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Abstract. Recently, the attention of many researchers has been attracted by raw materials that contain flavonoids. The purpose of the research is to summarize the results of studies of the composition of flavonoids of a non-fat sea buckthorn meal, which is a secondary raw material for the production of sea buckthorn oil. In this case, the sum of the five identified flavonoid compounds was isolated by extraction and subsequent adsorption column chromatography. At this stage, the actual problem of identifying and determining individual isolated flavonoids was solved using chromatographic (TLC, HPLC) and spectroscopic methods in comparison with reliably known samples. During the study, the flavonoids were identified: isorhamnetin, kaempferol, myritin, quercetin and its glycoside rutin, quercetin and isorhamnetin predominate. Ultimately, the effective parameters of the method for the improvement of the competitiveness of dietary supplements are established.

1. Introduction

Modern trends in the development of the agro-industrial complex, the development of the food industry and the use of new promising materials stimulate the task of increasing the competitiveness of products. The global practice focuses manufacturers on minimizing the volume of waste through the introduction of technologies for the deep processing of vegetable raw materials to produce a complex of biologically active substances, food ingredients and additives. In this regard, non-fat meal is a scientific interest and practical significance. Non-fat meal is a large-capacity waste from the production of sea buckthorn oil, bioconversion of which results in obtaining of dietary fiber, water-soluble vitamins, amino acids and other valuable compounds. That will reduce the cost of the target product of sea-buckthorn fruit - fatty oil, to expand the range manufactured products and increase their competitiveness [1].

Interest in fat-free sea buckthorn meal is not accidental, since this secondary raw material resource (HRV) not only has a high biological value, but also has a relatively stable chemical composition and is available most of the year [2, 3]. The valuable components of sea buckthorn meal include bioflavonoids – a class of natural phenolic compounds that are distinguished by structural diversity, high and varied biological activity in the absence of toxicity [4, 5]. The importance of these substances is primarily due to the P-vitamin activity and the associated antioxidant potential [6, 7], given relatively low cost of

obtaining extracts from plant materials, including HRV. The processing of sea buckthorn meal in order to give out the complex of bioflavonoids is promising and relevant.

The proposed article shows the possibility of separating compounds of flavonoid nature from sea buckthorn meal with the rationale for the need to separate them into individual compounds and use as effective antioxidants.

2. Materials and methods

The objects of study were as the follows: defatted sea buckthorn meal, polyphenol sum extract obtained from defatted sea buckthorn meal, standard flavonoid samples: quercetin (*Cayman Chemical*), CAS: 117-39-5; isorhamnetin (*USP Standart*), CAS: 480-19-3; kaempferol (*Cayman Chemical*), CAS: 520-18-3; rutin (*Cayman Chemical*), CAS: 207671-50-9-R5143, myrcetinum (*Cayman Chemical*), CAS: 529-44-2.

The authors used thin-layer chromatography (TLC), column chromatography; high-performance liquid chromatography (HPLC) with photometric detection on a «Waters 2695 Alliance» instrument, and quantitative determination of flavonoids in sea buckthorn meal was carried out using the Folin-Chocalteu spectrophotometer using a «Shimadzu UV-1800» spectrophotometer. antioxidant activity was determined on a «CvetIauz» analytical liquid chromatograph. Pre-prepared sea buckthorn meal was subjected to exhaustive extraction with ethyl alcohol in a Soxhlet apparatus, the resulting extract was evaporated under vacuum to dryness and then subjected to chromatographic separation. The chromatographic column (silica gel L 40/100) was eluted to remove simple phenolic compounds with water and then with 40% ethyl alcohol with increasing concentration. Control over the separation of flavonoids was performed using TLC analysis on TLC Silica gel 60 F254 plates in ethyl acetate-ice acetic acid-water (7.5: 1.5: 1.5) and n-butanol-ice acetic acid-water (4: 1: 5) systems . As a control, working standard samples (RNO) of flavonoids served, the detection of adsorption zones was carried out in visible light and UV light at a wavelength of 254 nm.

3. Determination of the composition of polyphenolic sea buckthorn meal complex

In the composition of fat-free sea buckthorn meal the content of substances of polyphenolic nature, determined spectrophotometrically, ranges from 3.5% to 4.5% and depends on the time and place of raw material procurement, storage time and technological modes of obtaining the target products. Based on the literature data and the results of our own research, it was established that the polyphenol complex of sea buckthorn meal is mainly represented by flavonols: isorhamnetin, kaempferol, myrsetin, quercetin and its glycoside rutin, exhibiting P-vitamin activity, which is expressed in strengthening the walls of capillaries and reducing their permeability [6, 8, 9].

In connection with the different solubility of flavonoids in organic solvents, we examined the extraction of prepared raw materials with polar (ethanol) and non-polar (chloroform) solvents. At the same time, as expected, the component composition and the sum output of polyphenols are different. In the chloroform extract, the yield of which was 1.4%, quercetin, kaempferol and isorhamnetin were detected by HPLC. Alcohol extract (yield 3.1%) is represented by rutin, its aglycone quercetin, kaempferol, isorhamnetin, myritin and an unidentified compound, presumably glycosidic in nature. The composition of chloroform and alcohol extract of polyphenols sum is shown at the chromatograms on Figures 1 (1 – quercetin; 2 – kaempferol; 3 – isorhamnetin) and 2 (1 – unidentified substance; 2 – myrcetin; 3 – rutin; 4 – quercetin; 5 – kaempferol; 6 – isorhamnetin), respectively.

The identification of the compounds obtained was carried out by retention time in comparison with the RSO of flavonoids. The content of components in the obtained samples of the extracts is presented in table 1. According to the experimental data obtained, the predominant flavonoid in chloroform and ethanol extracts of sea buckthorn meal is isorhamnetin – 58.87% and 40.67%, respectively. However, the yield and total content of polyphenols in ethanol extract is higher than in chloroform and is 3.1% (in terms of a.c.s.) and 92% (in terms of rutin).

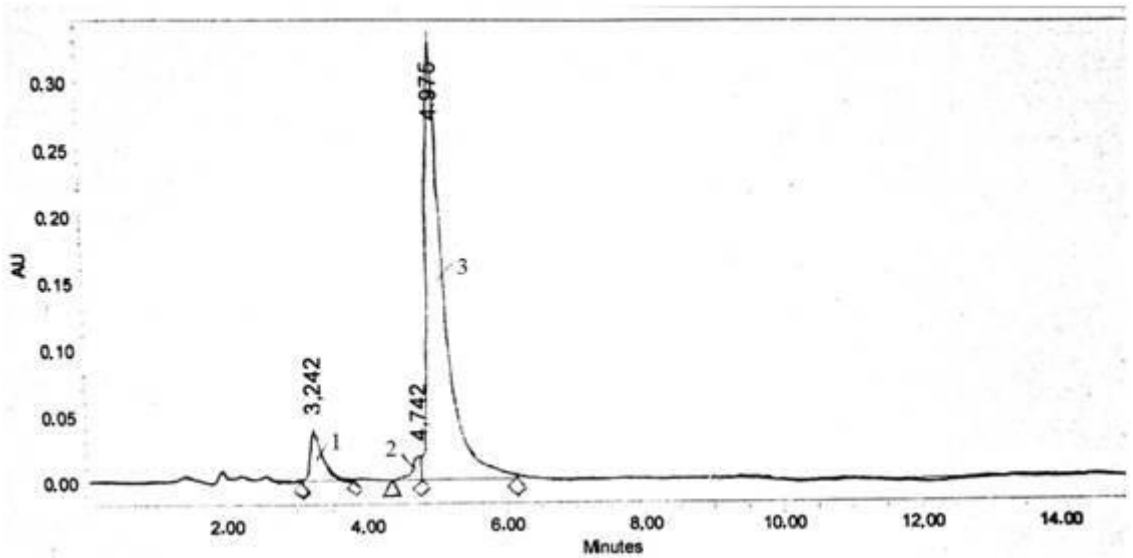

Figure 1. Chromatogram of chloroform extract of sea buckthorn meal (HPLC method); at a wavelength of 365 nm

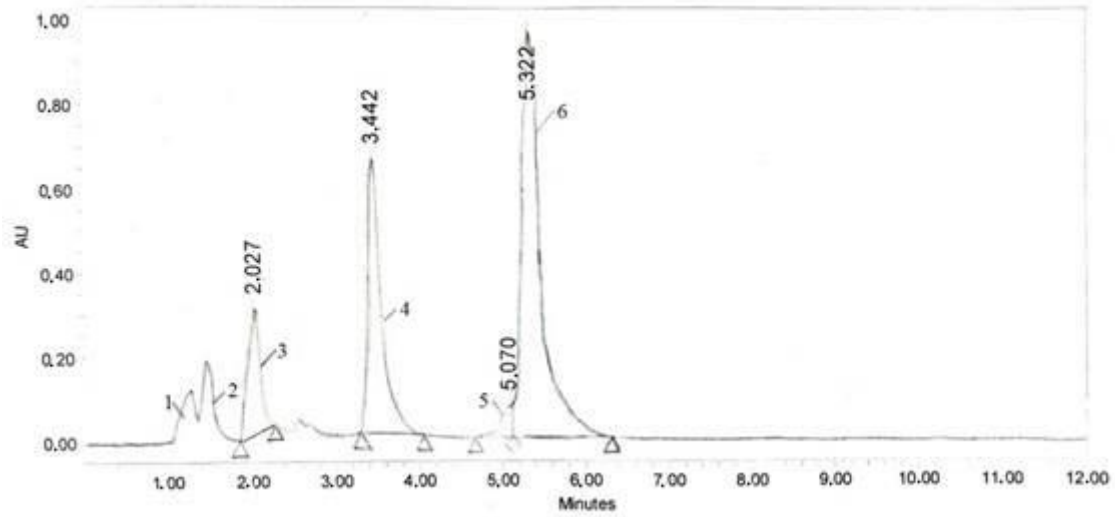

Figure 2. Chromatogram of ethanol extract of sea buckthorn meal (HPLC method); at a wavelength of 365 nm

Table 1. Retention time and content of flavonoids in extracts

Compound	Chloroform extract		Ethanol extract	
	Retention time, min	Content, %	Retention time, min	Content, %
Rutin	-	-	2.027	15.49
Quercetin	3.242	38.36	3.442	26.30
Kempferol	4.742	2.77	5.070	1.93
Isorhamnetin	4.975	58.87	5.322	40.67
Myricetin	-	-	1.108	8.47
Not identifiable. substance	-	-	0.936	7.14

Based on the conducted research, the content of flavonoids in sea buckthorn meal was calculated and the values of antioxidant activity (AOA) of RSO flavonoids were determined (Table 2).

Table 2. The content of flavonoids in sea buckthorn meal and their AOA

Compound	The content of the amount of polyphenols, %	Content in meal, %	The value of the AOA, mmol / 100 g
Rutin	15.49±0.02	0.33±0.01	2.6±0.1
Quercetin	26.30±0.02	0.55±0.01	4.4±0.1
Kempferol	1.93±0.02	0.04±0.01	4.0±0.1
Isorhamnetin	40.67±0.02	0.85±0.01	4.1±0.1
Myricetin	8.47±0.02	0.16±0.01	4.3±0.1

From table 2 it follows that isorhamnetin and quercetin are the predominant flavonols in sea buckthorn meal, and glycosides have the least pronounced antioxidant effect, in the case of sea buckthorn meal polyphenols rutin. In the flavonol series, an increase in the AOA is observed as the number of hydroxyl groups in the B ring increases [10]; thus, myricetin and quercetin are superior to antioxidant properties of kaempferol and isorhamnetin.

4. Conclusion

The qualitative and quantitative composition of the polyphenol complex of sea buckthorn meal was studied by reverse phase HPLC, as a result of which the extract revealed the presence of five flavonoids, with quercetin and isorhamnetin being the dominant ones, the AOA of which exceeds the standard rutin more than 1.5 times. In this regard, extracts obtained from sea buckthorn meal can be an alternative substitute for routine in the pharmaceutical industry and the production of dietary supplements, and in general will increase the competitiveness of Russian manufacturers in the pharmaceutical market.

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