Study of aroma formation from lipids of the fruit raw material

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Keywords:

Aroma
Homogenate
Precursor
Enzyme
Substrate

Article history:

Received 22.09.2016
Received in revised form 23.11.2016
Accepted 27.12.2016

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DOI: 10.24263/2304-974X-2016-5-4-3

Abstract

Introduction. It was studied the formation of fruit flavor involving precursors and enzymes. It has a certain advantage over other methods.

Materials and methods. Lipid emulsions fruits (cucumber, squash, watermelon) were prepared in the Soxhlet apparatus according to the classical procedure using chloroform-ethanol solvent. The intensity of oxidative processes was evaluated using the developed technique based on the reactions of carbonyl compounds (CC) in the vapor phase with 2,4-dinitrophenylhydrazine. The particle size distribution (PSD), ζ-potential of the colloidal fraction was made on the analyser Malvern Zetasizer Nano ZS with a detection angle 173 °C. Recovery of fresh taste and aroma lost during thermal treatment was calculated by using organoleptic methods.

Results and discussions. The molecules of a precursor-compound can withstand the processing modes, while enzymes and aromatic compounds break down frequently. Most of the aromatic components are reaction intermediates formed between the substrate (lipid hydroperoxide derivatives, HPO) and the corresponding enzymes (hydroperoxide lyase HPL). The fruit and vegetable pretreatment conditions and subsequent environment in which enzymatic reactions take place can be considered as potential factors in the formation of fresh flavors.

The hanges of the plant aromatic components during heat or combined processing are associated with transformations of lipid components. The availability of these components for enzymatic reactions depends on the distribution of lipid particles according to their size and potential mobility. Pre-treatment of samples positively influences binding energy in the complex of enzyme-substrate. The change of iodine number in treated homogenates, as compared to fresh ones, shows isomerization of flavor precursors. The minimal quantity of homogenates introduced (up to 20 g) and the duration of aroma-restoring reaction (from 5 to 7 minutes) were defined.

During heating in vacuum (with underpressure 6±1 kPa, at temperature 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 change. These effects ensure multimolecular adsorption and biosynthesis of green leaf volatiles (GLVs) in the fruits after heat treatment.

Introduction

The studies in the field of aroma formation mechanisms have showed that fragrances are formed from precursors and enzymes. Amino acids, carbohydrates, and lipids can be flavor precursors of the plant raw material under different conditions (DeMan John M., 1999; Guentert M.,2007). It has been found that thioglycosides, conjugate S-cysteine in grapes, and alliin in garlic can be precursors of fresh fruits of cabbage family (Ho Ch.-T at el,1990; Yu T.H. at el,1994; Tominaga T. at el,1998). As a result of reactions between the precursors of lipid nature, polyunsaturated fatty acids (PUFAs) and enzymes, the "green flavor" of crushed leaves (GLVs - Green Leaf Volatiles) occurs (Kalbrener J. E, 1974; Yang, X. et al, 2011; Gigot C., et al., 2010). The formation of fruit flavor involving precursors and enzymes is important and has a certain advantage over other methods. This advantage ensures fast formation of food flavor shortly before a meal or in the mouth. The conditions of flavor formation by virtue of precursors of lipid nature and enzymes have been sufficiently studied at different pH, temperature, presence of specific isoforms of enzymes, localization in the cell of fresh fruits (Luning P. A., 1995; R. Davidovich-Rikanati, 2008; Song J., 2010). The possibility of formation of really different volatile components has been shown on the test solutions of PUFAs extracted from the plant raw material (Kalbrener, J. E, 1974).

Mechanical grinding is sufficient for carrying out enzymatic reactions involving lipids cells in fresh fruits (cucumber, tomato, banana, etc.). Changes in lipid structures after heat, physical or combined processing of fruits have been considerably investigated only from tpoint ohe f view of peroxide processes (Jiménez-Monreal, A. M., 2009). 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 (Reed G.,1966). The preconditions for the repetitive flavor formation from precursors in fruits after heat treatment have been previously investigated (Dateo G. P. at el., 1957; Hasselstrom T., 1962; Schwimmer S.,1963), 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 rescovery 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) (Damodaran S., Parkin K. L., 2008; Hui Y. H., 2010). 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.

Materials and methods

Materials. Cucumbers, pumpkins, watermelons, and basil leaves were purchased from Poltava farmers during mass harvesting in summer.

Fruit heat processing: hydrothermal processing such as convective processing (fruits were immersed in boiling water, kept for 20 minutes and cooled) and combined processing including vacuum one with microwave heating (in the vacuum plant for steam distillation combined with a Selekta microwave oven with capacity of 0.6 kW, frequency of 2450 Hz, vacuum depth of 6±1 kPa, the material temperature of 32±2 °C), freezing (at temperature of -18 °C) in a freezer, and defrosting in a microwave unit).

Aqueous suspensions of fruits were prepared from fresh or heat treated fruits by mixing them with water at room temperature in the ratio 1:1 followed by homogenization.

Lipid emulsions were prepared in the Soxhlet apparatus according to the classical procedure using chloroform-ethanol solvent.

Centrifugation of the lipid fraction was carried out on the laboratory centrifuge MPW-223e with the drum rotation speed of 300–4000 r/min.

Method for determination of hydroperoxides and diene conjugates is based on measuring the light absorption by diene hydroperoxides at 234 nm on the spectrophotometer SF-42 at room temperature, with a molar extinction coefficient $2{,}5 \cdot 10^4$ $M^{-1}cm^{-1}$.

Determination of aldehydes. The intensity of oxidative processes was evaluated using the developed technique based on the reactions of carbonyl compounds (CC) in the vapor phase with 2,4-dinitrophenylhydrazine. The plant raw material weighing 100 gr was transferred to a 500 ml volumetric flask. A special container of 5 cm^3 was filled with 1% alcoholic solution of 2,4 - dinitrophenylhydrazine and hung inside the flask.

The flask was sealed with a ground-in stopper and put in the thermo cabinet for 5 hours at 60 °C. The flask was cooled for 50 minutes on the glazed tile, the 1% alcoholic solution of 2,4-dinitrophenylhydrazine was transferred from the special container to a standard glass cuvette of 24 mm wide and 5 mm thick. The absorption spectra of light waves at 490 nm in photocolorimeter were determined. The concentration of C_6–C_9 carbonyl compounds in pairs of the plant product was defined with the help of the pre-constructed calibration line.

Analysis of volatile components was carried out on the chromatography-mass spectrometry system Agilent 6890N/5973 inert (Agilent Technologies, the USA). The capillary column: HP-5ms 30m × 0,25mm × 0,25mkm (Agilent technologies, the USA). Temperature mode: 50 °C (5 min.), the temperature gradient: 5 °C/min to 220 °C (5 min.), the second gradient: 5 °C/min to 300 °C (10 min.), carrier gas: helium, flow velocity through the column: 1.0 ml/min. The evaporator temperature is 250 °C, interface temperature is 280 °C, the sample was introduced in the mode of 1:25 flow separation. Registration of ions was performed in SCAN mode in the range of 30–420 m/z. The mixture components were identified using a mass spectra library NIST02.

The distribution of size, particle size distribution (PSD), ζ-potential of the colloidal fraction was made on the analyser Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK) with a detection angle 173 °C. All measurements were performed in a temperature-controlled cell by 25 °C using ditch/cuvette DTS0012. At least five replicate measurements were made to control results repeatability on each sample. Size distribution in terms of intensity was obtained from the analysis of the correlation functions using an algorithm of General-purpose software analyser Zetasizer Software 7.11.

The descriptive sensory analysis of flavor in aqueous suspensions was made by the session of qualified judges of 12 women of different age groups who had the experience in the field of sensory evaluation. The evaluated attributes (aroma identity, other flavors, correspondence to the fresh raw material, shades) were grouped into conventional categories by the distribution method. The team members practiced assessment of defining attributes and their intensity during training on the basis of the differential threshold such as the minimum perceptible presence or change of flavor shades. Every descriptive attribute was verbally described; the patterns of fruit sample flavors were submitted to judges at the time of preparation; identification of descriptors served as a guidemark during testing.

Results and discussions

In spite of the diversity of the plant volatile components, most of them belong to three main groups: terpenes, phenylpropanoids/benzenoids and PUFA derivatives. By the accumulation point, volatile components are divided into those concentrated in: protoplasm or cell sap, idioblasts, and in special structures such as receptacles of essential oils. 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 (Mactavish H. S., 1998). 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 1).

Table 1

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 (Damodaran S., Parkin K. L., 2008). 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.

Aromatic components of melons (cucumber, pumpkin and watermelon) are characterized by distinct sensibility to the thermal effect; they contain similar key and shade C_6-C_9 aldehydes, their alcohol derivatives (Bezysov A., 2015). Only trace amounts of aromatic components which are not recorded on aromograms were identified by smell in the samples of these fruits after freezing and hydrothermal treatment, as their concentration was below the detection threshold of chromatographs. Our earlier studies of the fruit lipids demonstrated that the fruit content was not changed after cooking but the accumulation of hydroperoxide derivatives of PUFA occurs. Also, as a result of heat processing, the accumulation of hydroperoxides (HPO) occurs after heat treatment in artichoke, asparagus, green beans, pepper, spinach, carrots and others (Jiménez-Monreal, A. M., 2009).

Hydroperoxides (HPO) of fatty acids are split by the enzyme of hydroperoxide lyase (HPL) (Feussner and Wasternack, 2002; Noordermeer et al., 2001). Depending on the characteristics of the carrying base, HPL_S can be classified into three groups (Matsui K. et al., 2001; Mita et al., 2005):

– 13-HPL which specifically splits fatty acids 13-HPO to form C6-aldehydes (polyhexenal (1) or (Z) -3-hexenal (2)) and 12-oxo- (Z) -9-dodecenoic acid;

– 9/13 HPL which can split both 13 and 9-HPO-HPO with virtually the same efficiency;

– 9-HPL which specifically splits 9-HPO to C9-aldehydes ((Z) -3-nonenal (3) or (Z, Z) -3,6- nonadienal (4)) and 9-oxo-nonanoic acid.

Hydroperoxide lyase HPL belongs to membrane bound enzymes. 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:

$$9/13\ NRA + 9/13HPL \rightarrow C_6\text{-}\ C_9\ \ aldehydes + C_9\text{-}C_{12}\ oxoacids$$

The reactions of hydroperoxide lyase in fresh fruits are sufficiently studied (Klee, H. J., 2010; Lanciotti R. et al, 2004), and their participation in the thermally processed raw material causes aroma-forming reactions. 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 2).

Table 2

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	10000…5000	5000…1000	1000…500
Total content of hydro-peroxide 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 (Nakauma et al., 2008). 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 (Tsukagoshi K.,1996; Radko S., 2000). 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 2) are the conditions for maximum effect of HPL. The expected result of this action is the reduced amount of hydroperoxides and increased concentration of C_6-C_9 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, single lipids don't exist in cell membranes but lipid nanodomains do (Eggeling C. et al., 2009), 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 (Figures 1–4).

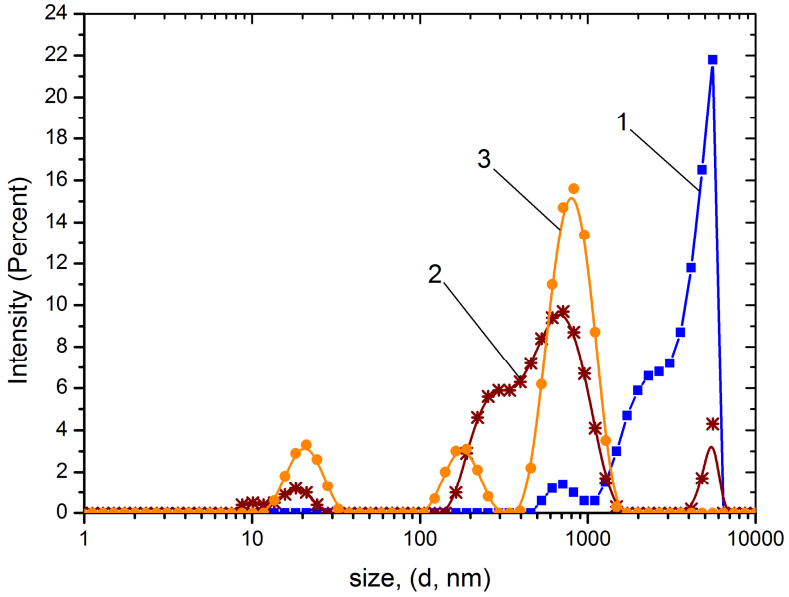

Figure 1. PSD of lipids from the fruits after hydrothermal processing: 1 – without separation, 2 – separation for 10 minutes at frequency of 1500 rev/min, 3 – separation for 10 min at frequency 4000 rev/min)

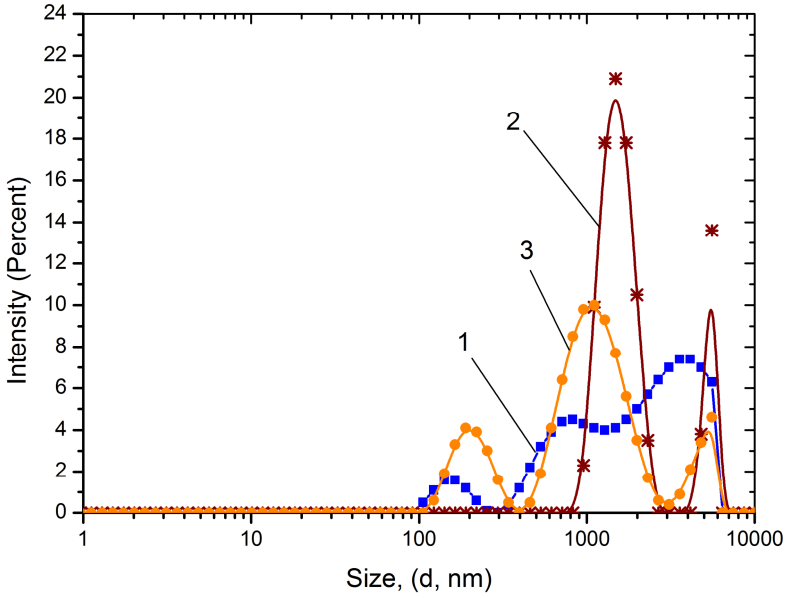

Figure 2. PSD of lipids from the fruits after hydrothermal processing: 1 – without separation, 2 – separation for 20 minutes at frequency of 1500 rev/min, 3 – separation for 20 minutes at frequency of 4000 rev/min

Lipid domains in the extract that was not separated in the centrifugal field are mainly composed of particles with a hydrodynamic diameter of 5000 nm. Separation in the centrifugal field for 10 minutes at frequency of 1500 rev/min decreases a hydrodynamic diameter of lipid domains to 2000 nm and separation at frequency of 4000 rev/min reduces it to 1000 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 (Figure 2).

Lipid extracts that were not separated in the centrifugal field are mainly composed of particles with a hydrodynamic diameter of 6000 nm. Separation in the centrifugal field for 20 minutes at frequency of 1500 rev/min decreases a hydrodynamic diameter of lipid domains to 800 nm and 25 nm and separation at frequency of 4000 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.

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 (Van Buggenhout S., 2006). 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 (Figures 3–4).

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 1000 nm and 100 nm.

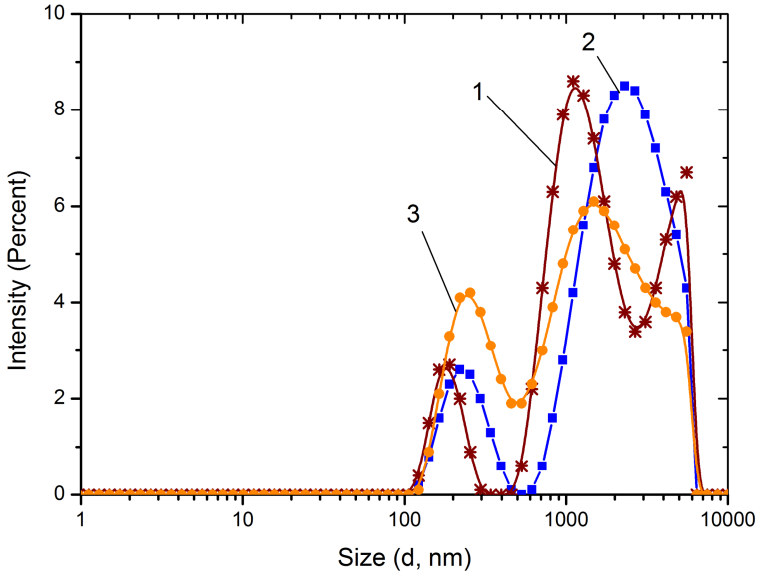

Figure 3. PSD of lipids from the fruits after vacuum treatment:
1 – without separation, 2 – separation for 10 minutes at frequency of 1500 rev/min,
3 – separation for 10 minutes at frequency of 4000 rev/min

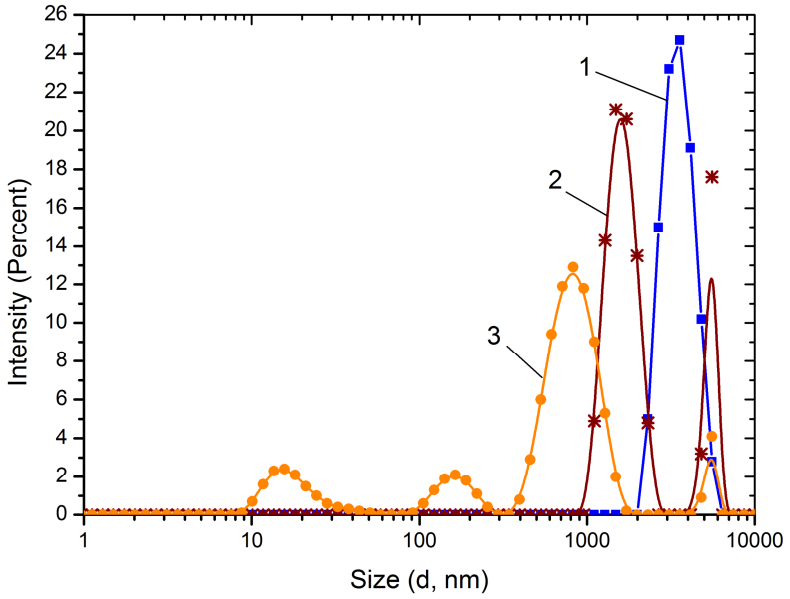

Figure 4. PSD of lipids from the fruits after vacuum treatment:
1 – without separation, 2 – separation for 20 minutes at frequency of 1500 rev/min,
3 – separation for 20 minutes at frequency of 4000 rev/min

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 (Figure 3) 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 Ca2 + 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 C_6-C_9 aldehydes were registered (Figures 5,6).

The intensity and peak area of aromatic components indicate the formation of C_6-C_9 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.

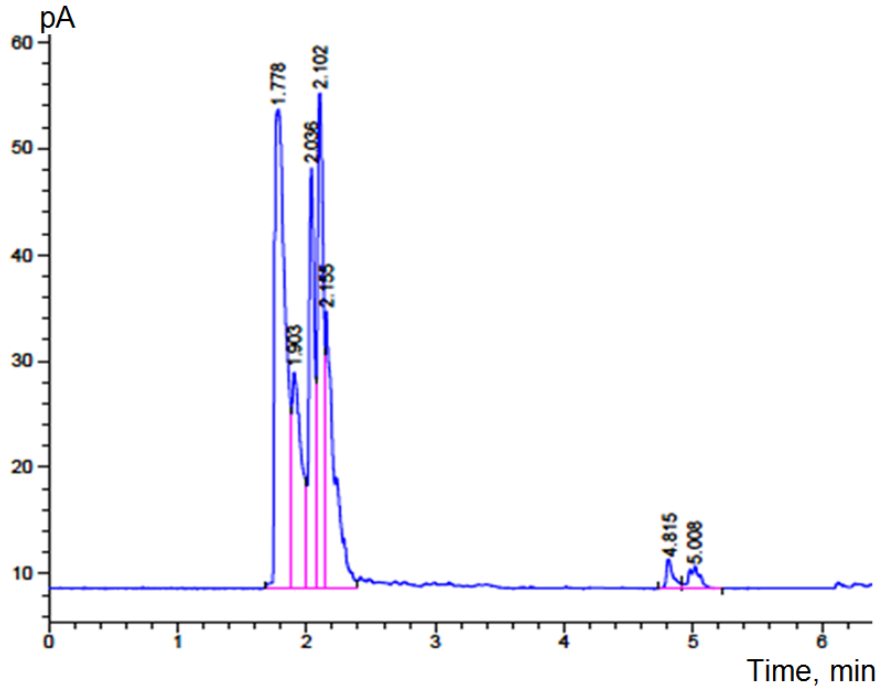

Figure 5. Aromatogram of the lipid extract after combined processing of fruits with active HPL

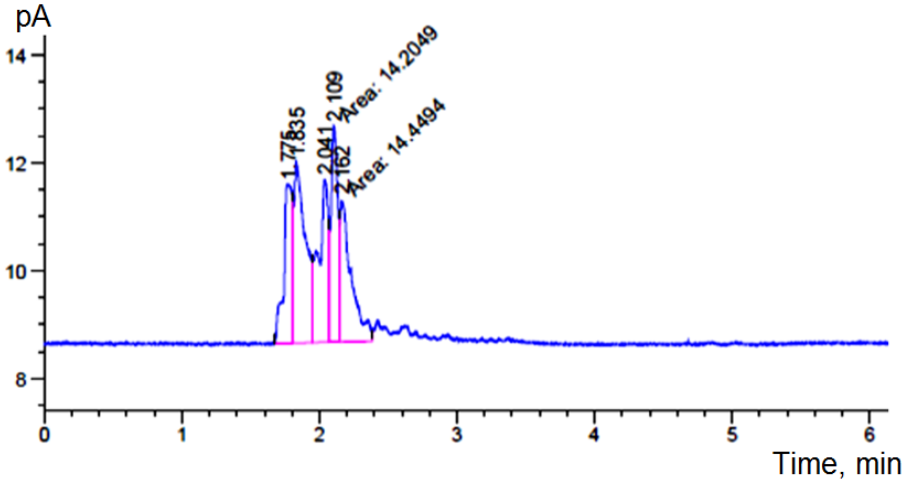

Figure 6. Aromatogram of the fruit lipid extract with inactive HPL

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 statistical analysis of experimental data on the effect of vacuum depth and processing temperature mode fruit pulp is represented as the following relationship:

$$Y = 2,94 - 0,0125 \cdot X_1 – 20,73/X_2$$

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, ºC. Upper level is 46ºC, lower level is 20 ºC, variation interval is 3 ºC

Y – number of flavor, relative units

The received data are valid according to the value of the Student's coefficient for the mathematical model.

Thus, regardless of the method of heat input, the functional activity of membrane proteins and dynamic properties of the membrane lipid matrix contributing to conformational flexibility of enzymes were shown. Therefore, vacuum processing of watermelon flesh which lost its flavor results in aroma recovery due to the repeated enzyme-substrate interactions down to measurable concentration which can be recorded on a chromatograph (Figure 7). The peaks responsible for fresh flavors of Nonadienal, 3-hexenal, 3-nonenal, 12-oxo- (Z) -9-dodecenoic acid, 5-Nonanol corresponding to enzymatic

hydrolysis of peroxide compounds of fatty acids were identified among the compounds (2-Propenoic acid, 2-hydroxyethyl ester, Propane, 2- (ethenyloxy) - 2-Propanamine, Adenosine, 4'-de (hydroxymethyl) -4 '- [N-ethylaminoformyl], 2-Furanmethanol, Propanoic acid, 2-oxo-, methyl ester, Ethyl 3-hydroxy-3-methylbutanoate, dl-Glyceraldehyde dimer, S-Ethyl ethanethioate, 2-Butanone, Cyclohexanone, 2,4-Dihydroxy-2,5-dimethyl-3 (2H) - furan-3-one, Propane, Cyclopropanecarboxamide, ethyl ester, Furyl hydroxymethyl ketone, Propanal, 4-Mercaptophenol, 1,2,3-Propanetriol, 1-acetate).

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.

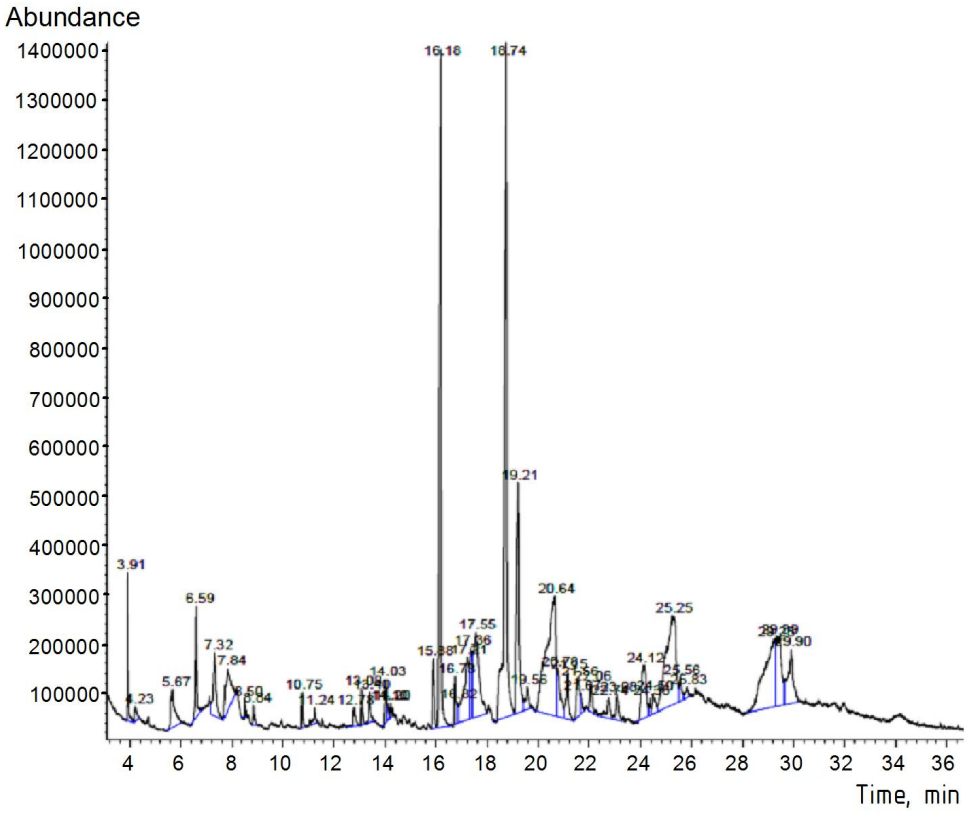

Figure 7. Aromatogram of the restored flavor of watermelon samples

Conclusions

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 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.

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.

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