THE ANALYSIS OF SUPERCRITICAL FLUID CO_2-EXTRACTS OF *SALVIA OFFICINALIS L.*

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INTRODUCTION

The modern production of herbal remedy and biologically active is notable for high temp of scientific-technological progress of which main directions are creation of little waste technology, a complex usage of valuable natural materials, an increase of variety of products obtained out of one herb. Some methodic of industrial processing of herbal raw materials widely applicable in the food industry is of great interest for investigations. The extraction by liquefied gases, in particular, the well studied, technology of CO_2- extraction is among them. Such a technology may became the method to receive new original remedies from many species of herbal raw material [1].

One of the features of the liquated gases extraction is an ability of the parameters (pressure, temperature) to widely vary what allows to receive from a raw material products different by composition and properties last years the most attention is attracted to so called solvent fluid state which has the unique properties and high solvency is among them. Therefore the fluids achieve the most application in processes of supercritical fluid CO_2. In the multicomponent systems with an appoximathion of thermodynamic parameters of the solvent to the critical point begins a precritical interphase redistribution of substances, what allows to educe pure compounds, obtain a fractionation by means of gradient pressure falloff, easily carry out a transition from extraction to re-extraction, and etc. In the precritical region multicomponent systems may be divided in to the components by the simple change of temperature and pressure. This phenomenon is used for separation such complex systems as the biological active substances [2, 3].

There are some results of research on supercritical fluid CO_2- extract of *Salvia Officinalis L.*, cultivated in Ghunib Plateau, Daghestan presented in the work. An extract have been educed in the experimental device, permitting to carry out the complex researches of conditions for extraction of the raw materials, the thermodynamic properties and a phase behaviour of complex systems in a wide range of state parameters including the critical region [4].

I–MATERIAL AND METHODS

During the investigations we have educed three fractions of supercritical fluid CO_2-extract of officinal sage in the isothermic regime at the temperature $31,5^{o}$ C and pressures up to 10 MPa, 20 MPa and 30 MPa in consecutive order. The educed extracts differed by mass outward appearance, density and consistency. The first fraction of 2.65 g has been extracted out from 100g of sage leaves reduced to particles of 0.1-0.2 mm at the pressure 10 MPa. The fraction was a mobile, transparent yellow- brownish liquid with strong smell of the sage essential oil. Next the second fraction of 1.99g has been obtained from the same material at

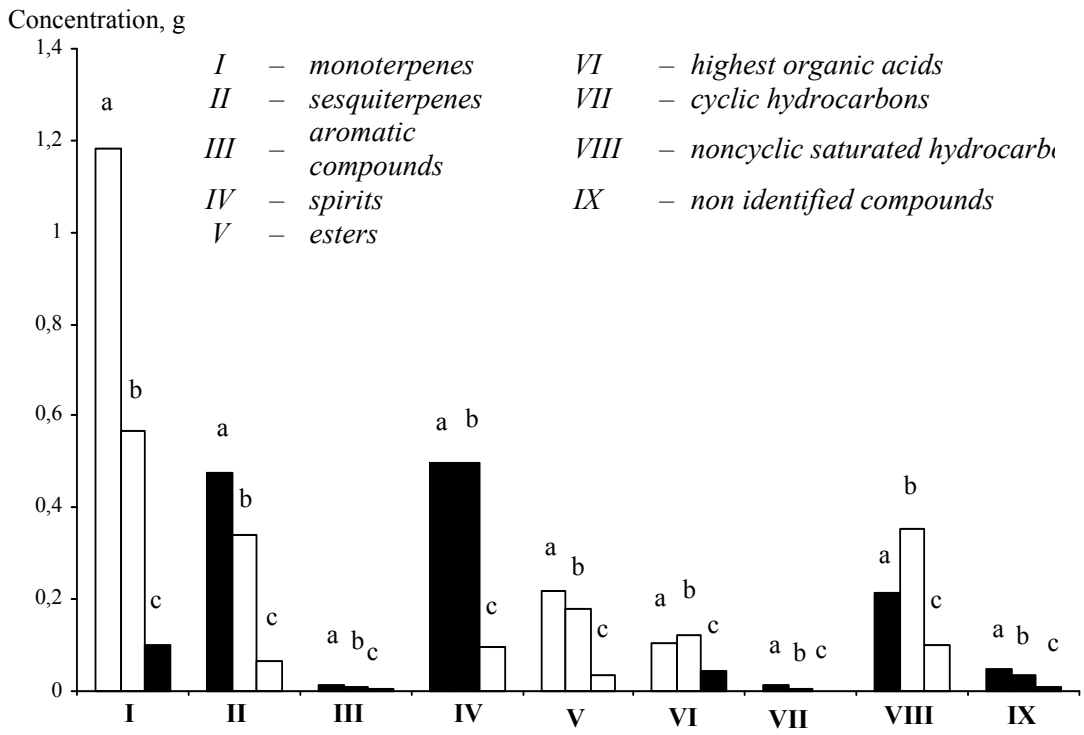

Figure 1. The dynamics of extraction of different compound classes out of Salvia Officinalis L. leaves by the supercritical fluid CO_2 at pressures 10 MPa (a), 20 MPa (b), 30 MPa (c).

the pressure 20 MPa. The second fraction consisted of two phases water (about 0.5 ml) containing in the residual amount in the raw material and dense, immovable, brown mass with a smell of the sage oil. The third fraction of 0.45g extracted at 30 MPa was a dense, ointment, dark brown mass with a weak smell of sage.

The analysis of fractions extracted has been carried out by a method of the gas chromatography with mass-spectral and UV detection in the device Saturn 2000 (Varian) and column Stabilwax which length is 30m, inner diameter is 0.32 mm, and thickness of the stable phase is 0.5 micron.

In figure 1 there is shown a dynamics of the extraction of different compounds from sage leaves by the supercritical CO_2 at 10 MPa, 20 MPa and 30 MPa. A composition and correlation of main compounds in the fractions of the supercritical fluid CO_2- sage extract educed consecutively at the abovementioned pressure are presented in figure 2.

In our recent works we have shown a fractionation, phase redistribution in the visual cell under sub- and supercritical conditions [5]. The figure 3 presents a measuring tube of the device where the fractionation of supercritical fluid CO_2 – extract of sage can be seen. A fraction distribution in the cell occurs in the molecular mass in such a way that high molecular compounds (hydrocarbons, derivative, lipid acids, resins) create a dark coloured region in the lower part of the tube, and components of the ether oil dominated in a composition of CO_2-extract are in the middle part.

Let's consider each fraction separately: I- fraction is presumably resins (high molecular spirits and acid), di- and triterpene. The carnosic acid (salvin) among them is more characteristic for the sage, however in gas-liquid chromatography analysis, which is carried out at a high temperature, it is not observed in a native condition by reason of easy distructionality. In this fraction are also presented the highest organic acid and spirits (saturated and unsaturated), octadecatrienoic acid (1,56%), octadecenaic acid (0,64%), n-

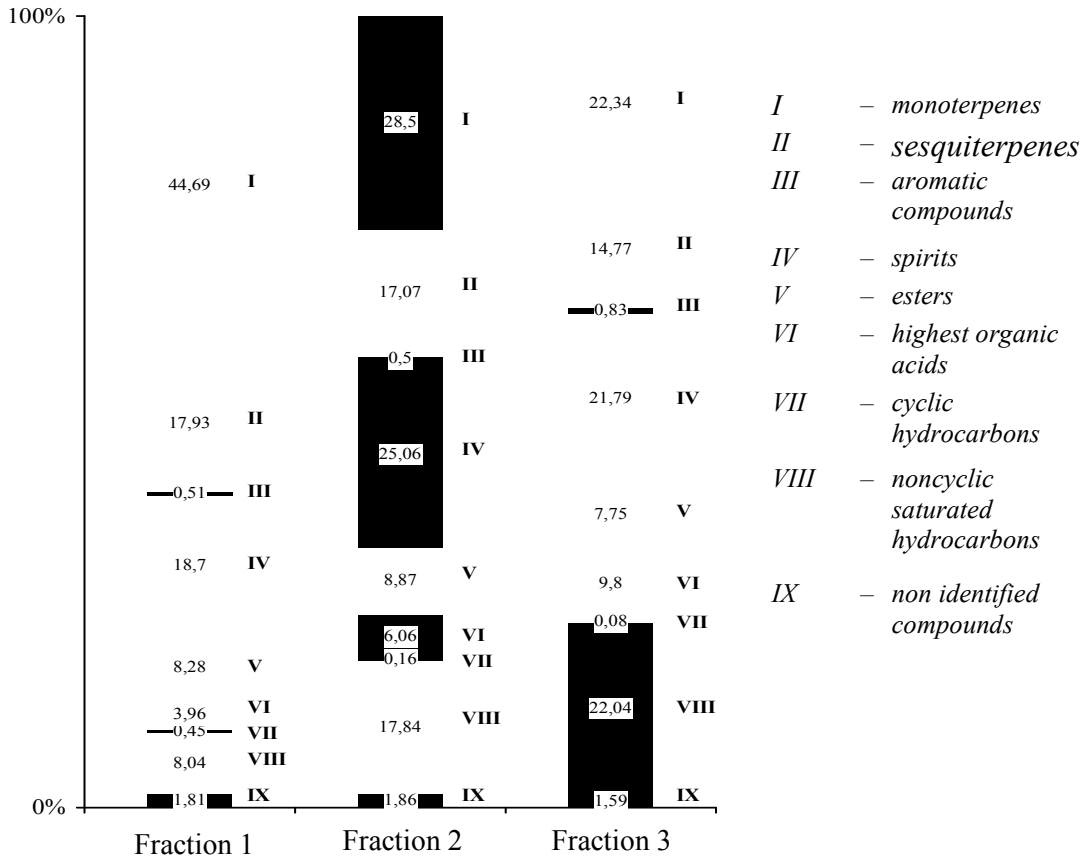

Figure 2. Composition and correlation of main compound classes in fractions of supercritical fluid CO_2 – extraction of Salvia Officinalis L. obtained at pressure 10 MPa, 20 MPa, and 30 MPa consecutively.

Figure 3. Fractionating of supercritical CO_2-extracts of Salvia OfficinalisL.

Hexadecanoic acid (1,322%), etc. are among them. Amongst spirits the manool (over 17%) is more prevails. II-proceeding from molecular mass probably its is sesquiterpens. A group of substances is of extreme interest for pharmacology. Among the sesquiterpens are found compounds with anticancer activity. In *Salvia Officinalis L.* it may be noted azulene (5,94%),

1- cyclopropylazulenol-4 (6,15%), caryophyllene (4,72%), caryophyllene-oxide (0,54%), and etc.

I- and II fractions include noncyclic saturated hydrocarbons (paraffins). They are not active from the point of view of pharmacology, but essentially influence on bio-accessibility of the rest of substances. Mainly they contain the fractions from $C_{10}H_{22}$ to $C_{18}H_{38}$.

III- according to molecular mass it is probably esters (acid esters and spirits including highmolecular ones) for instance here a prevaile role may be belong to cyclopropylazulenol, as it has to do with esters. Besides it the bornyl acetate (1,24%) is presented in the great number.

IV, V, and VI – in all fractions the main part belongs to monoterpenes (the main class of compounds in an ether oil composition). Probably the fraction IV belongs to monoterpenes spirits for example, to borneol and eucalyptol (or cineol) and to aromatic spirits, myrtenol and eugend. Also here present components from fraction III, possessing conjugated double bounds (impart light yellow colouring).

V- is monoterpene hetone. They are prevalence substances both in a composition of ether sage oil and in a composition of CO_2 –extract. Here both isomer is alpha- beta- thujone, and alpha-, beta- camphor.

VI- is a mixture of alpha- thujone (dominating isomer) and the rest of none limiting monoterpenes – alpha- , beta- pinene camphene, limonene, terpinolene, etc. All these compounds have phitoncide, antimicrobe, antivirus properties, add the typical coniferous sage smell, light-volatile, i.e. storing an extract at the open air leads to notable decrease of concentration of substances.

VII- is not identified.

In practice the fractiation of complex compound mixtures, which are the herbal extracts, is achieved by several methods. One of them is carried out in a number of experimental devices and consists in consecutive eduction of fractions from the slightest to the heaviest one, what to the best advantage does for extraction of pure individual photo- and themolabile compounds. According to another methodic a sample of herbal raw material is consecutively worked out by one solvent in several technologic regime, and form the products different in physical- technological properties (outward appearance, density, etc.).

The first methodic of extraction is more technologic, but the second one is considerably accessible and applicable as for the most part of herbal extracts it is assume some attendant substances similar in a structure in a composition.

CONCLUSION

The data obtained show the correlation of biological active components of the sage ether oil and indifferent hydrocarbons to be changed in the dependence on a stage and parameters of the extraction process. So proceeding from summary eduction of each fraction it may be concluded that in order to obtain a biological active extract of the sage by the supercritical fluid extraction method it is sufficient to use the second stage of extracting (P=20 MPa) initially up to total deplete of the raw material.

An application of higher pressure is experiment as a technique for removal of lipophilic imparities exerting interfering influence in the technology of total polyphenol complex out of sage leaves.

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