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THE CONTENT OF MICRO- AND TRACE ELEMENTS IN COMMERCIAL AGAROSES AND THEIR RAW MATERIAL

Abstract. Samples of algae, agars and agaroses were analysed for inorganic elements by using neutron activation analysis (NAA). Special attention was paid to the alga *Ahnfeltia tobuchiensis* and its technological products.

In the course of agarose isolation and purification various elements get detached very differently. No strong dependence upon an element's position in the periodic table is observed. Some of the light elements have a considerable affinity to the agarose matrices and they cannot be removed below a certain level during the purification process.

Commercial agarose preparations are analysed and details of such analyses are discussed.

In recent years, the role of algae in marine biotechnology has become considerable [1–3]. One of the main applications of macroalgae is the utilization of gelling hydrocolloids obtained from certain red seaweed (Rhodophyta) species. From this practical point of view, *Ahnfeltia tobuchiensis* (Kanno et Matsub.) Mak. (the Sea of Japan, Pacific) [4] is the most important alga species on the whole territory of Russia and the CIS [5]. It is the only alga species of wide occurrence along the vast coastline from Chukot to Vladivostok which is suitable for the manufacture of high-quality agar and agarose.

This polysaccharide skeleton is promising in biotechnology [6]. The matrix is suitable for the manufacture of immunosorbents, being on a pilot production level for the separation and purification of human plasma proteins [7].

In human blood serum the normal concentration of trace elements varies in the range of 10^{-6}—10^{-9} g/ml. These ranges of values are relatively small, being approximately ±20% of the average [8]. Consequently, it is important to research the micro- and trace element contents and their changes in the course of manufacturing and utilizing the carriers. In the present paper, the elements content of algae and agaroses, as well as the distribution of elements in the agarose technology have been dealt with.

Literature data on the elementary composition of algae are available, but it is often difficult to compare the results of different authors [9]. Beyond doubt, the elementary composition of seaweeds is dependent not only on the species (within one genus it varies in the range of some orders, see Fig. 1), but also on natural conditions (habitat, season), the preparation of materials, etc. As the trace elements content of algae is

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very low, the rules of operating with extremely low concentrations should be followed to get adequate results [10]. Methods involving fewer chemical operations (or being entirely non-destructive) in the course of which there is always a danger of contamination or unexpected adsorption on the glassware walls [11] seem to be more preferable.

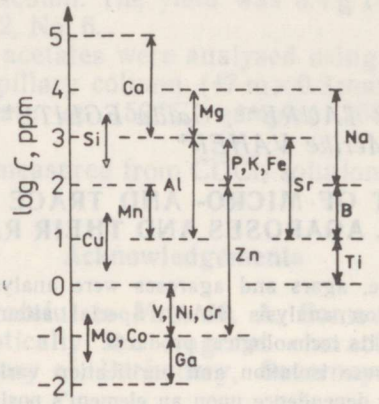

Fig. 1. Variations in the inorganic elements concentration C (0-level: parts per million).

The elementary composition of algae from the Sea of Japan has been examined by Yamamoto and coworkers [9, 12], Gryzhankova et al. [13], and others. The composition of algae *products* has been investigated to a lesser extent.

The species *Ahnfeltia tobuchiensis* and the agar produced have been studied by Saenko et al. [14]. They paid main attention to the waste products of the agar industry, in the high-brand agar the content of only 6 elements has been determined.

In recent years, a close relationship between the chemical composition of seawater and marine algae [15] and also the role of some elements in algal biosynthesis [16, 17] has been established. Ultratrace biotransformations seem to have been strictly determined too [18]. Apparently, even noble metals play a certain role in algal biochemistry [19, 20]. Therefore, multielemental analysis of seaweeds may contribute to an understanding of such subtle mechanisms.

The choice of the analytical method [21] is very important for such kind of analyses. Surely, there are wide possibilities of concentrating trace elements [22]. But as it is better to avoid additional operations, the most sensitive methods are preferred. For a good deal of elements (but not for all) some radioanalytical methods have the highest sensitivity. In some cases, neutron activation analysis (NAA) has a detection limit of up to 10^{-13} g [23]. It has the advantage of being a multielement method which acts in a very wide range of concentrations and can even give results for elements that were not expected to be present in the sample.

Experimental

Materials. Seaweeds *Ahnfeltia tobuchiensis* (from both habitats, see Table 1) were obtained from the agar factory at Tafuin (Juzhno-Morskoy), Primorye, Russia, in an air-dry state and washed thoroughly with distilled water. If not mentioned otherwise, all the samples of algae, agar and agarose were dried at 80° and (as noted, part of them) gradually ashed at 500 °C. *Ahnfeltia* agar and agarose were obtained from the Pilot-Pro-

Table 1

The content of some elements in *Ahnfeltia tobuchiensis* from various habitats (results of NAA)

Sample No.	Habitat	Preliminary treatment	Component content, % of dry alga											
			Ash content	10^{-1}		10^{-2}		10^{-3}			10^{-4}		10^{-5}		
				Fe	Br	Al	Cu	V	Mn	Zn	Cr	Ce	Sc	Co	Th
1	The Sea of Japan, region near	drying at 105 °C	24	1.3	4.5	5	2.1	1.5	5	2	3.9	3	2.4	6	1.8
2	Vladivostok	ashing		1.1	4.1	5.8	1.8	1.13	4.8	1.9	3.6	2.9	2.3	5.3	1.5
3	The Pacific, Kunashir Island	at 500 °C	30	0.96	2.8	6.6	1.2	1.0	**132**	**0.21**	2.1	6.3	3.6	**102**	2.3

Table 2

The nuclides used and their characteristics

Element	Nuclide	$T_{1/2}$	E (KeV)	Element	Nuclide	$T_{1/2}$	E (KeV)
Na	^{24}Na	15.1 h	1368.6	Br	^{82}Br	1.47 d	554.3
Mg	^{27}Mg	9.46 m	1014.4				776.5
Al	^{28}Al	2.31 m	1778.8	Rb	^{86}Rb	18.7 d	1076.6
Cl	^{38}Cl	37.29 m	1642.3	Sr	^{85}Sr	64.8 d	514.0
Ca	^{47}Ca	4.53 d	1296.9	Ag	^{110m}Ag	250 d	657.8
Sc	^{47}Sc	3.44 d	159.4	Sb	^{124}Sb	60 d	602.7
	^{46}Sc	83.8 d	1120.5				1691.0
			889.3	I	^{128}I	54.99 m	442.9
Ti	^{51}Ti	5.8 m	320.1	Cs	^{134}Cs	2.06 y	795.8
V	^{52}V	3.76 m	1434.4	Ba	^{131}Ba	11.8 d	496.3
Cr	^{51}Cr	27.7 d	320.1	Ce	^{141}Ce	72.3 d	145.4
Mn	^{56}Mn	2.58 h	1811.0	Eu	^{152}Eu	13.6 y	1408.0
Fe	^{59}Fe	44.5 d	1099.2	Tb	^{160}Tb	73.2 d	879.4
			1291.6	Yb	^{175}Yb	4.2 d	396.3
Co	^{60}Co	5.27 y	1117.3		^{169}Yb	31.8 d	177.2
			1332.5	Hf	^{181}Hf	42.9 d	482.2
Ni	^{58}Co	71.3 d	810.8	Au	^{198}Au	2.7 d	411.8
Cu	^{66}Cu	5.10 m	1039.0	Th	^{233}Pa	271 d	311.8
Zn	^{65}Zn	246 d	1115.5				

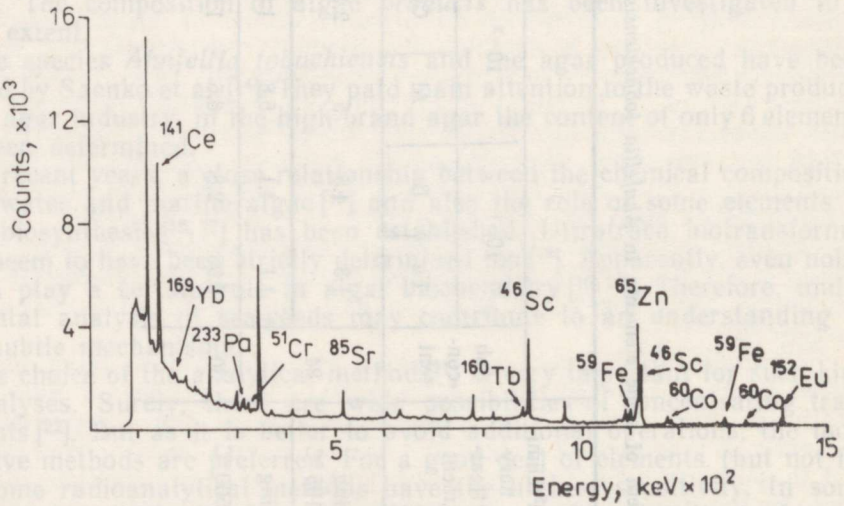

Fig. 2. A typical gamma spectrum of the irradiated agaroses.

duction Plant of Organic Synthesis and Biopreparations of the Institute of Chemistry, Estonian Academy of Sciences.

Equipment and conditions. The samples were irradiated in a Nuclear Research Reactor [24] of the Institute of Physics, Latvian Academy of Sciences. A pneumatic tube conveyer was used to transport samples to and from the reactor. The nuclides formed, their half-lives and the gamma-ray energies used for the determinations are listed in Table 2.

For the determination of Mg, Al, Cl, Ti, V, Mn, Cu and I the short-lived radionuclides were used. The samples were irradiated in horizontal

Table 3

Changes in the micro- and trace elements content in agarose processing

Element	Elements content, ppm of ashed material/product			Purification multiplicity		
	alga	agar	agarose	from alga to agar	from agar to agarose	total
Na	2160	16.5	52.4	131	0.315	41.2
Mg	310	39	76.2	7.95	0.512	4.07
Al	580	33	27.7	17.6	1.19	20.9
Cl	134	2.85	1.1	47.0	2.59	122
Sc	0.23	0.0024	0.00048	95.8	5.0	479
Ti	14	38	1.85	0.368	20.5	7.57
V	11.3	0.045		251		
Cr	3.6	0.87	0.48	4.14	1.81	7.50
Mn	48	0.975	0.778	49.2	1.25	61.7
Fe	1100	39	13.2	28	2.95	83.3
Co	0.53	0.0825	0.0291	6.42	2.84	18.2
Ni	13	1.2		10.8		
Cu	180		3.5			51.4
Zn	19	4.65	23.5	4.09	0.198	0.809
Br	4100	8.4	2.0	488	4.2	2050
Sr	166	2.85		58.2		
Ag	0.5		0.0185			27.0
Sb	0.17	0.204	0.015	0.833	13.6	11.3
I	790	0.09	0.059	8780	1.53	13400
Au		0.00375	0.0034		1.1	
Hg	0.38	0.036	0.0053	10.6	6.79	71.7
Th	0.15	0.00465	0.002	32.3	2.32	75
Ash content, %	23.8	1.5	0.27	15.9	5.56	88.1

channels at a flux of 1.6×10^{13} ncm^{-2}s^{-1} for 30 s. The gamma-rays from the irradiated samples were measured using a Ge(Li) detector connected to a 4000 channels analyzer (NTA-1024, Hungary).

When long-lived nuclides were used for analysis, the irradiation of samples was performed in vertical channels at two regimes: with reactor neutrons at a flux of 1.4×10^{13} ncm^{-2}s^{-1} for 24 hours, and with resonance neutrons at a flux of 1.3×10^{11} ncm^{-2}s^{-1} for 72 hours. In both cases, the irradiated samples were measured twice: 4 days and 3 weeks after irradiation. The gamma-activity initiated was measured using a Ge(Li) detector connected to a 4000 channels analyzer (LP-4900, Nokia, Finland).

The results obtained are presented in Tables 1 and 3—5. A typical gamma spectrum is shown in Fig. 2.

Results and Discussion

Table 1 shows that the volatility on ashing at 500 °C is only a little higher than on drying at 105 °C and the difference in the content of some elements (Mn, Zn, Co) in algae depends on their habitat only.

The course of removal of elements in the agarose technology is presented in Table 3. To get comparable results, the samples are taken from successive technological stages of the same process.

Difference in the effect of the eluting of *Ahnfeltia* agarose with a complexing agent and distilled water

(element content of dry matrix, ppm; NAA of ashed samples)

Element	Agarose eluted with		Detection limit (d. l.) in matrix	Statistical error, %
	20mM Na_2EDTA	dist. water		
Na	1060	120	2	1
Mg	0.81	1.1	0.2	23—55
Al	8.1	11	0.4	1—2
Cl	5.3	3.8	4	50—100
Ca	140	1260	40	2—16
Sc	0.0011	0.0013	0.00004	2—11
Ti	d. l.	2.9	2	29—100
V	0.024	0.02	0.008	8—100
Cr	0.57	0.46	0.002	1—19
Mn	11	0.4	0.4	1—27
Fe	0.14	20	0.3	1—9
Co	0.032	0.025	0.0004	1—4
Ni	0.84	1.34	0.04	1—24
Cu	3.3	2.5	1	27—100
Zn	2.6	2.1	0.02	1—3
Br	3.2	2.1	0.1	1
Rb	d. l.	0.034	0.02	9—21
Ag	0.021	0.013	0.001	1—17
Sb	0.023	0.008	0.0008	2—14
I	0.099	d. l.	0.08	16—100
Ba	1.0	2.3	0.2	3—25
Ce	0.01	0.013	0.001	1—17
Hf	0.001	d. l.	0.0002	9—19
Au	0.0042	0.040	0.000002	1
Hg	0.0057	0.016	0.0004	3—11
Th	0.0017	0.0013	0.0002	2—17

In the process algae → agar → agarose the total mineralization decreases by a factor of about 88 (this number characterizes an average purification degree), but the separation of individual elements proceeds very differently. No clear dependence upon the element's position in the periodic table has been observed. (Elements in Tables are arranged in the order of increasing atomic numbers.)

As the composite number <1 indicates the enrichment process, the higher affinity of the element to the agarose matrix (in given conditions) should be supposed. If enrichment takes place in the second stage of purification (from agar to agarose), which involves thorough washing with distilled water, some functional relations of those elements with the polysaccharide matrix may be supposed. Such elements are Na, Mg and, especially, Zn (see Table 3). Also, elements whose concentration increases in the first stage (Ti, Sb) may be removed in the next one.

On the other hand, halogens (especially iodine) are easy to separate and they should not have a noticeable affinity to the matrix. Consequently, the unusually high concentrations of bromine and iodine in algae should be related to the other cell structures (the biological function of these elements in algae is unknown [25]).

The content of some elements in commercial agarose preparations
(non-ashed, air-dry)

Element, its order of content, %		Chemapol agarose for electro-phoresis	SIGMA agaroses			LKB agaroses		
			Type I: Low EEO	Type V: Low EEO HGT	Type IV: Special High EEO	-L Low EEO	-M Medium EEO	-H High EEO
Na	10^{-1}	2.0	1.8	0.9	4.2	0.7	2.3	2.5
Mg	10^{-2}	1.2	0.1	4.2	d.l.	0.9	4.7	2.3
Al	10^{-3}	0.7	1.9	1.7	0.9	7.1	1.0	11.5
Cl	10^{-2}	0.2	2.0	0.5	1.6	0.5	6.7	5.2
Ca	10^{-2}	d.l.	6.4	4.0	d.l.	1.6	1.8	3.0
Sc	10^{-7}	1.9	4.7	7.3	4.4	3.2	2.4	8.4
V	10^{-4}	d.l.	0.2	8.9	0.3	0.6	0.1	1.0
Cr	10^{-4}	0.9	1.8	3.9	1.3	3.0	1.8	4.0
Fe	10^{-3}	3.0	3.0	5.3	2.4	1.7	1.7	2.1
Co	10^{-6}	2.6	3.8	1.2	3.7	2.3	0.9	d.l.
Zn	10^{-3}	1.8	1.7	1.4	0.3	0.3	0.3	0.2
Br	10^{-4}	8.1	4.4	4.9	3.3	2.3	2.0	3.0
Sb	10^{-5}	0.2	0.7	2.0	0.3	1.7	0.5	0.7
I	10^{-5}	26.6	1.0	2.9	15.0	7.9	0.3	10.6
Hg	10^{-6}	15.5	5.6	7.3	6.2	5.7	7.5	8.2

d. l. = detection limit

On long-term eluting with a complexing agent (20 mM Na_2EDTA, at 45 °C, for 20 h), as a rule, elements are not separated from the matrix (Table 4), only the calcium (barium to a lesser extent) content decreases considerably (calcium is replaced by sodium). Table 4 shows that in many cases the eluting with distilled water of the same volume and temperature has an even bigger effect. Regardless of this, the removal of water-insoluble calcium compounds has a very valuable clarifying effect on agarose gels.

In case of non-ashed samples (Table 5) the results have been used for comparison rather than for the sake of their absolute values because the element content is close to the detection limit. A number of elements (Ti, Mn, Ni, Cu, Rb, Ag, Cs, Ba, Ce, Tb, Yb, Hf, Eu, Au, Th) are not detectable in this way.

In conclusion, it may be said that the ashing of samples followed by NAA is an advantageous combination for multielemental analysis of the raw material and products of the agarose industry, providing a sufficient sensitivity as well as avoiding sample contamination. The ashing at 500 °C increases the element content in the samples by a factor of about 10^2, yet causes no essential volatility.

The results of parallel spectral analysis of the samples of the same ashing temperature are not so good.

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KOMMERTSAGAROOSIDE NING NENDE TOORME MIKRO- JA JÄLGELEMENTIDE SISALDUS

Tuumareaktori soojusneutroneid kasutades uuriti nii kommertsagaroose kui ka nende tootmise lähtematerjale — punavetikaid *Ahnfeltia tobuchiensis* (Kaug-Ida kahest kasvukohast) ja nendest ekstraheeritud agareid. Neutronaktivatsioonanalüüs (NAA) anorgaaniliste elementide sisalduse määramiseks tehti lühi- ja pikaealiste radionukliidide baasil; kasutati reaktori- ja resonantsneutroneid (kiirgusvood vastavalt suurusjärgus 10^{13} ja 10^{11} $ncm^{-2} \cdot s^{-1}$). Moodustunud isotoopide gammaspektreid registreeriti ja uuriti paljukanaliste Ge(Li)-detektoriga analüsaatorite abil.

Tuvastati, et agaritööstuse toorme ja vaheproduktide (agarite) elementkoostis võib partiide (ja vetikaareaalide) lõikes tugevasti varieeruda. Seetõttu tuleb elementide sisalduse dünaamika jälgimiseks tehnoloogilises protsessis analüüsida proove ühest ja samast tehnoloogilisest ahelast. Eri firmade kõrgkvaliteetsed agarid ja agaroosid erinevad tunduvalt mineraalosa sisalduse ja koostise poolest.

Agaroosi tootmisel kasutatavas puhastusprotsessis eralduvad polüsahhariid-maatriksist kõige kiiremini halogeenid (see viitab nende biofunktsionaalsele neutraalsusele maatriksi suhtes), kuid ka nende puhastumiserinevused on suured. Veel väiksem on seos perioodilisussüsteemiga ülejäänud elementide puhul. Leelismetallidel ja tsingil on märgatav afiinsus agaroosmaatriksi suhtes ja nad ei ole eemaldatavad allapoole teatavat sisaldustaset. Kompleksimoodustajaga (triloon B lahusega) voolutamine ei vähenda maatriksi raskmetallisisaldust.

Proovide tuhastamine koos järgneva NAA-ga on otstarbekas kombinatsioon agaritööstuse toorme ja produktide multielementseks analüüsiks, et tagada ühtaegu küllaldast tundlikkust ning vältida proovide saastumise ohtu.

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СОДЕРЖАНИЕ СЛЕДОВЫХ И МИКРОЭЛЕМЕНТОВ В КОММЕРЧЕСКИХ АГАРОЗАХ И ИХ СЫРЬЕ

С применением тепловых нейтронов ядерного реактора исследованы коммерческие агарозы и сырьевые материалы их производства — красные морские водоросли *Ahnfeltia tobuchiensis* (из двух местонахождений российского Дальнего Востока) и экстрагированные из них агары. Для количественного определения 30 неорганических элементов проведен нейтронно-активационный анализ (НАА) с использованием как реакторных, так и резонансных нейтронов (в потоках с размерностями 10^{13} и 10^{11} $нсм^{-2} \cdot с^{-1}$ соответственно). Гамма-спектры образовавшихся радиоизотопов зарегистрированы и исследованы при помощи многоканальных анализаторов с Ge(Li)-детекторами.

Установлено, что элементный состав сырья и промежуточных продуктов агаровой промышленности может значительно варьироваться в пределах разных партий (и местонахождений водорослей). Поэтому для исследования динамики содержания элементов в технологическом процессе необходимо анализировать пробы по одной и той же технологической цепи. Высококачественные агары и агарозы разных фирм мира значительно различаются по содержанию и составу минеральной части.

В производственном очистительном процессе выделения фракции агарозы быстрее всего от полисахаридной матрицы отделяются йод и бром (что указывает на биофункциональную нейтральность этих галогенов в отношении матрицы), но разница в степени очистки от них матрицы велика. В случае остальных элементов связь между расположением их в Периодической системе и степенью очистки еще незначительнее. Щелочные металлы и цинк обладают заметной афинностью в отношении матрицы агарозы, и они неотделяемы ниже определенного содержания в матрице. Элюация комплексообразователем (раствором трилона Б) не уменьшает содержание тяжелых металлов в матрице.

Метод озоления с последующим НАА оказался целесообразной комбинацией для многоэлементного анализа сырья и продуктов агаровой промышленности, обеспечивающей достаточную чувствительность и предотвращающей опасность загрязнения проб.