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project consulting for life science

Project Abstract for Marine-Biotechnology

2007

CHITIN
CHITOSAN
GLUCOSAMINE

CHITIN

White, horny substance found in the outer skeleton of insects, crabs, and lobsters and in the internal structures of other invertebrates. It is a polysaccharide consisting of units of the N-Acetyl Glucosamine. Chitin is one of the three most abundant polysaccharides in nature, in addition to cellulose and starch. It ranks second to cellulose as the most plentiful organic compound on earth. Chitin and its derivatives have many properties that make them attractive for a wide variety of applications, from food, nutrition and cosmetics to biomedicine, agriculture and the environment. Their antibacterial, anti-fungal and anti-viral properties make them particularly useful for biomedical applications, such as wound dressings, surgical sutures and as aids in cataract surgery and periodontal disease treatment. Research has shown that chitin and chitosan are non-toxic and non-allergenic, so the body is not likely to reject these compounds as foreign invaders. Chitin's biodegradable and anti-fungal properties are a plus for environmental and agricultural uses. Over the past decade, researchers in Japan, Europe and the United States have tested chitin and its derivatives in biomedical applications. Researchers also have focused on the food and nutrition arenas, including edible films and coatings to preserve the quality and texture of foods. Today, more than a million people worldwide take chitin and chitosan in dietary supplements

Nutritional Uses chitin:

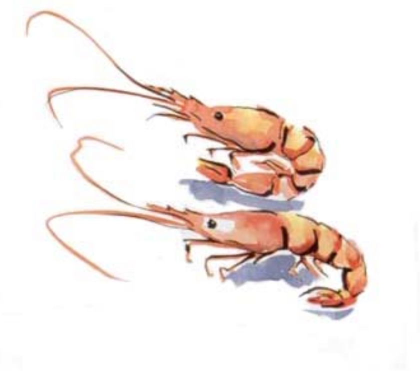
* Dietary Supplement
* Fiber Source

Food:

* Nutraceutical
* Food Film
* Flavor Preservation
* Flavor Enhancer
* Texture-Enhancing Agent

Biomedicine:

* Wound Healing
* Burn Healing
* Contact Lenses
* Sutures



Skin and Hair Care:

* Moisturizing Creams and Lotions
* Hair care products

Environment and Agriculture:

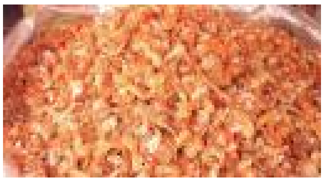
* Water Treatment
* Seed Treatment

Other:

* Undergarments
* Paper
* Textiles
* Beverage Clarifier
* Feed Additive

CHITOSAN

Chitosan is an all natural product which is derived from the Polysaccharide chitin. It is a Polysaccharide consisting units of the amino sugar D-Glucosamine. Chitin is found in the exo-skeletons of shrimp, crabs and other shellfish. The Chitosan has the unique ability to attach itself to lipids or fats. There are no calories in Chitosan since it is not digestible. Chitosan attaches to fat in the stomach before it is metabolized. The Chitosan traps the fat and prevents its absorption in the digestive tract. The fat binds to the Chitosan fiber and becomes a large mass which the body cannot absorb. This large mass is then eliminated from the body. Neutralizing fat after it enters the stomach spares the body from having to deal with the fat. Chitosan fiber differs from other fibers in that it possesses a positive ionic charge. This positive charge gives Chitosan the ability to chemically bond with negatively charged lipids, fats and bile acids.



Additional Benefits of Chitosan

This dietary fiber is a valuable addition to a properly balanced weight management program. Fibers also provide important cleansing attributes which aid in the digestive process and promote digestive tract health. Chitosan can also help to lower your bad cholesterol.

Applications of chitosan

Wastewater Treatment	- Removal of Metal Ions - Flocculant / Coagulant : Protein Dye Amino Acids
Food Industry	- Removal of Dye, Suspended solids etc. - Preservative - Colour Stabilization - Animal Feed Additive
Medical	- Bandages - Blood Cholestrol Control - Controlled Release of Drugs - Skin Burn - Contact Lens - etc.
Biotechnology	- Enzyme Immobilization - Protein Separation - Cell Recovery - Chromatography - Cell Immobilization
Agriculture	- Seed Coating - Fertilizer - Controlled Agrochemical Release
Cosmetics	- Moisturizer - Face, Hand and Body Creams - Bath Lotion - etc.
Pulp and Paper	- Surface Treatment - Photographic Paper
Membrane	- Permeability Control - Reverse Osmosis

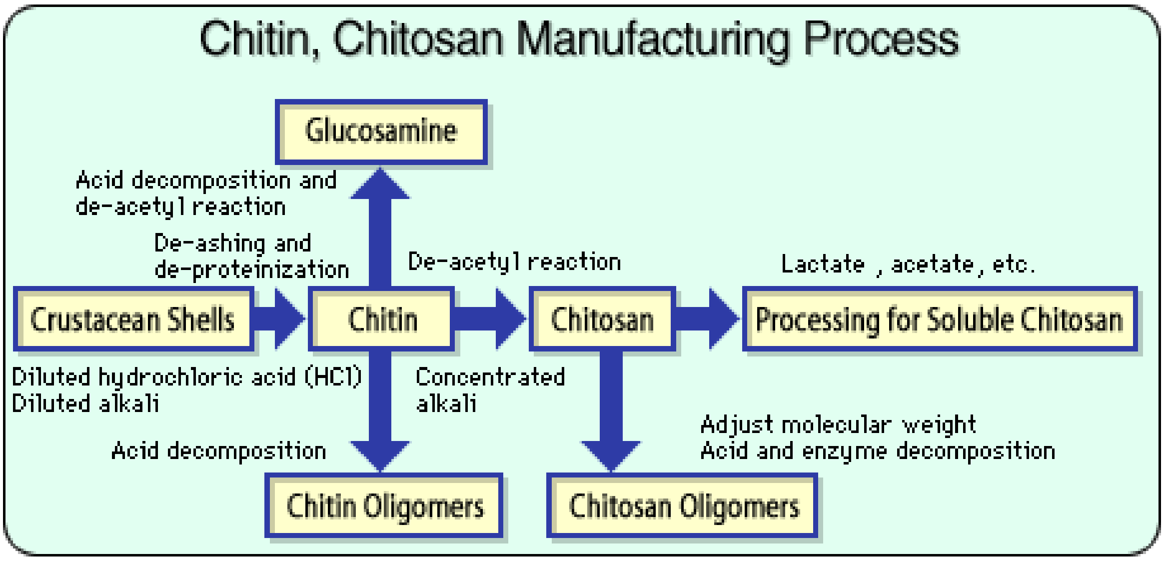
GLUCOSAMINE

D-GLUCOSAMINE HYDROCHLORIDE - CARTILAGE REPAIR

This product is natural amine sugar extracted from the Chitin of prawn-fish from fresh water and shrimp from seawater, as food additive and raw material for pharmacy. It provides the building blocks for the body to make and repair cartilage.

Production

- Chemical/Biochemical Process
- Start material head and shell of shrimp



CHITIN,CHITOSAN,GLUCOSAMINE

1. Product description
2. Biochemical introduction
3. Marketing and Feasibility study
4. Industrial use
5. Process Technology
6. Production Line
7. Technology Transfer
8. Environmental aspects

The Commercial Project and Marketing Report for Purchasing is available

Raw Material (Shrimp head and waste)

Calcium

Protein

Chitin

Pigment

GA

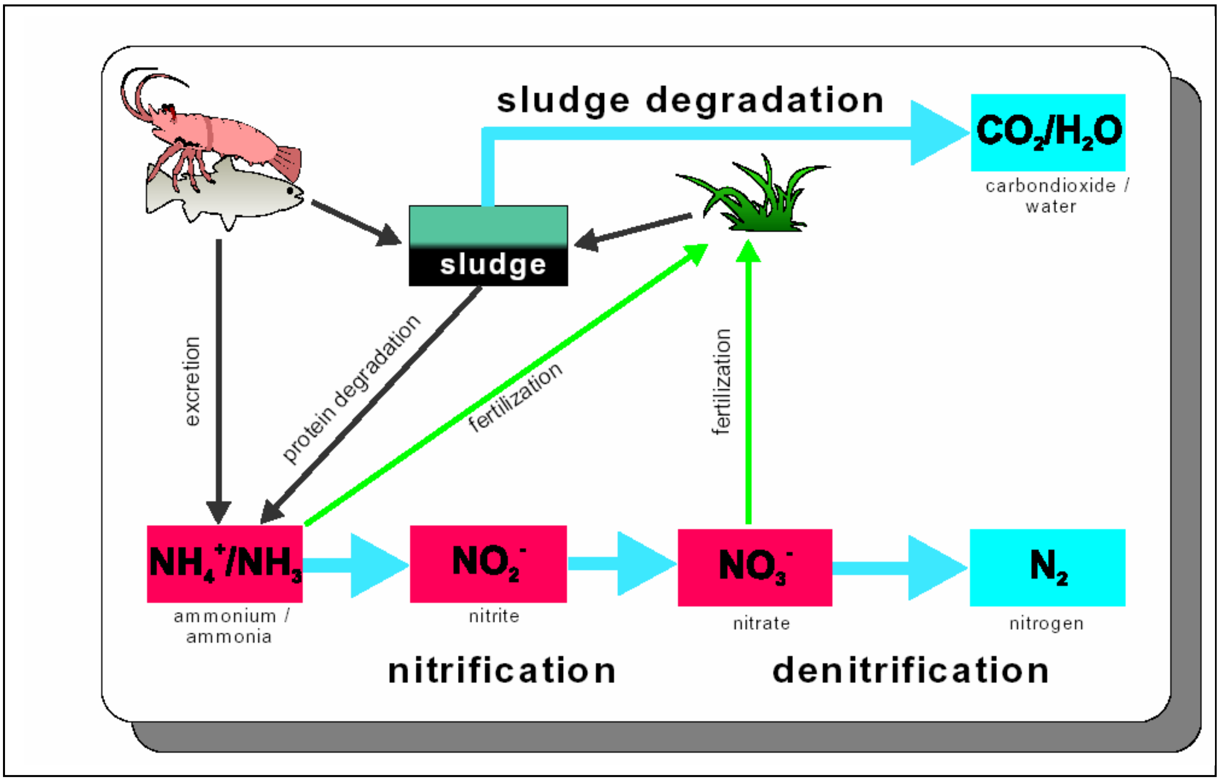
Chitosan

Activated Chitosan

Ensymm managed the potential of the shrimp Head waste in 5 diff. Products and by-products

The way ASA T works in aquaculture

In the open nature there are fish, plants, small living organisms and microorganisms which live
together in an aquatic system. Dead plants and animals are completely mineralized by the microorganisms. The end products of the biodegradation process, e. g. nitrates are taken up again by the plants as nutrients. There is an ecological balance. The water remain clean due to its biological self-purifying ability.



Dramatical deterioration of water quality caused by shrimp farming

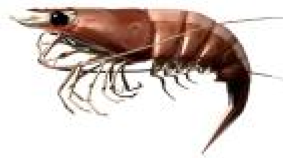
In prawn farming an excessive amount of inorganic substances (fertilizer) and organic waste (plant remains, animal excretion, left over feed-stuffs) find their way into the system then the self-purifying ability is not able to cope and the ecological balance is upset.

This leads to an increase in concentration of ammonia and nitrite, which are toxic to shrimps..

The increased formation of ammonium and nitrate stimulate the growth of filamentary and freefloating
algae, which increase the water turbidity. The dying algae cause an additional load of the water by organic waste.

Use of antibiotics in the shrimp aquaculture industry: far reaching ecological and economical risks and consequences

The organic material (starch, protein, fat, cellulose) are not completely degraded by the microbes and form an organic layer at the bottom of the pond. This sludge sediment is responsible for a further reduction of water quality and decrease of oxygen concentration. Furthermore it represents an ideal nutrient medium for pathogenic bacteria, f.e. *Vibrio spp.* . Especially *Vibrio harveyi* has been implicated as the main bacterial pathogen of shrimps In the Philippines the „luminous Vibrio disease“ caused the loss of the main part of the shrimp production in 1996. The responsible *Vibrio spec.* showed resistence to all antibiotics used including chloramphenicol, furazolidon, oxytetracyclin and streptomycin, and obviously, it was more virulent than in the previous years.



In order to protect shrimps against bacterial diseases precautionary antibiotics as chloramphenicoll are often added to the ponds. However, in many cases they are not effective and lead to the development of resistant bacterial strains as well as to the transfer of resistance genes to other species as human pathogens. (partially with dramatical consequences for the control of human infection diseases)
Additionally antibiotics damage the gut flora of the shrimps and, consequently, their digestion .
This weakens the animals and they become sensitive to viral infections, f.e. „White spot desease“ or „Yellow head disease“. Because virus are not influenced by antibiotics this led in some regions to viral epidemics and to high shrimp mortalities.
Since antibiotics can be detected in shrimps after the harvest, in the last years an increasing number of importing firms from USA, Europe and Japan decline lots with high contents of antibiotics, which leads to clear losses of income for the shrimp farmers.

Furthermore antibiotics and other bactericides are not effective in every case: experiences, made in Thailand in 1999, showed, that high dosages of norfloxacin in combination with colloidal silver in shrimp feeds were able to control bacterial infections. However, when the addition of bactericides was stopped all shrimps died within a few days. Obviously a high virulent strain of *Vibrio* had developed in response to the bactericides.
Also the use of chlorine seems to have negative influences, as it was observed, that the development of resistance genes was stimulated. Farmers from Thailand reported, that in ponds treated with chlorine a rapid increase of *Vibrio harveyi* occurred after removal of the chlorine

How does ASA T work ?

The microorganisms in **ASA T** improve the water quality and solve all these problems in a **natural way**:

"sludge degrading" microorganisms form the hydrolytic enzymes necessary for the degradation of the organic waste the microorganisms in **ASA T** repress pathogenic bacteria species by competing with them about the same nutrients nitrifying microorganisms convert ammonium, toxic ammonia and nitrite to nitrate denitrifying microorganisms change nitrate to gaseous nitrogen thus removing it from the body of water different species of green algae often associated with *Vibrio species* will be repressed

The positive effects of the use of beneficial microorganisms (as they are contained in **ASA T**) for the displacement of pathogenic bacteria was already made in the animal industry

Economical calculations

All data were calculated based on the results of our test ponds in India and Vietnam from the cultivation of *Penaeus monodon* (Black Tiger Prawn).
The sales prices for the shrimps were between 3,50 und 7,00 € per kg shrimps and dependent of the quality and region of production. Following from that for these calculations a sales price of 5,-00 € per kg Shrimps was estimated. However, for shrimps free of antibiotcs higher prices should be achieved.

Pond Size	7.500	m^2
Number of Shrimps	30	animals / m^2
Weight of Shrimps	34	g/animal
Survival Rate	70	%
Total Yield of Shrimps	5.350	Kg
Income per Pond	26.750,-	€
Income per tons of Shrimps	5.000,-	€ / t shrimps
Costs of ASA aqua-clean N/S	145,-	€ / t shrimps
Calcul. Costs of Antibiotics	50,-	€ / t shrimps

Other marine Products:

1. **Alginate**
2. **Collagen**
3. **Sponge**
4. **Microalge**
5. **Agar**

Alginate

What is Alginate?

Alginate is found in a wide variety of brown seaweeds and is present as a structural polysaccharide. Alginate is made up of a linear block copolymer of α-L-guluronic acid and β-D-mannuronic acid. The blocks vary in size and alternating M and G segments as well as random blocks may also be present. The type of structure is influenced by the seaweed source as well as the growing conditions of the weed. The block structure ultimately dictates the gelling properties of the alginate produced. Durvillea and ascophyllum species tend to be high in mannuronic acid and hence form softer gels whereas alginate sources such a laminaria hyperborea stems tend to have a higher guluronic acid content an hence form much more rigid gels.

Fucus type brown seaweed

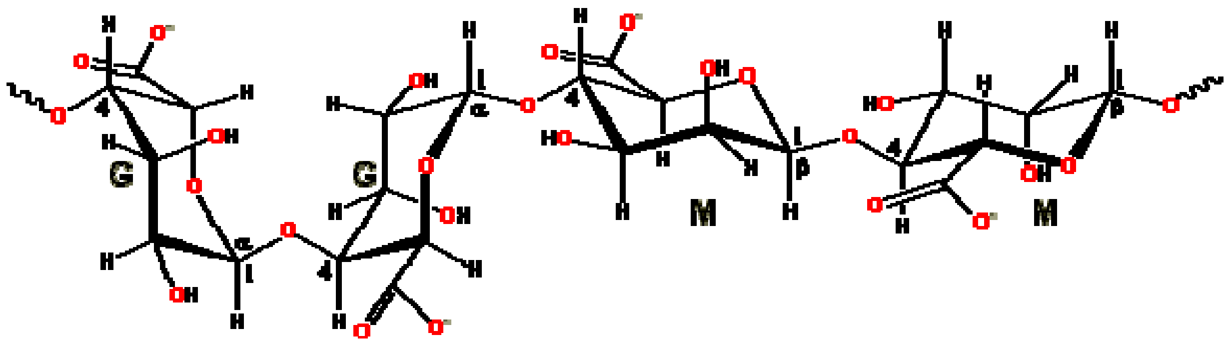
Alginate bearing weeds are typically found in temperate or cold water. Major commercial sources of alginates are the giant kelp from California (*Macrocystis pyrifera*) and *Ascophyllum Nodosum* from the north Atlantic. The major manufacturers are based near the weed sources in San Diego, Scotland and Norway. More recently manufacturers have been developing in Asia. Other weed sources from around the world are shipped to the major factories and include several types of Laminaria, Ecklonia, Lessonia and Sargassum.

Alginate can also be produced from a bacterial source (Azobacter Vinelandii). However the block structure in bacterial alginate tends to give a product with poor gelling characteristics and the expense of production means the product has never been commercialized.

Source

Alginates (E400-E404) are produced by brown seaweeds (*Phaeophyceae*, mainly *Laminaria*).

Structural unit



Production of Alginate

There are essentially two processes for the preparation of alginates. They all start off with similar extraction procedures but vary in the methods used to precipitate the alginate at the end of the process.

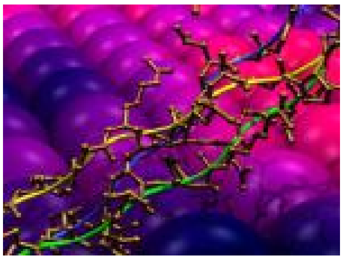
Alginate is classified as a hydrocolloid (a water-soluble biopolymer of colloidal nature when hydrated). The first scientific studies on the extraction of alginates from brown seaweed were made by the British chemist E.C. Stanford at the end of the 19th century, and the large-scale production of alginate was introduced 50 years later. Alginate is one of the most versatile biopolymers and is used in a wide range of food, pharmaceutical and specialty applications for:

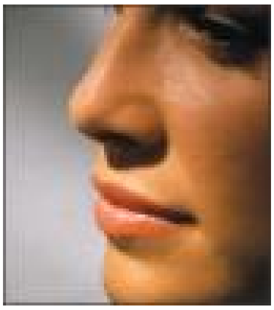
- **Thickening**
- **Stabilizing**
- **Gelling**
- **Film forming**

Collagen

What is Collagen?
The most important building block in the entire animal world, collagen is the tie that binds the animal kingdom together. Life is a string of complex molecules: polymers.
Nature's most abundant protein polymer is collagen. More than a third of the body's protein is collagen. Collagen makes up 75% of our skin. The more science learns about the body, the more integral we see collagen to be.

Collagen
Acts as a scaffolding for our bodies.
Controls cell shape and differentiation.
Is why broken bones regenerate and wounds heal.
Why blood vessels grow to feed healing areas.
Using in pharmaceuticals
Cosmetics
Creme and health care products
Beauty farms and beauty injection





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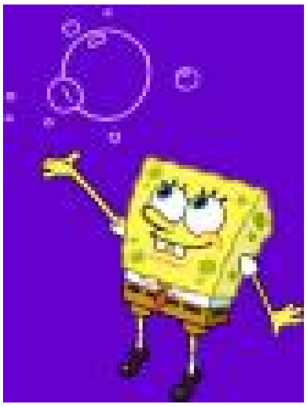
Sponge

Sea Wool Sponges
The world's finest natural sea sponges for bath, home, and general cleaning. These are the best, most durable for home and bath use. They are soft as silk when wet and available in all sizes.Typical sea wools can last 10 years or more.

Yellow Sea Sponges
The world's finest natural yellow sea sponges for bath, home, and general cleaning. These are durable for home and bath use. They are also very popular for all your cleaning needs, as well as sponge and faux painting. They are available in all sizes. These last about 4 years with normal care.

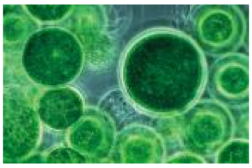
Silk Sponges
Silk Sponges are great for small cleaning jobs, cosmetics, and other purposes were a very fine grained soft sponge is required. These are Fine pored firm sponges also used in arts and crafts, pottery, and ceramics.

Vase Grass Sponges
Vase Grass Sponges are beautiful natural sponges that can be used for a variety of uses including in arrangements, planters, and other decorative uses.



Microalgae

Microalgae are an essential food source for marine bivalve mollusks (clams, oysters, scallops) throughout their life cycle, larval stages of some marine Gastropoda (abalone, snails), larvae



of some marine fish species, Peneidae shrimp and zooplankton. Choosing the microalgal specie to be used as food varies according to the nutritional value that the specie has for a specific organism and depending on its cellular size, digestibility, production of toxic compounds and biochemical composition. Another important use for massive phytoplankton culture is obtaining different chemical products of potential marketing interest generated by the metabolic activity of different types of Microalgae, such as vitamins, carotenoids, amino acids, antibiotics, bioflocculants, etc.

The Microalgal Laboratory has the objective of continuously and efficiently supplying microalgal species that research projects, graduate theses, agreements and/or external users demand, and provide research spaces adequate to the needs of experiments involving Microalgae.

Bi-products from microalgae

Microalgae products are nutritional microalgae(Chlorella, Spirulina and red algae) powder also extracts used as ingredients in health food formula and foods, or as ingredients in, and encapsulated for use as, dietary supplements. Additional applications of microalgae include currently marketed SPA and cosmetics products and the technologies to assist in special fry feeds for shrimp or fish culture and special fluorescent diagnostics in medical field.

- Microalgae are considered a source of a variety of bio-products which can be used as food additives, beverage, medicine, and biofuel. The overall objective of this research is the development of a commercially viable new rural industry for obtaining renewable hydrocarbons (petroleum products) from the alga, *Botryococcus braunii* (Bb), particularly suitable for the Murray Darling Basin, to augment the supplies of biodiesel for Australia and reduce the reliance on overseas oil imports. This project aims to better understand the operating parameters for the mass production of Bb under controlled conditions, particularly under increased salinity. Specifically, we will determine the salt tolerance of various strains of Bb and examine the link between salt tolerance, nutrient supply, light level and hydrocarbon production.

- *Gross composition.* Microalgae that support good growth in farmed animals may contain 20-25% protein, 5-30% carbohydrate and 5—25% lipid. The quality of these fractions is more important than the gross content in determining nutritional value (Brown and Jeffrey, 1992).

- *Amino acids.* All microalgae tested (more than 60 strains of all algal types) were rich in essential amino acids — arginine, histidine, iso-leucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan and valine. No algal species was deficient in any essential amino acid. Protein quality was thus highly conserved across all microalgal classes (Brown, 1991).

- *Carbohydrates.* In contrast, the sugar composition of complex carbohydrates varied across the species with glucose, mannose, galactose, arabinose, fucose, rhamnose, ribose and xylose being the most important. Carbohydrate differences could account for differences in nutritional value of the microalgae. Most algal polysaccharides are digested by animals, but more needs to be done to check microalgal polysaccharide availability to animals at all life stages.

- *Lipids.* Lipid quality is a key factor contributing to nutritional value of microalgae, and this is determined by the content of triacylglycerols, polyunsaturated fatty acids (PUFA), polar lipids, sterols, hydrocarbons, alkenones and pigments (carotenoids, chlorophylls, biliproteins). Of outstanding significance is the content of three essential polyunsaturated fatty acids-arachidonic acid, 20 : 4 n-6; eicosapentaenoic acid, 20 : 5 n-3(EPA) and docosa-hexaenoic acid, 22 : 6 n-3 (DHA).

This is a farm in Hawaii and they use the same condition like Red Sea for microalgae breeding!!

Microalgae contain a wide range of fatty acids in their lipids. Of particular importance is the presence of significant quantities of the essential polyunsaturated fatty acids, ω6-linoleic acid (C18:2) and ω3-linolenic acid (C18:3), and the highly polyunsaturated ω3 fatty acids, octadecatetraenoic acid (C18:4), eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6).

Agar

Agar is a galactose polymer (or agarose) obtained from the cell walls of some species of red algae or seaweed (Sphaerococcus euchema) and species of Gelidium and Gracilaria, chiefly from eastern Asia, Chile and California. It is also known as Kanten, Agar-Agar, or Agal-Agal (Ceylon Agar).

Chemically, agar is a polymer made up of subunits of the sugar galactose; it is a component of the algae's cell walls. Dissolved in hot water and cooled, agar becomes gelatinous; its chief use is as a culture medium for microbiological work. Other uses are as a laxative, a vegetarian gelatin substitute — a thickener for soups, in jellies, ice cream and Japanese desserts such as anmitsu, as a clarifying agent in brewing, and for paper sizing fabrics.

Uses in cooking

Agar-agar is typically sold as packaged strips of washed and dried seaweed, or in powdered form. Raw agar is white and semi-translucent. For making jelly, it is boiled in water at a concentration of about 0.7-1% w/v (e.g. a 7 gram packet of powder into 1 litre of water would be 0.7%) until the solids dissolve, after which sweeteners, flavouring, colouring, and pieces of fruit may be added. The agar-agar may then be poured into molds or incorporated into other desserts, such as a jelly layer on a cake.

Uses in microbiology

Main article: Agar plate

Nutrient agar is used throughout the world as a medium for the growth of bacteria and fungi, but not viruses. Though less than 1% of all existing bacteria can be grown successfully, the basic agar formula can be used to grow most of the microbes, whose needs are known. More specific nutrient agars are available, because microbes can be picky. For example, blood agar, which is generally combined with horse blood, can be used to detect the presence of haemorrhagic micro-organisms such as E.coli O:157 H:7. The bacteria digest the blood, turning the plate clear.

Agarose is also used in Agarose gel electrophoresis.

Selective Media

Selective media is agar specially treated to apply a selective pressure to organisms growing on it -- for example, to select for salt-tolerant, gram-positive, or gram-negative bacteria.

Differential Media

Differential media includes an indicator that causes visible, easily detectable changes in the appearance of the agar gel or bacterial colonies in a specific group of bacteria. For example, EMB (Eosin Methylene Blue) agar causes E. Coli colonies to have a metallic green sheen, and MSA (Mannitol Salt Agar) turns yellow in the presence of mannitol fermenting bacteria.

Hysteresis

Hysteresis describes the phenomenon of the differing liquid-solid state transition temperatures that agar exhibits. Agar melts at 85 °C and solidifies from 32-40 °C.

We thank you for your attention

ensymm

looks forward to a fruitful co-operation

between your company and our network

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