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Effect of Subcritical Difluorochloromethane on the Technological Parameters and Elemental Composition of Aerial parts of Yellow Bedstraw

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Abstract: For the first time it has been determined the elemental composition received after the extraction of raw material of yellow bedstraw by the subcritical difluorochloromethane. It has been identified 6 macro- and 13 microelements, among which there are a lot of K, Na, Ca, Si, Mg and P. It has been established that the raw materials processing by the subcritical difluorochloromethane has not influence on its elemental composition. It has been made the technological research of meal obtained after the raw materials extraction by the subcritical difluorochloromethane of yellow bedstraw crushed by different ways.
Key words: yellow bedstraw (Galium verum L.), technological characteristics, meal, elemental composition, difluorochloromethane.

INTRODUCTION

Actually the medicinal herbal raw material (MHRM) is the alternative source of biologically active substances (BAS), which are used in the treatment of various etiologies diseases[1]. A typical strategy of MHRM using in drug technology is shown in Fig. 1.

MHRM use in fresh or after the technological preparation (freezing, drying, milling, tableting, granulation, encapsulation) does not resolve the issue of the BAS bioavailability increasing because the last are contained in the immobilized condition in the plant cells and in bastard concentration[3].

This obstacle cannot predict the therapeutic activity of MHRM without any detailed researches because the BAS of different chemical groups can interact in three ways: synergism (sensitization, additive effect, potentiation), antagonism and indifference[4]. So the important point in the development of herbal medicines is the use of technological methods in the separation of BAS with different pharmacological characteristics and the subsequent manufacture of the dosage form (DF) (Fig. 1).

One of the advanced methods of BAS selection from MHRM is the extraction with the liquefied gases[5]. The usage in the capacity of the extracting medium liquefied by the difluorochloromethane has several advantages[6], because the lipophilic complex doesn't fall for the negative influence of high temperature during the solvent testing, and also it is provided the satisfactory microbiological purity of the obtained extract. It should be noted

also the economic advantage of liquefied gases using in the capacity of extracting medium[7]. In addition, the received meal can be used as raw materials to obtained the BAS of the another chemical nature, so there is the question of the complex processing of MHRM.

Continuing of the researches in the development of new herbal preparation, one of the stages of work was the studying of the technological properties of yellow bedstraw (Galium verum L.) meal, obtained after raw materials processing by the liquefied difluorochloromethane and evaluation of the elemental composition of raw materials, meal and extract, which was the purpose of this research.

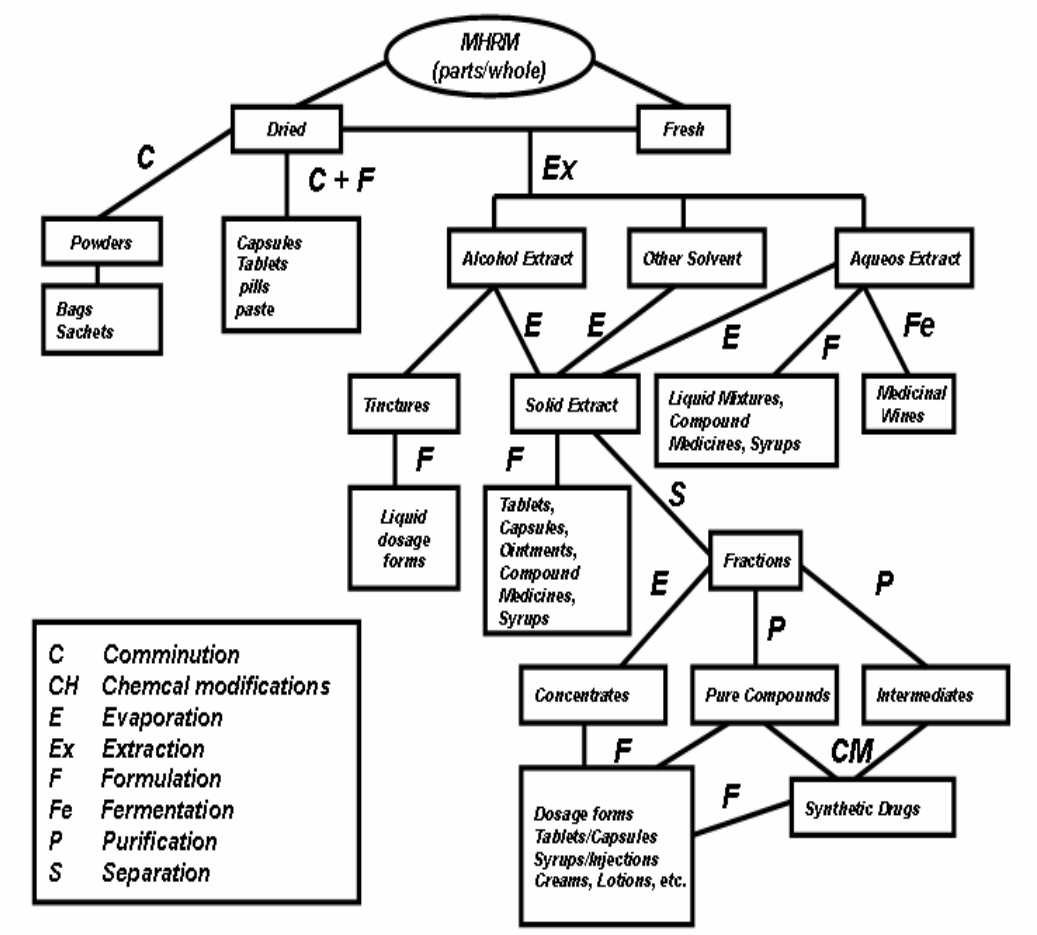

Figure 1: A typical strategy of MHRM use[2]

EXPERIMENTAL PART

The objects of the study was the meal of yellow bedstraw grass of 2007, 2008, 2009, 2010 and 2011 years of collecting, obtained after the extraction of the above mentioned raw materials by the subcritical difluorochloro methane in laboratory and industrial equipment[8].

The fractional composition was determined by a well-known method[9,10].

Determination of weight loss during the drying was made by the gravimetric method according to the SPU, 2.2.32[13,14]. The weighted amount was 1 g.

Fowability of crushed samples was studied by the method of SPU, 2.9.16.

Bulk density before the shrinkage ρ_b and after the shrinkage ρ_{1250}, the volume of researched objects after the shrinkage V_{10}, V_{500}, has been determined in accordance with the SPU, 2.9.15 in the weighted amounts of 50 g. The capacity for shrinkage was calculated by the difference V_{10}-V_{500}.

Hausner's ratio (HR) and Carr's index (CI) of samples were calculated by the formula[11,12]:

$$HR = \frac{\rho_b}{\rho_{1250}},$$

and:

$$CI = \left(\frac{\rho_{1250} - \rho_b}{\rho_{1250}} \right) \times 100\%.$$

The apparent density ρ_0 has been determined as follows. The exact weight of samples, approximately 5 g were dipped into the flasks with volume of 25 ml. From the dropping glass it has been add hexane to the mark, then the apparent density ρ_0 has been calculated as follows:

$$\rho_0 = \frac{m}{25 - V_s},$$

where: m - exact weight of raw material, g; V_s - volume of poured solvent, ml.

The P_m porosity has been calculated with the divergence of volume and bulk density:

$$P_m = \frac{\rho_0 - \rho_b}{\rho_0}.$$

The P_b porosity has been calculated after the formula:

$$P_b = \frac{\rho - \rho_0}{\rho},$$

where ρ – the true density of plant tissue which is 1,52 g/cm[317].

The elemental analysis of plant materials was made with the use of atomic and emission spectral analysis (SPU, 2.2.22) based on the complete evaporation of the substance in the alternative current arc discharge (field source - IBC-28) and the record of the emission spectrograph SFU-8.

The method is based on the evaporation of the previously concentrated plant sample from the craters of graphite electrodes in the arc burning at a current strength 16A, voltage - 220 V, exposure – 60 s. The spectrum area - 250-350 nm. It has been used the set of calibration samples (synthetic compounds) for the pass from the volumes of the analytical signals (blackening of lines of the determined elements) to the concentrations.

A series of calibration samples with the addition of certain elements $1 \cdot 10^{-2}$–$1 \cdot 10^{-5}$ wt % have been prepared by the way of thoroughly mixing of the basics of calibration samples (CS) and oxides or salts of the determined elements which correspond to the herbal composition. The base for the CS was the mixture of oxides and metal salts of the following composition: SiO_2 – 36 %; $CaCO_3$, K_2SO_4 - both 40 %; Na_2SO_4, KH_2PO_4 - both 30 %; KCl – 14 %; MgO – 10 %.

The analyzed samples were prepared as follows: a weighted amount of dry raw meal and extract with the mass not less than 3 g were placed in a quartz crucible, which has been moistened with 10 ml of 5 % solution of H_2SO_4, dried in an oven at 100 °C, and then on the electric plate till the absent of vapors of sulfuric acid. The crucible has been placed in a cold muffle furnace, the temperature was adjusted to 500 °C and it has been fried during 1 hour, then it has been cooled and weighed.

In this paper work it has been used the spectral graphite electrodes 7-3 of 6 mm diameter and a length of 50-60 mm. The calibration samples and the prepared samples were placed in the lower crater (depth of 4 mm, diameter 4,5 mm) and upper crater (depth of 5 mm, diameter 1,9 mm) of electrodes.

Reagents and Chemicals

All the materials were of analytical reagent grade.

RESULTS AND DISCUSSION

The experimental data of fractional composition of meal* according to the collecting years are represented at the figure 2 and in the table 1.

* - MOB – The meal obtained after the raw materials extraction, crushed in blander.

MORM 1 - The meal obtained after the raw materials extraction, crushed in roll mill 1 time.

MORM 2 - The meal obtained after the raw materials extraction, crushed in roll mill 2 times.

Fractional composition with the particle size 0,5 - 1,5 mm, % is represented in the table 1.

Information about determination of humidity, flowability, bulk density and shrinkage ability of the meal samples received after the extraction crushed by the various ways of the raw materials of yellow bedstraw liquefied by the difluorochloromethane is represented in the table 2, 3 and 4.

From the data obtained, there is a slight increase of humidity for samples MORM 2 in comparison with MORM 1.

The flowability of samples MORM 1 and MORM 2 was low, and it was absent for the sample MOB which can be explained by the anisometric particle form.

The bulk density ρ_b of meal samples depended from the crushing method of raw materials and according to the fractional composition. The Fig. 3 shows the changing of the arithmetic average bulk density of meal samples, depending on the conditions of shrinkage.

From the Fig. 3 we can see the ability to the shrinkage of the sample MOB which is significantly lower than the ability of MORM 1 and MORM 2.

The bulk density of samples MORM 2 was in average in 6% and 26% higher in comparison with the MORM 1 and MOB respectively.

The P_m porosity of samples was almost the same, but the P_b porosity of MOB was in 1,7 and 2,4 times higher than the porosity of MORM 1 and MORM 2 respectively.

Taking into account that the characteristics of crushed raw material of herbs are different from the characteristics of crushed crystalline powders, the determination of flowability as the angle of repose, the rate of shear, Hausner's ratio (HR) and Carr's index (CI), it is clear that, depending on the technological process of identifying of some of them are quite arbitrary. For example, for the powdered plant material, which is the subject to the further compaction, the determination of the angles of repose and the rate shear are obligatory at the same time they are secondary for the raw materials which should be treated with solvents.

In calculating of the HR and CI for the researched samples of meal (Fig. 4), it has been found that the patterns of flowability are not fulfilled.

Thus, even with values of HR < 1.25 and CI <15 (which correspond to the powders with the excellent flow properties) the flowability of meal samples is not satisfactory for the further compression. However, these indicators can be neglected for the extraction process because the download of extractors by the raw materials is made after the preparing of the extraction cartridges manually[14].

The next stage of our research was the determination of the influence of liquified difluorochloromethane on the elemental composition of yellow bedstraw. Previously, we have identified the macro- and microelement composition of samples of raw material (R) of yellow bedstraw of 2007-2011 collecting years, meal (M) obtained after extraction of raw materials by the subcritical difluorochloromethane and also the extract (E).

Table 1:The results of fractional composition determination with the particle size 0,5 - 1,5 mm of meal samples.

Collection year	MOB, %	MORM 1, %	MORM 2, %
2007	50,807	71,323	76,659
2008	48,548	74,291	75,529
2009	42,875	71,194	78,102
2010	44,719	71,625	75,423
2011	44,738	71,323	76,233

Table 2: Results of technological characteristic research of meal samples received after the extraction of the raw material samples of yellow bedstraw, crushed in blander, by the liquefied difluorochloromethane.

Technological parameter	MOB				
	2007	2008	2009	2010	2011
Humidity, %	7,49±0,43	8,37±0,36	9,80±0,38	8,24±0,63	8,02±0,15
Flowability, s/100 g	Absent	Absent	Absent	Absent	Absent
Bulk density ρ_b, g/cm^3	0,174±0,005	0,177±0,006	0,181±0,004	0,185±0,007	0,175±0,003
Volume $V_{10,}$ cm^3	263,0±0,5	268,0±0,5	273,0±0,5	264,0±0,5	269,0±0,5
Volume $V_{500,}$ cm^3	238,0±0,5	242,0±0,5	247,0±0,5	239,0±0,5	244,0±0,5
Volume $V_{1250,}$ cm^3	234,0±0,5	238,0±0,5	242,0±0,5	235,0±0,5	239,0±0,5
Shrinkage ability, cm^3	25,0±0,5	25,0±0,5	26,0±0,5	25,0±0,5	26,0±0,5
Apparent density ρ_0, g/cm^3	1,038±0,007	1,042±0,003	1,028±0,006	1,032±0,005	1,046±0,005
P_m porosity, g/cm^3	0,822±0,005	0,830±0,004	0,824±0,004	0,821±0,003	0,832±0,006
P_b porosity, g/cm^3	0,357±0,004	0,314±0,007	0,324±0,004	0,321±0,003	0,312±0,004

The results of the elemental analysis are shown in **Table. 5.**

In the Table. 5 the raw materials and the meal of yellow bedstraw contain the significant amounts of potassium, calcium, magnesium and silicon, a little amount of - sodium, phosphorus, aluminum, iron. Thus, for the studied raw materials, meal and extract we can set a following number of elements depending on the decrease of their content: for the raw materials : K> Si> Ca> Mg> P> Na> Al> Fe> Zn> Sr> Mn> Cu> Ni> Pb = Co> Mo> Cd = As = Hg; for the meal: K> Ca> Si> Mg> P> Na> Al> Fe> Mn> Zn> Sr> Cu = Ni> Pb = Co> Mo> Cd = As = Hg, for the extract : Si> K> Ca> Na> P> Mg> Al> Mn> Fe> Zn> Cu> Ni> Pb = Co> Mo> Sr> Cd = As = Hg. The value on the content of some micro elements are correlated with the available sources[15,16]. However, there is a significant variation of silicon from 0,5 mg/100 g[15] to 400 mg/100 g[16]. As reported in[17,18], the variations of the elemental composition is associated with the terms of the accumulation of macro- and microelements in the growing process and the method of their determination in plant materials.

At the same time, there are the low amounts of the same elements in the samples of extracts. This can be explained by the sufficiently lower extraction capacity of liquefied difluorochloromethane towards the mineral elements. Therefore, analyzing the obtained data we can affirm that the processing of raw materials by the subcritical difluorochloromethane virtually has no effect on its elemental composition.

Thus, it has been identified the main technical characteristics of the grass of meal of yellow bedstraw obtained after the extraction of the above mentioned raw materials by the subcritical difluorochloromethane in the laboratory and industrial equipment. It has been identified 19 elements in plant material, meal and extract.

The results of the research can be used to develop the industrial technology of the extraction medical drug on the basis of meal of yellow bedstraw or in the technology of solid dosage form which are used in the treatment of pathologies associated with disorders of mineral balance.

Table 3: Results of technological characteristic research of meal samples received after the extraction of the raw material samples of yellow bedstraw, crushed in roll mill 1 time, by the liquefied difluorochloromethane.

Technological parameter	MORM 1				
	2007	2008	2009	2010	2011
Humidity, %	8,34±0,56	9,17±0,26	9,43±0,38	9,21±0,44	8,92±0,73
Flowability, s/100 g	125,54* ±0,24	113,65* ±0,33	118,95* ±0,63	123,61* ±0,43	126,44* ±0,73
Bulk density ρ_b, g/cm^3	0,214±0,005	0,217±0,008	0,209±0,004	0,212±0,005	0,223±0,003
Volume $V_{10,}$ cm^3	225,0±0,5	221,0±0,5	219,0±0,5	221,0±0,5	218,0±0,5
Volume $V_{500,}$ cm^3	186,5±0,5	183,0±0,5	181,0±0,5	181,0±0,5	180,0±0,5
Volume $V_{1250,}$ cm^3	177,5±0,5	178,0±0,5	177,0±0,5	176,0±0,5	173,0±0,5
Shrinkage ability, cm^3	38,5±0,5	38,0±0,5	38,0±0,5	40,0±0,5	38,0±0,5
Apparent density ρ_0, g/cm^3	1,230±0,005	1,241±0,007	1,229±0,006	1,246±0,005	1,237±0,006
P_m porosity, g/cm^3	0,814±0,007	0,825±0,004	0,830±0,005	0,830±0,003	0,821±0,005
P_b porosity, g/cm^3	0,214±0,006	0,183±0,007	0,191±0,004	0,180±0,007	0,186±0,008

* - only with the powered on vibration motor

Table 4: Results of technological characteristic research of meal samples received after the extraction of the raw material samples of yellow bedstraw, crushed in roll mill 2 times, by the liquefied difluorochloromethane.

Technological parameter	MORM 2				
	2007	2008	2009	2010	2011
Humidity, %	8,56±0,33	9,29±0,74	9,13±0,72	9,34±0,17	9,36±0,56
Flowability, s/100 g	104,36* ±0,55	109,29* ±0,32	102,65* ±0,76	98,74* ±0,64	101,43* ±0,27
Bulk density ρ_b, g/cm^3	0,221±0,007	0,222±0,008	0,227±0,004	0,231±0,006	0,225±0,004
Volume $V_{10,}$ cm^3	223,5±0,5	223,0±0,5	221,0±0,5	224,0±0,5	223,0±0,5
Volume $V_{500,}$ cm^3	183,0±0,5	184,0±0,5	181,0±0,5	184,0±0,5	183,0±0,5
Volume $V_{1250,}$ cm^3	178,0±0,5	179,0±0,5	176,0±0,5	179,0±0,5	176,0±0,5
Shrinkage ability, cm^3	40,5±0,5	39,0±0,5	39,0±0,5	40,0±0,5	40,0±0,5
Apparent density ρ_0, g/cm^3	1,306±0,005	1,318±0,007	1,298±0,003	1,315±0,007	1,313±0,004
P_m porosity, g/cm^3	0,831±0,007	0,832±0,002	0,825±0,005	0,825±0,005	0,829±0,007
P_b porosity, g/cm^3	0,141±0,003	0,133±0,004	0,146±0,006	0,135±0,003	0,137±0,004

* - only with the powered on vibration motor

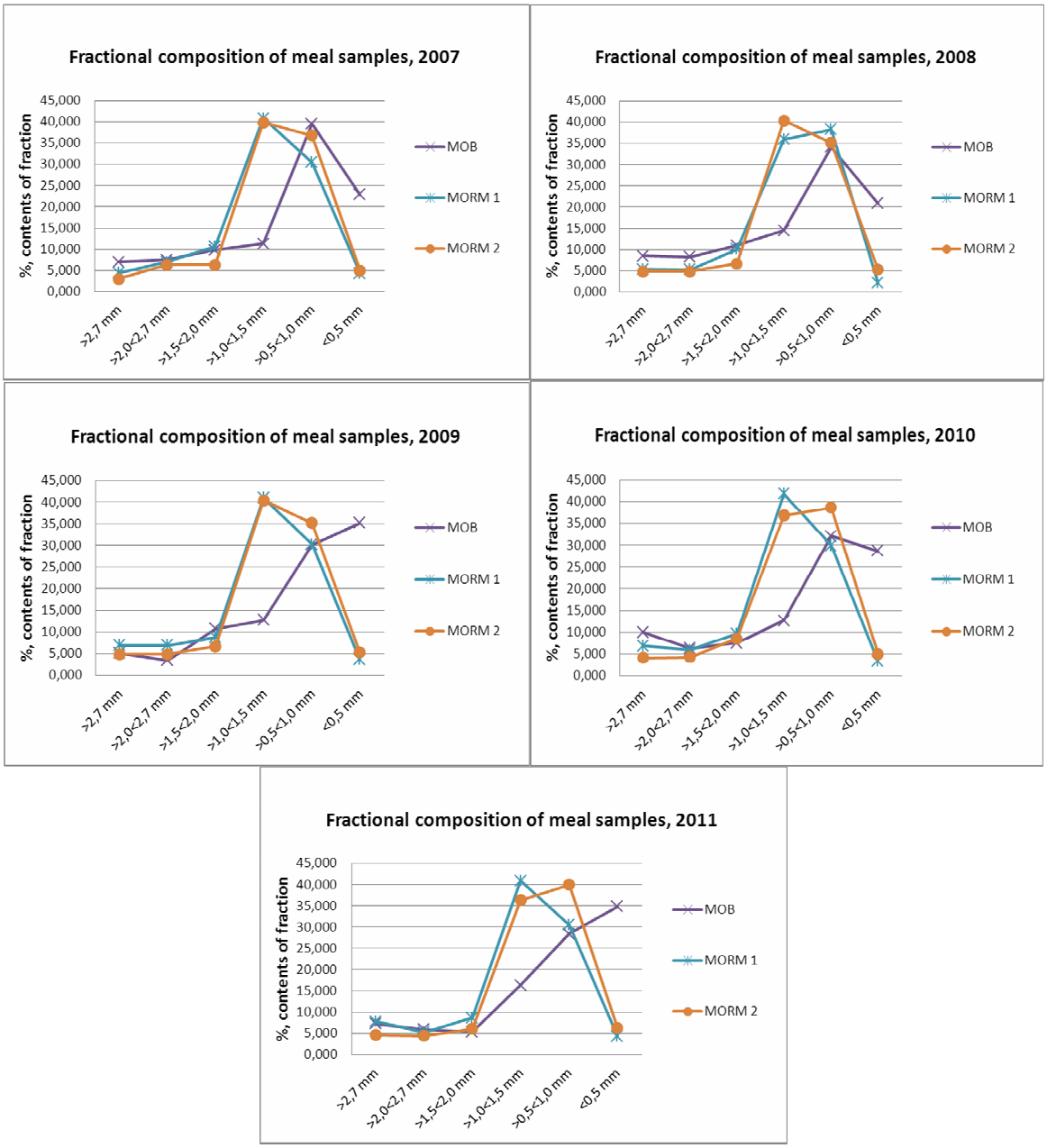

Figure 2: Fractional composition of meal samples according to the years

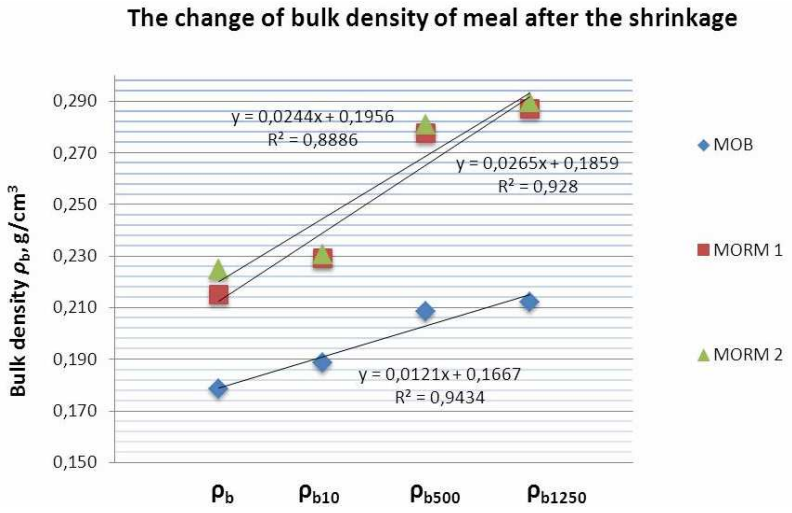

Figure 3: The change of bulk density of meal after the shrinkage

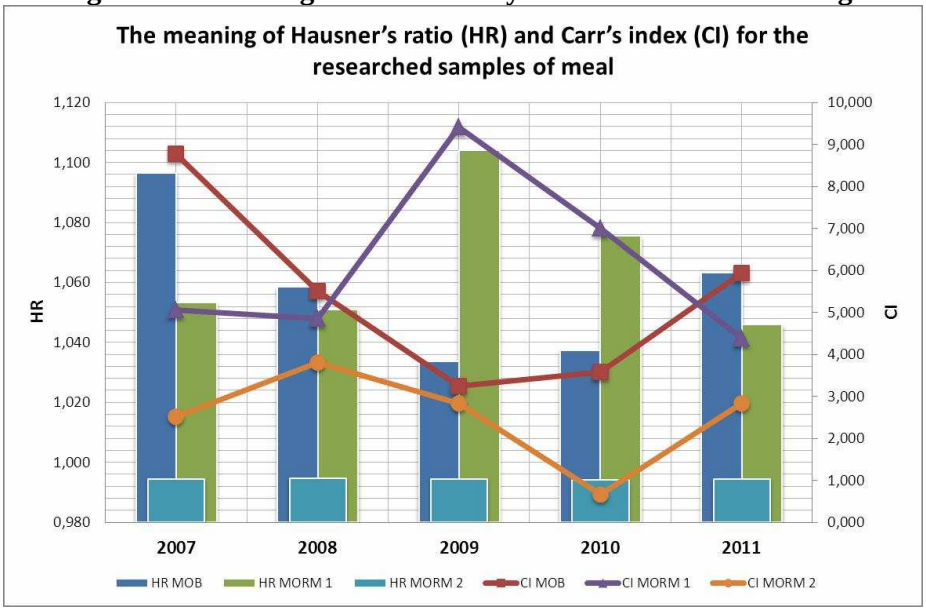

Figure 4: The meaning of Hausner's ratio (HR) and Carr's index (CI) for the researched samples of meal

Table 5: The results of the elemental analysis of yellow bedstraw raw materials, meal, received after the extraction by the subcritical difluorochloromethane and also the extract.

Element	2007			2008			2009			2010			2011		
	R	M	E	R	M	E	R	M	E	R	M	E	R	M	E
	Macroelements, mg/100g														
K	1560	1400	75	1670	1620	110	2250	2370	94	2520	2370	90	1910	1840	68
Na	127	115	8	87	77	12	75	69	17	84	74	9	90	85	13
Ca	290	264	33	350	330	38	420	400	50	670	590	45	420	380	36
P	100	78	22	90	80	15	100	96	27	140	125	45	93	82	21
Mg	40	65	12	200	185	10	350	150	20	250	220	18	135	130	12
Si	430	400	80	380	360	130	550	530	140	600	590	180	380	320	120
	Microelements, mg/100g														
Fe	16,1	4,9	0,5	32,9	12,8	1,3	39,2	13,5	1,1	22	2,5	1,4	11,4	4,7	0,7
Mn	12	8,4	1,1	6	3,5	0,7	1,2	1,1	0,4	3,7	4,2	0,3	1,5	1,2	0,2
Al	66	45	16	54	8,6	4,5	42	36	5,3	37	1,7	13	23	14,5	9
Sr	1,5	1,2	0,01	0,61	0,56	0,01	0,33	0,28	0,01	0,74	0,84	0,02	1,2	1,1	0,02
Ni	0,12	0,08	0,05	0,13	0,12	0,07	0,21	0,14	0,13	0,42	0,15	0,2	0,22	0,13	0,11
Cu	0,8	0,7	0,07	0,15	0,12	0,02	0,6	0,57	0,06	0,44	0,32	0,15	0,37	0,21	0,08
Zn	6,3	5,8	0,3	8,0	6,3	1,2	2,4	1,8	0,6	0,8	0,7	0,4	3,2	2,8	0,5
Co	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03
Mo	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02	<0,02
Pb	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03	<0,03
Cd	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
As	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
Hg	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01

CONCLUSIONS

At the base of the results of our research and also at the base of data which we found in the literature, it was determined that the factors which significantly affect at the technological parameters of meal of the yellow bedstraw is the humidity, the crushing method of raw materials and the particle size of the researched material. It has been established that during the calculation of HR and CI for the researched samples of meal the generally accepted patterns of flowability are not fulfilled.

For the first it was determined the elemental composition of meal obtained after extraction of raw materials of yellow bedstraw by the subcritical difluorochloromethane. It was identified 6 of macro-and 13 microelements, among which there is the largest number of K, Na, Ca, Si, Mg and P. The average content of heavy metals in the extracts is within the MPC. It was established that the processing of raw materials by the subcritical difluorochloromethane virtually has not effect on its elemental composition.

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