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APPLICATION OF WHEY FOR PLANT BIOLOGICALLY ACTIVE SUBSTANCES EXTRACTION

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Abstract

The relevance of plant raw materials application for functional food design is due to the wide range of biologically active substances included in its composition. A promising direction of functional dairy product technology development is the enrichment of plant extracts rich in phenolic compounds. Phenolic compounds have antiviral, anti-inflammatory, bactericidal, hepatoprotective, and capillary-strengthening effects. Whey is a source of a wide range of biologically active substances (BAS). The beneficial effect of using a combination of plant raw materials with secondary dairy raw materials containing valuable whey proteins has been proven. The introduction of whey into the beverage formulations in combination with plant extracts allows the resulting product to add nutritional value, as well as original organoleptic characteristics. This research aimed to study the efficiency of whey application as an extractant for BAS recovery from plant raw materials.

Targets of research were cheese whey, plant raw materials (milk thistle, peppermint, sage, purple coneflower), their water, and whey extracts. The research was carried out in the scientific laboratory of "Food and Industrial Biotechnology" of North Caucasus Federal University and the scientific laboratory of "Applied Issues of Biology" of Belarussian State University. ABTS radical antioxidant activity and total concentration of phenolic compounds of plant extracts were determined using spectrophotometric methods (reducing power method and Folin-Denis technique respectively). The quantitative and qualitative composition of extracts was studied by gas chromatography with mass spectrometry detector method.

The following parameters of the BAS extraction process from plant raw materials were established: temperature: 55 - 60 ^{0}C; duration - 2 hours; the oscillation amplitude of the thermoshaker (RM) = 70 min^{-1}. The optimal values of the ratio of solid and liquid phases for studied types of plant raw materials have been established: milk thistle and peppermint - 1 : 8; sage and purple coneflower - 1 : 10. It was found that the antioxidant activity of whey extracts of plant BAS is greater in comparison with water extracts.

The analysis of experimental data allows us to conclude that the application of whey as an extractant for BAS extraction from plant raw materials is promising in terms of its organoleptic and physico-chemical parameters and the possibility of additional enrichment of extracts with valuable components. The results of the study allow us to recommend the use of plant extracts for fermented dairy products technology development.

***Key words**: Whey, plant extracts, Biologically active substances, Antioxidant activity, Phenolic compounds.*

1. Introduction

One of the main ways for biologically active substances of plant origin to enter the human body is the consumption of functional products based on plant raw materials. The relevance of the use of plant raw materials in functional products is due to the wide range of biologically active substances included in its composition. Such substances are vitamins, flavonoids, antioxidants, tannins, ascorbic acid, and macro and microelements [5, 13].

Antioxidants are the most important regulators of intracellular free radical processes [1, 9]. Recent studies have shown that phenolic compounds are the most effective antioxidants. Among the biologically active components of plants, the most significant are flavonoids, which are part of the group of phenolic compounds (polyphenols) [3, 7].

Phenolic compounds are a numerous class of biologically active compounds of plant raw materials, which are found in almost all plants. Currently, about 4,000 species of natural flavonoids of various structures are known, but there are representatives of the plant world, especially rich in these compounds [2, 10]. The largest number of phenolic compounds accumulate in plants of the mint family (*Labiatae*) [4, 7, and 12]. Most phenolic compounds produced by plants are natural antioxidants and are capable of neutralizing free radical processes. Phenolic compounds have antiviral, anti-inflammatory, bactericidal, hepatoprotective, and capillary-strengthening effects [2, 6, and 8].

A promising direction for improving the technology of functional products based on dairy raw materials is the inclusion of extracts of plant origin rich in phenolic compounds in the composition. Whey is a source of a wide range of biologically active substances (BAS). Fermented dairy products with the addition of plant extracts are becoming increasingly important [11, 15].

The beneficial effect of using a combination of plant raw materials with secondary dairy raw materials containing valuable whey proteins has been proven. The introduction of whey into the fermented product formulations in combination with plant extracts allows the resulting product to add nutritional value, as well as original organoleptic characteristics. The enrichment of fermented dairy products with biologically active components of plant raw materials contributes to the improvement of many physiological processes in the human body [14, 15].

2. Materials and Methods

The purpose of this research was to study the efficiency of whey application as an extractant for BAS recovery from plant raw materials.

The following subjects of research were used:
- samples of cheese whey produced by JSC "Dairy Plant "Stavropol", Russia;
- distilled water;
- milk thistle fruits, produced by JSC Firm "Krasnogorskleksredstva", Russia;
- crushed peppermint leaves, produced by JSC Firm "Krasnogorskleksredstva", Russia;
- sage leaves, produced by JSC Firm "Vitaukt-prom", Russia;
- purple coneflower herb, produced by JSC Firm "Vitaukt-prom", Russia.

Pre-dried and crushed plant raw materials were mixed with the extractants at different ratios of solid and liquid phases (hydromodule), kept in a thermoshaker at the specified process parameters, and filtered at the end of extraction.

The research was carried out in the scientific laboratory of "Food and Industrial Biotechnology" of North Caucasus Federal University and the scientific laboratory of "Applied Issues of Biology" of Belarussian State University. Total solids concentration in plant extracts was measured by the refractometry method. pH values of extracts were determined using a potentiometric technique. The method of cryoscopy was applied for extract freezing point evaluation. ABTS radical antioxidant activity and total concentration of phenolic compounds of plant extracts were determined using spectrophotometric methods (reducing power method and Folin-Denis technique respectively). The quantitative and qualitative composition of extracts was studied by gas chromatography with mass spectrometry detector method.

3. Results and Discussion

The following parameters of the BAS extraction process from plant raw materials were established based on the results of preliminary experiments: temperature: 55 - 60 ^{0}C; duration - 2 hours; the oscillation amplitude of the thermoshaker (RM) = 70 x min^{-1}. The effect of the hydromodule on the physicochemical parameters (Total Solids concentration, TS, %; pH; freezing point, ΔT, ^{0}C) of milk thistle, peppermint, sage, purple coneflower water, and whey extracts was studied (Tables 1, 2).

The optimal values of the ratio of solid and liquid phases for studied types of plant raw materials have been established: milk thistle and peppermint - 1 : 8; sage and purple coneflower - 1 : 10. Sage and purple coneflower were recommended for further study taking into account higher yields of total solids in their extracts in comparison with extracts of milk thistle and peppermint.

The influence of the hydromodule on the concentration of phenolic compounds in aqueous and whey extracts of sage and purple coneflower is shown in Table 3. It was established, that the highest yield values of phenolic compounds are achieved with a ratio of raw materials to extractant 1 : 8. Concentration of total phenol compounds in sage extracts is significantly more than ones in purple coneflower. There is no significant difference in the amount of total phenol compounds in aqueous and whey extracts.

Table 1. Physico-chemical parameters of aqueous extracts at different values of the hydromodule (p ≥ 0.95)

Hydromodule	ΔT, °C	pH	TS, %
Milk thistle			
1:2	-0.106	6.50	0.57
1:8	-0.104	6.54	0.55
1:12	-0.086	6.55	0.50
Peppermint			
1:2	-0.200	6.28	1.35
1:8	-0.198	6.26	1.35
1:12	-0.195	6.26	1.30
Sage			
1:8	-0.352	5.66	3.20
1:10	-0.241	5.77	2.40
1:12	-0.197	5.87	2.20
Purple coneflower			
1:8	-0.312	5.90	3.90
1:10	-0.303	5.97	2.90
1:12	-0.244	6.09	2.40

Table 2. Physico-chemical parameters of whey extracts at different values of the hydromodule (p ≥ 0.95)

Hydromodule	ΔT, °C	pH	TS, %
Milk thistle			
1:2	-0.489	6.46	1.5
1:8	-0.455	6.46	1.4
1:12	-0.451	6.48	1.4
Peppermint			
1:2	-0.249	6.25	2.7
1:8	-0.238	6.24	2.6
1:12	-0.230	6.24	2.6
Sage			
1:8	-0.877	5.50	10.4
1:10	-0.842	5.36	9.6
1:12	-0.790	5.31	9.2
Purple coneflower			
1:8	-0.915	5.61	10.1
1:10	-0.938	5.45	9.4
1:12	-0.868	5.45	9.2

Table 3. Concentration of total phenol compounds (mg of Gallic acid per ml) (p ≥ 0.95)

Hydromodule	Aqueous extracts	Whey extracts
Sage		
1:8	1.20	1.12
1:10	1.06	1.08
1:12	1.00	0.98
Purple coneflower		
1:8	1.06	1.07
1:10	1.02	0.97
1:12	0.94	0.92

The antioxidant activity of sage and purple coneflower extracts was studied. The dependence of ABTS radical scavenging activity of sage and purple coneflower extracts on hydromodule value is shown in Figures 1 and 2 respectively. The concentration of antioxidant compounds (μMol of trolox per mL) in sage and purple coneflower extracts at different values of hydromodule is presented in Figures 3 and 4 respectively.

It was found that the antioxidant activity of whey extracts is greater in comparison with aqueous extracts of BAS of studied plant raw materials, due to the chemical composition of the extractant. The antioxidant properties of whey are manifested by the presence of sulfur-containing amino acids (methionine, cystine, cysteine), as well as vitamins (ascorbic acid, thiamine, folic acid, and biotin). The efficient transport of BAS with antioxidant activity into whey extract can be explained by their interaction with hydrophobic sites of whey protein molecules.

The quantitative and qualitative composition of sage and purple coneflower extracts was studied by gas chromatography with mass spectrometry detector method. Results of organic compounds of extracts are presented in Tables 4 and 5.

Data obtained confirmed presence in whey extracts of some organic substances with high antioxidant activity (L-Alanine, N-acetyl-, methyl ester; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl; 2-Quinolinecarboxylic acid, methylester; Tetradecanoic acid; Hexadecanoic acid, methyl ester; 9-Octadecenoic acid (Z)-, methyl ester; 10-Octadecenoic acid, methyl ester; 10-Octadecenoic acid, methyl ester; 9,12,15-Octadecatrien-1-ol; 1,2,3-Benzenetriol; 2,5-Dimethylanisole; Trimethyl phosphonoacetate; 2-Propanol, 1-chloro-, phosphate; Pentadecanoic acid, 14-methyl-, methyl ester; Methyl stearate; 10(E),12(Z)-Conjugated linoleic acid; 9,12,15-Octadecatrienoic acid; Glycerol 1-palmitate) which are not typical for aqueous extracts.

The composition of purple coneflower whey extract is characterized by a wider range of biologically active organic compounds in comparison with a similar sage extract.

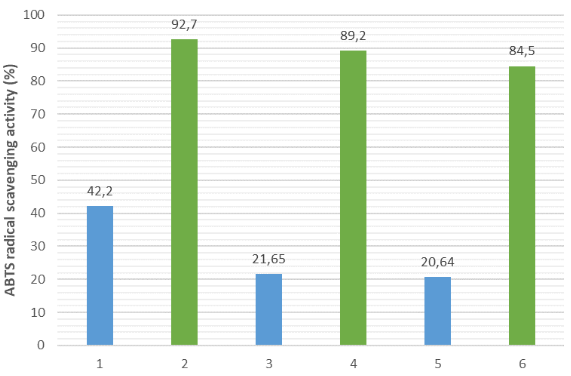


Figure 1. ABTS radical scavenging activity (%) of sage extracts at hydromodule value (p ≥ 0.95): 1 : 8 (1 - Aqueous extract, 2 - Whey extract); 1 : 10 (3 - Aqueous extract, 4 - Whey extract); 1 : 12 (5 - Aqueous extract, 6 - Whey extract)

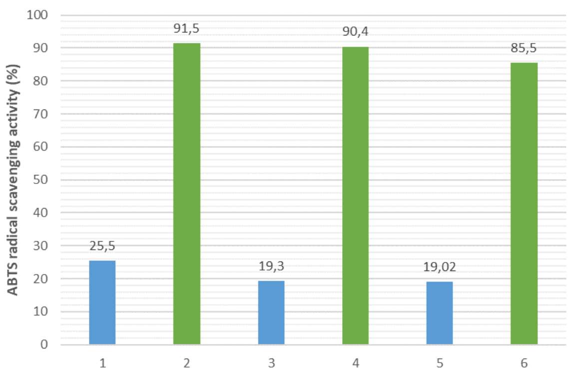


Figure 2. ABTS radical scavenging activity (%) of purple coneflower extracts at hydromodule value (p ≥ 0.95): 1 : 8 (1 - Aqueous extract, 2 - Whey extract); 1 : 10 (3 - Aqueous extract, 4 - Whey extract); 1 : 12 (5 - Aqueous extract, 6 - Whey extract)

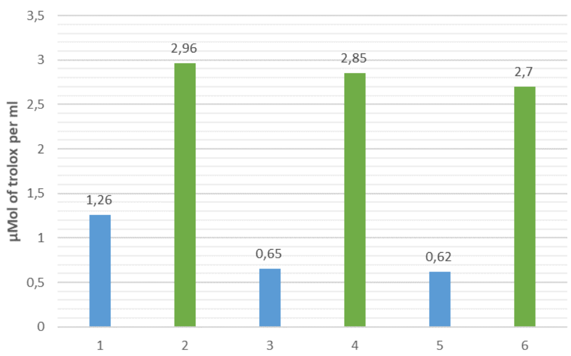


Figure 3. The concentration of antioxidant compounds (µMol of Trolox per mL) in sage extracts at hydromodule value (p ≥ 0.95) 1 : 8 (1 - Aqueous extract, 2 - Whey extract); 1 : 10 (3 - Aqueous extract, 4 - Whey extract); 1 : 12 (5 - Aqueous extract, 6 - Whey extract)

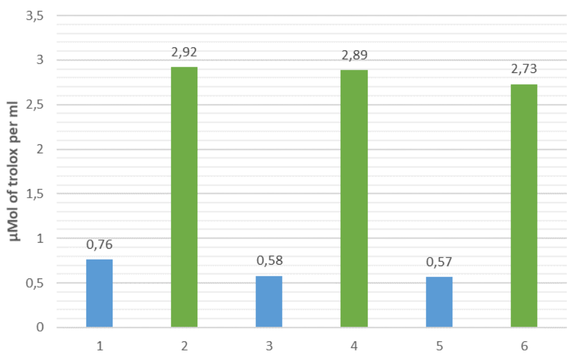
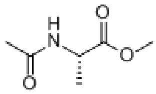
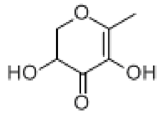
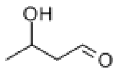
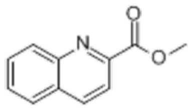
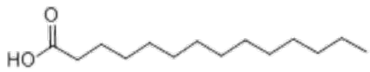
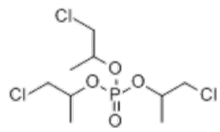
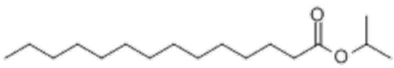
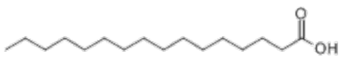
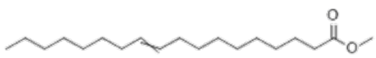


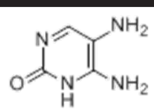
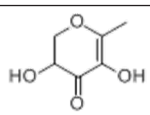
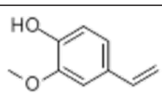
Figure 4. The concentration of antioxidant compounds (µMol of Trolox per mL) in purple coneflower extracts at hydromodule value (p ≥ 0.95) 1 : 8 (1 - Aqueous extract, 2 - Whey extract); 1 : 10 (3 - Aqueous extract, 4 - Whey extract); 1 : 12 (5 - Aqueous extract, 6 - Whey extract)

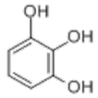
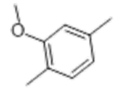
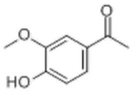
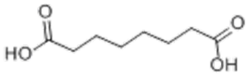
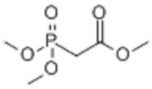
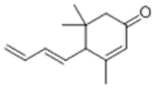
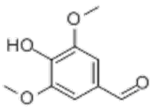
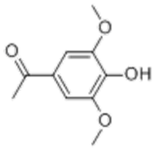
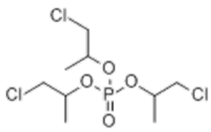
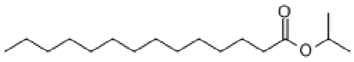
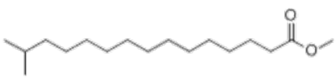
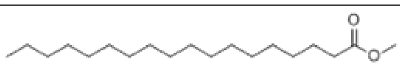
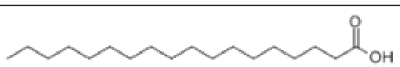
Table 4. Comparative analysis of the composition of biologically active substances of sage aqueous and whey extracts

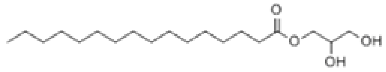
Substance, CAS	Formula	Molecular formula	Relative concentration (%) in extracts: Aqueous	Whey
L-Alanine, N-acetyl-, methyl ester, 003619-02-1		$C_6H_{11}NO_3$	- (*	4.78
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 028564-83-2		$C_6H_8O_4$	-	15.75
Butanal, 3-hydroxy-, 000107-89-1		$C_4H_8O_2$	0,99	1.50
2-Quinolinecarboxylic acid, methylester, 019575-07-6		$C_{11}H_9NO_2$	-	0.32
Tetradecanoic acid, 000544-63-8		$C_{14}H_{28}O_2$	-	0.66
2-Propanol, 1-chloro-, phosphate (3:1), 013674-84-5		$C_9H_{18}Cl_3O_4P$	0,82	0.91
Isopropyl myristate, 000110-27-0		$C_{17}H_{34}O_2$	0,99	0.51
Hexadecanoic acid, methyl ester, 000112-39-0		$C_{17}H_{34}O_2$	-	0.58
n-Hexadecanoic acid, 000057-10-3		$C_{16}H_{32}O_2$	0,68	1.12
10-Octadecenoic acid, methyl ester, 013481-95-3		$C_{19}H_{36}O_2$	-	0.32
9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-, 000506-44-5		$C_{18}H_{32}O$	-	0.37

(* Trace numbers

Table 5. Comparative analysis of the composition of biologically active substances of purple coneflower aqueous and whey extracts

Substance, CAS	Formula	Molecular formula	Relative concentration (%) in extracts Aqueous	Whey
L-Alanine, N-acetyl-, methyl ester, 003619-02-1		$C_4H_6N_4O$	-	4.03
4H-Pyran-4-one, 2,3-dihydro-3,5-hydroxy-6-methyl-, 028564-83-2		$C_6H_8O_4$	19.54	27.62
2-Methoxy-4-vinylphenol, 007786-61-0		$C_9H_{10}O_2$	7.85	7.81

1,2,3-Benzenetriol, 000087-66-1		$C_6H_6O_3$	-	1.74
2,5-Dimethylanisole, 001706-11-2		$C_9H_{12}O$	-	4.82
Apocynin, 000498-02-2		$C_9H_{10}O_3$	1.57	1.67
Octanedioic acid, 000505-48-6		$C_8H_{14}O_4$	-	1.25
Trimethyl phosphonoacetate, 005927-18-4		$C_5H_{11}O_5P$	-	0.66
Megastigmatrienone, 038818-55-2		$C_{13}H_{18}O$	6.00	2.25
Benzaldehyde, 4-hydroxy-3,5-dimethoxy, 000134-96-3		$C_9H_{10}O_4$	1.56	3.61
Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-, 002478-38-8		$C_{10}H_{12}O_4$	0.87	0.35
Tetradecanoic acid, 000544-63-8		$C_{14}H_{28}O_2$	-	0.67
2-Propanol, 1-chloro-, phosphate, 013674-84-5		$C_9H_{18}Cl_3O_4P$	-	1.34
Isopropyl myristate, 000110-27-0		$C_{17}H_{34}O_2$	0.33	0.25
Pentadecanoic acid, 14-methyl-, methyl ester, 005129-60-2		$C_{17}H_{34}O$	-	0.98
n-Hexadecanoic acid, 000057-10-3		$C_{16}H_{32}O_2$	0.53	2.64
9-Octadecenoic acid (Z)-, methyl ester, 000112-62-9		$C_{19}H_{36}O_2$	-	0.55
Methyl stearate, 000112-61-8		$C_{19}H_{38}O_2$	-	0.35
10(E),12(Z)-Conjugated linoleic acid, 002420-56-6		$C_{18}H_{32}O_2$	-	0.26
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, 000463-40-1		$C_{18}H_{30}O_2$	-	1.16
Octadecanoic acid, 000057-11-4		$C_{18}H_{36}O_2$	0.23	0.67

9-Octadecenamide, (Z)-, 000301-02-0		$C_{18}H_{35}NO$	0.46	0.94
Glycerol 1-palmitate, 000542-44-9		$C_{19}H_{38}O_4$	-	0.87

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4. Conclusions

- Results of research on the physico-chemical parameters of plant raw materials aqueous and whey extracts allow us to conclude, that optimal values of hydromodule are - 1 : 8 for milk thistle and peppermint and 1 : 10 for sage and purple coneflower.
- Sage and purple coneflower extracts were recommended for further study taking into account higher yields of total solids.
- The analysis of experimental data allows us to conclude that the application of whey as an extractant for BAS extraction from plant raw materials is promising in terms of its organoleptic and physicochemical parameters and the possibility of additional enrichment of extracts with valuable components.
- Data obtained confirmed the presence in whey extracts of some organic substances with high antioxidant activity which are not typical for aqueous extracts.

The composition of purple coneflower whey extract is characterized by a wider range of biologically active organic compounds in comparison with a similar sage extract.

- The results of the study allow us to recommend the use of plant extracts for fermented dairy products technology development.

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