

Higher educational establishment of Ukoopspilka
“POLTAVA UNIVERSITY OF ECONOMY AND TRADE”
(PUET)

H. Ye. Dubova

**ADVANCES IN
RESEARCH ON FOOD
AROMA RECOVERY**

2nd edition

MONOGRAPH

Edited by A. T. Bezusov

English Translation: V. I. Voskoboinyk,
N. I. Rudenko

**Poltava
PUET
2017**

УДК [664-0.35.66]-047.37

Д79

Recommended for the publication, placement in the electronic library and educational use by the Academic Council of the Higher Educational Establishment of the Ukoopspilka "Poltava University of Economics and Trade", according to 29.06.2017, protocol 6.

Author:

H. Ye. Dubova

Reviewers:

A. K. D'yakonova, Doctor of Technical Sciences, Professor and Chair, Department of Hotel and Restaurant Business, Odessa National Academy of Food Technologies;

I. K. Mazurenko, Doctor of Technical Sciences, Director of the research unit «Research and Design Institute for Standardization and Technology eco-friendly and organic products», National University of Life and Environmental Sciences of Ukraine;

V. H. Yukalo, Doctor of Biological Sciences, Professor, Department of Food Biotechnology and Chemistry, Ternopil Ivan Puluj National Technical University.

Editor:

A. T. Bezusov, Doctor of Technical Sciences, Professor and Chair, Department of Biotechnology, Canned Foods and Beverages, Odessa National Academy of Food Technologies.

English Translation:

N. I. Rudenko (Chapter 1.2–1.7), ***V. I. Voskoboynyk*** (Chapter 1.1, 2, 3, 4).

Dubova H. Ye.

Д79 Advances in Research on Food Aroma Recovery : monograph / H. Ye. Dubova ; edited by A. T. Bezusov ; English Translation : V. I. Voskoboynyk, N. I. Rudenko. – 2nd edition. – PUET, 2017. – 187 p.

ISBN 978-966-184-287-7

The monograph advances scientific discussion of the issues related to the development of food aromatization processes. The research introduces an original scientific concept - creation of fresh flavors in foods is based on reactions of flavor precursors. Fatty acid composition of plants, antioxidant activity, and oxidation reduction potential are described as factors influencing the course of reactions. The study includes practical examples of flavor restoration by using PUFA found in the raw plant material.

УДК [664-0.35.66]-047.37

ISBN 978-966-184-287-7

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LIST OF ABBREVIATIONS, SYMBOLS

PUFA – polyunsaturated fatty acids PUFA

FA – fatty acid

HPO – hydroperoxides

VC – volatiles, volatile component

LOX – lipoxygenase

TAG – triacylglyceride

MW – microwave field

LN – linolenic acid

LNL – linolenic acid

HPL – hydroperoxy of lyase

MVD – Microwave vacuum drying

SFME – Solvent-free microwave extraction

AOA – antioxidant activity/antioxidative

ORP – redox potential

CC – carbonyl compounds

DLVO theory – Deryagina-Landau-Verwey-Overbeek theory

PEP – plant enzyme preparations

INTRODUCTION

The second edition of the book “Advances in Research on Food Aroma Recovery” was supplemented with the study of the effect of flavorings on the functioning of salivary glands, recommendations for flavoring of dietary foods, and the prospects of using the *Push Top* packaging in the technology of application of flavor precursors. The original *Push Top* technology has a world-wide novelty and is presented by healthy drinks in a separate package for autonomous mixing. The specific understanding of food philosophy according to the facts of development of culinary technologies and growth rate of food range is given. As it has been proven by historical stages of production of flavorings, aroma is one of the important organoleptic ingredients for food developers. A review of food production based on the development of nanotechnologies, as well as promising and cautioning publications on nanotechnologies in the food sector is presented. On the basis of the literary analysis, the future impact of nanotechnologies on the evolution of the aromatization process of food products is predicted. It has been determined that the peculiarity of the development mentioned above lies in the use of plant enzymes and/or flavor precursors in a nanoscaled range. The example of enzymatic breakdown of polyunsaturated fatty acids of plant cell membranes as one of the ways of creating fresh flavor of many fruits, namely C₆–C₉ aldehydes and alcohols, is considered. It is noted that “green, fresh” aromatic ingredients are needed to improve the organoleptic profile of foods from heat-treated vegetables, melons and gourds. The following factors of development of food aromatization are defined: differentiation of principles of healthy nutrition into the “fast food” industry, repetition of natural processes of aroma formation, application of wild green leafy vegetables, and evolution of medical nutrition. The information on food aromatization by packing with autonomous mixing and their approximate assortment is given. The innovations in food aromatization are aimed at quality nutrition, time saving, recreation and entertainment, meeting specific needs (vegetarian dishes, restrictive diets).

One of the trend priorities in the flavoring industry is to ensure maximum level of original aroma in food products. This is evidenced by regular updates in the legislation provisions, regulations, and terms relating to flavorings. Relevant studies are oriented on reducing the amount of additive flavorants in food and, along with it, the search for alternative ways of improving the organoleptic profile. Modern scientifically-based production technologies of natural flavorants, the efficient use of raw material potential in restoring the lost flavor, development of new approaches to flavoring process are increasingly important. Flavorant business is profitable, innovative and still being studied. A human's reaction on food flavor is currently being studied, particularly, in the products that contain reduced content of salt, sugar and fat; this issue can be found in cross-cultural studies of food preferences, and neuro-gastronomy. Flavor perception is important for improving the appetite and secretion of gastric juice, human's health and preventing chronic diseases like obesity and diabetes. The trend towards healthy organic food development is being supported in the world where the use of non-organic aromatic substances is not acceptable.

The biosynthesis of the aroma compounds by lipoxygenase has been shown on tomatoes, cucumbers, olives, bell peppers, apples, citrus fruits, and strawberries. This approach is used in the microbial synthesis of C₆–C₉ aldehydes, alcohol-flavourings imparting specific “green, fresh” aroma – Green Leaf Volatiles (GLVs), which is made from vegetable oil wastes on an industrial scale. Systematic studies on food flavouring «in vitro» have not been found in publications. The implementation of this approach by using the original set of vegetable raw material enzymes is the task of the present research. Due to high activity and instability of their components aromatic substances react on even the most minor changes in the quality of raw materials and all the mistakes in the process of technological treatment.

Flavoring processes in most food technologies are necessary and system researches in this field are of sufficient demand. In the production of many food products a wide variety of approved food additives is used. These substances changing the appearance,

structure, chemical and physical properties, influencing the flavor and aroma slow down the microbial and oxidative damage. The most important requirement for many food products is the safety for human health. In a society there is an irrational fear of chemicals – “Chemiphobia” – the fear of “chemistry”. Today, biotechnology allows purposefully enhance the ability of a living organism to produce a particular reaction for getting the product without the use of food additives.

Enhanced demand on natural flavourings and flavoured products determines the relevance of research in this area. Natural flavoring components are obtained by physical methods (distillation, concentration in vacuum, supercritical CO₂ extraction, etc.). But the plant raw material potential is insufficiently used in this respect. Instrumental methods enable to extract only a limited amount of easy volatile fractions, the mass fraction of which in fruits is close to its maximum. Aromatic components present in fresh fruits are in low concentrations (1–10 mg%) and they are practically not trapped by modern instrumental methods, that is a specific problem for flavor identification.

Fresh flavor of many fruits and vegetables is partially lost or greatly changed after heat treatment. As for possible reformulation of fresh flavour in products there is a theory based on the enzymatic processes of their formation. According to this theory aroma reformulation depends on the presence of flavouring precursors and enzymes. The intensive study of the influence on enzymes flavour in genetically modified raw materials, the experience of fresh flavour reformulation in foods by enzymatic reactions has not been developed. Awareness of the reaction process mechanism allows managing the process of food products flavouring without the use of flavourants.

CHAPTER 1

CURRENT STATUS AND PROSPECTS OF SCIENTIFICALLY BASED PERSPECTIVES FOR FLAVOURING PRODUCTION

1.1. Study of factors affecting development of food aromatization

The growth of population and consumption is likely to contribute to the increasing global demand for food, at least during the next forty years [1]. These high growth rates require deeper understanding of the philosophy of food when using innovative technologies. The food production philosophy has been presented in the article by S. Rodgers “Technological innovation supporting different food production philosophies in the food service sectors” [2]. The author offers the general classification of food service sectors, namely: industrial cuisine, fast food and fresh food, and states that the difference between sectorial philosophies is gradually decreasing due to market pressure: elements of industrial cuisine are used for fast food and fresh food. There is a differentiation of the philosophy in each sector – the elements of fresh food are transferred to fast food. One can agree with the author that the use of food production philosophy can bring some benefits such as compensating for intensive processing in industrial cuisine, counteracting the negative image of fast food roasting and improving healthy selection of fresh food. For example, functional dishes with health-improving ingredients can be developed: salad dressing with stanols (for blocking resorption and lowering cholesterol levels), pasta with dietetic fibers, dessert with silver coffee (a drink from non-roasted coffee beans with antioxidants), sauce with lupine seeds (for cancer prevention) and orange juice with probiotic bacteria [3]. Smoothie King (USA, <http://smoothieking.com>) has already suggested numerous nutritional supplements for energy, muscle mass, women’s balance, caffeine charge, recovery of joints and tissues, increased immunity, as well as dietary, anti-stress, and multivitamin ones. Nowadays flavor has become a major organoleptic component for food developers; it gives attractiveness of various foodstuffs, restores food specificity lost during processing and allows developing new products of special taste. The monograph is to study factors affecting the development of food aromatization in terms of the use of

nanotechnology in the food industry, repetition of natural processes of flavor development in the nanoscale range, application of wild green leafy vegetables in cooking as the conditions decreasing differentiation of principles of healthy eating and fast food.

Development of food aromatization processes. The issue of the practical introduction of new food products is the matter of current interest; it is indirectly connected with aromatization processes. The relevant historical periods of developing aromatization processes have been presented by R. G. Berger [4]. The author has defined the following periods: old-fashioned period characterized by old-fashioned natural flavors based on natural extracts, essential oils, reaction flavors and few synthetic components (1950–1965); classical period of instrumental analysis and synthesis represented by natural isolates, synthetic and single natural aroma components (1965–1990); new age period of technology which became decisive for success of flavors in the marketplace and introduction of fashion food and food on-the-go (1990–1999); new century period when flavor is required to be added to food to produce taste and to make it healthy for consumers (2000-till now). Based on the analysis of the evolutionary and innovative development of the methods for obtaining aromatic components, we expect that the next period of evolution in food aromatization processes will be connected with the use of precursors (Fig. 1).

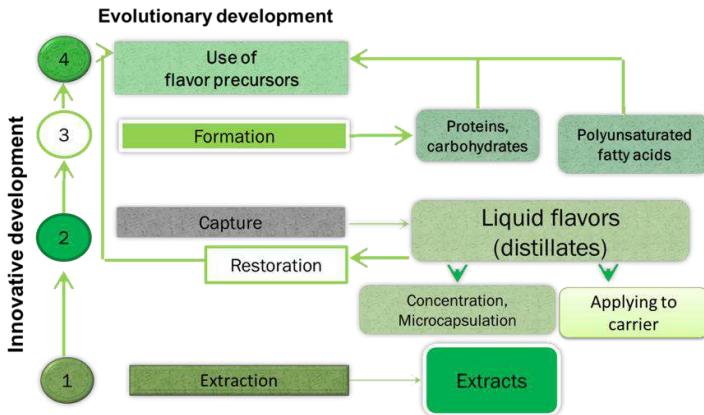


Figure 1 – Development of food aromatization methods

A precursor (from Latin *praecursor* – predecessor) is a substance that participates in the reaction resulting in the formation of the target substance. Under different conditions, flavor precursors in the plant material can be represented by amino acids, carbohydrates, and lipids [5]. Precursors of flavorings are different from flavorings and can be added to foods to create and / or modify flavors in accordance with Regulation (EC) N 1334/2008 of the European Parliament and of the Council on Flavorings and Certain Food Ingredients with Flavoring Properties. The detailed review of flavorings according to the specified Regulation is given in the article “Use of food flavorings in accordance with the EU regulations” by S. B. Verbitsky and T. V. Sheiko (Institute of Food Resources of National Academy of Sciences of Ukraine).

A considerable amount of research has been done to define the precursors of aromatic substances in meat, cheese, fish, beer, champagne, bananas, apples, coffee, peanuts, and other products. The nature of flavor precursors has not still been defined in some fruits [6]. Over the last few years, it has been shown that many flavors exist in the form of flavor precursors that are more stable than active flavors. For example, many flavors (especially in plant food) exist as glycoside precursors that are much more stable than the created flavor and have no taste properties. We are of the opinion that the most promising way of developing aromatization with the help of precursors is related to nanotechnology.

Application of nanotechnology in the food industry. Food products are not made by nanomachines as it is often meant while speaking about nanofood [7]. In the article “Safety of nanofood: A review”, nanofood is defined as food where nanoparticles or nanotechnology techniques or tools such as microarrays of DNA, microelectromechanical systems, microfluidics are used during cultivation, production, processing, or packaging of food (Ijabadeniyi O. A., 2012). It should be noted that there are several definitions of the term “nanofood”, but we think they do not significantly differ in matter.

Science and technology related to nanoparticles are the new frontiers of this century, so food nanotechnology is a new area. The

application of nanotechnologies in the food sector is relatively new compared to their use in medicine, in particular when transmitting drugs and pharmaceuticals to the body [8]. Despite the fact that successful application of nanotechnology in food is still somewhat limited, some main concepts based on the nanoscale have been unambiguously established [9]. In the food industry, nanotechnology is associated with two main applications: alteration of food products to nanosizes and providing of food matrix ingredients with nanostructures. Nowadays, food quality and safety assessment in the area of food production can be improved through nanotechnology. Food industry can be greatly enhanced in terms of smart nutrient intake by the body, protein bioseparation, rapid sampling of biological and chemical contaminants, nanocapture of nutraceuticals, solubilization, flavor and color in food systems. This is an incomplete list of new nanotechnology topics for food production and agriculture [10]. However, food nanotechnology as a new area requires consideration of both potentially adverse effects and many positive ones. The authors of the paper “Application of Nanotechnology in the Food Industry: Innovative Approaches, Opportunities and Uncertainties for the Global Market”, have described some developments in nanotechnology and their application in nutritional and nutraceutical systems; they have also identified a number of unsolved issues [11]. The review considers some of the nanoscale structures standing along in the food industry and various food production techniques that can be beneficial due to nanotechnology, as well as during the use of nanotechnologies in development and storage of food. Nanotechnology in the area of functional nutrition finds potential application of biological molecules thanks to the properties that are substantially different from those ones they have in nature [3]. The scientists discuss the examples of malignant and healing effects of nanoobjects on the human body confirming that the final effect doesn't depend on the nanotechnology itself, but on the ability to use it for own benefit [12].

The specific character of food nanotechnology is defined by both the tasks of food raw material processing and the characteristics of the raw material itself. The article “Energy analysis of food

nanotechnology” states that food nanotechnology can be developed in three directions. The first one is manipulation with nanoscale elements for composition of artificial foods (milk, meat, etc.). These technologies are based on the bottom-up mechanism. The first direction can be attributed to modification of individual complexes and providing them with new properties. The second direction involves managing transfer processes at the level of nanoscale objects of food raw materials, improvement of traditional manufacturing processes, food and their application through the full use of quantum properties and surface phenomena on the nanoscale. The third direction is a combination of the mentioned two ones so as to create unique patterns [13].

The increased funding possibilities and considerable interest in this area leads to more frequent and mainly liberal use of the term “nano” arousing some criticism in the scientific community. An ethical debate in the area of nanoscience, nanotechnology and its methods reflects NEST-ethics (ethics of new and emerging science and technology) [14]. Whether justified or not, one should understand that the entire area of nanosciences is essentially an eclectic derivative from the established disciplines such as chemistry, physics, micro-technology, etc. However, the use of the term “nano” allows researchers to emphasize the fact that processes (for example, nanomanufacturing) or material structures (e.g., nanomaterials) are designed and optimized in order to use specific properties and behaviors in the range of 10^{-7} to 10^{-9} m [3].

The functionality of many raw materials and successful food processing are determined by the presence, modification and generation of self-organizing nanostructured forms [15]. The examples include cellulose fibril accumulation in the walls of plant cells, crystalline starch structures, and processed starchy products that influence gelatinization and cause food benefits during digestion. Fiber structures are responsible for melting, gluing and texture of gels. Two-dimensional (2D) nanostructures formed at the interfacial water-water and air-water segregation and controlling stability of food foam and emulsions also illustrate the statement mentioned above [16]. Specifically, creation of foam or emulsions (sauces,

creams, yoghurts, oils, margarine) involves formation of bubbles of gas or drops of fat or oil in a liquid medium. This process requires formation of interfacial air-water or oil-water segregation; the molecules involved in this process determine its stability. Similar structures have one-molecule thickness being the examples of two-dimensional nanostructures. When there are mixtures of proteins and other molecules close to the nanoscale range in size, such as surfactants (soap-like molecules or lipids), the instability source potentially blocked by nanotechnology occurs in most food products at the interface [2]. New raw materials with new molecular structures and properties identified by nanotechnology require the permission for use in food. The discussion of ELSE issues (context of ethical, legal, social and environmental) of nanoscience and nanotechnology has already started in some developed countries [17].

The most discussions on nanotechnology and food have been caused by accidental or deliberate introduction of prepared nanoparticles into food products. These debates mainly concern risks and benefits, given the lack of knowledge about potential bioaccumulation and toxicity of nanoparticles. The report “Nanofood” published by Helmut Kaiser Consultancy (2004) evaluates the development of nanotechnology and patent applications related to food [18]. The number of nanofoods is growing rapidly. According to P. Ravichandran, the examples are as follows [11]:

- nanoparticles of carotenoids that can be scattered in water, which allows them to be added to fruit drinks, which provide improved bioavailability;
- nanosized micellar systems containing canola oil, which are claimed to provide a number of materials such as vitamins, minerals or phytochemicals;
- a spectrum of nano-products containing nanoclusters (as a means of formation, for example, a chocolate drink);
- mineral-based additives based on nanoparticles, such as Chinese nanoscale manufacturers, claim to improve selenium uptake by an order;
- patented nanodrop supply systems for encapsulating materials.

We believe that this list is not complete; it will be extended on a continuous basis in the long term.

The potential benefits of the food products made through nanotechnology are impressive. In the world where thousands of people starve every day, the necessity to increase manufacturing is the main reason to support nanotechnology worldwide [19]. Over the last few years, the food industry has invested millions of dollars in research and development in the area of nanotechnology. Some of the world's largest food producers, including Nestl'e, Altria, H. J. Heinz and Unilever, pioneer the way for nanofood, while hundreds of small companies follow them. But in spite of the potential benefits, nanofood is not very popular compared to other nanotechnology areas. Continuous disputes on security and regulations in the field of nanotechnology have slowed introduction of nanofood; but the research and development are being continued. It is interesting that most large companies work behind the scenes (when you look for the term "nano" or "nanotechnology" on the websites of Kraft, Nestl'e, Heinz and Altria companies, you will have no results) [11]. Although the risks associated with nanotechnology in other areas, such as cosmetology and medicine, are the same, the public is much less likely to jump on the nanotechnology platform when it comes to food [20].

A great number of scientific works has been devoted to the research on nanotechnology in food manufacturing. In particular, the authors of the article "Acceptance of nanotechnology foods: a conjoint study examining consumers' willingness to buy" point out that consumers attribute negative benefits to nanotechnology food, even when the food products have had a discernible advantage for them. The results show that consumers are interested in food with additional healing effects only when this effect is caused by natural additives. Public perception of nanotechnology will be crucial for technological advances in the field of food science. The change in the description of nanotechnologies in food manufacturing can lead to more positive consumer ratings [21].

Summarizing, one can agree that nanofood is indeed a global phenomenon [3]. In Australia, for example, nanocapsules are used to

add omega-3 fatty acids to one of the country's most popular white bread brands. According to the manufacturer, nano-caps of tuna oils added to TipTopBread provide valuable nutrients, while encapsulation prevents bread from being tasting. NutraLease, a start-up company at the Jewish University in Jerusalem, has developed new carriers for nutraceuticals in food systems [11].

In literary sources, the negative link between cheap highly processed food and obesity, poor health has been repeatedly demonstrated [22, 23]. Of course, there is no substitute for healthy natural food; but if nutritional nanotechnology could make the processed food less harmful to health, it would be a huge success for nanotechnology [20]. Designing and producing food products through formation of molecules and atoms is a promising future for the food industry globally.

Flavor formation in the nanoscale range. A review of scientific publications has shown that manipulations with aromatic components are not a direct object of nanotechnology. For example, filters that can sift out or let pass certain molecules according to their shape, not size, have been developed; this makes the aroma control process possible [24]. Nanotechnology can provide a unique advantage to food products processed in various ways. The programmed food considered to be the ultimate consumer's dream will have built-in design food, so the consumer will be able to create the food product of the desired color, flavor and texture with the help of specially programmed microwave ovens. The idea is to make this food at the production plant while providing it with millions of nanoparticles of different colors, flavors and nutrients; the consumer will be able to program an oven to activate only selective particles of the food according to his/her preferences, whereas others remain inert, thus achieving the desired food profile [11]. The leader of nanotechnology and development of the nanoscale industry is Kraft Foods Nanotek Consortium. The consortium focuses on "interactive" foods and drinks. These food products will fit into the individual consumer needs and tastes. The foods being developed include beverages that change colors and flavors, as well as foods that can recognize and adapt to consumer allergies or nutrition needs [24].

While investigating the issue of the reactions causing formation of aromatic components in plants, it is necessary to establish whether aromatic substances or components producing them are in over the range of nanosized units. The subject of research in food nanotechnologies are microorganisms, nanoporous and nanocapillaries of plant material, cell membranes, protein, polysaccharides, and water molecules [13]. Nutritional proteins are often globular structures 1–10 nm in size. Most polysaccharides and lipids are linear polymers less than nanometers in thickness. Synthesis of aromatic components of plant material is carried out on the basis of enzymes (size 10 ... 100 nm), polysaccharides (size 1 ... 10 nm), etc. Therefore, synthesis of fruit flavor *in vivo* is a sequence of certain reactions occurring in the nanometer range. The precursors of aromatic substances (proteins, carbohydrates, lipids) are not just a set of nanoscale objects: atoms and molecules are organized into hierarchical structures and dynamic systems, which are the result of experiments of nature for millions of years. Ions with a diameter of tens of nanometers, such as potassium and sodium, regulate the behavior of important biomolecules, including sugars, amino acids, hormones and DNA; they reside in the nanometer range. Most of the molecules of proteins and polysaccharides are of the nanoscale size. Each living plant exists due to the presence, absence, concentration, location and interaction of these nanostructures [3].

Among all nanofoods, enzymes are the most widely used ones in the manufacturing of food and beverages, since an enzyme is a nanosized protein molecule that acts as a catalyst for a chemical reaction. Nanotechnology also has the potential to improve the nutritional processes that use enzymes to gain health benefits while eating. For example, enzymes are often added to food to ensure hydrolysis of anti-nutritional components and increase bioavailability of essential nutrients, such as minerals and vitamins. To make these enzymes highly active and cost-effective, nanomaterials are used for enzyme support systems today [25].

Precursors of taste and flavor do not necessarily turn into aromatic substances *in situ*, even despite the presence of specific flavorase bienzyme (*S.G. Deng, C.H. Zhang, 1998*), since these

reagents can be physically separated from each other by cellular microstructures. The significance of cell integrity destruction for the biochemical changes in vegetables and fruit cannot be overestimated [26]; this destruction affects formation of taste and aroma, off-flavors and smells. We have investigated the impact of destruction of cell integrity to nanoparticles during vacuum heating (temperature 32 ± 2 °C, dilution 6 ± 1 kPa) on membrane-bound enzymes in suspended plant homogenates resulting in the increased duration of the production of aromatic components [27].

At first sight, it seems that in the case of nanoparticles, which are enzymes and precursors in this study, the same forces as in the classical problems of colloidal chemistry operate in the biological system. Indeed, it has been noted that Van der Waals forces, electrostatics, the influence of the solvent, and the hydrophobic effect act here. However, scientists point out that it is necessary to significantly adjust the preconditions with consideration of the nanoscopic scale of the ongoing processes and the presence of biological objects in the system. The peculiarity of nanosystems is that they contain a relatively small number of atoms; the nature of the interaction between particles will essentially depend on their mutual orientation and dielectric permeability [12]. We have shown that the value of the hydrodynamic diameter of particles and zeta potential, the effect of disjoining pressure are also of great importance for the interaction of aroma-forming precursors and enzymes in the nanoscale range [27].

The estimated values of usefulness of the information about flavor have shown that flavor naturalness is the most important consumer factor for this characteristic [11]. One of the ways of adding natural flavor to food is repetition of natural processes of its formation. Enzymatic degradation of polyunsaturated fatty acids is one of the methods to create fresh flavor of many fruits. The precursors of various aromatic compounds can be lipids, polyunsaturated fatty acids (PUFA). The reactions of α -, β -oxidation and enzymes of the lipoxygenase type are the main achievements in the study of the mechanisms of aroma formation in fruit *in vivo* [4]. Biosynthesis of aroma compounds by lipoxygenase takes place in

tomatoes, cucumbers, olives, bell peppers, apples, citrus, and strawberries. This particular way is used for microbial synthesis of C₆-C₉ aldehydes, alcohols – flavorings of the specific green and fresh aroma Green Leaf Volatiles (GLVs) made from oil waste on industrial scale. Scientific literature does not reveal systematic studies on food aromatization by lipoxygenase *in vitro*. The implementation of food aromatization with the help of a complex of enzymes of plant raw materials has been shown in our works [28, 29].

The use of green leafy vegetables in cooking. The following factor in the development of food aromatization processes is the potential benefits of the use of green leafy vegetables for health and dietary nutrition. This area has been recognized as an important sphere in the research of the use of green leafy vegetables, generally non-cultivated (wildlife). The use of wild plants in a daily diet based on local cuisine is potentially of considerable interest to research scientists in the field of nutrition because these plants are important as local products and have the potential to be the sources of new nutraceuticals [30]. Nutrition is a manifestation of belonging to the region; at the same time, it is often viewed as an opportunity for local enterprises to get profit working with small producers, sellers and, above all, restaurants offering specialized products. In addition to health benefits, these food products are also important elements for determining local or regional identity [31]. We consider the use of green leafy vegetables in cooking to be the factor contributing to the introduction of the principles of healthy nutrition into the fast food industry. The following advantages of application of the innovative aromatization approach for fast food have been defined:

- consumers flavor food independently by using plant concentrates with the increased flavor in/on food. It enriches the organoleptic profile of dietary dishes. This is an alternative way of industrial aromatization when the determined quantity of flavorings is added to the raw material;
- for products with a modified recipe (without fat, salt, sugar, or with their reduced content);
- consumption of clean label food (0 % fat, 0 % salt, sugar 0 %, 0 % colors, 0 % flavorings).

For example, in the fast food preparation technology we have proposed to use potato juice (for people with hyperacidity, ulcer, and other diseases) with flavor of green leafy vegetables, mashed melons and gourds with renewed fresh flavor, mashed cabbage (homogenized) with cucumber flavor.

The implementation of the innovative approach in the manufacture of flavored foods was able due to packaging with autonomous mixing; it is the original Push-Top technology, which has worldwide novelty and is represented by healthy drinks in separate packaging (the inventor of the development is S. V. Savinsky, patent “Packing and sealing machine for containers of autonomous mixing”). Autonomous mixing is based on separate packaging of beverage components in a two-chamber container. Before consuming food, you need to press the top of the cap and destroy the membrane between a container chambers; whereupon the plant enzyme extract and the fruit mixture with aroma nanoprecursors are autonomously blended and converted into the ready-to-use flavored food. But when this happens the container remains hermetically sealed.

Innovations in aromatization continue to evolve focusing on quality nutrition (natural flavorings), time saving (fast food preparation of flavored food), recreation and entertainment, meeting specific needs (vegetarian dishes, restrictive diet).

The following factors affecting development of food aromatization have been investigated:

- decreased differentiation of principles of healthy nutrition and fast food;
- development of nanotechnologies in the food industry;
- repetition of natural processes of aroma formation;
- use of green leafy vegetables.

The recommendations to enrich the organoleptic profile of food are especially needed for people who for some (long) time consume dietary foods or have a uniform menu due to life circumstances, including the military, the elderly, consumers with chronic diseases or after surgery, tourists, and lovers of food without nutritional supplements (among other things, religious restrictions).

1.2. The role of the aromatic substances in human nutrition and the development of neurogastronomy

Flavor perception is the thinnest of human sensations surpassing even the taste. During the centuries of evolution odours have played an important role in the human and animal's life when searching for food or warning of danger. In case of damaging the olfaction system a human organism suffers from destroying the nature of nutrition, hormone production, sexual development, and even memory [32, 33]. For a long time the mankind has used herbal aromas as means of pleasure and therefore health promoting. Awareness of the sense of smell importance in perceiving the flavour and forming cognitive and emotional responses to foods and beverages can help to improve health and prevent chronic diseases such as obesity and diabetes (Fig. 2).

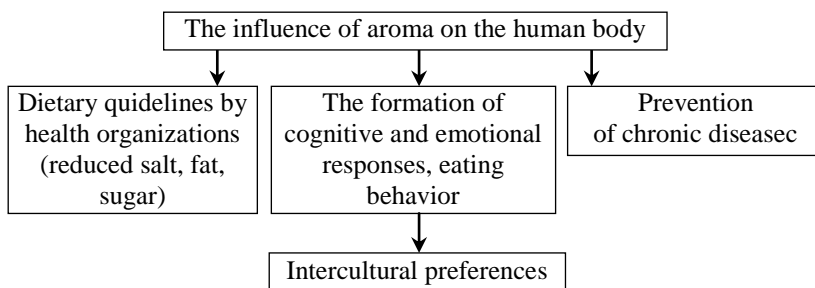


Figure 2 – Interaction between aroma factors and human behaviour

In food consumption pleasant sensation of flavour can cause abundant secretion of digestive enzymes and thus provide quick and good absorption of food nutrients. In 1924 Schested could prove through fistula introduced into the dogs' intestine that aroma producing substances stimulate chemically the secretion in the intestinal tract [34, 35]. Varieties of bread from the flour of large outputs or rye flour with its strong aroma cause higher secretion of saliva and gastric juice compared to wheat bread made of white flour [36]. Simultaneously amplified salivary amylase activity [37–39]. Bread prepared by baking at a shortened duration (low aromatic) leads to the deceleration of starch cleavage in the digestive tract [34].

The high content of aroma substances in frying foods promotes the secretion of the digestive enzymes. If we recognize that the contents of aroma substances affect the appetite anxiety (which affects the digestibility) and perception, it can lead to far-reaching conclusions.

The sense of smell can simultaneously change the taste perception in case of diabetic powdery confectionery and sugar free canned fruit consumption; it gives the impression of less expressed flavour, though the main aroma is kept in its usual amount. The same impression is produced by unsalted and too sour bread.

At a time when there were only intuitive presentations about the infections, certain smells were considered to be harmful carrying the infection and causing illness. The first attempts to use the scents of plants were taken in Ancient Egypt. At that very time the ancient Egyptians found the ways to maintain flavours, dissolving them in fat. The people of Middle Asia, as well as the Romans adopted the experience of the ancient Egyptians. The Hindus, Chinese, Japanese were developing their own ways of using aromatic plants for treatment, rituals and other needs. The alchemists' invention of a distillation cube gave a new impetus to the production of aromatics. It is believed that Avicenna was the first who produced rose water - the distillate of the rose. The Arabs introduced the musk into perfume that became an excellent fixer for essential oils. Around the second decade of XX century, in the period of discoveries of organic synthesis, the synthetic analogues of vegetable aromatic substances started to be widely used. Our sense of smell well distinguishes between unpleasant and pleasant doors. Pleasant smells improve health and mood; aromatic substances have antibacterial, anthelmintic, repellent and other properties. Later it was found that lavender oil inhibits the growth of tubercle bacilli, and thyme oil, cinnamon, lavender, cedar and angelica are active against glanders and yellow fever [40]. The servers of different religions are widely used the aroma treatment. The ancient Greeks and Romans knew how to excite the appetite or stimulate relaxation; appropriately used the art of psychotropic aroma effect could evoke psychological disposition to tears or laughter [41].

Essential oils have a wide range of biological activity and low toxicity. They are available for mass usage due to an established industrial production and well studied chemically. Inherent aromatic properties create additional opportunities of varied effect on the human body. Flavours fulfil a signalling function, affecting a person's memory, causing positive or negative emotions. The sense of smell so necessary for the surviving of a primitive man is manifested in contemporary children at their very birth or shortly thereafter [42].

Remembering smells is purely individual: the same smell can cause various associations in different people related to the conditions under which it was remembered (the smell of spruce needles evokes the memories of a New-Year tree and it also communicates with the smell of funeral wreaths).

Association between the flavors and their formation on the environmental background and other factors is a new trend in food industry, called "neurogastronomy". It has turned out the taste of food can be really affected, without changing its composition. Charles Spence Laboratory at Oxford University has been studying how different factors affect all the senses simultaneously, but not our taste, smell or sight separately. "Neurogastronomy" is based on the fact that everything we eat or drink, pass through all the senses that means it is multisensory [43].

At the symposium devoted to multisensory taste perception Eric van der Linden proposed to use the term "gastrophysics". Both neurogastronomy and gastrophysics are focused on the approaches of solving the problems of overeating and replacing the food "quantity" on food "quality". The solution for odors compatibility will significantly contribute to the hedonistic psychology and affective neuroscience [44]. The combination of food ingredients affects people, so the attempts to discuss science-based theory of odors compatibility have been made [45, 46]. Dr. Weurman started creating food volatile compounds (VCF) database, to be used by many scientists in the field of taste research. The theory was based on the idea of good combination of products with the same aromatic components, such as jasmine and pork liver containing indole or white chocolate and

caviar containing trimethylamine. Over time, this theory has not received the recognition and further development, although it was supported by some scientists (<http://www.foodpairing.com>), who studied the Western cuisine. The Western cuisine, in contrast to the Asian one, tends to use ingredients containing similar aromatics, that supports the hypothesis of food pairing.

Flavour perception and defining its character by different people can vary greatly. For example, the smell of methyl salicylate in the US and Canada is estimated to be very pleasant, and in England and Switzerland as smelly, and unpleasant. Flower fragrances are assessed differently not only in different countries, but also by the representatives of one nation. Thus, a sharp divergence in the assessment of the same smell by people of different genders, ages, and health status has been defined. This and other factors suggest a large proportion of subjectivity in assigning a particular smell by a particular group.

Anthropologists and scientists are looking for cross-cultural similarities and differences in odour preference and provide the basic patterns in the behaviour of consumers in the world market. Despite a large number of scientific approaches, neither approach has provided a reasonable explanation of human preferences of odours. The research data suggest that (a) there are flavor preferences based on the previous experience, and (b) individual olfactory experience is unique. It means that everyone experiences the smell differently.

Flavour (specific flavour notes) can define the product success or its failure. This could explain why the material sold in Japan has less intense flavour than the one sold in Germany. Olfactory experience is partly influenced by the evolutionary heritage. The human sense of smell is based on the interaction between physiological, chemical, cognitive and socio-cultural factors within the individual [47].

Studies have shown that a mixture of smells in pumpkin pie or lavender enhances sexual arousal by 40 %, while the famous musk enhances the potency by only 7 %. Businesses have long used aroma to increase sales. Smell acts as advertising in sense of a powerful motivational stimulus [48, 49].

1.3. Human sense of smell: physiology, theory of perception and behavioural aspects

The human sense of smell is a complex system existed to capture thousands of chemicals. According to statistics, about half of the adult population cannot perceive the odour of some products or groups of components and almost every second person is an anosmic to a certain scent. Anosmia is usually explained by a defect or lack of a molecular receptor that is responsible for detecting odours. Flavour ratings reflect the smell of personal emotional reaction to a situation in which the smell was perceived for the first time. Events, objects or other factors may be associated with the odour. Pleasant smells are processed more slowly on the basis of momentary affective reactions, emotions and the evaluations are generated involuntarily. Hedonic responses to scent are formed quickly, if the smell is unpleasant [47].

Despite the achievements of analytic techniques, the possibilities of the advanced gas chromatographs are far from the ability of human senses that are able to determine and record the trace amounts of many substances, combining them into a complex of sensations. An untrained person can by using olfactory sense to distinguish 2000 different fragrances, a qualified taster – about 10 thousand [50]. The nose of one person perceives differently the same smell: it is more pleasing to the right nostril.

Perception of odour occurs in the upper part of the nasal cavity in the olfactory epithelium (*regio olfactoria*). This area of nasal mucosa has a surface area from 5 to 10 cm² and contains from 3 to 50 million receptors responsible for the sense of smell. Volatiles reach the receptors either through the nose or through the oral and retro-nasal cavity where they become volatile in the process of chewing, saliva soaking and food heating. Aromatic substances reach human olfactory neurons that form neuro-epithelium, by the line of projections (shells) in the side walls of the nasal cavity. Shells are a series of folds of the lateral extensions of bone.

The first step in smell perception is odorant binding with special protein. Specialized cells of the olfactory epithelium in the nasal

cavity enabled to detect trace amounts of volatile odorants are responsible for almost unlimited variations of odour intensity and quality perception.

Overall process of odour perception at the molecular level consists of three consecutive stages eventually determining a taster's perception. These stages include reception, transduction and neural processing (encoding the information into electrical impulses). For odorants the primary act of perception includes selective binding (it is believed to be subject to a conceptual structure "key-lock" model) of molecules of aromatic substances with a specific receptor protein in the membrane of the corresponding receptor cell. When binding molecules of highly aromatic substances with the receptor protein chemical energy is converted into electricity by means of a specific cascade of biochemical reactions [32, 51, 52].

Neuroscientists believe that in this way the aromas directly affect the human subconscious. Nerve impulse from the olfactory bulb can penetrate almost any part of the brain but which one the neuroscientists cannot yet predict. One and the same scent evokes the activity of different parts of the brain in different people. The following conclusion can be made: people react in the same way only on the most primitive, life meaningful smells. For example, hydrogen sulphide released during the decomposition of protein tissue sickens everyone that effect helped the ancestors to survive. The perception of more complex odours is closely linked with the subconscious. The reactions enshrined in the brain as a result of experience are dominated in this case. It is important to note that not only one's own experience, but the ancestors experience is remembered [53].

A human's ability to odor remembering is much less developed than memorization of colours and sounds. Visual impressions more quickly penetrate into our consciousness (for example, rapid response to traffic). This may explain the fact that coming across with the difficultly determinable smells we try to use "external factors". One reason for this phenomenon lies in the fact that the organs of hearing and vision, in contrast to the organs of smell, are continuously trained, being used in everyday life [54].

There are several systems and processes involved in smell assessment. Unpleasant and pleasant odours are processed separately. Scientists distinguish smell as a memory, caused by other stimuli on the basis of the emotional activity. The emotional factor of the human sense of smell is well-presented in the psychological publications [50, 53, 54]. Familiar smells usually score higher than pleasant, but unfamiliar.

Emotional reactions and odour assessment are direct, while the assessment control occurs at a deeper level of cognition and understanding. Flavour preferences, after the first recognition, remain throughout the life. Scientific data suggest that people begin to discover the olfactory stimuli in their mothers' womb, and they may be the base for the formation of early olfactory preferences. The mother's food odour preferences affect the odour preferences of their infants.

The inclusion of cultural aspects in research of odour preferences has become constructive. As biological and socio-cultural beings we are moving through the world and create our own odours preferences, which can be changed, at least, if they are not finally formed. After only 15 minutes of moderately positive emotional experience, an unpleasant odour is subsequently measured as much more acceptable, and this improvement is maintained throughout the week. In particular, the smells, which are not strongly attached to a particular source, can be verbally manipulated with the words used for description [55]. In addition, the concept of natural and synthetic fragrances is a powerful mediator in smell preference.

Natural and synthetic flavours have a lot in common, and often the consumer is not able to distinguish between them, but the synthetic fragrances are assessed as more attractive than natural. A mixture of natural and synthetic components of flavour was evaluated by the flavouring tasters as positive, believing that these were 100% natural ingredients. This suggests that the consumers will be satisfied with the product, containing an equivalent amount of the artificial and natural ingredients. The labels' information on fragrances also affects what samples will be sniffed first i.e., emotions are the means of motivation in relation to natural flavours.

The psychological and behavioural aspects of olfactory function in children are being studied. This expands the knowledge of daily use of olfactory signals and highlights the variability of smell behaviour from the very childhood. The sense of smell has a significant impact in the regulation of social relations, food fun, and emotional self-regulation. In addition, the importance of daily mood odours was higher in girls than in boys. The results showed that girls were much more focused in the sense of smell than boys, especially in relation to human odours, themselves and the environment.

In general, the sense of smell is significant for children of 6–10 years old everywhere. Children's ability to describe the odour and percept the world is the same among the children of 6 and 10 years. In connection with the facts when the children, getting used more to "identical to natural" flavours, do not perceive the smell of fresh fruits and berries as more pleasant and give preference to the processed food. The study of behavioural importance of odours in children contributes to the expansion of our understanding of these problems [56].

With age the human ability to recognize odours deteriorates. In this connection it has been ascertained dietary calcium deficiency, copper, magnesium, pantothenic acid, potassium, selenium and zinc. When the flavour was improved and expanded in food, it was found that the older people began to eat more food. Increase in flavour of food for the elderly people in the retirement home has resulted in improved immune function, which was not associated with the changes in food nutrients or its biochemical status. Flavoured food intake for 3 weeks has resulted in an increase in the arm strength and increase of the bond strength in both hands. These data are consistent with the conclusion that the food is not the only factor in the functioning of the immune system in the elderly and may be associated with stimulation of the taste of the limbic system, the effects of endogenous endorphins [57].

Endorphins are multifunctional peptides that regulate the emotional and physiological well-being. Short-term studies of young people have shown that the pleasure responses to food flavours are mediated by endogenous opioid activity [58, 59]. The activities of

endorphins are associated with eating and the immune system functioning [60, 61]. Improving the immune status associated with the improvement in food flavour involves the opioid activity and related increase of immunity.

There are about 17 thousand substances containing odour. The nature of smell physiology has not been studied yet. There are many hypotheses (stereochemistry, quantum, enzymes, absorption, dipole, etc.), trying to explain physical and chemical interaction of odoriferous substances and receptors. Thus, the stereo-chemical theory is based on the assumption that it is the shape and size of the molecule substance having odour, cause the sensations of smell, and the shape and size of the odour receptors is such that the chemical substances with odour act as “keys” to “locks”.

Stereo-chemical theory has not given an unambiguous answer to the question concerning the activation mechanism of the human’s olfactory receptors. According to the quantum (vibration) theory the receptors react not on the shape and size of the molecules but on their vibration properties and on the mutual motion of the atoms of molecules. The theory is called “magnetic map” and is based on the fact that the aromatic components due to their different masses have different vibration energy of carbon-hydrogen bonds. However, one of this theory tenets saying that the olfactory receptors should have neither agonists nor antagonists has been recently refuted by the Japanese scientists.

In 2012 the Israeli physiologists discovered the phenomenon in the olfactory signals categorization mechanisms which showed that with the increase in number of mixture components its fragrance is becoming less apparent. The perception of smell and taste is influenced by the “white noise” [62]. From receptors the signals, through the olfactory nerve, enter the olfactory bulb containing numerous glomeruli. Each glomerulus receives signals from only one type of receptor, thereby different smells lead to excitation of various glomeruli set.

Olfactory bulb breaks all the variety of incoming signals on larger categories, which are sent to the cortex olfactory center. According to the nature of fragrances to evoke the perception they are shared

by: the threshold of revealing, the threshold of stimulus (detection threshold, stimulus threshold); the threshold of recognition (recognition threshold); the threshold of differentiation (differential threshold); the verge of limitation, the threshold of saturation (boundary threshold, saturation threshold); supr-sub- threshold stimuli.

Overall process of odour perception at the molecular level consists of three consecutive stages eventually the perception of the taster. These stages include reception, transduction and neural processing (encoding the information into electrical impulses). However, the similarity in molecule structures of aromatic substances does not always mean the similarity of their odours. In addition, the smell is strongly influenced by isomers (e.g., cis- and trans-isomers may be very different in smell) – isovanillin unlike vanillin does not smell [63, 64].

In recent years, the researches of aromas are gradually moving from the stage of determining volatile components and their ability to release to another stage associated with perception and variability. Based on aroma stimuli required to form perception it is possible to overcome some problems with food of good quality but reduced calorie or low fat content.

Sufficient level of aroma compounds in products increases the digestive enzymes secretion, endorphins content, and immunity strength. However, the perception of flavour, its identification, appraisal, and emotional reactions are individual and finally unpredicted.

1.4. Localization of aromatic substances in plants, and synthesis reaction of aromatic components

Despite of the plant VC diversity, the majority of them belong to three main groups: terpenes, phenylpropanoids / benzenoids and fatty acid derivatives. Volatile organic molecules of different classes are secondary plant metabolites that are not vitally essential, but serve important functions in the life of plants: protecting, signalling, enabling, inhibiting, etc. Most aromatics are the result of

decomposing reactions and internal reorganization of the plant cell walls in the process of maturation [65]. This process of reorganization allows the related enzymes to attack different substrates usually not accessible to enzymes and results in the formation of many low molecular weight products (volatile aroma compounds). The processes of flavour development do not take place at the early stage of fruit growing but rapidly develop during the climacteric respiratory rise when the exchange of substances in foetus changes to catabolism. A slight amount of lipids, carbohydrates, proteins and amino acids is enzymatically converted into simple sugars or acids and volatile compounds. The rate of flavour development in foetus with climacteric ripening reaches its maximum in the post-climacteric phase [66].

Carbohydrates, fat acids and amino acids are natural precursors for the aroma compounds [67]. The enzymes affecting the formation and regulation of volatile aromatic compounds have been described [65, 68] for apples, strawberries, tomatoes, bananas, melons. Despite the intensive efforts of experts in chemistry, biochemistry, molecular biology, most of the ways leading to the biosynthesis of aromatic volatile substances have not been determined yet.

Nowadays it is considered that metabolism of branched fatty acids and amino acids can serve as a precursor for the biosynthesis of volatile aroma components in most fruits (Radiel, 2002). Fatty acids play an important role in the synthesis of esters consisting of 2-, 4- and 6-carbon chains arising mainly by β -oxidation of fatty acids [69]. De novo synthesized free fatty acids contribute to the formation of esters in many fruits (Bangert and Song, 2003). It is assumed that lipoxygenase (LOX) contributes to the breakdown of long chain fatty acids up to C_6 -aldehydes, which are then converted into alcohols by the aldehyde hydrogenase [26, 69].

A characteristic feature of the secondary metabolites of plants is the ability to accumulate in rather high concentrations and, sometimes, in the organs they were not synthesized. Since many secondary metabolites possess high biological activity, accumulation of the substances occurs in special, usually extracellular structures to avoid toxic effect on the plant itself [70]. In addition, many odorous

substances are accumulated inside the plants in the glycosidic forms (carbohydrates phytoessence complex) sesquiterpene lactones or carotenoids that can subsequently be hydrolyzed [69]. For example, 2-phenylethyl- β -D-glycoside is stored inside the rose petals being the main source of the volatile compounds as 2-phenylethanol. Fragrances may exist in the cytoplasm or the cell sap, accumulate in the idioblast or concentrate in the special structures called essential oils receptacles. These structures are divided into exogenous located in outside tissues and spatially connected with the epidermis and endogenous [65].

The former include glandular stains, hairs and scales, to the second – glandular cells and glandular secretion containers. The essential oil is distributed unevenly on the plant body. Most often, it is concentrated in one organ (leaves, flowers, roots, fruits). These components of odoriferous substances are synthesized in the plant in a relatively small number of metabolic pathways that are normally duplicated (I – mevalonate pathway of isoprene synthesis, II – metileritritol phosphate, the “alternative”, pathway of isoprene synthesis, III – shikimate pathway of the compounds synthesis with an aromatic ring, IV – synthesis of fatty acid derivatives). Biosynthesis of the terpene series flavours has been studied as an enzymatic process involving monoterpene, sesquiterpene, and diterpene synthases of the plant origin [71].

Saturated and unsaturated fatty acids, in their turn, serve as precursors for many plant volatiles. The derivatives of fatty acids are usually significantly modified (oxidize, methylate, esterify, etc.), producing many odours, including watermelon, cucumber, pumpkin, mown grass. “Green odour” similar to green leaves appears from the volatile aldehydes and alcohols of C₆–C₉ compounds that later is synthesized from linolenic and linoleic acids through corresponding hydroperoxides [72, 73]. These reactions occur as a protective mechanism of the plant in response to physical damage or pathogens attack by producing various kinds of oxylipins [74].

Recently collected data on the growth promoting, fungicidal, repellent, anti-tumour and other characteristics speak in favour of oxylipin positive properties [75, 76]. Some of these oxylipins are

used in industry in fungicides, lubricants, and thickeners, and in this case present as precursors of green notes of aromatic compounds such as leaf aldehyde (2E) -hexenal, leaf alcohol (3Z) -hexenol [77]. Enzymes that catalyze the biosynthesis of lipoxygenase and hydroperoxide lyase usually form fresh green, fruity aroma and flavour of fruits and vegetables, which in turn is widely used as flavouring of foods and beverages, especially in processed food. The market of these compounds is estimated at about US \$ 20–40 million per year [78].

Synthesis of green leaf flavour (Green Leaf Volatiles, GLVs) usually includes all the aldehydes and alcohols produced by hydroperoxide lyase (HPL). Thus, GLVs are derived from the LOX leaf metabolism. First, the lipids are hydrolysed of free fatty acids by various types of lipase. Subsequently, oxygen catalyzes the stereo specific oxidation of unsaturated free fatty acids. (9Z, 11E, 15Z) -13-hydroperoxy-9,11,15-octadecatrienoic acids (13-HPOT) are prepared from linoleic acid and further HPL is metabolized to form 12-oxo- (Z) -9-dodecenoic acid (traumatol precursor) and (Z) -3-hexenal.

Another type of LOX can also synthesize (10E, 12E, 15Z) -9-hydroperoxy-10, 12, 15-octadecatrienoic acids (9-HPOT) from the linolenic acid. These products can also be converted by HPL into C₉-oxo- acids and C₉ aldehydes [79]. Many of these volatile compounds are produced in trace amounts that are below the threshold of most analytical instruments but they can be detected by the human sense of smell.

1.5. Influence of food matrix components on flavour volatility and tangibility

Human perception of the volatile compounds is determined by two main factors: the concentration of volatile compounds in fruits and the threshold of human scent perception. The thresholds of flavour volatile compounds help to link their physical and chemical properties with human perception (perception threshold, the upper absolute threshold of perception, sensory threshold, threshold of

absolute perception, differential threshold of perception, lower absolute threshold of perception, relative threshold, operational threshold) [80]. For flavour identification and evaluation an important factor is the characteristic of food environment: substances comprising the flavour of food products manifest themselves in varying degrees on the background of nucleotides, sugars, acids and salts. Trans-non-2-enal is one of the key components of cucumber flavour in the concentration of 0.9 ppb, although at a concentration of 0.5 ppb in beer, this component is perceived as a strong flavour of the old cardboard [68].

The role of lipids in the flavour matrix interactions in complex liquid model systems has been studied. Extraction of nine aromatics (methylbutanal, hexanal, heptanal, octanal, hexanol, benzaldehyde, nonanal, pinene, and octene-3-one) have been investigated in a liquid solution of fat, collagen, chitin, cellulose gum. It has been shown that fat is an energy-dependable component affecting the flavour retention and binding. An important role in the release of CC from the food matrix is played by gelatin. Moreover, aldehydes are desorbed from the finished product to a greater extent than ketones, by 80 % and 30 % respectively. It is believed that ketones may enter into specific interaction with gelatin [81].

In “water- flavouring and aromatic compound” model systems the proteins adding cause the decrease in concentration of flavouring compounds in the free gas space above the product due to their binding with proteins. The mechanism of binding depends on the moisture content in the protein sample, but typically, these interactions are not covalent. The degree of binding in flavouring compounds by the hydrated proteins depends on the number of available hydrophobic parts on the surface of the protein molecule [81]. In liquid products and products with high moisture content the mechanism of binding the flavouring compounds with protein is determined by the interaction of non-polar parts (ligands) of the latter with hydrophobic pores or parts of the protein surface. In addition to hydrophobic interactions the flavouring compounds with polar groups in the "head" (hydroxyl and carboxyl) may interact with proteins both through hydrogen bonding and electrostatic interaction.

After binding to the hydrophobic parts of protein surface the aldehydes and ketones can diffuse into the hydrophobic internal part of a protein molecule. “Protein flavouring compound” interaction is typically fully reversible, but aldehydes are covalently linked to the amino group of lysine side chain, and this interaction is not reversible. Adding flavour and aroma to protein products can be fulfilled exclusively by non-covalently associated fractions [26].

Flavouring ligands comprising of reactive groups (e.g., aldehyde) can covalently bind with the amino groups of lysine residues, modify the overall protein charge and cause thereby unwinding of its molecule, which in its turn can provide the appearance of new hydrophobic parts for binding the ligands. Denatured proteins generally provide more binding sites characterized by small constants of association [26]. As volatile flavouring compounds interact with hydrated proteins primarily through hydrophobic interactions, any factors which affect the interaction or the degree of hydrophobicity of a protein molecular surface alter binding of flavouring compounds. Methods for measuring the binding of proteins to flavouring compounds have been described in the corresponding studies [82, 83].

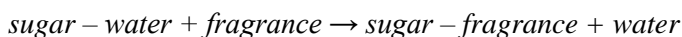
The temperature slightly affects the process of binding until there is a significant unwinding of the protein molecule. This is due to the fact that the process of association is primarily determined by the entropy and not by enthalpy. Enhanced ability to bind flavouring compounds was revealed by the thermally denatured proteins, but their binding constant is usually less active than that of the native proteins. Salt binding effect on flavouring compounds is due to the result of in- salting and out- salting. In-salting salts destabilizing hydrophobic interactions reduce binding of flavouring compounds, while out- salting salts increase their binding.

Typically, binding under alkaline condition is stronger than under acid, since in the alkaline medium the proteins are more denatured. Disruption of the disulfide bridges in the alkaline pH range, resulting in protein molecule unwinding leads to binding amplification. Proteolysis leads to a decrease in the amount of hydrophobic parts of the protein molecule and, respectively, to a less binding of flavoring and aromatic compounds.

Aromatic descriptors for binding in different environments vary quantitatively and qualitatively. Contribution of the fifteen volatiles (acetone, geranylacetone, hexanal, trans-2-hexenal, cis-3-hexenal, cis-3-hexenol, β -ionone, hexanol, 3-methylbutanal and 3-methylbutanol, 6-methyl-5-heptene -2-one, 2-phenylethanol, trans-2-pentenal, 1-penten-3-one, 2-isobutylthiazole, ethanol metanol and cis-3-hexenal) in the aroma of fresh tomatoes was studied in deionized water, water-alcohol mixture and deodorized tomato homogenate [84].

Among the odor thresholds of primary tomato aroma VC the cis-3-hexenal exhibited the highest odor level in all three media. The odor thresholds for all compounds were lower in deionized water than in water-alcohol mixture, and even less than in deodorized tomato homogenate for most compounds. Hexanal, cis-3-hexenal, trans-2-hexenal produce, depending on the environment, the range of aromas from green, grass, apple, vine up to celery, and citrus in deionized water, water-alcohol mixture, deodorized homogenized tomato mass.

The properties to fix the smell have got large carbohydrate molecules, forming a membrane around these substances; they prevent the absorption of moisture and the loss of it due to evaporation and chemical oxidation. Bond ability of the disaccharides aromatics is expressed to a greater extent than of monosaccharides. Good flavour retainers are the cyclodextrins. The process of aroma preservation via carbohydrate is in changing of interactions [85]:



Carbohydrates do not only possess good binding properties, retain the flavourings during processing, but also participate in the formulation of new odour components during heat treatment (non-enzymatic browning reaction). The kinetics of flavourings release has been studied in two complex matrices (carbohydrates: D-glucose, pectin and starch lipids and triolein) with different composition and the same rheological properties. The effect of release from a matrix with carbohydrates was more complicated: ethylhexanoate and trans-2-hexenal were saved and diacetyl, 2-pentanone and cis-3-hexenol were “reflected” from the matrix with a reduction in the initial rate,

which is attributed to the variation of diffusion and / or retention of carbohydrates. Comparison of the carbohydrates release rate from a matrix and a complex lipid matrix shows the role of lipids, a comparison of the release rates from water and the complex matrix – the combined effects of texture and lipids. Reducing the initial release rate is more important in the presence of lipids than in the presence of carbohydrates [86].

Volatile compounds produced by the reaction of either lysine, glycine or with aldehydes formed by lipids oxidation (hexanal, (E)-2-hexenal, or (2E,4E)-decadienal) have been analyzed in both glucose presence and its absence. The main reaction products identified in these models were a mixture of CC as a result of an amino acid catalysed reactions of aldol condensation [87]. Several 2-alkylfurans have been found, although some reaction products were the result of amino acids condensation with lipids – the aldehyde derivatives. In this case the amino acid plays an important role as a catalyst of degradation and further reaction of these CC. These results show that degradation of amino acids and further reaction of lipid products peroxidation may be important in thermally processed foods.

By choosing the reaction mixtures and process conditions simulating the course of reactions in conventional food technology, the production of flavour concentrate is taking place. Selected ingredients usually including reducing sugar, amino acid, and sulphur compounds are subjected to processing at higher temperatures with forming a specific flavour profile [88]. A common ingredient is thiamine which nitrogen and sulphur atoms are already available as part of the ring. Some of the most pleasant flavours, resulting from the process reactions, are caused by maltol, isomaltol, cyclotene, and furanones. These compounds provide caramel flavours and have been identified in many processed foods. Cyclotene, for example, is widely used in the synthesis of “maple syrup” flavour and maltol as a sweet flavour enhancer for food and beverages. Furaneol (4-Hydroxy-2, 5-dimethyl-3 (2H) -furanone) is sometimes called because it was firstly isolated from a recycled pineapple flavour in which it plays a specifically important role. Furaneol is also

biosynthesized, bringing a note of ripe strawberry, typical for freshly picked berries. 3-Hydroxy-4, 5-dimethyl-2, 5-dihydrofuran-2-one is sometimes called “sugary furanone” due to its specific flavour easily detected over sucrose in a gaseous medium.

Furthermore, furanones are found in the cooked meat, where they enhance the meat flavour and odour. The structure of these compounds (either in the form of the enol located in one plane or in the form of a cyclic ketone) is usually due to the sugars precursors, and this structural component is apparently responsible for the caramel flavour. For maltol, a planar shape of enolone is more preferable than a shape of cyclic ketone, as the shape of enolone allows a strong intermolecular hydrogen bonding [89].

In plant food products dimethyl sulphide is biosynthesized out of the molecules especially out of 8-methylmethylioninsulphone salts which heat resistance is insufficient and during cooking dimethylsulphide is easily released. Dimethylsulphide gives a characteristic smell to a freshly brewed and canned sweet corn as well as to tomato juice, tomato and other heat-treated products.

The most reactive compounds in the mixtures for the process flavourings are hydrogen sulphide and ammonia which are often included in the model systems for stimulating definite reaction mechanisms. Thermal cleaving of cysteine leads to the formation of ammonium hydrogen sulphide and acetaldehyde. In the subsequent reaction of acetaldehyde with mercapt derivative of acetoin (by the Maillard reaction) is formed thiazoline which is involved in the formulation of a boiled beef smell. Alkylpirazines are the most important sources of flavour for all kinds of fried, roasted or the like heat-treated food. The most direct way of their formation is the interaction of α -decarbonise compounds (the intermediates of the Maillard's reaction) with amino acids by the Shtrecker's cleavage. The transfer of amino groups in dicarbonyl allows the amino acid nitrogen integrate into small compounds involved in the condensation reactions. Methionine is the amino acid taken for the Shtrecker's cleavage reaction as it contains a sulphur atom enabling to form methional – the compound producing a specific aroma of boiled potatoes and cheese crackers. Furthermore, methional easily

decomposes to form methylmercaptan which oxidizes to dimethyl-disulphide that is a source of reactive low molecular weight sulphur compounds involved in the overall system of flavour development.

1.6. Methods of the lost flavors recovery

It is considered that the aroma recovery means reformulation of aromatic components lost in the process of juice concentration [90–93]. The aroma components are being captured during water evaporation from juice or other foods and collected in the distillate. Flavorings as concentrated in the final distillate product are added to restore the original flavor and aroma, since the treatment process of food and beverages results in a change of flavor [93].

Distillates are the solutions obtained by dividing liquid flavoring components on boiling points or separating liquid from difficultly evaporating substances, i.e. by distillation [94]. To distil most amounts of the aromatics from the pome fruit juices it is necessary to evaporate for about 10 % of moisture under atmospheric pressure, and under vacuum from 15 % to 85 % [95]. Concentrated distillates (FTNF, WONF) are in demand in food industry due to their long term storage and small volume.

Concentration of aroma components during diffuse evaporation through the membrane, when not all aroma compounds are concentrated, but only the key ones obtained from natural sources has got intensive development in evaporation processes [96, 97]. The use of the concentrated flavours has its disadvantage due to the lack of the original natural flavour in the process of reformulation. This problem is also related to the difficulty of preserving the relative concentrations of various aromatic compounds, and their proportions. According to the publications, none of the existing methods of concentration is able to accurately restore the original taste and flavour of the product. These methods have been improving and, nevertheless, there remains a need to further control of the aroma reformulation process [98].

The flavour recovery process of low-calorie fermented beverages is of interest [99]. Ethanol in these beverages is removed by the

dialysis. Then, alcohol is separated from the dialysis liquid by vacuum distillation and the remaining liquid with aromas is returned to the main product. Flavour in the finished product, such as non-alcoholic wine, remains in the initial concentration and is as impressive as before the distillation. In most cases, the addition of only a key component does not solve the problem of aroma reformulation, and the use of distillates for this purpose requires further development. In practice solving the problem of flavour reformulation had some drawbacks: it was reduced to identification of the detected flavoring substances, i.e. defining their qualitative components. It is absolutely unclear whether the found substances in their extremely low concentration can participate in the formulation of food flavour or they are just physiologically imperceptible accompanying substances. Many substances are simple in structure flavourings found in almost all the foods. These, for example, include simple alcohols (from C_2 to C_6), acetaldehyde, hexanal, acetone, ethyl acetate and acetic acid. All foods subjected to expensive researches with using modern methods of detecting flavouring substances have demonstrated their simultaneous presence of the entire series of saturated fatty acids from C_2 до C_{18} . It is doubtful that all the numerous components still identified as flavours, actually create flavour only in some food products. Flavours are divided into the following components: **core** (defining the basic organoleptic properties and characteristic aroma), **additional** (enhancing specificity of simulated flavour, although the organoleptic properties may not necessarily be close to it) and **tint** (giving certain shades to flavour) **substances**. For many flavours the key substances have not been identified. The mainstream of flavour is often defined by more than one substance [80].

One and the same substance in different flavours can be a key substance as well as an additional or a tint one. For example, on the basis of 16 common components the flavour recipes with a changed concentration of the key component have been presented for an apple, banana, pear and pineapple [100]. Based on the data of food flavour composition analysis there can be distinguished some homogeneous (by chemical structure) groups of substances that are

significant for specific aroma formulation. These groups include: aliphatic aldehydes – for citrus flavour; amines – for seafood flavour; diones (maltol, cyclotome, furaneol) – butter flavour, and caramel; lactones – for apricot flavour, coconut, peach, cream and others. Aromatic substances are grouped, as a rule, not by chemical classes but by their characteristics. “Wheels of aroma” classification allows getting an idea of the most important aromatic tones and related chemical compounds. The ingredients needed to objectivise the optimum properties of the aromatic constituents, as a rule, cannot be used as food flavourings in their pure form. The correct description of the flavour profile is actually a recipe for its reconstruction. The perception of flavour can be conditionally divided into a number of “pure” tones, and then reformulated through recombination of these tones in the correct proportion [101].

Another way to restore flavour could be the use of flavours or flavour precursors (a term introduced in the FL / 06/47 Project). Since 2011 in the EU the Regulation № 1334/2008 has become obligatory, the term “identical to natural” and “synthetic” in labelling is no longer indicated [102]. Technical Regulation in Ukraine from 11.02.2011 requires using the term “natural” but only to natural flavours. The current legislation does not determine the maximum level of using flavourings and only indicate an approximate level of their concentration. Due to the frequent use of flavourings the new methods of calculation for reducing their amount are being worked over [103]. To further promotion of the scientific approach to the evaluation of chemical effects on food, the European Commission in 2008 funded a FACET (Flavours, Additives and Food Contact Materials Exposure Task) focusing on food additives in foods and flavourings [104]. Evaluating the safety of new flavours, the EU Expert Committee on Food Additives considers it necessary to study additives metabolic and biological transformation into substances with certain toxicological properties.

Also the level of using flavours is set for various foods, in particular, for the natural ones, comparing the flavour chemical structure with certain compounds and their biochemical and toxicological properties. A new concept of assessing the safety of food flavourings

has been suggested for defining the “thresholds” of toxicity, carcinogenicity and effects on metabolism [105]. When using certain aromatic compounds in high doses a carcinogenic effect was detected in animal experiments. Methyl eugenol, estragole, pentane-4-dion were excluded from the register due to their genotoxic properties. The original computer program for calculation allowed getting the threshold of carcinogenicity for the 15 flavours used in the US food industry.

Flavouring precursors (Natural sources flavourings) differ from the flavouring and can be added to foods for the purpose of flavour creation and/or its modification in accordance with the “The European Parliament and Council Regulation on Flavourings”. Under different conditions in plant materials flavour precursors may be in the form of amino acids, carbohydrates, and lipids [106]. Extensive work has been done on defining of flavouring precursors in meat, cheese, fish, beer, champagne, banana, apple, coffee, peanuts and other products. In some fruits the nature of flavour precursors has not been still defined [107].

Over the past few years it has been shown that many flavours exist as flavour precursors, and are more stable than the active aroma. For example, many flavours (particularly in plant foods) exist as glycoside precursors, which are much more stable than the formulated fragrance and have no taste properties. When food is crushed, during cooking or chewing, glycosidase enzymes act on glycosides as generating molecules of flavour. Investigation of the role of non-volatile flavour precursors in fresh, dried, boiled, baked and fried garlic has showed the presence of not one, as is commonly believed, but a number of groups forming specific aroma [108]. Volatile compounds were divided into four groups: produced by thermal degradation of non-volatile precursors of garlic flavour; generated by thermal interactions of sugars and volatile precursors of garlic flavour; derived from thermal interactions of lipids and volatile flavour precursors; obtained by thermal interactions of sugars, lipids, and non-volatile flavour precursors.

The aroma of dried vegetables found in products can be restored by adding to the processed products the liquid extract of fresh raw

materials [109]. In their groundbreaking research dated back to 1957, a group of researchers from the Armed Services Technical Information Agency examined the nature of a specific substance found in the raw materials, which they described as a precursor of flavor. They found that thioglycoside from the cabbage family of vegetables can be used as precursors. The additional findings, as the result of the full synthesis of this group of precursors, demonstrated that synthetic thioglycosides can be as effective under the influence of specific enzymes, as those found naturally in cabbage. The most important observation made in their consecutive studies was that the main potential of flavor precursors is their ability to be converted into a fresh flavor under the influence of their own enzymes. In order to recover the fresh flavor they added isolated enzymes during the food preparation [109]. In 1962 they studied the possibility of restoring the flavor of dried cabbage using the same method [110].

To continue the line of research, Schwimmer demonstrated that blanched, dried or canned beans, peas, broccoli, carrots, tomatoes, and cabbage change their flavor under the influence of enzymes isolated from fresh raw vegetables, genetically related to the main product or from mustard [111]. Thus, he formulated the theory of possible aroma recovery based on enzymatic processes. According to this theory, aroma recovery is dependent on the presence of the precursors and on the availability of enzymes that then specifically form natural aromas from these precursors [112].

One of the major achievements in understanding the mechanisms of flavor formation is this knowledge of the reactions of flavor precursors. Extensive work has been done since to identify flavor precursors of meat, cheese, fish, beer, champagne, bananas, apples, coffee and other products.

Fresh fragrance formulation can be achieved by rapid formation of aromatic fractions obtained from the precursors and enzymes just before eating. Taylor et al. defined flavour-emitting compositions containing micro-emulsions and/or hydrated reverse micelles [113]. These compositions are suitable not only for use as flavouring agents in food products, but also for the enzymatic synthesis of various flavours and flavour precursors in vitro. A typical composition has

80 % of oil, 15 % of surfactant (phosphatidylcholine, phosphatidylethanolamine, monoglyceride, a sorbitan complex ester), 3 % of ethanol <3 % of water, 1 % of precursor and 0.5 % of enzyme. Fragrance precursor and enzyme are located in the micro emulsion core which upon hydration or water activity increase and deformed water micro droplets participates in the enzyme and precursor reaction with obtaining the desired flavour.

Micro emulsions can be used to maintain flavour precursors stability until consumption, at the same time ensuring rapid formation of aromatic fragments from the precursors in the mouth or shortly before a meal. The WO 99/62357 Patent describes flavouring compositions containing micro emulsion droplets of water-in-oil emulsion and/or reversible hydrated micelles containing latent flavours. Such compositions may be used as flavouring systems for food products wherein the active aroma is generated by the action of enzymes.

In preferred embodiments the preferred molar ratio of water to surfactant is less than the value of 10, preferably less than 5. In this connection these systems should be referred to reversible hydrated micelles rather than to micro emulsions. In the only example it is described the preparation of micro emulsion containing 3.0 % of water [114].

The aroma as well as sweet things is the most important indicator of all the melons' quality. The most powerful aromas of melon are the compound esters formed from the amino acids [115]. In a fresh melon there have been identified 18 amino acids with dominating aspartic acid, glutamic acid, arginine, and alanine. O. Lamikarna et al. (2000) have shown that the total content of amino acids in a freshly cut melon rapidly decreases at 20 °C [116].

According to O. Lamikarna et al. the composition of freshly sliced by 3 mm pieces melon after 2 days of storage at 20 °C changes and the total content of amino acids is reduced by about 40 %. These studies have found that for the first 2 days the most significant loss was in aspartic acid, glutamic acid, asparagines, glutamine and serine. Many of the other amino acids including arginine, histidine, proline, and phenylalanine in melon were also reduced at 20 °C, but to a

lesser degree. The loss of soluble solids under these conditions is 17 %, and the sugar content is practically unchanged. Reformulation of the melon aroma lost components may be studied in connection with the destruction of amino acids.

For the purpose of refusing from chemical components, such as sodium glutamate, in the USA a new method was developed to enhance flavour in foods by exposing food products to the low-frequency acoustic wave's transducer. Acoustically processed foods can be converted from low quality and less expensive foods into the ones of best performance at the tasting. The product can be immersed in the liquid or may be located in the range of about ¼ inch to about 20 feet. The food product or raw material is exposed to the waves at a frequency in the range from about 1 Hz to about 1000 Hz, optimally of 600 Hz for about one minute to 24 hours, optimally for about 30 minutes [117].

Interest in physical processes for producing aromatic compounds solely from plant material and sometimes animal tissues in their natural state or in the processed form, attempts to reproduce their synthetic analogy have led to the decline of interest to the direction described above. Further development of biotechnology, genetically modified products including the ones with predetermined aromatic properties has shown that the enzymes involved in the formation of aromas and their reformulation is an important and promising area of research.

1.7. Flavouring enzymes and substrates

1.7.1. Characteristics of lipid flavour precursors

Rancid or repellent odour is a typical description of the products of lipid oxidation reactions. The formation of such odours is the result of spontaneous decomposition of hydroperoxides or enzymatic reactions with them, the bearers of rancidity being volatile aldehydes and ketones. However, in the event of rancidity, typical substances, at the same time, are considered to be an important component of flavour in some foods: hexanal for wheat bread, apple juice, peanuts, hexenal for chopped strawberries, bananas, nonadienal for cucumbers decadienal for crisps.

The products of lipid peroxidation are involved in the formation of the volatile aroma composition in roast poultry, potatoes, dry breakfast cereal, and many kinds of cheese. 41 out of 193 components in the aroma of roasted chicken is derived from lipid oxidation of the aldehydes, dominated by hexanal and 2,4-decadienal [118]. One of the most important flavours in roasted products, poultry, and deep-frying is 2,4-decadienal having a lower aroma threshold (0.00007 mg/kg) compared with hexanal (0.0045 mg/kg). Oxidation of 2,4-decadienal leads to the formation of trans-4,5-epoxy-2-decenal that is one of the strongest white bread aromas [34]. A small amount of lipid oxidation products may be necessary for the flavour profile in some foods. The aldehydes formed by the oxidative cleavage of the fatty acid can react to nucleophilic components of food; they react to sulphhydryl groups, and protein amines, altering the functional properties of the latter and, consequently, the flavour of the final product [65].

The “lipid oxidation” term refers to a complex of lipids successive chemical changes due to their interaction with oxygen. The type of lipid degradation products depends on the fatty acid composition in the food product; lipid oxidation in different ways affects its organoleptic properties: oxidation of vegetable oils mainly composed of ω -6 fatty acids will give a “grassy” or “beanie” side smell and oxidation of macromolecular ω -3 fatty acids – a “fishy” smell [26]. Interaction of lipids and oxidants has free radical character and consists of three stages adequately described for many lipid-containing products (vegetable oils and fish products) [119, 120].

Applying this knowledge to the actual food products is often limited, as lipids are distributed as discrete phases, dispersed within the structure of heterogeneous food matrix. D. MacKlements and E. A. Decker analyzed lipid emulsions such as “oil- in-water” and showed the influence of the concentration, physical state of the lipid emulsion droplets, their orientation to the interface, size distribution, specifics of boundary interaction, and other factors [121].

When removing water from the food system lipid oxidation rate usually decreases due to the decrease of the reactants mobility. In

some foods moisture removal results in accelerating of lipid oxidation which is caused by decrease in the protective layer of the solvated water around lipid hydroperoxides. Depending on the rate of lipid oxidation in products the concentration of CC and flavour associated with them, may vary substantially.

The aroma in vegetables, herbs and leaves is associated with lipid transformations of PUFAs – linoleic and linolenic acids [65]. The narrow range of lipids in plants results in a relatively small number of primary products of the oxidation reaction. The variety of fruit flavours formulation is the result of PUFAs hydroperoxide cleavage by the appropriate enzymes and subsequent transformations. The experiments in model solutions with linoleic, linolenic acid and the enzyme complex show the possibility of a large number of volatile components reformulation [122].

More than 99 % of fatty acids in plant and animal materials are esterified with glycerol. Free fatty acids in living tissues are not very common because of their cytotoxicity, i.e. the ability to destroy the structure of cell membranes. Glycerol esterified with fatty acids lose surface activity and, hence, cytotoxicity [123]. Known for anti-microbial effect the lipid classes can be arranged in series by increasing their activity: triglycerides → phospholipids → fatty acid salts → fatty acids → monoglycerides → products of PUFA oxidation [124].

Sometimes small amounts of diglycerides and monoglycerides occur in a lipid composition. There are 2 groups of compound esters formed by 2 molecules of fatty acids and 2 hydroxyls of glycerol in diglyceride, and 1 esterified group in monoglycerides. The isomers of mono- and diglycerides differ for the location of free hydroxyl (α - and β -glycerides). Each molecule of natural triglycerides is formed by different fatty acids. Triglycerides are composed of a mixture of isomers differing in the place of fatty acid radicals' location and their quantitative ratio. Therefore, from 5–8 different fatty acids it is possible to build ten times more triglycerides.

The composition and structure of fatty acids constituting triglycerides determine their physical and chemical properties. The most important qualities of vegetable fats distinguishing them from

animal fats are unsaturated fatty acids constituting them. The origin of unsaturated phenomenon and its biological significance have been the subject of many years research [125]. The oxidation of unsaturated lipids can produce an enormous complex mixture of volatile substances that in very small amounts can have a significant impact on the organoleptic properties of food.

E. Frankel's monograph deals with the data on the odour's volatility and compounds flavour derived from the lipids [126]. According to the author, the study of sources of volatile lipid oxidation products are still controversial, they are often difficult to interpret. In subsequent studies E. Frankel and colleagues analyzed in detail the sources and the chemistry of volatile lipid oxidation products [127–129] to provide a basis for a better understanding of the taste changes mechanisms which affect these VC. Some separate lipid compounds present in trace amounts can have such a strong smell that their influence on the overall aroma and flavour of food is of sufficient importance. For example, in the study of hydroperoxide produced by oxidation of methyl-9,15 octadecadienoate it was discovered a very strong cucumber-melon scent spread throughout the area of the laboratory premises.

Aroma resembling a green melon previously was ascribed to a mixture of *cis* and *trans*-6-nonenal, that was presumably obtained by the decomposition of 10- hydro peroxide in the oxidized 9,15-octadecadienoate. Although the hydroperoxide isomer is found in relatively small amounts (11 %), its decomposition results in the formation of aldehyde with predominant influence among the volatile substances obtained from other hydroperoxides detected in the oxidized 9,15-octadecadienoate. *Trans*-6-nonenal actually has one of the smallest threshold values of 0.0003 ppm in oil. Other examples of potent aromatic compounds include *cis*-4-heptenal of cod muscle lipid with a flavoured threshold of 0.0005-0.0016 ppm in oil, and 1, 5-*cis*-octadiene-3-one in milk fat with a taste threshold of 0,00002 ppm. There have not been proposed any satisfactory mechanism for appearing of these volatile compounds.

The attempts to link the molecular structure with the flavour intensity have not led to simple generalizations [130]. Except

nonanal, 2-alkenals have a higher threshold than the corresponding alkanals of the same chain length. For the 2-alkenals and trans-, cis-alkadienals, homologous series of an odd number of carbon atoms have lower thresholds than an even carbon number. A reverse tendency is observed in the series of trans, trans-alkadienal. Alkenals with an isolated double bond have more intense flavour than the corresponding 2-alkenals. The effect of the double bond configuration in isolated alkenals is inconsistent. On the one hand, cis-3-hexenal (0, 11 ppm) and cis-4-heptenal (0.0005-0.0016 ppm) are much more intense than the corresponding trans-3-hexenal (1, 2 ppm) and trans-4-heptenal (0.1–0.32 ppm). On the other hand, cis-6-nonenal has a higher threshold value (0,002 ppm) than the corresponding trans-6-nonenal (0, 0003 ppm) [131].

Volatile aldehydes derived from the oxidized linolenates and fatty acids with ω -3 double bond have a low threshold value. Unsaturated aldehydes with ω -3 double bond have particularly low thresholds, such as 3,6-nonadienal (0,0015 ppm), 2,6-nonadienal (0,002 ppm), 2-pentenal (0,046 ppm), 2,4-heptadienal (0,055 ppm), 3-hexenal (0,09 ppm), and 2,4,7-decatrinal (0,15 ppm). Hydrogenated soybean oil also produces particularly low volatility thresholds because they are derived from the oxidation isolinoleate, producing aldehydes with remote double bonds, such as cis-6-nonenal (0,002 ppm), cis-7-nonenal (0,0003 ppm), 2,6-nonadienal (0,002 ppm), and 2,7 decadienal (0,02 ppm). Recently, much attention has been paid to the correlation between the analysis of volatile components by gas chromatography and flavour evaluations [65].

Unsaturated aldehydes and ketones produced as the decomposition products from the primary hydroperoxides are the obvious sources of additional volatile products due to their susceptibility to further oxidation. Any products of secondary oxidation with one or more hydroperoxide can promote further oxidation of the volatile products formed from the lipids. Monohydroperoxides may also form dimers and polymers and react with unsaturated lipid substrates [132].

The double bonds and isomerisation (conversion of cis-, trans- and trans, trans- conjugated hydroperoxides) in the products of

oxidation is another important factor influencing the change in the flavour of foods containing lipids. The configuration of the obtained unsaturated aldehyde affects the flavour properties. Although the double bond in the $\alpha\beta$ -unsaturated aldehydes is always in the stable trans- configuration, the non-conjugated aldehydes are usually in the natural cis- configuration. This cis- double bond is easily isomerised to thermal oxidation in a trans- and in conjunction with a carbonyl group. This positional and geometric isomerisation of double bonds significantly affects both the quantitative and qualitative taste responses of volatile oxidation products [133].

None of the reliable methods is yet available to predict the stability of lipid-containing flavour and aroma in food oils and products. There are many variations in describe different compounds by various researchers however they testify to a subjective nature of the test panel. Further complications arise from the additive and antagonistic interactions between mixtures of volatile compounds [134].

Many volatile compounds are formed by further oxidation of the secondary products. Many of these secondary products of oxidation have a significant effect on the aroma and flavour of foods containing lipids. Although hydroperoxides of fatty acids are usually odourless and tasteless, their decomposition products have a great impact on flavour. Some volatile aldehydes cleavage products are extremely powerful and influence the aroma in the concentrations less than 1 part per million. The decomposition of hydroperoxides includes a very complex set of reactions by which a plurality of non-volatile and volatile products is formed [135].

Much attention was paid to the problems of measuring lipids oxidation and flavour formation in natural conditions In recent years this question has been considered in connection with the development of genetic engineering. When linoleic acid was added to the crude homogenate derived from transgenic tomato there was formed a large number of C₉-aldehydes, whereas the number of C₆-aldehydes remained the same. This result indicated that the 13-hydroperoxides of fatty acids are preferably formed from

endogenous substrates. In contrast, 9-HPO is formed of exogenous fatty acid substrate [136].

Lipid metabolism is associated with a variety of plants activity: photosynthesis, development, cell permeability, mineral elements exchange, plants organs transition to the state of rest, resistance to endure adverse of environmental conditions, etc. The research of unsaturated fatty acid structure of plant lipids is associated with considerable difficulties due to their great variety. The results of numerous studies carried out earlier are not enough detailed and reliable [137].

In many TAG of vegetable nature fatty acids are concentrated in the second (sn-2 - secondary hydroxyl group of glycerol) position. Cocoa butter has 85 % of oleic acid is in the sn-2 position and palmitic and stearic acids are evenly distributed by the sn-1 and sn-2 positions. Fatty acids in the sn-2 position are generally more unsaturated than the ones in the sn-1 position. Unsaturated fatty acids in the sn-2 position can be released by phospholipase using the enzymes of cyclooxygenase and lipoxygenase (LOX) as a substrate. Due to the surface activity of phospholipids they can be used to alter the lipids physical properties since they act as emulsifiers and affect the behavior of typical lipids in crystallization [26].

The molecules of cis- isomers in unsaturated (olefin) acids at the location of the double bond have a clearly expressed bend found at the X-ray study. In the molecules of trans- isomers the bend is replaced only by some ledge. The number of twists in the molecule of unsaturated acid cis-isomer corresponds to the number of the double bonds. In the fatty acids with multiple double bonds the cis-configuration attaches carbohydrate chain a curved truncated form. In vivo an arachidonic acid molecule has a hairpin configuration [68].

Some authors believe that the terminal methyl groups of fatty acids are close to the ether linkage which may lead to the steric obstacle effects for lipase. High bending effect of eicosapentaenoic acid (20 carbon) and docosahexaenoic acid (22 carbon) due to the presence of 5 and 6 double bonds respectively enhance steric interference effect [138].

In polyunsaturated fatty acids (with more than two double bonds) double bonds in most cases are separated by a methylene group. Such a configuration is referred to pentadienoic system in which two double bonds are located at the 1-st and the 4-th carbon atoms. In other words, these double bonds are not conjugated, but instead, they are broken by the methylene group. Such a structure in linoleic and linolenic acids is metilen-broken or divinilmetan:



In most cases the oxidation rate is doubled in case of adding one carbon atom limited by the methylene groups. In the linoleic acid (C18: 2) there is one carbon atom limited by methylene fragments, in the linolenic acid (C18: 3) there are two, and in the arachidonic acid (C20: 4) there are three. Thus, linolenic acid is oxidized twice as fast as linoleic and arachidonic – twice as fast as linoleic and four times faster than linoleic. When linoleic acid is attacked by singlet oxygen, the hydroperoxides with double bonds are formed at all carbon atoms. It means that hydroperoxides are formed both at the 9th and 13th carbon atoms as in the case of oxidation initiated by free radicals; moreover, hydroperoxides are formed at the 10th and 12th carbon atoms.

The presence of the double bond in fatty acids affects the melting point and the double bond in the cis-fatty acid configuration imparts a curved configuration, it means unsaturated fatty acids are non-linear that hampers their orientation in a compact “package”. Because of such steric hindrance the Van-der- Waals interactions between the unsaturated fatty acids are weaker that allows them to maintain a liquid state at the room temperature while their melting and solidification temperature is relatively low.

As the number of double bonds of the molecule becomes more curved the Van-der-Waals forces reduce and the melting temperature lowers even more. Fatty acids with double bonds in the trans-configuration are more linear than in the cis-configuration that leads to a more compact packing of the molecules and a higher melting point. For example, the approximate melting point of stearic acid (octadecanoic) is 70 °C, oleic (cis-9-octadecenoic) – 5 °C, and elaidic (trans-9-octadecenoic) – 44 °C [139].

It requires some energy for a hydrogen atom separation of from the methylene group of the fatty acid radical. This energy can be the light energy of (photochemical initiation) or heat (heating) in case of the liquid phase oxidation – exposure to ionizing radiation [140]. In the absence of additional sources of energy or initiators of oxidation the free radicals can be formed from molecules, the atoms bond of which is weakened as a result of their high kinetic energy compared with an average level of the molecules energy in the system.

Due to the fact that the formation of free radicals requires energy for separation of the bonds in the carbon and hydrogen atoms of the methylene group the oxidation reaction must first join the molecules in which such bonds are weakened or the energy of their separation is relatively small. To weakening of these connections, contributes, for example, the chain branching and the presence of double bonds. It is experimentally proved that the hydrogen separates from the secondary carbon atom four times faster than from the primary and from the trisubstituted 19 times faster than from the secondary one. Methyl esters of stearic, oleic, linoleic and linolenic acids at 100 °C oxidize at a rate of 1 : 11 : 114 and 179, and at 20 °C – at a rate of 1 : 100 : 1200, 2500 respectively [135].

The breaking energy of the CH bond of unbranched paraffins is 93 kcal/mol, an olefinic hydrocarbon of the CH bond breaking energy of the methylene group being in the α -position of double bond is 77 kcal/mol. Consequently, in unsaturated radicals of fatty acids the connections between carbon and hydrogen are weakened in the methylene groups located near to the double bond. Such links (CH–) are even more diluted in methylene groups located between the double bonds. In this regard, in a mixture of fatty acid or glycerides primarily are oxidized the molecules with unsaturated radicals [135].

Lipid cell structures are affected by chemical, enzymatic or physical methods to enhance or attenuate hydrophobic interactions, covalent bonding or Van-der-Waals forces. The lipid content in the fruits is low and they require less water as a means of transportation, the lipids mobility is sufficient to form an enzyme-substrate complex [141]. Considerable amount of the linoleum and linolenic acid are contained in the plant chloroplasts [142]. When the fruits ripen, they

lose their green colour due to the degradation of chloroplasts, which then free the membrane lipids, rich in these key precursors of flavour. Lipids in tissue cells are very finely dispersed, so that they by a very large surface contact with many substances that are part of cellular structures, including other lipids. These precursors then form, using a plurality of plant lipoxygenases, a large amount of esters and CC which characterize the fruit flavour. The hypothesis of a small amount of fatty acid de novo biosynthesis (free FA) has been proposed as a limiting factor for the biosynthesis of aroma with too early harvested fruit. This hypothesis is also supported by the evidence of a close link between low aromas of volatile foods, low content of FA and ATP in the foetus of apples. The role of FA oxidative degradation or free FA obtained by biosynthesis as the precursors responsible for the formation of a direct chain of compound esters in many fruits participating in the formation of flavour needs to be clarified [143].

Thus, the study of volatile products of lipids oxidation has found that the type of flavor obtained from a number of volatile substances may depend on their complex interactions, concentration range, and the environment in which they are examined. The great complexity of flavors and the need for reliable and universal test panels have become a major obstacle to progress in this area. Many studies of the threshold and minimum detectable levels for a number of VC of the primary and secondary lipid oxidation have been registered but there remains a problem of interaction of many compounds combination and permutation. Not all the products of reactions within the group of C₆–C₉, aldehydes, ketones and alcohols have the same odour as there are many factors affecting the odour biogenesis, in particular, the differences in the ratio of LN and LNL, as well as their concentration in free state.

1.7.2. Enzymes that produce fresh vegetable flavours

Flavour formation by lipid degradation is based on the reactions of α -, β -oxidation or oxygenase transformations. The chain of transformations from the activation of lipases to the formation of

other VC is performed at high speed. Typically, aromatic compounds are produced from fatty acids by enzymatically catalyzed degradation. Fatty acids enzymatic oxidative degradation precedes the action of acyl hydrolases that release free fatty acids from the lipids (acylglycerols). Changing the relationship between linoleic and linolenic acids in plant cells, reducing the activity of lipolytic enzymes was studied during the soybean leaf development [144]. It is believed that lipases and preceding with their participation a reversible reaction of hydrolysis are the mediate for the volatile esters formation. There is no evidence of such lipases action in the fruit but these reactions occur during the fermentation of the yeasts; a faint fruity odour may be due to the presence of lipases, they also contribute to the flavour in mature cheese. Lipases used to form savoury flavour of ripened cheese, likely have a limited effect in the enzymatic reactions of flavour formation. First of all, lipids hydrolysis by the action of lipases results in the increased level of free fatty acids, i.e. cytotoxic situation to the foetus. Free fatty acids modulate the activity of phospholipases, ion channels, ATP, G-proteins, and protein kinase; they also regulate the phosphoinositide and sphingomyelin cycle, hormonal signal and genes transcription of [145].

Some studies have shown that a preferential substrate for the production of GLVs (green and fresh scent) is galactolipids (Geimel, 1987). If lipase activity is extremely high, phospholipids and, finally, mono-, di- and triglycerides are also metabolized (Ishiguro et al., 2001). Galactolipids are present in tialkaloid tissues of membranes and contain a large amount of trienoic acids such as C18: 3 and C18: 2, while the phospholipids form the bulk of the plant cell membrane. Typically, galactolipase (GL) and other levels of lipase activity are naturally very low in plants. Rapid release of free fatty acids from galactolipid such as monogalactosyldiacylglycerol (MGDG) may be associated with some of inducible defense system.

Polyunsaturated fatty acids and their derivatives (i.e. mono- and diacylglycerols, amides and oxylipins) function as effectors of biological activity. Furthermore, enzymatic oxygenation of unsaturated

fatty acids leads to a highly active broad spectrum of oxylipins that function as signaling molecules. As oxylipins are synthesized from polyene fatty acids in response to various biological stimuli, their assessment gives a quantitative reflection of the cells and tissues state. Most of the oxidized lipids that are present in the biofluids and tissues are specifically biosynthesized by PUFA under the effect of strictly regulated enzyme (s). The number and types of oxylipins in biofluids were used to denote inflammatory, damaged and clearly disease states [146].

It is known that the enzymatic processes under the influence of lipases do not dependent on the mass fraction of water [147]. An important process involving lipases is a directed redistribution of lipid acyl groups which allows obtaining lipids with a given structure. This process is carried out in the “microwater” environment, i.e. in an organic solvent or in a medium of the lipid substrate, performing the role of a “solvent” (water content in the medium is less than 1 %). Under these conditions, lipases catalyze not the hydrolysis of esters but their (re) synthesis. It is known that the lipases do not interact with the substrate as long as its concentration exceeds their solubility and on the surface of phases division starts the formation of colloidal aggregates of the micelle type.

Many of the aliphatic esters, alcohols, acids, and CC found in the fruits are derived from the oxidative degradation of linoleic and linolenic acids. Formation of the C₆-aldehydes is changed with the development of leaves: young leaves (high concentration of linoleic acid) produce high levels of C₆-aldehydes, mainly consisting of hexanals. As the leaves (PUFA composition change, an increase in linoleic acid), the level of C₆-aldehydes formation decreases considerably, thus increasing the content of hexenals [148].

Some of the volatile compounds derived from the enzyme – catalyzed oxidative degradation of unsaturated FA may also be prepared by the autoxidation [149]. Oxidation and autoxidation of lipids lead to different final products, especially in the food raw materials, in this case not only lipids may react with lipid free radicals, hydroperoxides, aldehydes [150].

Analysis of the aromas obtained by catabolism of fatty acids (FA) in the cytoplasmic membrane shows differences associated with the isomeric forms of enzymes and substrates. In the last few years under stress factors it has been found that oxygenated derivatives of polyunsaturated fatty acids are released from the membrane phospholipids and glycolipids. The membrane fluidity parameters are crucial for the functioning of a plurality of membrane-bound enzyme systems, and plant hardiness [151]. Cold causes the decrease of membrane fluidity, which can be compensated by the desaturation of fatty acids membrane lipids. One of the mechanisms of plant adaptation to lower temperature is through increasing the degree of instauration of the fatty acid residues in the cell membranes. The introduction of additional double bonds in the hydrocarbon chains of the lipid bilayer leads to a reduction in phase transition temperature from the liquid crystal to the solid state and provide the necessary fluidity of the membranes at low temperatures [152]. The double bonds formed in the fatty acids residues catalyze the fatty acid desaturase. Fatty acid desaturase (FA) are the enzymes which catalyze the conversion of a single bond between the carbon atoms in the acyl chains (C–C) into double bonds (C=C). In the ripe isolated fruits the decrease in ambient temperature by 10 °C leads to only 3 % change in the molecular mobility of lipids [153]. To some extent this is enough to activate the desaturation of FA membrane and after some time to notice the changes in the physical properties of the cytoplasmic membranes. Wang et al. (1996) have shown that the successful introduction of the yeast Δ -9 desaturase in transgenic tomato plants lead to an increase in palmitoleic acid levels, 9, 12 – hexadiene acid and linoleic acid, accompanied by the reduction of palmitic acid and stearic acid [154]. The changes in fatty acid profile are associated with the change of certain aromatic compounds derived from fatty acids, particularly the cis-3-hexenol, 1-hexanol, hexanal, and cis-3-hexenal. In isolated fruits the enzymes continue for some time to influence the reaction of the aroma precursors formation. C. Zhang, S. Tian showed that lipid rearrangement in plasma membranes occurs in response to low temperature, and this process is accompanied by adaptive change of the biophysical

membrane properties. Therefore, the peaches are better stored at 0 °C than at 5 °C [155]. The authors have shown that C_{18:3} linolenic acid of the lipid membrane plays a vital role in the process of cooling fruits because the higher the level of C_{18:3} the more conserved the membrane fluidity is and fruits are more tolerant to cooling as the molecular mobility is proportional to the absolute temperature in case no phase transition occurs. Storing fruits at the temperature from 5 °C to 0 °C can alter the kinetics of the aromatics formation (Lineweaver, 1939; Schwimmer et al, 1955). Under prolonged storage the unwanted flavourings, accumulation of less volatile elements, and emergence of flavour may be received.

Herbal lipoxygenase (LOX) plays an important role in cell protection and aroma formation. It was first isolated in 1995, out of soybeans. LOX is one of the most studied enzymes in fruits and vegetables [156]. LOX is considered to be the enzyme responsible for the formation of the secondary volatile compounds that primarily influence the human perception of the aroma. LOX is classified as linoleate: 13 oxidoreductase (EC 1.13.11.12) oxygen. Nevertheless, many isoenzymes of LOX, which have been thoroughly characterized, catalyze the oxidation of other substrates such as linoleate: 9-oxidoreductase oxygen. There is no differentiated or common nomenclature for these enzymes. In plants LOXs are found in almost all the tissues with some exceptions. LOX, is generally regarded as a soluble enzyme, located in the cytoplasm, but recent studies have shown a much more complicated structure [157]. Lipoxygenase activity in a homogenates suspension from tomatoes, cucumbers, olives, peppers, apples, basil, strawberries, and bananas is decisive for the process of aroma formation [158]. Homogenates of vegetable raw materials allow using enzymes without prior laborious isolation and purification, without energy consumption for dialysis or lyophilization. This has been confirmed by a study on the characteristics of the enzyme both with pre-extraction from the sweet pepper substrates of polyunsaturated fatty acids and without it [159]. Analysis of aromas obtained by fatty acids catabolism of the cytoplasmic membrane shows differences in the enzymes action. The enzyme activity differs depending on the stage of fruits maturity. For

example, bell pepper lipoxygenase activity is higher in the green maturing stage, whereas in tomato conversely in the mature stage [160]. The action of lipoxygenase in ripe and unripe strawberries is equal. Lipoxygenase isomeric forms may be located in certain places of the cell's various compartments and have temporarily differentiation in activity [161]. A number of studies on the use of exogenous forms of lipoxygenase in homogenates with conflicting results concerning the changes in volatile substances concentrations have been carried out. Some researchers believe that the inactivation of vegetable raw material lipoxygenase positively affect the flavour characteristics of foods from tomatoes, green beans, soy milk and others. Another view is that the acceleration or deceleration of enzymatic reactions can cause undesirable changes in the product flavour [162]. Even the minimum duration of blanching leek, basil, and spinach leads to maximum destruction of hexanal and hexenal. Therefore, the enzymes involved in the oxidation of lipids, their hydroperoxides and other products of their reactions are being thoroughly studied in the world. I. Aguylío Aguio, D. Olyu have shown that even a small saving in lipoxygenase activity in the prepared strawberry juice (fruit fine technology) greatly improves the flavor of the final product in contrast to the samples subjected to heat treatment [163]. E. Yilmaz, K.Tandon have proved that there is little or no correlation between the activity of the lipoxygenase enzymes, hydroperoxide lyase, dehydrogenase alcohol and the concentrations obtained from the lipid composition of volatile flavours. Predictive models have been compiled for hexanal, trans-2-heptanal, pentenona, cis-3-hexenal, trans-2-hexenal, cis-3-hexenol, and methanol as a function of one or more enzymes activity. Lipoxygenase and hydroperoxide lyase activity is related to the model of hexanal, trans-2-heptenal and pentenon formation, while cis-3-hexenal is produced in connection with the sufficient hydroperoxide lyase activity, and trans-2-hexenal is formed under lipoxygenase activity in the models. The correlation coefficients relating to the enzyme activity and volatile concentration of aroma components show that the relations are not direct [164]. K. Viljanen, and M. Lille have analyzed the changes in smell of tomatoes in the processing at two temperature regimes (20 and 60 °C) and pressures (atmospheric and 800 MPa)

[165]. The authors have found that treatment at 800 MPa and 60 °C leads to a significant increase in the intensity of smell in prepared tomato beverage due to the lipoxygenase activity. Rodrigo et al. have shown that tomato juice lipoxygenases treated for 12 min. at 60 °C were completely inactivated. On the other hand, tomato lipoxygenases activity increases with increasing pressure of (400 MPa). This may be due to an increase of the lipoxygenases output from the membranes [166]. Recently, in installations with a high hydrostatic pressure and low temperature of processing it is possible to keep the activity of lipoxygenase and hydroperoxide lyase, which leads to the conservation of natural aroma in the final product [167].

The lipoxygenase action effects obtaining of the representative aromatic extracts from fresh plant material due to the possible changes in the volatile profile [168]. GX analysis comparison of the volatile profile obtained by the dynamic extraction of free space from fresh basil leaves blanched in water has shown numerous volatile C₆ and C₉ aldehydes in fresh samples, while these compounds were in trace amounts in the blanched leaves. The lipoxygenases have been isolated from the seeds of chickpea, barley, rice, fruits and eggplants, tomato, kiwi, etc.

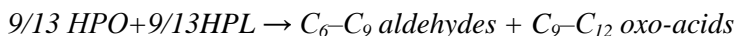
The LOXs from different sources are different by their isoforms, pH optimum, regio and stereoselectivity. Many LOX in plant raw material are regioselective in the oxidation of the carbon atom in the C₉ linoleic and linolenic acid (soybean). Other isoforms have no regio-stereoselective properties (pea seeds) due to their low affinity to the FA. Selectivity of the lipoxygenase reaction also depends on whether the fatty acid is esterified, in what form it is presented (micellar, complexed with a detergent or in a salt form), and what pH value it has that determines the degree of dissociation of the carboxyl group. Antioxidants do not have a direct effect on lipoxygenase but inhibit the secondary oxidation reactions. Only a few compounds directly inhibit lipoxygenase (catechols and esculetin, etc.). The effect of a pulsed electric field (PEF) on the inactivation of lipoxygenase (LOX) in tomato juice prepared for a long-term storage have been studied [169]. It was revealed that an active energy to inactivate LOX by PEF was 35.7 kJ/mol.

The effect of localization of enzymes and tissue maceration should be taken into account under modification of the volatile entities. The beans with high content of this enzyme (varieties of shelling beans, soybeans, peas) are particularly susceptible to oxidative damage. Mash beans (mung beans; lat. *Vigna radiata*) have been investigated as a new source of lipoxygenase in connection with obtaining natural «green» flavors. The mung pH profile has a wide range (optimal pH is 6.5) of lipoxygenase-2 and lipoxygenase-3 isozymes whereas for soybean only isozymes of lipoxygenase-1 and lipoxygenase-2/3 (optimal pH is 9–10) were observed. As compared to soybean, mung lipoxygenases were a good substitute for the formation of hexanal since they produced 76 % and soya – 60 % [170]. The physico-chemical conditions on the flow of linoleic acid oxidation reaction by 5-lipoxygenase of the potato tubers have been analyzed [171]. Many studies have been devoted to the research of lipoxygenase activity during the germination of seeds or grains. It was noted a decrease in the activity of LOX 2 and LOX 3 in the first 24 hours of germination and its increase it in the period of 5–7th day; in the first three hours of imbibitions and up to seven hours of germination the bean LOX activity increases by half compared with the dry grain, and after 18 hours its activity level drops to the baseline; in sunflower seeds the activity peak appears on the fourth day of germination, in watermelon –on the sixth day [172, 173].

The article of M. Gargori provides literature overview on the use of lipoxygenase enzyme for the production of flavoring molecules (hexenal, nonenal, nonadienal and the corresponding alcohols) from vegetable oils. This approach is used for the vegetable oil rich in PUFAs. The cascade reactions with various enzyme systems, raw materials as well as intermediate and final products with different properties in bioconversion have been shown [174]. LOX-produced HPO hydroperoxides are highly active compounds. The further stage of fragrance formulation requires several enzyme systems realization in the reaction medium.

Fatty acids hydroperoxides (HPOs) are cleaved by the enzyme of hydroperoxide lyase (HPL) which refers to the membrane-tied enzymes [175, 176]. Green bell peppers, guava, some leaves

(spinach, tomatoes, tea, mint and watermelon), sprouts (alfalfa, soybeans) and some algae, or fungi, are known as sources of lyase hydroperoxide. Reactions of lyase hydroperoxide of fresh fruit have been sufficiently studied [177,178]. Schematically hydroperoxide catalysis can be represented as follows:



Depending on the substrate characteristics the HPL_S can be classified into several groups. The number of such groups is not definitively established, in general, the following ones can be identified [179, 180]:

- 13-HPL specifically cleave the fatty acid of 13-HPO to form C₆ aldehydes (poli-hexasanal or (Z) -3-hexenal) and 12-oxo- (Z) -9-dodecenoic acid;
- 9/13 HPL which can cleave both 13-HPO and 9HPO with almost equal efficiency;
- 9-HPL which specifically cleaves 9-HPO and C₉-aldehydes ((Z) -3-nonenal or (Z, Z) -3, 6-nonadienal) and 9-oxo-nonanoic acid are formed from C₁₈ fatty acids;

For example, although the major product of the lipoxygenase action in tomato fruits is 9- HPL, the specificity of hydroperoxidlyase (the velocity ratio of its interaction with the 9- and 13- HPL is 1: 62) leads to the formation of the C₆ and C₁₂ fragments in the damaged tissues. Conversely, the cucumber hydroperoxidlyase has a low specificity (the velocity ratio of its interaction with the 9- and 13- HPL is 2: 1) and the dominance of the C₉-fragment among the fatty acid oxidation products is primarily determined by the selectivity of lipoxygenase. It has been shown that 13-HPO fatty acids formation is preferably produced from endogens substrates in contrast to 9- HPO that is formed from hexogen fatty acids substrates [179].

Formulated aldehydes may be subjected to further transformation by the dehydrogenase enzymes to alcohols which generally have similar odour profiles to aldehydes. This way of flavour formulation

was first demonstrated on the banana flavouring reformulation and enzymes involvement in this process was proved on the example of cucumbers and tomatoes. Changing the ratio of these aldehydes and the corresponding alcohols produces “green odour” in different kinds of plants, depending on their environment and the season. Lyase hydroperoxide has very low activity during the development of soybean and during their storage, in contrast to lipoxygenase. This enzyme is relatively thermo-labile losing its activity at lower temperatures than lipoxygenase. The ability to use enzyme activity from a variety of sources to perform sequential reactions is one of the advantages of using plant enzymes.

1.7.3. Modifications of enzyme-substrate aroma-formulating reactions

The aroma formulating mechanisms in lipid degradation reactions are based on α -, β -oxidation or LOX transformations. To alpha oxidation are subjected the fatty acids with a very long chain – more than 20 carbon atoms (in this case one carbon atom is cleaved from the FA) or a FA with a branched carbon chain like phytanic acid in which the β -oxidation is not possible due to the presence of the methyl groups at every third carbon atom. The process of β -oxidation (Knoop-Linen cycle) is a specific way of fatty acids degradation carried out only under aerobic conditions. As a result two-carbon fragments are cleaved sequentially from the fatty acid molecule of the carboxyl group.

Oxygenation or lipoxygenase pathway leads to the appearance of the hydroperoxide radical $-OOH$. The double bond in the oxygenation reaction is shifted by one position to form a conjugated diene. If a double oxygenation of linolenate happens it may form a conjugated triene ($-CH=CH-CH=CH-CH=CH$). In lipid oxidation reactions free radicals are central. They are the molecules or atoms with unpaired electrons in their structure. These free radicals differ significantly in their energy (Fig. 3).

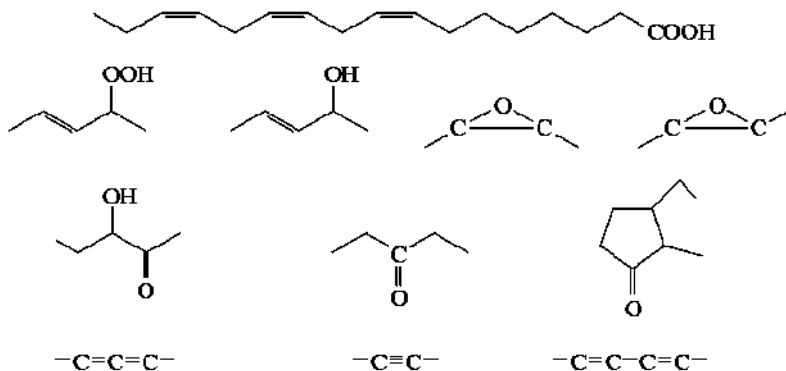


Figure 3 – The formation of linolenic acid radicals [72]

Fatty acids oxidation can be divided into three main stages: initiation, development and oxidation completion. Modern views on the mechanism of lipids oxidation is based on the Baja Engler's peroxide theory, the theory of the chain degenerate-branched reactions by Semenov, and on the Framer's hydroperoxide (migration of double bonds) theory.

Revision of publications has shown that the possible way to structure the process of flavoring reformulation can be characterized by:

- the methylene group status, the status of hydroperoxide radical;
- the double bonds number and position in the PUFA (position of double bonds: isolated, conjugated,);
- the position of PUFAs in a free state or as a TAG, phospholipids, galactolipids component;
- the ratio of 9 and 13 hydroperoxides;
- the presence of enzymes and their isomers stereospecificity.

Most plant LOX use free fatty acids as their substrates. However, for the two plant 13-LOX, such as soybean LOX and cucumber LOX it was demonstrated the activity towards the PUFA in the composition of phospholipids that corresponds to the assumption of LOX participation in the process of penetration through the membrane.

The changes of the membrane physicochemical properties by modifying its fatty acid residues lead to assimilates and ions penetration into the cell. The 13-LOXs, such as barley seed LOX, vegetable soybean leave LOX it was established the activity towards fatty acids in the composition of neutral lipids - triglycerides [181]. The oxidation induced by lipoxygenase may take place not only by the PUFA cleavage, but also by the formation of free fatty acid radicals initiating the autooxidation process (autooxidation). The spontaneous formation of the lipid aromatic derivatives received by autooxidation leads to the formation of oxoacids from fatty acid molecule, but they apparently have no effect on the odour. The formation of free radicals may be the result of ionising radiation and active hydroxyl radicals ($\cdot\text{OH}$) received from water are capable of separating hydrogen as well as proteins molecule and the DNA from the lipids. Irradiation of food products, especially with high-fat and pro-oxidants may enhance oxidative rancidity – the effect of photo-oxidation (Fig. 4).

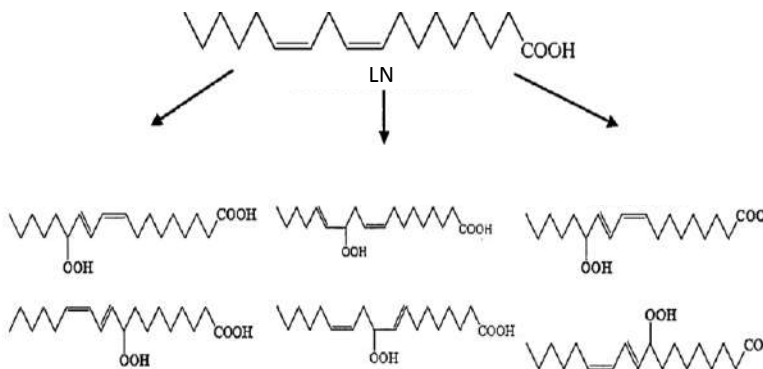


Figure 4 – The ways of linoleic acid transformation

Homogenization or mechanical grinding is enough to carry out the enzymatic reactions with the lipids of fresh fruit cell (cucumber, tomato, banana, etc.). It has been shown that the amount of volatile compounds increased after breaching fresh fruit integrity as the enzymes came into contact at the surface [182]. LOXs are soluble

proteins found in the chloroplasts stroma, vacuoles, cytosol, mitochondria and lipid vesicles (oleosoma). In contrast, LOX substrates are poorly soluble in aqueous medium at the physiological pH. It is assumed that a possible mechanism for the activation of soluble LOX is their optional Ca^{2+} -dependent association with cell membranes, where hydrophobic substrates of LOX are localized [183]. Since the lipid precursors have a hydrophobic nature and the enzymes have hydrophilic, the enzymatic reaction *in vitro* conditions occur at a very low speed. The water-lipid systems use surfactants (surfactant) to increase the contact surface area in the lipid-substrate reaction and decrease the water shell thickness. The surface area is a factor affecting the rate of lipid oxidation. Increasing the surface area of the fat phase accelerate the rate of lipid oxidation, as it enlarges the contact area with oxygen and pro-oxidants.

The kinetics of lipids oxidation in foods is often characterized by a delay phase (lag phase) after which begins the exponential increase in the rate of oxidation. Both free fatty acids and acyl groups may be oxidized. In most foods after a lag phase there is a rapid exponential increase in the products oxidation. This suggests that there are some lipid oxidation reactions in which additional free radicals are formed. Since there are numerous positions for forming hydroperoxides in unsaturated fatty acid chains, β -cleavage reaction results in the production of many foods.

The dynamic properties of the membrane lipid matrix provide conformational flexibility of the enzymes. The properties of the lipid matrix are associated with the structural rearrangement in the biological membranes. For example, in frozen fruit water crystallization induces the activation of the membrane-bound lipolytic enzymes and, as a consequence, a significant change in the structure and physicochemical characteristics of the membrane lipids fatty acids. Thermal treatment of the membrane lipids affects physical properties of the lipids and the oxidation process by the endogenous enzymes. Thermal effect, freezing, electrical breakdown, and osmotic pressure are the factors driving structural adjustment and activity of endogenous enzymes [184].

The fatty acid hydroperoxides, the primary products of the lipoxygenase reaction, rapidly metabolize in two main ways: lipoxygenase and peroxigenase cascades (formation of hydroxyl- and epoxy- derivatives catalyzed by dependent oxygenase (peroxigenase) making transfer of one oxygen atom from the hydroperoxide fatty acid radicals to the double bond of oleate or linoleate with the epoxide formation [185]). A. Grechkin and staff have found more than 15 previously unknown derivatives of linoleic and linolenic acids [186].

The lipoxygenase-2 is the main producer of hexanal in the homogenized soybean [187]. In the presence of isoforms 1 and 3 or both, the isoforms ability to form 2-hexanal is reduced indicating the hydroperoxide fatty acids dependance on the type of their producing isoform. The isoform-2 also causes the formation of undesirable volatile substances due to adding the legume flour even in the amount of 1 % into the dough. It is known that there is a LOX of dual specificity [188] in pea and potato products catalyzing the formation of a mixture of – 9- and 13-hydroperoxidase-derivatives in various ratios.

The aldehydes and ketones formed by the action of lipoxygenase are converted into the corresponding alcohols which are typically characterized by a higher detection threshold and more saturated odour than the starting CC. The present cis-trans-isomers convert the cis-bounds of aldehydes into the trans-bounds and as a result of these structural changes the aldehyde odour varies. Generally, the compounds with six carbon atoms give a green flavour (“fresh cut grass”), with nine – the smell of cucumber and melon, and with eight – the smell of mushrooms, violet leaves and geranium. The work of M. Gregory shows that using a pure substrate or a 9-or 13-hydroperoxy fatty acids makes the synthesis of specific volatile compounds efficient. Rapid disposal of hydroperoxide lyase enzyme, alcohol dehydrogenase and secondary flavour transformation eliminated by the effective allocation is the barrier for the aroma biosynthesis.

Lipid oxidation is initiated or accelerated by the prooxidants, which are not true catalysts (e.g., singlet oxygen is converted into hydroperoxide and ferric ion turns to oxidized state). Prooxidants can accelerate lipid oxidation either through direct interaction with unsaturated fatty acids forming hydroperoxides (for example, singlet oxygen or lipoxygenase) or by inducing the formation of free radicals (for example, hydroperoxide decomposition, activated by the transition metal or UV radiation). It has been noted that lipid hydroperoxides do not participate in the formation of unpleasant tastes and odours [189]. Water-soluble products of lipid peroxidation have an inhibitory effect on the enzyme activity [190]. Structural adjustment in biological membranes occurring during the peroxidation of endogenous membrane lipids contribute to the change of membrane-associated enzymes activity. PUFAs cleavage products often contain double bonds, and in some cases intact pentadienoic systems. These double bonds systems can be subjected to separation of the hydrogen atom or singlet oxygen attack that results in formation of additional degradation products and unique flavours. If HPO (hydroperoxide) localized at the 9th carbon or the 13th-carbon atom and the β -cleavage occurs from the methyl end of the molecule, HPO (hydroperoxide) first is decomposed to alkoxy radical, and then to two reaction products of 9 oxononanoate (ethyl 9-oxononanoate) and vinyl radical at the 9th carbon atom of the olefin radical. These vinyl radicals often interact with hydroxyl radicals forming aldehydes and thus producing 3-nonenal [191]. The linoleic acid hydroperoxide may be exposed to β -splitting also by the carboxyl end of a fatty acid, when after the formation of alkoxy radical the octanoate and ethyl 2, 4-decadienal are formed [192]. The role of the lipid decomposing intermediates (e.g., isomers of hydroperoxides, Table 1) in the flavour formation is being studied in the physiology and biochemistry of the plants [193].

During the cooking process naturally occurring aromatic compounds can be eliminated or modified and new compounds can be formed.

Table 1 – Aldehydes corresponding to hydroperoxides isomers [5]

<i>Fatty Acid</i>	<i>Methylene Group Involved*</i>	<i>Isomeric Hydroperoxides Formed from the Structures Contributing to the Intermediate Free Radical Resonance Hybrid</i>	<i>Aldehydes Formed by Decomposition of the Hydroperoxides</i>
Oleic	11	11-hydroperoxy-9-ene	octanal
		9-hydroperoxy-10-ene	2-decenal
Linoleic	8	8-hydroperoxy-9-ene	2-undecenal
		10-hydroperoxy-8-ene	nonanal
		11-hydroperoxy-9,11-diene	hexanal
Linolenic	11	11-hydroperoxy-9,12-diene	2-octenal
		9-hydroperoxy-10,12-diene	2,4-decadienal
		16-hydroperoxy-9,12,14-triene	propanal
Arachidonic	14	14-hydroperoxy-9,12,15-triene	2-pentenal
		12-hydroperoxy-9,13,15-triene	2,4-heptadienal
		13-hydroperoxy-9,11,15-triene	3-hexenal
	11	11-hydroperoxy-9,12,15-triene	2,5-octadienal
		9-hydroperoxy-10,12,15-triene	2,4,7-decatrienal
		15-hydroperoxy-5,8,11,13-tetraene	hexanal
13	13-hydroperoxy-5,8,11,14-tetraene	2-octenal	
	11-hydroperoxy-5,8,12,14-tetraene	2,4-decadienal	
	10-hydroperoxy-5,8,10,14-tetraene	3-nonenal	
	10-hydroperoxy-5,8,11,14-tetraene	2,5-undecadienal	
10	8-hydroperoxy-5,9,11,14-tetraene	2,4,7-tridecatrienal	
	9-hydroperoxy-5,7,11,14-tetraene	3,6-dodecadienal	
	7-hydroperoxy-5,8,11,14-tetraene	2,5,8-tetradecatrienal	
7	5-hydroperoxy-6,8,11,14-tetraene	2,4,7,10-hexadecatetraenal	

The aromatic compound can be administered during or at the end of the cooking process to preserve a natural note. It is very important for the lipid-containing products. Aromatic lipid oxidation products have a lipophilic composition and due to its little solubility in the aqueous phase can be distributed in the air to utmost. The C₆–C₉ concentration of CC at the air phase can be changed in case the given ability of these components to dissolve in fats and the similarity of their partition coefficient in the water: oil system are taken into consideration. If desired such oxidation products are removed from the surface, such as the aquatic ones [194]. By comparing the release kinetics in different matrices (in the presence of lipids and carbohydrates) the kinetic study on the rate of the six aromatic compounds release has been carried out [195]. Reducing the initial release rate (diacetyl, 2-pentanone and cis-3-hexenol acetate,

ethylhexanoate, trans-2-hexenal) was more important in the presence of lipids.

Differences in the 9-LOX and 13-LOX enzymatic properties, kinetic parameters of the environment and influence of the substrates physicochemical properties are critical to the reactions of free fatty acids and TAG, the formation of the volatile short-chain aldehydes and different composition alcohols. To achieve optimum efficiency in the production methods of volatile compounds by lipoxygenase it is necessary to use plant extracts as a source of lipoxygenase and / or lyase hydroperoxide. The best source of fatty acid is the hydrolyzate of seed oil, and of alcoholdehydrogenase is the yeast.

1.8. Modern aspects, methods and perspectives in food flavouring

Natural aromatic compounds present in plant essential oils are obtained by steam distillation of the plant material, and followed by fractional distillation. This extract method has different problems. Fresh raw material contains low concentrations of the target compounds, which makes extraction lengthy. This increases the cost by 10–100 times. An alternative method for the natural synthesis based on microbial biosynthesis or bioconversion has been described in the works of M.Gargori [196]. The biotechnological approaches can be divided into two classes: the microbiological and enzymatic. Microbiological methods used for the synthesis of natural fragrances are divided into *de novo* synthesis and biotransformation. The first is the aromatics formulation after the cell metabolism via a simple cultivation media, while biotransformation relates to the use of microbial cells to convert the precursor into the desired product. The *de novo* synthesis should therefore be used for complex mixtures or product mixtures, while biotransformations are capable of performing a single step process. Through the use of exogenous glycosidases it was possible to increase the flavour intensity in fruit juices and wines. Aromatic compounds can be presented in a free state in fruits and/or in the form of glycosides. Much of important flavors in most fruits accumulates in a non-volatile fraction and is

known as a glycosidic aroma precursor found in various parts of the plant: the green parts, roots, rhizomes and seeds [197]. Gonzalez-Pombo et al. worked to increase the wine aroma with the immobilized *A. niger glycosidase* and demonstrated the importance of exogenous glycosidases to enhance the wine taste. The muscat wine biocatalyzation treatment for 20 days significantly increased the amount of free monoterpenes (from 1119 to 2132 g / l) [198].

The main reason of low productivity and efficiency of the biosynthesis is the accumulation of hydrophobic aromatic compounds having inhibitory effect in a culture medium [199]. The removing of these products is a powerful tool to overcome these limitations. The aroma reformulation separately from both the fermentation medium and other biological mediums is not a simple task, since the fragrance is usually diluted in a complex matrix. The use of the organophilic evaporation (OPV) has received a lot of support as a potentially suitable technology for the continuous removal of the volatile compounds. The number of OPV research has increased over the past few years. It has always been considered on the basis of the membrane technology having the great potential for a moderate reformulation of natural aromatics. The term pervaporation refers to a membrane process for separating liquid mixtures. OPV can be regarded as a process of the vacuum distillation in which a water or vapour mixture containing fractions of volatile compounds contacts with a hydrophobic non-porous polymer membrane. The vacuum is established for providing a driving force for selective mass transfer of raw material. In general, the pervaporation is technologically acceptable for the selective removal of the small amounts of volatile substances (MW <200 Da) in mass quantity. Pervaporation affects the selectivity not only by differences in vapour pressure but also by differences in the polymer membrane components solubility and diffusivity. In general pervaporation is the technology suitable for the selective removal of the small amounts of volatile substances (MW <200 daltons) [200].

Despite of a large number of technological developments and techniques it is still difficult to extract the flavour close to natural,

and mostly due to the lack of “fresh notes” in liquid flavours. There are some ways for the original flavour reformulation:

- evaporating and sending 10 % of water to the first fraction of ready distillate;
- using the technique of a “separation effect”;
- using by evaporation not only juice, but also aromatic extract (alcohol), fruits freezing and fruits enzyme treatment (Dzh.Merori);
- applying modern approaches to the issue: (allocating of fresh ingredients by pervaporation, supercritical fluid CO₂ extraction, extraction by low polarized water (PLPW), using water coherence, inseparability and separation as an anomalous property of water).

Interest in water is caused by its unique ability to change physico-chemical parameters such as dielectric constant, viscosity, specific heat, the diffusion coefficient and density depending on pressure and temperature. Water in these conditions behaves like a polar organic solvent. Water properties enable to use it as a medium for dissolving /extraction of the organic agent present in the plant material on the one hand and as a reactant in the chemical reaction occurring in the environment, and chemical properties of which are controlled by the temperature, pressure and catalysts on the other hand. Extraction of subcritical water bioflavonoids, alkaloids, and other. Biologically active substances [201]. Water temperature affects the extraction in two ways: firstly by changing the dielectric constant of water, and thus, the solubility of the target compounds and secondly violating the interaction between the analytes and the matrix.

Recently, the use of microwave energy has become the object of much research in terms of a real alternative to a conventional extraction procedure [202]. Dissolution in microwave extraction (Solvent-free microwave extraction – SFME) is based on a combination of heating in a microwave oven and dry distillation carried out at the atmospheric pressure. SFME has already been applied to extract the essential oil from a number of fresh vegetable raw or soaked-dried materials in the food and pharmaceutical industries encouraging a high potential for future applications [203]. In the attempt of studying the extraction of flavor in microwave two

theories have been formulated: ΔT and the vapor pressure theory. In the framework of the first theory pure fragrance molecules were experimentally developed to predict the release of the compounds due to their thermal capacity and dielectric properties (Shaath and Azzo, 1989). However, after food products having been tested the correlation between ΔT and flavor evaporation has not been found.

Furthermore, the amount of fat or water aromatic compounds with high microwave absorption and higher levels of microwave heating was not predominantly due to microwave (MW) heating. This theory is not applicable to flavor compounds in foods because the food properties not the flavor compounds that affect thermal characteristics. At the same time, the number of evidences in favour of vapor pressure theory has been increasing. [204, 205]. It is known that no specific processes occur in the MW [206], but most researchers indicate the difference in the products flavour after processing them in the CF [194, 207]. The MW heating differs from the convective heating by the selectivity towards the lipid and hydrate components and releasing of free fatty acids from the lipids [208]. During microwave heating the enzyme activity increases, consequently affecting the quality and quantity of the aroma biosynthesis products [209]. MW heating increases the mobility of the components, their diffusion, which may affect the likelihood of effective communication and help avoid decomposition of thermally unstable compounds. Overheating of polar solvents and heating the points without solvents – are the requirement to accelerating the reaction in the MW field [210]. The combination of ultrasound and MW is used to extract aromatic components out of the plants; the method advantage is in saving time and improving the extract quality [211,212]. The extraction time depends mainly on the diffusion; the ultrasound considerably accelerates the process destroying the cell walls and tissues.

Vacuum dryer with microwave heating is used for drying timber, medicines, sensitive to high temperatures. The technology for drying fruit with soft consistency (strawberry, banana, leaves) has recently been developed. Scientific research in this area is related to the drying mode, its intensity and finished product quality. The quality

retrieved is higher than in other drying methods [213]. During vacuum drying with microwave heating the easy volatile aromatic components allocated together with the water vapor are tried to be kept in the original material. The issue of capturing these aromatic components is missing for several reasons:

- described technical limitations related to the use of vacuum drying and collecting condensate;
- not high vapour temperature (approximately 40 °C) at the SFME exit does not involve the process of their condensation;
- not accumulated sufficient material on the composition and properties of the water vapour and easy volatile aromatic components (there may be a misconception in terms of whether water drops contain the aroma and flavour is stored only in the air phase).

Water vapor extracted from the raw material after condensation during the is the water with aromatic components. The problem is that this water vapour and aroma components are not trapped at the industrial plants. Modern SFME desiccators are set at the exit of water vapour for the dehydration of the exhausting air.

Concurrent with the demand today for food products that are tasty and safe, is the additional requirement that these products contain lower levels of sugar, fat and salt or have “clean label” (0 % conservative, flavorings, sugar, salt, fat). Adding salt to the food results in a reduction of water activity (a_w) due to the formation of strong ion-dipole interactions between the salt ions and water ; this leads to the reduced availability of water molecules for solubilisation of aromatic compounds. This phenomenon known as the “salting-out” effect, leads to an increased release of aroma from food due to the decreased availability of water molecules for the solubilisation of aromatic compounds [214].

The “salting out” effect is observed in vegetable soups with different concentrations of flavour and colour. The sensory data showed that the “green color”, “sweet” and “pepper” attributes were associated with low salt soup, and the soup with the “salty”, “yellow”, “carrot flavor” attributes was defined as of the conventional salinity and higher concentrations of limonene, p-cymene, beta-caryophyllene and isopropyl disulphide [215].

Natural and synthetic flavours are a mixture of several chemicals used to replace the flavor of the foodstuff. In most cases, these compounds mimic the natural flavor. Some additives of this group also have various functions in foods as antimicrobial activity, gelling property and others. There are more than 1,700 natural and synthetic compounds that are available to impart the flavor to product. In this large amount the acidifiers play an important role by reducing the total pH in food. Most of the acidulants are the organic acids, some of them are not limited in use: acetic acid (E260), lactic (E270), malic acid (E296), citric acid (E330), propionic acid (E280), succinic acid (E363), while the others require ADI level permission (mg/kg of the body weight): fumaric acid (E297), tartaric (E334), adipic acid (E355) [216].

The laws and regulations necessary for the consumer's safety and security influence sufficiently on the aroma production. For example, potential allergens are prohibited for use. Over time, additional laws have appeared on labelling, which perform an informative function not only for the security matter but also for the product nature, its religious status, organic origin, genetic transformation, sodium glutamate and others.

Conclusion to Chapter 1

1. Currently, flavors make up more than a quarter of the world market of food additives and represent an industrial size of almost 7 billion US \$ per year. Most of them are provided by extraction from natural sources or by traditional methods of chemical synthesis. There is a growing demand for natural products, rather than synthetic ones.

2. Aroma participates in human sanogenesis and has a positive role in the regulation of vital functions. The ability of a person to recognize odors depends on the physiological characteristics of the epithelium, age, traditions, psychological association with odor, and other factors. Aromatic components can be retained by the food matrix and desorbed at different intensities, so the introduction of flavorings into product recipes is not always justified.

3. Oxidative reactions from the main pathway for the synthesis of fragrances in plants responsible for a specific “green, fresh” aroma are carried out by a group of aroma-forming enzymes and PUFAs. The conditions of such aroma formation reactions are intensively studied on waste products of fruit, vegetable oils in the processes of biosynthesis of de novo synthesis and biotransformation.

4. The precursors of the fragrance may be glycosides, amino acids, carbohydrates or PUFAs, which because of the frequency and special features of use have obtained the status of a food supplement (flavor precursors, active elements) in some countries. Management of enzymatic reactions in situ is an alternative to the use of traditional flavors.

5. Isomers of polyunsaturated fatty acids as well as peculiarity of their reactions and concentration of oxidation products (primary and secondary) resulting from these reactions play an important role in the characterization of the product flavor. The study of flavor formation from lipids in vitro presents a number of methodological difficulties, so the lipid transformation by means of thermal, physical or combined treatment of plant material has been given little attention in scientific publications. There has been no published studies that would clearly define the role of desaturase in flavor formation in vitro by means of PUFA transformations. One of the main advantages of the use of plant enzymes in flavor recovery is the possibility of using enzymatic activity from a variety of sources to perform sequential reactions.

6. The participation of enzymes in the formation of fragrances and their recovery is an important and promising area of research. The increase in the concentration of aromatic components in the product, based on enzymatic processes, is advisable during drying in vacuum dryers with microwave heating, which facilitates the extraction of aromatic components. Under these conditions, there is a well-marked need for trapping the escaping fumes and using captured aromatic components.

7. In the world, the most important ability of aromas is their ability to adapt the taste sensations to reduce the amount of salt, sugar, fat in food. The change in the aromatic profile of plant raw

materials is associated with a decrease in organoleptically important amino acids, therefore studies of the restoration of aromatic components in the product by using legumes as carriers of aromatic precursors are promising.

8. In the development trends of flavors, an important role belongs to the study of neurohistronomy, intercultural preferences, organicity, the development of universal aroma testing panels, the development of flavored products for different age categories of consumers. The development of food science proves the urgency of developments aimed at the retro-nasal effect, i.e. On sensation of aromas mainly in the oral cavity. The flavors of trends important role belongs to the study neyrogastronomii, cross-cultural preferences, organic, development of universal test panels fragrances, scented products development for different age categories of consumers.

9. Innovations in aromatization continue to evolve, focusing on: quality food (natural flavors), time saving (fast food preparation), recreation and entertainment, meeting specific needs (vegetarian dishes, restrictive diet).

The factors of flavoring foods, such as:

- differentiation principles of healthy eating in the industry “fast food”;
- development of nanotechnologies in the food industry;
- repetition of natural processes of formation of aroma;
- the use of green leafy vegetables.

Aromatic components inherent in the Ukrainian nature are of great interest, especially in the vegetable sector.

CHAPTER 2

AROMA RECOVERY BY DISTILLATES

Sanogenesis is a dynamic complex of protective and adaptive mechanisms of the physiological nature. This term is one of the newest in a physiological science. The research of sanogenesis as the recovery process of the body self-regulation violations is associated with the use of flavoring agents. The world around us is an ocean of flavors and the sense of smell helps us find our way around in it. It gives an idea about any substance without any direct tactile or visual contact with it. The complex effect of aroma on the human body has been discussed in many research works, in particular in connection with the development of biosemiotics, the use of the concepts *Umwelt* introduced by Jakob von Uexküll and expounded by Thomas A. Sebeok. *Umwelt* (pl. *Umwelten*, means *the environment, the world* in German) is the biological basis for the study of communication species (including humans) [217]. According to Jakob von Uexküll, animals due to biological characteristics can have different *Umwelten* despite the unity of the physical habitat. In addition, a man is actually the only living species who doesn't have a certain *Umwelt*.

Therefore, it is important for the human body to have both food with natural aromas, and domestic flavors that are very close to them. Forecasting popular electronic devices of the future demonstrates the perspective of using capsules with concentrated aromas of nature, home comfort and other specific fragrances for use in homes and offices.

Johnson F. studies have showed that the confidence in the correct identification of the proposed order is expressed as a subjective probability that is on average higher than the real percentage of correct identification of order. There is a proven hypothesis that the more intense the order is, the researchers are more confident in its identification. This paradigm can be related to properties of aromas and emotional variables including a man's metamemory [218]. Therefore, the goal of the work is not only to chemically recover aromatic components, but to endue food with improved consumer properties.

This section discusses the issues of comparison and evaluation of industrial flavoring agents and prospects of liquid flavoring agents obtained in a microwave vacuum drier, specific processes of isomerism in aromatic components and the flow of enzymatic processes in the microwave field.

2.1. Characteristics of industrial liquid flavoring agents (distillates), and products with them

The problem of aroma identity and products marked by aromas of certain fruits has been discussed in the scientific literature in terms of psychology, metamemory, valence, neurogastronomy and others. According to the stoichiometric theory of flavor perception, the interaction of molecules of aromatic components with receptors is determined by geometrical factors. Despite the fact that this theory has not uniquely been accepted in classical studies, however, it is one of the most applied ones [219]. The melon liquid flavouring agent by the firm “GLCC Co” was analysed according to the size of micro particles in the concentrated form and after dilution 1 : 10 and 1 : 100 (Fig. 5).

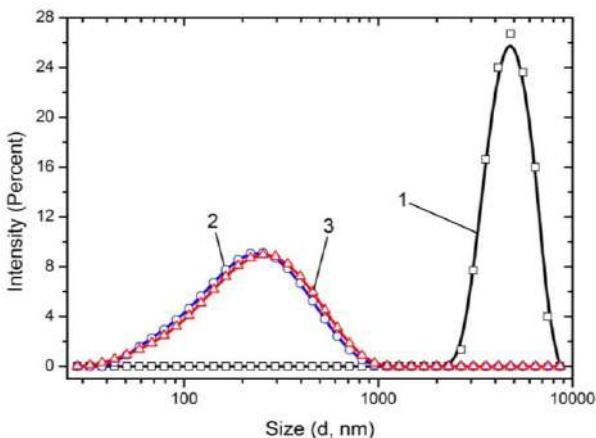


Figure 5 – Changes of the size of ether particles in the melon industrial flavouring agent: 1 – in concentrated form; 2 – at 1:10 dilution; 3 – at 1: 100 dilution

The analysis of the obtained results shows that after 1:10 dilution of the melon aroma concentrate, dimensional properties changed towards the reduction of hydrodynamic diameter from $4.138 \pm 0.274 \text{ nm} \pm 2,5$ to 185 nm , the polydispersion slightly increased from 0.220 ± 0.057 to 0.253 ± 0.014 . Further 1 : 100 dilution virtually does not change hydrodynamic diameter ($196 \pm 24 \text{ nm}$), but a slight increase in polydispersion to 0.327 ± 0.148 was observed that confirms homogeneity of the fractional composition of samples.

Changes of hydrodynamic diameter during 1 : 10 and 1 : 100 dilution can be associated with the presence of the solvent 1,2-propylene glycol in the composition of the flavouring agent. Obviously, the dilution of more than 100 times is not desirable in the context of flavour perception and the absence of significant changes in the size of micro particles of the flavouring agent.

Aroma in the samples of industrial flavoring agents in the various pH medium is characterized by rich floral and caramel flavors, has expressed shades that are not typical for melon, watermelon, pumpkin and similar to flavors of pears and flowers. The watermelon aroma (identical to the natural one) of VAPE company (OOO “Midgard”, Ukraine) and the fresh watermelon pulp was compared in beverages using an electronic nose (Fig. 6).

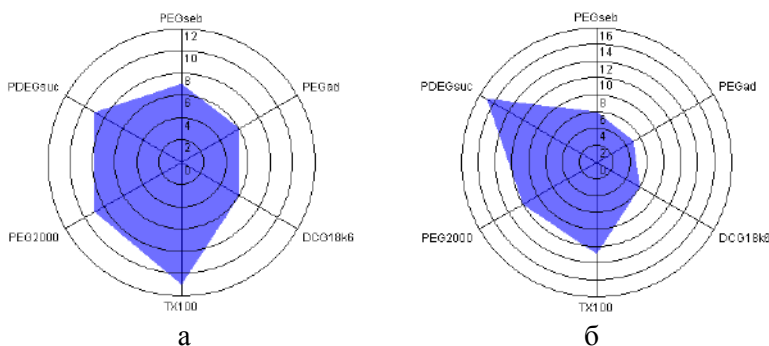


Figure 6 – Watermelon flavor:
a – beverage with a flavoring agent identical to the natural one (S = 167,58); b – beverage with fresh pulp (S = 206,11)

In radar charts of different copyprints, their area shows differences in aromatic components of the samples. A parallel comparison of flavors using sensory analysis indicates the absence of fresh notes specific to watermelon in the industrial sample of watermelon flavor. Watermelon is a member of the cucurbits family which includes squashes, melons, pumpkins and cucumbers. Aromas of cucurbits fruits have not been studied enough, although some of them were studied earlier, for example, CC of fresh cucumbers (*Cucumis Sativus*) were studied nearly 20 years ago. The most striking feature of volatile components of these fruits is diversity and magnitude of 9-carbon compounds. These compounds include nonanol, nonanal, various nonenols, nonadienols, nonenals and nonadienals. The identification of main volatile components of fresh and cooked pumpkin showed that almost all six carbon aldehydes and alcohols were lost in the cooked pumpkin [220]. The description of the products with pumpkin flavor (liquid pumpkin flavoring agent, coffee with pumpkin flavor, extract with pumpkin flavor, cake with pumpkin flavor, candle with pumpkin aroma, room aromatizer) carried out together with the taste panel indicates a large deviation between the samples with industrial and natural flavoring agents of pumpkin.

The products with a pumpkin flavoring agent were described by the taste panel as caramel, cream and fruit due to the high concentration of 2-methyl butanal (corresponding to the descriptors of caramel, sweet). The highest scores for fruit flavor were associated with the highest number of esters responsible for fruit notes, some sweet aromas of ripe fruits such as pears, melons [221]. The identification of pumpkin failed in all samples, both in e food products and household goods. This may be due to the lack of a key component (*Z, Z*)-3,6-nonadien-1-ol (pumpkin, cucumber, fresh).

Thus, industrial liquid fruit flavoring agents by performing the function of saturating a product with aroma typically give only some tones, such as fruit, caramel ones. Separation of liquid flavoring agents by raw material is conventional and does not fully reflect the flavor of the fruit from which it has been extracted. Obtaining liquid flavoring agents through pervaporation is the most common way; it

leads to accumulation of certain aromatic components. These components, being the key ones, do not correspond to the flavor of the original product after concentration and addition to it.

2.2. Response of the human body to differences in flavor

The issue of a person's flavor perception is being constantly discussed, thus it is important to study it more carefully. Many works have been devoted to the impact of food and other incentives to the flow rate and composition of human saliva [222]. The perception of food sensory attributes as specific irritants has been learned regarding the flow of saliva of parotid gland [223]. Hvinad D. et al have concluded that the parotid salivation can affect the rate of flavor release from a chewing-gum [224]. There are no definite conclusions about the stimulating effect of vanilla in custard desserts on the secretory function of salivary glands [225]. At the same time, flavor is referred to chemical stimuli, which innervate salivary gland through parasympathetic and sympathetic nerve fibers, along with mechanical and thermal stimuli. One aspect of this issue which has not been studied enough is the impact of various food flavors on the secretion of salivary glands. Among other reasons are both a large assortment of flavors added to foods, and the method of their production. The lack of compliance with the flavor of the original product leads to a distorted perception, disorder of food preferences and other sensory abnormalities.

Shefferdom has given an overview of recent advances in the area of brain mechanisms of flavor perception and has also suggested several hypotheses to integrate these mechanisms into the existing neural theories in order to justify the complex multi-system mechanism of flavor perception [32]. A series of studies has resulted in revealing subzones that are activated by food flavoring agents. They include orbitofrontal cortex (OFC), hippocampal gyri, anterior fusiform gyrus, and cingulate gyri. These are mainly areas of cerebral cortex or centers that are closely connected with them. Besides, it has been found that the association with pleasant food

products activates the medial OFC; bad foods activate the lateral OFC [32]. The signals from olfactory bulbs go to brain faster than any others (Fig. 7). Neuroscientists believe that aromas directly affect human subconscious in this way. The nerve impulse from olfactory bulb can go to almost any of the brain regions; but neuroscientists cannot predict yet to which one. It has been shown that the most favorable response is associated with the presence of aldehydes in flavor.

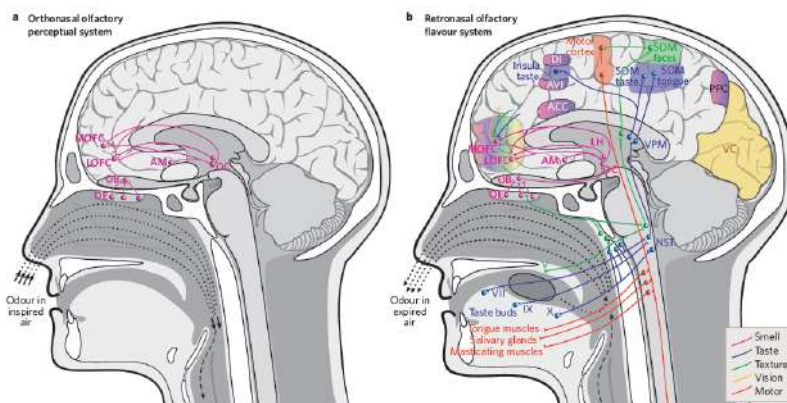


Figure 7 – Scheme of orthonasal perception and retronasal perception [32]

Stimulation of salivary glands by aromatic components is considered as orthonasal (extraoral) and retronasal (intraoral). The neural way of retronasal perception of flavor is much more complicated than orthonasal one [32]. The change of the length of the neural signal path may be caused by various factors, such as presence or absence of natural flavor of fresh fruit. Increased neural signal path can be explained by complexity of perception and recognition of flavor. The increase in neural signal path may correlate with the change in salivation rate, namely its reduction. The investigation of the process of the body response (salivation) to different flavors (solutions of liquid industrial flavoring agents, fresh fruit flavor, foods with natural flavorings) is an important factor for man.

The research started from a potentially stronger irritant to which we have referred flavor of fresh fruit. It was shown that the most favorable response in the OFC is associated with the presence of aldehydes in aroma [226]. A significant number of C₆-C₉ aldehydes are contained in the flavor composition of the pumpkins that were selected as research samples. All participants of the research rated flavor of fresh fruit as “++”; sialometry index was on average 1,25–1,45 ml/min. Compared to the reference values, these values are 2.0–2.2 times bigger. Additionally, it should be noted that all participants of the experiment described flavor of fresh fruit as stable, bright and expressive. The rapid identification and evaluation of flavor of fresh fruit resulted in increased salivation.

The next group of irritants included a – 0.1 % solution of flavoring agents extracted from cucumber, watermelon, and melon by pervaporation and vacuum distillation. When using vacuum distillation, the maximum number of C₆-C₉ aldehydes moves to the distillate, unlike pervaporation [37]. Previously it has been shown that the conditions of vacuum distillation activate enzymatic processes in fruits; it approximates the extracted flavor to the raw fruit material to the uttermost. The participants of the experiment indicated that liquid flavors cause different feelings and associations; they divided them into groups (Tab. 2).

Table 2 – Changes of salivation rate during testing solution of flavorings

Group number	1–4	5–6	7–8	9–10	11–12
Industrial flavorings					
Rate ml/min	0,95±0,05	1,15±0,05	0,75±0,05	0,65±0,05	0,5±0,05
Evaluation of flavor (+/-)	+	++	+-	-	--
Laboratory flavorings					
Rate ml/min	1,15±0,05	0,95±0,05	0,95±0,05	0,75±0,05	0,75±0,05
Evaluation of flavor (+/-)	++	+	+	+-	+-

The analysis of the data from Table 2 has showed that laboratory flavorings received more positive assessments and salivation rate (average for all participants) was 0.91 ml/min. The index of the secretory function of salivary glands is 1,23 times bigger than the reference value. Salivation rate during retronasal aroma perception of the solution of industrial flavoring depended on the recognition and pleasant associations, namely memory. The participants who could not identify aroma with some fruit perceived it rather negatively, looking for some characteristics. In such circumstances, salivation rate significantly decreased and was 0.8 ml/min (average for all participants); this value was 1.23 times bigger than the reference value. It can be noted that participants perceived food products with industrial flavorings more complicated, mainly because of smaller distinctiveness. While evaluating flavor as “– –”, salivation rate decreased by 10–12 % in comparison with the control value. Solutions of industrial flavorings had an expressed “top” note and very weak “lower” notes. This is due to presence of a small set of aromatic components in samples. Thus, flavor distinctiveness, presence or absence of natural flavor of fresh fruit in the solution of a flavoring agent can affect the way of the neural signal and may change it.

Saliva performs the following functions in the body: hydrolytic (breakdown of carbohydrates), bactericidal (due to lysozyme), protective (dilutes, buffers, helps to eliminate harmful and inedible substances), motor (wets and covers food with saliva, provides swallowing). The role of the secretory function of salivary glands is very important in the process of eating. For this reason, for an objective understanding of retronasal perception of flavor, drinks with laboratory and industrial flavorings were tested. The full assessment of the impact of a flavoring on organoleptic properties of food is only possible when the results of tasting the finished product are used [227]. The participants of tasting were conventionally divided into three groups according to the degree of emotional perception of drinks, as presence of essences for some of them was an unpleasant fact or the essence of flavor was not perceived during the research (Tab. 3).

Table 3 – Changes of salivation rate during testing drinks with flavorings

Group number	1–4	5–8	9–12
Industrial flavorings			
Rate ml/min	0,95±0,05	0,75±0,05	0,65±0,05
Evaluation of flavor (+/-)	+	+ -	-
Laboratory flavorings			
Rate ml/min	1,15±0,05	0,95±0,05	0,75±0,05
Evaluation of flavor (+/-)	++	+	+ -

Salvation rate during retronasal perception of flavors of drinks depended on a set of multisensor senses: recognition, pleasant associations, flavor saturation, its distinctiveness and others factors. Salvation rate during drink testing was higher than the level of the control sample. Sialometry rate (on average) of laboratory flavorings was 20 % higher than of industrial ones.

Thus, a group of natural flavorings affects the functioning of salivary glands as a chemical irritant, regardless of the method of their production. In a product (drink), strength of stimulus of flavor is much smaller compared with aroma of fresh fruit. This fact should be taken into account during aromatization, giving priority in this regard to fresh fruit. It has been found to some extent that recognition and pleasant associations are the factors that can affect the neural signal path length and response rate (salivation). Increased neural signal path can be explained by complexity of perception and recognition of flavor, its low distinctiveness, lack of identification with a particular fruit. So proximity of laboratory flavorings to fruits in composition has simplified their testing, evaluation and perception. As this study has not had any similar ones, final conclusions can be drawn after repeated retronasal testing of participants from different age groups and their perception.

So, the influence of natural flavorings on the functioning of salivary glands during retronasal perception has been shown. It has been determined that the flow rate of saliva is the most pronounced during retronasal perception of fresh fruit; it is 1,8–2,0 times higher than the control measurements. Sialometry index for natural flavorings is 1.2–1.3 times higher than the control measurements if a flavoring belongs to the group of well-known or pleasant ones.

2.3. Characteristics of distillates obtained in vitro

The methods for making aromatic concentrates both from juices and fruit marc have been developed in several stages. In a vacuum pan, fruit and berry marc is filled with water and steamed in order to transfer volatile fractions into distillates; as adverse qualitative changes rapidly occur in them they are processed by fractional distillation to produce a concentrate. This process is accompanied by a significant loss of flavor components; so the peculiarity of the heat-treated fruit marc is the actual absence of esters, except from ethyl acetate. The process of obtaining concentrated flavors depends on the characteristics of the marc (by acid indicator, alcohol content, features of growing mouldy) and the proper choice of their processing modes (degree of their humidity and the expected duration of the process) [228]. Therefore, the use of MVD to obtain aromatic distillates may be the best option for studying the aroma recovery process using distillates from fruit marc.

Physical and chemical parameters and sensory evaluation of distillates are an important indicator in the process of manufacturing alcoholic drinks, juice [229]. The distillate formed during drying is an accumulator of the volatile components emitted from the raw material into the air space [230, 231]. To make the analysis of aroma changes objective, we investigated the balance of aromatic components in the raw material itself: fresh one, with altered pH, frozen one, dehydrated in the microwave vacuum drier and in vivo. According to the content of essential oils, the raw materials were chosen as follows: cucumber marc <pepper leaves <walnut leaves <basil. The raw material was put into the microwave vacuum drier minced as strips of 10–15 mm, cucumber marc was not subject to additional grinding. Drying fresh leaves to reach the moisture content of 15–20 % lasted for 12–14 minutes; duration of drying cucumber marc was 20–23 minutes. The vacuum was adjusted in the range of 30, 40, 50 kPa (Tab. 4).

Table 4 – Change in the number of flavors in the raw material and distillates, mg Na₂S₂O₃/1g

Names of samples	Cucumber marc		Pepper leaves		Walnut leaves		Basil	
	dry residue	condensate	dry residue	condensate	dry residue	condensate	dry residue	condensate
Fresh raw material (drying under pressure (control)								
30 kPa	6,2	17	4,0	19,2	5,6	18	3,5	33
40 kPa	5,0	12	3,3	14,5	3,0	15	3,5	22
50 kPa	5,5	9	3,5	12,8	3,8	13	3,8	22
Raw material dried naturally	10	–	14	–	18	–	25	–
Frozen raw material (drying under pressure 30 kPa)	3,8	18	2,8	20	3,5	19	3,0	34
Raw material processed by alkaline solution pH = 9	3,8	10	2,8	14	3,3	12	3,0	14
Raw material processed by acid solution pH = 3,5	3,5	15	2,5	16	3,3	16	3,0	19

The depth of 40–50 kPa vacuum affects the yield of aromatic components in the condensate: the number of flavor decreases in the dry residue and increases in the condensate at 40 kPa. The vacuum of 30 kPa activates intracellular processes during which the flavor content simultaneously increases both in the dry residue and in the condensate. The number of flavor in the distillate is not dependent on the initial content of essential oils in the raw material as it increased by 7 mg Na₂S₂O₃/1g in the distillate of cucumber marc, by 5 mg Na₂S₂O₃/1g in pepper, by 8 mg Na₂S₂O₃/1g in basil. In the frozen raw material (–18 °C), the number of aroma in distillates increased by 2.1 mg Na₂S₂O₃/1g due to mechanical disruption of cell membranes and release of aromatic components. When processing the raw material with an alkaline solution, deterioration of distillate

quality and unnatural flavors were marked. Yield of aromatic components and qualitative composition of distillates during acid treatment is higher compared with alkaline processing. At the same time, the dry residue has low values of flavor number as compared with the raw material without acid treatment. This indicates termination of enzymatic processes in the raw material with a low pH, and on the contrary testifies about activation of intracellular enzymes from natural pH raw material.

The increased number of flavor in the condensate during MVD is explained by enzymatic synthesis of new aromatic components because there are precursors of volatile compounds in the raw material, the enzymes necessary for the reaction of formation of aromatic components are activated. The temperature of the process at 30 kPa (not higher than 40 °C) contributes to the activation of aroma-forming enzymes.

Physical and chemical properties of laboratory distillates are the following: light transparent slightly yellowish liquid, 4.5–5.0 pH, unit weight of 0,833–0,915, well soluble in alcohol and other organic solvents. The content of soluble solids in the condensate is 0.1 %. They can be stored at the temperature of about 4 °C for 2 months, be resistant to microbial spoilage, preserve flavor unchanged during the whole storage period in a hermetically sealed container. We have found that distillates were concentrated by freezing out water (cryotreatment). Optimal cryotreatment parameters depend on the amount of organic components and their qualitative composition determining flavor richness. The optimal freezing temperature for flavor number of 2.0–2.5 mg Na₂S₂O₃/1g is minus 12 °C and for the range of 2,6–3,0 mg Na₂S₂O₃/1g is minus 18 °C. The conducted organoleptic evaluation of aromatic concentrates has confirmed viability of concentration.

We have found that the flavor distillates obtained by MVD at 30 kPa are similar to the flavor of the raw material by organoleptic characteristics but contain several times more aromatic components than the raw material. This feature of distillates is connected with the enzymatic formation of aromatic components during processing in the microwave vacuum drier.

Easily volatile components of melon and cucumber are mainly represented by ethers, alcohols, aldehydes, and acids transformed into the distillate during distillation. Aldehydes, alcohols, and acids dissolve due to condensation; esters, lactones, diethers of hydrates aldehydes (acetals) occur in the form of insoluble micro particles in the obtained distillates. These micro particles are soluble in alcohols and organic solvents, but they create definite dispersion in the aqueous medium. The number of carbon atoms in insoluble micro particles affects their molecular weight, size, and aroma serving as a specific identifier in studies. Sizing of esters and acetals in distillates is a promising area of the analysis of the exhalation processes of volatile components during distillation.

Flavour of esters, acetals, and lactones depends on the number of carbon atoms in initial compounds. Aliphatic esters in different combinations play an important role in many fruit aromas; in addition, they are carriers of floral and fruit, caramel flavours, and a variety of shades: apple, strawberry, pear, pineapple ones and others. The major aroma components of melon distillate are esters of hexyl octanoate, hexyl acetate, propyl acetate, and ethyl butyrate.

The main components of cucumber flavour are water-soluble aldehydes (E, Z)-2,6-nonadienal and (E) -2-nonenal. Aldehydes are highly reactive components and together with alcohols they create a strong aroma of fresh fruit, vegetables, and new-mown grass. However, the share of ester (cis-3-hexenylacetate) in flavour of new-mown grass is about 40 % of the total number of aromatic components. Insoluble acetals which are typical for cucumber flavour are represented by (E, Z) -2, 6-nonadienal diethyl acetal, di-(Z) -3-hexen-1-yl acetal. Cucumber distillates gain their aromatic shade due to insoluble ester ethyl 3- (methylthio) propionate and divinyl esters of PUFA. The chromatographic analysis of melon and cucumber distillates showed the presence of complex esters and acetals about 18–20 %; their composition was somewhat different depending on the type of fruit, the maturity stage and growing conditions. The study of distillates during convective distillation demonstrated the presence of water-insoluble particles with different hydrodynamic diameter (Fig. 8).

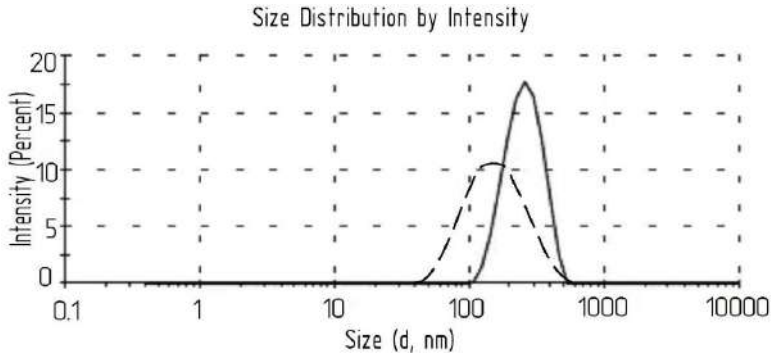


Figure 8 – Distribution of distillates particle by size

The molecular weight of acetals in the cucumber distillate on average is slightly bigger than the molecular weight of esters in melon which is reflected in polydispersive properties of particles. Hydrodynamic diameter of particles in the cucumber distillate is 336 ± 36 nm with polydispersion $0,436 \pm 0,083$. Esters in the melon distillate have particle sizes 153 ± 2 nm and polydispersion $0,211 \pm 0,022$. The molecular weight of acetals (E, Z)-2, 6-nonadienal diethyl acetal, di- (Z)-3-hexen-1-yl acetal is 212,3 and 226.4, respectively, and the molecular weight of esters in melon on average is 118,2 [232]. Due to the bigger molecular weight, most acetals have slight flavour or no flavour at all. The flavour intensity in the melon distillate was higher than in the cucumber one, which corresponds to the difference in their polydispersion. It should be noted that depending on the variety, growing location, maturity, storage conditions and other factors, the numerical values of hydrodynamic diameter in cucumber and melon distillates could have other values different from the mentioned above. But described interrelations remain the same.

It has been found out that more than one peak and correspondingly different values: 200 nm, 300 nm, 400 nm and 600 nm were observed in distillates under repeated measurements of hydrodynamic diameter of particles. This indicates that micro particles are more likely to have an irregular form of droplets or micelles. The study of

the micro particle behaviour during dilution in water and alcohol has proven the hypothesis about the particle droplet structure and hydrophobic nature. For example, interfusion of the cucumber distillate on a magnetic stirrer causes the change in the hydrodynamic size from 456 ± 121 nm and polydispersion $0,546 \pm 0,058$ to 273 ± 18 nm and polydispersion $0,454 \pm 0,049$. These changes are the result of intermolecular interactions; so they depend on the behaviour of the dissolved substance on the molecular level.

Customer benefits are represented in the laboratory samples, as their flavor is close to the one of the used raw material by richness of volatile substances, in contrast to industrial samples which have dominant components. The use of the developed method for obtaining and concentrating aromatic components is promising to implement due to peculiarities of intermolecular interactions.

Hydrophobic groups of added solutes interact weakly with neighbouring water molecules, as if they prefer a non-aqueous medium. However, these weak interactions can have profound structural after-effects. The formation of special structures in water next to incompatible nonpolar substances is called “hydrophobic hydration” (fig. 9, tab. 5). If there are two isolated non-polar groups, their incompatibility with an aqueous medium encourages their association, thereby reducing interfacial surface “water-nonpolar substance”. This process is thermodynamically favourable and is partly reverse to hydrophobic hydration; it is called “hydrophobic interaction”.

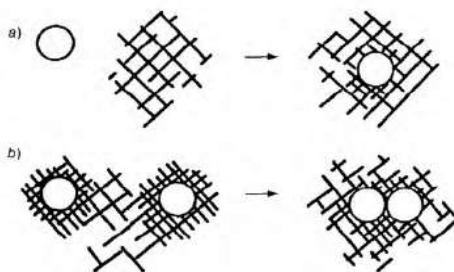


Figure 9 – Schematic map of hydrophobic hydration (a) and hydrophobic association (b). Circles represent hydrophobic groups, and the shaded area – water (Franks F., 1975)

The processes of hydrophobic hydration and hydrophobic interaction of distillates were investigated and visualized during dilution of distillates in bidistilled water at the ratio of distillate : water 1 : 1, 1 : 10, 1 : 20, and 1:40 at 25 °C (tab. 5). Taking into the consideration that the behaviour and size of micro particles depend on the concentration and nature of the solvent, the samples of distillates were mixed with alcohol (96 % vol) at 0.1 % concentration at temperature 25 °C (Fig. 10.1–10.4).

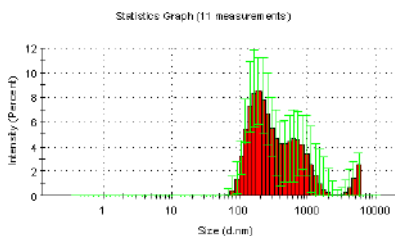


Figure 10.1 – Melon distillate (with an alcohol)

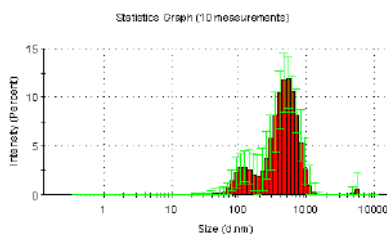


Figure 10.2 – Mellon distillate with changed pH (citric acid solution, 1 %)

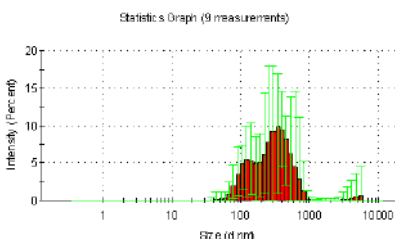


Figure 10.3 – Cucumber distillate (with an alcohol)

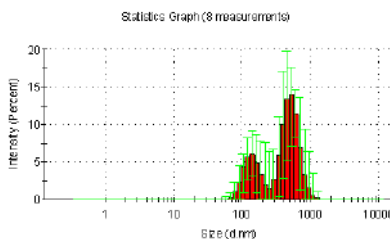


Figure 10.4 – Cucumber distillate with changed pH (citric acid solution, 1 %)

Changes in the hydrodynamic size and polydispersion of droplet micro particles in the alcoholic solution shown in the figures indicate the coacervation process. The mass in melon and cucumber distillates is divided into two fractions with different hydrodynamic sizes. Larger droplets are coacervate; they are a multimolecular

complex or drops with higher concentration of the colloid (dissolved substance) than in the rest of the solution with the same chemical composition.

The initial pH of distillates is 7 and aroma is conveyed with the intensity similar to fruit. To analyse aroma shades when changing the pH, the distillate was concentrated as qualitative and quantitative changes of flavour occur with different pH values. Together with the pH, the perception of the same volatile compounds in different food mediums also changes. Acetals and esters are converted into the initial aldehydes, acids, and alcohols in the acid and alkaline medium. During the research, flavour changes were analysed in the weak acid medium pH = 6,0 and acid medium pH 3,5. Acidity was adjusted using certain concentration of citric acid. Salts of corresponding acids are formed in the alkaline medium; they bind aromatic components and help remove flavour from the analysed medium, which contradicts the research objectives.

Table 5 – Characteristics of Distillates

Name	Melon Distillate	Cucumber Distillate
Size of the particles, nm		
Dilution (water) 1 : 1	283±0,51	282±20
Dilution (water) 1 : 10	246±31	253±51
Dilution (water) 1 : 20	310±14	260±28
Dilution (water) 1 : 40	390±22	291±31
Flavour		
Concentrated distillate (pH = 7)	Melon, with honey shade	Vegetable, with mushroom and cucumber shades
Distillate (pH 6,0)	Fruit with saturated/rich melon shade	Cucumber with vegetable shades
Distillate (pH 3,5)	Melon with extrinsic shades	Fresh saturated/rich cucumber

Changes of the particle hydrodynamic size in the distillate when water diluted prove the assumptions given above about their hydrophobic nature and the presence of the results of such processes as

coacervation, hydrophobic hydration, and hydrophobic interaction. Since water and nonpolar groups are antagonistically related, the structure of water is adapted to minimize contact with nonpolar groups. Two aspects of this antagonistic relationship are considered: formation of clathrate hydrates and association of water with hydrophobic groups.

The distillate concentration allowed to perceive flavour in samples better and to give them the characteristic that serves as a comparison standard. Changes of the medium pH and the solvent nature effect organoleptic properties of the distillate during distillation. Substantial transformations in the acid medium are recorded in cucumber samples. We can admit that acetals (E, Z) -2, 6-nonadienal diethyl acetal, di- (Z) -3-hexen-1-yl acetal were converted into aldehydes from which (E, Z) -2,6-nonadienal, (E) -2-nonenal, and cis-3-hexenal were formed. The changes of the medium pH in the melon distillate also resulted in a variety of aromatic reactions: melon flavour was more intensive and vivid in the weak acid medium; but it was less distinct with prevalence of non-typical shades in the acid medium.

The shelf life of some products was prolonged and significantly increased due to certain natural compounds, such as phenols, aldehydes, and organic acids present in plant extracts and spices, which demonstrated antimicrobial effects [233]. Caccioni et al. (1997), Hardin et al. (1997) reported about antimicrobial effects of C₆ aldehydes obtained from plant tissues by lipoxygenase oxidation. Toxicity of C₆ aldehydes protects the injured area from the microorganisms causing their decomposition; it shows effectiveness of hexanal as an exchange fungicide. Interconversion of other aromatic volatiles from hexanal is minimum (Guerzoni, 1997). The effects of hexanal as a packaging atmosphere component affecting shelf life and evolution of natural populations of microorganisms in fresh apple slices during storage at 4 and 15 °C [234] have been shown. The research of the content and composition of fractions in seeds, leaves and fruits containing aldehydes have demonstrated that hexanal and nonanal were the most active compounds among short-chain aldehydes. The study [235] has shown that saturated aldehydes

did not have significant antibacterial activity, but unsaturated aldehydes demonstrated wide spectrum of antimicrobial activity.

The study of melon fruits is basically focused on the content of sugars, amino acids, and colorants. Key components of juice are usually considered in the study of aromatic components. In this regard, we have studied the content of aldehydes in the flavor components of the fresh red and white melon pulp and melon rinds by analyzing them using a gas chromatograph “Color 100”. According to our investigation, the highest concentration of C₆–C₉ aldehydes (85 % on average) in the general content of aromatic components was established in the white pulp (90.6 mg/kg). The content of aromatic components was 68.7 mg/kg in the red pulp, 77 mg/kg in rinds, whereas aldehydes accounted up to 55 % on average.

Aldehydes are concentrated mainly in the watermelon white pulp and partly in its red pulp. The samples of distillates were extracted in the microwave vacuum drier after 20, 30, 40, 50 minutes to determine the effect of the aldehyde concentration on the microbiological contamination: each sample was analyzed after 30 days of storage at 4 °C in a sealed container (table 6). The control sample – watermelon pulp (white and red) that had not been processed in the microwave vacuum drier – was tested for microbiological contamination after 15 days of storage.

Table 6 – Distillates of watermelon white and red pulp

Time of extracting aldehydes	Watermelon red pulp		Watermelon white pulp	
	Aldehydes, mg/100 g	Microbiological contamination	Aldehydes, mg/100 g	Microbiological contamination
Control	22	10 colonies	50	4 colonies
20	2	7 colonies	30	2 colonies
30	2	7 colonies	32	1 colonies
40	7	5 colonies	34	1 colonies
50	10	3 colonies	40	1 colonies

The decontamination effect of the microwave field (compared to control) and the presence of C₆–C₉ aldehydes in the distillate of watermelon white pulp ensures extra microbiological stability of the

samples. When increasing concentration of aldehydes (the duration of extraction) and the action of the microwave field, this effect enhances.

The effect of C₆–C₉ aldehydes in the condensate from watermelon white pulp, cucumber marc, and sprouted wheat on the reduction of microbiological contamination of curd-based products has been established. To determine quality and safety of the developed recipes of appetizers “Spring breath”, “Vitaminous”, microbiological parameters were defined and compared with regulatory documents and compared with the control sample “Cheese pasta with green onions” (Fig. 11.1, 11.2).

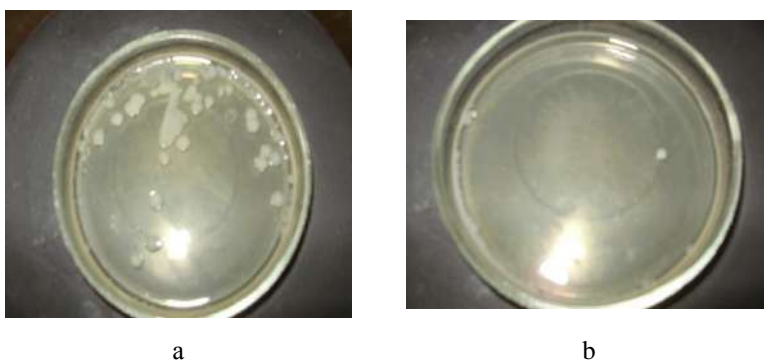


Figure 11.1 – Microbiological contamination: a – “Cheese pasta with green onions”, b – appetizer “Spring breath”

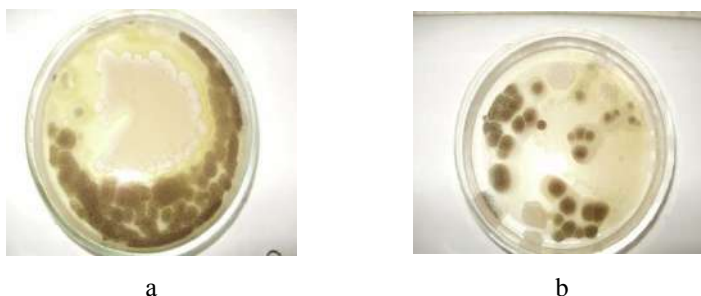


Figure 11.2 – Fungi: a – “Cheese pasta with green onions”, b – appetizer “Spring breath”

The results of the study indicate that the developed recipes have better microbiological parameters compared to the analogue recipe. These studies confirm antiseptic properties of aldehydes. The amount of microbial contamination decreases three times when adding distillates and 1.4 times when adding fungi. So adding the distillate improves both organoleptic and microbiological parameters of ready meals.

It has been established that optimal cryotreatment parameters of distillates depended on the amount of organic components and their qualitative composition determining richness of flavor. The optimal temperature of freezing out for the number of flavors 2.0–2.5 is minus 12 °C and for the range of flavors 2,6–3,0 is minus 18 °C.

2.4. Change in the aromatic profile of the raw material and distillates in the microwave field

The conditions for transition of volatile substances in the distillate during distillation depend upon a variety of factors and determine their diverse composition [236]. The main operating parameters, including the extraction time (30–38 min), capacity of microwave radiation and relative humidity weight, were investigated when extracting the essential oil from bayberry fern (*Dryopteris fragrans*) in SFME [237]. Essential oils of basil, garden mint and thyme extracted by SFME for 30 minutes were similar in quantity (yield) and quality (the aromatic profile) to the results obtained by conventional hydrodistillation for 4.5 h. SFME method yields an essential oil with higher amounts of more valuable oxidized compounds compared to hydrodistillation (HD) [238]. Oxygen-containing compounds are very fragrant and more valuable than monoterpenes. Their higher proportion in essential oils from SFME is related to the decreased thermal and hydrolytic effects, compared with HD.

The changes in the cellular structure of basil during convective and microwave treatment have been investigated. The leaves were treated for 15 minutes in the microwave field and for 15 minutes in the convection oven at 80 °C. Both types of heat treatment led to structural changes in basil leaves in comparison with the fresh sample (Fig. 12).

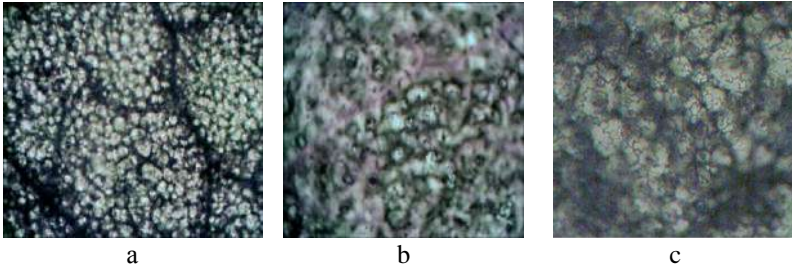


Figure 12 – Microstructure of basil leaves:
 a – after microwave treatment,
 b – fresh leaves, c – leaves after convective drying.

The effect of microwave treatment resulted in a significant reduction of cell sizes and consolidation of cells. During convective treatment, the samples, on the contrary, increased in size. Atrophic changes have opposite values in the samples. Li X.J. et al. Similar studies of *Dryopteris fragrans* have revealed that perforation was clearly observed on the surface of a leaf and most of the sample was completely destroyed after SFME (30–38 min); but only few gaps appeared in the sample after HD extraction (5 hours) [237]. This difference in changes of the leaves during microwave treatment is connected with initial moisture of leaves of *Dryopteris fragrans* and basil, as during preparation the leaves of *Dryopteris fragrans* were soaked for one hour, and the basil was used without soaking.

Microwave radiation has a significant impact on all components of the cell wall, which is proved by chemical analysis, UV and IR spectroscopic studies [239]. The formation of aromatic components of different chemical composition or isomeric forms under microwave and convective heating can be studied using awakened seeds. It is known [240] that seeds contain a certain amount of lipids: watermelon (43–48 %), melon (25–34), cucumber (32–37), tomato (24–31), carrot (10–15 %), flax (33–51 %). Hydration and awakening of seeds lead to the activation of numerous enzyme systems, including lipases, lipoxygenases, hydroperoxide lyase, etc. Numerous reactions begin to take place during subsequent seed grinding in an aqueous medium (ratio 1 : 3) and heating the obtained suspension to

the temperature of 43 ± 2 °C, including aroma formation from PUFAs of seeds. The maximum formation of flavor from PUFAs is usually 20–30 minutes, because longer incubation results in extraction of polyphenols of episperm, inhibitors of oxidative reactions. Therefore, the suspension contains aromatic components after a specified time interval. The particular method for supplying heat to the aqueous solution of aromatic components causes differences in the sensory evaluation of the flavor produced by enzymatic reactions from lipids. The suspension of awakened seeds was heated by convective method and in the microwave field to the boiling point three times with an interval of 10 minutes. Since in an aqueous medium aromatic components are divided into easily volatile (azeotropes that can escape from the mixture with the solvent) and semivolatile (nonazeotropes), the flavor was evaluated at a time interval (table 7). The aroma of the suspension without heating (the control sample) is described for all samples as slightly sour, milk, without specific feature.

Table 7 – The aroma of aqueous suspension of seeds

Aqueous suspension of seeds	Duration (minutes) of heating and method of heat supply					
	10		20		30	
	Convective heating	Micro-wave heating	Convective heating	Micro-wave heating	Convective heating	Micro-wave heating
Water-melon	similar to control	fresh	fruity	green	boiled fruit	grassy
Melon	fresh	similar to control	cooked cereal	raw bean	fruity, sweet	pineapple with sugar
Cucumber	similar to control	fresh		grassy	woody	cucumber
Carrot	similar to control	fresh	harsh	green kernel	grassy	carrot
Tomato	similar to control	fresh	no	fruity, sweet	woody	fruity, sweet

The flavor of the samples of seed suspension in the microwave field was different from the one of convectively heated samples.

During the first 10 minutes of microwave heating, fresh shades dominated in the samples (except from melon seeds) due to the formation and separation of volatile C₆- aldehydes. Subsequently, there were flavor changes due to residual lipoxygenase activity and the accumulation of lipid hydroperoxides as a result of heat treatment of the aqueous lipid suspension of seeds, availability of acids and proteins. The appearance of woody flavor is explained by influence of the microwave field on lignin-like substances. Hydrolysis of polysaccharides into glucose in the microwave field affects the aromatic nature of components. The efficient emission of macromolecular structural components facilitates rapid changes in plant cells and formation of flavor during the first stage. The combination of awakened seeds in different proportions when heated gives a bouquet of aromatic component and a wide range of new perceptions.

The processes of cis-trans isomerism can occur in the microwave field. Acetals and esters have chiral centres and, therefore, potential stereoisomers. So while analysing the efficiency of the heat mode (convection and microwave ones) we should take into account the formation of enantiomers (mirror-shaped molecules). Enantiomers (optical isomers) have the same physical properties (boiling temperature, vapour pressure, identical vibrational spectra, etc.), but different aromatic characteristics. For example, carvone, limonene, 1-octene-3-ol, 3-methylbutanal have various flavours in different spatial configurations. In the distillates under consideration, acetals and esters may form optical isomers (fig. 13).

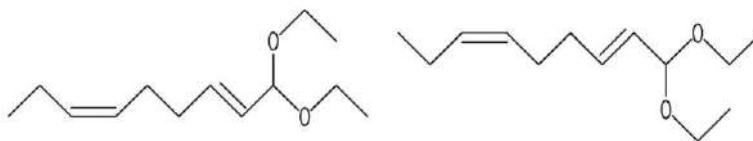


Figure 13 – Cis- and trans-isomers
of 2, 6-nonadienal diethyl acetal

Sizes of the particles of ethers and acetals insoluble in water in distillates differ neither in convective distillation, nor in microwave

one. During dilution of samples, the nature of changes under volumetric heating remains the same as under convection one. The aromatic distillate profile obtained in the microwave heating plant was different from the samples with convection heating of the suspension (table 8).

Table 8 – Characteristics of distillates flavour

Name of characteristics	Cucumber distillate		Melon distillate	
	Convection	Microwave	Convection	Microwave
Characteristics of main aroma	Vegetable aroma, cooked/boiled tone	Fresh cucumber aroma	Melon aroma, fruit syrup tone	Melon aroma, pear tone
Identified shades – (except main aroma)	Grassy, green, vegetable	Fresh, sweet pepper, green rind, fresh pumpkin, kiwi	Carrot, fruit, ether, sweet	Pineapple, wild strawberry, honey, banana
Aldehydes, % mg	0,035	0,084	0,026	0,028
Aroma number (sensory)	2,0	3,1	2,1	2,4

The reaction products of biosynthesis and aromas resulted from these reactions depend on the position of the hydroperoxide group in fatty acid and enzyme isoform that vary in the microwave field. The splitting products of PUFA in cucumber suspensions contain double bonds and intact pentadienoic systems. These systems of double bonds undergo the hydrogen atom abstraction that results in formation of additional splitting products and intense flavours inherent in cucumbers. The presence of C₆–C₉ carbonyl derivatives is less important for melon flavour than for cucumber one, so the difference in the microwave and convective heating of the homogenate is not so notable.

Ethers and acetals in distillates affect fruit tones, have a synergistic effect, increase and emphasize fullness and complexity of flavours. Their presence contributes to the distillate quality improvement by providing bright and fresh shades. Many flavour chemi-

cals can exist in one of the several isomeric forms or as mixtures thereof. Molecules with similar structure do not always produce the same shades of flavours (such as vanillin and isovanillin). Conversely, there are compounds with similar flavour but different molecular structure. For example, both benzaldehyde and tiglic aldehyde have almond aroma. Selective heating in the microwave field allows regulating the composition of volatile components which form quality of the aromatic distillate to be obtained. The effectiveness of isolation and quality of aromatic distillates depend on specific stereochemical positions.

2.5. Enzymatic production of aromatic components in distillates

Isolation and extraction of the plant aromatic complex depends on the mode and intensity of the heat effect on aroma-containing structures of the raw material. The brief exposure at high temperature (80 °C) is the most commonly used in industry when there is an intense flavor separation from one or more accumulation points, depending on their sensitivity to temperature. The method of heat treatment at low temperature (32±5 °C) and the prolonged exposure was developed in order to increase the concentration of aromatic components by activating the specific enzymatic complex of the raw material [241]. The mechanism of enzymatic processes is specific and has many particular features; so with this method, flavor of the final product will be different at various heat input methods (volumetric and surface) to the product. For example, various heat treatment of basil leaves contributes in each case to occurrence of special components of aroma. Both identical components (eucalyptol, heptan-2-one, eugenol, phenol, 1,6-octadien-3-ol, mono (2-ethylhexyl) ester, 1,2-benzenedicarboxylic acid), and specific ones appropriate to this type of pretreatment were identified in the samples with preliminary freezing (№ 1), convective processing (№ 2) and combined processing (№ 3) (Table 9).

Table 9 – Aromatic components and characteristics of basil flavor

№	Distinctive aroma components	Flavor characteristics
1	1,3,6-octatriene, 1-Hepten-6-one, 7-Octen-2-ol, 2-methyl-6-methylene, acetic acid, hexyl ester, limonene, (E)-2-butenylcyclopropane, 1,3,6,10-Dodecatetraene, 1,4-methanophthalazine, 1-Cyclopentene, 6,6-dimethylcycloocta-2,4-dienone	Basil, ethereal, spicy
2	2-aminobenzoate, 3-cyclohexene-1-methanol, 4-trimethyl-, (S)-p-menth-1-en-8-ol, octahydro-7-methyl-3-methylene-4-(1-methylethyl), 2-methylenecyclopropyl	Grassy, hay, of specific bitterness
3	1,5-heptadiene, 1,3,6-heptatriene, trans-isobornyl acetate, 1-naphthalenol	Saturated basil, fresh, clove

The identified aromatic components of basil leaves differ from the well-known ones due to the heat treatment temperature range and heating method. In the sample with leaves pre-frozen and then defrosted in the microwave field, the presence of ether shades is explained by specific enzymatic processes during and after thawing [26]. In the sample with convective and microwave processing, the sequence of thermal effects on various enzyme-containing structures is different. The shade of fresh hay prevails with convective processing, and a pleasant clove shade dominates with microwave processing. Thus, the way of heat input and the method of heat treatment at low impact temperatures on the raw material influence the product overall aroma profile.

During the combined treatment in the microwave vacuum drier, emission of aromatic components with water vapor in the condensate is carried out according to the following pattern: aromatic components are emitted discretely during a uniform exudation of moisture (Fig. 14, 15).

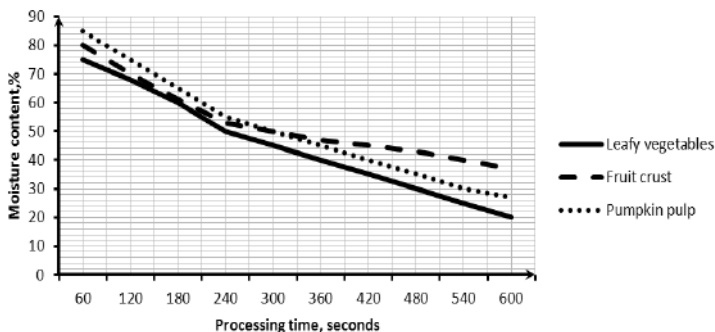


Figure 14 – Dynamic exudation of moisture during dehydration in the microwave vacuum drier

The difference in the emission of aromatic components during aroma extraction in the distillate is dependent on certain conditions:

- activity of certain enzymes and the time required for flavor production;
- timely removal of the group of azeotropes from the raw material; these azeotropes are the end product of enzymatic reactions inhibiting their flow at a certain concentration.

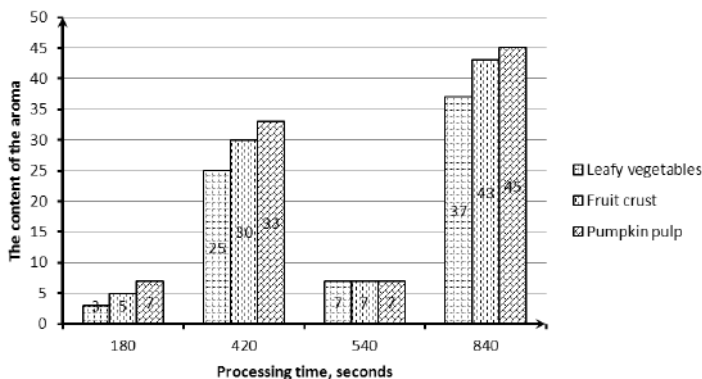


Figure 15 – Dynamics of extraction of aromatic components during dehydration in the microwave vacuum drier

During the first period of dehydration in the microwave field (5–8 minutes) by heating, intercellular gasses containing easily volatile ethereous substances become less dense. In this case, the division of aromatic components into azeotropes (ethers, acetals) and noneazeotropes (aldehydes, alcohols) is likely to occur. The first ones when separated from the raw material were condensed in refrigerators in the amount of 20–22 % of the total weight of the condensate. In the second period (9–15 min), aldehydes, organic acids were extracted through the cytoplasmic membrane; their availability slows down the evaporation of volatile components increasing flavor durability.

We used [242] different distillation methods of components (E, Z) -2,6-nonadienal (NDE), as well as (E) -2-nonenal (NEA) from cucumber juice, which have not been considered successful. The stability of aldehydes in the distillate was achieved after slowing down the action of the alcohol dehydrogenase enzyme containing zinc by the sequestrant of 1,10-phenanthroline (PTL). The effect was observed not only in slowing down the destruction of aldehydes, but also in the reduction in the speed of alcohol formation. Due to the lack of efficient natural sources of obtaining nonadienals (NDE), their synthetic analogues are mainly used. Therefore recovery and condensation of C₉ aldehydes from natural sources in distillates is an alternative to artificial flavoring agents.

The main advantage of the use of microwave radiation is the rapid extraction process [243–245]. We have developed the method for preparation of a multifunctional malt additive in the microwave vacuum dryer where the process of dough fermentation is accelerated due to extraction of amylolytic enzymes. Selective heating of the components of cell walls in the microwave field is one of the important factors that distinguishes this type of heating. For membrane-bound enzymes (lipoxygenase, hydroperoxide lyase), the substrate is components of the cytoplasmic membrane – a bilayer of lipids. Specific heat capacity of the substrate is lower than heat capacity of water, so the lipid layer can be heated rapidly in a microwave oven, faster than water; it is very important for hydrophobic-hydrophilic reactions of PUFAs with enzymes.

From this point of view, for the cytoplasmic membrane where PUFAs and enzymes are concentrated, the effect of heat capacity becomes the determining factor for enzymatic reactions of aroma formation to take place. The advantages of microwave activation of enzymes were investigated in the following way:

- Moisture from spice-aromatic herbs (chopped into strips of 7–10 mm) in the amount of 50 % was removed in several ways: in the microwave field, by pressing (control) and by cryodestruction.

- The partially dehydrated raw material was mixed with salt (salt and herbs ratio was 1 : 1) and dried to a final moisture content of the herbs on the average about 10 %. Drying was carried out by heating the raw material to the temperature of 60 °C in a convection drier and a microwave oven.

- Herbs were blown away from the surface of salt by air separation and the salt aromatization process was evaluated after one hour and 72 hours, as well as after dissolution in water (desorption) (Tabl. 10).

Table 10 – Number of flavor (mg Na₂S₂O₃/100r g) of edible salt

Type of treatment of salt and prepared herbs	Pretreatment of herbs		
	Microwave	Cryodestruction	Pressing (control)
Microwave: 1 hour	120	150	100
72 hours	120	100	80
Desorption in water	100	70	55
Convective: 1 hour	85	88	78
72 hours	70	73	68
Desorption in water	50	52	50

A control sample with drying herbs and salt in the convective field as the most commonly used one in food industry showed the lowest value of flavor. The parameters of the control sample in the microwave field firstly increased but the process of flavor sorption at salt did not take place, probably due to a low concentration of aromatic components during convective heating (flavor evaporates into the environment). The combination of microwave pretreatment

and microwave final drying of herb with salt showed the highest number of salt flavor. Pretreatment by dehydration reduces the number of the easily volatile fraction which to some extent inhibits aroma formation processes *in vitro*. Their lack contributes to the activity of the raw material enzymes connected in the membrane and the emission of the sufficient number of aromatic components to adsorb at salt.

Conclusion to Chapter 2

1. The following results have been achieved under experimental conditions in a laboratory:

- properties of aromatic components vaporized during MVD of plant materials have been studied;
- ways of increasing the output of aromatic components in the condensate without damaging the dried raw material have been investigated;
- quantitative characteristics of the aromatic components transition from the raw material into the condensate have been discovered;
- issues of the condensate concentration have been analyzed;
- properties of dried foods during microwave vacuum drying have been studied.

These results have showed the relationship between sensory properties of fruit distillates, dispersion and the method of fruit heat treatment in the convective and microwave field. The differences in flavour shades of melon and cucumber in fruit distillates demonstrate the advantage of the microwave effect during distillation of aromatic substances. The results of these studies can be used to improve pervaporation processes and membrane selection criteria for concentration of aromatic components, as well as in the technology of microcapsulation of aromatic components.

Lost aromas are restored through adjusting the medium pH in which particles of insoluble distillates are put. In the acid medium (pH = 3), transformation of acetals of cucumber distillates into aldehydes results in full restoration of fresh flavour as in this case

aldehydes are the key components. In weak acid medium (pH = 6.0), positive transformations of flavour occur with components of the melon distillate. These results contribute to economic competitiveness of distillates compared with other types of flavouring agents.

Technologies that allow obtaining more saturated distillates at the first stage of flavour isolation without re-concentration during preparation of aromatic concentrates with the predominance of C₆–C₉ aldehydes and alcohols are considered to be very promising. These technologies are based on the processes of de novo biosynthesis or biotransformation and the use of microwave energy. Obtained flavour distillates are made from vegetable raw materials; by their organoleptic properties they are closer to the natural raw material than the existing industrial analogues.

Processes leading to the change in polydispersion of particles, isomerization, and formation of enantiomers when isolating aromatic components require further research.

CHAPTER 3

ENZYMATIC METHOD OF AROMA RECOVERY

Fresh watermelon, cucumber, melon, and pumpkin pulp is aromatic for a limited period of time (up to several hours after grinding); after heating it acquires only boiled pumpkin flavor. For this reason, salting and pickling are usually used for storage and processing of watermelons and cucumbers; these methods ensure aroma formation using lactic acid bacteria. The presence of much water and tender texture of these fruits make it difficult to use the method of low-temperature storage and subsequent processing. As a result, the range of products from watermelons, cucumbers, and melons is limited; the fruits are mainly used fresh and with additional aromatic components (spicy seasonings, citrus fruits, etc.). In general, the rate of destruction of aromatic components is related to enzymatic processes in the fresh raw material.

Aroma-forming enzymes can be obtained by extraction of the fresh raw material. In this case, emission of enzymes is not always necessary. This section shows that the lost flavor can be restored by using the finely ground raw material (Fig. 16); flavor can be restored after having been lost as a result of heat processing (freezing, canning or drying) by using aqueous extracts of the fresh raw material.

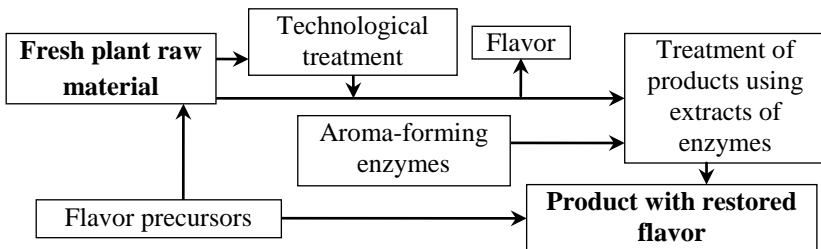


Figure 16 – The diagram of aroma recovery in products

This chapter shows that aroma recovery depends on the availability of precursors in the treated food products and the

presence of enzymes that specifically form aromatic components from precursors. The plant raw material should contain these enzymes in sufficient concentrations for the enzymatic formation of aroma, which occur due to successive hydrolytic and oxidative processes. The process of enzymatic aroma recovery takes place under certain conditions.

3.1. Changes in aromatic components of the heat-treated melons

Aromatic components of melons and gourds (cucumber, pumpkin and watermelon) are characterized by specific sensitivity to heat treatment; they contain similar key and shade C₆–C₉ aldehydes, alcohols, and their derived spirits [68]. Trace amounts of aromatic components were identified in the samples from these fruits after freezing and hydrothermal treatment. In our studies, aromatic components of melon fruits were not recorded on aromograms after heat treatment because their concentration was below the threshold of detection of chromatographs. Therefore, the cucumber and watermelon pulps were analyzed using an electronic nose using various processing methods (Fig. 17–22).

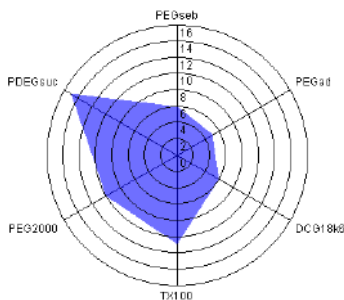


Figure 17 – Fresh Watermelon
S = 206,11

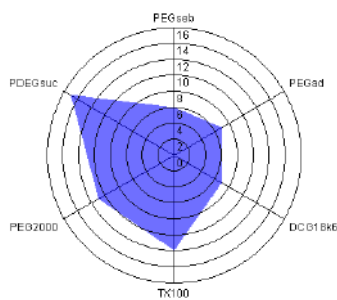


Figure 18 – Fresh Cucumber
S = 243,35

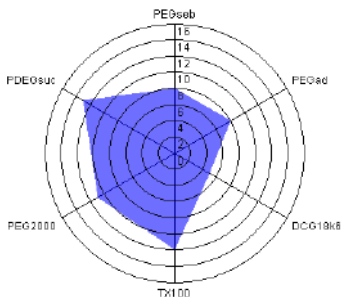


Figure 19 – Watermelon flesh
cooked S = 235,13

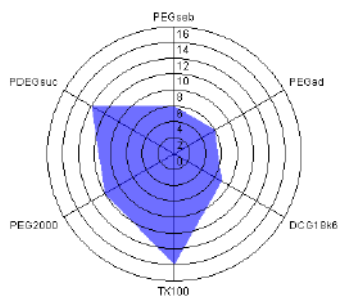


Figure 20 – Cucumber cooked
S = 219,97

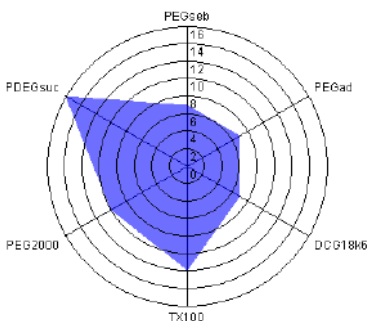


Figure 21 – Watermelon pulp
frozen S = 248,55

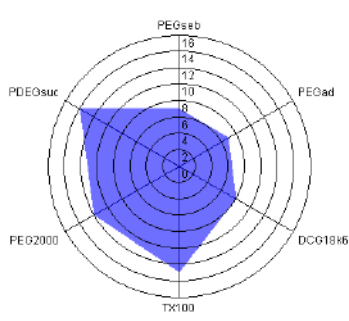


Figure 22 – Cucumber frozen
S = 273,23

Maximum flavor changes were registered in the fruits after hydrothermal treatment on a radar chart. Minimum flavor changes as compared with the fresh raw material were observed in the frozen raw material. Some studies have shown that lipoxygenase isomeric forms may have certain places in different cell compartments with temporarily differentiation activity. R. H. Buescher, R. V. Buescher indicate the increased number of lipoxygenases in cucumber exocarp (unlike mesocarp and endocarp) which also contains a large number of cellular PUFAs. Therefore, when cucumbers are frozen or blanched the system producing fresh flavors dies beyond resource [242]. Thus, the tested melon fruits do not always contain or activate

necessary aroma-forming enzymes after thermal effect to give the product the desired flavor.

The presence of a boiled tone distinguishes melon products after heat treatment from other fruits. To investigate the mechanism of destruction of volatile compounds of carbonyl nature, different parts of watermelon were chosen (rind with white flesh, juice, red pulp). The juice was separated from the red pulp by centrifugation for 20 min. The content of CC (fig. 23) in fresh samples and the samples after heating at 100 °C for one hour was compared.

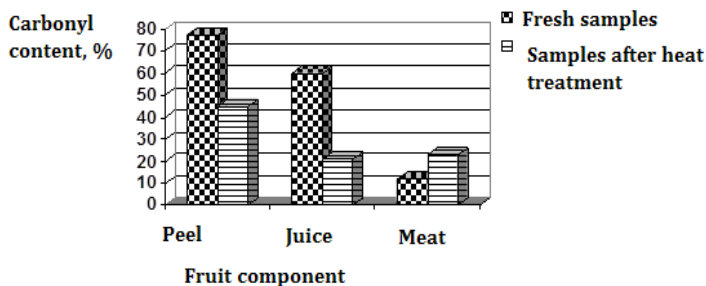


Figure 23 – The content of carbonyl compounds in watermelon products

Fig. 23 shows that watermelon pulp contains 3.2 times less CC than rind and 2.4 times less carbonyl compounds than juice. After heat treatment of watermelon products, the amount of CC in juice and rind after heating is reduced by 1.5-2 times, and new groups of volatile compounds are formed in watermelon pulp. But the flavor of watermelon pulp after thermal processing is different from the pleasant watermelon aroma by acquiring a vegetable “boiled” tone. In this case, the main flavor compounds are the result of the second stage of Maillard reaction (till the formation of dark-colored products, Fig. 24), which takes place in the different way: 1-amino-1-deoxy-2-ketosis experiences irreversible enolization in the positions 2-3 and eliminates amine from C_1 forming an intermediate methyl dicarbonyl compound which is further divided into C-methyl aldehydes, keto aldehydes, dicarbonyl compounds and reductones (1,2 endiols).

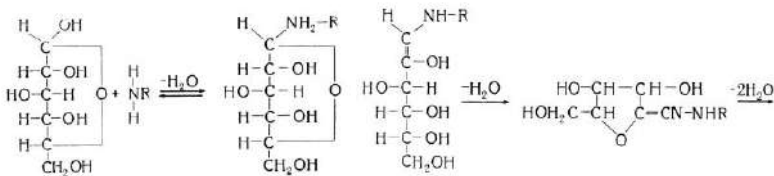


Figure 24 – Scheme element grouping Amadori

The first stage of the reaction is followed by the second one – dehydration of sugars and destruction of amino acids, or formation of reductones. In subsequent studies, a number of CC after hydrothermal processing decreases in the samples (cucumber, banana, watermelon, currant, sweet pepper, melon, and zucchini), but mostly in melon fruits. The total number of aromatic components virtually does not decrease; it even increases in berries, bananas, and tomatoes. At the same time, melon fruits lose, along with CC, the total number of flavor. Thus in comparison with other fruits, melon fruits lose overall flavor due to the destruction of CC after heat treatment. The increased number of flavor in berries, bananas, tomatoes is explained (unlike melons) by several aroma formation mechanisms in which the reaction with PUFAs are minor. Flavor components of melon fruits such as cucumbers are synthesized mostly from LNL acids. Therefore, after destruction of main flavor products, the formed aroma is perceived from amino acids and sugars – by Amadori cycle [246]. The number of sugars (on average 40 %), amino acids (22 %) and pH during heating in melon fruits causes similarity of flavor end products after heating. The amount of other flavor precursors in addition to PUFAs is different: melon has the higher amino acid content; cucumber, watermelon and pumpkin contain 2 mg/100 g (on average) of flavonoids, polyphenols, etc.

3.2. Argumentation of plants objects choice to restore the aroma

Ways of lipid conversion into desired flavours depend on the combined action of specific enzymes (lipoxygenase and hydroperoxide lyase), and on the presence of ancillary enzymes. The

mechanism of interaction of lipids and oxidants has free radical character that natural antioxidants inhibit. Their antioxidant activity is dependent on the concentration of these substances, and the physico-chemical properties. These include the polyphenols, L-ascorbic acid, carotenoids, vitamin K, ubiquinone, tocopherol. Only reduced polyphenols are active antioxidants, oxidized, quinone forms do not possess antioxidant properties. The ability of natural antioxidants in the form of a phenol or quinone, L-ascorbic or dehydroascorbic acid, determine their potential to exhibit antioxidant activity (Fig. 25). During the cooking process, these antioxidants can be lost due to the potential of lipid oxidation at high rates, so antioxidant are added during food processing.

The reasons why antioxidant activity of some vegetables after cooking changes are the following [247]:

- the release of high amounts of antioxidant components due to the destruction of cell walls and subcellular compartments;
- the thermal inactivation of oxidative enzymes;
- the formation of new compounds or antioxidants.

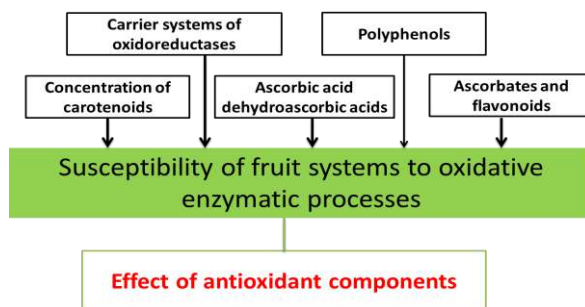


Figure 25 – Susceptibility of fruit system to enzymatic processes

The increase of the level of lipid peroxidation products and antioxidant system malfunction, as a rule, are directly dependent. Specifically, that the main effect of antioxidants in the peroxidation study, is not indicative because of the characteristics of an antioxidant in the aquifer/lipid systems, both inside and on the surfaces. Many antioxidants are found in extracellular fluids, they can initiate or prevent penetration of lipid peroxy radicals and involvement in

proliferation phase [248]. Antioxidants may be in water soluble form (ascorbate, glutathione, albumin) and in a lipid phase (alpha and gamma tocopherols, ubiquinone, lycopene, lutein). The mechanism of their activity is different, some affect singlet oxygen access to some other radical species. Ascorbates and flavonoids act as pro-oxidants under certain conditions and can have the dual biochemical and pharmacological effects [249]. Measurement of antioxidant activity (AOA, %) of the samples after sample preparation showed the differences between the results of fresh fruit (I), boiled (II), after defreezing (III) and enzymatic transformations (IV) (Fig. 26).

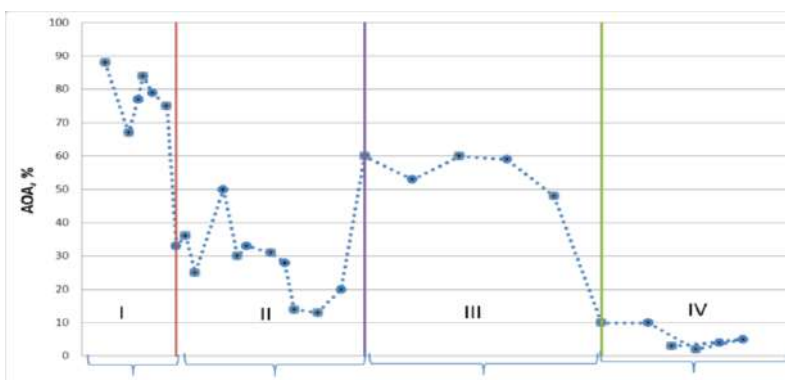


Figure 26 – Antioxidant Activity

The presence of several endogenous antioxidant systems, in fresh ground fruit (I), resulted in the highest values AOA. Possibly, antioxidant activity was less obvious due to the violation of some of the antioxidant systems in refrozen fruit (III). Boiled fruits (II), generally have values of AOA equal 10–20 %, but in black currants and sweet pepper, this figure is higher, and is 45–50 %. Thus, the hydrothermal treatment of cucumbers, pumpkins, watermelons, lowering the value of AOA, facilitates further oxidation reactions, including reactions that involve enzymes. The lowest AOA indices in the samples with an extract of enzymes (IV) are associated with both the pro-oxidant properties of the components and the occurrence of oxidative processes.

The consequences of technological processing affect the ability of oxidation reactions in the fruit system. The ORP is the indicator of normal growth, development and operation of the plant [250]. ORP parameters are particularly important for grapes and products of its industrial processing in flora. Phenolic substances grapes are rich in play an important role in redox processes. ORP indicator shows the activity of certain enzymes, which is an important in the selection of process variables. In animal origin products – one of the indicators of the quality of lipids in the meat, which indicates the quality of technology, packaging and storage [251–253]. It was shown that both, high values of ORP, and the decrease of this figure indicates a low rate of oxidation reactions or their lack [254].

To carry out enzymatic oxidation reactions in the fruit system ORP value can be quite informative in terms of substrate availability. Many substances of polyphenol nature, pigments, ascorbic acid, having antioxidant properties, perform a protective function in stressful for the fruit conditions (Suslow, 2004). To measure ORP the samples after hydrothermal treatment (II) and freezing (III) were used. As a result, test samples were arbitrarily divided into 3 groups (Fig. 27): melons and gourds (W), enzyme extract (E), black currant, cherries, pepper (D).

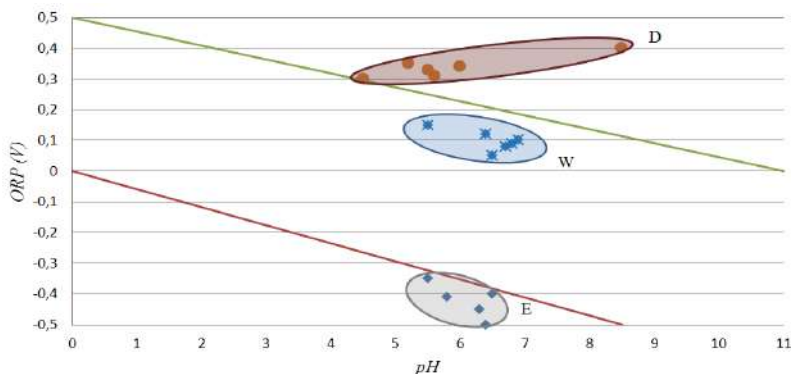


Figure 27 – Changes of ORP in fruits and enzyme extract

Analysis of the results showed that the fruit after hydrothermal treatment and freezing can be theoretically divided into groups with high and low value of ORP. Melons and gourds belong to the group with a low value of ORP 50–170 mV. The range of values for fruits with strong antioxidant system (currant, cherry, sweet pepper) is in the range of 300 mV and higher. A separate area is the system-carrier of enzymes, class of oxidoreductases – from -330 mV and lower. These ORP differences mean that the activity of electrons in enzyme extract is much higher than that of electrons in the fruit system. ORP, as an indicator of the electrons activity has a significant impact on the functional properties of electroactive components of biological systems.

Participation of defatted soybean meal (soybean solvent cake) lipoxygenase in oxidative processes is explained by low values of ORP, and specific values ORP melons and gourds. The ability to use the fruit lipoxygenases with high ORP, from the viewpoint of the energy balance, is difficult. This is confirmed by the results of experiments in which enzymes extract was added to berries, melons and gourds after heat treatment to restore the lost flavour. In the first case, the flavour changes did not occur, as suggested because of the presence of antioxidants. In the second – aroma is recovered at a sufficient contact area between the substrate and enzyme.

3.3. The influence of the lipophilic nature of melon fruit precursors on flavor change

The content of lipids in the vegetable feed (non-oleiferous) is about 1–1.5 %. Products of vegetable feed lipids oxidation are involved in the formation of volatile aroma composition and have a different impact on its organoleptic properties.

The type of the fatty acids decomposition and the aroma correspondingly depends on foodstuff fatty-acid composition. Analysis of FA of melons and gourds is needed for forecasting and modelling of enzymatic processes involving lipoxygenase, splitting up FA into hydroperoxides (HPOs) in Table 11.

Table 11 – Composition of melons and gourds FA, % of the amount

Names of FA	Composition of melons and gourds FA, % of the amount			
	Watermelon rinds	Watermelon pulp	Pumpkin	Cucumber
Palmitic 16: 0	15,35	15,29	25,1	26,3
Stearic 18: 0	4,551	5,012	3,3	7,3
Oleic 18: 1	16,928	17,27	7,2	11,1
Linoleic 18: 2	29,14	31,81	23,1	41,0
Linolenic 18: 3	11,77	9,15	40,0	14,0
The amount of saturated FA	35,80	34,74	28,4	33,6
The amount of unsaturated FA	57,84	58,23	70,3	66,1

Linoleic and linolenic acids make 30–40 % of the total fatty acids in lipids extracted from watermelon pulp, rinds, pumpkin and cucumbers. Unsaturated fatty acids and their isomers form 2- heptanal, hexanal, 1-octen-3-one, (E)-2-(Z)-6-nonadienal and (E)-2- hexenal. These compounds give odours characteristic for fresh mushrooms and tomatoes, and cucumbers etc. The list of aromatic compounds, which are formed as a result of free radical reactions PUFA, includes a large number of items [26]. Depending on the rate of lipids oxidation in food, the concentration of CC and flavour associated with them varies.

The reactions of enzymatic formation of aroma components from the lipids differ greatly and depend on if the substrate-precursor is a linoleic acid or linolenic acid. Furthermore, the system of double bonds PUFA sometimes varies by isomerization in conjugated configuration, for example, 9-cis, 11-trans linoleic acid. Isomers are formed during the hydrogenation and characterized by different biological effects, including the oxidation.

Conjugated double bonds are produced rapidly at the beginning stage of oxidation of polyunsaturated fatty acids during the separation of the hydrogen atom. Detection of diene conjugation is a

sensitive test for the occurrence of HPOs (Gardner, 1975). 9,13-hydroperoxy is referred to as primary product of PUFA lipid oxidation in plants. As a result of the splitting of the hydroperoxides by appropriate enzymes and subsequent reactions, multiple fruit aromas are formed: aldehyde and alcohol derivatives with shortened chain (4-hydroxy-2-nonenal, hexanal and hexenal, 2-octenal, 2,4-decadienal, propanal, 2-pentenal, 2,4-heptadienal, 3-hexenal, 2,5-octadienal, 2,4,7-decatrienal, 2-octenal, 2,4-decadienal, 3-nonenal), low molecular products (ethane, pentane), epoxides and malondialdehyde (MDA). Sometimes, the content of conjugated diene and lipid hydroperoxides is used interchangeably since many lipid hydroperoxides contain conjugated diene systems.

Because there are numerous positions to form HPO in the chains of unsaturated fatty acids, many different products are formed as the result of the β -splitting reaction. The analysis of diene conjugates and HPOs, and malondialdehyde formation, was carried out after different methods of processing treatment, including enzymatic (Table 12). Measurements were made during 1–2 minutes after finishing sample preparation.

Table 12 – Primary and secondary products of lipid oxidation

Name	Hydroperoxides and Diene Conjugates	Malondialdehyde
Pumpkin fresh	$D_{233} = 0,078$	$D_{532} = 8,27$
cooked	$D_{233} = 0,05$	$D_{532} = 15,79$
frozen	$D_{233} = 0,071$	$D_{532} = 10,13$
Black currant III	$D_{233} = 0,72$	$D_{233} = 27,70$
Sweet pepper III	$D_{233} = 0,55$	$D_{233} = 18,50$
Cherry III	$D_{233} = 0,64$	$D_{233} = 21,65$
Cucumber fresh	$D_{233} = 0,016$	$D_{532} = 4,51$
cooked	$D_{233} = 0,038$	$D_{532} = 9,02$
frozen	$D_{233} = 0,025$	$D_{532} = 6,85$
Watermelon pulp fresh	$D_{233} = 0,096$	$D_{532} = 27,00$
cooked	$D_{233} = 0,015$	$D_{532} = 13,57$
frozen	$D_{233} = 0,128$	$D_{532} = 35,04$

In fresh fruits, hydroperoxides are formed under the action of tissue lipoxygenases, which, after the intensive milling of raw

material are involved in enzymatic reactions. In defrozen fruits the formation HPOs hydroperoxides coincides with enzyme complex activation including hydroperoxide lyase (HPL) which undergo a reaction fast. Therefore, the values obtained can only partially reflect the actual accumulation of hydroperoxides (HPOs) after defreezing. Lipid oxidation products are produced in the greatest quantity after treatment with exogenous enzymes of defatted soybean meal (soybean solvent cake). In fruits of cucumber and pumpkin the ratio of linoleic and linolenic acids is different, so the low value of the PUFA oxidation products in cucumbers may indicate that linolenic acid primarily takes part in oxidative processes. The values of MDA are the fact that confirms that – MDA output during the oxidation of lipids depends on the fatty acid composition, and more unsaturated fatty acids give more MDA. The fact that polyphenols, anthocyanins, carotenoids and L-ascorbic acid to a certain extent do not prevent oxidation processes of the test samples and determine their direction draws our attention.

In heat-treated raw materials due to thermal inactivation of tissue enzymes hydroperoxides practically were not formed as in a watermelon pulp so and in black currants, cherries, sweet pepper. Hydroperoxide content increased in pumpkin, possibly due to the involvement of carotenoids in oxidative processes.

The primary oxidation products are non-volatile and therefore are not directly involved in the formation of odors. Hydroperoxides concentration decreases in the later stages of oxidation. The rate of hydroperoxides splitting and the formation of aromatic components increases when the rate of their formation slows down. PUFA splitting products often contain double bonds and (in some cases) intact pentadiene systems. These double bonds systems may undergo hydrogen atom separation or attack of singlet oxygen that results in formation of additional splitting products and unique flavours. If HPO is localized at 9th carbon atom or 13-carbon atom and the β -splitting occurs by the methyl end of the molecule, then, at first, HPO is decomposed to form alkoxy radical, and then to form the two reaction products – 9-oxononanoate (ethyl 9-oxononanoate) and vinyl radical at the 9th carbon atom (olefin radical). These vinyl radicals often react with hydroxyl radicals to form aldehydes, thus giving 3-nonenal (Eriksson, 1975). Linoleic acid hydroperoxide can

be subjected to β -splitting and the carboxyl end of fatty acid, where ethyl octanoate and 2,4-decadienal are formed after the formation of alkoxy radical [26].

3.4. Investigation of the conditions of flavor formation from lipids of raw fruits

3.4.1. Lipid transformation during cooling

The prospects for the use of enzyme theory have been described in patents on including enzymes in processed foods immediately before eating for strengthening taste and aroma. Lipid degradation and availability of polyunsaturated fatty acids for enzymatic oxidation reactions are important factors for the formation of certain flavor components. First, lipids are hard to reach due to their location in the cytoplasmic membrane. Segregation of lipids is correlated with its destruction by means of heating, freezing, or other physical methods. It is plausible to assume that freezing yields better outcomes, since it results not only in destruction of cytoplasmic membrane of the cell, but also in special isomerization of lipids. Furthermore, decreasing the temperature causes a decrease in membrane fluidity, offset by desaturation of membrane lipid in fatty acids caused by desaturases. Such processes in isolated fruits are yet to be studied.

The formation of 9,13-hydroperoxides from PUFA as the starting point for biosynthesis of aromatic components with the help of plant enzymes (Fig. 28).

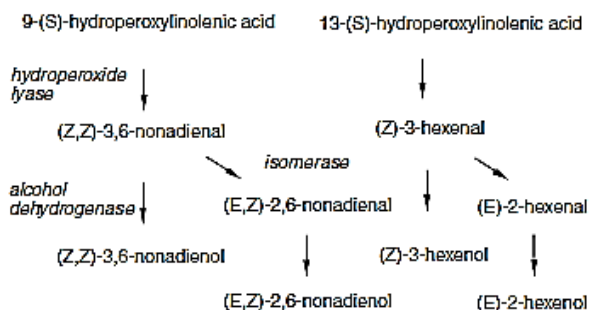


Figure 28 – Enzymatic formation of flavor from Linolenic acid C 18: 3 [231]

Most of the enzymes are still active after the harvesting of fruits and vegetables and this continuing enzymatic activity upon storage affects their quality (Zhang & Tian, 2010). The 10 °C drop in the environmental temperature for isolated ripe fruits is sufficient for activation of the membrane PUFA desaturation and over time one can observe a change in physical properties of the cytoplasmic membrane, such as an increase in the degree of unsaturation of the fatty acid residues in the cellular membranes (Los, Mironov, & Allakhverdiev, 2013). Wang et al. (1996) demonstrated that the successful introduction of the yeast Δ -9 desaturase in transgenic tomato plants leads to an increase in levels of palmitoleic acid, 9, 12 – hexadienoic acid, and linoleic acid being accompanied by a decrease in palmitic acid and stearic acid. Change in the profile of fatty acids is due to a change in certain aromatic compounds derived from fatty acids, namely the cis-3-hexenol, 1-hexanol, hexanal, and cis-3-hexenal. The findings show the change in iodine number in test samples of the fresh homogenates after a short storage and freezing (Fig. 29).

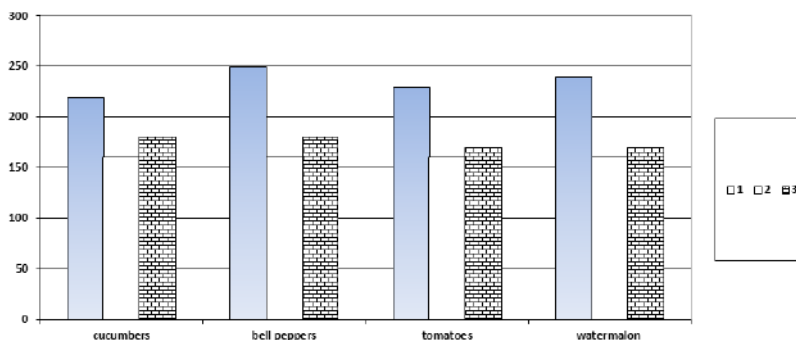


Figure 29 – Changes in iodine number of homogenates after freezing (1), fresh (2), after a short storage (3)

The average 75 g increase of iodine number in frozen homogenates as compared to fresh ones is the evidence for the arrangement of double bonds in PUFA and enhanced activity of desaturases. When the cellular systems are damaged by freezing,

most of the enzymes exhibit significant activity upon thawing. The reason for this increase in the rate of enzyme-catalyzed reactions is the rupture of the membranes, organelles, particularly sensitive lysosom, and failure in insulation of enzymes, enzyme substrates, and enzyme activators. Some studies report that lipoxygenase exhibit significant activity after freezing and thawing [167]. Lipoxygenase and hydroperoxide lyase are so closely associated in lysosomes that any extract from homogenates upon thawing contained a critical concentration of both enzymes. In part this increase in the activity of enzymes can be explained by the change in properties of the components of the cytoplasmic membrane. Preliminary preparation by cooling results in adequacy of the enzyme and substrate which positively influences binding energy.

Desaturases prepares the substrate to be influenced by the aroma-forming enzymes and as the result fresh flavor is formed de novo (Fig. 30). Careful thawing plays an important role in these processes, for instance in microwave oven with low power setting in order not to inactivate enzymes. Lipase, lipoxygenase, and hydroperoxide lyase are then released from lysosomes and mitochondria, while being less accessible in fresh raw materials even after a fairly thin homogenization.

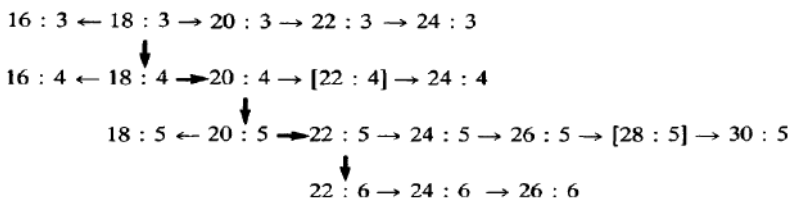


Figure 30 – Change in the position of double bonds [5]

The treatment of fresh homogenates in the microwave field at low power (up to 70 W) causes the change in binding energy influencing the enzyme-substrate complex. The specific heat capacity of a substrate is lower than the heat capacity of water, therefore lipid layers can be rapidly heated in a microwave oven, faster than water. The selective heating of cellular wall components in the microwave

field is one of the important factors distinguishing this type of heating.

This paper proposes a flavoring method consisting of several steps (Fig. 39). These recommendations are based on the previous studies by this author on the recovery of fresh flavor in heat-treated suspensions of watermelons, cucumbers, sweet peppers, strawberries, and tomatoes.

In the experiments, the flavor-creating complex of enzymes (lipase, lipoxygenase, hydroperoxide lyase) results in the formation of fresh flavors from PUFA cytoplasmic membranes. The enzymatic nature of these processes is confirmed by a small amount of homogenates introduced and a brief reaction time until a persistent flavor.

The prepared watermelon suspension containing the 20 g active complex of enzymes is able to restore the lost aroma in 250 gs of the thermally-treated pulp. The reaction time depends on the form of PUFA (micellar, as a detergent complex or as salt) and values of pH determining the degree of carboxyl group dissociation. The reaction time of recovery at optimal combination of all factors is 5–7 minutes.

3.4.2. Overcoming a hydrophilic-hydrophobic barrier under conditions *in vitro*

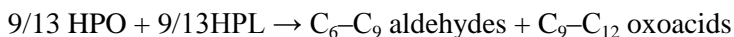
The study of aroma formation from lipids *in vitro* is connected with large methodological difficulties; so lipid transformation processes due to thermal, physical or combined effects on the plant raw material are not much discussed in scientific publications. According to the theory of flavor restoration by G. Reed, with the availability of precursors, flavor can be restored in the fruit raw material after heat treatment when interaction conditions are created. The preconditions for the repetitive flavor formation from precursors in fruits after heat treatment have been previously investigated, but the participation of lipid substrates in these processes is not still clarified (Gargouri M., 2008; Oey I., 2010).

Since lipid precursors are of hydrophobic nature and enzymes are of hydrophilic one, enzymatic reactions under *in vitro* conditions

occur at very low speed. Surface active agents (surfactants) are used in aquatic lipid systems to increase the contact surface area in lipid-substrate reactions and to decrease water shell thickness. Similar effects can result from the physical effect and lead to the recovery of the lost flavor. In this study, enzyme catalysis is studied to a greater extent from physical perspective, since enzymes alter energy levels of the system intermediates using nonvalent interactions.

The theory of enzyme kinetics considers enzymatic reactions to be multistage ones accompanied by the formation of temporary intermediates. Most of the aromatic components are reaction intermediates formed between the substrate (lipid hydroperoxide derivatives, HPO) and the corresponding enzymes (hydroperoxide lyase HPL). Therefore, changes of aroma components can fairly quickly reflect the results of enzymatic hydrolysis *in vitro*. The study of structural changes in flavor lipid precursors will enable to determine the optimum conditions of physical effects on the enzyme-substrate system.

Without exception, all cellular membranes are thin lipoprotein films consisting of a double layer of lipid molecules that includes protein molecules. When freezing, heating, or undergoing microwave exposure, protein components of the raw material are coagulated. The main factors determining the behavior of particles in the coagulated structure are as follows: size of particles, the hydrophilic-hydrophobic balance of the particle surface, overall and electrokinetic potential of the surface. The activity of HPL using insoluble substrates such as hydroperoxides essentially depends on the dynamic properties of the general lipid phase of membranes. Most hydroperoxides in thermally processed fruits are formed from linoleic and linolenic acid. The hydroperoxide catalysis can be represented schematically as follows:



The reactions of hydroperoxide lyase in fresh fruits are sufficiently studied, and their participation in the thermally processed raw material causes aroma-forming reactions (Klee, H. J., 2010; Lanciotti R. et al, 2004). To identify and understand the conditions of

catalysis with the participation of hydroperoxide lyase, the comparative analysis of physical and chemical parameters of the lipid system of fresh and thermally processed samples of the cucumber extract was made (Table 13).

Table 13 – Physical and chemical characteristics of lipids in the cucumber extract

Index name	Fresh	Frozen	Hydrothermal processing
ζ -potencial, mV	-2,87±0,15	-4,11± 0, 30	-5,50±0,22
Size, nm	10 000...5 000	5 000...1 000	1 000...500
Total content of hydroperoxide compounds	8	12	18
Characteristics of aroma of aqueous suspension	Intense cucumber	Weak vegetable, mushroom shade	Grassy, soup
Total aldehydes, mg/g	0,079	0,055	0,043

According to the traditional view, the greater the absolute value of ζ is, the greater the electrostatic repulsion between droplets is, and hence the more sustainable the system stability is [255]. Experimental measurements of ζ -potential indicate steric repulsion of particles in the hydrocolloid system and characterize stabilizing properties of the emulsifier represented by phospholipids in cell walls (Ferezou, J., & Bach, A. C., 1999). The samples ζ -potential ranges from -2,5 to -5,5 mV. As a comparison, ζ -potential range of industrial flavorings after enzymatic processes ranges from -22 to -25 mV. According to these data, we can characterize the stability of the studied emulsion of triglycerides; ζ -potential in the fruit raw material may indicate the system instability and the behavior of reactions involving enzymes. For the test samples, ζ -potential is distributed in the following order: fresh raw material < frozen raw material < hydrothermal processing. Dimensions of triglycerides extracted from cucumber are distributed in reverse sequence relative

to ζ -potential. Thus, with the increase of the particle size their mobility rises. A similar pattern was described for liposomes [256, 257]. The samples of pumpkin and watermelon after heat treatment showed identical distribution patterns of ζ -potential and the particle hydrodynamic diameter.

Dynamic properties of the membrane lipid matrix provide conformational flexibility of enzymes. The properties of the lipid matrix are associated with structural rearrangement in biological membranes. For example, water crystallization in frozen fruits induces activation of membrane-bound lipolytic enzymes and, subsequently, results in significant changes in structure, physical and chemical characteristics of fatty acids of membrane lipids. Thermal processing of membrane lipids affects physical properties of lipids and the oxidation process by endogenous enzymes. Thermal effects, freezing, electrical breakdown, and osmotic pressure are factors determining structural adjustments and the activity of endogenous enzymes (Gonzalez, M. E. et al, 2010). Maximum number of hydroperoxides in the extracts of fruit lipids after hydrothermal processing and the minimum amount of aldehydes (table 11) are the conditions for maximum effect of HPL. The expected result of this action is the reduced amount of hydroperoxides and increased concentration of C₆–C₉ aldehydes.

The data on nanodimensional areas are a powerful approach to the study of the dynamics of biomolecules. The size of single molecules of the plant lipids is about 5–200 nm. According to some studies [258], single lipids don't exist in cell membranes but lipid nanodomains do, with average size of 710 nm. It is shown that the size of more than 700 nm testifies about the presence of cluster proteins, the hydration shell, and hydrophobic hydration which hinder detection of lipids through electron microscopy and their subsequent diffusion. To isolate lipid domains from extracts and analyze their hydrodynamic diameter, the samples were examined before and after centrifugation at different frequency and amount of time of the emulsion separation (Fig. 31–34).

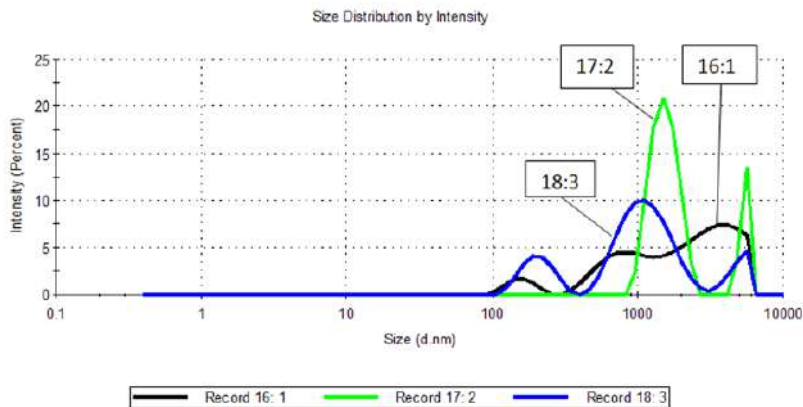


Figure 31 – PSD of lipids from the fruits after hydrothermal processing (16: 1 without separation, 17: 2 separation for 10 minutes at frequency of 1 500 rev/min, 18: 3 separation for 10 min at frequency 4 000 rev/min)

Lipid domains in the extract that was not separated in the centrifugal field are mainly composed of particles with a hydrodynamic diameter of 5 000 nm. Separation in the centrifugal field for 10 minutes at frequency of 1 500 rev/min decreases a hydrodynamic diameter of lipid domains to 2 000 nm and separation at frequency of 4 000 rev/min reduces it to 1 000 and 300 nm. In order to understand the impact of the physical effect of the centrifugal field on protein-lipid associates, the separation time in the centrifugal field was increased to 20 minutes (Fig. 32).

Lipid extracts that were not separated in the centrifugal field are mainly composed of particles with a hydrodynamic diameter of 6 000 nm. Separation in the centrifugal field for 20 minutes at frequency of 1 500 rev/min decreases a hydrodynamic diameter of lipid domains to 800 nm and 25 nm and separation at frequency of 4 000 rev/min reduces it to 900, 250 nm and 25 nm. The increase of separation time in the centrifugal field to 20 minutes results in the decrease of a hydrodynamic diameter of lipid domains.

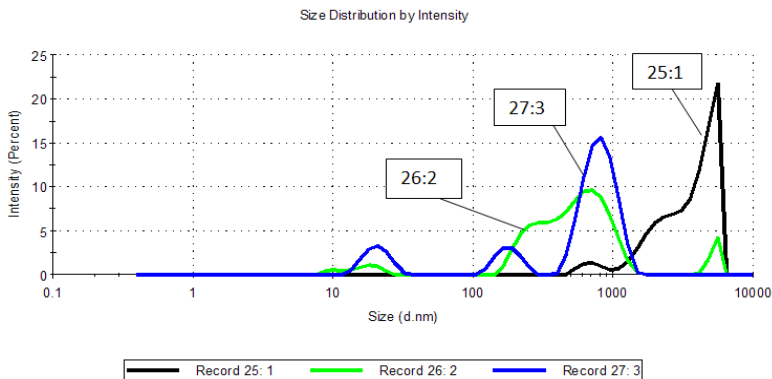


Figure 32 – PSD of lipids from the fruits after hydrothermal processing (25: 1 without separation, 17: 2 separation for 20 minutes at frequency of 1 500 rev/min, 18: 3 separation for 20 minutes at frequency of 4 000 rev/min).

The impact on the cell lipid structures to enhance or diminish the hydrophobic interactions, covalent links, and Van der Waals forces is made using chemical, enzymatic or physical methods. The combination of the physical impact and enzymatic processes leads to the preservation of natural flavor that was shown in plants with high hydrostatic pressure and low temperature. Intermolecular forces are components of disjoining pressure which depends on the thickness of the lipid film, temperature, the composition and properties of the interacting phases (bodies). The study of disjoining pressure forms the basis of the theory of stability of hydrophobic colloids – DLVO theory; it explains many surface phenomena.

Overcoming positive disjoining pressure preventing thinning of the film under the influence of external forces leads to adhesion or fusion of contacting bodies. It means coalescence or coagulation of the particles of the dispersed phase in the context of colloidal systems; in this study it denotes the enzyme-substrate interaction. The combined processing of fruits in vacuum with depth of 6 ± 1 kPa is accompanied by the optimal temperature mode for activation of fruit enzymes (32 ± 2 °C).

Changes in properties of lipid structures of plant cells and membrane-bound enzymes in vacuum comply with DLVO theory (Gennis R. B., 2013; de Jesus, S. S., & Filho, R. M., 2011). This theory considers the combined effect of several components of surface energy. According to this theory, colloidal protein-lipid particles can loosely approach each other until they come in contact with their watery diffuse shells or layers. In these conditions, there are no interaction forces between them. For the reaction approximation of particles of enzymes and lipids, it is necessary to achieve deformation of diffuse shells to reach their mutual overlapping (or penetration into each other). While the thickness of the liquid layer or film is greater than the total thickness of boundary layers with special structure, the influence of these layers is manifested only through the relevant changes in electrostatic and molecular components of disjoining pressure.

The implementation of these processes causes some change of the PSD profile described above. Hydrodynamic diameters of lipids in the extract are distributed more evenly due to the combined processing (Fig. 33–34).

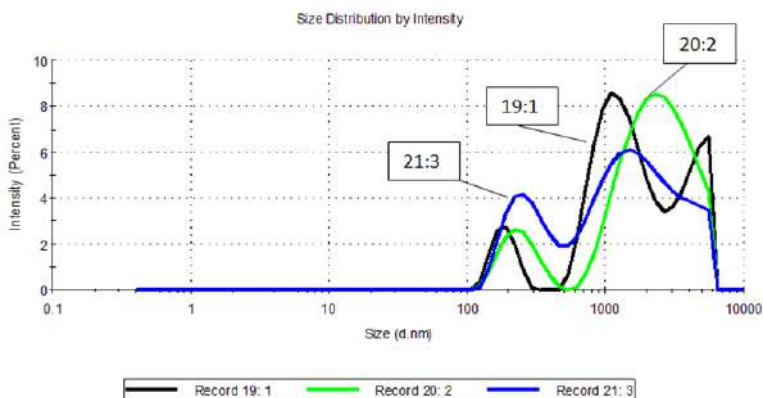


Figure 33 – PSD of lipids from the fruits after vacuum treatment (19: 1 without separation, 20: 2 separation for 10 minutes at frequency of 1 500 rev/min, 21: 3 separation for 10 minutes at frequency of 4 000 rev/min)

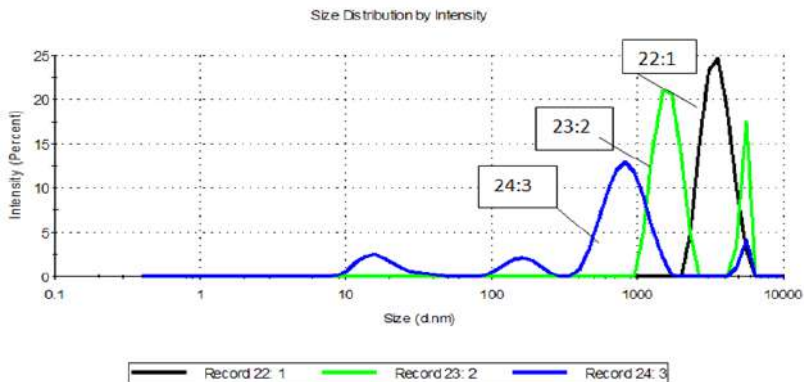


Figure 34 – PSD of lipids from the fruits after vacuum treatment (22 : 1 without separation, 23 : 2 separation for 20 minutes at frequency of 1 500 rev/min, 24 : 3 separation for 20 minutes at frequency of 4 000 rev/min)

The comparison of the results of the samples after hydrothermal and combined processing testifies about the system greater permanence and stability, which is expressed in the adjustment of a hydrodynamic diameter to the range of 1 000 nm and 100 nm.

The analysis of the PSD profile of the samples after hydrothermal and vacuum processing with separation for 20 minutes shows approximate results and the similar stability after the impact of the centrifugal field. Thus, the increase of the physical impact on lipid nanodomains is efficient under certain conditions. The expansion of the local zone of the lipid layer surface leading to adjustment of a hydrodynamic diameter (Fig. 34) and favorable conditions of enzymatic hydrolysis is observed in vacuum. Strengthening of the further physical impact on this system changes the conditions of disjoining pressure; so the particles demonstrate repulsion resulting in the decreased hydrodynamic diameter and different PSD profile.

In the case of peripheral proteins (membrane bound enzymes of HPL), the described bilayer modifications lead to their activation. For example, when Ca^{2+} ions or products of lipid peroxidation are added to membrane fractions, the activation of mitochondrial phospholipases is observed (Halliwell B., & Chirico S., 1993; Adibhatla R. M., & Hatcher J. F., 2008). After combined processing, due to changes in the activity of hydroperoxide lyase HPL,

alterations in the fruit flavor occurring during the accumulation of C₆–C₉ aldehydes were registered (Fig. 35, 36).

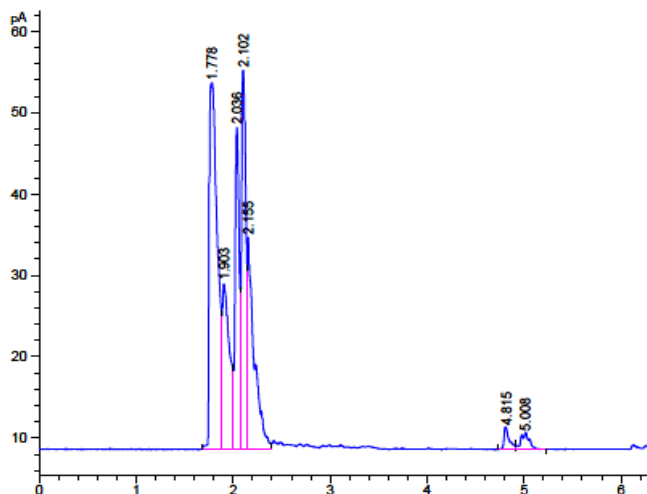


Figure 35 – Aromatogram of the lipid extract after combined processing of fruits with active HPL

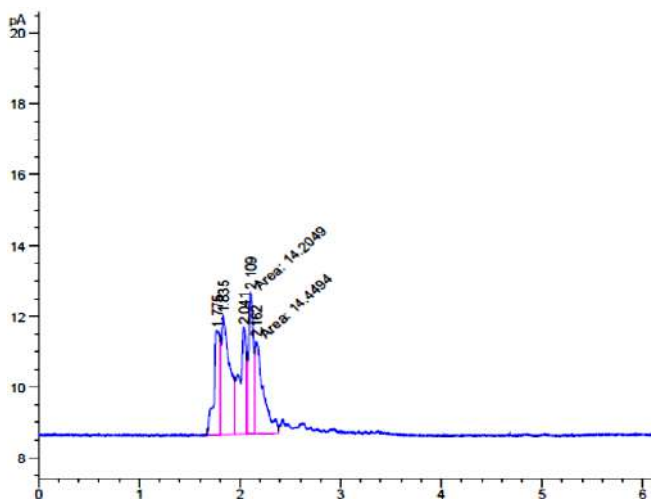


Figure 36 – Aromatogram of the fruit lipid extract with inactive HPL

The relationship between physical properties of the substrates and the final products of enzymatic aroma formation reactions *in vitro* has been examined. It has been found that the availability of components for reactions is determined by a complex indicator – the stability of lipid emulsions n_y (hydrodynamic particle size and ζ -potential). The change of n_y has been established to affect the potential mobility of particles and the number of flavors.

The intensity and peak area of aromatic components indicate the formation of C₆–C₉ aldehydes of the GLVs profile from lipid hydroperoxide compounds. The effectiveness of combined processing of fruits in the context of reactions implies activation of hydroperoxide lyase and reactions between hydrophilic enzymes and hydrophobic precursors. Under disjoining pressure in vacuum, the sheet of water in the interphase interlayer of the lipid bilayer, hydration shells around polar portions of lipids and membrane proteins is sufficiently reduced to make these reactions possible.

The physical effect of vacuum on the colloidal solution causing changes in the size of the diffuse layers and the value of ζ -potential depends on the method of heat input. In the discussed combined plant, microwave heating in a range of 32±2 °C was used, which was replaced by convective heating in subsequent experiments. The purpose for replacing the heat input method to convection is the focused study of conditions for interaction between lipids and their oxidation products and enzymes in the fruit system. The samples of watermelon pulp processed in vacuum with convective heating were studied (Fig. 37). The statistical analysis of experimental data on the effect of vacuum depth and processing temperature mode is represented as the following relationship:

$$Y = 2,94 - 0,0125x_1 - 20,73 / x_2,$$

where x_1 – pressure, kPa. Upper level is 91, 3 kPa, lower level is 1.3 kPa, variation interval is 10 kPa.

x_2 – temperature, °C. Upper level is 46 °C, lower level is 20 °C, variation interval is 3 °C

Y – number of flavor, relative units

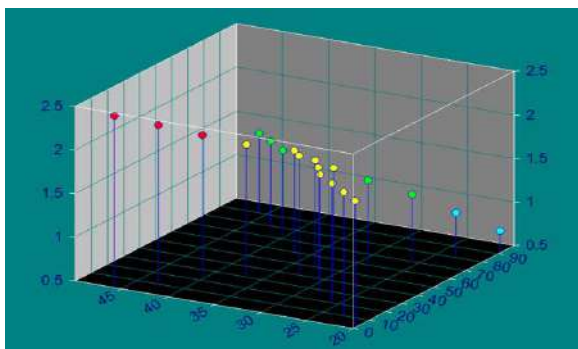


Figure 37 – Mathematical model of aroma recovery

It is known that the protein molecule may be fixed in the bilayer using various types of interactions including electrostatic ones (at the level of polar heads of lipids) and hydrophobic ones (in the bilayer thickness). During heating fruits or aqueous suspensions in vacuum (underpressure is 6 ± 1 kPa, temperature is 32 ± 2 °C), substrate-enzyme interactions are the most intensive because of the conditions of interphase activation, when there is a change in mobility, structure and spatial position of lipid domains. These effects ensure multimolecular adsorption and biosynthesis of GLVs in the fruits that lost their flavor after thermal processing.

3.4.3. Overcoming a hydrophilic-hydrophobic barrier in gelatin jelly

A special feature of watermelon pulp is presence of flavor precursors such as lipids of cell cytoplasmic membranes. Destruction of free lipids and release of strongly bound lipids during thermal treatment makes them more accessible to the action of aroma-forming enzymes. Carrying out reactions with enzymes is difficult due to the contact area of a hydrophobic substrate and a water-soluble enzyme. These reactions can take place when there are conditions of interfacial activation – a large contact surface between the substrate and enzymes. The probability of a significant increase in the activity of enzymes in the gelatin solution is quite high, since

its protein molecules are natural surfactant nanoparticles that have the properties inherent in nanosystems.

Initially, the most common method of pouring hot (90 °C) gelatin solution onto the prepared products (in this case, watermelon pulp) was used. The enzyme extract was added to the mixture of gelatin and watermelon pulp in a ratio of 1 : 5 to the conventional gelatin concentration of 10 %. The process step was carried out after cooling the mixture to 40 °C to avoid heat inactivation of enzymes. For uniform distribution of the extract, the mixture was mixed, cooled at room temperature first to allow polymer molecules to form a frame, and then at the temperature of 0...8 °C. The time for jelly formation was 2.5...3 h.

Strongly bound lipids of watermelon fibers in hot gelatin solution are better distributed at interfaces of stages providing greater accessibility to membrane bound aromatic precursors. The effects causing disintegration of the membrane structure facilitate the oxidation activation of unsaturated lipids that affect the oxidative formation of volatile compounds. However according to the experts who have analyzed organoleptic characteristics of the finished jelly, *de novo* synthesis of aromatic substances didn't occur, the flavor of the boiled watermelon pulp dominated.

The study of the microstructure of fresh watermelon fibers and the jelly (Fig. 38) prepared according to the above mentioned technique showed the change in color of lipid components on the electron microscope, as a “crushed drop” specimen studied at 100-fold magnification.

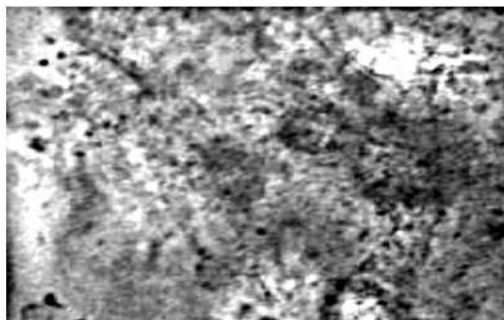


Figure 38 – Intermycelial space between watermelon fibers

The intermycelial space between watermelon fibers is filled with drops, painted in orange and yellow colors. The use of hot gelatin solution promotes emission of lipids and lycopene dissolution in lipids; lycopene has antioxidant properties and inhibit oxidation reactions. As a consequence of these processes, there were no changes in flavor of the end product.

The unique properties of gelatin gels due to the spiral configuration of polypeptide chains, stabilization effect of hydrogen bonds can successfully perform enzymatic synthesis of flavor. The procedure of enzyme immobilization in gelatin solution was as follows: a complex of enzymes was added to warm gelatin solution at 45...42 °C in a ratio of solution : extract 3 : 1; stirred for 2–3 minutes; allowed to stand for 5 minutes; added to the prepared watermelon pulp; cooled (the test sample). The extract of enzymes was replaced by water in the same ratio in the control sample of jelly.

The organoleptic analysis of the control sample and jelly using the extract of enzymes showed a significant difference in flavor characteristics. The test sample had a delicate aroma of fresh fruit, a sweet shade and key notes specific for fresh watermelons. The approximation of flavor of the end jelly to fresh flavor was assessed by experts as 87–90 %. The flavor of the end product didn't change in the control sample because of lack of active enzymes.

In the test sample, enzymes are likely to cover micelles of a gelling agent with a thin layer. The extract of enzymes is a part of the molecularly disperse system of aqueous gelatin solution; it is involved in the formation of a three-dimensional frame defining mechanical properties of the system. Gelatin jelly having the properties of polyelectrolyte may ensure electrostatic attraction of the substrate molecules. In addition to the electrostatic mechanism of binding the substrate and enzymes in jelly, a specific and mechanical adhesion can be very important. The role of this factor can be illustrated in a different jelly preparation technology, when the enzyme extract is added not to gelatin solution, but to watermelon pulp prepared at 30 °C. After stirring for 15 minutes, gelatin solution is poured. Recovery of the lost flavor occurs in the end jelly, however, largely due to adhesion processes.

The following ultrastructural changes were observed: spherical microbodies, vacuoles, pigmented globules, as well as other structural organelles are differently distributed in jellies. In Fig. 39 (b), fiber-ordered distribution of components dominates which is typical for watermelon pulp without enzymatic treatment. In Fig. 39 (a), the tendency to self-organization in volume, mutual flocculation and concentration of structural units in reaction sites is observed. The analysis of other micrographs carried out for the studied samples by a 3-fold repetition is consistent with the results described above. This confirms that there is an interaction between protein and positively charged protein associates of gelatin-enzyme and negatively charged pulp particles; this interaction becomes more intensive as gelatin solution changes into the structure with triple molecular chains. Obviously, adhesion processes play a minor role in aroma recovery.

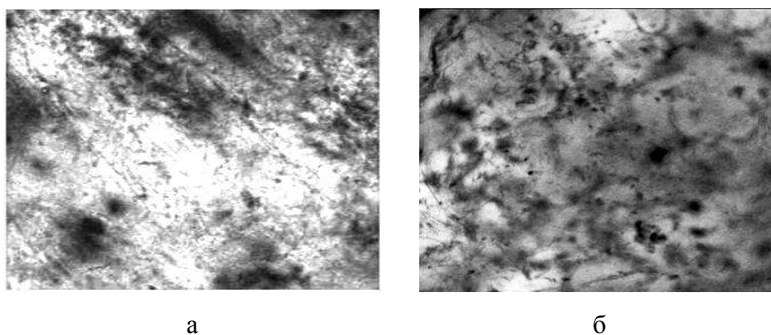


Figure 39 – Intermycelial space between watermelon fibers and gelatin jelly

The method of adding the extract of enzymes in gelatin solution and watermelon pulp significantly affected the rate and depth of flow of the flavor formation reaction. The advantage of using gelatin solutions is the ability to immobilize enzymes from an aqueous extract due to surfactant properties, to repeatedly increase the contact surface of the enzyme-substrate and to ensure electric attraction of particles. These benefits should be considered in the technology of enzymatic aromatization of gelatin jellies.

3.5. A general flavor recovery scheme

The enzymes catalyze the formation of flavors associated with molecules of precursor. We selected watermelon pulp as a substrate for the restoration of flavor, from which we removed the cell sap and boiled it for 15 minutes (Fig. 40, curve 2). This pulp was then mixed with a complex of flavor forming enzymes from fresh plants as well as oxidized fatty acids and lipoxygenase [7]. Due to changes in the natural enzyme activity that led to a rapid sensory reaction, the flavor of boiled fruit pulp was restored (Fig. 40, curve 1).

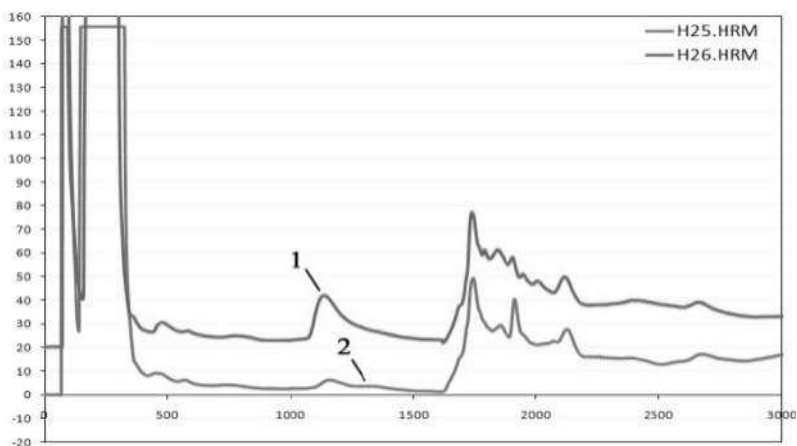


Figure 40 – The degree of natural flavor recovery in watermelons (1 – flavor of recovered substrate 2 – flavor of heat-treated substrate)

In order to secure a persistent and pronounced flavor it is necessary to ensure the interphase activation – a large contact surface interaction between the substrate and enzyme. This author used gelatin solutions for the experiments because of their surface-active properties and potential to provide maximum availability of the cytoplasmic membrane substrate to the active centers of enzymes. The probability of a considerable increase of lipase activity in gelatin solution is high enough because molecules of protein in gelatin solution represent natural surface-active nanoparticles possessing

properties of nanosystems. Therefore, the final product can be represented by flavored films from gelatin solutions or foam thereof (Fig. 41).

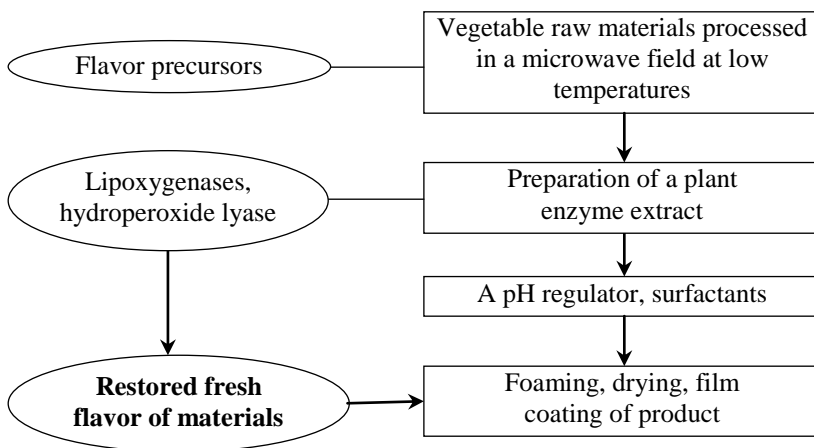


Figure 41 – General schematic diagram of the lost flavor recovery

According to our research, restoring melon flavor is more of the non-enzymatic nature. Studies by O. Lamikarna and co-authors have found out that 3mm sliced fresh melons had the most significant loss of aspartic acid, glutamic acid, asparagine and glutamine during the first two days. We have investigated the degree of destruction of CC, which are the most responsible for fresh flavor of melon, for two days with intervals in 12 hours with varying degrees of melon grinding, as well as in combination with the puree cooked from beans and chickpeas. As the amino acid composition of chickpea and bean differs, the role of amino acids in maintaining fresh melon flavor must be important.

The comparison of aromatic components in the system of homogenates “melon-beans” on the chromatograph confirms the different role of amino acids in recovery of aromatic components after 48 hours (Fig. 42).

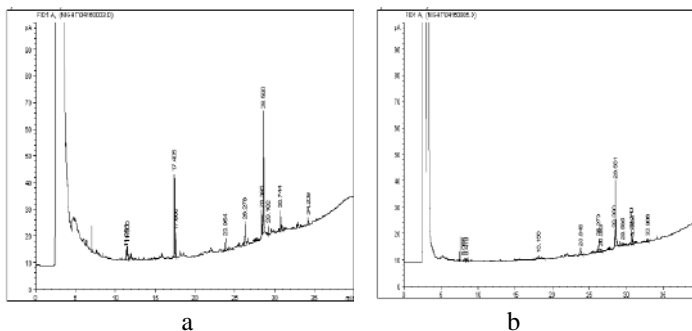


Figure 42 – Chromatogram of the vapor phase of the complex “melon-chick” (a), “melon-bean” (b)

The concentration of compounds in the free gas space above the product indicates that the ability to binding carbonyl (aromatic) components by legumes (beans, chickpeas) appears differently. The peak analysis demonstrated that nonadienol retained in both samples, but to a lesser extent in beans; nonenal, oksononanal and nonadienal were available largely only in the samples with chickpeas. The related components of melon flavor such as butyl acetate, isopropyl acetate, methyl propionate, ethyl hexanoate, ethyl propionate had the approximately equal concentration in the samples with legumes.

The participation of amino acids in preservation and restoration of fresh melon flavor by compensating them from homogenates of legumes is confirmed by the experiments with the pattern samples.

Conclusion to Chapter 3

1. The predisposition of raw material components to lipid oxidation is the basis of selection criteria of plants for restoration of the lost aroma. The antioxidant capacity and the oxidation-reduction potential of fresh fruit and fruit after cooking analysis showed some trends of oxidizing processes. These two characteristics determine to a certain extent the ability of fruits to the repeated formation of aromatic components. It was found out that the melons and gourds (watermelons, pumpkin, and cucumbers) have sufficient potential to restore flavour by exogenous lipoxygenases. Analysis of the lipid composition of the fruits showed a sufficient amount of unsaturated fatty acids, which produce hydroperoxide compounds.

2. Many structurally simple aroma-forming substances found in certain foods affect the completeness of sensation and perception of the product as being delicious, fresh and fragrant. Their presence or absence can be regulated by introducing small quantities of fresh homogenized material. Aroma recovery is the enzymatic process which depends on availability of enzymes and flavor precursors in foods. In order to improve enzyme activity, the material may not necessarily be fresh, but also frozen or stored at low temperatures, and processed in the microwave field. More experiments are needed on recovery of fresh flavor by using enzymes from plant materials, especially in creation of flavored food glazes and foam. Reinforcement of the flavor profile will be reflected in reducing the amount of salt or sugar used in preparation of products and in manufacturing fat-free products. The increased demand for organic foods and flavors should support the interest in this area of research.

3. Heat processing (convection and microwave) of the raw material in vacuum allows purposefully affect the aromatization enzymatic process. The availability of flavor precursors of lipid nature for enzymatic reactions can be assessed by the distribution of their hydrodynamic diameter and ζ -potential mobility. Changes in these parameters of the plant lipid components during processing affect the concentration of aromatic components. The efficiency of aroma restoration depends on the number of formed 9-, 13-hydroperoxides that serve as a substrate for aroma-forming enzymes. New aspects in the formation of aromatic components are data on the primary and secondary products of lipids oxidation of fresh raw materials and raw materials after heat treatment.

4. The influence of triglyceride dimensional characteristics on the possibility of aroma-forming reactions involves the change in the conditions of the contact interaction of hydrophilic-hydrophobic colloidal systems. It has been established that during vacuum heating (underpressure is 6 ± 1 kPa, temperature is 32 ± 2 °C) of the suspended plant homogenates, substrate-enzyme interactions are the most intensive because of the conditions of interphase activation when the hydrophobic interaction, covalent links, and Van der -Vaalsovyh forces are weaken. These effects ensure the multimolecular adsorption and biosynthesis of GLVs in the fruits after thermal processing.

CHAPTER 4

Technologies of flavoring agents and flavored products, substantiation of their production

The theoretical principles of formation and recovery of aromatic components became the basis of the technology of manufacturing restaurant products. The main areas of the developed technologies are to obtain liquid and emulsion flavoring agents, to restore lost flavors in products. Each area is a system with a variety of subsystems, priorities, timeliness and appeal to the consumer and the manufacturer (table 14).

Table 14 – Characteristics of main research areas

System	Subsystems	Priority	Timeliness	Attractiveness
Liquid flavoring agents	Condensate	Additional source of aromatic components	Fresh ingredients, full profile	Improvement of the profile of flavoring agents FTNF, WONF
	Condensate concentrate	Full identity with the raw material	Recovery of aldehydes from acetal	New flavor compositions in food, household flavoring agents
	Dry raw material	Use of cross-modal mixtures	Non-traditional sources (tops, leaves)	Flavoured salt, products for special purpose
Emulsion flavoring agents	Sprays	The formulation includes vitamin D, chlorophyll, lycopene	For products with a modified formulation (without fat, salt, sugar, or with the reduced content)	The consumer aromatizes food by himself/herself on the basis of his/her experience and preferences. Alternative by the method of entering into \ on the product
	Foams			

Continuation of the Table 14

System	Subsystems	Priority	Timeliness	Attractiveness
Restoration of lost flavors	Vacuum processing and cooling	Use of the capacity of the raw material, after heat treatment	Lack of non-natural additives	Fresh water-melon and cucumber flavors in heat-treated products
	Gelatin films	Products with a “clean label”	Immobilization of plant enzymes	Combining technological operations
	Tempura	Amino acids of chickpeas and beans in the formulation	Expanding the range of culinary products	Adaptation of Slavonic products and Japanese cooking technologies

Recommendations on flavoring dietary food products

Consumer category	Name of food	Flavored products
Hepatitis, obesity, pancreatitis	Soups on vegetable broths, sauces	Sprays, foams, distillates
Patients with peptic ulcer and related diseases, dyspeptic symptoms, hepatitis	Toast, pureed dishes from pumpkin and cabbage	Flavored oil, flavored salt
Patients with functional indigestion, after resection. Children	Fruit jelly, cold soups, potato juice	Flavored jelly, cold soups. Gluten-free bread. Dietary bread
Elderly	Sauces, broths, pureed dishes, tempura	Flavored salt, dried raw material, distillates, minarines, oils, films

All subsystems of aroma recovery, except from tempura products, are designed for carrying out the main process – enzymatic reactions involving PUFAs (Fig. 44).

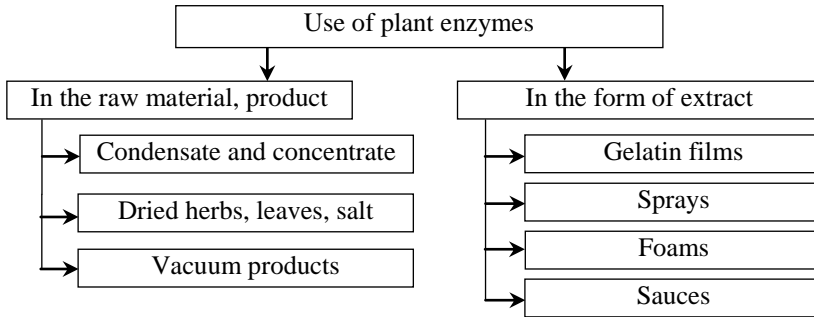


Figure 44 – The use of enzymes in aromatization processes

The enzymatic system in the raw material and extract provides the aromatization effect expressed within the range of several hours in the food product to two months in distillates. Therefore, technological solutions for the use of liquid flavoring agents and flavored products are not universal and differ depending on the method. The big advantage of the technologies using enzymes is natural and available components, the correspondence of flavor to the raw material, the use in the restaurant industry and others. The research results have been implemented by several ways.

1. Preparation of flavoring agents in the microwave vacuum drier and technologies of their use: to implement dehydration of moisture vapor by capturing water droplets in the form of the condensate, or adsorption (on dry food surface), or via membranes; to foster increased aromatic components accumulation in the product and outgoing vapors through enzymatic processes occurring during drying in microwave vacuum driers; to develop recommendations on the concentration and use of the collected aroma components.

The inserted condensate immersed in dry ice and a drop catcher is supposed to be used on the way to a “vacuum pump”. To obtain a non-food (household) flavoring agent, a desiccator should be replaced by the insert filled with magnesium sulfate, which absorbs aromatic components and dehydrates air flows from the microwave vacuum drier. A condensate trap made of polypropylene (this material is not heated in the microwave field) can be placed in the

machine processing chamber, attached to the body of the microwave radiator or mounted to a vacuum pump at the pipeline outlet. Depending on the design of a microwave vacuum drier, options of devices for collecting the condensate may be different. The developed technology uses the condensate and dried product as an end product (Fig. 45).

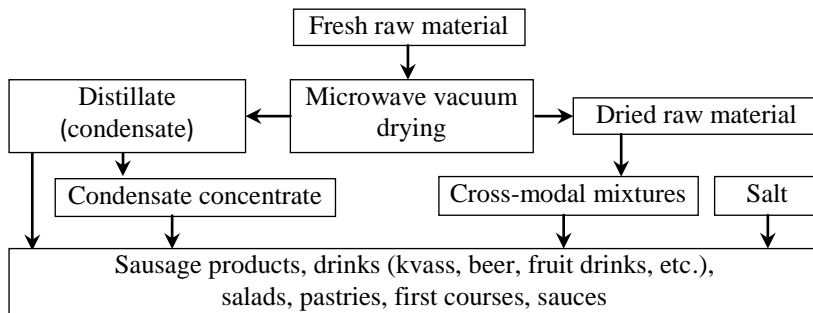


Figure 45 – End products of the microwave vacuum drier

The duration of treatment in the microwave vacuum drier is the following: 14–16 minutes for beet leaves; 11–13 minutes for tops of carrots; 10–12 minutes for pepper leaves, nuts, currants, mint; 8–10 minutes for marc of cucumbers, berries; 16–20 min for watermelon rinds, melon rinds; 14–17 minutes for pumpkins. The distillates were obtained by drying the raw material or the mixture according to the following procedure: Preparation of leaves, tops, marc, rinds → Grinding → Putting the raw material in the chamber → Setting the vacuum mode of 7–9 kPa → Setting the microwave heating mode of 0.6–0.8 kW → Drying the raw material and collection of the condensate → Making cross-modal mixtures (from dried material) and Concentration and implementation of the condensate.

MVD is promising not only for obtaining the condensate of aromatic components. Cross-modal mixtures were prepared from the following fresh raw material: rinds of watermelons, melons and cucumbers; leaves of pepper, currants, walnut and basil; tops of beets and carrots; these mixtures were used for developing the formulation of flavored salt “Sol’ka” or separately as seasonings, additives to

bakery products [401]. The implementation was carried out according to the following scheme:

- preparation of spice-aromatic herbs;
- drying to the moisture content of 55 % in the microwave field (0.3 kw);
- mixing with salt (1 : 1);
- heating in the microwave field (0.6–0.8 kW);
- grinding, sieving.

The most rational combination of cross-modal mixtures for aromatization of salt and obtaining the relevant condensates:

- leaves of pepper and basil (ratio of 5 : 2);
- walnut leaves and beet tops (ratio 1 : 1);
- carrot tops and leaves of currant (ratio 2 : 1).

The use of a flavored salt «Sol'ka» allows reducing the formulation quantity of salt without loss of the flavor profile.

2. Obtaining emulsion flavoring agents: the flavored base for emulsions is produced by vacuum processing of precursors and enzymes in a special device (Fig. 46).



Figure 46 – Vacuum marinators

To obtain concentrated emulsions by flavor biosynthesis, special suspensions were developed. The mixture for suspensions is composed of flavor precursors or substrates 5–25 %, aqueous extracts of enzymes LOX from the plant raw material 45–65 %, fruit marc (source HPL) 10–35 %, or activators of the enzymatic formation of aromatic components (Tabl. 15).

Table 15 – Biosynthesis of aromatic components

Flavor of emulsion	Aromatic components	Substrate / precursor, activator	Enzymes
Fruit, watermelon	Geranyl acetate, ionone, 2-methyl-2-heptene-6-on	Watermelon white or red pulp, soy oil meal	Wheat bran (LOX), watermelon fresh pulp (HPL)
Fresh greens, cucumber	Hexanal, hexenal 2,4-decadienal, nonenal and others	Corn, linseed oils, waxes (available PUFAs),	Soybeans, wheat bran (of LOX), fruit marc (HPL cucumber, watermelon white pulp , sweet pepper)
Fresh pumpkins, sweet pepper	Resorted complex of fruit raw material	Heat treated fruit raw material	Marc of pumpkin, cucumber, pepper
Meat flavoring agent	Flavor of boiled meat	Broth, vitamin D	Soybeans, wheat bran (LOX)
Fresh greens	Fresh	Corn, linseed oils	Sprouted grains (wheat)

The obtained bases are suspensions or thick puree products, which are treated in a vacuum marinator after mixing. Just before cooking the base of a flavoring agent, the sources of extracts of enzymes are cooled or heated in a microwave oven.

The methods of obtaining emulsion flavoring agents can be an example of molecular design when fragments of naturally occurring structures form an aromatic component using covalent bonds. The following equipment is necessary to use the emulsion:

- a pump atomizer for oil (a sprayer for vegetable oils) – ½ of the bottle is filled with the emulsion “oil in water” and ½ of the bottle is filled with the extract of enzymes (Fig. 44, items 1–3).
- an espuma machine (Fig. 44, item 4.).
- a syringe for meat products (Fig. 44, item 5).



Figure 44 – Equipment for emulsion flavoring agents

Industrial protein-based foams are prepared from oleoresin where certain aromatic components dissolve in oil. The condensate concentrate or emulsion base is used in the developed method, instead of oleoresins. The pressure vessel is filled with homogenates and a flavor precursor in espuma machines. The gas can be of two types – nitrogen oxide N_2O , and carbon dioxide CO_2 . If nitrogen oxide is supplied, the content is foamed and kept longer. If carbon dioxide is supplied the liquid bubbles, becomes carbonated. The product is brought to the state of a liquid puree, and then under the influence of nitrogen oxide in the pressure vessel is converted into a foam-like substance. This mixture activates the taste buds. The flavored emulsion with oil, soy extract and vitamin D can be used for pumping meat products or preparations of spray products.

Formulations of singlet oxygen foams were developed for enteral oxygen therapy in schools, sports and recreation complexes, cafes and juice bars; they are a complex with the following components: 1) singlet oxygen, 2) foaming mixture, 3) phyto preparations or mineral water, and 4) juice. An aqueous solution of lecithin (60 %), the suspension of enzymes from wheat bran (10 %) and homogenates from the cooled samples (30 %) of watermelon, cucumber, pepper and pumpkin were used as a blowing agent in this study. Singlet oxygen is produced in special or domestic devices such as VORWEKS Thermomix with parallel grinding and pressing fruit.

3. Flavored oils, films. It is advisable to use flavored oils during cooking toast in sandwich makers with two heating surfaces or toasters. The used enflourage technique is used to extract the aromatic components that are sensitive to even the slightest heat. Dissolved in oil, a flavor carrier is not destroyed by heating for some time. So the flavored toasts covered with an oil film with cucumber, strawberry flavor retain fresh flavor within an hour. The presence of unsaturated fatty acid with three double bonds causes the ability of rapid drying in linseed and corn oils. After application of a thin layer of the flavored oil, bread is recommended to be blown by hot air for several minutes to reduce the induction period of film drying. Flavored oils can be used for dressing salads, adding to dough before baking. While cooling, the flavored cocoa butter goes into the solid state within 4 hours so to use it for glazing extruded products (crackers, snacks, corn sticks) is promising.

The possibility of enzymatic processes in homogenates can be considered during aromatization of plant oils. The most important feature of oils is dissolution and protection of aromatic components from destruction by heat. The use of different flavors can significantly expand a range of food glazes. The processes with formation of aroma of freshly baked bread, roasted nuts mainly occur when adding oxidation products and linolenic acid to the offal suspension.

4. Products of flavored emulsions from sprouted wheat. Among the most famous products, alfalfa and green shoots of sprouted grain cereal (sprouts) are characterized by the high content of C₆–C₉ carbonyl compounds. We have found that sprouts from wheat, barley, and oats contained 5–8 times more C₆–C₉ carbonyl compounds than apple, cucumber, and pumpkin juices. Having undoubted health benefits, a high content of active chlorophyll, iron, sprouts and juices from them are not very appetizing due to a specific flavor. The strong predominance of grassy shades complicates the use of these dietary products. The companies specializing in production of dietary supplements from sprouted cereals solve the problem of excessive grassy shades by adding flavoring agents such as peppermint oil.

Diluting products from sprouted grains in water does not bring the desired effect of reducing grassy flavor. This is explained by the fact that because of small solubility in the aqueous phase flavor lipophilic composition is distributed in the air to the uttermost [10]. It is possible to reduce concentration of C₆–C₉ carbonyl compounds in the air phase, given the ability of those components to be dissolved in fats and the similarity of their partition coefficient in the system of water: oil. These features have been used for preparation of flavored emulsions for salads using sprouted grains. We have found that by 11–12 days of sprouting, the length of wheat germs was about 8 cm and contained maximum amount (for the whole growth period) of chlorophylls and C₆–C₉ carbonyl compounds. Grinded wheat germs of this period are the homogeneous dispersion medium which is resistant to disintegration due to the sufficient content of general lipids, phospholipids and free fatty acids. The deodorized vegetable oil (corn, sunflower, and soybean) was mixed with the grinded sprouted mass in different ratio, organoleptic parameters were evaluated. The taste panel stated that intensity of grassy flavor in the mixture was reduced in proportion to the amount of added oil. Residual “green” shades and the corresponding color were harmoniously combined in a ratio (wheat germs: oil) from 1 : 1 to 1 : 4. The shelf life of this mixture was several days at low temperatures.

There are some factors that contribute to lipid oxidation reactions in which chlorophyll and its pheophytin derivative act as prooxidants. In particular, chlorophyll may act as a sensitizer forming singlet oxygen – prooxidant of oxidation reactions of PUFAs. Iron and copper in the composition of chlorophylls and sprouts accelerate lipid oxidation by stimulating decomposition of hydroperoxides. The effect of these factors is eliminated by preliminary blanching of the plant raw material before basic processing. However, hydrolysis of plant lipids during blanching and acidification contribute to degradation of chlorophyll to pheophytin and manifestation of pheophytin oxidative properties.

The content of general lipids in sprouted cereals after 15 days of germination decreases; flavors are predominantly accumulated not in a liquid portion but in a solid residue. This is explained by the fact

that there is a significant redistribution of fatty acid composition during sprouting; it is proven by an iodine number of samples. Basic conversions of flavors after 15 days of cereal germination occur with participation of hydroperoxide lyase, which is concentrated in cell membranes. The organoleptic evaluation showed that the juice extracted from wheat germs after 15 days contained subtle flavor components, which did not have a sufficient capacity to be dissolved in oil. However, sprouted grains have fresh flavor in water, fruit and vegetable juices, fancy bread, and margarine. Adding a small amount of juice from sprouted grains to the heat-treated vegetable puree significantly improves its aromatic profile by restoring specific plant notes. The foods with an acidic medium stimulate the reaction of splitting chlorophyll and formation of pheophytin. This reaction is accompanied by the change in color (from green to olive); it narrows the scope of application of the flavored additives containing chlorophyll.

Considering the recovery of lost flavors in canned and dried foods as the enzymatic process, it is advisable to take into account oxidative processes in lipid components. Recovery of aromatic components involves blocking antioxidant properties of carotene, adding lipoxygenase and hydroperoxide lyase, and carrying out enzymatic reactions with their participation.

Thus, to attain a full flavor profile, presence of fresh green flavors is desired at certain concentrations and the relevant food matrix; in the products after industrial processing it is a need. The ways of lipid transformation into flavoring agents depend on the combined action of enzymes, their peculiarities as well as pro-oxidants and the relative amounts of auxiliary components. As a result of lipid oxidation reactions, a set of CC is produced; it imparts fresh flavor to the product or is able to restore it at the certain concentration.

Plant enzyme preparations: a natural PEP is proposed to use for improving organoleptic properties of end salads (Fig. 45, tabl. 14). The unpleasant order is produced during cooking and stewing vegetables; it reduces consumer demand for ready meals. The PEP

gives fresh pleasant flavor to vegetable salads, dietary products, krudite (mixture of boiled vegetables).

Table 16 – The range of dishes using natural flavoring agents

Name of dishes	Thermal processing	Ingredients	Flavoring agent (flavor enhancer)
Cabbage rolls (uncooked food)	Freezing	Cabbage, vegetable stuffing	Solid, during freezing
Soups	Boiling	Cabbage, root vegetables, tomatoes, onions	Liquid, before heat treatment
Salads	Fresh	Cabbage, tomatoes, onions, root vegetables, green herbs, spices, cucurbits	Dry, during mixing ingredients
Vegetable side dishes	Boiling, stewing	Cabbage, tomatoes, onions, root vegetables, herbs, spices, cucurbits	Liquid, before heat treatment
Baked vegetables	Baking	Cabbage, tomatoes, onions, root vegetables, herbs, spices, cucurbits	Liquid, before heat treatment
Hotpot (uncooked food)	Freezing	Pumpkin, peas, carrots, turnips, cabbage, macaroni	Solid, during freezing
Hotpot	Fresh, boiling	Pumpkin, peas, carrots, turnips, cabbage, macaroni	Dry, during mixing ingredients

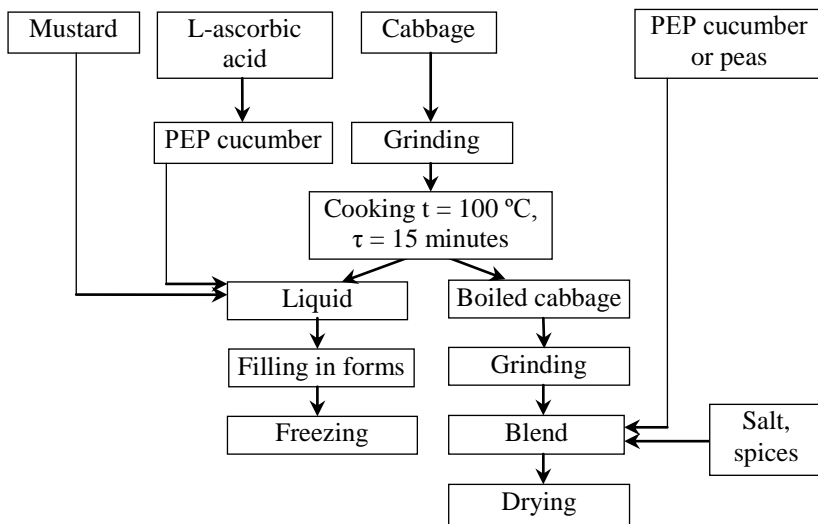


Figure 48 – Scheme manufacturing PEP

The formulations and the process production scheme for sauces of functional use have been developed during the research. The essence of the process is based on the ability of extracts from soy enzymes to give the boiled pulp of melons and pumpkins the aroma of special exotic additives (rose petals, lime flowers, etc.) that is lost during boiling. The optimal concentration of introducing enzyme extracts has been determined to be 5 % in relation to the amount of fruit puree, 10 % in relation to the amount of aroma components.

6. Tempura: Amino acids and the majority of unsaturated CC are destroyed by heating, so the change in flavor of tempura products testifies about its effect in aromatization processes. To this end in the formulation of tempura batter, 20 % tempura flour was replaced by melon homogenate with beans (1 : 1 ratio) and finely grinded prepared chickpeas and beans. Squashes and pumpkins were subjected to thermal processing: they were cut into 5–7 mm pieces, which were dipped in batter and fried in a deep frying pan with hot corn oil. The peculiarity of squash and pumpkin is the lack of pleasant flavor after heating, both in a liquid medium and in other

media. The analysis of the end products was performed organoleptically, after cooling to room temperature (Table. 15). The samples for analysis were prepared in the form of fried batter without products from tempura and wheat flour (classic batter).

Table 15 – Predominant flavors of fried products

Batter ingredient	Product in tempura batter		Batter (without product)	
	Squash	Pumpkin	Tempura	Classic
Chickpea-melon	Fresh	Cucumber	Cucumber	Bean
Chickpea	Popcorn	Bean	Nutty	Nutty
Kidney bean-melon	Apple	Fruity	Fruity	Bean
Kidney bean	Popcorn	Potato	Bread	Bread

The organoleptic evaluation and prevailing descriptors of separately fried batter, squash and pumpkin in different batter indicates that melon homogenate affects the end flavor in some way. The distinctive properties of starch in classic batter are that it has the lower ability to binding melon flavor. Therefore, a complex of unpleasant flavors is preserved when frying squash and pumpkin. Amino acids of beans and chickpeas cause formation of almost identical flavors in the ready-to-serve batter from tempura and wheat flour. So it can be concluded that the increased concentration of amino acids in Maillard reaction makes it possible to simulate the flavor of the ready-to-serve batter in a range of descriptors “bread”, “nutty”, “popcorn”.

7. The original Push Top technology has a world-wide novelty and is presented by healthy drinks in a separate package for autonomous mixing. It is based on separate packaging of the beverage components in a two-chamber tank.

The range of products for the Push Top packaging:

1. Watermelon puree (homogenized) with fresh watermelon aroma.
2. Melon puree (homogenized) with fresh melon aroma.

3. Potato juice (for people with increased gastric acidity or peptic ulcer) with fresh vegetable flavor.
4. Juice of wheat germs with fresh cucumber flavor.
5. Cabbage puree (homogenized) with fresh cucumber aroma.
6. Concentrated juices with restored fresh aroma.
7. Strawberry puree with fresh flavor.

Practical application of the results of in vitro aroma formation studies has been discussed in terms of flavoring potato juice.



The basic technological scheme of production of flavored products with autonomous mixing.

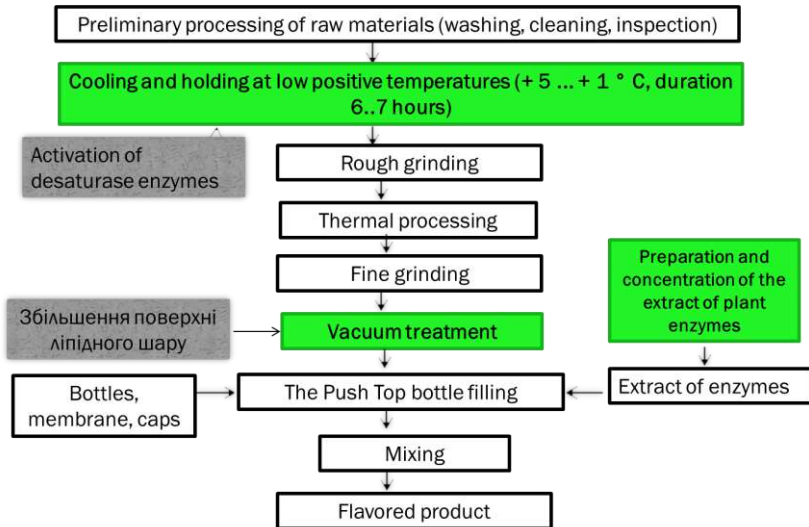


Figure 49 – The basic technological scheme of production of flavored products with autonomous mixing

Main results.



Emulsion flavorings (from precursors)

- Foam
- Sprays
- Emulsions



Restoration of lost flavors (on precursors)

- Vacuum processing and cooling
- Gelatin films
- Tempura
- Frozen flavorings



Aroma from precursors

- Salt
- Mixes from non-conventional raw material
- Concentrates of aroma



CONCLUSIONS

The manuscript is devoted to theoretical and experimental substantiation of the scientific principles of repetitive flavor formation or recovery in food systems. The systematized information on the participation of enzymes and flavor precursors has showed the prospects for developing processes of formation *in vitro* for Green Leaf Volatiles (GLVs) flavors. The scientific and practical approach called the technology of food products flavored *in situ* has been developed on the basis of the results. The use of aromas of lipid emulsions extracted from fresh or pretreated fruits and aqueous suspensions of plant homogenates in the biosynthesis has been studied.

The relationship between physical properties of the substrates and the final products of enzymatic aroma formation reactions *in vitro* has been examined. It has been found that the availability of components for reactions is determined by a complex indicator – the stability of lipid emulsions n_y (hydrodynamic particle size and ζ -potential). The change of n_y has been established to affect the potential mobility of particles and the number of flavors.

The conditions for multimolecular adsorption and biosynthesis of GLVs in emulsion flavors have been defined. Processing of enzyme-substrate complexes in vacuum results in approximation of lipid particles and enzymes, deformation of diffuse membranes and their mutual penetration. It has been first established that during vacuum heating (underpressure of 6 ± 1 kPa, temperature of 32 ± 2 °C), membrane-bonded enzymes of HPL in suspended plant homogenates are not inhibited in heat-treated fruits by excessive HPOs. This can be explained by the conditions of interphasic activation of enzyme-substrate complexes with underpressure of 6 ± 1 kPa, when Gibbs free energy changes, hydrophobic interaction, covalent bonds and van der Waals forces weaken. The importance of DLVO theory and disjoining pressure in the process of enzymatic aroma recovery has been demonstrated.

The conditions for activation of the enzymatic system of raw materials through its pretreatment have been identified: cooling

($t = 1...3$ °C, 5 hours), vacuum processing (7–9 kPa) and combined pretreatment (microwave heating and vacuum processing) necessary for formation of volatile components. Melons and gourds (watermelons, pumpkins, cucumbers, and melons) have been found to maximally restore flavors after heat treatment as compared with other fruits. The comparative analysis of the concentration and qualitative composition of PUFAs in the lipid extract of fresh melons and melons after cooking has showed the preconditions for fresh aroma recovery. The analysis of antioxidant activity (AOA), oxidation-reduction potential (ORP) of fresh melons and melons after heat treatment has showed that these two indicators largely determine the ability of fruit to repetitively form aromatic components. Hydrothermal treatment of melon fruit by reducing AOA values by 60–80 % causes subsequent oxidation reactions involving enzymes and prooxidant components of fruit.

It has been shown that processed fruits (hydrothermal treatment, processing in vacuum and microwave field, freezing) form lipid peroxidation products in varying degrees and can be conditionally divided into groups with high and low ORP values. Melon fruit belongs to the group with a low ORP value of 50–170 mV. Carrier systems of the oxidoreductase group of enzymes represent the area from –330 mV or lower. The activity of electrons in the enzyme extract is much higher than in the fruit system; it affects the process of aroma recovery that is retained in food from one to three hours. The possibility of prolonged preservation of C₆–C₉ carbonyl compounds has been studied in the oil extract from wheat germs with an optimal ratio of germs and oil.

The properties of the distillates obtained in a vacuum microwave dryer have been investigated. It has been shown that differences in the distillate particle size affect the perception of the aromatic profile. Recovery of the lost flavors involves control of the pH in the medium the distillates are introduced to. It has been established that fresh cucumber flavor is restored by converting acetals from distillates in the acidic medium (pH = 3) into key aldehydes. In the faintly acid medium (pH = 6.0), flavor is positively transformed due to the components of the melon distillate.

Aroma recovery of heat-treated melon fruits has been established to depend on changes in the amino acid composition. Chickpeas and beans are considered to be sources of amino acids restoring aroma of melon and watermelon. The chickpea ability to renew the amino acid composition of melon is stronger by 25 % compared with beans. The use of chickpeas as a component of tempura flour increases the proportion of aromatic components in the final product and gives it a distinctive flavor.

The participation of isomeric forms of PUFA waxes (bee and vegetable waxes), fish oil, lipids of raw meat, and linseed oil in the formation of the volatile flavor composition as precursors has been shown. The foam heterogeneous system has turned out to represent changes in the key and coloring flavor components to the uttermost.

The index of the enzymatic reaction rate and ways to increase it have been studied which is an important condition for technological processes. The ability of vitamin D to activate lipolytic enzymes and the ability of singlet oxygen to reduce the lag time of oxidation processes of PUFA have been defined. Activators of aroma recovery processes are substances with oxidizing power such as pheophytin, cis isomers of lycopene, and desaturation reactions of PUFAs.

Interphasic activation of enzymatic reactions is carried out through increase of the contact surface area in the “enzyme-substrate” system, dispersal on the product surface and intermolecular interaction in gelatin jelly by foaming. The advantage of the use of gelatin solutions in aromatization *in situ* due to their ability to immobilize enzymes from an aqueous extract has been identified.

The manuscript provides scientific and practical recommendations for cooking flavored foods (foam, jelly, salt «Sol'ka», tempura, sauces, distillates, fillings and stuffings, emulsions, oil). The foods flavored *in situ* overcome sensory deficiency associated with reduction of salt, sugar and fat in food. The social implication of new aromatization ways is to develop foods for children, the elderly and people who are prone to chronic diseases.

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Наукове видання

ДУБОВА Галина Євгеніївна

**РОЗВИТОК ДОСЛІДЖЕНЬ
ВІДНОВЛЕННЯ ХАРЧОВИХ
АРОМАТІВ**

(Англійською мовою)

Монографія

Головний редактор *М. П. Гречук*
Комп'ютерне верстання *О. С. Корніліч*

Формат 60x84/16. Ум. друк. арк. 10,9.
Тираж 300 пр. Зам. № 120/897.

Видавець і виготовлювач
Вищий навчальний заклад Укоопспілки
«Полтавський університет економіки і торгівлі»,
к. 115, вул. Коваля, 3, м. Полтава, 36014; ☎(0532) 50-24-81

Свідоцтво про внесення до Державного реєстру видавців,
виготівників і розповсюджувачів видавничої продукції ДК № 3827 від 08.07.2010 р.