirements because of less pronounced secondary structures and no cross links due to the lack of SH groups.

The apolar “tail” of this flexible molecule is adsorbed by the oil phase of the boundary layer and the polar “head”, which projects into the aqueous medium, prevents coalescence. The solubility and emulsifying capacity of some proteins can be improved by limited enzymatic hydrolysis.

Emulsifiers are used to obtain a homogeneous and stable mixture from two immiscible phases, an oily phase and an aqueous phase. These molecules have a polar part (which have an affinity for water) and a non-polar part or lipophilic (with an affinity for oil). Such molecules can be placed at the interface between the oil phase and the water phase (monoglyceride, diglyceride, phospholipids etc.).

The main emulsifiers which had been used ordinarily in foodsystems are shown in table 2. They can be divided into two categories:

• Small molecules: Mono and diglicerides, Sucrose Esters, Sorbitan Esters (SPAN),Polysorbates (TWEEN), Stearoyl Lactylates, Lecithin and erivatives

• Macromolecules: proteins such as bovine serum albumin, b-lactoglobulin, lysozyme,and ovalbumin. Only food emulsifiers defined as food additives are usable by law.

Table 2. Food emulsifiers used in food systems

Name	Common Name
Glycerin Fatty Acid Esters	Monoglyceride (MG)
Acetic Acid Esters of Monoglycerides	Acetylated Monoglyceride (AMG)
Lactic Acid Esters of Monoglycerides	Lactylated Monoglyceride (LMG)
Citric Acid Esters of Monoglycerides	CMG
Succinic Acid Esters of Monoglycerides	SMG
Diacetyl Tartaric Acid Esters of Monoglycerides	DATEM
Polyglycerol Esters of Fatty Acids	PolyGlycerol Ester (PGE)
Polyglycerol Polyricinoleate	PGPR
Sorbitan Esters of Fatty Acids	Sorbitan Ester (SOE)
Propylene Glycol of Fatty Acids	PG Ester (PGME)
Sucrose Esters of Fatty Acids	Sugar Ester (SE)
Calcium Stearoyl Di Laciate	CSL
Lecithin	Lecithin (LC)
Enzyme Digested/Treated Lecithin	EDL or ETL
Proteins	

a. Proteins as emulsifiers: Protein functionality has been defined as: «any property of a protein, exception of its nutritional ones that affects its utilization». When proteins are used to generate emulsions, the system becomes highly complex. They are responsible for the creation of a new surface area. The high energy state is relieved by rapid coalescence of fat globules. For prevention of coalescence, protein molecules need to diffuse to the fat/ water interface and then unfold and coat the surface. When enough of the new surface is covered, coalescence ceases. With proteins, the rate of diffusion to the interface is a significant variable in the amount of protein that absorbs to the interface during the emulsion formation.

If something tends to decrease the rate of diffusion of the protein molecules, the protein load decreases.

Proteins are included in emulsions to aid in their formation and to increase their stability. They are much larger and more complex than other simple emulsifier molecules and the formation of a protein stabilized emulsion requires that the protein molecule must first reach the water/ lipid interface and then unfold so that its hydrophobic groups can contact the lipid phase. To illustrate the forces involved, the situation of a protein molecule approaching a static water/lipid interface will first be considered. In native proteins most of the non-polar amino acid side chains are located in the interior of the molecules. Proteins have charged groups at the surface of the molecule which are in contact with water molecules. The favorable interaction of water with surface charge lowers the total energy of the protein molecule. The hydrophobic groups are removed from contact with the aqueous phase while charged groups maximize solvent contacts. As a protein molecule approaches the interface, there is less opportunity for the charged groups to interact with the solvent. In the extreme case, charged groups are removed from the aqueous phase and enter the lipid phase. This is energetically unfavorable and these groups are repelled from the interfacial area. If the groups closer to the interface are in a region of the protein molecule that contains some flexibility, the molecule may begin to unfold. This unfolding causes the exposure of hydrophobic groups to the surface. If these groups are exposed to the aqueous environment, there is an increase in total energy and random fluctuations in protein structure cause these groups to return to the inner part of the molecule. If the exposure occurs at an interface, the state of lowest free

energy depends on the nature of the interface. In the case of a protein un-folding near lipid, the hydrophobic groups are inserted into the lipid phase. This insertion has a very lowenergy of activation and proceeds spontaneously.

b. Classification of protein based surfactants

The three types of protein-based surfactants are: (1) Amino acids, (2) peptides, both of them derived from synthesis and hydrolysis of the (3) proteins.

- ✓ The amino acid-based surfactants are composed of an amino acid as the hydrophilic part and a long hydrocarbon chain as the hydrophobic part. The hydrophobic chain can be introduced through acyl, ester, amide, or alkyl linkage.

  Examples of these kind of surfactants are the long-chain Na acyl amino acid derivatives from pure amino acids or protein hydrolysated, which have been extensively used in the cotton chemical industry. N-Acylsarcosinate salts are suitable for cosmetics, toothpaste, wound cleaners, personal care items, shampoo, bubble-bath pastes, aerosols and synthetic bars. The many kinds of amino acid-based surfactants have a potential wide application in the cosmetic, personal care, food, and drug industries.
- ✓ The peptide surfactants are derived from the condensation of dipeptides or tripeptidesand hydrophobic chains such as fatty acids. Most of the surfactants in literature have been chemically synthesized, although some have been biosynthetically produced. Examples of these surfactants are the diethanolamides (DEA) of N-lauroyl dipeptidesof various molecular structures.

c. Molecular basis of protein surfactants

- ✓ Hydrophobic Interactions: one of the main mechanisms by which proteins diminishtheir free energy involves the removal of hydrophobic groups from the aqueous environment. This may provide the greatest single decrease in free energy of all the types of binding that occur within proteins. The strength of hydrophobic binding is, however, very sensitive to changes in temperature and the dielectric constant, thus, the changes in these parameters strongly influences protein structure.

The distribution of hydrophobic groups is also important. In proteins such as β-lactoglobulin, the hydrophobic groups are evenly distributed throughout the molecule. There are no large portions of the molecule where hydrophobic amino acids are grouped, nor are there large sections of the molecule that do not contain charged amino acids. This makes it difficult to find portions of the molecule that are sufficiently hydrophobic or to find residues that do not contain amino acids with charged groups that would resist their removal from the aqueous phase. In molecules such as b-casein there are large sections of the protein that contain hydrophobic amino acids without the presence of charged groups. The molecule has such an uneven distribution of charge and hydrophobic groups that it is amphipathic. It is easy to find portions of thismolecule that contain at least six non-polar amino acids and no charged groups.

- ✓ <u>Electrostatic interactions:</u> electrostatic interactions play a major role in the determination of the molecular structure of a protein. Proteins contain a number of amino acids that can ionize to form either positively charged ions (e. g., arginine, lysine, proline, histidine and the terminal amino group) or negatively charged ions (e. g. glutamic and aspartic acids and the terminal carboxyl group). If the protein contains many similarly charged groups, it is more likely to adopt an extended configuration because this increases the average distance between the charges and therefore minimizes the unfavorable electrostatic repulsions. If, on the other hand, the protein contains many oppositely charged groups, it is more likely to fold up into a compact structure that maximizes the favorable electrostatic attractions. As a result, proteins are often extremely compact at their isoelectric point and unfold as the pH is either increased or decreased. Electrostatic interactions also play an important role in determining the aggregation of proteins in solution. Similarly charged proteins repel each other and therefore tend to exist as individual molecules, whereas oppositely charged proteins attract each other and therefore tend to aggregate (depending of the strength of the various other types of interactions involved). The binding of low-molecular weight ions, such as Na^{+} and Ca^{2+}, is also governed by electrostatic interactions and may influence the strength of the hydration repulsion between proteins in solution.

- ✓ Hydrogen bonding: proteins contain monomers that are capable of forming hydrogen bonds. These bonds are a relatively strong type of molecular interaction, and therefore a system attempts to maximize the number and strength the formed hydrogen bonds. The protein may adopt an arrangement that enables it to maximize the number of hydrogen bonds which are formed between the monomers within it, which leads to the formation of ordered regions such as helixes, sheets and turns. Alternatively, a protein may adopt a less ordered structure where the monomers form hydrogen bonds with the surrounding water molecules. Thus, a part or all of the protein may be found in either a highly ordered conformation (which is entropically unfavorable) with extensive intramolecular hydrogen bonding or in a more random- coil conformation (which is entropically more favorable) with extensive intermolecular hydrogen bonding. The type of structure formed by a protein under certain sets of environmental conditions is governed by the relative magnitude of the hydrogen bonds compared to the various other types of interactions, most notably hydrophobic, electrostatic and configurational entropy.

- ✓ Disulfide bonds and protein flexibility: flexibility is an important feature affecting the emulsifying properties of proteins. In aqueous solution, the hydrophobic domains of a protein are generally buried in the interior of the molecule. To stabilize an emulsion, the hydrophobic domains of the protein should ideally be oriented toward the oil phase. The ease with which a protein is able to unfold (i. e., denature) to expose its hydrophobic domains, therefore, affects its emulsifying properties. The three-dimensional structure of proteins can be stabilized by both covalent and non-covalent interactions. Covalent interactions consist of disulfide bonds, both intra- and intermolecular. Several approaches have been used to modify disulfide bonds and to test whether the resulting protein has enhanced emulsifying properties.
- ✓ Molecular conformation and aggregation: the conformation and aggregation of proteins depend on the relative magnitude of the various attractive and repulsive interactions which occur within and between molecules, as well as their configurational entropy. It has shown the protein aggregation processes is an important factor in the emulsifying properties of proteins like wheat glutenins and soy globulins. Segments of one protein may be capable of forming strong hydrogen bonds with segments on

another protein molecule, which causes the molecules to aggregate. These junction zones usually involve hydrogen-bonded helical or sheet-like structures. Hydrogen bonded junction zones tend to be stable, which stabilizes the oil globule covering web at low temperatures but dissociate as the temperature is raised above a certain value because the configurationally entropy term dominates.

d.Characterization of protein emulsifying properties

The determination of meaningful emulsion data with complex food products is difficult. Much of the experimental work with model systems has been done in very dilute solutions. The surface pressure or interfacial tension is often the quantity measured. The situation in food products is also complicated by the presence of other surface active molecules in addition to the proteins present. A variety of tests has been applied to indicate the value of a protein in an emulsion. A range of experimentally controllable parameters will alter the measured emulsification properties of a protein being evaluated. These include: type of equipment used to produce the emulsion, energy input into the system, amount of protein used, phase volumes used, ionic strength, pH and type of oil used. In table 3 some of the properties and tests used in the evaluation of the protein emulsifying properties are shown.

Table 3. Tests used for the evaluation of proteins emulsifying properties

Property	Test
Emulsifier Efficiency	Emulsifying Capacity Emulsion Stability Index Interfacial Tension Interfacial Rheology
Microestructure and Droplet Size Distribution	Microscopy Static Light Scattering Dynamic Light Scattering Electrical Pulse Counting Sedimentation Techniques Ultrasonic Spectrometry NMR Neutron Scattering Dielectric Spectroscopy Electroacoustics
Dispersed-Phase Volume Fraction	Proximate Analysis Density Measurement Electrical Conductivity Alternative Techniques
Droplet Crystallinity	Dilatometry NMR Thermal Analysis Ultrasonics
Droplet Charge	Electrophoresis Zetasizer© Electroacoustics

Chapter II: Scission reactions

II.1. Hydrolysis of carbohydrates

During wet heat treatment, as in blanching, boiling and canning of vegetables and fruits, there is a considerable loss of low molecular weight carbohydrates (i.e. mono- and disaccharides) as well as micronutrients, into the processing water. For example,in the blanching of carrots and swedes (rutabagas) there was a loss of 25% and 30%,respectively of these carbohydrates. With subsequent boiling another 20% was lost. In peas, green beans and Brussels sprouts the loss was less pronounced - about 12%following blanching and another 7-13% at boiling.

II.1.1. Hydrolysis of lactose: this is carried out either chemically (pH 2 and at a temperature close to 100°C) or enzymatically in the presence of a β-galactosidase (figure 4) from moulds (genus *Aspergillus*) or yeasts (Kluveromyces).

a. Enzymatic hydrolysis of lactose

The process of lactose hydrolysis does minimal damage to the nutritional components in milk and is highly specific. Two processes, batch and aseptic, are used in producing lactose-free milk. In the batch process, neutral lactase is added to milk under slow stirring until lactose is fully hydrolyzed, after which the milk is pasteurized, homogenized, and packaged. The batch process lacks pasteurization during the hydrolysis phase, and enzyme dosage is relatively high because the reaction occurs at low temperatures to prevent microbial spoilage. Research on cold-active β galactosidase has improved this defect. In the aseptic process, milk is first sterilized using the UHT procedure, after which sterile lactase is injected into the milk just before packaging. Although the lactase dosage decreases compared to the batch process, the aseptic process requires special equipment, and process control is absent since the hydrolysis reaction continues after packaging.

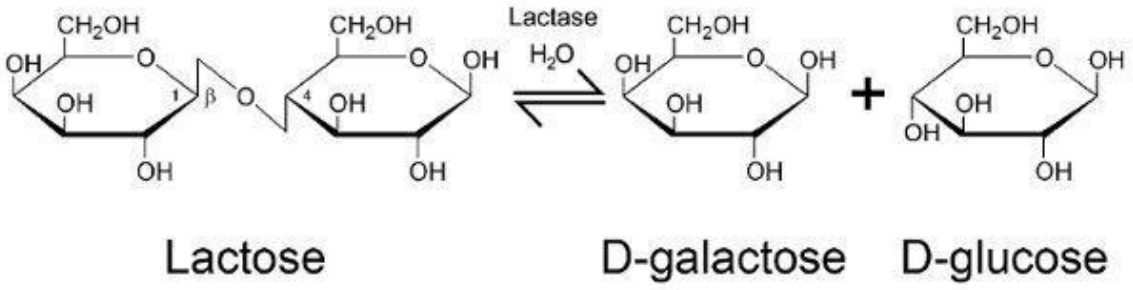


Figure 4. Enzymatic action of lactase.

Hydrolysis is carried out with free enzymes, which must be inactivated by treatment or eliminated by filtration (figure 4). The hydrolysis of lactose into glucose and galactose helps improve the quality of food for people with lactose intolerance to increase the nutritional value of its solubility and sweetening power and double its reducing power.

- Industrial applications include hydrolyzed lactose milk
- Secondary processing: chocolate, biscuits, confectionery, etc.

- **Production of lactose-free dairy products**

  The development of lactose-free and low-lactose dairy products made it possible for lactose-intolerant people to utilize the rich nutrients in milk, which is one of the effective ways to improve lactose malabsorption and lactase intolerance. Currently, there are three processes commonly used to reduce lactose content: enzymatic hydrolysis of lactose, membrane filtration, and fermentation. Studies have tried to combine several of above processes. Lactose-free milk can be further processed into lactose-free/low-lactose yogurt, cheese, milk powder, ice cream and other dairy products (figure 5). Many studies have confirmed that most consumers diagnosed with lactose intolerance can tolerate up to 10 g/day lactose and have no observable adverse reactions to 2 g/day lactose.

Although there are no common standards on allowable lactose threshold, in most countries, lactose content of low-lactose and lactose-free products are within 1 g/100 g and 10 mg/100 g, respectively.

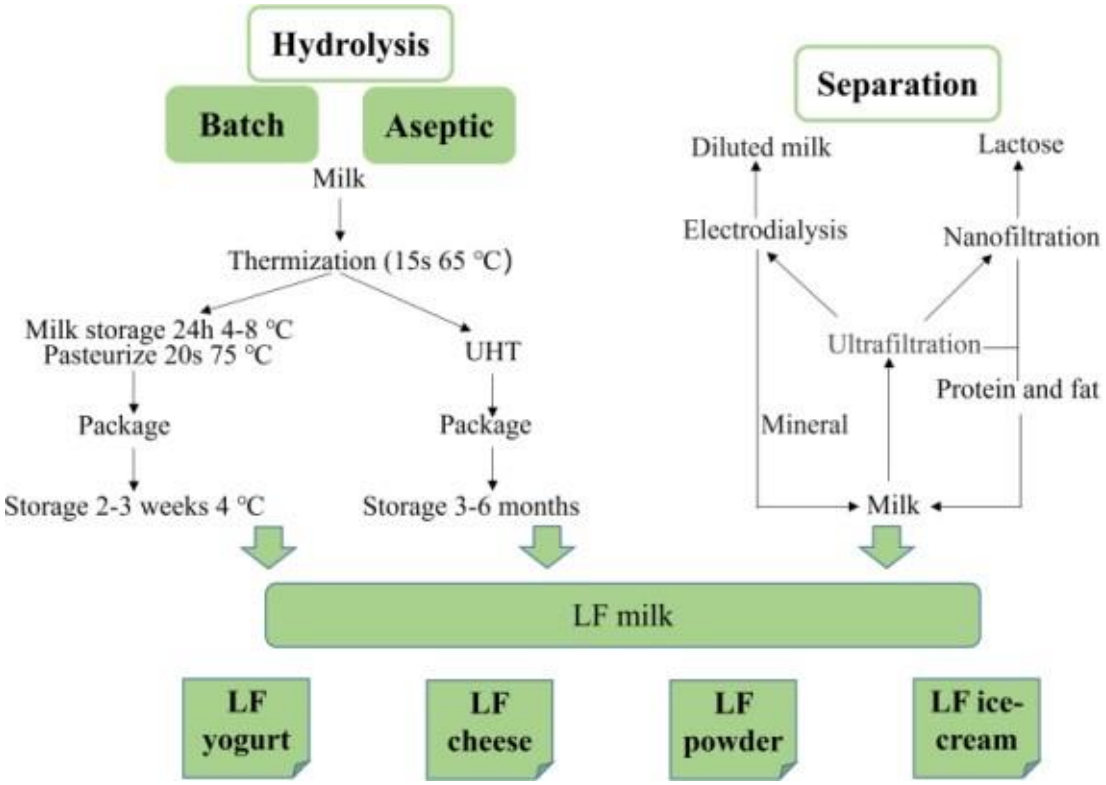


Figure 5. Production of lactose-free (LF) dairy products.

b. Separation of lactose

Among methods of separating lactose, the most well-known and widely used are methods of membrane separation, including ultrafiltration, nanofiltration, reverse osmosis, and electro-dialysis. In different fields of the dairy industry, membranes are applied to shelf life extension of milk, whey processing, cheese industry, milk protein processing, fractionation of milk fat and desalting or demineralization. The key problem in the removal of lactose from milk is the separation of proteins and lactose, and the broad particle size distribution of proteins in milk reduces the separation efficiency of proteins and lactose. Protein and fat are blocked, while lactose and small molecules are allowed to pass through in ultrafiltration and nanofiltration. Polymer ultrafiltration membranes are usually used in industrial practice because of their ease of preparation and cost-effectiveness.

II.1.2. Sucrose hydrolysis

Sucrose hydrolysis can be carried out by invertase (from yeast) or by acid hydrolysis at high temperatures. The product formed is called "inverted sugar". This is called

inversion because the rotatory power of the solution with respect to polarized light is reversed by hydrolysis from αD=66.5-20°. The inversion leads to an increase in the dry weight of the solution, which in turn leads to an increase in the temperature of the solution, sweetness and an increase in the solubility of sugars (solubility of fructose). This possibility of increasing the concentration of sugars in solution is particularly useful in the production of sugar syrups. Sucrose inversion can be achieved by acid hydrolysis or by using invertase or β-fructofuranosidase, responsible for sucrose inversion yielding d-glucose and d-fructose as shown in figure.6

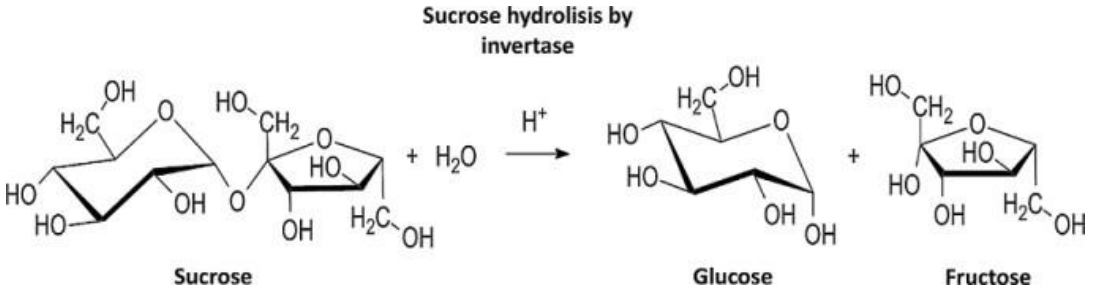


Figure 6. Invertase activity of sucrose hydrolysis.

Sucrose hydrolysis yields an equimolar mixture of fructose and glucose, commercially known as invert sugar. The invert sugar syrup is sweeter than sucrose and easier to incorporate in food and pharmaceutical preparations because it does not show the crystallization problems of its precursor in highly concentrated solutions. The enzymatic process produces food-grade syrups without brown color and undesirable by-products such as hydroxymethylfurfural obtained through non enzymatic methods. In view of the great demand for FOS, levan, and invert syrups as food ingredients in the food industry, the opportunity exists for the screening and identification of novel strains capable of producing new enzymes with transfructosylation activity and for developing improved and less-expensive production methods. In this review, we discuss fructosyltransferases and invertases as well as the beneficial effects of FOS, levan, and invert sugar and how they can play a key role in the food market. Bear in mind, though, that more effective and less costly production methods can be a main advantage in the food industry.

- **Invert syrups in food Industries**

The most significant application of invertase is to produce sugar syrups. The production of high fructose syrup, high fructose corn syrup, and high glucose syrup extensively involves invertases. The production of glucose or fructose syrups by acid treatment has several drawbacks in the finished product, such as texture and taste alteration and formation of the cancerigen 5-hydroxymethyl furfural. Therefore, enzymatic hydrolysis is preferred due to the benefits of greater specificity, clear products, higher yields, and a greater degree of purity. Because fructose is sweeter than sucrose, commercial invertas have a high value in confectionery industries for invert sugar production. In addition, the traces of fructoligosaccharides that are generated as a result of the transfructosylation reaction add beneficial medicinal properties for diabetics and increase the absorbance of iron in children. This sugar has the same properties of honey, so that as it does not crystallize, it is ideal for sweetening ice cream because it remains malleable and creamy with a very soft texture. Therefore, it is also used in chocolate confectionery, namely, fondant, jellies, caramels, and toffees. It is also used in pastries and bakery items as it helps fermentation and retains moisture so that makes the breads or cakes remain tender for longer. It is widely used in the food industry, especially in the manufacture of ice cream through its anti-crystallizing power.

Invert sugar is also used in the ice cream shop because of its against freezing power, that is, it avoids recrystallization, such as glucose or dextrose, providing the ice cream with a soft, smooth, and creamy texture. In addition, it is also useful in manufacturing drug formulae or drugs such as cough syrups, digestive aid tablets, nutraceutics, baby foods, and formulations of animal feed such as food for livestock and honeybees.

II.1.3. Hydrolysis of starch

Starch is the predominant chemical entity in our foods. It makes up 70% or so of the cereals and tubers (dry weight) that are such a large part of our diets. In addition, starch is found in much lower quantities in many fruits and in beans and other legumes. Starch in uncooked food occurs as small packets or granules. The size and shape of the granules vary with the source. In many cases they vary even within a single source. For example, wheat starch varies both in size and shape. The large

starch granules in wheat is about 35 pm across and is lenticular. The small granules are only 2 to 5 pm in diameter and are spherical.

The term gelatinization is often used to describe all changes that occur when we heat starch and water, however, it should be restricted to the loss of birefringence. Once the starch has lost its birefringence, continued heating to higher temperature with water brings about a continuum of changes. These changes can be described best as solubilization.

At 95°C and 0.5% solids concentration, nearly all wheat amylose (29% of starch) is solubilized. The onset of gelatinization occurs between 55-70°C, with initial swelling detectable viscometrically in the presence of hydrocolloids like carboxymethyl cellulose. Resistant starches prepared from wheat starch show higher water absorption at 25°C and 95°C compared to commercial resistant starches from high-amylose maize starch. The gelatinization process follows first-order kinetics until reaching a constant value, which increases with temperature and water content

Factors such as annealing and heating rate can influence amylose yield during leaching. As most food systems are heated to less than 100°C and with limited water, our cooked foods are a mixture of solubilized and insoluble starch.

II.1.3.1. Starch structure

The molecules in starch granules are ordered. Because of this order the granule detracts plane-polarized light in the characteristic maltose cross pattern. This property is called birefringence. The birefringence is often confused with crystallinity. However, material can be birefringent (ordered) but not crystalline. Starch, it turns out, is both birefringent and partially crystalline. The crystallinity can be seen by X-ray diffraction. Starch consists of two large polysaccharides, amylose and amylopectin. Amylose is an essentially linear molecule comprising α (1,4) glucosyl linkages. Its molecular weight is typically in the range 10^5 to 10^6 (ie 500-5000 glucose residues) with a small number (9-20 per molecule) of α (1,6) branches. The structure of amylopectin is much more complex than that of amylose in that it contains three types of α (1,4) glucosyl linked chains; A, B and C, that are linked via α (1,6) branches to form a molecule of weight in the order of 10^7 to 10^8.

Starch is made up of two polyosides: amylose, a linear D-glucose chain linked in theα (1-4), and amylopectin, an α-linked D-glucose chain (1-4) (on which there are α- branched chains (1-6).

Starch is widely distributed in various plant organs as a storage carbohydrate. As an ingredient of many foods, it is also the most important carbohydrate source in human nutrition. In addition, starch and its derivatives are important industrially, for example, in the paper and textile industries. Starch is isolated mainly from the sources listed in table IV. Starch obtained from corn, potatoes, cassava, and wheat in the native and modified form accounted for 99% of the world production in 1980. Some other starches are also available commercially. Recently, starches obtained from legumes (peas, lentils) have become more interesting because they have properties which appear to make them a suitable substitute for chemically modified starches in a series of products. Starches of various origins have individual, characteristic properties which go back to the shape, size, size distribution, composition, and crystallinity of the granules. The existing connections are not yet completely understood on a molecular basis. Most starches contain 20–30% amylose and listed in table 4.

Table 4. Raw materials for starch

Raw material	Starch production 1980[a]
Raw materials of industrial importance	
Corn	77
Waxy corn	
Potato	10
Cassava	8
Wheat	4
Rice	
Waxy rice	
Other raw materials	
Sago palm	Kouzu
Sweet potato	Water chestnut
Arrowroot	Edible canna
Negro corn	Mungo bean
Lotus root	
Taro	Lentil

[a] % of the world production.

New corn cultivars have been developed which contain 50–80% amylose. The amylose can be isolated from starch, e. g., by crystallization of a starch dispersion, usually in the presence of salts (MgSO4) or by precipitation with a polar organic compound (alcohols, such as n-butanol, or lower fatty acids, such as caprylic or capric), which forms a complex withamylose and thus enhance its precipitation.

Normal starch granules contain 70–80% amylopectin, while some corn cultivars and millet, denoted as waxy maize or waxy millet, contain almost 100% amylopectin. If an aqueous starch suspension is maintained for some time at temperatures below the gelatinization temperature, a process known as tempering, the gelatinization temperature is increased, apparently due to the reorganization of the structure of the granule. Treatment of starch at low water contents and higher temperatures results in the stabilization of the crystallites and, consequently, a decrease in the swelling capacity.

The changes in the physical properties caused by treating processes of this type can, however, vary considerably, depending on the botanical origin of the starches. On wetheating, the swelling capacity of both starches decreases, although to different extents (table 5).

Table 5. Physicochemical properties of starches before (1) and after (2) heat treatment in the wet state (T =100 ◦C, t = 16 h, H2O = 27%)

Property	Wheat starch		Potato starch	
	1	2	1	2
Start of gelatinization (°C)	56.5	61	60	60.5
End of gelatinization (°C)	62	74	68	79
Swelling capacity at 80 °C (ratio)	7.15	5.94	62.30	19.05
Solubility at 80 °C (%)	2.59	5.93	31.00	10.10
Water binding capacity (%)	89.1	182.6	102.00	108.7
Enzymatic vulnerability (% dissolved)	0.44	48.55	0.57	40.35

II.1.3.2. Starch enzymatic hydrolysis

Starch hydrolysis can be acidic, enzymatic or combined. Acid hydrolysis is a random attack on the starch chain. It leads to formation of linear or branched chains with few free D-glucose molecules; conversely, enzymes have a specific action (figure 7):

- Endo-enzymes= α-amylase (hydrolysis of α-bonds (1-4))
- Exo-enzymes= hydrolysis of non-reducing ends
- Debranching enzyme= pullulanase (hydrolysis of α-linkages (1-6)).

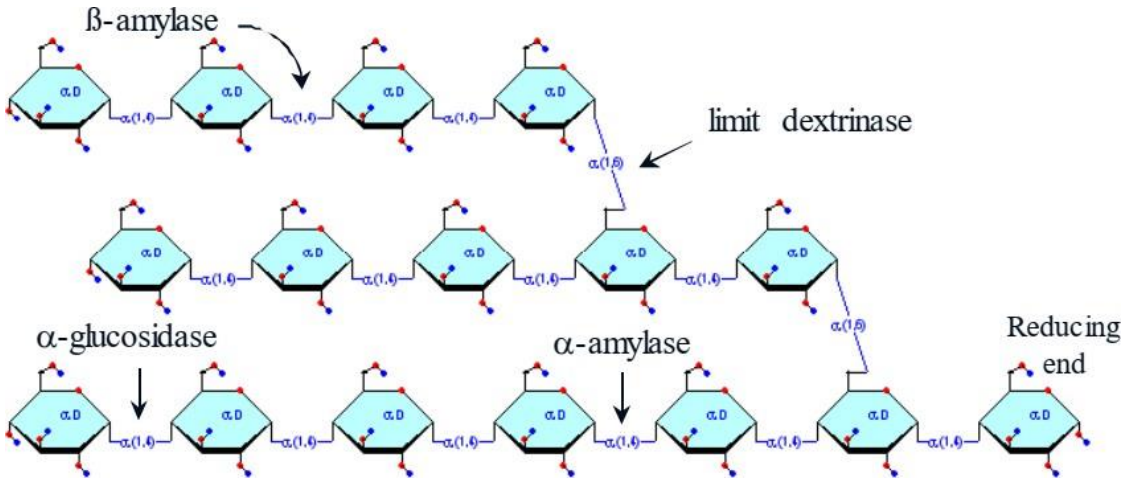


Figure 7. Schematic representation of starch hydrolysis.

α-amylases catalyze the endo-hydrolysis of starch while β-amylases (E.C. 3.2.1.2; GH 14) catalyze the exo-hydrolysis. This difference in the position of polysaccharide hydrolysis is resulted from the two slightly varied but distinct mechanisms of action, namely retaining (α-amylases; Figure. 8A) and inverting (β-amylases; Figure. 8B), where both involve the displacement(s) of nucleophiles.

α-amylase has a general five-step reaction mechanism which involves double nucleophilic displacement, giving the popularly known α-retaining double displacement mechanism in hydrolyzing its substrate. There are two key amino acid residues which are usually a pair of acidic amino acids (aspartic acid and/or glutamic acid) separated at an approximately 5 Å distance. The first amino acid acts as a catalytic nucleophile which attacks the anomeric center of the substrate while the second amino acid donates a proton to the glycosidic oxygen at the anomeric center, catalyzing the removal/departure of aglycone (ROH).

An oxocarbenium ion-like transition state is formed during the glycosylation step before the addition of water molecule and aglycone departure. The proton donation of

the second amino acid renders itself as a general base which subsequently catalyzes the second nucleophilic displacement in the deglycosylation step of the covalent glycosyl-enzyme intermediate. Both nucleophilic displacements before glycosylation and after deglycosylation steps involve the oxocarbenium ion-like transition states as aforementioned, thus the α-retaining double displacement mechanism β-Amylases, however, catalyze the hydrolysis of starch into maltose inside then polysaccharide chain (endo-). Although the key catalytic residues are similar with α-amylases, which are commonly a pair of carboxylic acids, these acidic residues are distant from 6–12 Å. The more distant acidic amino acids enable the accommodation of the water molecule and substrate at the active site, where the cleavage of scissile glycosidic linkage is performed via general-base catalyzed nucleophilic attack of water molecule on the anomeric center and general-acid assisted aglycone departure. Compared to the retaining mechanism of α-amylases, this inverting mechanism involves only a single nucleophilic step, where the nucleophile from the acidic amino acid attacks the water molecule which subsequently attacks the substrate's anomeric center.

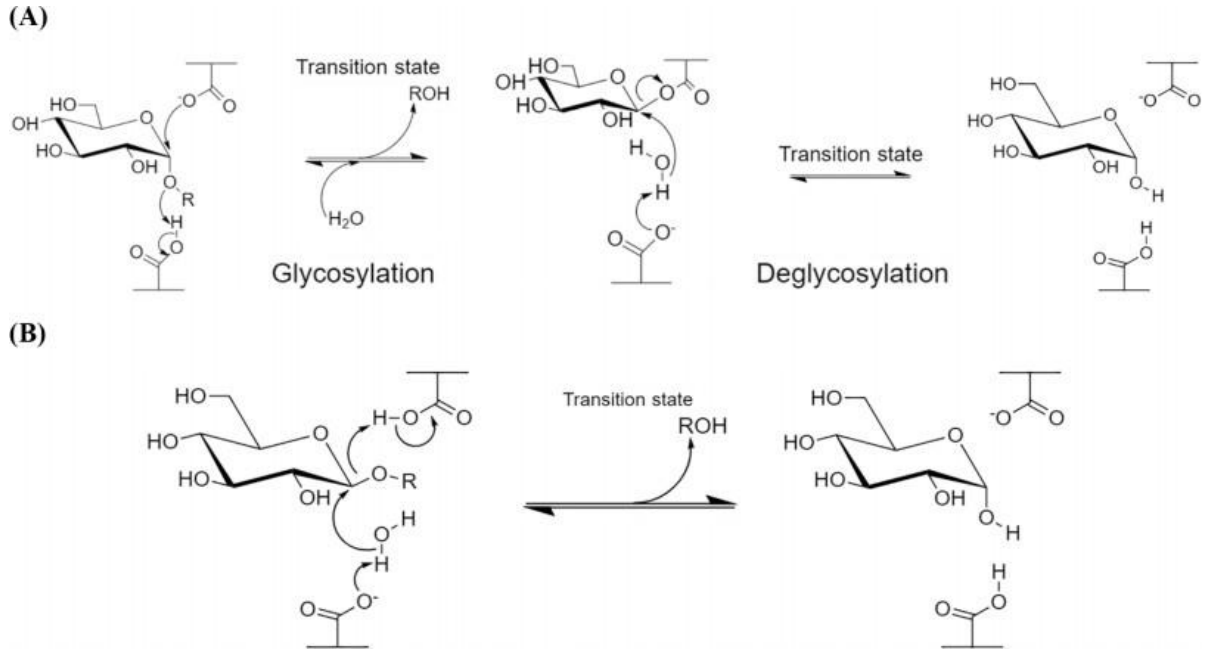


Figure 8. Reaction mechanisms of amylases. (A) α-Retaining double displacement mechanism of α-amylases. (B) Inverting single displacement mechanism of β- amylases.

The enzymes comprise the combined activity of α -amylase, β-amylase, α-glucosidase and limit dextrinase whose concerted action hydrolyse the α (1,4) and α (1,6) glucosyl linkages in starch (Figure 8) into fermentable sugars (ie glucose, maltose, etc), dextrins and limit dextrins.

The actions of the DP enzymes are summarised as follows:

1. α-Amylase cleaves α (1,4)-linkages internally (endo-acting) to primarily produceoligosaccharides, limit dextrins and some fermentable sugars.
2. α-Amylase cleaves α (1,4)-linkages from the non-reducing ends (exo-acting) toproduce maltose.
3. Limit dextrinase hydrolyses internal α (1,6)-linkages (endo), to remove branch-points in amylopectin or α-limit dextrins.

α-Glucosidase primarily cleaves α (1,4)-linkages from the non-reducing ends to produce glucose.

Generally, it is considered that α-amylase, β-amylase and limit dextrinase are of most importance in determining starch degrading potential while in the absence of contraryevidence it is considered that the contribution α-glucosidase is minimal

II.1.3.3. Hydrolysis starch products

Among starch degradation products:

- **Maltodextrins:** are obtained by acid, enzymatic or combination of They are made up of a mixture of different sugars: glucose and maltose, maltotriose, oligosaccharide, polyoside) resulting from the hydrolysis of starch in proportions that depend on their degree of hydrolysis, which is measured by dextrose equivalent (DE). Dextrose is represented by D-glucose; the more the hydrolysis is the higher the simple sugar content. The DE limit for maltodextrin is 20%. Beyond this limit, the product is glucose syrup".Applications: bread fermentation, brewing (table 6 and 7).

Table 6. Starch hydrolysis degree

60, 90, dan 120 minutes of hydrolysis time.

Samples	Hydrolysis time (min)	Dextrose Equivalent
Maltodextrin of	60	9.94
cassava starch	90	12.25
	120	16.53

Table 7. Dextrose equivalent of some samples

Starch sample	Dextrose equivalent	Glucose	Isomaltose
Corn[b]	96·0 ± 1·9	105·50 ± 2·30	1·16 ± 0·25
Cassava[c]	97·2 ± 2·3	105·20 ± 2·80	1·60 ± 0·10[a]
M. rumphii[c]	94·5 ± 1·5	103·50 ± 1·90[a]	1·41 ± 0·27
M. rumphii II[c]	94·4 ± 1·6	104·70 ± 1·30	1·28 ± 0·10
M. sagu[c]	95·3 ± 1·4	104·50 ± 1·30	1·48 ± 0·13
A. microcarpa[c]	95·6 ± 1·1	104·20 ± 2·00	1·35 ± 0·15
A. pinnata I[c]	86·2 ± 1·1[a]	94·00 ± 3·60[a]	1·21 ± 0·06[a]
A. pinnata II[c]	95·6 ± 1·1	104·60 ± 1·50	1·33 ± 0·05

[a]Significantly different from corn at the 5% level.
[b]Based on six determinations.
[c]Based on four determinations.

Based on the conducted research experiments, it can be concluded that the best starch hydrolysis time is at 120 minutes, which results in the optimal DE value. The higher the amount of maltodextrin added, the solubility and vitamin C content of the guava juice powder extract will increase as well.

Low DE maltodextrins (≤12) are only slightly hydrolyzed and have a broad molecular weight distribution. They exhibit higher glass transition temperatures and viscosities than high DE maltodextrins (≥21) consisting mostly of oligosaccharides. We showed that the viscosity of maltodextrins can be described by the ratio of glass transition temperature to the system temperature Tg/T (figure 9).

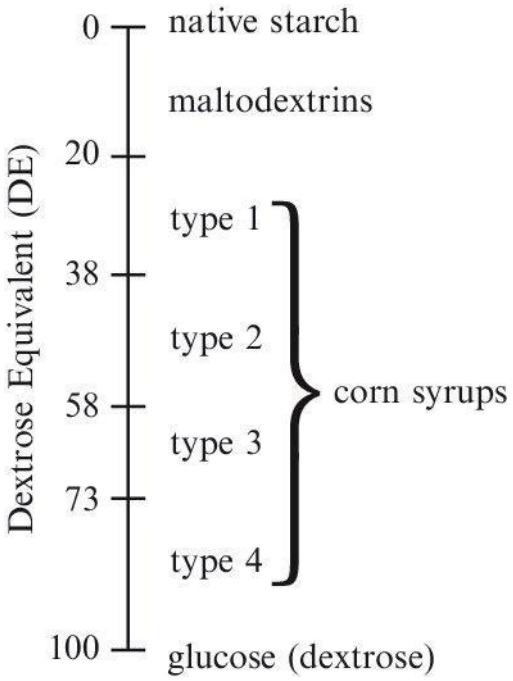


Figure 9. Classification of starch hydrolysates based on the dextrose equivalent (DE) value.

II.1.3.4. Starch - heat-induced effects

Raw (uncooked) starch does not absorb much water (about 30% of its dry weight). Evidently, the crystallinity of the starch limits its swelling and thus limits the amount of water it can absorb. With gelatinization, the crystallinity is lost and the granules are free to swell. With swelling the granule is free to take up much more water. Also, with gelatinization the starch loses its raw starch taste and becomes smooth or cooked.

Cooking starch or starchy foods in water leads to an increase in the viscosity of the cooked product, gravies and sauces being good examples. The rather large increases in viscosity can be followed conveniently with an amylograph. In the amylograph a dilute starch: buffer (4% starch) solution is heated at a constant IV 2°C/min and the relative viscosity recorded. The uptake of water by the starch granules decreases the amount ofwater remaining in the system. The starch that is solubilized during the heating then isdissolved in less total water resulting in a more viscous system. In limited water systems, such as cake batters or bread doughs.

Heating of starch (<15% of water) to 100–200 ◦C with small amounts of acidic or basic catalysts causes more or less extensive degradation. White and yellow powders are obtained which deliver clear or turbid, highly sticky solutions of varying viscosity. These products are used as adhesives in sweets and as fat substitutes.

a. Pregelatinized starch

Heating of starch suspensions, followed by drying, provides products that are swellable in cold water and form pastes or gels on heating. These products are used in instant foods, e. g., pudding, and as baking aids.

b. Thin-boiling starch

Partial acidic hydrolysis yields a starch product which is not very soluble in cold water but is readily soluble in boiling water. The solution has a lower viscosity than the untreated starch, and remains fluid after cooling. Retrogradation is slow. These starches are utilized as thickeners and as protective films.

- **Gelatinization**

Gelatinization refers to the irreversible loss of the crystalline regions in starch granules that occur upon heating in the presence of water. The temperature ranges during which the crystalline structure of the starch granule is dependent on the water content, and on the type of starch. The gelatinization dramatically increases the availability of starch for digestion by amylolytic enzymes.

Usually, the starch granules are not completely dissolved during food processing, and a food can be regarded as a dispersion in which starch granules and/or granular remnants constitute the dispersed phase. The degree of gelatinization achieved by most commonly used food processes, however, is sufficient to permit the starch to be rapidly digested. Consequently, even food processes which result in a low degree of gelatinization (e.g. steaming and flaking of cereals), produces a postprandial blood glucose and insulin increment similar to that with completely gelatinized food.

The temperature at which starch gelatinization occurs depends first on the source of starch. For example, potato starch gelatinizes at a lower temperature than does corn starch.

Generally, we must speak of a gelatinization temperature range rather than a specific temperature. With most starches, the temperature range over which gelatinization occurs in excess water is 7-10°C. As water becomes more limiting the temperature range becomes much broader. The temperature at which starch starts togelatinize does not change as water is limited. The second major factor that affects starch gelatinization temperature is which and how many molecules are dissolved in the available water. Small molecules dissolved in water will decrease the water activity.

As water activity is decreased gelatinization temperature is increased. Sugars, of course, will affect water activity. In addition to their effect on water activity, sugars, will cause starch gelatinization to occur at higher temperatures apparently by an additional mechanism. In many of our food systems this is used to control the temperature at which the product sets.

- **Retrogradation**

Gelatinized starch is not in thermodynamic equilibrium. There is, therefore, a progressive re-association of the starch molecules upon ageing. This recrystallization is referred to as retro gradation, and may reduce the digestibility of the starch. The retrogradation of the amylopectin component is a long-term phenomenon which occurring gradually upon storage of starchy foods. Amylose, however, re-associates more quickly. The crystallinity of retrograded amylopectin is lost following re-heating to approximately 70°C, whereas temperatures above 145°C are required to remove crystallinity of retrograded amylose. This is a temperature well above the range used for processing of starchy foods. This implies that retrograded amylose, once formed, will retain its crystallinity following re-heating of the food.

- **Par-boiling**

During par-boiling of rice, the kernels are subjected to a pre-treatment involving heating and drying. This process reduces the stickiness of the rice, possibly by allowing leached amylose to retrograde and/or form inclusion complexes with polar lipids on the kernel surface. Parboiling also affects the final cooking properties of the rice.

c. Starch - texturization

In pasta products, gluten forms a viscoelastic network that surrounds the starch granules, which restricts swelling and leaching during boiling. Pasta extrusion is known to result in products where the starch is slowly digested and absorbed. Available dataon spaghetti also suggest that this product group is a comparatively rich source of resistant starch. The slow-release features of starch in pasta probably relates to the continuous glutenous phase. This not only restricts swelling, but possibly also resultsin a more gradual release of the starch substrate for enzymatic digestion. Pasta is nowgenerally acknowledged as a low glycemic index food suitable in the diabetic diet. However, it should be noted that canning of pasta importantly increases the enzymic availability of starch, and hence the glycemic response.

II.1.4. Dietary fiber modification

During milling of cereal grains to refined flours the outer fiber-rich layers are removed, and resulting in a lower content of total dietary fiber. This reduction is due mainly to a decrease of insoluble fiber. An increased temperature leads to a breakage of weak bonds between polysaccharide chains. Also glycosidic linkages in the dietary fiber polysaccharides may be broken. These changes are important from analytical, functional and nutritional points of view.

A decreased association between fiber molecules, and/or a depolymerization of the fiber, results in a solubilization. If the depolymerization is extensive, alcohol soluble fragments can be formed, resulting in a decreased content of dietary fibre with many of the currently used fiber methods. Moderate depolymerization and/or decreased association between fiber molecules, may have only minor influence on the dietary fiber content, but functional (e.g. viscosity and hydration) and physiological properties of the fiber will be changed. Other reactions during processing that may affect the dietary fiber content and its properties are leakage into the processing water, formation of Maillard reaction products thus adding to the lignin content, and formation of resistant starch fractions. Also, structural alterations in the cell wall architecture are important to follow during processing as these are highly correlated to sensory and nutritional characteristics.

The cross-linking of constituent polysaccharides and phenolics within the cell wall is important in determining the properties of the fiber matrix, as the solubility of the fiber is highly dependent on the type and amount of cross-links present. During heat-treatment the cell-wall matrix is modified and the structural alterations that occur may be important not only for the nutritional properties of the product but also for its palatability.

With extrusion-cooking of wheat-flour, even at mild conditions, the solubility of the dietary fiber increases. The solubilization seems to be dependent on the water content used in the process, and the lower the content of water, the higher the solubilization of the fiber, at least for whole-grain wheat flour and wheat bran. The screw speed and the temperature had minor effects in those experiments. An increased solubility of the fiber has also been obtained with 'severe' popping of wheat, whereas baking (conventional and sour-dough baking), steam-flaking and drum-drying had only minor effects on dietary fiber components. One reason why popping caused an increased solubility of the fiber was that the outer fibrous layers were removed and the content of insoluble fiber decreased

II.2 . Lipid hydrolysis and other modifications

Research reports on enzyme-induced changes in lipids in foods are abundant. In general, they are concentrated on changes in the unsaturated fatty acids or the unsaturated fatty moieties in acylglycerols (triglycerides). The most studied are linoleate (linoleic acid) and arachidonate (arachidonic acid) as they are quite common in many food systems. Because of the number of double bonds in arachidonic acid, enzymatic oxidation can occur at various sites, and the responsible lipoxygenases are labeled according to these sites.

- **Changes in lipids during chees manufacturing**

Milk contains a considerable amount of lipids and these milk lipids are subjected to enzymatic oxidation during cheese ripening. Under proper cheese maturation conditions, these enzymatic reactions starting from milk lipids create the desirable flavor compounds for these cheeses. These reactions are numerous and not completely understood, so some general reactions are provided (table 8). The ester bonds can be broken either chemically or enzymatically develops mainly under the action of heat and humidity.

Table 8. Changes in lipids during chees manufacturing

Enzyme or Actions	Reaction
Lipolysis	
Lipases, esterases	Triglycerides → β-keto acids, acetoacetate, fatty acids
Acetoacetate decarboxylase (EC 4.1.1.4)	Acetoacetate + H^+ → acetone + CO_2
Acetoacetate-CoA ligase (EC 6.2.1.16)	Acetoacetate + ATP + CoA → acetyl CoA + AMP + diphosphate
Esterases	Fatty acids → esters
Conversion of fatty acids	
β-oxidation and decarboxylation	β-Keto acids → methyl ketones

II.2.1. Non-enzymatic hydrolysis

Lipids can be hydrolyzed in an acid medium (sulphuric acid) but the method The most commonly used method involves heat treatment (80-100°C) in an alkaline environment (soda or potash) to give the alcohol and the fatty acid in salt form (soaps). The saponification reaction is represented in figure 10.

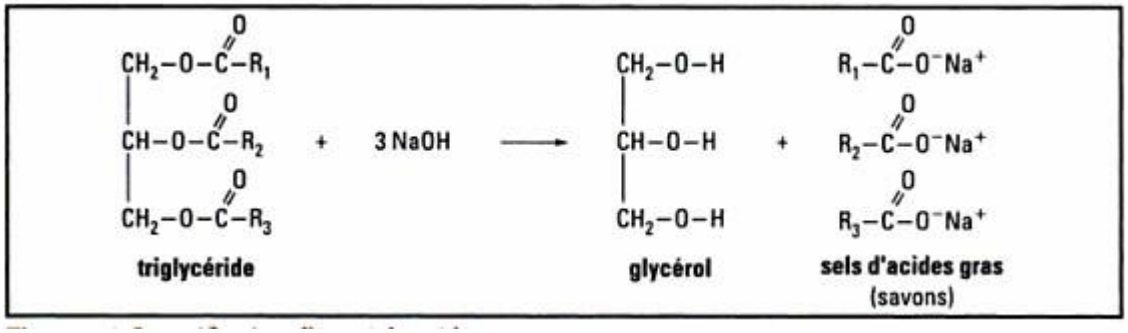


Figure 10. Saponification of a triglyceride.

In the longer term, after the actual drying process, hydrolysis of ester bonds may disconnect fatty esters from the triglyceride glycerol backbone. The figure 11 schematically illustrates hydrolysis, which may occur at each fatty ester leading to glycerol with two remaining fatty esters (diglyceride,DAG), glycerol with one fatty ester (monoglyceride- MAG) or a fully hydrolyzed triglyceride with all fatty esters detached.

Hydrolysis is one of the main degradation routes of fatty ester polymer systems. In thepresence of water, an ester bond in a triglyceride is broken via hydrolysis and a carboxylic acid is formed on the side of the free fatty ester and alcohol on the side of glycerol.

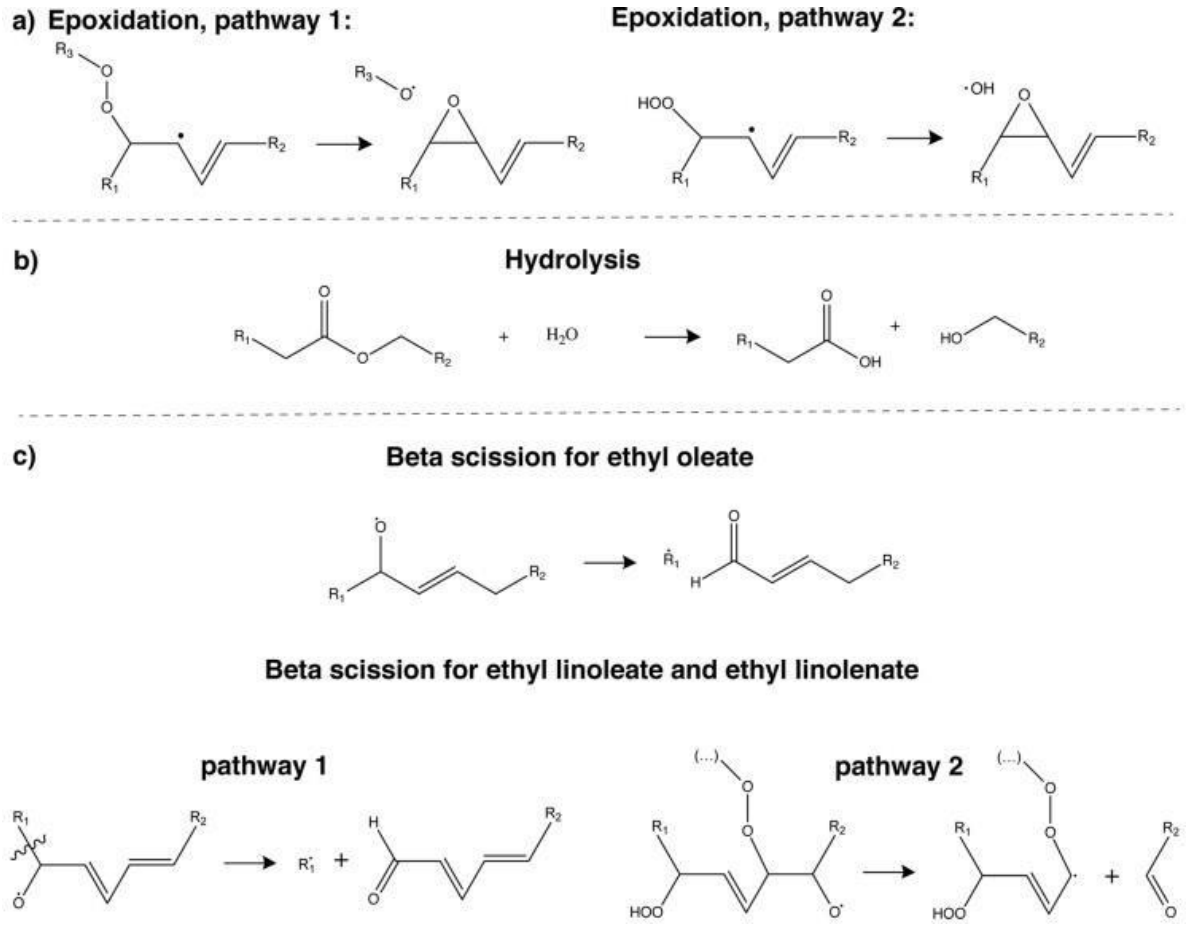


Figure. **11**. Epoxidation from a species with peroxy crosslink (1) and from a species with hydroperoxide (a). Hydrolysis of an ester bond between glycerol and fatty acid (b). β-scission pathways for oleic fatty ester (top) and for linleic and linolenic fatty esters (bottom) (c).

Some researchers studied the origins of carboxylic acids in oil paints with zinc oxide at various relative humidity (RHs). They measured the rate of hydrolysis over a decade under varying conditions concerning metal pigments, RH, and temperature and examined the resulting mechanical properties.

a. β-scission

Breaking the β-bond to an alkoxyl radical, β-scission propagates the free radical and forms an olefinic species. β-scission is common in thermal cracking, but at ambient conditions it is of limited importance. Products from scission reactions in drying oil systems vary significantly in carbon chainlength, as the location of β-bonds next to alkoxyl radicals may vary in oleic, linoleic and linolenic acids.

Products of β-scission in linseed oil degradation include volatile n-aldehydes such as ethanal, propanal, butanal, pentanal, hexanal, heptanal and octanal.

b. Peroxy crosslink decomposition

It was demonstrated that a gradual decrease in peroxy crosslink concentration. This is attributed to slow peroxy crosslink decomposition. In contrast to hydroperoxide decomposition is apparently not catalyzed by transition metal driers and occurs with an activation energy of 40kcal/mol.

II.2.2. Enzymatic hydrolysis of lipids

Hydrolases, which cleave acyl lipids, are present in food and microorganisms. The release of short-chain fatty acids (<C14), during the hydrolysis of fat milk, has a effect on food aroma. Lipolysis is undesirable in fresh milk since the free C4–C12 fatty acids are responsible for the rancid aroma defect. On the other hand, lipolysis occurring during the ripening of cheese is a desired and favorable process because the short-chain fatty acids are involved in the build-up of specific cheese aromas. Likewise, slight hydrolysis of fat milk is advantageous in the production of chocolate.

Linoleic and linolenic acid released by hydrolysis and present in emulsified form affect the flavor of food even at low concentrations. They cause a bitter-burning sensation. In addition, they decompose by autoxidation or enzymatic oxidation into compounds with an intensive odor. In fruits and vegetables enzymatic oxidation in conjunction with lipolysis occur, as a rule, at a high reaction rate, especially when tissue is sliced or homogenized (table 9). Also, enzymatic hydrolysis of a small amount of the acyl lipid present cannot be avoided during disintegration of oil seeds.

Table 9. Lipid hydrolysis occurring during potato tuber homogenization

	µmoles/g[a]	
	Acyl lipids	Free fatty acids
Potato	2.34	0.70
Homogenate[b]	2.04	1.40
Homogenate[b] kept for 10 min at 0 °C	1.72	1.75
Homogenate kept for 10 min at 25 °C	0.54	2.90

[a] Potato tissue fresh weight.
[b] Sliced potatoes were homogenized for 30 sec at 0 °C.

Since the release of higher fatty acids promotes foaming, they are removed during oil refining. Enzymes with lipolytic activity belong to the carboxyl-ester hydrolase group of enzymes.

a. Triacylglycerol hydrolases (lipases)

Lipases hydrolyze only emulsified acyl lipids; they are active on a water/lipid interface. Fats and oils are esters of the alcohol glycerol, which are built of long-chain fatty acids (monocarboxylic acids). Ninety-eight per cent of all natural fats and oils are mixtures of different triglycerides, whereby each of the three hydroxyl groups of glycerol is esterified with one fatty acid. During hydrolysis, lipases (esterases) enzymatically split fats into glycerol and the individual long-chain fatty acids. Consequently, fats can be completely hydrolyzed, but mostly only at low decomposition rates (figure 12).

The hydrolases responsible for the hydrolysis of triglycerides are widely involved. The release of short-chain fatty acids during butter hydrolysis has the following effects on the aroma. Lipolysis is not desired, particularly in milk, but It is essential for the maturing of cheeses.

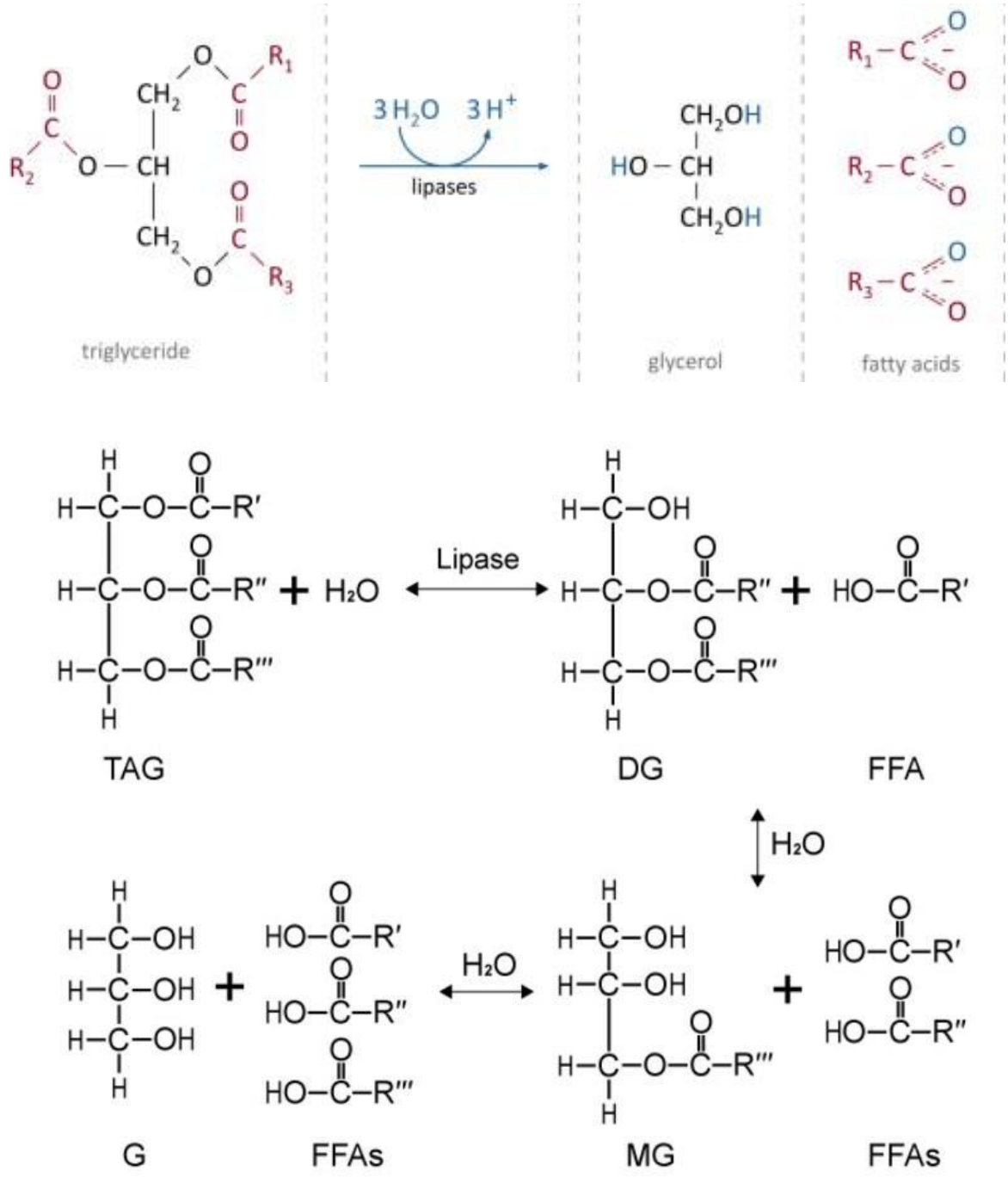


Figure 12. Hydrolysis of lipids.

Lipases in the food industry and nutraceutical production can be used in aqueous extracts and purified, immobilized, or whole cells to exploit the available raw material and increase their economic and nutritional value. These enzymes can be used to modify fats and oils and synthesize structured lipids or antioxidants with increased antioxidant power or modified lipophilicity, flavors, and aromas.

Lipases differ from esterase enzymes since the latter cleave only water-soluble esters, such as triacetylglycerol. Lipase activity is detected, for example, in milk, oilseeds (soybean, peanut), cereals (oats, wheat), fruits and vegetables and in the digestive tract of mammals. Many microorganisms release lipase type enzymes into their culture media. As to their specificity, lipases are distinguished according to the criteria. The lipase secreted by the swine pancreas has been the most studied. Its molecular weight is Mr = 48,000. The enzyme cleaves the following types of acyl glycerols with a decreasing rate of hydrolysis: triacyl- >diacylmonoacylglycerols. Table 10 shows that pancreatic lipase reacts with acyl residues at positions 1 and 3. The third acyl residue of a triacylglycerol is cleaved only after acyl migration, which requires a longer incubation time.

The smaller size of the oil droplet, the larger the oil/water interface and, therefore, the higher the lipase activity are characteristics relationship which should not be ignored when substrate emulsions are prepared for the assay of enzyme activities. Enzymes have different specificities, those that hydrolysis all the bonds and those that hydrolysis all the molecules. Those that preferentially hydrolysis fatty acids esterified in positions one and three of the same glycerol.

Table 10. Origin of lipase

Hydrolysis of triglycerides	**Origin of lipases**
1, 2 and 3	*Aspergillus flavus*
1 and 3	*Penecillium roqueforti*

II.3 Modification of proteins

II.3.1. Effects of thermal processing on proteins

Proteins could be denatured during heating, depending on the temperature and the protein in question, causing the loss of their quaternary and tertiary structures and forming unfolded random shapes. Additionally, thermal treatment (90 °C, 2 h) of proteins gives rise to the formation of isopeptides, lysinoalanine, and racemization, alter proteins' allergenicity and stretching of some amino acids along with their peptide bonds in the primary structure.

Proteins during heat processing are stimulated to interact with other components in the food system. Maillard reaction is one of the most important, which involve proteins and contribute greatly to the nutritional and sensory properties of foods. The reactions are initiated by interactions between reducing sugars and amino acids and they continue with a large set of chain reactions.

These reactions may affect the color, flavor, and aroma of the food product, cause the formation of toxic compounds (e.g., acrylamide, furans, and hydroxyl propyl furfural), and decreased the digestibility and nutritional value**.** Unlike denaturation, which only affects the quaternary, tertiary and hydrolysis affects the primary structure of proteins, leading to the formation of other, sometimes undesirable products. This degradation can be enzymatic or non-enzymatic.

II.3.2. Effects of no thermal processing on proteins

a. Surface hydrophobicity

Table 11 shows a summary of the effects of non-thermal properties on proteins and amino acids. The number of hydrophobic groups on the surface of proteins determines their hydrophobicity. Some functional properties of proteins (emulsifying, foaming and gel-forming) as well as their stability and conformation, are dependent on the hydrophobic interactions. Non-thermal treatments can affect the surface hydrophobicity of proteins by imposing conformational changes, such as unfolding (partial or full unfolding, depending on the processing conditions) and displacement of hydrophilic and hydrophobic groups.

Table 11. Effect of non-thermal processing on proteins and amino acids.

Treatment	Substrate	Condition	Results
Ultrasound	Corn gluten meal	40 kHz, pulsed on- 10 s and off 3 s, 40 min and 20 °C.	- Molecular unfolding and exposure of hydrophobic groups - Decrease in α-helix and increase in random coil contents after heat/ultrasound and ultrasound/heat treatments
	Soy protein	20 kHz, power 65 W, 0.5, 1, 5 & 15 min	Protein extraction yield enhanced due to increasing in the solubility
	Beef proteins	2.39, 6.23, 11.32 and 20.96 Wcm^{-2}, 30, 60, 90 and 120 min	- Increase in S0 and decrease in -SH groups - Myosin aggregation and formation of higher molecular weight polymers - Decrease in α-helix and increase in β-sheet contents
	Myofibrillar proteins	200, 400, 600, 800 and 1000 W, 88, 117, 150, 173 and 193 Wcm^{-2}	Increase in S0, decrease in particle size
	Squid (*Dosidicus gigas*) mantle proteins	20 kHz, 0, 20, and 40%), 0, 30, 60, and 90 s	- Hydrophobicity was increased - The content of reactive sulfhydryl didn't change - Better emulsifying ability
	Chicken myofibrillar protein	240 w, 0, 3, 6, 9, 12 and 15 min)	- Increase in -SH groups - No changes in primary structure - Increase in β-turn and decrease in α-helix and β-sheet structures - Decrease in particle size, narrow size distribution
	Duck liver protein isolate	24 kHz, 266 W by a pulsed on-time of 2 s and off-time of 3 s for 42 min	- Increase in S0 - No changes in -SH content, primary structure and peptide bonds - Decrease in α-helix and random coil and increase in β-sheet and turn structures - Decrease in particle size
	B-Lg In Cow Milk	9.5 W, 135 W/cm^2	No significant alteration in allergenicity
	Tropomyosin from shrimp	30 Hz, 800 W for 30–180 min	Allergenicity was reduced
High pressure processing	Hongqu Rice wines	200 and 550 MPa, 25 °C, 30 min	Free amino acids content was decreased after 6 months storage
	Brown rice	0.1–500 MPa,10 min	Free amino acids especially essential ones were increased
	Tropomyosin from shrimp	200, 400 and 600 MPa at 20 °C for 20 min	- Conversion of α-helix structure into β-sheet and random coil - Free sulfhydryl content was decreased - Surface hydrophobicity was increased by increasing the pressure from 200–400 MPa and decreased at the range of 400–600 MPa - Allergenicity was decreased
	Soy allergen (Glycinin)	100, 200 and 300 MPa for 15 min	- Polyelectrolysis was increased
	Brussels sprouts	200 and 800 MPa for 3 min, 5 °C	- The total free amino acids content was constant - The concentration of glutamin and asparagine were increased

The sensitivity of a protein toward a denaturation treatment (thermal or non-thermal) also depends greatly on its structure. The stability of tertiary and/or secondary protein structures in denaturation treatment is related to hydrogen interactions between the polar groups and interactions of non-polar groups (hydrophobic interactions) through the surrounding water molecules, which form cages around hydrophobic groups. Electrostatic bonds and Van der Waals interactions are also involved in the denaturation process, although to a lesser extent. For example, α-lactoglobulin is moreresistant to high pressure treatment (and thermal treatments) when compared to β- lactoglobulin due to its four intramolecular disulfide bonds as compared to the two forβ-lactoglobulin and its linked calcium ion that also help to maintain its stability toward denaturation. In another example, β-sheet structures are more resistant to pulsed electric fields as compared to α-helical structure. β-sheet structures, whose peptide chains are arranged in a pleated way and are also stabilized by hydrogen bonds, are not as widespread. In comparison to in α-helical structures, the side groups of β-sheet inherent amino acids are very closely arranged to one another, such that similarly or bulky charged residues disturb this structure type. Consequently, larger areas of β- sheet structure may only be formed in the presence of small residues, as can be found in alanine or glycine, whereas larger residues lead to polypeptide chain α-helical formation.

The effect of ultrasound on myofibrillar proteins (MP) was studied and found that the cavitation phenomenon in ultrasound treatment denature the hydrophobic amino acids from the interior parts to the surface, and thus increases the surface hydrophobicity. A positive correlation was reported between the surface hydrophobicity of the proteins and their solubility. It should be noted that the correlation between these two factors (protein surface hydrophobicity and protein solubility) depends on the strength of the intramolecular interactions within a protein and the involvement of exposed hydrophobic groups in these interactions. For example, more participation of exposed hydrophobic amino acids in intramolecular interaction results in less protein solubility. The presence of electrostatic interactions may explain some of these contradictory results. For instance, stronger electrostatic interactions (as compared to intramolecular ones) can prevent the aggregation by keeping proteins at a far distance from each other, and thus increasing the protein solubility.

The number of thiol (-SH) groups was decreased in broiler proteins, as affected by high intensity ultrasound, which is probably due to the formation of intermolecular disulfide bonds (S-S). Hydrogen peroxide, which was produced from water molecules during ultrasound treatment, can oxidize thiol groups to disulfide bonds.

The effect of different types of non-thermal treatments, including ultrasound, high pressure, cold plasma, and irradiation, on some of the protein properties was discussed. Protein hydrophobicity can be increased after non-thermal treatments due to the unfolding and exposure of hydrophobic amino acids. Different hydrophobic and disulfide interactions could occur as a result of exposing the thiol groups and hydrophobic amino acids to the surface. Changes in the proportion of different types of secondary structures as a result of non-thermal treatments have been reported in various investigations. Protein solubility and particle size could be altered in different ways (e.g., an increase in size and decrease in solubility due to aggregation/decrease in size, because of turbulent flow/increase in solubility due to more interaction points with water after unfolding) after non-thermal treatments.

Hydrogen bonding, and thus the gel-forming ability of proteins can also be changed by non-thermal treatments. The relationship between the reported modifications and sensory aspects by consumers are rarely addressed and future research should address this gap. Some researchers reported the decrease of the allergenicity of some proteins after non-thermal treatments due to changes in the antibody's binding ability. The content of amino acids and their concentrations has been also reported to changein some studies. The consequence nutritional impact of the changes in amino acids profile will affect the bioavailability, limiting amino acid as well as sensorial aspects asfree amino acids could change the flavor of food, and these effects are yet to be determined. Further studies are needed to determine the effect of other non-thermal processing methods, such as pulsed light on the physicochemical properties of proteins and amino acids. Moreover, most of the studies were performed on the laboratory-scale and for a commercial pathway; it is important to evaluate their effectson the industrial scales.

Overall, the non-thermal processing technologies are providing the food industry with opportunities to modify food and impart changes beyond the traditional food safety that can be of great importance to consumers and industry.

II.3.3. Non enzymatic degradation of proteins

The nature and extent of the chemical changes induced in proteins by food processingdepend on a number of parameters, for example, composition of the food and processing conditions, such as temperature (cooking), pH or the presence of oxygen. As a consequence of these reactions, the biological value of proteins may be decreased:

• Destruction of essential amino acids

• Conversion of essential amino acids into derivatives which are not metabolizable

• Decrease in the digestibility of protein as a result of intra- or interchain cross-linking. Formation of toxic degradation products is also possible. The nutritional/physiological and toxicological assessment of changes induced by processing of food is a subject of some controversy and opposing opinions.

a) Isopeptide formation

Heating proteins in a dry state at neutral pH results in the formation of isopeptide bonds between the ε-amino groups of lysine residues and the β- or γ-carboxamide groups ofasparagine and glutamine residues; by heating of intra- and inter-molecular bridges between the terminal amine functions of lysine and C-terminal functions of glutamine;the consequence being loss of digestibility. Transglutaminase (TG) makes a covalentbond between the side chains of glutamine and lysine to yield the protease resistant isopeptide bond and ammonia (figure 13). These isopeptide bonds are cleaved during acidic hydrolysis of protein and, therefore, do not contribute to the occurrence of unusual amino acids. A more intensiveheat treatment of proteins in the presence of water leads to a more extensive degradation. Organophosphorus pesticides and the nerve agent VX induce a covalent bond between the side chains of glutamic acid (or aspartic acid) and lysine to yield the isopeptide bond and water

Transglutaminase generates isopeptide crosslinks between proteins that stabilize structures important for brain function Glutamine and lysine residues susceptible to transglutaminase-catalyzed crosslinking are commonly identified by incorporating fluorescent transglutaminase substrates such as dansyl-cadaverine and dansyl-aminohexyl into target proteins.

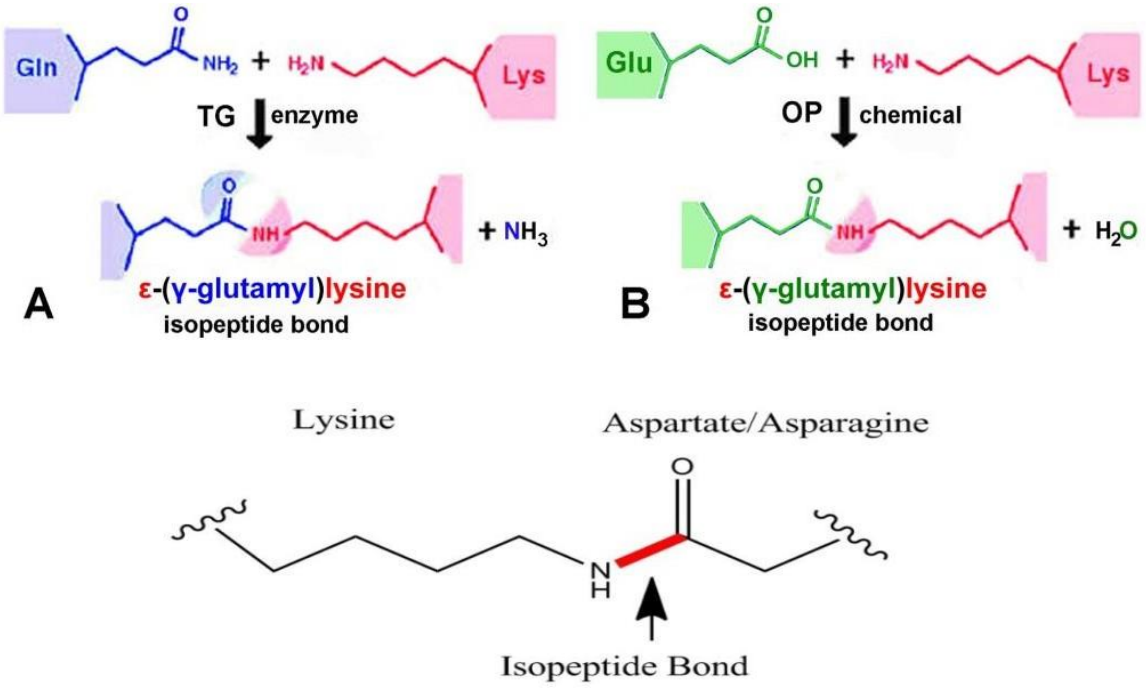


Figure 13. Isopeptide bond between glutamine and lysine formed by the action of thetransglutaminase (TG) enzyme with release of ammonia. B) Isopeptide bond between glutamic acid (or aspartic acid) and lysine formed by exposure to organophosphorus chemicals (OP) with release of water.

b) Formation of lysinoalanine: this is an unusuel amino acid resulting from the bonding of lysine and alanine residues at the end of their chains. Severe thermal and/or alkaline processing can induce protein–protein interaction, new bonds formation in the same protein chain or among nearby chains, which may formnew compounds naturally absent in foods, such as lysinoalanine (N6-(DL-2-amino-2-carboxyethyl)-L-lysine; possible toxic effect), lanthionine ornithoalanine, etc., isopeptides that can be grouped under the LAL family (Lysosomal acid lipase) which encompasses several proteins with diverse functions (figure 14).

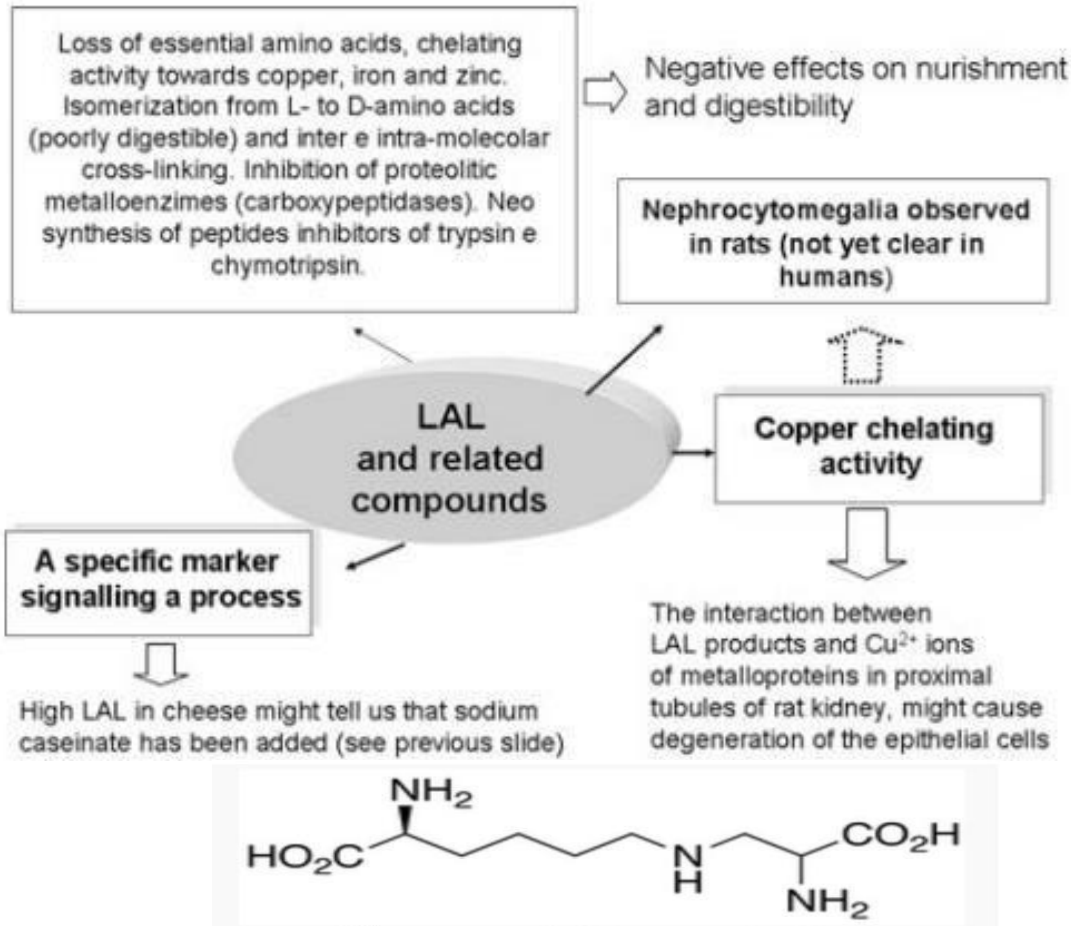


Figure 14. Lysinoalanine: chemistry, nutrition, and safety.

Sulfur amino acids, like methionine and cysteine, are particularly sensitive to oxidation, with severe nutrient loss, as they are essential amino acids. Development of a “cooked” taste in milk is caused by whey protein denaturation, with sulphydric acid building up, and lactone and methyl ketone formation from lipids

When fat normally present in food or added as a dressing undergoes thermal processing, a range of alterations takes place, the extent of which depends on processing conditions such as temperature, length of time, fat composition, presence of metals, and antioxidants. Such modifications may include oxidations, hydrolysis, and pyrolysis reactions, which may result in breakage of the chemical structure of fat components; these ruptures can cause production of low-molecular-weight compounds, isomerization reaction of essential polyunsaturated fatty acids, which involve double-bond shifting and conjugate diene formation.

The contents of lysinoalanine are obviously affected by the food type and by the processing conditions. In the radiation of food, o-hydroxyphenylalanine called o tyrosine is formed through the reaction of phenylalanine with OH-radicals. In hydrolysates, the compound can be detected with the help of HPLC (fluorescence detection or electrochemical detection). It is under discussion as an indicator for food radiation. The amount formed depends on the irradiated dose and on the temperature. In samples of chicken and pork, fish and shrimps, <0.1 mg/kg (non-radiated controls), 0.5–0.8 mg/kg (5 kGy, −18 °C) and 0.8–1.2 mg/kg (5 kGy, 20 °C) were found (figure 15).

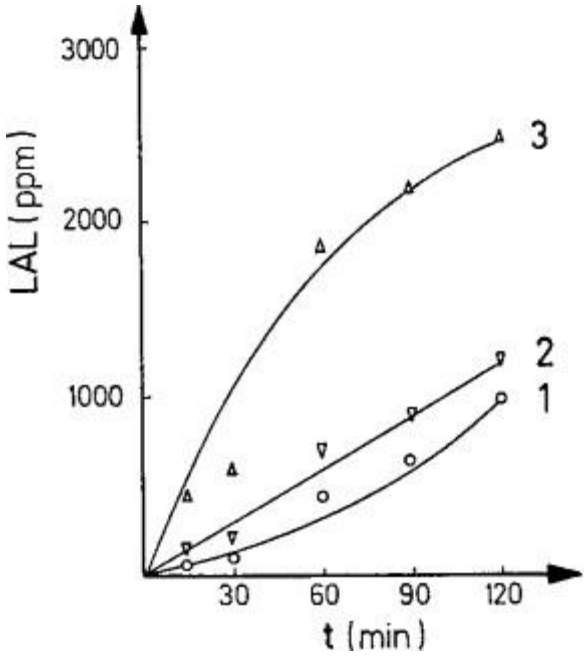


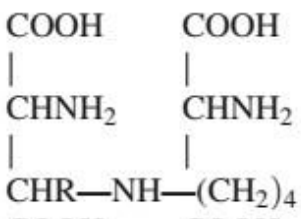
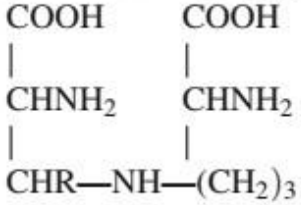
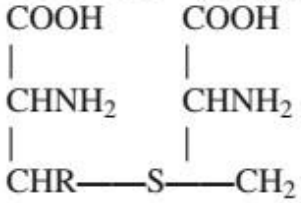
Figure 15. Formation of lysinoalanine by heating casein.

c) Formation of other unusuel aminoacids

At higher pH values. Hydrolysates of alkali treated proteins often contain some unusual compounds, such as ornithine, β-aminoalanine, ornithinoalanine,lanthionine, methyllanthionine and D-allo isoleucine, as well as other D-amino acids (table 12). The formation of these compounds is based on the following reactions: 1,2-elimination in the case of hydroxy amino acids and thio amino acids results in 2-amino-acrylic acid (dehydroalanine) or 2-aminocrotonic acid (dehydro-aminobutyric acid): in the case of cystine, the eliminated thiolcysteine can form a second dehydroalanine residue:

Formation of D-amino acids occurs through abstraction of a proton via a C2-carbanion. The reaction with L-isoleucine is particularly interesting. L-Isoleucine is isomerized to D-alloisoleucine which, unlike other D-amino acids, is a diastereo isomer and so has a retention time different from L-isoleucine, making its determination possible directly from an amino acid chromatogram (figure 16).

Table 12 . Formation of unusual amino acids by alkali treatment of proteins

Name	Formula
3-N^6-Lysinoalanine (R = H)	
3-N^6-Lysino-3-methyl-alanine(R = CH_3)	
3-N^5-Ornithinoalanine (R = H)	
3-N^5-Ornithino-3-methylalanine (R = CH_3)	
Lanthionine (R = H)	
3-Methyllanthionine (R = CH_3)	
3-Aminoalanine (R =H)	
2,3-Diamino butyric acid (R = CH_3)	

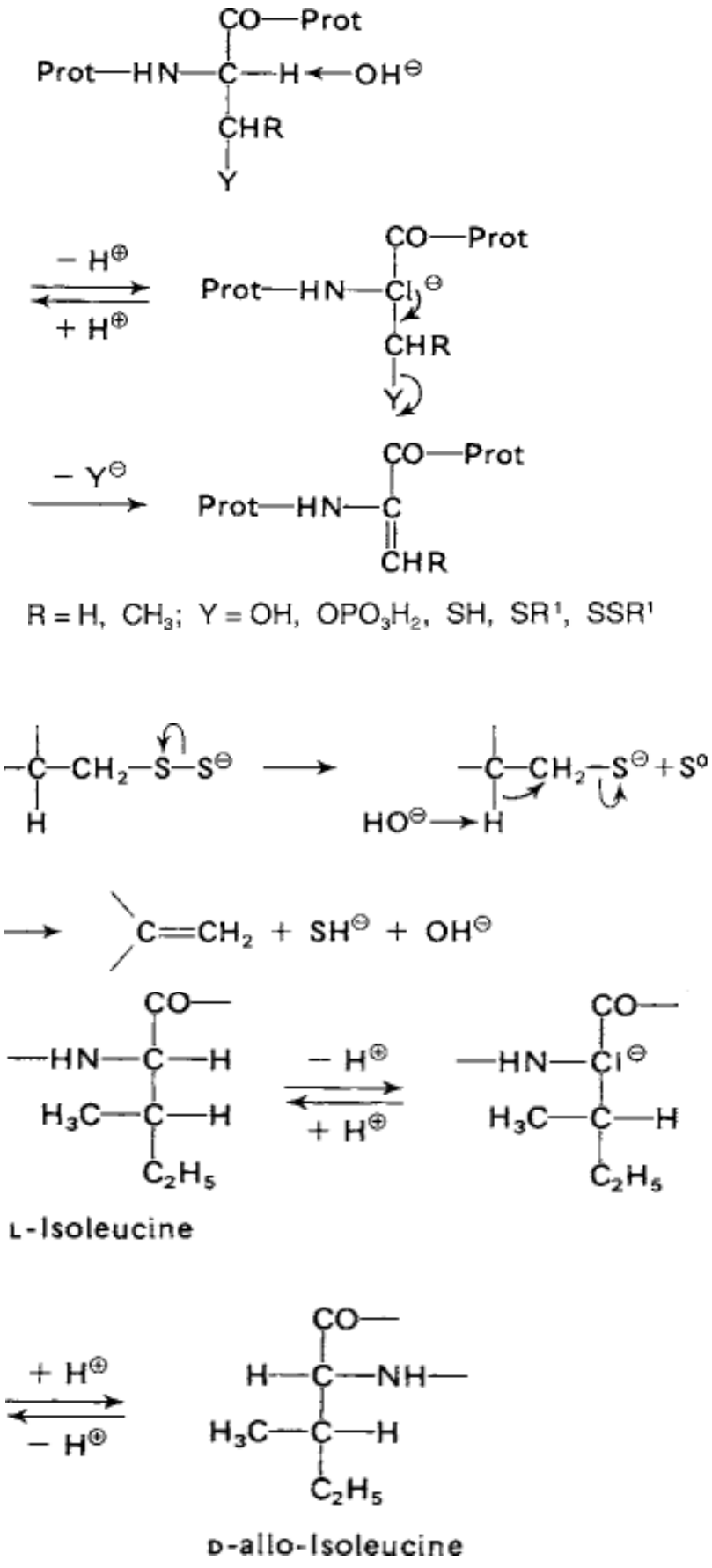


Figure 16: Formation of D-amino acids.

d) Formation of Maillard reaction products: this is a reaction between the carbonyl function of the reducing sugar and the amine function of the amino acid, responsible for the formation of odors, aromas and pigments in cooked foods. It reduces the value nutritional value of foods following the loss of essential amino acids. Maillard is the name of the process. The process, which is a no-enzymatic browning reaction, normally moves quickly between 140 and 165 °C (280 and 330 °F). Several recipes specify an oven temperature that will guarantee the Maillard reaction takes place. Higher temperatures result in more pronounced caramelization and pyrolysis (the last breakdown that causes burning and the emergence of harsh tastes).

Maillard reaction mechanism

- N-substituted glycosylamine and water are produced when the carbonyl group of the sugar interacts with the amino group of the amino acid.
- Amadori rearrangement of the unstable glycosylamine results in the formation of ketosamines.
- The ketosamines can further react in a number of ways:
- Make two water molecules, as well as reductones.
- There can be formation of diacetyl, pyruvaldehyde, and other short-chain hydrolytic fission products.
- Make melanoidins and brown nitrogenous polymers (figure 17).

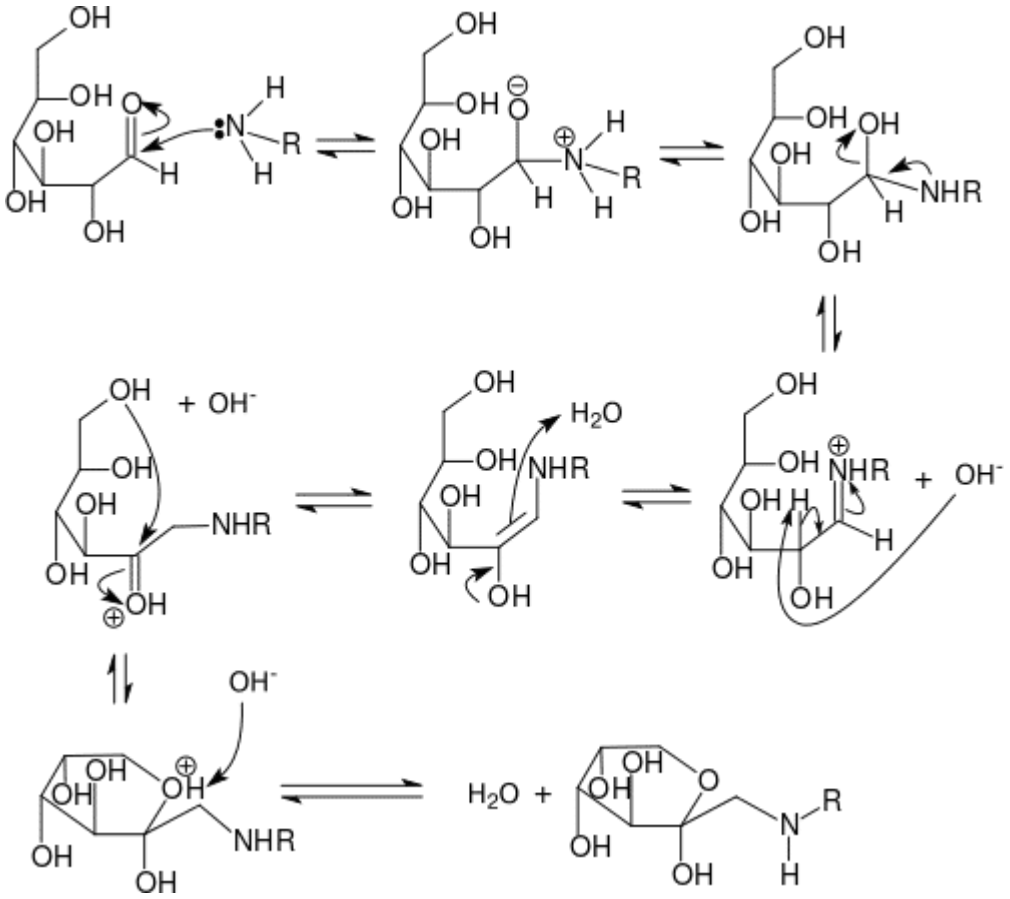


Figure 17. Maillard reaction mechanism.

Examples of Maillard reaction

The browning of different meats when seared or grilled, the browning and umami flavor in fried onions, and coffee roasting are all results of the Maillard reaction. It affects the color and flavor of dried and condensed milk, toffee, black garlic, chocolate, toasted marshmallows, and roasted peanuts, as well as the darkened crust of baked goods, the golden-brown hue of French fries and other crisps, the browning of malted barley.

- The biscuit- or cracker-like flavor found in baked foods like bread, popcorn, and tortilla products is caused by 6-acetyl-2,3,4,5-tetrahydropyridine.
- The scent and natural occurrence of the structurally related molecule 2-acetyl-1-pyrroline, which also arises without heating, are comparable.
- The substance is responsible for the distinctive aromas of many types of cooked rice and the herb pandan.

- The odour thresholds for both substances are less than 0.06 nanograms per litre.
- It takes a variety of chemical events, including the breaking of the tetrapyrrole rings in the muscle protein myoglobin, to produce the complicated browning reactions that happen when meat is roasted or seared.
- With dried fruit, Maillard reactions also take place.

Despite the fact that the results of the two processes can occasionally be identical to the naked eye, caramelization and Maillard browning are completely separate processes (and taste buds).

Browning may occasionally result from caramelization in the same foods where the Maillard reaction takes place, but the two processes are separate. The Maillard reaction involves amino acids, whereas caramelization is the pyrolysis of specific sugars. They are both induced by heating.The Maillard reaction takes place throughout the silage-making process, which lowers the quantity of energy and protein accessible to the animals that consume it.

- **Maillard reaction: important facts**

The nucleophilic amino group of the amino acid combines with the reactive carbonyl group of the sugar to generate a complex mixture of poorly understood compounds that are responsible for a variety of tastes and fragrances.

- The amino groups (RNH + 3 –> RNH2) are deprotonated in an alkaline environment, increasing their nucleophilicity and speeding up this process (for example, when lye is used to darken pretzels; see lye roll).
- Many of the formulations used in the flavouring industry are based on this response. A potential carcinogen known as acrylamide can develop at high temperatures.
- By heating at a lower temperature, using asparaginase, or introducing carbon dioxide, this can be prevented.
- Hundreds of distinct taste compounds can be produced during the cooking process by Maillard reactions, which are influenced by the chemical composition of the food, cooking temperature, cooking duration, and the presence of air.

- These chemicals frequently split apart to create new taste compounds. Over the years, flavor experts have created synthetic flavors using the Maillard.

II.3.4. Hydrolysis and other modifications of proteins

Protein hydrolysates are commonly used as an alternative protein source in commercial products. They consist of a mixture of different proteins and peptides which is formed by the hydrolysis of intact proteins. During this process, peptide bondsof intact proteins are broken (figure 18 A) which results in the formation of a range of peptides of different sizes. Depending on their properties, protein hydrolysates are applied in different products. Mildly hydrolyzed proteins are, for example, used in clinical and sport nutrition to support digestibility, while extensively hydrolyzed proteins are used in infant formulas as a hypo-allergenic alternative for intact cow's milk proteins (figure 18 B).

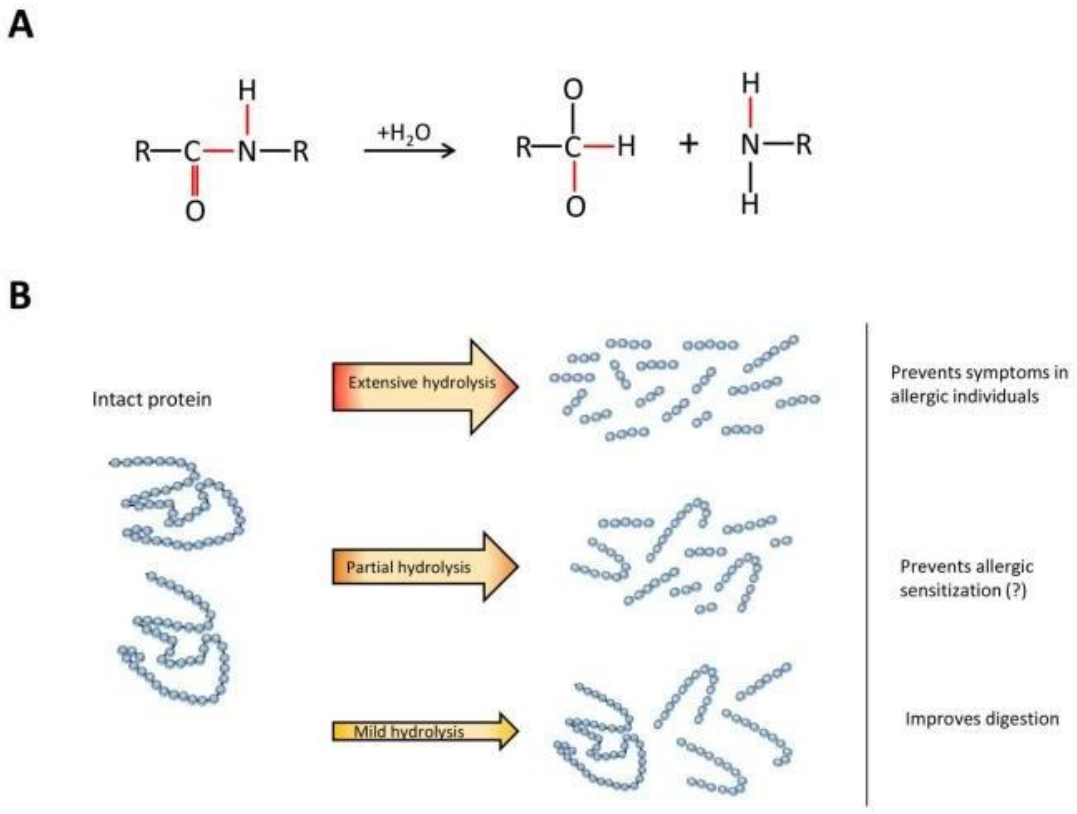


Figure 18. The process of protein hydrolysis and its products. (A) chemical reaction ofprotein hydrolysis; (B) different hydrolysates.

Furthermore, protein hydrolysates are recognized as a potent source of bioactive peptides. Different peptides with, for example, anti-thrombotic, anti-hypertensive, anti-microbial, anti-cancer, anti-oxidative, and many immunomodulatory effects have been identified. Consuming protein hydrolysates containing these peptides might be helpfulin the management of many western diseases. Since many of these diseases are immune-related, immunomodulatory products have gained special attention from both academical and industrial researchers for the management and amelioration of, for example, inflammatory bowel diseases, allergies, and diabetes.

II.3.4.1. Proteolytic enzyme modification

Processes involving proteolysis play a role in the production of many foods. Proteolysis can occur as a result of proteinases in the food itself, e. g., autolytic reactions in meat, or due to microbial proteinases, e. g., the addition of pure cultures of selected microorganisms during the production of cheese. This large group of enzymes is divided up as shown in table 13.

Table 13. Proteolytic changes during food processing

Action and Enzymes	Reaction
Coagulation	
Chymosin (rennin, EC 3.4.23.4)	κ-Casein → Para-κ-casein + glycopeptide, similar to pepsin A
Proteolysis	
Proteases	Proteins → high molecular weight peptides + amino acids
Amino peptidases, dipeptidases, tripeptidases	Low molecular weight peptides → amino acids
Proteases, endopeptidases, aminopeptidases	High molecular weight peptides → low molecular weight peptides
Decomposition of amino acids	
Aspartate transaminase (EC 2.6.1.1)	L-Asparate + 2-oxoglutarate → oxaloacetate + L-glutamate
Methionine γ-lyase (EC 4.4.1.11)	L-methionine → methanethiol + NH_3 + 2-oxobutanolate
Tryptophanase (EC 4.1.99.1)	L-tryptophan + H_2O → indole + pyruvate + NH_3
Decarboxylases	Lysine → cadaverine
	Glutamate → aminobutyric acid
	Tyrosine → tyramine
	Tryptophan → tryptamine
	Arginine → putrescine
	Histidine → histamine
Deaminases	Alanine → pyruvate
	Tryptophan → indole
	Glutamate → α-ketoglutarate
	Serine → pyruvate
	Threonine → α-ketobutyrate

The two subgroups formed are: peptidases (exopeptidases) that cleave amino acids or dipeptides stepwise from the terminal ends of proteins, and proteinases (endopeptidases) that hydrolyze the linkages within the peptide chain, not attacking the terminal peptide bonds. Further division is possible, for example, by taking into account the presence of a given amino acid residue in the active site of the enzyme.

Modification of proteins is still a long way from being a common method in food processing, but it is increasingly being recognized as essential, for two main reasons:

- Firstly, proteins fulfill multipurpose functions in food. Some of these functions can be served better by modified than by native proteins.
- Secondly, persistent nutritional problems over the world necessitate the utilization of new raw materials.

Modifying reactions can ensure that such new raw materials (e. g., proteins of plant or microbial origin) meet stringent standards of food safety, palatability and acceptable biological value. A review will be given here of several protein modifications that are being used or are being considered for use. They involve chemical or enzymatic methods or a combination of both. Examples have been selected to emphasize existing trends. Table 14 presents some protein properties which are of interest to food processing. These properties are related to the amino acid composition and sequence and the conformation of proteins. Modification of the properties of proteins is possible by changing the amino acid composition or the size of the molecule, or by removing or inserting hetero constituents. Such changes can be accomplished by chemical and/or enzymatic reactions.

From a food processing point of view, the aims of modification of proteins are:

• Blocking the reactions involved in deterioration of food (e. g., the Maillard reaction)

• Improving some physical properties of proteins (e. g., texture, foam stability, whippability, solubility)

• Improving the nutritional value (increasing the extent of digestibility, inactivation of toxic or other undesirable constituents, introducing essential ingredients such as some amino acids).

Table 14. Food protein properties

Properties with	
nutritional/physiological relevance	processing relevance
Amino acid composition Availability of amino acids	Solubility, dispersibility Ability to coagulate Water binding/holding capacity Gel formation Dough formation, extensibility, elasticity Viscosity, adhesion, cohesion Whippability Foam stabilization Emulsifying ability Emulsion stabilization

a. Alcoholic fermentation

Alcoholic fermentation is a complicated biochemical procedure during which yeasts convert sugars to carbon dioxide, ethanol and other metabolic byproducts that promote to the chemical composition and sensorial characteristics of the fermented foodstuffs. Alcoholic fermentation is the outstanding science of the fermentation processes and is active in several chief transformation, stabilization and conservation techniques for sugar-rich substrates, such as fruit, and vegetable and fruit juice. In this fermentation practice, yeast is mainly used as a bio-culture and aqueous solution of monosaccharide (raw materials) as the culture media for the production of beverages. Alcoholic fermentation starts with the breakdown of sugars by yeasts to form pyruvate molecules. The appropriate control of the dosage of the amino acid addition and the application of mixed amino acid supplementation may be a technique to adjust the fermentation kinetics and volatile compound modulation in soy whey alcohol fermentation. Alcoholic fermentation is the base for the manufacturing of alcoholic beverages like beer and wine, and control of fermentation is usually considered as a prerequisite to demonstrating the quality of the final product. Under anaerobic conditions, the pyruvate can be converted to ethanol.

The breakdown of proteins into alcohol is a process that is involved in the alcoholic fermentation of fruit and cereals; For example: yeast uses amino acids (a source of nitrogen); this activity leads to the formation of alcohol. Furtheremore, the aminopeptidases play important roles in diverse cellular processes such as protein modification, protein degradation, cellcycle control, and hormone level regulation. Therefore, these enzymes play a significant role in many pathophysiological conditions from infections to cancer.

Microorganisms are the major sources of high yield production of medically important aminopeptidases with economic feasibility. Pharmaceutical applications of aminopeptidases are directed to control the pathophysiological effects, in a way helps in the development of diagnostic tools such as biomarkers of these physiological pathways. Microbial aminopeptidases are potential targets for structure based drug design since these enzymes have high economic feasibility, high yields, regular availability, ease of modification, and high catalytic efficiency. Microbial aminopeptidases and their human counterparts share structural and functional similarity (Figure 19).

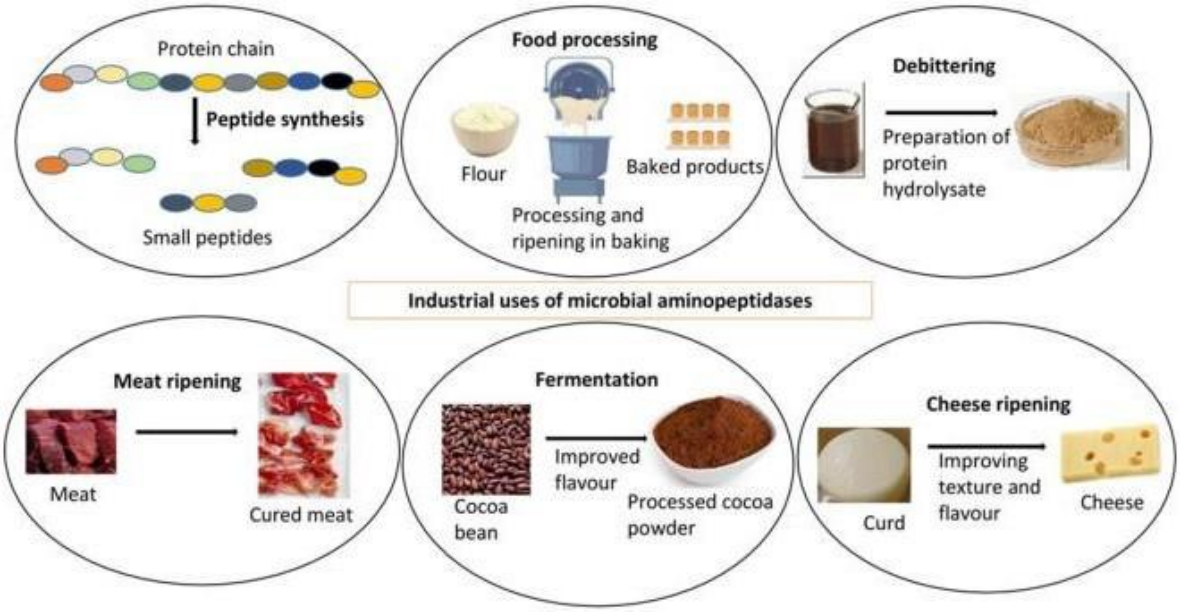


Figure 19. Illustration of various industrial applications of microbial aminopeptidases.

b) Bioactive peptides

Bioactive peptides are fragments of specific proteins, composed of 2 to 20 amino acid residues, have a molecular weight lower than 6000 Da, and stand out for promoting positive health effects in the consumer, which occur when the peptides are released as they are encrypted and inactive in the parental protein. Furthermore, they are associated with important biological activities such as antihypertensive, antioxidant, anti-inflammatory, immunomodulatory, hypolipemic, antidiabetic, anticancer, antiadhesive, etc. Therefore, they are considered therapeutic agents for treating and preventing certain diseases, with high specificity, a broad spectrum of action, low toxicity, high structural diversity, and small size. For all these reasons, bioactive peptides are ideal candidates to be applied as nutraceuticals or functional foods. However, to obtain this recognition, they must meet specific requirements: to maintain their bioactivity, be absorbed, have low or no toxicity, preserve an acceptable taste, and submit to the country's regulations. There are currently bioactive peptides on the market sold in food and drink, tablets, capsules, powders, and liquids.

- **Bioactive peptides characteristics**

Bioactive peptides are characterized by a bitter taste, implying that their acceptability is low in the society that consumes them in therapeutic or functional products; the bitter taste is positively correlated with the general hydrophobicity of peptide molecules. Furthermore, it is also reported that the intensity of bitterness is related to molecular mass, as 4 kDa peptides were shown to have higher bitterness than those of 1 kDa. Another factor that influences this flavor is the length of the peptide chain since it has been observed that the longer it is, the bitterness also increase. And the presence of amino acids with α-amino groups is also a determinant. Different solutions have been developed to reduce bitterness, such as the use of enzymes to reduce the bitter peptide content, the "removal" of these peptides using specific techniques (gel separation, alcohol extraction, silica gel chromatography, and isoelectric precipitation) individually or in combination, and the transformation, modulation, or masking ofthe flavor, through the use of flavor modifying agents such as sugars, salts, and nucleotides or through the fermentation of the product since during this process the flavor changes.

- **Enzymatic bioactive peptide formation**

This method involves incorporating commercial enzymes to obtain bioactive peptides since these are responsible for cleaving the peptide bonds established in the protein and thus releasing the encrypted peptide. For the enzyme to carry out its activities, it is essential that it first binds to the substrate and then continue with enzymatic catalysis. For this, the enzyme has specific active sites containing residues that form temporary bonds with the substrate and residues that catalyze the reaction with the substrate. In this way, binding sites and catalytic sites are formed, respectively. The bonds forming the enzyme-substrate complex are usually hydrogen bonds, hydrophobic bonds, or Van der Waals interactions. Finally, when the enzyme-substrate complex is in a specific conformation, it can ensure protein hydrolysis (figure. 20).

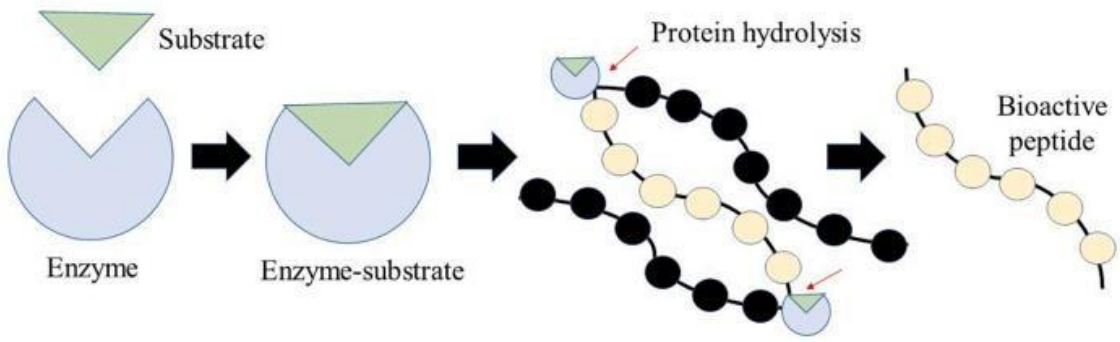


Figure 20. Enzymatic hydrolysis process for the release of bioactive peptides.

Enzymatic hydrolysis can generally be carried out in three ways:
i) under traditional batch conditions,
ii) using immobilized enzymes, or
iii) using ultrafiltration membranes.

The least used, due to its disadvantages, is the one carried out under traditional batch conditions. Since the cost of enzymes is high, it has low yields and productivity, and undesirable secondary metabolites are obtained due to the enzymatic autolysis generated.

c) Degradation of food protein (biogenic amines formation)

The formation of biogenic amines (BA) occurs during the microbial degradation of protein- rich foodstuffs (putrefaction of meat and fish, maturation of cheese, various fermentations). This reaction of decarboxylation of amino acids is catalyzed by enzymes (decarboxylases)

Biogenic amines are responsible for a number of food poisoning outbreaks, the most common of which is food poisoning. Scombroid poisoning is the most dangerous which is caused by ingestion of products such as fish containing high levels of histamine.

Histamine is formed in the flesh of many fish as a result of the decarboxylation of this reaction is catalyzed by the enzyme histidine decarboxylase. Biogenic amines (BAs) are nitrogenous compounds derived from microbial decarboxylation of amino acids (AAs) and from the amination or transamination of ketones and aldehydes following specific microbial enzymatic pathways (figure 21).

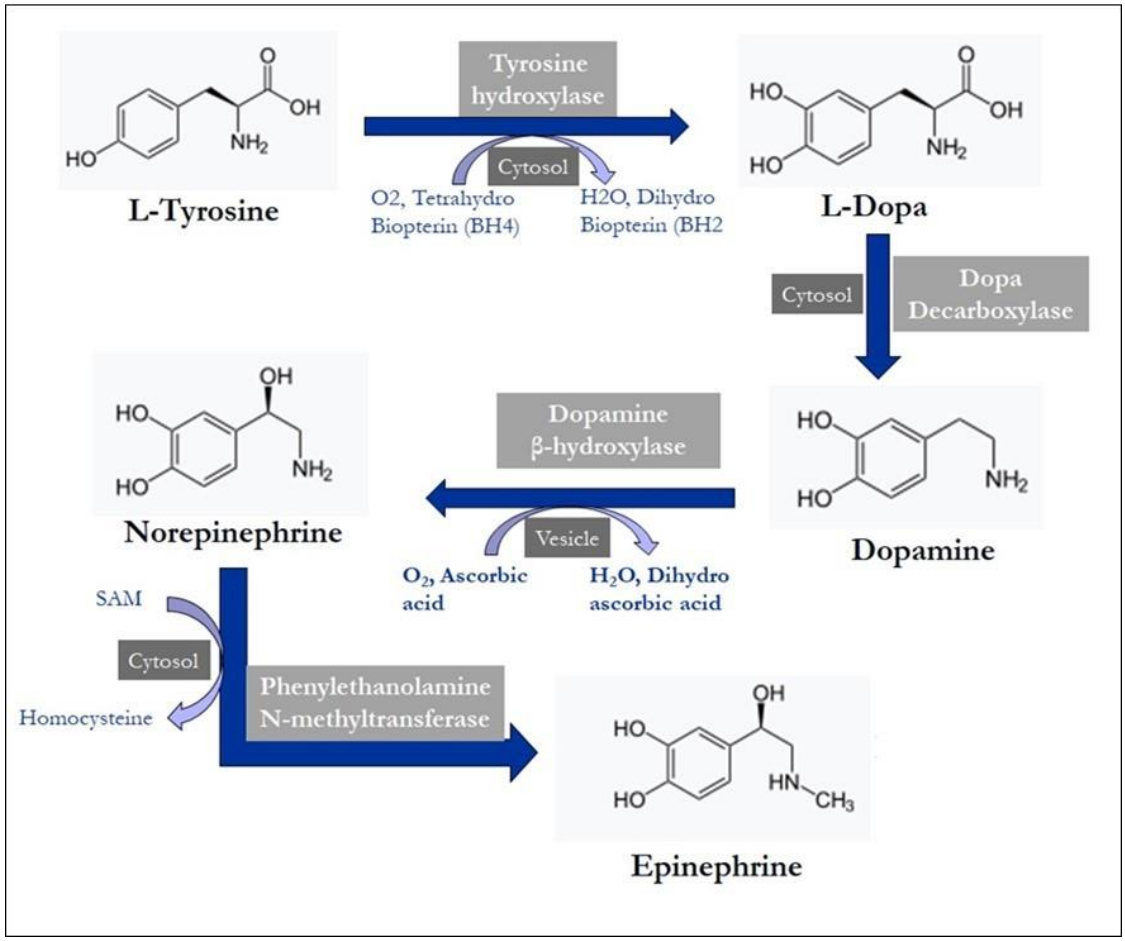


Figure 21. Degradation pathways of amino acids leading to the formation of biogenic amines.

These compounds can be divided into three groups according to their chemical structure: aliphatic amines (agmatine, cadaverine, putrescine, spermidine, and spermine), aromatic amines (phenethylamine and tyramine), and heterocyclic amines(histamine and tryptamine) (figure 22).

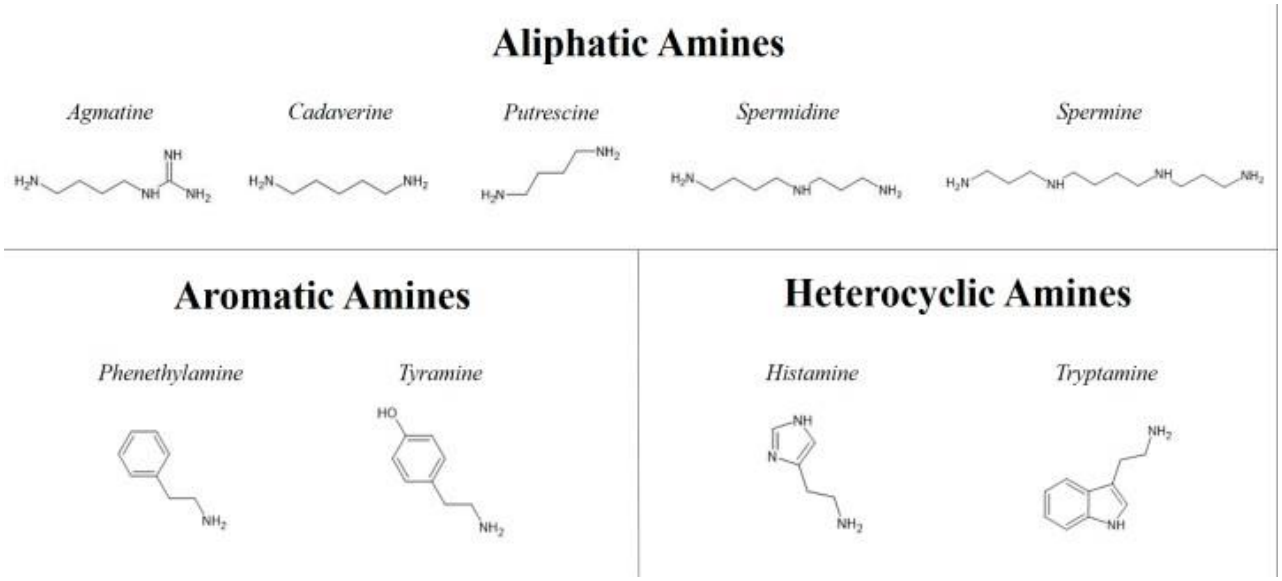


Figure 22. Classification of biogenic amines by their chemical structure.

According to the number of amine groups they can be also divided into: monoamines (phenethylamine and tyramine) and polyamines (di-, tri-, tetra-amines) (agmatine, histamine, cadaverine, putrescine, spermidine, spermine, and tryptamine). Both animals and humans naturally produce BAs; they are involved in natural biological processes such as controlling blood pressure, synaptic transmission, allergic response, and cellular growth. Some BAs may also have beneficial health effects. For example, at physiological concentration, tryptamine and phenethylamine play a role as endogenous neuromodulators of central monoaminergic neurotransmission and also act indirectly as sympathomimetic amines by causing noradrenalin release from sympathetic neurons. Additionally, agmatine has been shown to have different functions in the body as it displayed anti-atherosclerosis, anti-inflammatory, antioxidative, neuroprotective, and cell proliferation inhibition properties.

Several studies have reported its potential therapeutic effect and positive role in vascular, neuronal, and metabolic functions. However, it has also been reported that agmatine can react with nitrites to form nitrosamines, which are carcinogenic compounds. Due to the low activity of arginine decarboxylase in mammals, the amounts of agmatine found in their tissues can only be minimally attributed to de novo synthesis by arginine decarboxylase, while high concentrations can result from the diet (figure 23).

The endogenous production of BA can also derive from an excess of undigested amino acids which can be used as a substrate for microbial degradation in the intestine, where different toxic compounds are formed from nitrogen, such as ammonia and amines. In addition to the endogenous concentration of biogenic amines, dietary supplementation of these compounds can cause significant animal health problems. In fact, these nitrogenous compounds can also be found in food as a result of microbial contamination and proliferation, and if BA levels in food or drink reach a critical threshold, they could be harmful to health.

The raw materials most commonly used for the production of dry pet food are fresh meats and meat meals deriving from meat industry products not intended for human consumption. Meat meals are often used due to their high-protein content and are obtained by the rendering process. The lack of respect for good pet food manufacturing and handling practices could result in a favorable condition for the growth of microorganisms capable of decarboxylation processes. BAs can therefore be an alarm bell for possible microbial contaminations as they can occur in the final product as a result of microbial decarboxylation processes; this has been observed indry dog chews and feeds, in sealed packages and in bulk.

The aim of this work is therefore to evaluate the presence of BAs and free Aas (FAAs) in three different formulations of chicken-based dry pet foods: kibbles based on chicken fresh meat (CFM), chicken meat meal (CMM), and a mix of the two (CMix).

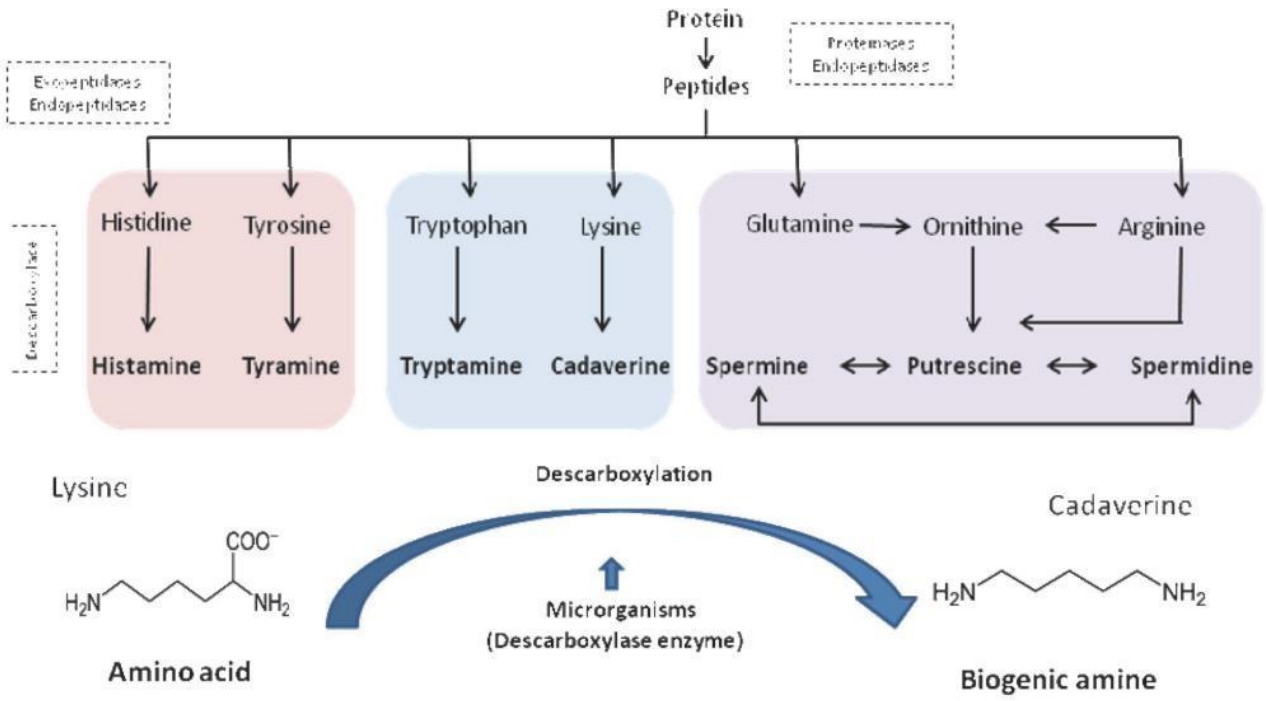


Figure 23. Biogenic amine formation and decarboxylation process.

- **Biogenic amines as quality indicator**

BAs have been used as a quality index and indicator of unwanted microbial activity in meat and cooked meat products. A combination of putrescine and cadaverine has been suggested as an index of acceptability in fresh meat because their concentrations increase prior to spoilage and correlate well with the microbial load. The concentrations of tyramine, putrescine, and cadaverine normally increase during the processing and storage of meat and meat products, while spermidine and spermine decrease or remain constant. The usefulness of BAs as a quality indicator depends on the nature of the product; the results tend to be more satisfactory in fresh meat and heat-treated meat products than in fermented products. Several factors affect the levels of Bas, including types and degrees of contamination of raw materials, manufacturing practices, certain processing stages (maturation, cooked, etc.), and the use of culture starters.. All these factors vary according to the nature of the product and, in some cases, can mask changes in the type and concentration of BAs through the different phases of treatment and storage, delaying visible signs of spoilage and/or off-odor development.

- **Biogenic amine analysis**

Several methods to analyze BA in food based on thin layer chromatography (TLC), gas chromatography (GC) and liquid chromatography (LC) have so far been described and used by researchers. TLC is simple and does not require special equipment, but most published methods suffer from the excessive time needed for analysis and/or inaccuracy of obtained results (semi-quantitative). GC is not so often applied for the determination of BAs. LC with pre- or post-column derivatization is by far the mostly frequently reported technique for BA separation and quantification.

Extraction is the most critical step in the detection procedure of BAs. According to the method, this step negatively affects the analytical recoveries. The extraction of BAs from a solid matrix is usually carried out with acids such as hydrochloric acid, perchloric acid, trichloroacetic acid or with methane sulfonic acid. Nevertheless, organic solvents such as methanol, acetone, acetonitrile or dichloromethane can be used. The extraction is a process influenced by many factors such as type of acid, type of organic solvent, salt used for saturation, pH at which amine extraction (liquid-liquid partition with the organic solvent)is carried out, and the time and type of stirring. Improper techniques may cause erroneous results, so strict validation of separation methods, especially recovery studies, is necessary to ensure the accuracy and precision of the sample-preparation processes.

- **Fish, shellfish and fish products**

Histamine intoxication is probably the principal sanitary problem associated with the high content of BAs in fish. Scombroid fish species, such as tuna, bonito, saury, and mackerel, as well as non-scombroid species, such as mahi-mahi, sardines, pilchards, anchovies, herring, marlin, salmon, amberjack, and bluefish have high levels of histidine in their flesh. However, some cases of food poisoning from fish with low contents of histamine indicate that other substances, as toxicity potentiators, might be involved. Histamine was the prevailing BA throughout storage. However, a great increase in cadaverine and tyramine and a slight increase in putrescine were also observed. The researchers proposed the index of BAs from the sum of histamine, tyramine, cadaverine, and putrescine which showed good correlations with time of storage.

- **Meat and meat products**

BA content in meat can be considered a freshness marker or a bad conservation marker. In particular, the study of BA quantities in meat as a function of conservation time could be a useful tool to control meat spoilage. In fact, the formation of some amines and concentration increase of those already existing in meat are due to degrading processes in food.

High amounts of BAs can be found in fermented foods derived from raw material withhigh protein content, such as dry and semi-dry fermented sausages. Dry fermented sausages can potentially support the accumulation of BAs due to the presence of significant levels of spermidine and spermine but also for microbial growth. Nevertheless, a great variability characterizes the BA content in fermented meat products.

BAs levels of sucuk (Turkish dry fermented sausage) were determined by using HPLC method with diode array detector after pre-column derivatization with dansyl. Levels of putrescine and cadaverine were detected as 93% and 87% of the samples, respectively.

Spermine and spermidine were detected in ranges from not detected to 16.4 and fromnot detected to 10.7 mg kg^{-1}, respectively. Histamine was found to be between 50 and100 mg kg^{-1} as 17% of the samples. Tryptamine was detected in the range of 1.2–mg kg^{-1}. Tyramine contents of all samples were within the acceptable level. Phenylethylamine was found in 17 of the 30 samples and levels in all detected samples were found to be lower than 25 mg kg.

There are numerous studies on the determination of biogenic amines in dry and semi- dry fermented sausages. A developed HPLC UV method to simultaneous determination of Bas and their precursor amino acids after a precolumn derivatization with dansyl chloride. The HPLC-UV method was validated and applied to the analysis of salami and other foods. All matrices showed recoveries always >92% and relative standard deviation always <5%. It has demonstrated that these products had conditions for BAs production principally for the use of culture starter cultures and spoilage bacteria, reaffirming the need for the meat industry to pay special attention to the use of microorganisms and specific control of all processes.

- **Chicken meat**

Many authors had reported several methodologies to determinate these substances in chicken. A developed HPLC-UV method to dansyl chloride derivatives with good precision, recovery values > 93%, relative standard deviation between 1.47% and 2.94% and detection limits for all amines, and confirmed that very low quantities of BAs could be detected in real samples.

A validated HPLC method to determine tyramine, putrescine, cadaverine, spermidine and spermine in chicken using perchloric acid (5%) extraction and 40Ml benzoyl chloride, followed by homogenization in vortex for 15 sec and kept at room temperature (25 ± 2°C) for 20 min. The biogenic amines were collected through liquid partitioning with 1000 µL of diethyl ether, which proceeded twice. The layers containing amines were evaporated to dryness under nitrogen stream, resuspended in 1000µL of mobile phase (acetonitrile:water) and stored at 4 ± 1°C. Separation was performed in C18 column, in isocratic condition of water:Aceronitrile 42:58 (v/v). The chromatography conditions were: flow rate of 1 mL.min-1, injection volume of 20µL, column temperature of 20°C, detector wavelength set at 198 nm and total run time of 15 min. A similar technique was used but with some changes: gradient system, 254nm and internal standard (1,7-diaminoheptan). Chicken-based products such as mortadella, frankfurters, sausage, meatballs, hamburger, and nuggets contain bioactive amines. In general, these products had lower BA levels and were considered unlikely to elicit direct adverse effects inconsumers.

- **Honey**

A simple HPLC analytical method was proposed for the simultaneous analysis of amino acids and BAs in liquid food matrices:a precolumn derivatization with OPA in the presence of 2-mercaptoethanol, performed in the sample injection loop, and fluorescence detection. Only residual levels of BAs were detected in the analyzed samples. It was reported a new, simple, rapid and economical method for routine determination of 24 amino acids and BAs in honey. The method was validated in the range 0.25–10 mg/L; repeatability was less than 3% RSD and the intermediate precision ranged from 2 to 7% RSD. The method was shown to be linear by the ‘lack of fit’ test and the accuracy was between 97 and 101%. The LOQ was 100µg/L for putrescine and cadaverine.

II.3.4.2. Other protein chemical modifications

Among other protein chemical modifications which can occurred in foods, the acylation (table 15); the treatment with succinic anhydride generally improves the solubility of protein. For example, succinylated wheat gluten is quite soluble at pH 5. This effect is related to disaggregation of high molecular weight gluten fractions. In the case of succinylated casein it is obvious that the modification shifts the isoelectric point of theprotein (and thereby the solubility minimum) to a lower pH. Succinylation of leaf proteins improves the solubility as well as the flavor and emulsifying properties.

Table 15. Chemical reactions of protein significant in foods.

Reactive group	Reaction	Product
$-NH_2$	Acylation	–NH–CO–R
$-NH_2$	Reductive alkylation with HCHO	$-N(CH_3)_2$
$-CONH_2$	Hydrolysis	$-COOH$
$-COOH$	Esterification	$-COOR$
$-OH$	Esterification	$-O-CO-R$
$-SH$	Oxidation	$-S-S-$
$-S-S-$	Reduction	$-SH$
$-CO-NH-$	Hydrolysis	$-COOH + H_2N-$
		Foam stabilization
		Emulsifying ability
		Emulsion stabilization

Chapter III: Oxidation reactions

III.1. Introduction

Food can be profoundly damaged by the oxidation of their molecules. The degradation of a food can range from a drop in acceptability of the intensity of the flavour to the point of total rancidity, making it unsuitable for consumption.

III.2. Oxidation factors influencing

➢ Light irradiation

➢ High temperature (cooking)

➢ Oxygen concentration

➢ Nature of lipids, in particular fatty acids, and state of hydrolysis oftriglycerides

➢ Presence of other products with a slowing (antioxidant) or accelerating effect onthe reaction (prooxidant: metal ions such as copper).

III.3. Oxidation impacts on food quality

- Nutritional quality: degradation of essential fatty acids and certain vitamins (E, C).
- Organoleptic quality: development of unpleasant flavors, change of color, marketability…: loss of market value.
- Health impact: secondary oxidation compounds show effects cytotoxic and mutagenic.

III.4. Oxidation of fatty acids

The reaction mechanism of triglycerides is an autoxidation process that proceeds under the influence of oxygen and light. It predominantly consists of the following reactions: oxidation, crosslinking and scission.

III.4.1. Autoxidation of fatty acids

The autoxidation process consists of a very complex scheme of radical reactions occurring repeatedly to the functional groups of the organic linseed oil molecule. Multiple reactions may occur rapidly and essentially simultaneously, leading to products that are not easily isolated in experiments. The autoxidation of linseed oil is characterized by two competing mechanisms: formation of the polymer network and breakdown of the molecules due to β-scission, unstable crosslink decomposition and hydrolysis. The schematic representation of the autoxidation process is shown in figure. 24.

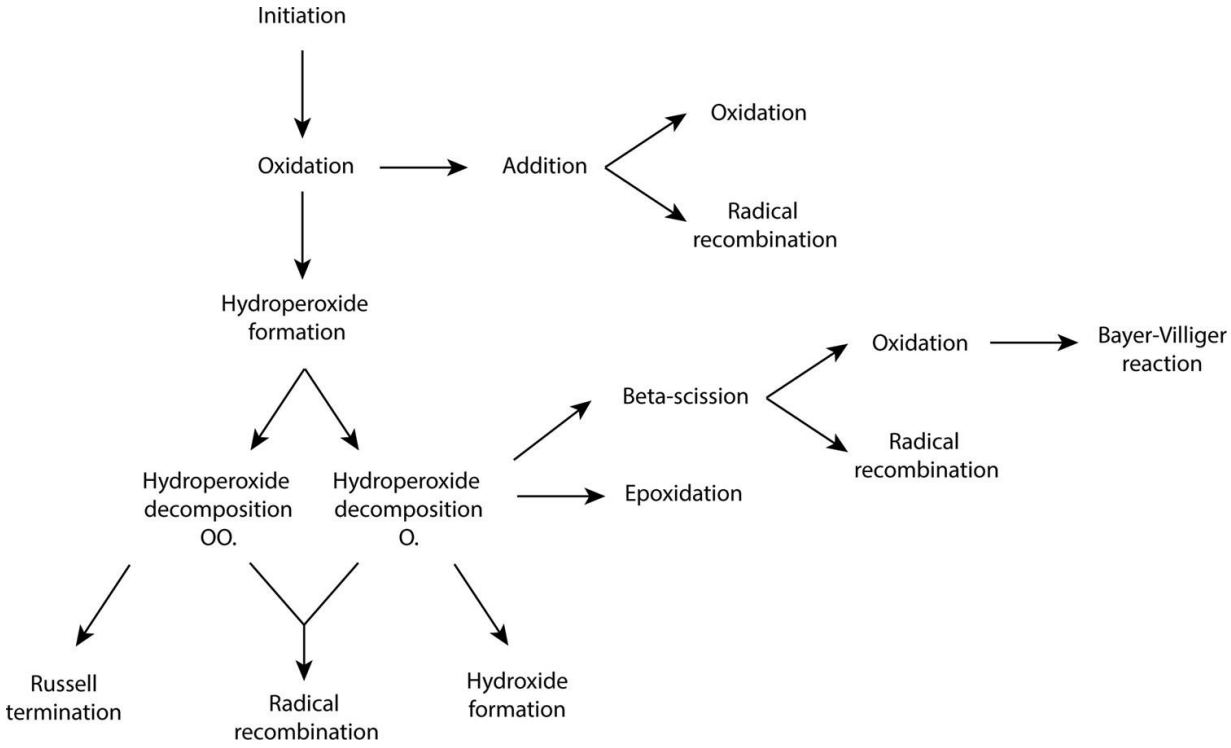


Figure. 24. A schematic representation of the reactions occurring during autoxidation.

The four main stages of free-radical polymerization will now be discussed: (1) initiation; (2) propagation; (3) termination; (4) degradation. In the case of drying oils, these reaction steps lead to a variety of intermediate and end products. We provide many reported reactions for each fatty ester at various stages of drying. Moreover, where possible, the reactions will be reported together with kinetic rate parameters, which influence the speed of reactions as a function of temperature. It must be noted that kinetic parameters cited in the review possess uncertainty, as there is both experimental and computational error in the values.

III.4.1.1. Initiation

Autoxidation begins with an initiation step involving a tiny amount of radicals that may be created in several ways. During the subsequent propagation step a comparatively higher concentration level of radicals prevails in the system. In linseed oil, the initiation of a radical can be delayed by the presence of natural antioxidants, causing an induction period before propagation starts. Differences in induction period are observed in the temperature ranges above 130 °C, 84–130 °C and below 84 °C. The induction period is short above 130 °C, while in the range of 84–130 °C the length of induction increases slowly as the temperature is lowered; below 84 °C the induction period is long and independent of temperature.

Initiation happens in two steps: primary initiation and secondary initiation. Primary initiation creates a tiny amount of peroxyl radicals that subsequently take part in the secondary initiation. During secondary initiation the radical created by primary initiation abstracts a hydrogen atom from a bis-allylic carbon of a linoleic or linolenic fatty ester tail, forming a delocalized pentadienyl or heptadienyl radical, respectively. The alkyl radical rapidly reacts with molecular oxygen and forms a peroxyl radical. Allylic hydrogen abstraction happens predominantly at elevated temperatures. For the resonant structures after allylic and bis-allylic hydrogen abstraction, (figure 24).

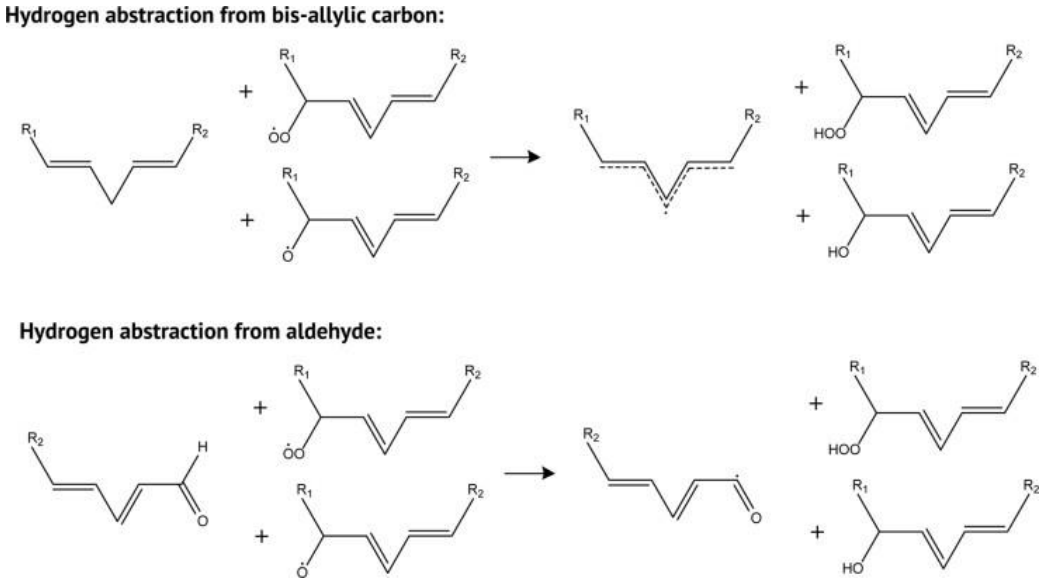


Figure 25. Propagation of radicals via hydrogen abstraction from bis-allylic carbon (top)and from an aldehyde (bottom) by alkoxyl and peroxyl radicals.

Initiation can occur spontaneously, or with the aid of UV light, singlet oxygen or activemetal catalysts. Spontaneous initiation is rationalized by the fact that linseed oil will cure in air without using a catalyst, although slowly. It has been shown that UV irradiation lowers the activation energy of curing of alkyd resins. Singlet oxygen is much more reactive thantriplet oxygen and is capable of attaching to the bis-allylic as well as to allylic carbon and forming a hydroperoxide via a concerted cyclic addition mechanism (figure 26). Singlet oxygen is formed from molecular oxygen in the presence of photosensitizers causing the excited state of oxygen by an uptake at least 22.4 kcal/mol.

Metal driers are added to oils to speed up the decomposition of hydroperoxides andinitiate autoxidation. A common transition metal catalyst is cobalt (II) 2-ethylhexanoate that undergoes primary initiation by reacting with molecular oxygen forming a peroxylradical with a rate coefficient in the order of 10-1 L/mol*s, while CoII becomes CoIII. The peroxyl radicals follow secondary initiation and abstract hydrogen to form bis- allylic or allylic radicals. Cobalt hydroperoxide then changes back to CoII and splits offa hydroperoxyl group and then may continue its catalytic activity.

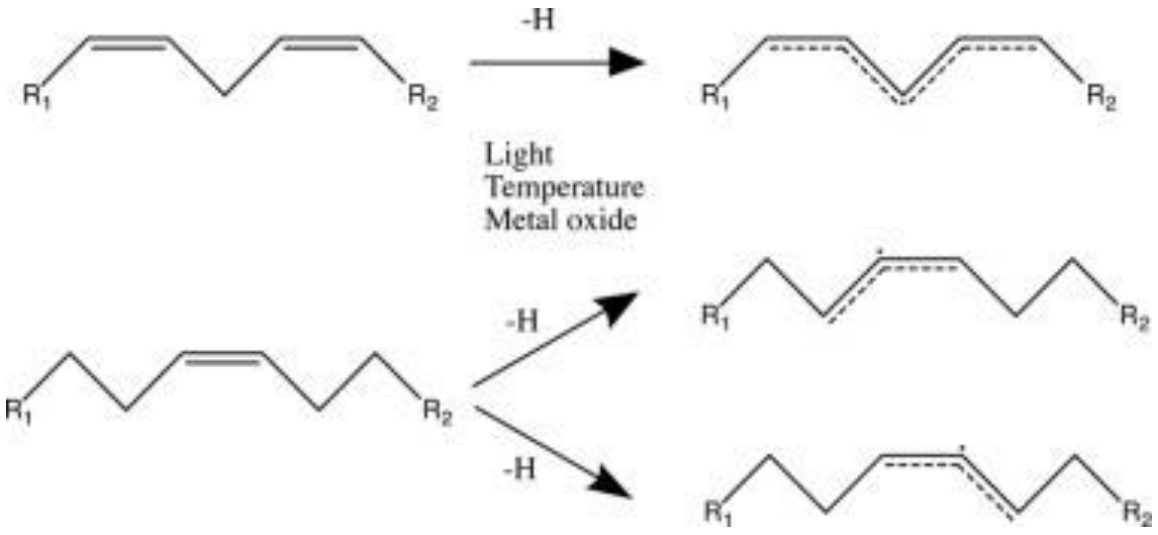


Figure 26. Possible initiation of allylic and bis-allylic carbons.

The step of initiation is slow, corresponding to the elimination of a hydrogen radical on a carbon adjacent to an unsaturation and release a highly reactive molecule (R°). This step may be spontaneous or catalyzed by metal cations, light or the light, and enzymes.

The abstraction of bis-allylic hydrogen occurs at a rate coefficient on the order of 10-1L/mol*s, while allylic hydrogen is 105 times less reactive. The energy barrier for hydrogen abstraction in the case of oleate is even higher, 80 kcal/mol. During the autoxidation mechanism, alkoxyl, peroxyl and alkyl radicals are produced, which also take part in hydrogen abstraction. Peroxyl radicals play a major role in hydrogen abstraction as they exhibit a rather long half-life (up to 7 s) in comparison with alkoxyl (10-6 s) and hydroxyl (10-9 s) radicals. The rate coefficient of hydrogen abstraction by peroxyl radical is estimated to be 6.6 L/mol*s. The speed of initiation can be measured experimentally by tracking the decrease of bis-allylic carbon concentration as, after oxygen addition, they disappear while the non-conjugated configuration of the double bonds changes into a conjugated configuration.

III.4.1.2. Propagation

The repeated hydrogen abstraction by peroxyl radicals and subsequent reaction with oxygen is responsible for a continuous production of radicals, which in polymer reaction engineering is therefore called the propagation step; this step is fast; this is the oxygen oxidation stage. It is characterized by the formation of peroxides and hydroperoxides; it takes place intwo stages:

a) It corresponds to the appearance of peroxyl from unstable free radicals. At this stage,the flavour of rancid may not be perceptible; therefore the merchantability of the product is not guaranteed and not yet altered.

b) It results in the evolution of peroxide radicals into hydroperoxides

$$ROO^{\circ}+R'H \Longrightarrow ROOH^{\circ}+R'^{\circ}$$

Alkyl radicals formed via hydrogen abstraction add oxygen, forming peroxyl radicals. The oxidation mechanism is shown in figure. 27. The reaction of oxygen and alkyl radicals happens very rapidly, with a rate constant on the order of 108 L/mol*s and an activation energy of 0 kcal/mol.

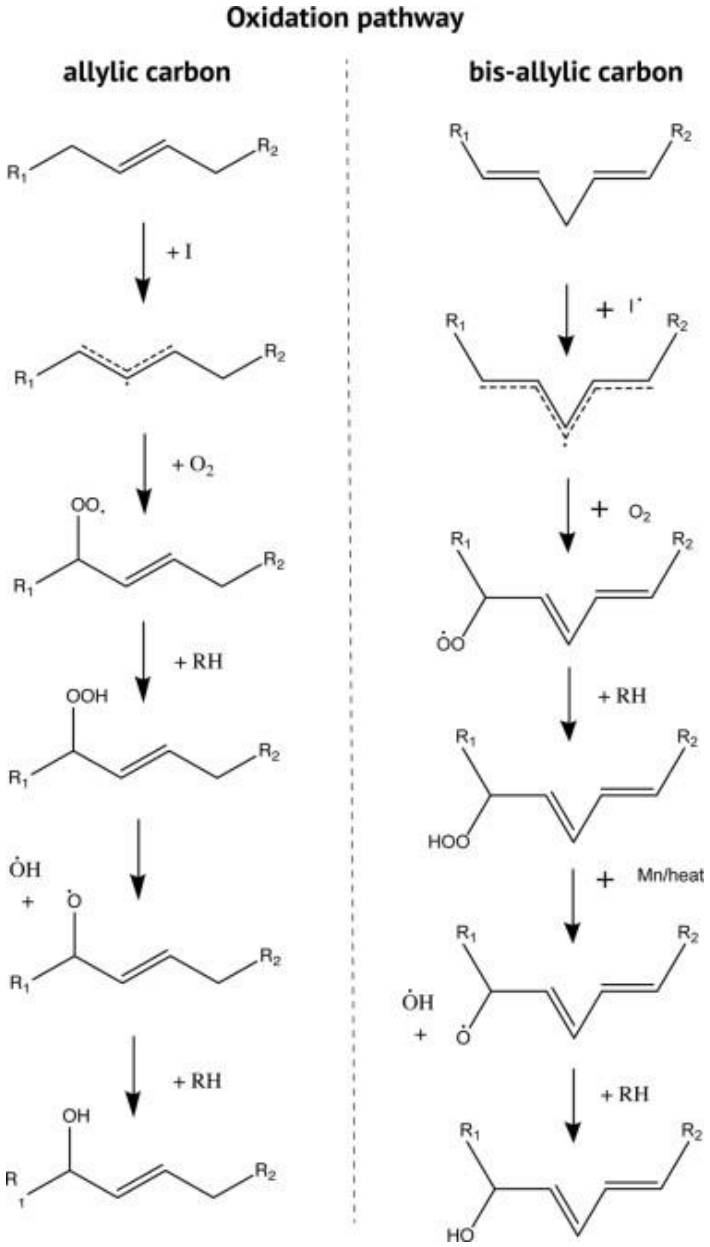


Figure. 27. Oxidation pathways of fatty esters containing allylic (left) and bisallylic (right)carbons that are found in fatty esters

The rate of oxidation varies depending on conditions, such as temperature, the presence of pro-oxidants (oxidizing agents), antioxidants and dissolved oxygen, and the rate also depends on the degree of unsaturation. As mentioned in the work by Wexler, the oxidation rates of triolein, trilinolein and trilinolenin are increasing in order of degree of unsaturation: 1:20:330. When adding to the resonant structure of the backbone, as in linoleic esters, oxygen prefers the position on the 9th or 13th carbon atom, and the resulting functional group is called an outer peroxide. Inner peroxides (C-10 and C-12) appear in minor amounts, possibly resulting from the formation of cyclic peroxides (endoperoxides). In the case of linolenic acid, hydroperoxides are found at the external as well as at internal positions.

a. Hydroperoxide, hydroxide formation

Hydroperoxides are formed from peroxyl radicals after they perform hydrogen abstraction. Being stable intermediates, hydroperoxides mark the initial stage of the autoxidation process. Decomposition of hydroperoxides is a slow unimolecular reaction with a rate constant on the order of 10-13 s-1 and an activation energy of 24.6 kcal/mol. A hydroperoxide decomposes into alkoxyl and hydroxyl radicals, and the reaction is accelerated under the influence of light and drying agents containing transition metals like cobalt.

After hydroperoxide decomposition, the alkoxyl radicals may proceed along several reaction pathways including secondary scission of alkyl chains, recombination with another radical or hydrogen abstraction. In the case of the latter, an alkoxyl radical becomes a hydroxide, which is one of the stable end products of the autoxidation process. The concentration of hydroxides in a linseed oil film reaches a constant value between 200 and 300 h of drying time.

b. Addition

Unsaturated fatty esters participate extensively in hydrogen abstraction, and the addition of oxygen to the alkyl radical is facile, while the previously unsaturated double bonds are rearranged into a conjugated state. The conjugated double bonds are also reactive and are consumed via addition of a peroxyl radical forming a dimer.

Thereby,the radical is transferred from the peroxide on one fatty ester to the carbon on the other fatty ester (figure. 28). The previously conjugated structure turns into a resonatingdouble bond between two carbon atoms until the radical reacts with oxygen or undergoes termination. The activation energy of peroxyl radical addition to a conjugated double bond is 6.7 kcal/mol.

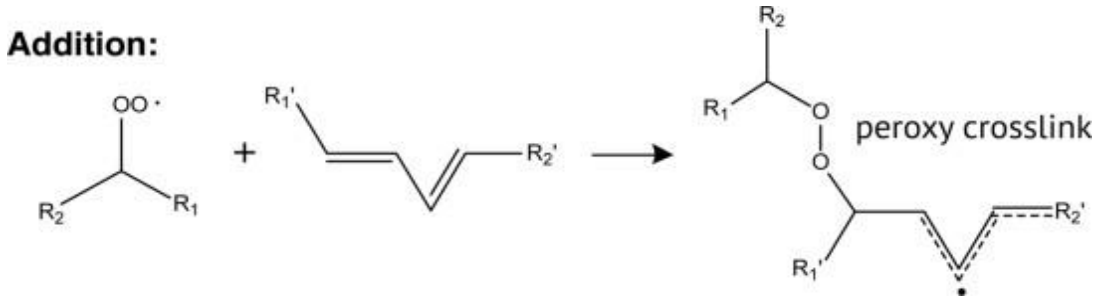


Figure 28. Addition of a peroxyl radical to a conjugated double bond resulting in a peroxy crosslink and a resonant double bond.

- **General behavior of double bonds**

As described above, a significant rearrangement of the double bonds occurs during the initial autoxidation steps. In the case of oleic fatty, esters, there is a trans–C=C (double bond), while the polyunsaturated lipids start with cis–C=C, which is further consumed via initiation and is turned into a conjugated double bond. Following the addition reaction, one of the conjugated double bonds is broken and the remaining double bond adopts a trans configuration. Thus, the cis–C=C initially present is consumed fast and completely. Thereafter, the concentration of the remaining trans–C=C reaches a constant level.

C. Epoxidation

Epoxides are the simples and cyclic species possible with one oxygen and two carbon atoms. It was observed the formation and slow decomposition of epoxides during drying of ethyl linoleate. Using quantum chemical calculations, it was elucidated the epoxidation of lipidradical species and found that allylic radicals on β positions to peroxides undergo epoxidation at ambient conditions. The epoxidation proceeds by two pathways representative for the autoxidation of unsaturated fatty esters (Fig. 29 a) having energies of activation of 17.7 kcal/mol and 19.3 kcal/mol, respectively.

At elevated temperatures epoxides readily decompose due to the ring-strain, while at ambient conditions, it was captured the subsequent epoxide-ring consumption with the decreased concentration of epoxide groups over 500 days. There is little consensus of the ring-opening mechanism; thus, the review does not provide a detailed account of this reaction.

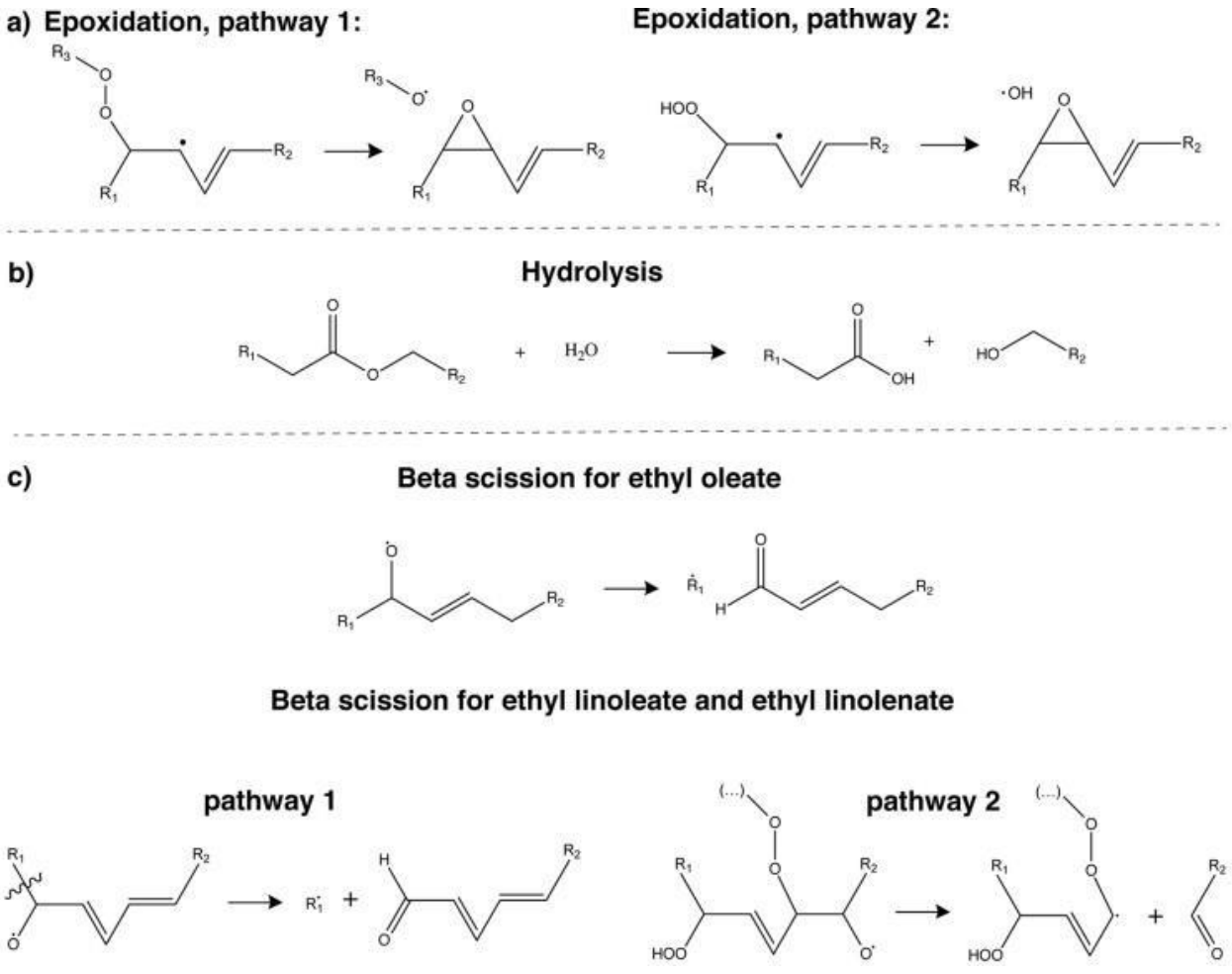


Figure. 29. Epoxidation from a species with peroxy crosslink (1) and from a species withhydroperoxide (a). Hydrolysis of an ester bond between glycerol and fatty acid (b). β- scission pathways for oleic fatty ester (top) and for linleic and linolenic fatty esters (bottom) (c)

III.4.1.3. Termination

The final step of the free-radical polymerization process is radical termination. In this step, two radicals react and either form a single, non-radical product (recombination) or two distinct non-radical products (disproportionation). It corresponds to the disappearance of peroxyl radicals and the accumulation of secondary oxidation (total oxidation of the substrate). This phase occurs when the concentration of radical species is high, leading to the production of a stable product after the reaction of the radicals with each other and the oxidised products resulting from the splitting of the initial fatty acids (alcohol, aldehyde, ketone, etc.)

a. Recombination

In the linseed oil system, the three main radical types are alkyl, alkoxyl, and peroxyl. When they recombine, they form crosslinks. This is one of two ways crosslinks are formed in the linseed oil polymer network. All radical recombination permutations are shown in figure. 29a.

The distribution of crosslinks in the resulting polymer system depends on oxygen concentration. Under conditions of facile oxygen transport, peroxy and ether crosslinksare formed in equivalent amounts, whereas alkyl crosslinks are formed in a minor amount. Radical recombination is mostly diffusion-controlled; as the polymer becomes more crosslinked, the increased viscosity restricts further radical collisions and thus recombination events. In exothermic reactions, the phenomenon of decreasing rates of termination due to viscosity increase is known as auto-acceleration or the Trommsdorff-Norrish effect and can lead to dangerous runaway reactions during polymerization of certain synthetic polymers.

The rate of radical recombination varies based on the polymer system. For example, the rate coefficient for radical termination in an acrylate system is on the order of 109 L/mol*s, whereas rate coefficients for recombination in the linseed oil system ranges from 107 to 108 L/mol*s.

The existence of dimers that cannot be split by hydrogen iodide is a marker for the presence of carbon–carbon, or alkyl crosslinks, the amount of which is shown to be minor. Ether links are formed via recombination of alkyl and alkoxyl radicals, the latter being formed after hydroperoxide or polyperoxide (polymer with peroxy crosslinks) decomposition. The peroxy crosslinks remain constant for over a year, but a net generation of ether crosslinks is detected, mainly originating from the slow decrease of double bonds. It was also quantified the concentration of alkyl (C–C) crosslinked species from dimers, finding 1% of alkyl crosslinks in pure unreacted EL, and 5% of alkyl crosslinks in EL in the presence of cobalt after five days of drying. Dimers with all three types of crosslinks are shown in figure. 29a.

b. Disproportionation

Disproportionation is a second termination reaction, a redox reaction in which the oxidation state of the reactants changes into two different oxidation states of the products. For radical disproportionation, this is commonly in the form of a terminal alkane and a terminal alkene group.

In the case of oxygen-rich species, oxygen-centered radicals may collide and form oxidation products such as alcohols and ketones via Russell termination. However, Russell termination is not undisputed for linseed oil polymerization as it has too high energy barrier for the recombination of secondary alkyl peroxyl radicals. Further kinetic studies are required to elucidate and validate the mechanism that leads to oxidation products. Another type of disproportionation is the Baeyer-Villiger reaction, where a peroxy acid reacts with an aldehyde, producing two carboxylic acids.

- **β-scission reaction**

Breaking the β-bond to an alkoxyl radical, β-scission propagates the free radical and forms an olefinic species. β-scission is common in thermal cracking, but at ambient conditions it is of limited importance.

Products from scission reactions in drying oil systems vary significantly in carbon chain length, as the location of β-bonds next to alkoxyl radicals may vary in oleic, linoleic and linolenic acids. Products of β-scission in linseed oil degradation include volatile n-aldehydes such as ethanal, propanal, butanal, pentanal, hexanal, heptanal and octanal. Through quantum chemical calculations, the β-scission was more likely to happen according to the scheme shown in figure. 29c.

- **Peroxy crosslink decomposition**

A decrease in peroxy crosslink concentration. This is attributed to slow peroxy crosslink decomposition. In contrast to hydroperoxide decomposition this peroxy crosslink decomposition is apparently not catalyzed by transition metal driers and occurs with an activation energy of 40kcal/mol.

III.4.2. Enzymatic fatty acids oxidation

III.4.2.1. Structure of lipoxygenases

Lipoxygenase or linoleate oxygen oxidoreductase, a haem iron protein, catalyzes theoxidation by molecular oxygen of polyunsaturated fatty acids with non-conjugated double bonds. These are dioxygenases, monomers formed in average of 900 amino acid residues and a molecular weight of 94-100KDa in plants. The optimum pH for lipoxygenase is between 5.5 and 9. The lipoxygenase presents a maximum activity at70°C, the lipoxygenase keeps 50% of its activity.

A lipoxygenase (linoleic acid oxygen oxidoreductase, EC 1.13.11.12) enzyme occurs in many plants and also in erythrocytes and leucocytes. It catalyzes the oxidation of some unsaturated fatty acids to their corresponding monohydroperoxides. These hydroperoxides have the same structure as those obtained by autoxidation.

III.4.2.2. Reaction mechanism

Lipoxygenase oxidizes only fatty acids which contain a 1-cis,4-cis-pentadiene system.Therefore, the preferred substrates are linoleic and linolenic acids for the plant enzyme, and arachidonic acid for the animal enzyme; oleic acid is not oxidized.

Lipoxygenase is a metal-bound protein with an Fe-atom in its active center. The enzyme is activated by its product and during activation, Fe^{2+} is oxidized to Fe^{3+}. The catalyzed oxidation pathway is assumed to have the following reaction steps (figure. 29):

- Abstraction of a methylene H-atom from the substrate's 1,4-pentadiene system and oxidation of the H-atom to a proton.
- The pentadienyl radical bound to the enzyme is then rearranged into a conjugated diene system, followed by the uptake of oxygen.

- The peroxy radical formed is then reduced by the enzyme and, after attachmentof a proton, the hydroperoxide formed is released.

In the rate-limiting step of catalysis, the isoenzyme LOX 1 from soybeans abstracts the pro-(S)-hydrogen from the n-8 methylene group of linoleic acid. Molecular oxygen is then introduced into the fatty acid present as a pentadienyl radical from the opposite side at n-6 with the formation of the 13S-hydroperoxide (figure. 30b).

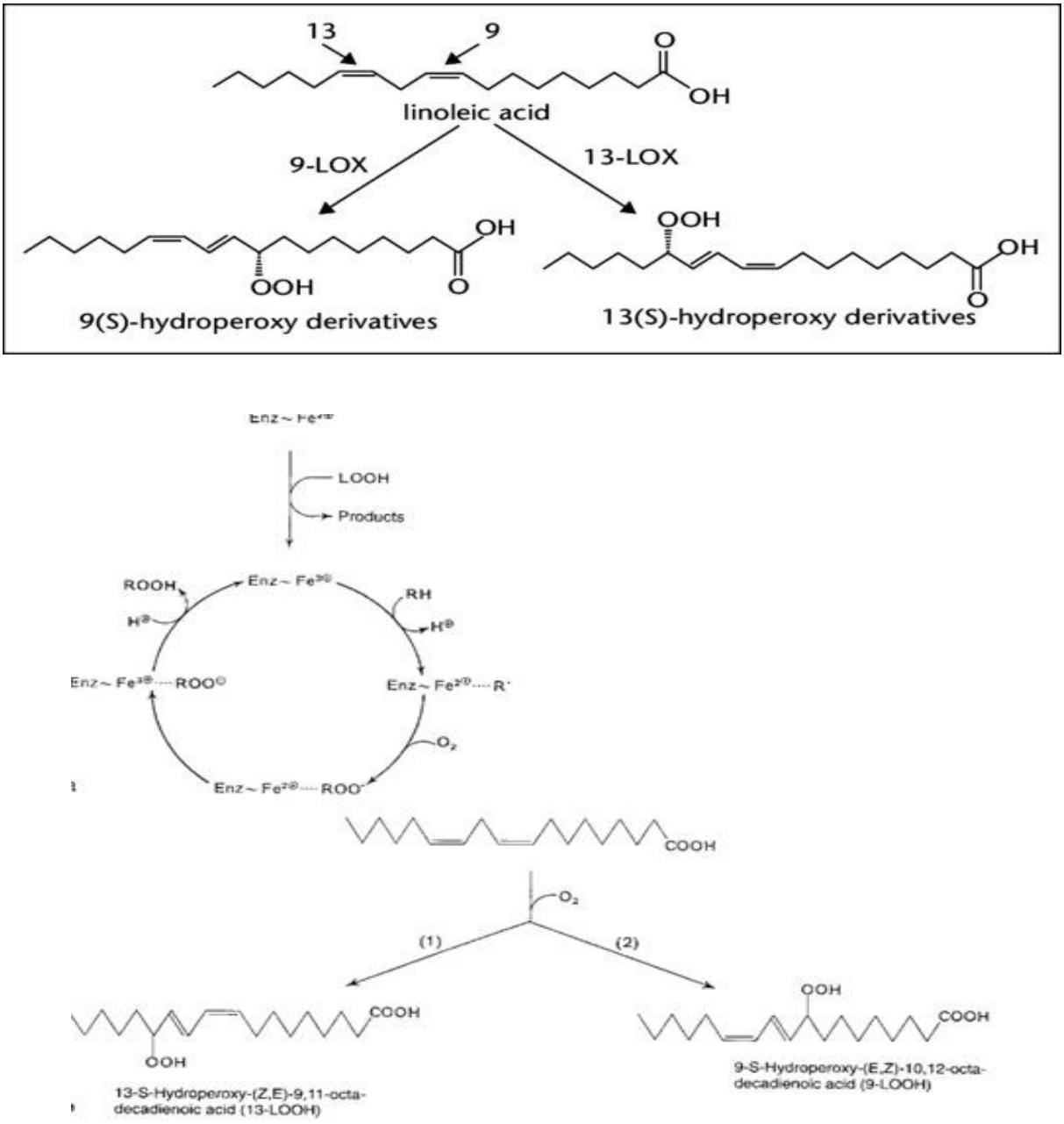


Figure 30. Lipoxygenase catalysis.

Another group of LOX, to which the enzyme from tomatoes belongs, abstracts the pro- (R)-hydrogen. This results in the formation of a 9S-hydroperoxide (figure. 29b) if the oxygen coming from the opposite side docks onto C-9. Unlike autoxidation, reactions catalyzed by lipoxygenase are characterized by all the features of enzyme catalysis: substrate specificity, peroxidation selectivity, occurrence of a pH optimum, susceptibility to heat treatment and a high reaction rate in the range of 0–20 ◦C. Also, the activation energy for linoleic acid peroxidation is rather low: 17 kJ/mol.

Linoleic and linolenic acids in fruits and vegetables are subjected to oxidative degradation by lipoxygenase alone or in combination with a hydroperoxide lyase. The oxidative cleavage yields oxo acids, aldehydes and allyl alcohols. Among the aldehydes formed, hexanal, (E)-2-hexenal, (Z)-3- hexenal and/or (E)-2-nonenal, (Z)-3-nonenal, (E,Z)-2,6-nonadienal and (Z,Z)-3,6-nonadienal are important for aroma Frequently, these aldehydes appear soon after the disintegration of the tissue in the presence of oxygen. A part of the aldehydes is enzymatically reduced to the corresponding alcohols. In comparison, lipoxygenases and hydroperoxide lyases from mushrooms exhibit a different reaction specificity (table 16).

Table16. Occurrence and properties of various hydroperoxyde lyase

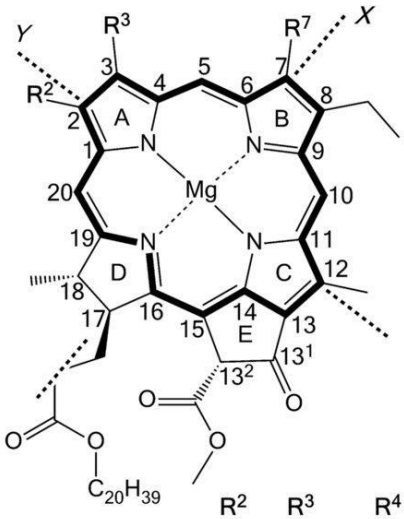
Occurrence	Substrate	Products of the catalyses
Apple, tomato, cucumber, tea leaf (chloroplasts), soy beans, grape	13(S)-hydroperoxy-9-cis,11-trans-octadecadienoic acid (13-LOOH)	hexanal + 12-oxo-9-cis-dodecenoic acid
Apple, tomato, cucumber, tea leaf (chloroplasts), soy beans, grape	13(S)-hydroperoxy-9-cis,11-trans, 15-cis-octadecatrienoic acid (13-LnOOH)	(Z)-3-hexenal + 12-oxo-9-cis-dodecenoic acid
Cucumber, pear	9(S)-hydroperoxy-10-trans, 12-cis-octadecadienoic acid (9-LOOH)	(Z)-3-nonenal + 9-oxo-nonanoicacid
Cucumber, pear	9(S)-hydroperoxy-8-trans, 12-cis, 15-cis-octadecatrienoic acid (9-LnOOH)	(Z,Z)-3,6-nonadienal + 9-oxononanoic acid
Champignon	10(S)-hydroperoxy-10-trans, 12-cis-octadecadienoic acid (10-LOOH)	1-octen-3(R)-ol + 10-oxo-8-trans-dcenoic acid (Z)-1,5-octadien-3(R)-ol+ 10-oxo-8-trans-decenoicacid
Champignon	10(S)-hydroperoxy-8-trans,12-cis-15-cis-octadecatrienoic acid (10-LnOOH)	

- **Applications**

End products from the lipoxygenase pathway such as aldehydes and alcohols are characterized by specific aromatic properties. Volatile compounds characterize several plants (green plants) responsible for the fresh smell of grass

III.5. Oxidation of chlorophylls

Chlorophyll is the derivative of dihydroporphyrin, chelated with centrally located magnesium atom and are ubiquitous in most of the higher plants (figure 31). It isassociated with carotenoids, lipids and lipoproteins and present in the chloroplast of leaves, a photosynthesizing part. Chlorophyll a and b found mostly in the ratio of 3:1 in green parts of the plant systems and differs in carbon C-3 substitution as the presence of methyl and formyl group, respectively.



	R2	R3	R4	Absorption maxima (nm) Soret	Q_y
1. Chlorophyll *a*	CH_3	$CHCH_2$	CH_3	433	665
2. Chlorophyll *b*	CH_3	$CHCH_2$	CHO	468	652
3. Chlorophyll *d*	CH_3	CHO	CH_3	454	697
4. Chlorophyll *f*	CHO	$CHCH_2$	CH_3	405	707

Figure 31. Chemical structure of chlorophylls.

The presence of phytol groupesterified to propionate at the carbon C-7 position makes the chlorophyll hydrophobic. The presence of intense phytol peak in the gas chromatogram as verified through mass spectroscopy support the disorganization of chlorophyll membrane array at subjected higher dehydration temperatures of 80°C as initial temperature.

As per the attained dehydrating radish leaves attained a temperature at wet bulb temperature lied nearly the temperature required for activation of chlorophyllase (chlorophyll chloropyllido - hydrolase, EC 3.1.1.14), and chlorophylidine as phyllin bright green pigment to some extent was formed leaving some of the chlorophyll intact in the leaf cells during the initial phase of dehydration.

As per the properties of reduction of moisture during the process of dehydration in falling rate period the raised temperature and reduced the moisture to a greater extent arrested the mobility of reacting entities and thus arrested the further conversions of chlorophyll during the initial phase. The reduction of dehydration temperature in the finishing phase has possibly maintained in the form of phyllin The characteristics of radish powder thus obtained by the improved technique at combination temperature of dehydration was subjected to fractionation into course and fine fractions using 60 BSS mesh following the particle size distribution pattern of mixed powder. The powders were analyzed for water absorption capacity, water solubility index with proximate composition, chlorophyll content and anti-oxidant activity as free radical quenching capacity.

Biological and chemical modification of chlorophyll. a Chl b is enzymatically reduced to Chl a via a 7-hydroxymethyl intermediate by Chl b reductase (CBR) and 7-hydroxychlorophyll a reductase (HCAR). The reverse reaction may also occur which is catalysed by Chl a oxygenase (CAO). Chemical reduction of the formyl group of Chl b is achieved with the reductant NaBH4. b Oxidation of the 3-vinyl or 2-methyl group of Chl a into a formyl group (–CHO) yields Chl d or Chl f, respectively. Both of these enzymatic reactions utilize molecular oxygen. The enzyme for Chl f synthase has been suggested as a modified D1 protein of photosystem II, super-rogue Psba4 and renamed ChlF. The chemical conversion of Chl a to Chl d requires the presence of a thiol compound such as β-mercaptoethanol (BME) and oxygen.

There are two main ways of degradation:

a. Occurs through the loss of the magnesium ion, leading to the formation of pheophitin(the magnesium ion is removed by being replaced by protons).

b. Leads to the formation of pheophorbide by cutting the phytol chain, the final chlorophyll cleavage causes the green colour to change.

a. Photo-oxidation: chlorophyll pigments play a role in stability oxidation of oils; these pigments are photosensitisers which, by in the presence of light produce oxygen. Thisexcited oxygen species is very responsive;

The products of photo-oxidation of AGIs are unstable hydropersoxides, which can decompose to give volatile compounds, at low PM very which cause the oil to go rancid. The photo-oxidation of edible oils induced by chlorophyll and their immediate degradation products, the pheophytins, is the source of the radicals necessary to initiate auto-oxidation reactions. In the last two decades, there has been growing interest in the effect of chlorophyll pigments on the oxidative stability of virgin olive oil. These pigments are photosensitizers which, in the presence of light, produce gold oxygen. This excited oxygen species is very reactive and reacts approximatively 500 times more rapidly with elinoleic acid than with atmospheric oxygen atmospheric oxygen are unstable hydroperoxides, which can decompose to give volatile decompose into volatile, low-molecular-weight, highly odorous compounds. Odoriferous and which are the origin of the hardening of virgin olive oil that the photo-oxidation of vegetable oils, induced by chlorophylls and their induced by chlorophylls and their immediate degradation products, pheophytins free radicals required to initiate autoxidation reactions.

α-tocopherol and β-carotene, natural products of olive virgin oil, are capable of retarding the negative effects of a photo-oxidation by activating oxygen. In the dark, the specific effects of chlorophylls and phenophylls on the oxidative stability on the oxidative stability of plant oils is poorly understood, and the previous work published on this subject are far from unanimous. Chlorophylls in the dark catalyze the auto-oxidation esters of soybean oil.

b. Mechanism of photo-oxidation by chlorophyll pigments

Chlorophyll pigments in virgin olive oil solution, act as photosensibilizers, (Sens) and catalyze the production of singlet oxygen according to the reaction scheme shown in figure 32.

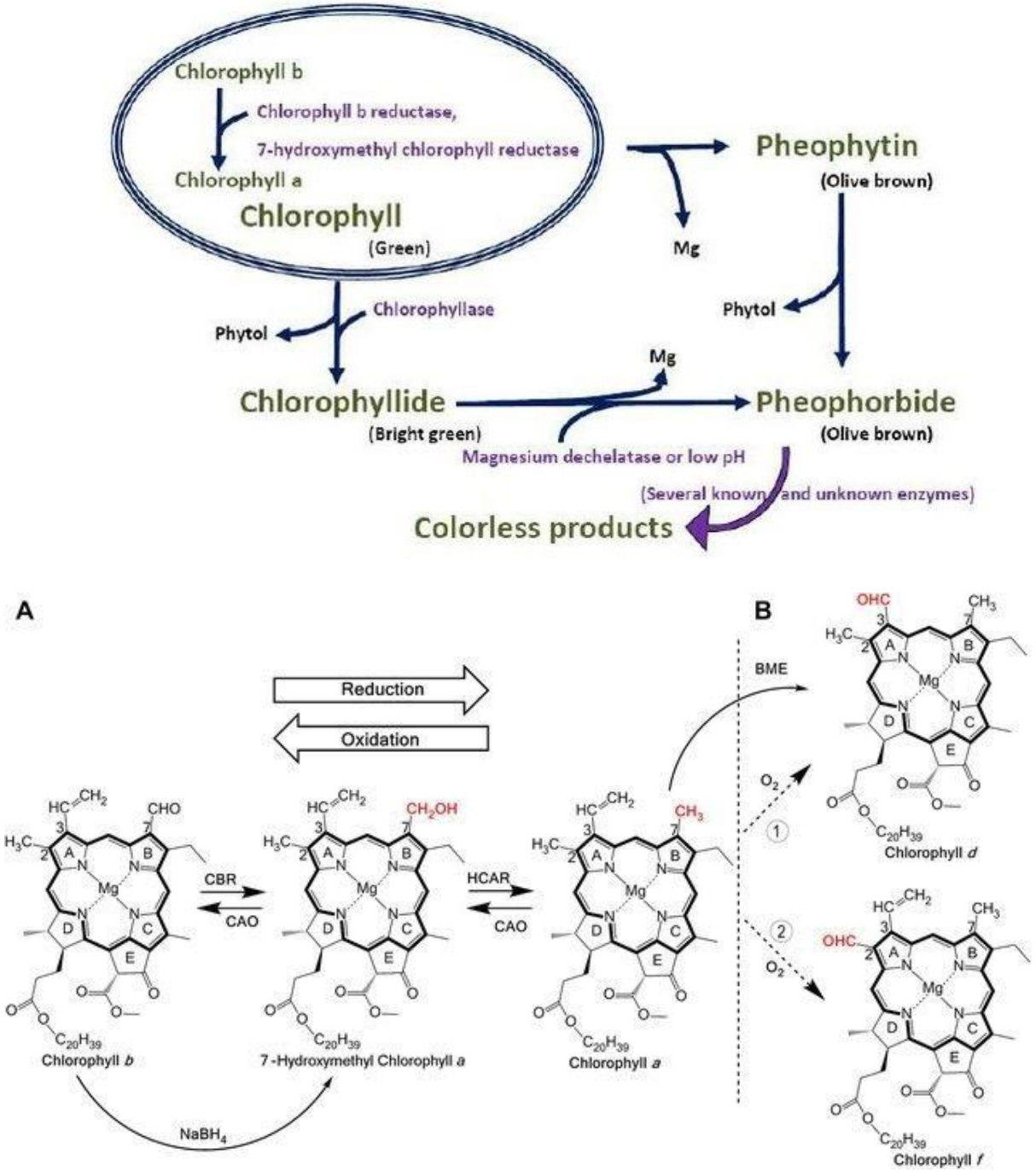


Figure 32. Oxidation and reduction of chlorophylls.

After absorption of a luminous photon (hv), the chlorophyll or pheophytin molecule pheophytin passes from a fundamental light state (lsens) to an excited light state (tSens'). (tSens') and then, by electronic transmission, to a triple excited state (fSens). meta-stable state is short-lived and tends to revert to the singlet state state by transferring the excess excitation energy to atmospheric oxygen dissolved in the oil to give the Oxygen singulet. Excitation energy was of about 22kca|/mole relative to atmospheric oxygen. The oxygen-ingredients produced react directly, on each carbon of the double bond of the unsaturated fatty acid of the oil (so-called "ene" reaction). "ene" reaction to give hydroperoxides (figure 33 and 34) for each double bond, which explains the number of hydroperoxides obtained, which is always twice the number of double (s) bond(s) in the unsaturated fatty acid, the substrate for photo-oxidation.

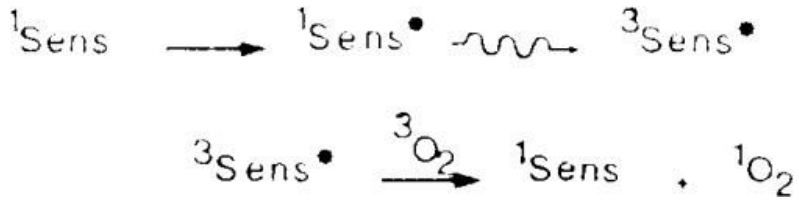


Figure 33: Production of oxygen by chlorophyll pigments.

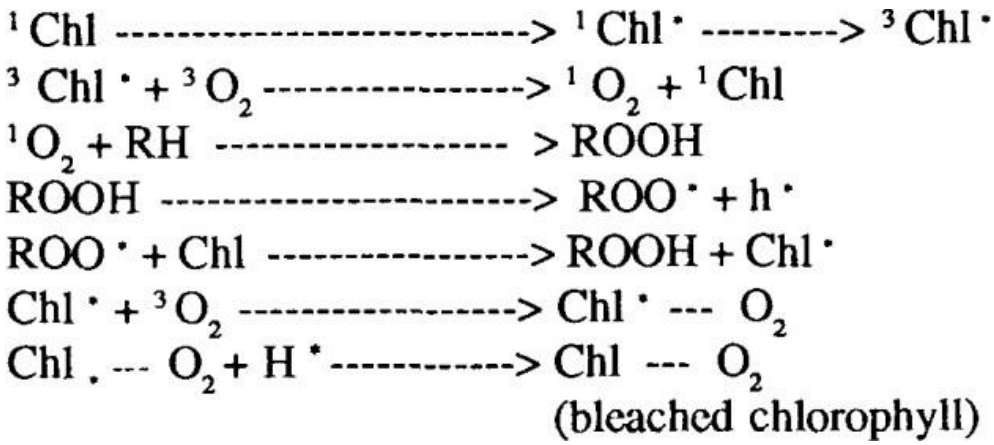


Figure 34. Interaction of chlorophyll and oxygen singulet.

The relative reactivities of unsaturated fatty acids to be in a ratio to the number of their double bonds. This is not quite because the reactivities of oleate, linoleate and methyl linolenate relative to oxygen linolenate are; 1.7 and 2.3 respectively.

Unlike radical auto-oxidation reactions, which produce only conjugated hydroperoxide isomers, photo-oxidation induced by chlorophyll pigments produces both conjugated hydroperoxide and non-conjugated hydroperoxide diesters. For a food oil with a low elinolenic acid content, as is the case for virgin olive oil, the presence of a substantial quantity of the 12- hydroperoxide isomer is an indication of photo-oxidation sensibility.

The pheophytin has the highest activity, in the light as a prooxidant, and in the dark as an antioxidant. The photosensitizing role of chlorophyll pigments in virgin olive oil has been demonstrated in a series of experiments. Much more unsaturated than virgin olive oil, these two oils prove to be more resistant to the action of daylight. This result may be explained by the photosensitizing effect of the chlorophyll pigments in the virgin olive oil than those of the other two oils.

The influence of a number of phenolic antioxidants on the photo-oxidation rate of virgin olive oil revealed that after six months' of a storage in transparent containers, in daylight and at room temperature and at room temperature, the level of peroxidation in samples containing the antioxidants was similar to that in samples without these antioxidants. This suggests that polyphenols which are the natural antioxidants in olive oil of virgin olive oil, are unfortunately ineffective against the oxidation process. According to the researcher's work, the speed of photo-oxidation of olive oil increases with the concentrations of chlorophyll or pheophyrin. Similarly, virgin olive oils show greater resistance to photo-oxidation than oils from the beginning of the season, which are generally richer in chlorophyll pigments.

The pro-oxidant action of chlorophylls and pheophytins in olive oil was found to be as follows pheophyne b > pheophytine a > chlorophyll b > chlorophyll a. When assessing the photo-oxidative stability of a virgin olive oil not only its total content of chlorophyll pigments, but also the concentration of each pigment type of chlorophyll or pheophytin.

III.6. Oxidation of carotenoids

III.6.1. Carotenoid structure

Carotenoids are polyene hydrocarbons biosynthesized from eight isoprene units (tetraterpenes) and, correspondingly, have a 40-C skeleton. They provide the intensive yellow, orange or red color of a great number of foods of plant origin (table 17). As well as for the provitamin A activity, which some of them possess. Most carotenoids are 40-carbon terpenoids having isoprene as their basic structural unit. A general subdivision is into:

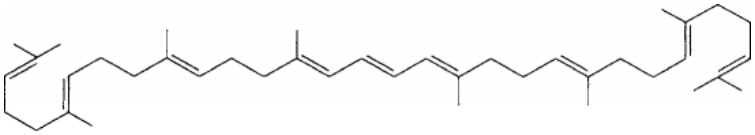
- "**carotenes**" which are strictly hydrocarbons (α- and β-carotene, lycopene) and
- "**xanthophylls**" (lutein, bixin, capsanthin etc), which contain polar ends groupsreflecting an oxidative step in their formation.

Table 17. Carotenoids amount in various food

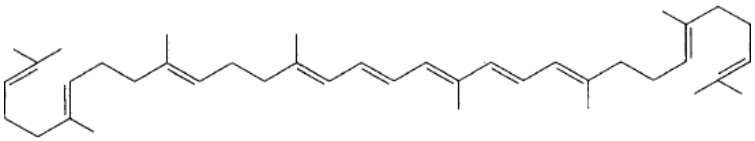
Food	Concen-tration (ppm)[a]	Food	Concen-tration (ppm)[a]
Carrots	54	Peaches	27
Spinach	26–76	Apples	0.9–5.4
Tomatoes	51	Peas	3–7
Apricots	35	Lemons	2–3

[a] On dry weight basis.

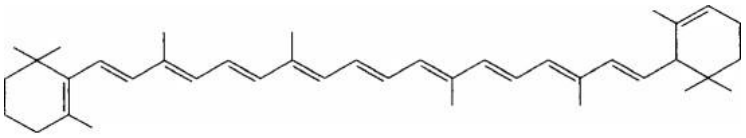
They are synthesized only by plants. However, they reach animal tissues via the feed (pasture, fodder) and can be modified and deposited there. A well known example is the chicken egg yolk, which is colored by carotenoids. The carotenoids are divided into two main classes: carotenes and xanthophylls. In contrast to carotenes, which are pure polyene hydrocarbons, xanthophylls contain oxygen in the form of hydroxy, epoxy or oxo groups. Some carotenoids of importance to food (figure 35).



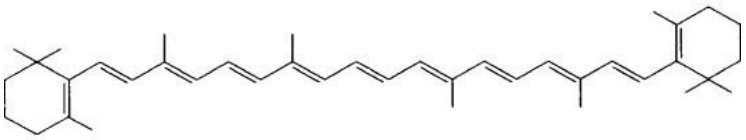
Phytoene (I)



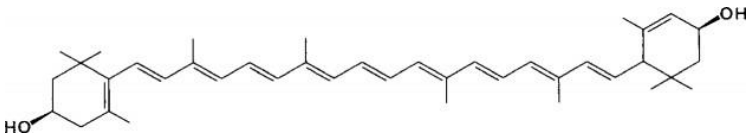
Phytofluene (II)



α-Carotene (β, ε-carotene) (VI)



β-Carotene (β, β-carotene) (VII)



Lutein (β,ε-carotene-3,3-diol).

Figure 35. Some food carotenoids structure.

III.6.2. Chemical properties

Carotenoids are highly sensitive to oxygen and light. When these factors are excluded, carotenoids in food are stable even at high temperatures. Their degradation is, however, accelerated by intermediary radicals occurring in food due to lipid peroxidation. The oxidation phenomena in the presence of lipoxygenase are particularly visible. Changes in extent of coloration often observed with dehydrated paprika and tomato products are related to oxidative degradation of carotenoids.

Such discoloration is desirable in flours (flour bleaching). The color change in paprika from red to brown, as an example, is due partly to a slow Maillard reaction, but primarily to oxidation of capsanthin and to some as yet unclear polymerization reactions.

III.6.3. Carotenoid chemical reactions

a. Enzymatic cleavage of carotenoids

Enzymatic carotenoid cleavage by specific carotenoid cleavage dioxygenases (CCDs) and nine-cis-epoxy-carotenoid dioxygenases (NCEDs) in plants can result in highly specific cleavage into apocarotenoids, exerting explicit functions either directly or aftermodification. Additionally, the same diversity of apocarotenoids is also formed by non enzymatic carotenoid degradation, resulting from the antioxidant properties of carotenoids. The two processes occur simultaneously and apocarotenoids undergo further derivatization.

The dynamic equilibrium between degradation and biosynthesis in plant tissues determines the steady-state carotenoid levels. Accordingly, high carotenoid levels in plant tissues are the result of high biosynthetic activity and/or by attenuated degradation, the latter often being achieved by carotenoid sequestration. Interestingly, if carotenoid-sequestrating structures are absent and carotenoid biosynthesis rates are low—for instance during storage of ripe seeds, vegetables, and fruits—β-carotene (provitamin A) contents steadily decrease at varying rates.

b. Non enzymatic cleavage of carotenoids

Non enzymatic degradation of carotenoids is due to the highly unsaturated hydrocarbon backbone being susceptible to oxidation, generating epoxy- and peroxide derivatives as well as polymeric aggregates. Upon oxidation, carotenoids decompose into apocarotenoids and finally form a plethora of products partially identical to those formed enzymatically. Several lines of evidence suggest that non enzymatic oxidation rates surpass those for enzymatic cleavage, i.e. degradation rather than enzyme- driven catabolism appears to be the main determinant of carotenoid levels, besides carotenoid biosynthesis. In chloroplasts, photo-oxidation is the predominant carotenoid degrading process and produces apocarotenoids involved in light stress signaling. However, levels of apocarotenoids are by far not quantitatively equivalent to carotenoid losses.

Therefore, it can be hypothesized that these primary cleavage products might be further metabolized by secondary cleavage reactions targeting the remaining double bonds, by formation of conjugates and of polymeric aggregates as well as by so far unknown reactions.

- **Antioxidant activity of carotenoids**

A large body of scientific evidence suggests that carotenoids scavenge and deactivate free radicals both *in vitro* and *in vivo*. It has been reported that their antioxidant action is determined by:

• Electron transfer reactions and the stability of the antioxidant free radical;

• The interplay with other antioxidants;

• The carotenoid structure and oxygen pressure of the tested system

Moreover, the antioxidant activity of carotenoids is characterized by literature data for

• Their relative rate of oxidation by a range of free radicals, and

• Their capacity to inhibit lipid peroxidation in multilamellar liposomes.

The antioxidant activity of carotenoids is a direct consequence of the chemistry of their long polyene chain: a highly reactive, electron-rich system of conjugated double bonds susceptible to attack by electrophilic reagents, and forming stabilized radicals. Therefore, this structural feature is mainly responsible for the chemical reactivity of carotenoids towards oxidizing agents and free radicals, and consequently, for any antioxidant role.

- **β-carotene antioxidant action mechanism**

β-carotene has received considerable attention in recent times as a putative chain breaking antioxidant. Although it does not have the characteristic structural features associated with conventional primary antioxidants, its ability to interact with free radicals is well-documented. In fact, the extensive system of conjugated double bonds makes carotenoids very susceptible to radical addition, a procedure which eventually leads to the free radical form of the carotenoid molecule.

According to this particular mechanism, β-carotene is capable of scavenging peroxyl radicals (React-3). The resulting carbon centered radical (ROO-β-CAR∗) reacts rapidly and reversibly with oxygen to form a new, chain-carrying peroxyl radical (ROO-β-CAR-OO∗). The carbon-centered radical is resonance stabilized to such an extent, (React-4) that when the oxygen pressure is lowered, the equilibrium of this reaction shifts sufficiently to the left, to effectively lower the concentration of peroxyl radicals and hence reduce the amount of autoxidation in the system.

Furthermore, the β-carotene radical adduct can also undergo termination by reaction with another peroxyl radical (React-5).

β-CAR + ROO∗ → ROO-β-CAR∗ (React-3)

ROO-β-CAR∗ + O2 → ROO-β-CAR-OO∗ (React-4)

ROO-β-CAR∗ + ROO∗ → inactive products (React-5)

To understand the mechanism of antioxidant activity of carotenoids it is also important to analyze the oxidation products that are formed during their action as antioxidants. A relationship between product-forming oxidation reactions and carotenoid antioxidant effects has been proposed. In more details, it has been reported that the epoxide forming reaction (homolytic cleavage) releases an alkoxyl radical and would not be expected to produce an antioxidant effect. However, heterolytic cleavage would lead to carbonyl fragments, a di alkyl peroxide and other non-radical products, so that β-carotene oxidation by this pathway results in the consumption of two peroxyl radicals and thereby to an antioxidant effect (Figure 36).

The activity of carotenoids is also strongly influenced by the oxygen pressure of the experimental conditions. Natural carotenoid mixtures inhibited the azo-initiated oxidation of sunflower oil-in-water emulsions (operated rapidly under low in terms of both primary and secondary oxidation products. However, other studies concluded that carotenoids not only did not inhibit aerial lipid autoxidation but even exerted a pro-oxidant character, a phenomenon also observed at high carotenoid concentrations. In both cases a lack of the antioxidant effect may be due mainly to a more increased formation of carotene- peroxyl radicals, promoting the propagation of autoxidation.

The addition of various carotenoids in foods containing unsaturated oils can improve their shelf life. The mechanism by which carotenoids energy state (React-7,and act so effectively, that one carotenoid molecule is able to quench up to 1000 molecules of singlet oxygen.

$^1O_2$ + β-carotene → 3 β-carotene∗ + $3O_2$ (React-6)

3β-carotene∗ → β-carotene + heat (React-7);

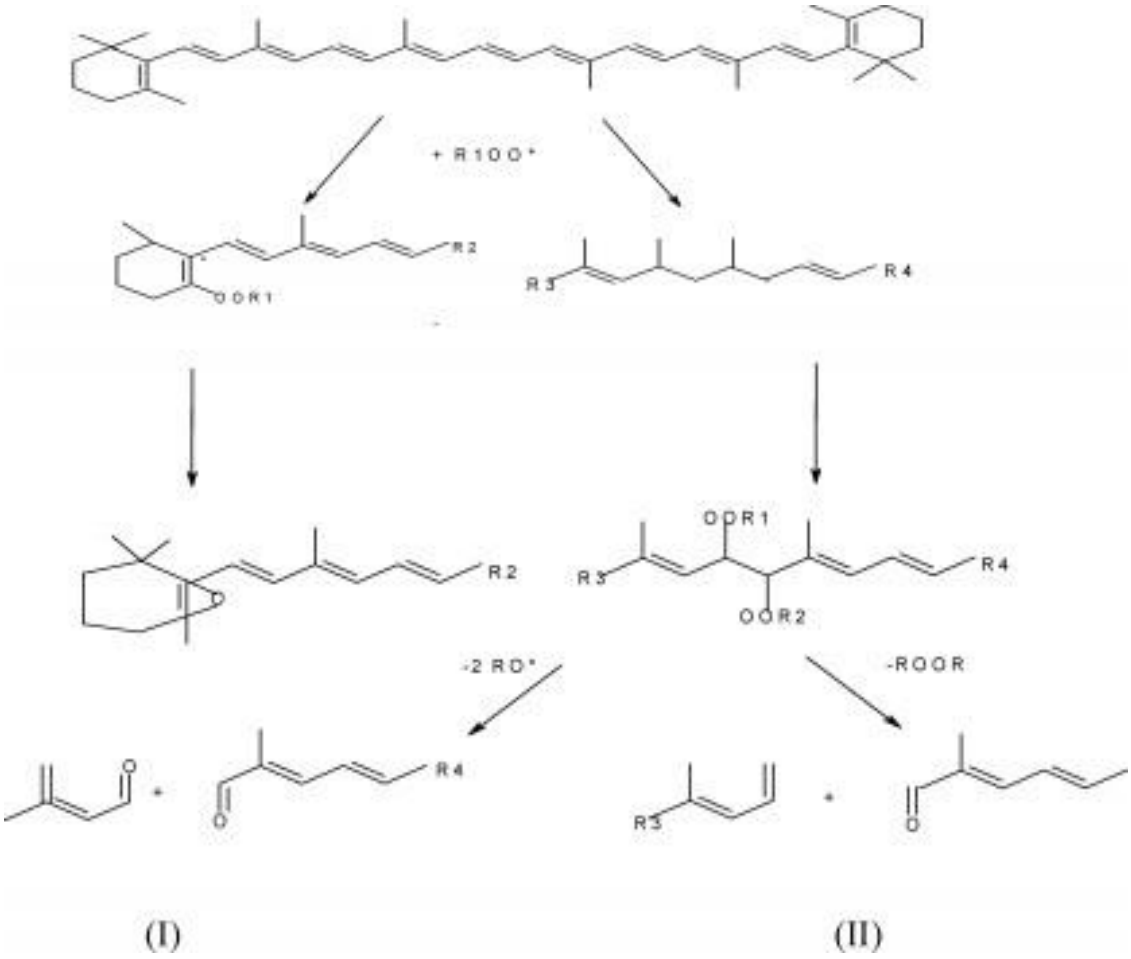


Figure 36. Oxidation of β-carotene by a peroxyl radical.

During chlorophyll sensitized photoxidation of soybean oil, the carotenoid antioxidant effect was enhanced with an increasing concentration and number of double bonds. Capsanthin, a carotenoid containing 11 conjugated double bonds, a conjugated keto- group and a cyclopentane ring, has been reported to exert a higher anti-photo-oxidative activity than β-carotene, which has the same number of double bonds but neither of the functional groups.

Autoxidation of β-carotene in benzene or tetrachloromethane in the dark at 30◦C under one atmosphere of oxygen or by bubbling oxygen through the solvent was found to occur with an induction period of less than one hour, followed by rapid production of oxidation products. Under these conditions, β-carotene was completely consumed within 30 hours. A combination of HPLC, FT-IR, and GC-MS were used to monitor the reaction and identify over twenty oxidation products (figure 37).

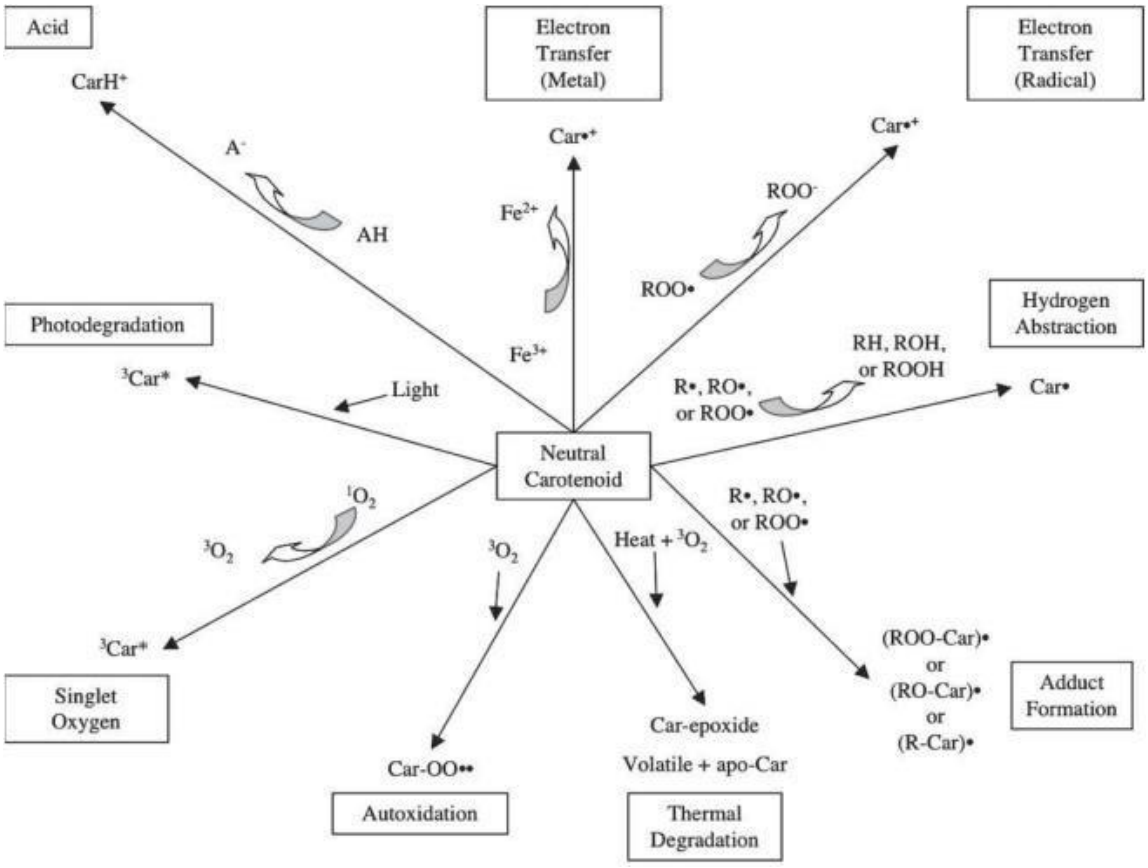


Figure 37. Mechanisms of carotenoid oxidation and initial products.

- **Thermal degradation**

Thermal treatment to carotenoids in the presence of oxygen results in the formation of volatile compounds and larger non-volatile components. The heating pure β-carotene at 180°C for two hours resulted in the formation of a number of cis isomers as well as oxidation products. This work also showedthat the level of air circulation in the sample increased the degradation of β-carotene because of the greater likelihood of β-carotene and oxygen interaction. The resulting compounds found in this work led the researchers to conclude that all of the double bonds of β-carotene could be oxidized and that the breakage of these bondsis likely to occur starting at the terminal end of the molecule and proceeding towards the center of the molecule.

Under thermal oxidation conditions, various radical species are formed and can react with oxygen to form peroxyl radicals, which can undergo propagation reactions with additional carotenoids. Even under vacuum, higher temperature treatment (240°C) of crystalline β-carotene resulted in the formation of toluene, m-xylene, pxylene, ionene, and 2,6-dimethylnaphthalene as determined by gas-liquid chromatography and infrared, nuclear magnetic resonance, and mass spectroscopies. At lower temperatures (60°C), with a stream of oxygen being passed through β-carotene in toluene, that β-carotene terminal double bonds could be broken, producing various epoxides as determined by chromatography followed by absorption spectra determination. There was no lag phase in the decomposition of β-carotene, ruling out autoxidation as a mechanism for degradation. The reaction of oxygen with β-carotene might be catalyzed by metals.

- **Photodegradation**

Light exposure degrades carotenoids, and several mechanisms of action have been proposed. Photo-oxidation produces species thought to be carotenoid radical cations Laser flash photolysis studies have produced evidence to suggest that rapid bleaching of β-carotene in some solvents like chloroform, can occur due to light exciting the β-carotene molecules, which then instantly react with the solvent (chloroform in this case) to form either a carotenoid-solvent free radical adduct or a β-carotene radical (due to hydrogen abstraction). The same work has also shown that the β-carotene molecules in the excited state may return to ground state, where they may be attacked by radical by-products created during the above reaction and undergo a slower degradation process thought to possibly form β-carotene radical cations.

In a mechanism similar to carotenoid excitation described in photo-degradation, light can also excite sensitizers like chlorophylls, leading to the formation of singlet oxygen ($^1O2$). As seen in Eq. 1, singlet oxygen may then react with neutral carotenoids to produce excited state carotenoids (3Car*). Once in an excited state, the carotenoid may return to ground state by releasing energy by vibrational and rotational interactions with the surrounding solvent (Eq. 2)

$1O_2 + Car \rightarrow 3O_2 + 3Car*$ (1)

$3Car* \rightarrow Car + Heat$ (2)

Additionally, a computational study using density functional theory has concluded that while the physical quenching pathway described in Eqs. (1 and 2) is the most favored mechanism for carotenoid and singlet oxygen interaction, the excited state carotenoids may also follow a chemical degradation pathway that is still not well understood.

- **Free radicals**

In some systems, such as foods, free radicals may already be present in the medium due to reactions such as lipid oxidation. Carotenoids have been found to confer both antioxidant and prooxidant effects in systems containing pre-formed radicals depending on the type and level of carotenoid used, oxygen concentration present, and the polarity of the solvent. When radicals are present in a system, several mechanisms of carotenoid interaction are possible. These reactions include electron transfer, hydrogen abstraction, and addition of radical species to form carotenoid radical adducts.

- **Electron Transfer**

Some studies suggest that neutral carotenoids are capable of participating in electron transfer reactions with radicals as well as with metals like iron as discussed previously. In these reactions, carotenoid radical cations are formed (Eq. 6). β-carotene, canthaxanthin, zeaxanthin, astaxanthin, and lycopene have been shown to form radical cations by electron transfer reactions with tryptophan radical cations in a pulse radiolysis study using carotenoids in micelles. Pulse radiolysis has also shown that radicals of nitrogen dioxide react with carotenoids in this manner in lycopene, lutein, zeaxanthin, astaxanthin, or canthaxanthin in tert-butanol/water mixtures. The same reaction mixtures and pulse radiolysis techniques also found thiyl-sulphonyl radicals to produce carotenoid radical cations, but an uncharacterized intermediate (possibly and ion-pair) was detected as well. The likelihood of electron transfer reactions taking place may depend on the type of carotenoid and radical. For instance, there is evidence from laser flash and steady-state photolysis studies that β-carotene is not likely to interact with peroxyl radical in this manner, but instead are more likely to undergo adduct formation or hydrogen abstraction reactions;

ROO • +Car → ROO− + Car•+ (6)

If carotenoid radical cations are formed, a number of reactions may occur. One path they may follow is to interact with oxygen to form a carotenoid peroxyl radical cation (Eq. 7), which might then be reduced by another carotenoid or ferrous iron, if present,to create a peroxide form of the carotenoid (Eqs.8 and 9).

If iron is present, reactions previously covered in discussion of Eq. 5, may occur. One study has also found that bulk electrolysis of canthaxanthin in CH_2Cl_2 with ferric chloride at low temperature (–10°C) followed by irradiation with UV-vis light for 1.5 minutes, can lead to the formation of carotenoid dimers. This is thought to occur by the formation of a radical cation that then reacts with neutral carotenoids to form dimers under these conditions. Pulse radiolysis studies have also found radical cations to decay in a bimolecular process, although the products were not determined;

Car •+ +O2 → CarO2•+ (7)
CarO2 •+ +Fe2+ → CarO2 + Fe3+ (8)
CarO2 •+ +Car → CarO2 + Car•+ (9)

A final reaction series that carotenoid radical cations have been found to undergo in solvent systems is deprotonation. This reaction has been studied using canthaxanthin and β-carotene in dichloromethane using electrochemistry and optical. The deprotonation reaction (Eq. 10) was found to be enhanced by the presence of water in the solvent system. Density functional theory applied to this reaction has led to the conclusion that for β- carotene radical cations, deprotonation at the 5 or 5′ methyl group on the cyclohexenering would result in the most stable product.

Car•+ ↔ #Car • +H+ (10)
#Car • +O2 → #Car − OO• (11)

(# indicates one less proton)

It has been suggested that deprotonated carotenoid radicals may be capable of reacting with oxygen to form additional radicals (Eq. 11). In addition, the deprotonated radical has been found to react with other deprotonated radicals to form didehydrodimers (Eq. 12).

#Car • +#Car• → (#Car)2 (12)

As already discussed, carotenoid dications may be formed by reaction of carotenoid radical cations with iron (Eq. 5). Electrochemical oxidation of β-carotene has also been shown to produce dications in tetrahydrofuran, dichloromethane and dichloroethane solutions as determined by EPR. Whether radical cations or dications are the predominant species formed, may depend on the structure of the carotenoid. found that β-carotene and β-apo-8′-carotenal form predominantly dications in experiments using simultaneous electrochemical-EPR of carotenoids in dichloromethane, while canthaxanthin produced predominantly radical cations. If dications are produced, two additional decay reactions have been proposed. The dication may be able to react with a neutral carotenoid in the system, creating two radical cations (Eq. 13), or it may be deprotonated (Eq.14), in a pathway like that shown in Reaction 10, which is especially enhanced in the presence of water

Reactions (13 and 14). A mixture of carotenoid radical cations and dications was produced using varied amounts of ferric chloride (1-2 equivalents) to oxidize canthaxanthin in dichloromethane, suggesting that Reaction 13 was occurring in this system.

Car2+ + Car ↔ 2Car•+ (13)

Car2+ ↔ #Car+ + H+ (14)

The deprotonation reaction (Eq. 14) has been studied using electrochemistry, optical spectroscopy. MALDI-TOF spectroscopy, and EPR spectroscopy, using canthaxanthin, and β-carotene.

- **Hydrogen abstraction**

Reaction of neutral carotenoid with a radical can result in the radical abstracting a hydrogen from the carotenoid. This results in the formation of a resonance stabilized carotenoid radical as seen in Eqs. 15 and 16.

ROO • +Car → ROOH + Car• (15)OH • +Car → H2O + Car• (16)

It has been proposed that the newly formed carotenoid radical might then encounter an additional alkoxyl or peroxyl radicals in the medium and react to form a non-radical product (Eqs. 17 and 18).

Car • +RO• → Car-OR (17)

Car • +ROO• → Car-OOR (18).

- **Adduct formation**

Finally, radicals can also react with carotenoids to form radical adducts. This may occur with alkyl, alkoxyl, and peroxyl radicals as seen in Eqs. 19–21. Laser flash and steady state photolysis studies of β-carotene indicate that at least for this carotenoid, reaction with peroxyl radicals occurs much more slowly than with alkyl or alkoxyl radicals. Acety lperoxyl radicals produced during laser flash photolysis have also been shown to produce adducts with β-carotene in aerated benzene at 20°C. Pulse radiolysis has also shown that glutathione thiyl radicals and 2-mercaptoethanol thiyl radicals react with carotenoids in a first-order reaction to produce adducts followed by bimolecular decay of the adducts in solutions of 10 μM lycopene, lutein, zeaxanthin, astaxanthin or canthaxanthin in tert-butanol/water mixtures.

Car + R• → (Car-R)• (19)

Car + RO• → (Car-RO)• (20)

Car + ROO• → (Car-ROO)• (21)

Reaction with additional radicals is thought to result in addition products. Reaction 22 was proposed to be the mechanism by which β-carotene addition products, detected by atmospheric pressure chemical ionization mass spectrometry, formed in reaction with AMVN-derived alkyl and alkoxyl radicals. The addition product in Eq. 23 was suggested to be the main product of β-carotene and lutein oxidation at low oxygen concentrations.

(Car-RO) • +RO• → RO-Car-RO (22)

(Car-ROO) • +ROO• → ROO-Car-ROO (23)

An additional pathway that radical adducts might follow involves reaction with oxygen. In what is thought to be a reversible reaction, oxygen may add to the radical adduct, producing a resonance stabilized, chain carrying peroxyl radical (Eq. 24). As as well as hydrogen abstraction as potential mechanisms by which β-carotene oxidation products were formed during oxidation with AMVN radicals in benzene at 60◦C based on atmospheric pressure chemical ionization mass spectrometry results. It was observed reversible oxygen addition to neutral 7,7′-dihydro β-carotene radicals and neutral β-carotene radicals utilizing phenylthiyl radicals produced by laser flash photolysis.

(Car-ROO) • +O2 ↔ ROO-Car-OO• (24)

The reaction described in Eq. 24 is likely governed by oxygen pressure. In experiments with β-carotene, rates of carotenoid autoxidation were found to decrease when oxygen levels were lowered. Similarly, as oxygen levels were lowered, β-carotene had a greater antioxidant effect in the AIBN-initiated oxidation of Tetralin and methyl linoleate in chlorobenzene at 30°C. As oxygen is lowered (often to levels similar to those in animal tissues), Reaction 24 shifts to the left and production of peroxyl radicals is reduced. At higher oxygen pressures, however, the reaction is driven to the right and autoxidation rates increase due to hydrogen abstraction reactions like that shown in Eq. 25.

ROO-Car-OO • +RH → ROO-Car-OOH + R• (25)

Peroxyl radical adducts, however, are also likely to follow other pathways. One path these radical adducts might follow is decay to carotenoid epoxides and new alkoxyl radicals (Eq. 26), which may further the extent of oxidative degradation as the newly formed radicals attack other oxidizable substrates in the system. Acetylperoxyl-β-carotene radical adducts to decay with a first order rate constant of 1.35 × 103 s−1,. Additional evidence for this type of pathway in the reaction products formed by AMVN-initiated peroxidation of β-carotene in methyl linoleate or benzene.

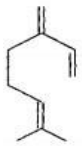
a. Oxidation of terpenes

The mono- and other terpenes in fruits and vegetables, herbs and spices are presented in figure 38. These compounds stimulate a wide spectrum of aromas, mostly perceived as very pleasant. The odor thresholds of terpenes vary greatly. Some of terpenes occur in flavoring plants in such large amounts that in spite of relatively high odor thresholds, they can act as character impact compounds. Terpenes are organic compounds derived from the basic structure isoprene.The most volatile compounds are monoterpenes (C10), which are made up of chains of Unsaturated hydrocarbons with one or more double bonds; Terpenes react with radical molecules such as hydroxyl radicals which leads to several oxidation products (figure 39).

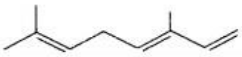
Monoterpenes with hydroxy groups, such as linalool, geraniol and nerol, are present in fruit juice at least in part as glycosides have been found in juice grapes. Terpene glycosides hydrolyze, in the production of jams, either enzymatically (β-glucosidase) or due to the low pH of juices. The latter process is strongly accelerated by a heat treatment. Under these conditions, terpenes with two or three hydroxyl groups which are released undergo further reactions, forming hotrienol (IV) and neroloxide (V) from 3,7-dimethylocta-1,3-dien-3,7-diol in grape juice, or cis- and trans-furan linalool oxides (VIa and VIb) from 3,7- dimethylocta-1-en-3,6,7-triol in grape juice and peach sap.

Monoterpenes

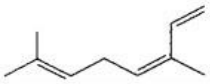
Acyclic (including cyclic derivatives)



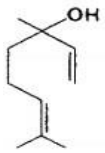
Myrcene (I)



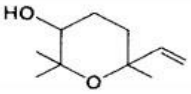
trans-Ocimene (II)



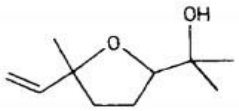
cis-Ocimene (III)



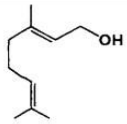
Linalool (IV)



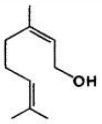
2,6,6-Trimethyl-2-vinyl-5-hydroxytetrahydro-pyran[a] (IVa)



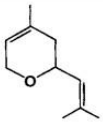
2-Methyl-2-vinyl-5-hydroxyisopropyltetra-hydrofuran[a] (IVb)



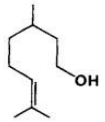
Geraniol[b] (V)



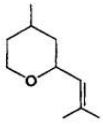
Nerol[b] (VI)



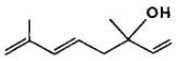
Neroloxide (VIa)



Citronellol[b] (VII)

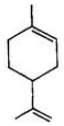


Rose oxide (VIIa)

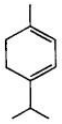


Hotrienol[c] (VIII)

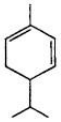
Monocyclic



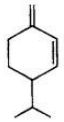
Limonene (IX)



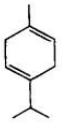
α-Terpinene (X)



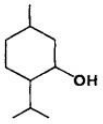
α-Phellandrene (XI)



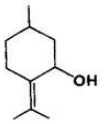
β-Phellandrene (XII)



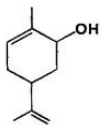
γ-Terpinene (XIII)



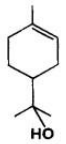
Menthol (XIV)



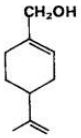
Pulegol (XV)



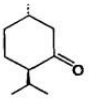
Carveol (XVI)



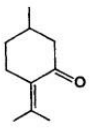
α-Terpineol (XVII)



Perilla alcohol (XVIII)



Menthone (XIX)



Pulegone (XX)

Figure 38. Terpenes in foods.

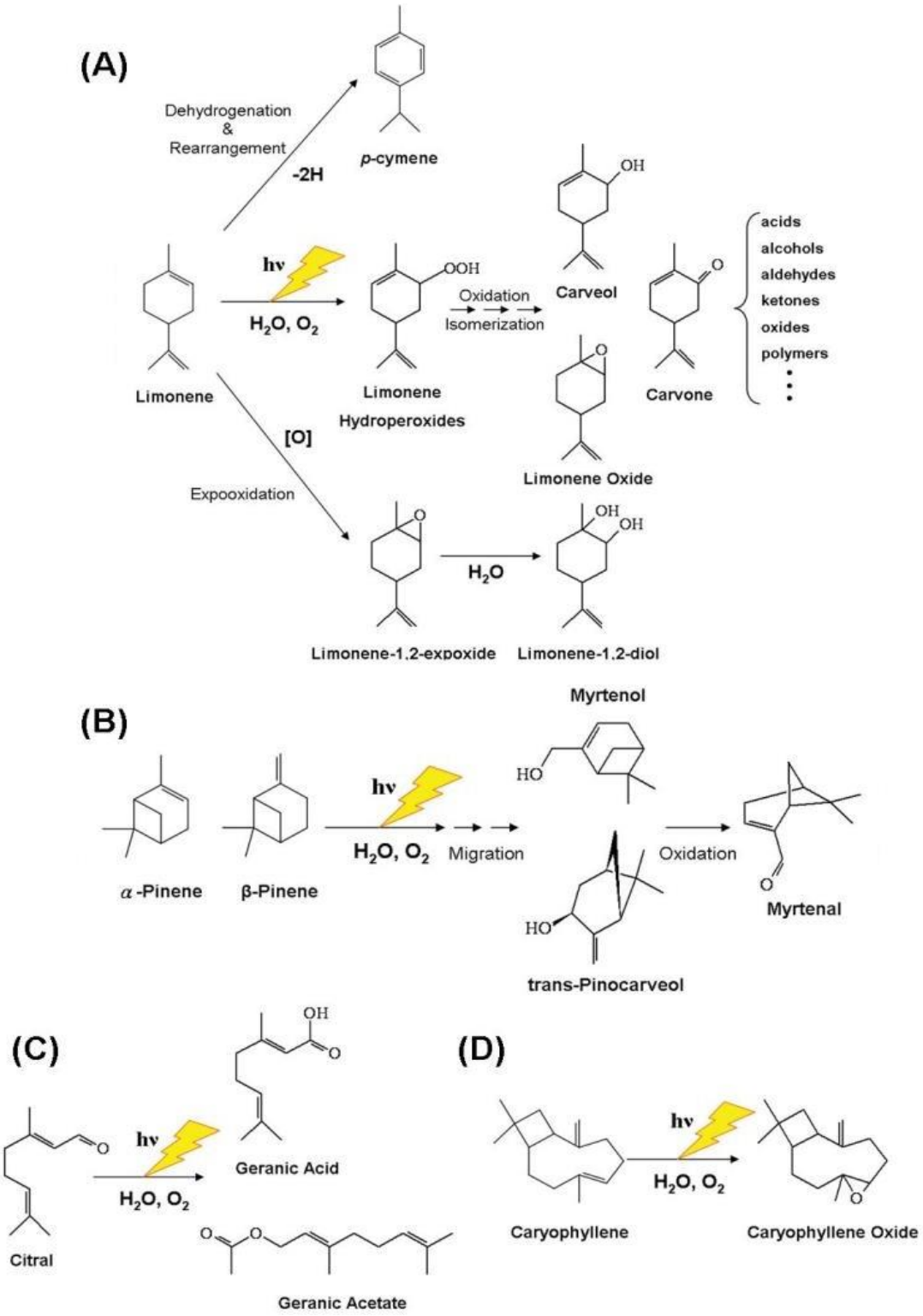


Figure 39. The reaction pathways of terpenes. (A) oxidation of limonene into various oxidative products, (B) the hydroperoxidation pathway of α-pinene and β-pinene , (C) oxidation of caryophyllene into its stable product, caryophyllene oxide, (D) citral oxidative and esterified products.

The oxidation of the terpenoids valencene (1), α-ionone (2), β-ionone (3) and theaspirane (figure 40), produces derivatives of huge interest in the flavor and fragrances industry. Ionone and theaspirane, composed of thirteen carbon atoms, are natural organic molecules derived from carotenoid degradation either through chemical/physical mechanisms or through enzyme(s)-catalyzed reactions.

These are relevant flavors or fragrances, and their accessibility represents an important economic resource for chemical companies. As mentioned above, their market value is not only related to the manufacturing cost. Indeed, the consumers' preference creates two different markets for the same chemical compound depending on its origin. In fact, the possibility to classify the product as artificial or natural influences its final cost. The oxidation products derived from compounds 2–4 were found to be components of different natural flavors such as saffron, tobacco and black tea.

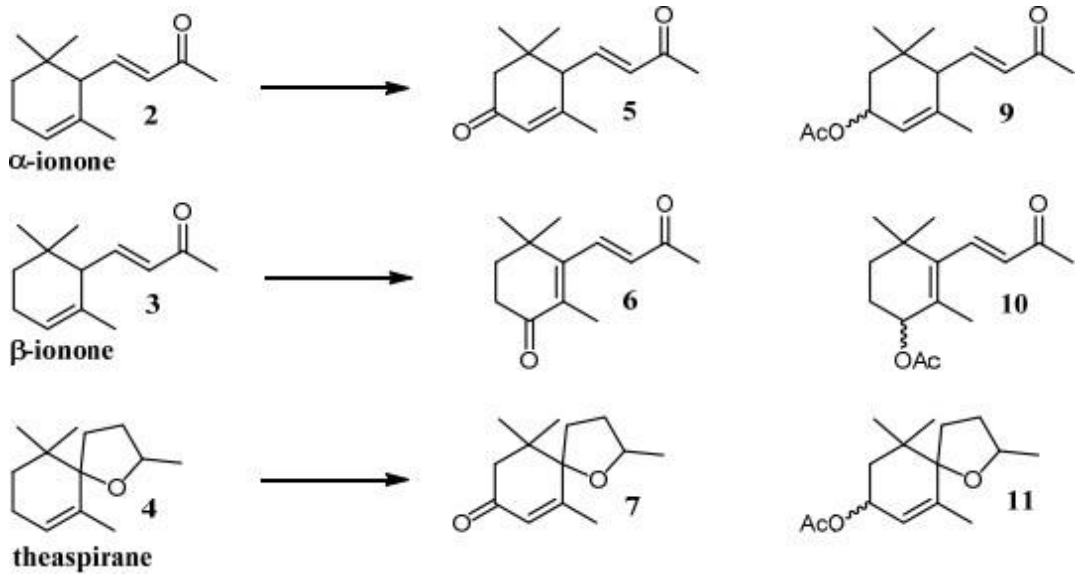


Figure 40. Oxidation products derived from α-ionone (2), β-ionone (3), and theaspirane (4)named 3-keto-α-ionone (5); 3-acetoxy-α-ionone (9); 4-keto-β-ionone (6); 4-Acetoxy-β-ionone (10); theaspirane (7) and 3-acetoxy-theaspirane (11), respectively.

Essential oils (EOs) are complex mixture and highly concentrated hydrophobic liquid containing volatile plant secondary metabolites belong to terpenoids and aromatic groups. They are usually extracted from various parts of plants (flower, leaf or fruit) by different methods such as steam/water distillation, solvent extraction or cold expression, etc. The natural source and pleasant flavor characters make Eos widely used in the medicine, cosmetic, household products and food industry. EOs was also reported to contain many bioactive compounds, such as terpenoids, alkaloids, flavonoids and carotenes. These components make EOs are extensively represent a green alternative in the pharmaceutical, nutritional and agricultural field due to their antimicrobial, antiviral, insecticidal, antioxidant, anti-inflamatory and stress-repellent properties.

References

Bamforth, C.W., (2003) Barley and malt starch in brewing: a general review. Master Brew. Ass. Am. Tech. Quart., 40: 89-97.Biotechnol. 2021, 25, 54–60.

Badoud, R., Löliger, J., Etournaud A., Bauer W.J.(2010). Science et technologie des aliments principes de chimie des constituants et de technologie des procédés. Presses polytechniques romandes, Lausanne. 720p.

Björck, I., Nyman, M., Asp, N-G. Extrusion-cooking and dietary fiber: Effects on dietary fiber content and on degradation in the rat intestinal tract. *Cereal Chemistry* 1984,61,174-179.

Bouglé, D.; Bouhallab, S. Dietary bioactive peptides: Human studies. Crit. Rev. Food Sci. Nutr. 2015, 57, 335–343. [CrossRef] [PubMed] 5. Kiewiet, M.B.G.; Gros, M.; van Neerven, R.J.J.; Faas, M.M.; de Vos, P. Immunomodulating properties of protein hydrolysates for application in cow's milk allergy. Pediatr. Allergy Immunol. 2015, 26, 206–217

Boura, K.; Kandylis, P.; Bekatorou, A.; Kolliopoulos, D.; Vasieleiou, D.; Panas, P.; Kanellaki, M.; Koutinas, A. New generation biofuel from whey: Successive acidogenesis and alcoholic fermentation using immobilized cultures on γ-alumina. Energy Convers Manag. 2017, 135, 256–260.

Castello, F.; Fernandez-Pachon, M.-S.; Cerrillo, I.; Escudero-Lopez, B.; Ortega, A.; Rosi, A.; Bresciani, L.; Rio, D.D.; Mena, P. Absorption, metabolism, and excretion of orange juice (poly) phenols in humans: The effect of a controlled alcoholic fermentation. Arch. Biochem. Biophys. 2020, 695, 108627.

Chua, J.-Y.; Tan, S.J.; Liu, S.-Q. The impact of mixed amino acids supplementation of Torulaspora delbrueckii growth and volatile compound modulation in soy whey alcohol fermentation. Food Res. Int. 2021, 140, 109901.

Coelho, A.L.S.; Orlandelli, R.C. Immobilized microbial lipases in the food industry: A systematic literature review. Crit. Rev. Food Sci. Nutr. 2021, 61, 1689–1703.

Damodaran, S. "Amino acids, peptides and proteins", In Food Chemistry, 3rd ed. Fennema, O. R. Ed., Marcel Dekker, New York, 1996, p 321.

Dekker, P.J.T.; Koenders, D.; Bruins, M.J. Lactose-free dairy products: Market developments, production, nutrition and health benefits. Nutrients 2019, 11, 551.

Denkov P.N., Tcholakova S., Politova-Brinkova N. Physicochemical control of foam properties.Current Opinion in Colloid & Interface Science, 50 : 101-376.

Dhaval, A.; Yadav, N.; Purwar, S. Potential Applications of Food Derived Bioactive Peptides in Management of Health. Int. J. Pept. Res. Ther. 2016, 22, 377–398.
Dickinson, E.; McClements, D. J. “Advances in Food Colloids”, Chapman & Hall, London, 1995.
Dumay, E.; Picart, L.; Regnault, S.; Thiebaud, M. Biochim Biophys Acta. 2006, 1764, 599-618.
El-Hofi M, Ismail A, Nour M, Ibrahim O. Isolation, purification and characterisation of transglutaminase from rosemary (Rosmarinus officinalis L.) leaves. Acta scientiarum polonorum Technologia alimentaria. 2014; 13(3):267–78
Emulsifying properties of proteins. Bol. Soc. Quím. Méx. 2008, 2(2), 80-89. 2008, Sociedad Química de México
FEDIAF. Guide to Good Practice for the Manufacture of Safe Pet Foods; FEDIAF: Brussels, Belgium, 2018.
Floros, J.D.; Newsome, R.; Fisher, W.; Barbosa-Cánovas, G.V.; Chen, H.; Dunne, C.P.; German, J.B.; Hall, R.L.; Heldman, D.R.; Karwe, M.V.; et al. Feeding the World today and tomorrow: The Importance of food science and technology. Compr. Rev. Food Sci. Food Saf. 2010, 9, 572–599. [CrossRef] 13. What is a Processed Food? You Might be Surprised! Understanding Our Food Communications Tool Kit; International Food Information Council Foundation: Washington, DC, USA, 2010.
Friberg, S. E. In: *“Food Emulsions”*, Friberg, S. E.; Larsson, K. (Ed.). Marcel Dekker. Inc., New York**.** 1997.
Fu, J., Li, L., & Yang, X. Q. (2011). Specificity of carboxypeptidases from actinomucor elegans and their debittering effect on soybean protein hydrolysates. Applied Biochemistry and Biotechnology, 165(5–6), 1201–1210. https://doi.org/10.1007/ s12010-011-9338-4 Fu, Y.u., Chen, J., Bak, K. H., & Lametsch, R. (2019). Valorisation of protein hydrolysates from animal by-products: Perspectives on bitter taste and debittering methods: A review. International Journal of Food Science and Technology, 54(4), 978–986
GENÇCELEP, H.; KABAN, G.; AKSU, M. İ.; ÖZ, F.; KAYA, M. Determination of biogenic amines in sucuk. Food Control, v. 19,n. 9, p. 868-872, 2008.
Girio, T.M.S.; Filho, A.N.; Junior, O.D.R.; Amaral, L.A.; Girio, R.J.S. Microbiological Quality of Dog Feed Sold in Sealed Packages and in Bulk. Ars Vet. 2012, 28, 036–040.

Gobert, A.; Tourdot-Marechal, R.; Sparrow, C.; Morge, C.; Alexandre, H. Influence of nitrogen status in wine alcoholic fermentation. Food Microbiol. 2019, 83, 71–85.
Granfeldt, Y., Hagander, B., Björck, I. (1995) Metabolic responses to starch in oat and wheat products. On the importance of food structure, incomplete gelatinization or presence of viscous fibre, *European Journal of Clinical Nutrition* 49: 189-199.
Grijalva-Vallejos, N.; Aranda, A.; Matallana, E. Evaluation of yeasts from Ecuadorian chicha by their performance as starters for alcoholic fermentations in the food industry. Int. J. Food Microbiol. 2020, 317, 108462.
Gullo, M.; Zanicehlli, G.; Verzelloni, E.; Lemmetti, F.; Giudici, P. Feasible acetic acid fermentations of alcoholic and sugary substrates in combined operation mode. Process. Biochem. 2016, 51, 1129–1139.
Hagander, B., Björck, I., Asp, N-G, Efendic, S., Holm, J., Nilsson-Ehle, P., Lundquist, I., Scherstén, B. (1987) Rye products in the diabetic diet- postprandial glucose and hormonal responses in non-insulin-dependent diabetics as compared to starch availability in vitro and rat experiments, *Diabetes Research and Clinical Practice* 3:85.
Holm, J., Koellreutter, B., Wursch, P. (1992) Influence of sterilization, drying and oat bran enrichment of pasta on glucose and insulin responses in healthy subjects and on the rate and extent of in vitro starch digestion, *European Journal of Clinical Nutrition* 46: 629.
Hull, P., 2010. Glucose Syrups: Technology and Applications. Wiley-Blackwell, West Sussex, UK. https://doi. org/10.1002/9781444314748
K˛epi´nska-Pacelik, J.; Biel, W. Microbiological Hazards in Dry Dog Chews and Feeds. Animals 2021, 11, 631.
Kato A.; Tanimoto S.; Muraki Y.; Oda Y.; Inoue Y.; Kobayashi K. J. of Agric. Food Chem. 1994, 42, 227-230.
Kato, A.; Komatsu, K.; Fujimoto, K.; Kobayashi, K. J. Agric. Food Chem. 1985, 33, 931.
Kato, A.; Yutani, K. Protein Eng. 1988, 2, 153.
Kazimierska, K.; Biel, W.; Witkowicz, R.; Karakulska, J.; Stachurska, X. Evaluation of Nutritional Value and Microbiological Safety in Commercial Dog Food. Vet. Res. Commun. 2021, 45, 111–128
Kim, S. H.; Kinsella, J. E. J. Agric Food Chem. 1986, 34, 623-627.

Kim, S. H.; Kinsella, J. E. J. Food Sci. 1987, 52, 128-131.
Kong, C.-L.; Ma, N.; Yin, J.; Zhao, H.-Y.; Tao, Y.-S. Fine tuning of medium chian fatty acids levels increases fruity ester production during alcoholic fermentation. Food Chem. 2021, 346, 128897.
Kong, C.-L.; Zhu, D.-Y.; Zhao, Y.; Zhao, T.-Y.; Tao, Y.-S. Spent yeast polysaccharides in mixed alcoholic fermentation between Pichia kluyveri, Pichia fermentans and Saccharomyces cerevisiae retarded wine fruity ester hydrolysis. J. Food Compos. Anal. 2022, 105, 104200.
Kumar, P.; Sharma, N.; Ranjan, R.; Kumar, S.; Bhat, Z.; Jeong, D.K. Perspective of membrane technology in dairy industry: A review. Asian-Australas. J. Anim. Sci. 2013, 26, 1347.
Ladero, V.; Calles-Enriquez, M.; Fernandez, M.; Alvarez, M.A. Toxicological Effects of Dietary Biogenic Amines. Curr. Nutr. Food Sci. 2010, 6, 145–156.
LÁZARO, C. A.; CONTE-JÚNIOR, C. A.; CANTO, A. C. V. C. S.; MONTEIRO, M. L. G.; COSTA-LIMA, B. R. C.; MÁRSICO, E. T.; MANO, S. B.; FRANCO, R. M. Biochemical changes in alternative poultry meat during refrigerated storage. Revista Brasileira de Ciência Veterinaria, v. 19, n. 3, p. 195-200, 2012b.
Lehninger, A. L.; Nelson, D. L.; Cox, M. M. “Principles of Biochemistry”, 2nd ed., Worth Publishers, New York, 1993.
Leiva, A.; Molina, A.; Redondo-Solano, M.; Artavia, G.; Rojas-Bogantes, L.; Granados-Chinchilla, F. Pet Food Quality Assurance and Safety and Quality Assurance Survey within the Costa Rican Pet Food Industry. Animals 2019, 9, 980.
Li, H.; Jiang, D.; Liu, W.; Yang, Y.; Zhang, Y.; Jin, C.; Sun, S. Comparison of fermentation behaviors and properties of raspberry wines by spontaneous and controlled alcoholic fermentations. Food Res. Int. 2020, 128, 108801.
Li, X.; Zhao, Y.; Lai, X.; Nong, J.; Zhao, G.; Xiao, X. One-pot biocatalytic synthesis and antioxidant activities of highly lipophilic naringin derivatives by using bi-functional whole-cells. Food Res. Int. 2020, 136, 109291. [CrossRef]
Li-Chan, E.C.Y. Bioactive peptides and protein hydrolysates: Research trends and challenges for application as nutraceuticals and functional food ingredients. Curr. Opin. Food Sci. 2015, 1, 28–37.
Lincoln, L., More, S.S., 2017. Bacterial invertases: occurrence, production, biochemical characterization, and significance of transfructosylation. J. Basic

Microbiol. https://doi.org/10.1002/jobm.201700269.
Malin, E. L.; Brown, E. M.; Wickham, E, D.; Farrell, H.M. J. Dairy Sci. 2005, 88(7), 2318-28.
Mangino, M. E. In “Protein Functionality in Food Systems”, Hettiarachchy, N. S.; Ziegler, G. R. Marcel Dekker Inc.,Chicago, Illinois. 1997.
Mauron, J. Influence of processing on protein quality. J. Nutr. Sci. Vitaminol. 1990, 36 (Suppl. 1), S57–S69.
McClements, D. J. “Food Emulsions: Principles, Practice and Techniques”, Ed. CRC Press LLC, 1999.
Mhasker, S. Y.; Lakshminerayana, G. J. Am. Oil Chem. Soc. 1992, 69, 643.
Montegiove, N.; Calzoni, E.; Cesaretti, A.; Alabed, H.; Pellegrino, R.M.; Emiliani, C.; Pellegrino, A.; Leonardi, L. Comprehensive Evaluation of Lipidic Content in Dry Pet Food Raw Materials: Comparison between Fresh Meats and Meat Meals. Sci. Bull. Ser. F Biotechnol. 2020, 24, 122–129.
Montegiove, N.; Calzoni, E.; Cesaretti, A.; Pellegrino, R.M.; Emiliani, C.; Pellegrino, A.; Leonardi, L. Soluble Protein Content Assessment in Dry Pet Food Raw Materials: Comparison between Fresh Meat and Meat Meal Formulations. Sci. Bull. Ser. F
Montegiove, N.; Pellegrino, R.M.; Emiliani, C.; Pellegrino, A.; Leonardi, L. An Alternative Approach to Evaluate the Quality of Protein-Based Raw Materials for Dry Pet Food. Animals 2021, 11, 458.
n. 1-2, p. 435-443, 2008.
Nadeem, H., Rashid, M.H., Siddique, M.H., Azeem, F., Muzammil, S., Javed, M.R., Ali, M.A., Rasul, I., Riaz, M., 2015. Microbial invertases: a review on kinetics, thermodynamics, physiochemical properties. Process Biochem.
Ochando, T.; Mouret, J.-R.; Humbert-Goffard, A.; Aguera, E.; Sablayrolles, J.-M.; Farines, V. Comprehensive study of the dynamic interaction between SO2 and acetaldehyde during alcoholic fermentation. Food Res. Int. 2020, 136, 109607.
PEREIRA, V.; PONTES, M.; CÂMARA, J. S.; MARQUES, J. C. Simultaneous analysis of free amino acids and biogenic amines in honey and wine samples using in loop orthophthalaldeyde derivatization procedure. Journal of Chromatography A, v. 1189,
Phillips, L. G.; Whitehead, D. M.; Kinsella, J. In: “StructureFunction Properties of Food Proteins”, Academic Press, New York, 1994, pp 75-106.

Pommet, M.; Redl, A.; Guilbert, S.; Morel, M. H. J Agric Food Chem. 2005, 53(10), 3943-3949.
Qi, T.; Yang, D.; Chen, X.; Qiu, M.; Fan, Y. Rapid removal of lactose for low-lactose milk by ceramic membranes. Sep. Purif. Technol. 2022, 289, 120601.
Rahaman, T.; Vasiljevic, T.; Ramchandran, L. Effect of processing on conformational changes of food proteins related to allergenicity. Trends Food Sci. Technol. 2016, 49, 24–34.
Ralet, M.C., Saulnier, L., Thibault, J.F. Raw and extruded fibre from peahulls. Part II: Structural study of the water-soluble polysaccharides. *Carbohydrate Polymers* 1993, 20, 25-34.
Ralet, M.C., Thibault, J.F., Delia Valle, G., Influence of extrusion-cooking on the structure and properties of wheat bran. *Journal of Cereal Science* 1990, 11, 249-259.
RICHARD, N.; PIVARNIK, L.; ELLIS, P. C.; LEE, C. Effect of matrix on recovery of biogenic amines in fish. Journal of AOAC International, v. 91, n. 4, p. 768-776, 200
Saha, B. C., & Hayashi, K. (2001). Debittering of protein hydrolyzates. Biotechnology Advances, 19(5), 355–370.
Sánchez, A.; Vázquez, A. Bioactive peptides: A review. Food Qual. Saf. 2017, 1, 29–46.
Schorn-Garcia, D.; Cavaglia, J.; Giussani, B.; Busto, O.; Acena, L.; Mestres, M.; Boque, R. ART-MIR spectroscopy as a process
Schwartz, A. M.; Perry, J. W. “Surface Active Agents: Their Properties and Technologies”, Krieyer, R. E. Ed.; Huntington, New York, 1978, p.35.
Siljeström, M., Westerlund, E., Björck, I., Holm, J., Asp, N-G., Theander, 0. The effects of various thermal processes on dietary fibre and starch content of whole grain wheat and white flour. *Journal of Cereal Science* 1986, 4, 315-323.
SORIANO-SANTOS, J. Dietary products for special populations. In: GUERRERO-LEGARRETA, I.; HUI, Y. H. (Ed.). Handbook of poultry science and technology. Hoboken: John Wiley & Sons, 2010. p. 275-289.
SUZZI, G.; GARDINI, F. Biogenic amines in dry fermented sausages: a review. International Journal of Food Microbiology, v. 88, n. 1, p. 41-54, 2003
Thompson, A. Ingredients: Where Pet Food Starts. Top. Companion Anim. Med. 2008, 23, 127–132.
Townsend, A. A.; Nakai, S. J. Food Sci. 1983, 48, 588. Haskard, C.; Li-Chang, E. C.

Y. J. Agric. Food Chem. 1998, 46, 2671-2677.
Van Boekel, M.; Fogliano, V.; Pellegrini, N.; Stanton, C.; Scholz, G.; Lalljie, S.; Somoza, V.; Knorr, D.; Jasti, P.R.; Eisenbrand, G. A review on the beneficial aspects of food processing. Mol. Nutr. Food Res. 2010, 54, 1215–1247.
Vargas-Trinidad, A.S.; Lerena, M.C.; Alonso-del-Real, J.; Esteve-Zarzoso, B.; Mercado, L.A.; Mas, A.; Querol, A.; Combina, M. Effect of transient thermal shocks on alcoholic fermentation performance. Int. J. Food Microbiol. 2020, 312, 108362.
VINCI, G.; ANTONELLI, M. L. Biogenic amines: quality index of freshness in red and white meat. Food Control, v. 13, n. 8, p. 519-524, 2002.
Weaver, C.M.; Dwyer, J.; Fulgoni, V.L.; King, J.C.; Leveille, G.A.; MacDonald, R.S.; Ordovas, J.; Schnakenberg, D. Processed foods: Contributions to nutrition. Am. J. Clin. Nutr. 2014, 99, 1525–1542.
Xia, J.; Nnanna, I. A. In: “Protein-Based Surfactants”, Xia, J. and Nnanna, I. A. Ed.; Marcel Dekker Inc. New York, 2001. Chapter1.
Yuichi, Y. T. Y. Patent 02, 237, 911; CA, 114:128, 807h (1991).
Zhang, J. H., Tatsumi, E., Ding, C. H., & Li, L. Te. (2006). Angiotensin I-converting enzyme inhibitory peptides in douchi, a Chinese traditional fermented soybean product. Food Chemistry, 98(3), 551–557. https://doi.org/10.1016/j. foodchem.2005.06.024 Zhang, Y., Lu, H., Lin, Y., & Cheng, J. (2011). Water-soluble polypeptides with elongated, charged side chains adopt ultrastable helical conformations. Macromolecules, 44(17), 6641–6644