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NEW METHODS OF PLANT SELECTION FOR FOOD AROMA RECOVERY AIDED BY OXIDATION PROCESSES

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Abstract: This paper presents a novel approach to the selection of plants to restore the lost aroma based on the oxidation processes. The predisposition of raw material components to lipid oxidation is the basis of selection criteria. It was determined that the content of unsaturated fatty acids in the lipid extract of watermelon, pumpkin, cucumbers is 30-40%, the ratio of linoleic and linolenic acids in fruit is different. The formation of diene conjugates and hydroperoxides, malondialdehyde after various processing treatment methods is shown. The efficiency of aroma restoration depends on the number of formed 9-, 13- hydroperoxides that serve as a substrate for aroma-forming enzymes. The antioxidant capacity and the oxidation-reduction potential of fresh fruits and fruits after cooking have been analyzed. These characteristics determine the fruit ability to repeated formation of aromatic components. It has been ascertained that gourds have sufficient potential to restore aroma by exogenous lipoxygenases.

Key words: flavor, oxidation, fatty acids, enzymes

INTRODUCTION

Aromatization is the important way of improving food quality. Historically, the main research focus has been on the aroma qualities rather than the mechanisms of its formation. The development of aroma industry in the last decade was connected with the analysis of natural compounds and finding new aromatic components from which natural aromas could be restored (Guentert, 2007, Ahn et al, 2011). The increased demand for natural flavours has renewed the interest in this research, which began in the 50s of the last century. The knowledge about the ways of fruit and vegetable flavour

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formation has been accumulating slowly over the years. The reactions between substances – precursors of volatile compounds, have become the main achievements in the study of aroma formation mechanisms in fruits.
The aroma of many fruits and vegetables is partially lost or greatly changed as the result of a thermal treatment. One of the approaches to aroma recovery focuses on enzymatic processes foods. According to this theory, aroma recovery depends on the presence of precursors and on the availability of enzymes that specifically form natural aromas from these precursors. In some cases the aroma can be restored by adding the liquid extract of fresh raw materials to the processed product (Reed, 1966). The possibility of developing a list of fruits to which the theory of aroma recovery can be adapted remains relevant. It has been found out that thioglycosides from the cabbage family could be used as precursors in fresh fruits (Hasselstrom at el., 1962). The main potential of flavor precursors is the ability to be converted into a fresh aroma under the influence of its own enzymes. Isolated enzymes were added to food during preparation for the restoration of flavour. To continue the line of this research, Schwimmer demonstrated that blanched, dried or canned beans, peas, broccoli, carrot, tomatoes, and cabbage change their aroma under the influence of enzymes isolated from fresh raw materials of vegetables, genetically related to the main product, or from mustard (Schwimmer, 1963). The strengthening of taste and flavour of the food or beverage comprises contacting the food or beverage with composition of enzymes. The composition profile consists of enzymes of glucosidase, lipase, amylase, glucoamylase, xylanase, and pectinase (Jolly at el.,2007).
The derivatives of aromatic compounds that can be formed from lipids via α-β-oxidation and lipoxygenase enzymes have been intensively studied recently (Reineccius, 2005, Oey, 2010). Unsaturated fatty acids of fruit have been proved to be precursors of many aromatic substances. Biosynthesis of aromatic components by lipoxygenase has been established for tomatoes, cucumbers, olives, sweet pepper, apples, citrus fruits, and strawberries (Oey, 2010, Leone at el., 2006). In previous studies, we have shown that lipids in fruits and vegetables, particularly tightly bound ones, can be a spare "depot", which has the potential for restoring the lost aroma (Dubova, 2014). 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.
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 effect of vegetables cooking methods (boiling, microwaving, pressure-cooking, griddling, frying, and baking) on their antioxidant activity is determined. Analysis of the radicals scavenging capacity (lipoperoxyl and hydroxyl) of the different vegetables showed that the highest losses have occurred in garlic with all the methods, except microwaving. Depending on the vegetable in question, griddling and microwave cooking produced the lowest losses, while pressure-cooking and boiling lead to the greatest losses and frying occupies an intermediate position (Jiménez-Monreal at el., 2009).
The reasons why antioxidant activity of some vegetables after cooking changes are the following:
- 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.
Natural bioantioxidants polyphenols, ascorbic acid, carotenoids inhibit the molecular oxygen oxidation of unsaturated fatty acids, some amino acids and carbohydrates. However, they allow you to keep the nutritional value of raw materials. To restore the lost flavour it is required to increased peroxidation of fatty acids, which are the aroma precursors. Since the formation of aroma occurs during few minutes, profound changes in the fatty acids does not occur.
The aim of the research is to investigate the reliability of the parameters that determine the ability of processed fruit to restore aroma and to establish the influence of the redox potential and fruit antioxidant properties on formation of hydroperoxides from unsaturated fatty acids of the cell cytoplasmic membrane.

MATERIALS AND METHODS

Materials (objects of the research) are melons and gourds (cucumber, watermelon, pumpkin), sweet pepper (source of vitamin C), currants (source of polyphenols), and strawberries (source of anthocyanins).
The method for determination of fatty-acid composition: lipids were extracted from 1g of the fruit sample, which was freeze-dried in the cold by isopropanol, then by isopropanol and chloroform mixture (1:1) and twice by chloroform and methanol mixture (1:1). The amount of fatty acids in the total lipid fraction was determined by gas-liquid chromatography as methyl esters.

The analysis of fatty acid methyl esters has been carried out by gas-liquid chromatography using a gas chromatograph GC-16A "Shimadzu".

Sample preparation. Fresh feedstock is subjected to the following types of processing: I – was finely ground, II – was finely ground and boiled for 30 minutes, III – was frozen at -18° C, stored 3 months, defrozen at room temperature, IV – melons and gourds (watermelon pulp, rinds, pumpkin, cucumber) were finely ground, after water-soluble enzymes extract from defatted soybean solvent cake(defatted soybean meal) was added, they were thermostating at 37 C, during 10 min, V – water-soluble enzymes extract from defatted soybean solvent cake (defatted soybean meal), received by strategy, described by R. Scopes (Scopes,1985).

Antioxidant activity of lipid extracts (AOA) was investigated in the oxidation reaction Tween-80 (Merck) by atmospheric oxygen (Antolovich at el., 2002). Photocolorimetric calculations/analysis was performed to determine the concentration of rose coloured trimethine complex of oxidation products with 2-thiobarbituric acid at 532 nm in control and test samples. Antioxidant activity (AOA) of aqueous extracts of the samples was evaluated by the deceleration rate of accumulation of peroxidation products. AOA is calculated by:

$$\text{AOA} = \frac{Dk - Dop}{Dk} 100\,\%$$

where D_k, D_{op} – optical density in control and test samples, respectively.

Method for determination of hydroperoxides and diene conjugates is based on measuring the light absorption by diene hydroperoxides at 234 nm on spectrophotometer СФ-42 at room temperature, with a molar extinction coefficient 2,5 • 104 М-1см-1.

Malondialdehyde determination. The method is based on the reaction between malondialdehyde (MDA) and thiobarbituric acid and formation of coloured trimethine complexes with maximum absorption at 532 nm in a spectrophotometer.

Measurement of Oxidation- Reduction Potential. Change in the oxidation-reduction potential (ORP) in solution was monitored with a pWORP meter equipped with a platinum combination electrode.

RESULTS AND DISCUSSIONS

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.1.

Table 1. 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	15.4	15.3	11.7	16.3
Stearic 18: 0	4.5	5.0	6.0	7.3
Oleic 18: 1	16.9	17.3	20.9	11.1
Linoleic 18: 2	29.1	31.8	37.5	41.0
Linolenic 18: 3	11.7	9.2	12.3	14.0
The amount of saturated FA	35.8	34.74	28.4	33.6
The amount of unsaturated FA	59.8	58.23	70.7	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 (Oey, 2010, Damodaran at el., 2008). Depending on the rate of lipids oxidation in food, the concentration of carbonyl compounds 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-hexanal, 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.2). Measurements were made during 1-2 minutes after finishing sample preparation.

Table 2. Primary and secondary products of lipid oxidation

Name	Hydroperoxides and Diene Conjugates	Malondialdehyde
Cucumber I	3.52	4.51
Cucumber II	9.68	9.02
Cucumber III	4.80	6.85
Cucumber IV	10. 47	7.00
Pumpkin I	52.06	8.27
Pumpkin II	12. 00	15.79
Pumpkin III	44. 50	10.13
Pumpkin IV	49. 10	14. 80
Watermelon pulp III	84.00	27.00
Black currant III	72.00	27.70
Sweet pepper III	55.20	18.50
Cherry III	64.33	21.65

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 (Damodaran at el., 2008).
The consequences of technological processing affect the ability of oxidation reactions in the fruit system. The redox potential (ORP) is the indicator of normal growth, development and operation of the plant (Pandey at el., 2014). 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 (Cutter, 2002, Nam, 2002, Nam at el., 2002). 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 (Okouchi at el., 2002).
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 (Figure 1): melons and gourds (W), enzyme extract (E), black currant, cherries, pepper (D).

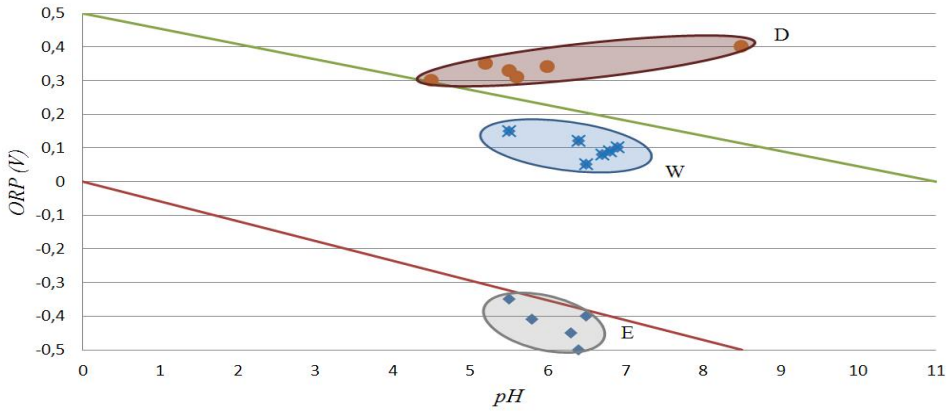

Figure 1. Changes of ORP in fruits and enzyme extract

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.

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 peroxyl radicals and

involvement in proliferation phase (Pinchuk at el., 2012). 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 (Stephanson at el., 2002). 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) (Figure 2).

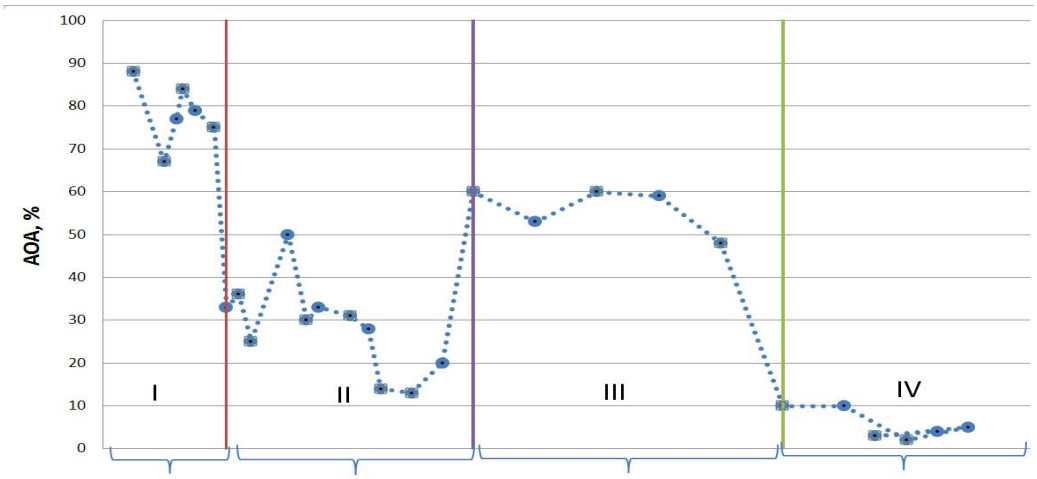

Figure 2. Antioxidant activity

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.

CONCLUSIONS

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. 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.

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