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## **BIOTECHNOLOGICAL UTILIZATION OF SOME MARINE RESOURCES AIMED AT MEDICAL AND INDUSTRIAL USES**

M. CRÂSMARU<sup>1</sup>, I. MORARU<sup>2</sup>, Mihaela BRATU<sup>3</sup>,  
Elena DUMITRESCU<sup>1</sup>, Daniela MIRCEA<sup>1</sup>, Daniela TIGANUS<sup>1</sup>

<sup>1</sup>National Institute for Marine Research and Development  
"Grigore Antipa" Constantza

<sup>2</sup>Medica Laboratories Ltd. Bucharest

<sup>3</sup>Dental Medicine and Pharmaceutics Faculty,  
"Ovidius" University, Constantza

### **ABSTRACT**

Biotechnology is the theory and practice of technological methods connected with biological processes. This includes complex applicative research; experiments for the characterization of the raw material, elaboration of processing, technologies, and characterization of obtained bioproducts.

Standing on the top of modern research, biotechnology has its roots in ancestral activities as fishing, hunting and agriculture.

Within the framework of Biotechnology, marine biotechnology is a specialized domain connected with the marine environment and which processes the "marine offer " including sea water and marine organisms.

According to this work and based on the NIMRD traditions in this field, the paper presents the utilization of marine resources in the medical-pharmaceutical domain.

First of all the qualities of sea water as support and vehicle for different bio-active substances are evaluated. Thus, some external adjuvants, for the treatment of respiratory diseases are achieved. The extension of the assortment of adjuvants and nutritive supplements, based on the adequate processing of some marine organisms - algae, molluscs, shellfish and fish - are further on intended.

All the presented activities are in agreement with the general policy of durable exploitation of marine resources and with E.U. legislation concerning the promotion of these nutritive and functional supplement and natural adjuvants.

**KEY WORDS:** marine resources, marine biotechnology, adjuvants, nutritive supplements

## INTRODUCTION

Biotechnology is a modern concept of implementation of many branches of science in order (round complete) to complement each other, the social environment with natural phenomena, by methods and techniques based on the knowledge of structure and function of **biosystems** (NICU, OPRITA, 1979).

The objects and directions for general of biotechnology research are:

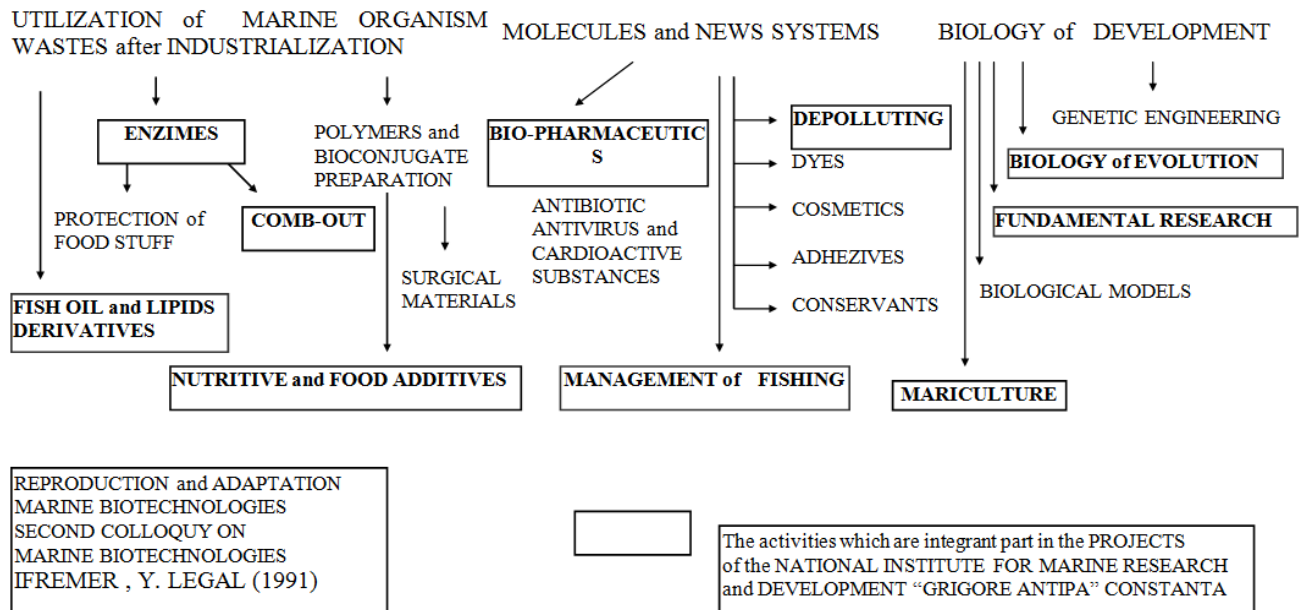
- \* the evaluation of availability and the free access to raw natural materials;
- \* the physical-chemical and sanitary characterisation of resources in order to establish profitable directions for their utilisation;
- \* the selection of the adequate technology, meaning the maintaining of bio-active natural properties and all requests regarding environment amelioration;
- \* the developing of the informational and organisational infrastructure in order to improve accurate connection **man - echosphere**.

**Marine biotechnology** concerns the environment and marine organisms revaluation, starting with their cultivation – **mariculture** - until their processing in order to obtain useful bioproducts in various branches (LE GAL, 1991) (Fig. 1).

Within the BIOTECH project “Biotechnological methods for utilisation of some marine resources used in medical and industrial fields “ NIMRD intends:

- \* to analyse the Black Sea offer - sea water and marine organisms;
- \* to render the above profitable by process them as **remedies, adjuvants, nutritive supplements**.

Fig.1. MARINE BIOTECHNOLOGY



The marine raw materials, in addition to their ordinary task for preventing, improving or control the specific effects for some functional troubles or affections, have following certain distinct peculiarities:

**Remedies** - represent resources and methods able to act under the most favourable circumstances on living organisms in order to heal or prevent an illness. The remedies could have psychical, (helio-therapy, breaker wave therapy and sun-therapy) or chemical (medicines and nutritive supplements) value.

**Medicine** - as per health medical science the medicine is any matter or combination of substances which alter a physiological or pathological process in order to bring the desired effect to those who use it.

**Nutritive supplements and complementars** - are the concentrated sources of nutritive or other substances with nutritive and physiological effects, alone or in combination (EU legislation).

As per nature and concentration of certain components, requested for health needs, these supplements became **functional food** or **fortifying food**.

EU legislation admits the existence of a huge diversity of nutritive food and sustains the efforts to enter the European market.

The requests regarding the sunogenic characteristics of above products impose that the substances nominated as **nutritive food**, **functional food** and **fortifying food** maintain and improve the health by protection and prophylaxy.

The **nutritive supplements** are obtained in the same way as **medicines**, by purification, concentration, additive technological process; they are marketed in special doses, with therapy instructions, mentioning that the **supplement food** has not to be used instead of medicines or food, but being supplements only, beside specific medicines.

The products named **marine algae**, **Epibiomer**, **Artromer**, **Reumamer**, may be integrated as **functional food**. Derived products of sea water with additive, **Nazomer**, **Nazomer forte**, **Apimer** may be integrated as **marine natural remedies**.

## 1. THE OFFER OF THE BLACK SEA AND ITS PROCESSING

### 1.1. Sea water as support and carrier for active biological substances

The **sea water** presence in natural sunogenic practices it is not a novelty, as it is well known its healing, anti-inflammatory and depurative effects in heliomarine cures.

It is also known that the sea water is good for health. In the same time, by additional bioactive natural ingredients (of vegetal origin) as

tinctures or hydroalcoholic extracts, sea water specific qualities can be much improved (CRÂSMARU, 2002).

However, there are some technical problems in order to obtain the homogeneous solutions on the sea water support and to identify and control the presence of additives.

The highly natural origin of the support and ingredients, the simple proceeding which allow preservation of the original qualities of components, allows a large use without any restrictions of obtained products, allowing them a favourable promotion.

These are some similar products obtained in other countries:

- \* RHINO-MER - ZYMA product - SWISSE - instillation;
- \* VICK -MER - VICKLAND SA CH - capsules, containing sea saline concentrate.

In our works the sea water processing is simple, easy to realise and control (Figs. 2, 3, 4, 5) and leads to various natural remedies with new items, as follows:

### **Nasal washable solutions - instillation**

- \* NASOMER - marine solution, aseptic filtrate, with rectified pH;
- \* NASOMER PROP - marine solution, aseptic filtrate, with rectified pH and propolis;
- \* NASOMER FORTE - marine solution, aseptic filtrate, with rectified pH and Echinaceae extract
- \* THINNING AGENTS and DEPURATIVE for nasal mucous membrane for allergic rhinitis , acute and chronic rhino-sinusitis.

#### **Gargles**

- APIMER - marine solution with propolis tincture;
- APIMER FORTE - marine solution with propolis and Echinacea tincture.

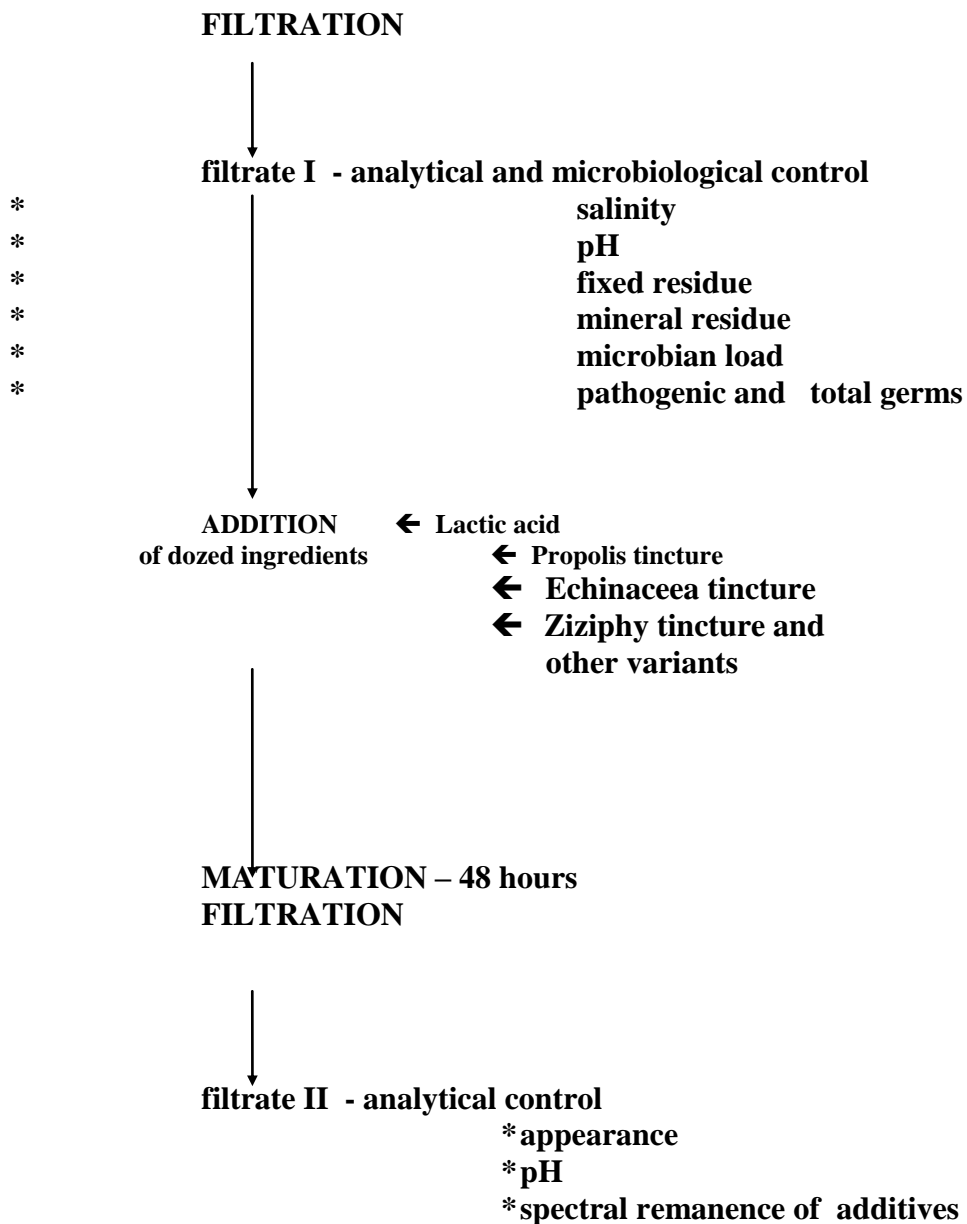
All products are recommended for mouth cavity affections, aphta (ulcer in the mouth), pharynges - amygdalitis, gingivitis - stomatitis, acute on chronic. Their specific characteristics are shown in Table 1.and the commercial presentation offer in Fig.5.

## **2. MARINE ORGANISMS**

### **2.1. Marine algae**

The regeneration of biotic components, including the seaweeds, ranges in the general tendency of improvement of the marine environment.

**Fig.2 - DRAFT of the PROCESS and  
ADDITION of MARINE WATER**





**Fig.3 COLLECTING POOLS FOR MARINE WATER**



**Fig.4 LABORATORY INSTALATION FOR FILTRATION AND  
ADDITION OF MARINE WATER**

**Fig.5. COMMERCIAL PRESENTATION OF SOME PRODUCTS;  
REMEDIES FROM ADDITIVATED MARINE WATER**



**NASAL WASHABLE – OROPHARYNGEAL HAEMOSTATIC  
HEALING, OROPHARYNGEAL ANTIINFLAMATORY, INCREASE  
OF THE LOCAL IMMUNITY ADJUVANT IN ALLERGICAL  
RHINITIS AND RHINOSINUSITIS (6)**



Table 1

Characterization of marine solutions for nasal washable and gargles

Physical-chemical characteristics	Instillations				Gargles	
	Nazomer	Nazomer prop.	Nazomer forte	Apimer	Apimer forte	Apimer plus
Aspect	Clear, colourless	Clear, yellowish	Clear, yellow-greenish	Clear, yellow-greenish	Clear, yellow-greenish	Clear, yellow-greenish
Salinity ‰	13.0 – 17.0	13.0 – 17.0	13.0 – 17.0	13.0 – 17.0	13.0 – 17.0	13.0 – 17.0
pH	6.5 – 7.0	6.5 – 7.0	6.5 – 7.0	7.0 – 8.5	7.0 – 8.5	7.0 – 8.5
Suspensions mg/l, bigger than 0.45 µm	Absence	Absence	Absence	Absence	Absence	Absence
Evaporation residue mg/ml	20.0 – 22.0	20.0 – 22.0	20.0 – 22.0	20.0 – 22.0	20.0 – 22.0	20.0 – 22.0
Mineral residue mg/ml	8.0 – 10.0	8.0 – 10.0	8.0 – 10.0	8.0 – 10.0	8.0 – 10.0	8.0 – 10.0
ADDITIFS						
Lactic acid mg/ml	1.0 – 2.0	1.0 – 2.0	1.0 – 2.0	1.0 – 2.0	1.0 – 2.0	1.0 – 2.0
Propolis	-	0.60 – 0.70	-	0.60 – 0.70	0.60 – 0.70	0.60 – 0.70
Echinaceae tincture ‰ vol.	-	-	5.0 – 10.0	-	5.0 – 10.0	-
Ziziphi tincture mg/ml	-	-	-	-	-	0.80 – 1.0
Ethanol % vol	-	0.2 – 0.3	0.2 – 0.3	0.2 – 0.3	0.2 – 0.3	0.2 – 0.3
MICROBIAL CHARGE						
Total germs /dm <sup>3</sup>	80 – 1000					
<i>Escherichia coli</i>	Absence					
<i>Staphylococcus aureus</i>	Absence					
<i>Pseudomonas aeruginosa</i>	Absence					
Fungi	absence					

There are a lot of organic deposits, containing marine macroalgae on beaches, after storms. This natural offer can be gathered in healthy conditions and used for therapeutics purposes, knowing already the qualities of these biota. (BARASCOV, 1963; SIMIONESCU *et al.*, 1974).

2003 estimates prove that natural deposits of marine algae on the Romanian seaside represent about many tones. Beside this availability it is necessary, of course, that the gathering be made under healthy conditions. It has to be mentioned that submerged marine algae are not taken in to account in order to conserve the marine habitats.

The marine algae mixture is composed by:

- \* **Enteromorpha spp** 80 - 90%;
- \* **Cladophora spp** 5 - 7%;
- \* **Ulva spp** 2 - 3%;
- \* **Ceramium spp** 2 - 2%.

This mixture brings an important contribution regarding the chemical-biochemical structure, improving the bioactive attributes as remedies and adjuvants.

All efforts in order to explore and utilise this huge quantity or raw materials are full rewarded, due to their therapeutic effects, already mentioned by the scientific literature, for medical treatment of lung, heart, thyroid gland, circulatory diseases, physical anaemia and physic asthenia.

The gathering, preserving and processing of that raw material must ensure that native qualities of the marine product will certainly remain unchanged.

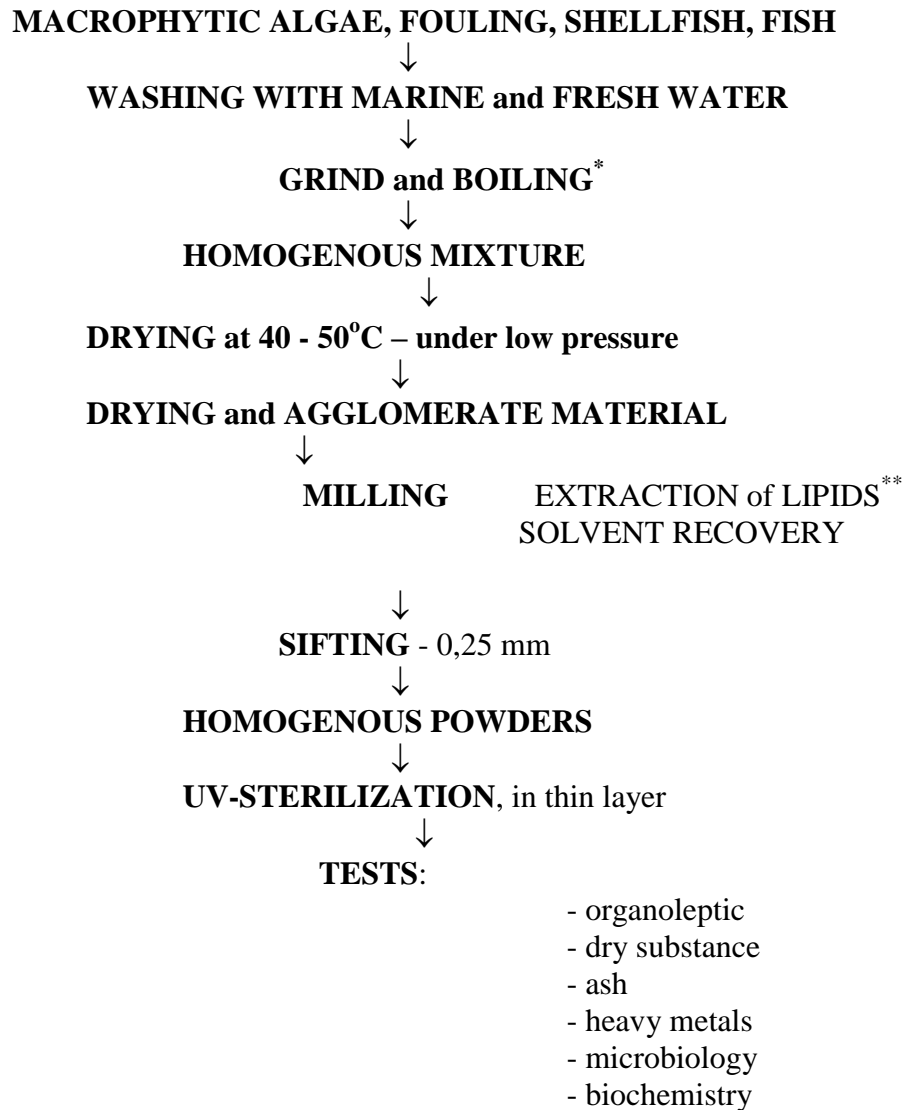
## 2.2 . Marine fouling

**Marine fouling** represents a life community formed by organisms which are fixed on submerged alive or not alive structures. In connection, **marine fouling**, represents a community of micro and macroscopic organisms, fixed on natural (cliffs), or artificial (dam, artificial reefs) supports.

In order to be used as biotechnological resource **marine fouling** is gathered from artificial submerged reefs, in sea water, having the following components:

- \* molluscs - *Mytilus galloprovincialis* and *Mytilaster* spp. 70 - 80%;
- \* shellfish – *Balanus* spp . 10 - 15%;
- \* algae - *Enteromorpha* spp. 10 - 15%;
- *Ulva* spp.
- *Ceramium* spp.
- *Polsiphonia* spp.
- other organisms 1 - 2%.

**Fig.6 GENERAL DRAFT OF THE PROCESSING OF RAW OF MATERIAL MARINE ORIGINE**



#### **PACKING of INTERMEDIARY PRODUCES**

LENGTH OF THE PROCESS FOR A SHARGE about 10 KG 72-96 hours

\* The boiling is used to the mussels crayfishes and shark meat

\*\* The extraction of lipids from marine fish and mussels

**FIG.7 PROCESSING OF MARINE ALGAE**



**COLLECTED ALGAE**



**DRY ALGAE**



**POWDER FROM  
MARINE ALGAE**



**NUTRITIVE – COMPLEMENT  
FROM MARINE ALGAE**

**ADJUVANT AND SUPPLEMENT IN THE RECOVERY OF THE  
MINERAL BALANCE AND ANTIOXIDANT RECOMMENDED IN  
MINERAL DEFICIENCY AND STATE OF TIREDNESS (6)**

**FIG.8 PROCESSING OF MARINE FOULING**



**COLLECTED MARINE FOULING**



**POWDER FROM MARINE FOULING**



**NUTRITIVE COMPLEMENT FROM MARINE FOULING**

**NUTRITIVE ADJUVANT OF THE CONVALESCENT AFTER THE  
EXTENSIVE PHYSICAL AND MENTAL EFFORT; PREVENTION OF  
AGEING PHENOMENA (4)**

**FIG. 9 PROCESSING OF MARINE MUSSELS**



**COLLECTED MARINE MUSSELS**



**POWDER FROM MARINE  
MUSSELS**



**NUTRITIVE COMPLEMENT  
FROM MARINE MUSSELS**

**DUE TO NUTRITIVE SUBSTANCES, THE PRODUCT IS: ADJUVANT,  
STIMULATOR OF CARTILAGES AND CONNECTIVE TISSUE  
METABOLISM, ANTIINFLAMMATORY RECOMMENDED IN ARTHROSIS,  
COXARTHROSIS DISEASES (9)**

**FIG.10 PROCESSING OF MARINE FISH**



**COLLECTED FISH FROM CAPTURE**



**DELIPIDATED FISH POWDER**



**LABORATORY EQUIPMENTS  
FOR EXTRACTION RECOVERY  
OF LIPIDS FROM FISH**



**NUTRITIVE COMPLEMENT  
SUPPLEMENT FROM  
MARINE FISH**

**CONTENT IN GLYCOSAMINOGLYCANS, GLYCOPROTEINS,  
OLIGOELEMENTS RECOMMEND THE PRODUCE AS  
ADJUVANT IN ARTHROSIS, SPONDYLOSIS, RHEUMATIC  
POLYARTREIS (6)**

**Table 2**

**Physical, chemical and biochemical characterization of capsulated powders**

<b>Product and commercial denomination / Analysis</b>	<b>Powder of marine macrophytic algae</b>	<b>Powder from marine fouling</b>	<b>Powder from mussels</b>	<b>Powder from fish ARTROMER</b>
<b>I. ORGANOLEPTIC</b> 1. Appearance 2. Colours 3. Odour 4. Taste	Powder, granulation 0.15-0.20 mm Brown-gray Typical marine Typical for marine grass	Fine powder, granulation 0.15 – 0.20 mm Brown-gray Typical of marine org. Light saline	Powder, granulation 0.8 – 1.0 mm Brown-gray Characteristic marine Characteristic marine	Powder, granulation 0.8 – 1.0 mm Brown Characteristic marine Characteristic marine
<b>II. PHYSICO-CHEMICAL CHARACTERISTICS</b> 1. Solubility % in distilled water 20°C 2. Dry substances % 3. Ash %	6.5 - 7.5 95.8 ± 0.5 14.5 ± 0.5	10.0 – 12.5 97.0 ± 0.5 85.0 ± 0.5	40.8 – 41.9 94.5 ± 0.5 19.5 ± 0.5	15.0 – 16.0 89.5 ± 0.5 16.1 ± 0.5
<b>III. ORGANIC SUBSTANCE %</b> Organic components % related to organic substance 1. Proteins 2. Glucides 3. Lipids	81.3 ± 0.5 8.5 ± 0.5 30.0 ± 0.5 12.0 ± 0.5	12.0 ± 0.5 8.5 ± 0.5 1.7 ± 0.5 1.2 ± 0.05	75.5 ± 0.5 66.9 ± 0.5 2.4 ± 0.5 0.1 ± 0.05	73.0 ± 0.5 70.0 ± 0.5 2.5 ± 0.5 0.4 ± 0.01
<b>IV. EVIDENCE OF SOME BIOACTIVE ATTRIBUTES</b> Pigments µg/g Vitamins µg/g Folic acid Biotin	0.75 – 0.80 1.1 – 1.4 0.09 – 0.1 1.7 – 2.2	4.2 - - -	0.5 – 1.0 - - -	- 1.5 – 1.7 1.5 – 1.7 0.6 – 0.7
Pantotenic acid Tiamine Riboflavine <b>ENZYMATIC ACTIVITY IN WATER EXTRACT</b> AMILOSIS u.i. ml/min. PROTEOLYSIS u.i./Anson LIPASE LYSIS u.i./mg/prot ALKALINE PHOSPHATASE u.i./mg/prot. SUPEROXIDISMUTASE u.i./mg/prot. CATALASE µmol H <sub>2</sub> O <sub>2</sub> /mg/	0.7 – 0.9 2.9 – 4.6 120 – 130 40 – 50 - - 0.05 – 0.15 26.5 – 27.5 -	- - 130 – 150 - 110 – 140 75 – 80 0.20 – 0.40 7.20 – 7.30 -	- - - - 3.9 – 5.7 3.10 2.20 – 2.24 3.11 0.65 – 0.7	0.8 – 1.2 2.0 – 3.0 - - -



prot.			40 – 55	40 – 50
ENZYMATIC INHIBITION %	-	-		
Raport enzyme/proteine 2:1,			40 – 55	40 – 54
Inhibition hyaluronidase activity	-			
Inhibition of protease activity	-		30 – 40	35 – 55
V. MICROBIAL CHARGE				
1. Total heterotrophic germs no./g	100,000	20,000	100,000	100,000
2. Total coliformes	10	absent	10	10
3. <i>Escherichia coli</i>	absent	absent	absent	absent
4. <i>Salmonella</i>	absent	absent	absent	absent
5. <i>Staphylococcus positive</i> <i>quaggy</i>	absent	absent	absent	absent
6. <i>Vibrio parahaemoliticus</i>	absent	absent	absent	absent
7. Sulphate-reduction bacteria	absent	absent	absent	10

\* Note: The commercial denomination of the products belongs to partner company  
firm S.C. LABORATOARELE MEDICA S.R.L. Bucharest

The estimated quantity that would be possible to be gathered is 10 kg/sqm. Beside the biological availability, the raw material is able in time to regenerate by itself. The marine fouling contains a diversity of chemical and biochemical components, giving a good recommendation in order to be used as nutritive adjuvants and natural remedies.

The scientific literature does not offer information regarding marine fouling utilities. However, there are many references regarding utilisation of its components such as algae, molluscs. This is very important in using this raw material for complex biotechnological processing.

The analytical data show a diversity of components based on natural association of vegetal and animal components, giving good quality for successful using of this resources. There are many mineral elements, hydrosoluble components and soluble elements in organic solvents. The pigments which are obtained from vegetal materials could be important for their antioxydising properties.

An adequate processing of this material fouling can conserve the requested qualities necessary for its use as remineralized and biostimulative product.

### **2.3 . Mussels (*Mytilus galloprovincialis*)**

**Mussels** represent one of the Black Sea mass species, inhabiting also the Romanian coastal waters. There are natural and cultivated population.

The mussels living along the Romanian seaside are not used at industrial level, till now. Nevertheless, the presence of that natural resource into the marine environment, and also some market products, used as food from some sporadic mariculture activities, prove and sustain the benefit of taking them into account as biotechnologies for new medical products (EROKHIN & CRÂSMARU, 1992).

The mussels are cosmopolitan, stationary, crowding in relatively stable populations, being accessible for collecting them. They are able to concentrate a large range of substances from the marine environment, more than  $10^2$  -  $10^3$  times their concentration in sea water.

The mussels have a seasonal cycle of somatic growing and the shells, gametogenesis and reproduction are important for choosing the optimum gathering time. Generally, the mussel production is used as marine food, according to their size.

Biotechnological processing refers to those mussels unsuitable as size, to biomasses growing on artificial reefs or in waste resulting from edible mussel cultures. The flesh represents 30 - 31% of total weight, shells - 40-41% and intestinal juice 24 - 26,3%. That means a reasonable and profitable interest for integral using of marine mussels (MITITELU & CRÂSMARU, 2001).

## 2.4 . Marine fish

Marine resources like goby (*Gobius niger jazo*) or hanus (*Mesogobius batrachocephalus*) are common species along Romanian seaside of the Black Sea, with recognised nutritive value, processed, generally, in fresh condition. Their meat qualities and active biological components are due to consumed crustaceans, molluscs and small fish which constitute their food. The raw material is undersize samples, left after the sort of fish caught for food.

Certainly, other species could also be used, as per season: whiting, groats, etc, with technological changes concerning the lipids extraction.

The processing of that marine resources in order to obtain the concentrates as complements and/or nutritive supplements is shown in Fig . 6.

The physical - chemical features show that processing has not altered the biochemical support of biochemical composition, meaning proteins, glucids, lipids content, managing in the same time a certain product sterilisation.

Fig. 7, 8, 9, 10 and Table 2 show the raw material and the obtained products.

## CONCLUSIONS

1. Technical analysis of the used proceedings point out that natural raw material was not altered concerning physical-chemical characteristics after the processing; the conservation of the most genuine qualities was assured.
2. Many raw material processing methods were investigated and selected in order to apply the optimum alternative, regarding costs, efficiency, etc.
3. The solutions and dry powder obtained as intermediate products are stable.
4. The sanitary parameters certify that the intermediary products have the appropriate content in heavy metals, pesticides and allowed microbial quantities.
5. The use and utility of these bioproducts, as **remedies** and **nutritive supplements** are due to maintain their native bioactive qualities.

6. The collected information allow to diversify the specific field knowledge: novelty in problem approach, originality of used proceedings and good qualities of the products already requested the market.

7. The contribution to general data regarding biotechnology and bioproducts consists of:

- originality of the methods for proceeding the raw material and the good quality of obtained bioproducts, useful information for scientific works and patents;
- the analytical results of the obtained bioproducts in some already certified products – **Alflutop, Mytilan, Chitosan**, point out that they have preserved their native qualities and capacity to be used as **adjuvants**, completing the already existing **remedies**;
- the sanitary certificate received for obtained bioproducts prove that the marine environment raw processed materials is ecologically stable.

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