STUDIES OF SOME ASPECTS IN THE PROCESS OF AROMA RESTORATION

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Abstract: The lack of data about the polydisperse nature of distillates and the impact of separate micro particles to recovery of the natural flavor in food still does not have a solution. Such properties of the distillates as dispersion and the size of the micro particles using a Zetasizer Software 7.11 are discussed at the article for the first time. It is established that the hydrodynamic diameter of the particles in the distillate values from 200 nm to 600 nm. Changes of the hydrodynamic particles size in distillate by water dilution confirms the assumptions about their hydrophobic nature and availability of results of such processes as coacervation, hydrophobic hydration, hydrophobic interaction. The differences in sensory characteristics to some extent is confirmed by the differences in the average hydrodynamic diameter of the sample: 150 nm and 190 nm, in the laboratory and industrial respectively. The interrelation between the sensory characteristics of fruit distillates, dispersion and method of heat treatment of fruits in the convective and microwave field is shown. The differences in the shades of the fruit flavours of melon and cucumber in the fruit distillates, manifested in the isomerization of the components of the flavour are given. It is shown that aroma restoration differs in different mediums by pH. In an acidic medium (pH = 3.0) converting of acetals of cucumber distillates to aldehydes leads to the full restoration of the fresh scent, because aldehydes are key components. In subacid medium (pH = 6.0), positive changes of an aroma are made to the components of melon distillate. These results contribute to the economic competitiveness of distillates compared with other types of flavouring materials.

Keywords: aroma, distillate, esters, acetals, dispersion, isomerization

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INTRODUCTION

It is believed that the restoration of flavour – a return of aromatic components lost during the juice concentration [1, 2, 3]. Aromatic components are caught during evaporation of water from the juice or other products, and is collected in the distillate. Flavouring materials in the form of concentrated distillates are added to the final product to restore the original flavours and aromas, as technological processing of food and beverages alter their flavours [4]. Distillates are solutions obtained by separating the liquid components of the aroma by the boiling points or the separation of liquid from substances difficult to evaporate, i.e. distillation [5]. To distillate most of the aromatic substances from pome fruit juices it is necessary to evaporate about 10% of moisture under atmospheric pressure, and from 15% to 85% under vacuum [6]. Concentrated distillates (FTNF, WONF) are in demand in the food industry due to long-term storage and their small volume.

The concentration of aromatic components during diffuse evaporation through the membrane, when only the key aroma compounds, obtained from natural sources, but not all are concentrated, got intensive development in pervaporation processes [7, 8]. The use of such concentrated flavours has such disadvantage as the lack of natural original flavour during the recovery process. This problem is also related to the difficulty of preserving the relative concentrations of various aromatic compounds, their proportions. According to the published facts, none of the existing methods of concentration is not able to accurately restore the original taste and flavour. These methods are improving and, nevertheless, there remains a need to further adjustment of the flavour during restoration process [9]. The lack of data about the polydisperse nature of distillates and the impact of separate micro particles on flavour restoration has led to this study, as a full-fledged restoration of the natural flavour in food products remains the problem, which is not completely solved.

The process of restoring the flavour in fermented beverages with low calorie content is of interest [10]. Ethanol is removed from these beverages by dialysis.

Then, the alcohol is separated from the dialysis liquid by vacuum distillation, and the remaining liquid with flavours is recycled to the main product. The fragrance in end products such as alcohol free wine remains in the initial concentration and is quite impressive as before the distillation. In most cases, the addition of only key components does not solve the problem of aroma restoration, and the use of distillates for this purpose requires further development.

The purpose of the research is to study the dispersion of particles of distillates, their stability and impact on the sensory characteristics. Realization of this goal will let determine the properties of some components of the distillate, the ability of target restoration of key aromas in the different pH mediums and assess the impact of isomerization during the restoration of melon and cucumber aromas.

OBJECTS AND METHODS OF STUDY

Distillates were obtained in the laboratory vacuum plant with microwave heating [11, 12] and the condensation of exhaust gases. The second laboratory vacuum plant is made similar to the first one, but with a convective heating. The process of vacuum distillation was performed at 10 kPa until the volume of homogenates was reduce by 20%. Microwave heating was performed under the power of 0.4 kW, convective heating under the temperature of 40°C. In laboratory plants the distance from the evaporator to the condenser – 450 mm. Concentration of the distillates was carried out by the addition of magnesium sulphate to oversaturation.

Homogenates for obtaining the distillates were prepared from fruit pomace (melon, cucumber), flavour precursors (linoleic and linolenic acid), aqueous extracts of enzymes from plants (soy lipoxygenase), a buffer solution, according to the developed method of the biosynthesis of flavour with shades of fresh fruit [13]. The mechanism of formation of aromatic components from homogenates is described with the help of such reactions as: oxidation of flavour precursor to 9,13-hydroperoxides, hydroperoxides cleavage to alcohols and aldehydes with six to nine carbon atoms, ester formation (from the acids and alcohols), acetals (from aldehydes and alcohols) [14].

The characterization of distillates was carried out according to the following parameters:

– The distribution of size of the colloidal fraction was made on the analyser Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., UK) with a detection angle 173°. 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 weight content of aromatic substances (was determined by dichromate method according to the quantity consumed by the titration of sodium thiosulfate);

– The concentration of aldehyde (was obtained with the help of spectrophotometer by the number of oxidized 2,4-dinitrophenylhydrazine);

– The number of aroma – the quotient of the mass concentration of the substance on its threshold concentration. The threshold concentration was determined organoleptically;

– Organoleptic, based on the grouping of samples of conventional categories, by the method of distribution. Categories of samples were based on a difference threshold – the minimum perceptible change of the intensity of the stimulus, i.e., availability of flavour shades.

10 women of different age took part in the sensory analysis. Round-table discussions were conducted prior to the beginning of the test to familiarize the panel with the test instructions and protocol. Partitioned booths were located in a temperature-controlled environment (20°C) and equipped with fluorescent lights. Minimal distractions were permitted to interfere with panellist judgments [15].

RESULTS AND DISCUSSIONS

Physicochemical parameters and sensory evaluation of distillates is an important indicator of the processes of production of alcoholic beverages, juices [16]. The distillate, formed during drying, stores volatile components isolated from the raw material in the airspace [17, 18]. Mainly ethers, alcohols, aldehydes, acids, which are transformed during the distillation into the distillate, represent volatile components of melon and cucumber. Due to condensation aldehydes, alcohols, acids dissolve and esters, lactones, diethers of hydrates aldehydes (acetals) in the obtained distillates are in the form of insoluble micro particles. These micro particles are soluble in alcohols, organic solvents and create definite dispersion in aqueous medium. The number of carbon atoms in insoluble micro particles affects their molecular weight, size, and aroma, which can serve as a specific identifier in the studies. Sizing esters and acetals in distillates is a promising direction in the analysis of the processes of separation of volatile components during the distillation process.

Odour of esters, acetals, and lactones depends on the number of carbon atoms in the starting compounds. Aliphatic esters in different combinations play an important role in many fruit aromas and in addition carry the floral-fruit, caramel flavours, and a variety of shades: apple, strawberry, pear, pineapple and others [19]. The major aroma components of the melon distillate are esters of hexyl octanoate hexyl acetate, propyl acetate, ethyl butyrate [20].

The main cucumber odor components are water-soluble aldehydes (E, Z)-2,6-nonadienal and (E)-2-nonenal [21]. Aldehydes are highly reactive components and together with alcohols they create a strong scent of fresh fruitage, vegetables, new-mown grass [22– 24]. However, the share of the ester (cis-3-hexenilacetate) is about 40% of the total number of aromatic components in the new-mown grass odour. Insoluble acetals, which are typical for the scent of cucumber, are represented by (E, Z)-2,6-nonadienal diethyl acetal, di-(Z)-3-hexen-1-yl acetal [25]. Cucumber distillates gain their aromatic trace due to insoluble ester ethyl 3-(methylthio) propionate and divinyl esters of PUFA.

The chromatographic analysis of melon and cucumber distillates, has showed the presence of complex esters and acetals about 18–20%; their composition was somewhat different depending on the type of fruit, maturity stage and growing conditions. Study of distillates during convective distillation showed the presence of water-insoluble particles with different hydrodynamic diameter (Fig. 1).

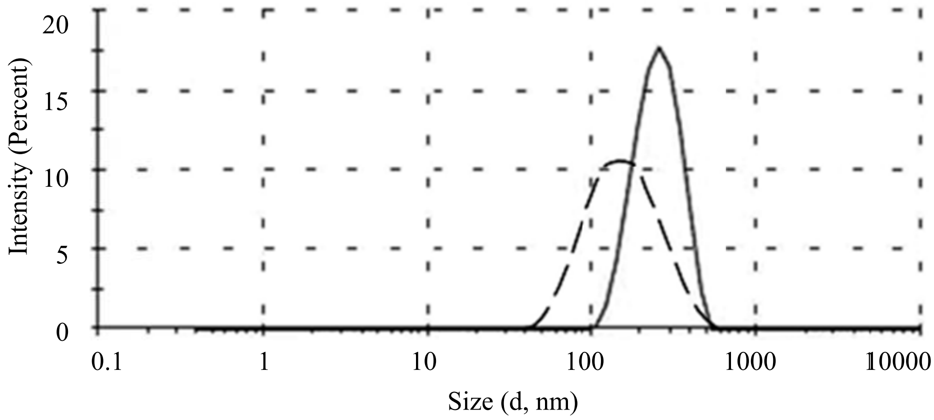

Fig. 1. Distribution of distillates particle by size.

The molecular weight of acetal in the cucumber distillate on average is slightly more than the esters in melon, which is reflected in particles polydispersion characteristics. Hydrodynamic diameter of particles in cucumber distillate is 336 ± 36 nm with a polydispersion 0.436 ± 0.083. Esters in melon distillate have particles sizes 153 ± 2 nm and a polydispersion 0.211 ± 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 esters in melon on average is 118.2 (Burdock, 2009). Due to the higher molecular weight, most acetals have negligible odour or are odourless. Flavour intensity in the melon distillate was greater than in cucumber, 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 the hydrodynamic diameter in cucumber and melon distillate could take other values different from the above. Taking into consideration that described the interrelations remains the same.

It was found out that not one, but several peaks and correspondingly different values: 200 nm, 300 nm, 400 nm and 600 nm are observed under repeated measurements of the hydrodynamic diameter of the particles in the distillates. This indicates that the micro particles have an irregular shape in the form of droplets or micelles. Studying the behaviour of micro particles at a dilution in water and alcohol the hypothesis about droplet particle structure and hydrophobic nature is confirm. For example, cucumber distillate stirring on a magnetic stirrer causes a change in the hydrodynamic size from 456 ± 121 nm and a polydispersion 0.546 ± 0.058, to 273 ± 18 nm and the polydispersion 0.454 ± 0.049. These changes are the result of intermolecular interactions and thus depend on the molecular level on the behaviour of the dissolved substance.

Hydrophobic groups added solutes interact weakly with neighbouring water molecules, as if they prefer a non-aqueous medium. However, these weak interactions can have profound structural aftermath [26, 27]. Special structures formation in water near incompatible nonpolar substances is called “hydrophobic hydration” (Fig. 2, Table 1). If there are two isolated non-polar groups, they are incompatible with an aqueous medium and it encourages their association, thereby reducing interfacial surface “water-nonpolar substance”. This process is thermodynamically favourable, partly reverse to hydrophobic hydration, and is called “hydrophobic interaction” [28].

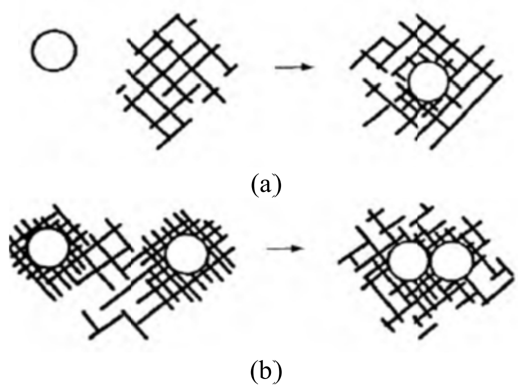

Fig. 2. Schematic map of hydrophobic hydration (a) and hydrophobic association (b). Circles represent hydrophobic groups, and the shaded area – water [29].

Research and visualization of processes of hydrophobic hydration, the hydrophobic interaction of the distillates was carried out at dilution of distillates in bidistilled water at a ratio of distillate: water 1 : 1, 1 : 10, 1 : 20, and 1 : 40 at 25°C (Table 1). Taking into the consideration that the behaviour and size of the micro particles depend on the concentration and nature of the solvent, the samples of distillates were mixed with an alcohol (96% vol) at a concentration of 0.1% under the temperature of 25°C (Fig. 3.1–3.4).

Table 1. Characteristics of Distillates

Name	Melon Distillate	Cucumber Distillate
Size of the particles, nm		
Dilution (water) 1 : 1 Dilution (water) 1 : 10 Dilution (water) 1 : 20 Dilution (water) 1 : 40	283 ± 51 246 ± 31 310 ± 14 390 ± 22	282 ± 20 253 ± 51 260 ± 28 291 ± 31
Odour		
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

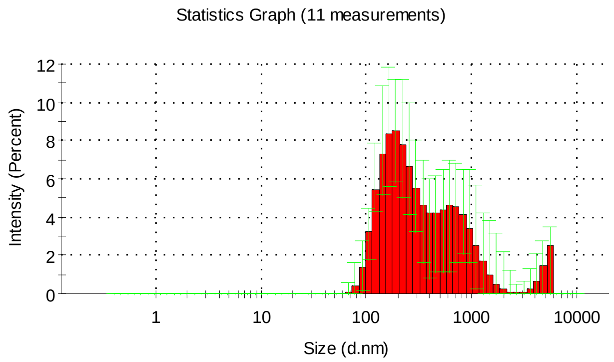

Fig. 3.1. Melon distillate (with an alcohol).

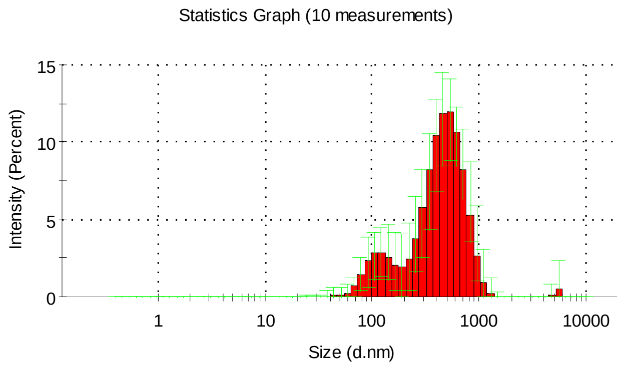

Fig. 3.2. Mellon distillate with changed pH (citric acid solution, 1%).

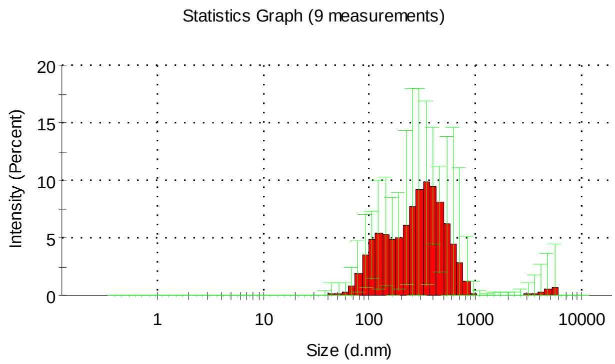

Fig. 3.3. Cucumber distillate (with an alcohol).

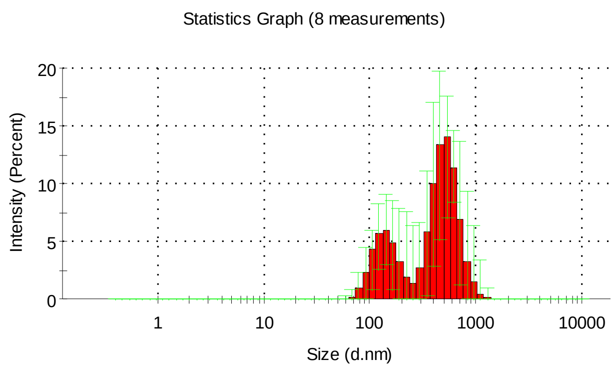

Fig. 3.4. Cucumber distillate with changed pH (citric acid solution, 1%).

Changing of the hydrodynamic size and the polydispersion of the droplet micro particles in an alcoholic solution shown in the figures indicates a coacervation process. The body is separated into two factions with different hydrodynamic size in melon and cucumber distillates. Larger droplets are coacervate – multimolecular complex or drops with a higher concentration of the colloid (dissolved substance) than the rest of the solution of the same chemical composition.

The initial pH of distillates is 7, aroma is conveyed with an intensity similar to the fruit. To analyse the aroma nuances, when changing the pH, the distillate was concentrated, as with different pH values qualitative and quantitative changes of flavour occur. Together with the pH, the perception of the same volatile compounds in different food mediums also changes [30]. Acetals and esters are converted to the starting aldehydes, acids, alcohols in the acid and alkaline medium. The change of the scent was analysed in mildly subacid medium pH = 6.0 and acid medium pH = 3.5 during the research. Acidity was adjusted with a certain concentration of citric acid. Salts of corresponding acids are formed in an alkaline medium, which bind aromatic components and help to remove the odour from the analysed medium, which contradicts the objectives of the study.

Changes of the hydrodynamic particles size in distillate by water dilution confirms the above assumptions about their hydrophobic nature and availability of results of such processes as coacervation, hydrophobic hydration, hydrophobic interaction. Since water and nonpolar groups exist in an antagonistic relationship, the structure of the water is adapted to minimize contact with the nonpolar groups. Two aspects of this antagonistic relationship are consider: the formation of clathrate hydrates and the association of water with hydrophobic groups [26].

Concentration of distillate allowed feeling better the flavour of samples and giving them a characteristic that serves as a comparison standard. Changing the pH of the medium and the nature of the solvent effect on the organoleptic characteristics of distillate in the distillation [31]. Substantial transformation in acid medium are recorded in cucumber samples. Admittedly acetals (E, Z)-2,6-nonadienal diethyl acetal, di-(Z)-3-hexen-1-yl acetal were converted to the aldehydes from which (E, Z)-2,6-nonadienal, (E)-2-nonenal, and cis-3-hexenal were formed. The changes of pH of the medium in melon distillate also led to a variety of aromatic reactions: in subacid medium melon flavour was more intense and vivid, and in the acid medium is less expressed, with prevalence of non-typical shades.

Liquid melon flavouring material of the firm "GLCS Co" was analysed according to the size of the micro particles in a concentrated form and after dilution 1 : 10 and 1 : 100 as in the previous samples (Fig. 4).

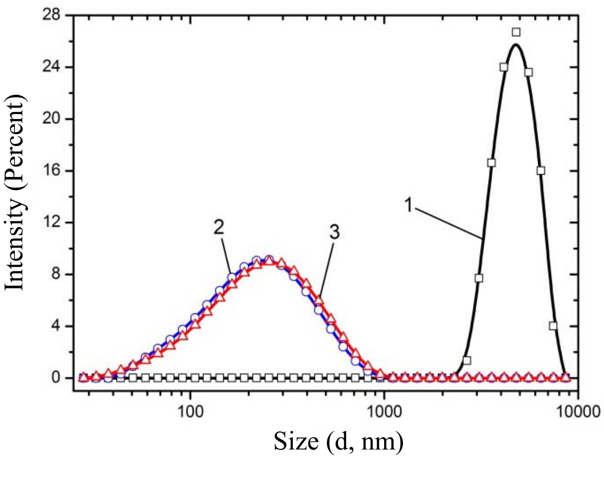

Fig. 4. Changing the size of ether particles in the industry melon flavouring material: 1 – in concentrated form; 2 – at a 1 : 10 dilution; 3 – at a dilution 1 : 100.

Analysis of the obtained results shows that, after dilution 1 : 10 of the concentrate of the melon odour dimensional characteristics has changed downward of the hydrodynamic diameter from 4.138 ± 0.274 mm to 185 ± 2.5 nm, the polydispersion has slightly increased from 0.220 ± 0.057 to 0.253 ± 0.014. Further dilution 1 : 100 in fact does not change the hydrodynamic diameter (196 ± 24 nm), but there was a slight increase in the polydispersion to 0.327 ± 0.148, which confirms the homogeneity of the fractional composition of the samples.

Changes of the hydrodynamic diameter at a dilution 1 : 10 and 1 : 100, can be associated with the presence of the solvent 1,2-propylene glycol in the composition of the flavouring material. Obviously, the dilution of more than 100 times is not desirable from the standpoint of fragrance tangibility and absence of significant changes in the size of the micro particles of the flavouring material.

The scent of the samples of industrial flavouring material in media with different pH is characterized by rich floral and caramel odour, has bright shades, not typical for melon, reminiscent of the odour of quince, pears and flowers. Based on organoleptic analysis the comparison of the aromatic profile of distillates and industrial melon flavouring material showed maximum approximation of laboratory samples to fresh, natural raw materials. The differences in sensory character-ristics to some extent is confirmed by the differences in the average hydrodynamic diameter of the sample: 150 nm and 190 nm, in the laboratory and industrial respectively.

Terms of transformation of volatile substances in the distillate during the distillation depends on many factors and determine their diverse composition [32]. Conversion of volatile fractions in distillate during the distillation process implementation depends, among other reasons, on the method of heating the raw materials. Microwave heating differs from convective selectivity with respect to for lipids and hydration components, free fatty acids release from lipids [33]. Free fatty acids in this study are involved in the enzymatic formation of aroma components, so the effect of the microwave heating has been studied in this aspect. The enzyme activity is increased under microwave heating [34] and, consequently, it affects the quantity and quality of the products of biosynthesis of fragrances. Microwave heating increases the mobility of the components, their diffusion, which could affect the probability of effective contacts, and avoid decomposition of thermally unstable compounds. Overheating of a polar solvent and heating of points, which do not contain solvents are the conditions to accelerate the reaction in a microwave field [35]. Changes in the surface strength of solutions were noticed in the microwave field [36]. The processes of cis-trans isomerism can occur in microwave field. Acetals and esters have chiral centres and therefore, the possible stereoisomers. Therefore, analysing the efficiency of the heat mode (convection and microwave) 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 qualities [37]. For example, the smell of carvone, limonene, 1-octene-3-ol, 3-metiltiobutanal in different spatial configurations have different odour. In the distillates under consideration, acetals and esters may form optical isomers (Fig. 5).

Dimensions of insoluble in water particles of ethers and acetals in distillates do not differ both in convective distillation, and in microwave. Under dilution of samples the nature of changes remains the same at the volumetric heating of, as at the convection. Aromatic distillate profile obtained with microwave heating plant differs from the samples with a convection heating of a suspension (Table 2).

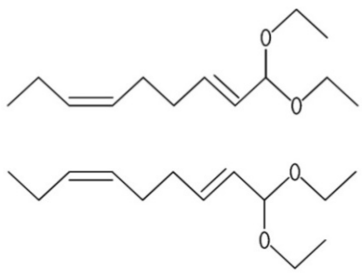

Fig. 5. Cis- and trans-isomers of 2,6-nonadienal diethyl acetal.

Table 2. 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

Reaction products of biosynthesis and flavours resulting from these reactions depend on the position of hydroperoxide group the in the fatty acid and enzyme isoform that vary in a microwave field. The cleavage products of PUFA in cucumber suspensions contain double bonds and the intact pentadienoic systems. These systems of double bonds undergo the abstraction of hydrogen atom that results in formation of additional degradation products and intense flavours inherent in cucumbers. For melon flavour the presence of C_6-C_9 carbonyl derivatives is less important than for the cucumber, so the difference in the microwave and convective heating homogenate is little palpable.

Ethers and acetals in distillates affect fruit tones, have a synergistic effect, increase and emphasizes the fullness and complexity of flavours. Their presence contribu.te to improving the quality of distillates, giving bright and fresh shades. Many flavour chemicals can exist in one of several isomeric forms or as mixtures thereof. Similar in structure molecules do not always produce the same shades of odours (such as vanillin and isovanillin). Conversely, there are compounds with similar odour but having different molecular structure. For example, benzaldehyde, and tiglinaldehyde, both having the almond odour. Selective heating in a microwave field allows regulating the composition of the volatile components forming the quality of the aromatic distillate to be obtained. The effectiveness of the isolation and the quality of the aromatic distillate depends on the specific stereochemic positions.

CONCLUSION

These results showed the relationship between the sensory characteristics of fruit distillates, dispersion and method of heat treatment of fruits in the convective and microwave field. Given differences, in shades of odours from the melon and cucumber in fruit distillates, show the advantage of microwave exposure during the distillation of aromatic substances. The results of these studies will be needed for the improvement of pervaporation processes, membrane selection criteria for the concentration of aromatic components, in the technology of microcapsulation of aromatic components.

Restoration of lost aromas consists in adjusting the pH of the medium in which the particles of insoluble distillates are put. In acid medium (pH = 3) the transformation of cucumber distillates acetals to aldehydes leads to full restoration of fresh odour as aldehydes in this case are the key components. In subacid medium (pH = 6.0) positive transformations of the flavour occur with components of melon distillate. These results contribute to the economic competitiveness of distillates compared with other types of flavouring materials.

Technologies that allow getting more saturated distillates in the first stage of extraction of flavours without re-concentration in the preparation of aromatic concentrates with a predominance of C_6-C_9 aldehydes and alcohols are promising. Such technologies are based on the processes of the biosynthesis *de novo* or biotransformation and use of microwave energy. Obtained flavours distillates are made from vegetable material, according to organoleptic characteristics closer to natural raw materials than the existing industrial analogues.

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

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