2

Agar

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2.1 Introduction

Agar-Agar, also called simply agar, was the first phycocolloid used as a food additive in our civilisation having been employed in the Far East over 300 years ago. Phycocolloids are those gelling products extracted from marine algae that are utilised in several ways solely because of their colloidal properties. The most important ones are agar, alginates and carrageenans that are produced in industrial quantities and presented in the form of clear coloured powders. In the Orient ‘natural agars’ in the old forms of strips and squares are still being used at home to prepare traditional dishes. Lately such types of agar have reached our dietetic and natural food stores while in Japan they are being substituted by powdered industrial agars prepared as tablets.

Agar is defined as a strongly gelling hydrocolloid from marine algae. Its main structure is chemically characterised by repetitive units of D-galactose and 3–6-anhydro-L-galactose, with few variations, and a low content of sulfate esters. We can also add that agar is also a mixture of polysaccharides made of dextro and levo galactoses united linearly.

2.1.1 Historical background

In Japan, where agar has been used for several hundred years, Tarazaemon Minoya is generally considered its discoverer in 1658. In fact, this phycocolloid had certainly been utilised much earlier than any other phycocolloid such as alginates or carrageenans, which it predates by 200 years. From Japan its use extended to other oriental countries during the seventeenth and eighteenth centuries.

Agar was introduced in the West by Payen (1859) as a Chinese foodstuff and its microbiological applications were presented by Koch (1882). Hence, it can be said that it was known in the Western world towards the end of the nineteenth century. Smith (1905) and Davidson (1906) contributed to its wider application by presenting very clear explanations of the raw materials and the manual processes involved in its production in Japan.
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Being the first phycocolloid used by man, it is one of the food ingredients first approved as GRAS (Generally Recognized As Safe) by the FDA. This was in 1972, based on the positive as well as lengthy experience acquired by its usage in the Far East for more than three centuries. It also passed all other controls in its toxicological (FDA 1973a), teratological (FDA 1973b) and mutagenic (FDA 1973c) aspects.

2.1.2 Agarophyte seaweed used for production

In the middle of the seventeenth century, agar was produced in Japan from Gelidium amansii exclusively, then China and Korea followed soon after. This ‘red’ (Rhodophyceae phylum) seaweed type was the one most abundantly available along their respective coasts. As Gelidium amansii was in short supply, efforts were made to utilise other Rhodophyceae as substitutes.

When Gracilaria seaweeds started to be used, agars with very poor gelling power were obtained and these were called agaroids. Only in 1938, when Yanagawa discovered the alkaline hydrolysis of sulfates, was it possible to increase the gel strength and produce stronger agars from Gracilaria. Gracilaria contains in its cells an agar content of a very weak nature which Yanagawa learned to transform into a stronger final product by using this alkaline hydrolysis processing method.

In Gelidium, Gelidiella and Pterocladia seaweed, there occurs a natural internal transformation through an enzymatic process that can be considered a maturing of the polysaccharide in the weed. In Gracilaria it is not converted in the needed amount during the weed’s lifetime so it becomes necessary to produce it industrially by means of a chemical method before extracting the agar from the weed.

In Table 2.1 we can observe the different agarophytes used in the world for agar production. Due to some confusion in taxonomy because of frequent name changes, we have decided to use the classical names applied normally for agarophytes in worldwide commerce. Once DNA determinations are done on each weed, hopefully a permanent description basis will be established. There are several types of agars available with very different applications due to the distinct characteristics of each one. They have been developed to satisfy diverse applications and they originate from different agarophyte algae that are produced by diverse technologies. Mainly we should distinguish ‘natural agars’ from ‘industrial agars’. The first have been produced by artisans and lack technical controls but are still sanitarially clean and proper for its main use in home cooking. The latter types are manufactured in modern factories and are utilised as industrial food ingredients, so are subjected to all kinds of established controls. Agars utilised for microbiology and biotechnology are also included in this category even though they comprise only 10% of the total volume.

Agar is a polysaccharide that accumulates in the cell walls of agarophyte algae. It is embedded in a structure of fibres of crystallised cellulose, constituting its polysaccharide reserve. For this reason, agar content in weed varies depending on the seasons. Initially an intermediate form of agar with low molecular weight and quite sulfated is secreted by the Golgi apparatus of the cell. Once deposited in the cellular wall it enzymatically polymerises and desulfates, being converted mostly into agarose that gives the agar its gelling power. The rest remains in the form of agarpectin. Matsuhashi (1990) suggested that agar could be linked to cellulose fibres by calcium ions. This would explain many phenomena that occur during the extraction process.

In Fig. 2.1 we can observe a section of Gelidium sesquipedale, the agarophyte found in Western Europe and Morocco. The image shows the enormous thicknesses of the cellular
walls where agar is found. The smaller cells or rhizoids contain agaroses of higher molecular weight. It so happens that the number of rhizoidal cells is higher where the water movements are stronger. Therefore the hydrodynamic conditions of the environment where Gelidium grows play an important role in the agar content in the weed as well as its gel strength characteristic.

2.2 Agar manufacture

Agar is called Kanten in Japanese, which means ‘frozen sky’, referring to the way it was first manufactured by artisans based on freezing and thawing in the open fields of the extracted agar gel. It is derived from the technique recorded by Tarazaemon Minoya in 1658 that has its fundamentals in the insolubility of agar when cooled. The traditional technique adapted by Minoya, developed towards the middle of the seventeenth century, is still in use marginally to produce ‘natural agar’ in the oriental craft industry in the forms of strip agar (Ito-Kanten) or square agar (Kaku-Kanten). This technique started with careful washings of Gelidium amansii employing similar devices to those used to wash tea-leaves.

The washed seaweed was selected by hand to eliminate any foreign body or extraneous seaweed. It was extracted in boiling water adjusting its pH. In olden days the

Table 2.1 Taxonomic classification of agarophytes (Armisén, R. 1995)

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Rodophyta.</th>
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<tbody>
<tr>
<td>Class</td>
<td>Florideophyceae.</td>
</tr>
<tr>
<td>Order</td>
<td>Gelidiales.</td>
</tr>
<tr>
<td>Family</td>
<td>Gelidiaceae.</td>
</tr>
<tr>
<td>Genus</td>
<td>Gelidium.</td>
</tr>
<tr>
<td>Species</td>
<td>G. sesquipedale*, G. amansii*, G. robustum*, G. pristoides, G. canariense, G. rex, G. chilense, etc.</td>
</tr>
<tr>
<td>Genus</td>
<td>Gelidiella.</td>
</tr>
<tr>
<td>Species</td>
<td>G. acerosa.</td>
</tr>
<tr>
<td>Genus</td>
<td>Pterocladia.</td>
</tr>
<tr>
<td>Species</td>
<td>P. capillacea*, P. lucida*</td>
</tr>
<tr>
<td>Order</td>
<td>Gracilariales.</td>
</tr>
<tr>
<td>Family</td>
<td>Gracilariaceae.</td>
</tr>
<tr>
<td>Genus</td>
<td>Gracilaria.</td>
</tr>
<tr>
<td>Species</td>
<td>G. chilense*, G. gigas, G. edulis, G. gracilis, G. tenustipitata*</td>
</tr>
<tr>
<td>Genus</td>
<td>Gracilariosiopsis.</td>
</tr>
<tr>
<td>Species</td>
<td>G. lamaneiformis, G. sjostedtii</td>
</tr>
<tr>
<td>Order</td>
<td>Ahnfeltiales.</td>
</tr>
<tr>
<td>Family</td>
<td>Ahnfeltiaceae.</td>
</tr>
<tr>
<td>Genus</td>
<td>Ahnfeltia.</td>
</tr>
<tr>
<td>Species</td>
<td>A. plicata.</td>
</tr>
</tbody>
</table>

* The most used agarophytes in industry.
adjustment was made with vinegar or sake but now diluted sulfuric acid is employed. The liquid extract was filtered while hot through cotton bags, poured into wooden trays and allowed to gel by cooling. The gels were cut into square bars (4 × 6 × 24 cm) or extruded to produce spaghetti-like strips 25–40 cm long. The gels prepared in this way were placed on bamboo grills and left to freeze all night in the open, usually in bluffs facing the northern winds. Once totally frozen during one or two nights, the agar was thawed during the daytime by sprinkling water over it. It was then sun dried and kept away from frosts. After the Second World War and up to the 1960s there were approximately 400 artisan plants in operation producing between 4 to 10 kg per day, climatic conditions allowing. It can be seen that this basic technique produces low standards and irregular qualities because the process is very dependent on climate. But it is reviewed here, as it is a simple way to comprehend the more involved processes that are performed now using more sophisticated mechanical means.

What are known as industrial agars are produced in modern plants in which fully standardised agars are obtained, assuring qualities that comply with physicochemical and bacteriological specifications in accordance with sanitary codes. The best installed plants comply with ISO-9000 norms, that assure the market that all processes are controlled and are traceable from raw materials to shipped finished goods. In Fig. 2.2 the block diagram shows a general industrial process that can be utilised to produce agar. It is to be noted that two different paths can be followed to dehydrate the gellified agar extracts.

2.2.1 Freezing-thawing method
This traditional method was first used for ‘natural agar’ production, and was not substantially changed until American Agar & Co. (San Diego, USA) started to manufacture agar industrially in 1939, in freezing tanks in the same way that ice bars are
made. Immediately after the Second World War the same technique was applied in Japan as well as in the new plants that were built in Spain, Portugal and Morocco. The seaweed extract, which normally contains between 1% and 1.2% of agar during the process, is concentrated after thawing and straining (normally by centrifugation) to contain 10% to 12% of agar which is a tenfold increase. The eluted water carries away oligomers, organic and inorganic salts as well as proteins from the algae including phycoerytrins that produce the red colour of the Rhodophyceae family. This process is one of purification.

2.2.2 Syneresis method
This method is based on the property of gelling colloids by which the absorbed water can be eliminated by means of a properly applied force. The technique was utilised in Japan solely for Gracilaria agar on a semi-refined scale. Gelled extracts packed in cloths with a closed mesh were pressed under stone blocks to push the water out from the gel. Afterwards, it was pressed by means of small hydraulic presses to eliminate the residual water.
In 1964, Prona, a forerunner of Hispanagar, innovated a modern syneresis technique to concentrate gelled extracts for all agarophytes (not only *Gracilaria*) building new plants in France, Mexico, Chile and South Africa as well as modernising those in Spain, Portugal and Morocco. It gave the company worldwide leadership in agar production, only shared with Algas Marinas (Chile) in the late 1980s and with INA (Japan) in the last decade. Concurrently Okazaki wrote in 1971 that syneresis was possible only for *Gracilaria*. The method, which allowed a semi-automated process, was later further improved to a totally automated one which is in use nowadays. Vertical mechanical presses have been substituted by horizontal hydraulic ones. This syneresis technique has spread rapidly all over the world due to its reduced energy cost. The freezing method requires an ice production of 80/100 metric tons to produce one ton of agar while the consumption of energy by the syneresis method is very low. Both methods can be mechanised today with a slight advantage in costs for syneresis. On the other hand, some buyers still prefer the quality of freeze-thaw agar for which they are ready to pay a premium.

Agar purity is increased in syneresis as the dry extract weight after pressing is as high as 20% compared to 11% in freezing which means that the latter has double the water content where the impurities stay while syneresis eliminates more soluble impurities. This is reflected in the lower ash content found in syneresis agars but again we state that many customers do not care about this.

From those operations in Fig. 2.2 we can consider the most important ones in addition to dehydration to be:

1. Treatment: usually an alkaline treatment is employed that allows a better extraction of the polysaccharide from the cell walls. For *Gracilaria* a stronger reaction is required to produce an alkaline hydrolysis of the sulfates in order to increase the agar gel strength.
2. Extraction: the agar contained in the cell wall is detached and dissolved in boiling water, often under pressure. Careful control of the pH is needed to obtain the best yields.
3. Filtration: this requires special care since clarity and purity will depend on this operation. Most standard filtering techniques can be applied.

### 2.3 Chemical structure of agar

Agar is a polysaccharide that formerly was considered to be formed by one unitary structure only having sulfate semiester groups linked to a few galactose oxydriles. Choji Araki (1937) showed that agar was formed by a mixture of at least two polysaccharides that he named agarose and agaropectin. Later in 1956 he assigned agarose the structure that can be seen in the upper part of Fig. 2.3. In 1938 Percival, Sommerville and Forbes, and independently Hands and Peat, discovered the existence of 3-6,anhydro-L-galactose as part of the agar molecule.

When diverse types of agars were carefully studied, the presence of agarobioses which we can observe in the lower part of Fig. 2.3 (Lahaye and Rochas, 1991) was proven. These 11 agarobioses can be produced in many variable forms by the different agarophytes depending on gender and species which depend on their genetic characteristics. It is influenced by a series of ecological factors such as the nutrient availability, substrate composition on which they grow and the habitat hydrodynamic
conditions. But of greater importance for the production of such agarobioses is the harvesting period as agar plants mature gradually through the summer season.

Agarobioses are the fractions of agar that essentially gel. They have high molecular weights, above 100,000 Daltons and frequently surpass 150,000 Daltons, as well as a low
sulfate content usually below 0.15%. The rest of the fractions are known as agaropectins. They have a lower molecular weight, usually below 20,000 Daltons, with 14,000 Daltons being most usual. Sulfates are in much higher content registering sometimes 5% to 8%. This is far below carrageenans which range from 24% to 53% and even furcellaran which is the less sulfated carrageenans at about 17%.

Agaropectins are fractions that have been studied less than agarose due to their lack of practical applications. Their properties do not match those of carrageenans for food applications and any process to produce them would be costly and complicated. As mentioned previously, some agaropectins are precursor polysaccharides for agaroses that are transformed internally by enzymatic polymerisation and desulfation processes.

As will be shown later, the diverse forms of agarobioses determine the physico-chemical characteristics of agar such as gelling and melting temperatures and reactivities or synergies of agars with other products.

2.4 Gelation of agar

Agar is a mixture of agarose and agaropectin fractions in variable proportions depending on the original raw material and the manufacturing process employed. Agar gelation occurs only by its agarose content that is produced exclusively by hydrogen bonds. Not needing any other substances to gel, it has an enormous potential in applications such as a foodstuff ingredient, for biotechnology uses, for cell and tissue culture or as support for electrophoresis or chromatography. Agarose produces ‘physical gels’ which means that these aqueous gels have all their structure formed only by the polymer molecules united solely by hydrogen bonds. Due to this unique gelling property, these gels hold in the interior network a great amount of water which can move more freely through the macroreticulum. Each molecule maintains its structure in complete independence so the process is not a polymerisation but a simple electrostatic attraction.

On the contrary, ‘chemical gels’ have the polymer molecules united by covalent bonds to form large macromolecules so we can consider this polymerisation to be caused by a chemical reaction that forms the gel. The most remarkable property of ‘physical gels’ is their reversibility. They melt just by heating but gel again upon cooling. These transformations can be repeated indefinitely in the absence of aggressive substances that could hydrolyse their agarose molecules or destroy them by oxidation. ‘Chemical gels’ such as polyacrylamides that have their molecules joined by covalent bonds are irreversible. Another basic property is the gelling mesh size that in the case of agarose is identified by very high exclusion limits. Exclusion limit is defined by the greatest globular protein size that can traverse the gel in an aqueous solution. In the case of a 2% agarose gel the exclusion limit is 30,000,000 Daltons. Considering that there is no protein of such a size for calibration it has been necessary to resort to subcellular particles such as ribosomes or viruses.

The agaropectin fractions present in agar narrow the reticulum reducing slightly its exclusion limit. Intermediate between the physical and chemical gels, we find those gels that require the presence of cations to form gel structures as in the case of carrageenans and alginites. In the case of gels formed by alginic acid with di- or tri-valent cations we face totally irreversible gels that will not melt by heating. These are gels that have formed ionic bonds that can be broken only by eliminating the bonding cation, which is normally calcium. It is done with the help of a complexing agent such as EDTA (ethylene diamine tetracetate). Hence, these gels can be considered as ‘ionic chemical gels’ as they form
ionic bonds and are irreversible. An important property of agar gels derived from their agarose content is the very high gelling hysteresis, defined as the temperature difference between its gelling (around 38°C) and melting temperatures (around 85°C).

In Fig. 2.4 we can observe the gelling and melting temperature graphs for regularly available agars. Concentrations are also typical (between 0.5% and 2%) being employed in agar applications. It shows a gelling hysteresis that in every case is above 45°C. As a comparison, the most gelling carrageenans have a hysteresis of 12°C that is 26% less than agar. These temperatures depend on the presence of agarobioses originally in the agarophyte seaweed from where the agar is extracted. Gel temperature is an indicator to identify the agarophyte used to produce an agar. Using Table 2.2 we can determine the origin of an agar by identifying its characteristic gelling temperature.

It has been proven that the gelling temperature is influenced by the methoxylation degree of the C6 of the agarobioses present in the agar, in such a manner that the more methoxylated corresponds to Gelidiella agaroses and the least to the Pterocladia ones. This is the same as saying that a greater methoxylation in carbon 6 will correspond to a higher gelling temperature. Curiously, the methoxylation of the rest of the carbons reduces the gelling temperature and its gel strength at the same time. This is due to the inability to establish hydrogen bonds by the hydroxyl group located in C6 because of their

**Fig. 2.4** Gelling and melting temperatures of agar gels: gelation hysteresis.

(Armisén, R. 1997)
position in the gelling helices while the rest of the hydroxyl group are bonding points where hydrogen bridges can be formed. The gelling process of agarose can be seen in Fig. 2.5, obtained from Medin (1995). This is an exothermic process which develops when agarose molecules are dissolved in water. It can be seen on the left that these molecules are real ‘statistical random coils’ subject to Brownian movements. When cooling down close to the gelling temperature, the next structures start to form.

According to Rees and Welsh (1997) we observe in the upper part of Fig. 2.5 how antisymmetric double helices (B1) are formed in their aggregation to form a macroreticulum as pictured in the upper side of C and D. In the lower part of Fig. 2.5 and according to Foord and Atkins (1989) we can see simple helices B2 that are joined by hydrogen bridges that produce folded structures (symmetric double helices) that will form the macroreticulum as can be seen in the lower side of C and D. It seems that both gelling processes can coexist and one or the other dominates depending on the cooling speed. A faster rate favours the first process. Both are based in the formation of hydrogen bridges and produce a macroreticular structure.

In Fig. 2.6 we can observe the spongy structure of a 2% agarose gel. The mesh cavities of the gel which can be seen are filled with solvent water that circulates freely through the mesh capillaries. The very high exclusion limit of agarose gels allow the passage of soluble macromolecules up to 30,000,000 Daltons of molecular weight. A characteristic property of an agarose gel is its syneresis capacity which relates to the capacity to eliminate water contained in its gel mesh. The ejection of aqueous solvents is speeded by

<table>
<thead>
<tr>
<th>Genus</th>
<th>1.5% solution, gel temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelidiella</td>
<td>42–45°C</td>
</tr>
<tr>
<td>Gracilaria</td>
<td>40–42°C</td>
</tr>
<tr>
<td>Gracilariopsis</td>
<td>38–39°C</td>
</tr>
<tr>
<td>Gelidium</td>
<td>36–38°C</td>
</tr>
<tr>
<td>Pterocladia</td>
<td>33–35°C</td>
</tr>
</tbody>
</table>

Fig. 2.5 Agarose gelation. (Medin, A. S. 1995)
pressure conveniently applied on a properly confined gel. In these conditions a 1% agar or agarose gel can eject a great proportion of the water soaked in the capillaries ending the process with a 20–25% dried extract which means that 95% of the water used to dissolve the agar/agarose has been eliminated. If the undried synergised gel is submerged in water it will recover its original size. The gel structure has been maintained during syneresis and upon rehydration it will recover exactly to the previous form. This is known as gelling memory.

2.4.1 Synergies and antagonisms of agar gels

We shall consider in this section the most important cases of blended products which modify agar by increasing its gel strength, modifying its texture or elasticity and the antagonist products that reduce the gel strength or block in any way the gelling process. Traditionally food agar gel strengths are controlled following the Japanese Nikan Sui method that measures the charge in grams that ruptures a gel by means of a cylindrical piston with a surface of 1 cm² after applying force for 20 seconds. This traditional method is used universally even though there are other more precise ones that operate with a growing load that also permits valuation of the elasticity module of the gel. No doubt these controls will be improved in the coming years but nowadays Nikan values are the basis for commercial transactions worldwide.

Agar-locust bean gum (LBG) synergies

Synergies with this gum are possible only with Gelidium and Pterocladia agars. It has practical applications because gel strength is increased and the gel texture is modified in a
way that rigidity is decreased and elasticity is enhanced becoming less brittle. As we can see in Fig. 2.7 a mixture of LBG and Gelidium agar dissolved at 1.5% gives Nikan gel strengths (g/cm²) which increase to a 9:1 ratio when maximum hardness is achieved, returning to the corresponding agar strength when the agar-LBG proportion is 4:1.

Contrary to this, Gracilaria agars do not show such synergy and upon substitution hardness falls in the same proportion as if the agar concentrations were reduced. The synergism agar-LBG is caused solely by the agarpectins contained in Gelidium and Pterocladia but not by those in Gracilaria.

Sugar reactivity
Reactivity with sugar (saccharose) is basically present in Gracilaria agars when dissolved in aqueous solutions with a high sugar concentration (around 60%). In agars with high gel strengths and low sulfate content the synergy is caused by the thread pitch of the gelling helices. It always requires the presence of agarose of high molecular weight (around 140,000 Daltons) and is a consequence of the 3-6,anhydro bridge in the L-galactose which is able to assist in the building of hydrogen bridges.

Gelling blockade by tannic acid (TA)
The presence of TA (pentadigaloil glucose) may inhibit agar gelation if the TA quantity is large enough. This acid is found in some fruits such as squash, apple and prune in variable concentrations. Adding glycerol in small amounts is usually enough to avoid this reaction.

Gelling blockade by chaotropic agents
Chaotropic agents which capture protons can also block agar gelation by avoiding the hydrogen bridges which form between the agarose molecules present. In any case, there is no problem associated with chaotropics for the use of agar in the food industry, as foodstuffs do not contain significant quantities of agents such as urea, guanidine, sodium thiocyanate or potassium iodide.

Fig. 2.7 Agar-locust bean gum synergies.
(Armisén, R. and Galatas, F. 1987)
Acid and alkaline hydrolysis
As with all polysaccharides, agar can suffer hydrolysis reducing its molecular weight and consequently losing its gelling power. Acid hydrolysis in agar appears more readily, as a result of lowered pH and the longer time the agar stays in dissolution at a high temperature. In general, hydrolysis is not an important problem unless the agar undergoes extensive heating at pH below 5.5. Alkaline hydrolysis is not important at pH below 8. Enzymatic hydrolysis is not relevant as there are few agarases (enzymes that break down agaroses) which are found only in marine bacteria, in a few bacilli and Esquiosaccharomyces that are not normally found in food products.

2.5 Applications of agar
Agar applications are fundamentally based on the enormous gelling power, high hysteresis and perfect gel reversibility which are unique properties conferred by its special ‘physical gel’ structure. Although agar has multiple applications, the traditional one is as a food ingredient accounting for 80% of its consumption. The remaining 20% is accounted for by biotechnological applications. A list of different uses and the corresponding type of algae required can be found in Table 2.3.

2.5.1 Agar in food applications
Agar is a food additive of universal use considered in the US as GRAS (Generally Recognized as Safe) by the FDA (Food and Drug Administration). In Europe it is considered an E406 additive. In the Register Service of the Chemical Abstracts it is registered as 9002-18-0. The synergies that were described when dealing with agar gelation are important. The synergy between agar-LBG shown by Gelidium and Pterocladia agars improves hardness as well as texture, making the mixture more palatable due to the elasticity conferred and the elimination of the brittleness period. The

| Table 2.3 Agar grades depending on their final uses and agarophytes used for their production (Armisén, R. 1995) |
|-----------------|---------------------|---------------------|
| Agar type applications | Agarophytes used |
| Natural agar | ‘Strip’ | Produced mostly with Gelidium by traditional methods |
| | ‘Square’ | |
| | Accustomed only on Far East traditional kitchen | |
| Industrial agar | Food grade agar used for industrial food production | Gelidium, Gracilaria, Pterocladia, Ahnfeltia, Gelidiella |
| | Pharmachological agar | Gelidium |
| | Clonic plants production grade | Gelidium or Pterocladia |
| | Bacteriological grade used for bacteriological media formulation | Gelidium or Pterocladia |
| | Purified agar used in biochemistry and in media for very difficult bacteria | Gelidium |
mixture of LBG-agar reduces the syneresis of the gels which makes them exude less liquid during handling, transportation and storage. In the same way, *Gracilaria* agars that show reactivity with sugar, experience an increase in gelling power when used with sugars with a high sugar content (60% or more) such as jams and jellies.

Adding glycerin or sorbitol to aqueous gels prepared with agar, reduces dehydration to such an extent that with sufficient quantities of these humectant products, the drying of gels exposed to air can be avoided. Obviously, the higher the relative humidity of the atmosphere, the added quantity of humectants should be lower. The ambient temperature changes during the expiration life of the product also affect this behaviour. Agar is tasteless and cannot be detected in foodstuffs with delicate flavours. In contrast, those gelling agents that need the presence of cations (calcium or potassium) to gel should be blended with foodstuffs with strong flavours to mask the characteristic flavour of said cations. Some agars with an elevated melting point such as those produced in Portugal by Iberagar are employed to prepare Mitsumame. This popular Japanese fruit salad mixes fruits and coloured gel cubes properly flavoured and, after canning, is subjected to heat sterilisation which the agar gel cubes withstand without melting. Normally agar is dissolved by adding it slowly to water with good stirring. In sweet products agar is usually premixed with a part of sugar, then adding it slowly later to avoid clumps which can form during dispersion.

If acidification is required in a process, it has to take place once all the agar has been dissolved and whenever possible at reduced temperatures to minimise hydrolysis risks. Nevertheless, agar has enough resistance to hydrolysis to the degree that, in meat preserves which require sterilisation in an autoclave over 121°C, it undergoes the treatment successfully withstanding hydrolysis. Aromas, especially volatile ones, should be administered at the final stage of cooling, just prior to moulding and packing of the product. In this way evaporation losses are avoided. In some industries it is customary also to add at this stage the colour ingredients. Given that agar is utilised in food as a minor ingredient (0.5% to 1.5%) relative to the total weight of the finished product, its nutritional contribution is low because the human digestive system hardly absorbs it. Agar has been consigned a digestibility below 10% of what is ingested. For this reason agar is used to prepare dietetic formulas and foods for diabetics since it does not depend on sugar to gel as with pectins. Formulations can be made employing edulcorators as substitutes for sugar together with agar which will create very low-calorie food products.

For many years agar has been included in the US Pharmacopoeia as a laxative as it has a convenient effect as a soft voluminator. In fact it is contained in several formulas for this purpose. It behaves just like natural fibre with the great quality of being totally soluble and resistant to hydrolysis. In addition the human digestive system produces no agarases, which contributes to the extremely low digestibility of agar. Today agar is included in the US National Formulary to be used as a slow-release ingredient for the slow absorption of pharmacological agents.

It is very interesting to realise that the alimentary applications of industrial agar differ depending on the cultural area that is considered. In Table 2.4 we can observe how they vary among geographical areas. We shall follow with some formulations used to prepare these products from our experience in this field and the bibliographical data available. In necessary cases indications will be given to prepare these products. We wish to state that in no circumstances can this information be considered as an aid to infringe patents or protected processes.
Some formulations for agar in human diet

Yokan

Matsuhashi, T. (1990)

Yokan, a traditional agar hard gelatin consumed usually in the tea ceremony but also on different occasions. Its ingredients are sugar, azuki bean purée, agar and water. Sometimes chestnuts are used instead of beans for higher priced Yokan. An approximate formula would be:

Agar 50 gm
Water 2000 to 3000 gm
Sugar 1000 to 2000 gm
Inverted sugar 1500 to 3000 gm
Citric acid 2 gm
Flavour and colour If desired
Azuki beans Variable according to each case but enough to produce a hard gel.

Agar is dissolved in boiling water with sugar and inverted sugar and maintained at 106°C for a few hours to reduce the volume. After brief cooling, the fruit purée previously prepared and the acid are added together with flavours and colourings. It is left to cool overnight at room temperature. This gel has a dried weight of 70–75%. It is placed in an oven at 55°C as long as needed to reach a dry weight of 84–86% and is cut in small pieces that are first folded in an oblate and later in plastic. This oblate is an edible paper made of starch and agar. Inverted sugar is hydrolysed saccharose that avoids crystallisation.
Sweet potato sweet (An Argentinian traditional dessert)
Fiszman, B. (personal communication towards 1975).

This is a very popular sweet in Argentina and Uruguay and also in some parts of Brazil, being part of its culture.

Components:
- Sugar 37%
- Glucose 21%, usually a syrup of 76° Brix
- Agar 0.181%
- Locust bean gum (LBG) 0.29%
- Fresh sweet potato with skin 80%.

As it is an elaborate process, it is recommended to:
1. Peel the potato.
2. Boil it in water.
3. Sieve it to a state of purée.
4. Mix the purée with sugar and glucose.
5. Concentrate by boiling until 60° Brix.
6. Add the agar that has previously been dissolved at 25%.
7. Bring the mixture to 58/60° Brix.
8. Add to this mixture the LBG that previously has been dry mixed at four times its weight in sugar and dissolved in cold water at 6–7 times the total weight.
9. Carry all of it to a 60.5° Brix, flavour and can while hot.

Flat cans of 25 cm in diameter and 5 cm in height are usually employed.

Sugar icings

Components:
- Agar 0.35%
- Salt (NaCl) 0.3%
- Emulsifier 0.4%
- Granulated sugar 15.0%
- Coating sugar (Glasee) 73.0%
- Water Enough to complete 100%

Different coatings are prepared with agar using several formulations depending on the solidification speed that is expected for each coating. These icings are prepared by boiling the agar, used as stabiliser, in a sugar solution followed by the addition of coating sugar. The icing heated up to 50–60°C is applied over the products to be coated in 60 seconds. Gelation times can be regulated, increasing or reducing the agar amounts.

2.5.2 Agar in insect culture media formulations

Agar is used for the breeding of larvae and other smaller sized animal species. It is applied for fattening silkworms year round in order to lengthen the season that was previously a limited one. The tiny worms could feed only on tender mulberry leaves that were produced at early budding. Agar for this purpose is dissolved and the feed, composed of carbohydrates and proteins, is suspended in the solution. Left to cool, the
mixture is extruded in the form of thin spaghettis with adequate sizes for the different worm constitutions from the egg eclosion period until the formation of the silk buds. Only agar could be employed for its composition because all other gelling agents have tastes that are rejected by silkworms.

Another classic use of agar is a similar application for feeding larval phases of flies such as *Drosophila melanogaster* used for genetic research. Obviously when technologies for biological insect plague contentions were developed, analogous methods for larval feeding were implemented using agar as a base. It dealt with insects that damaged intensive crops and modern methods were required for its control. In this way the Mediterranean fly that damages orchards or *Pectinophora glosipeii* caterpillars that destroy cotton farms are artificially grown in order to be subsequently sexually sterilised by γ (Gamma) radiation. The sterile individuals are kept hibernated and set free at the proper mating period for intercourse. Because these insects are able to copulate only once in their lifetime, their copulation capacity is frustrated rendering void their intercourse with a fertile mate. Without the need of organochlorated insecticides the species is thus controlled but not destroyed as some individuals can mate with fertile partners.

### 2.5.3 Vegetable tissue culture media formulations

The gelling properties of agar enhance the formulation of solid media for tissue culture growth originated in techniques developed to obtain orchid clones. Media has been formulated to reproduce plant specimens to grow identical plants free of viruses from each one. Usually vegetable meristems of plants to be cultivated are cultured in media of the adequate composition, enriched with vegetable hormones such as auxines or cytokines that are applied depending on the rooting desired for the plant and/or its growth velocity. Once the proper plant development is achieved, they are transferred to vegetable ground to continue its growth.

### 2.5.4 Culture media for microorganisms

Microbial cultivation began with R. Koch in 1882 and since then its use has been related to the development of microbiology in such a way that it has never been substituted, repeated efforts to the contrary notwithstanding. The special properties of the physical gels that agars form, together with their gelling and melting temperatures and especially due to their enormous gelling hysteresis and reversibility, all contribute to and offer characteristics which are unique for these applications and have found no substitute so far. Likewise their huge resistance to degradation by enzymes that damage other gelling agents and their ability to gel in the absence of cations, enable agars to support culture media with very well adjusted osmotic pressures to cell requirements whether red blood cells, bacteria, yeasts or moulds are to be grown.

### 2.5.5 Industrial agar application formula

#### Dental moulds

Meer, W. 1980

A formula for an industrial use of agar is offered as an example of a general application that is quite common in very diverse uses where high-precision moulding is sought. This composition is employed to prepare dental moulds in the US but in other countries it is
utilised to prepare plasters of archaeological pieces or moulds for sculptures. Police use it to preserve footprints or similar clues for criminal cases. The enormous reversibility of agar gels that change from solution to gel states by simply cooling them, is an incentive due to its convenient use.

Gels can be kept for long periods in tubes similar to toothpaste tubes. Before use, they are melted in boiling water baths and the solution is placed in a tray prepared for the dental imprint. Once the adequate temperature (39°C) is reached, the imprint is performed immersing the tooth (or the object to be copied) until the Gelidium agar gels at 36°C.

Other agars that gel at higher temperatures are not recommended as they can annoy patients. This is not the case for inanimate objects that may employ other agars. A common formulation is:

- Gelidium agar 13–17%
- Borates 0.2–0.5%
- Sulfates 1.0–2.0%
- Hard wax 0.5–1.0%
- Thixotropic materials (Bentonite type products) 0.3–0.5%
- Water Sufficient to arrive to 100%.

This formulation can be modified to achieve gels that will not dry in practice in normal atmospheric conditions. For this purpose sorbitol glycerin should be added or any other humidifier agent that will absorb the ambient dampness to compensate for the evaporation losses. In normal weather conditions 8–10% of glycerin is required but this percentage must be adjusted for practical uses depending on the maximum and minimum temperatures the product can withstand and on the relative humidities of the environment.

### 2.6 New developments

It has been a continuous goal to obtain an agar with good gelling properties but with a greater ease for solution. Specifically, efforts have been made to try to obtain agars that can be dissolved without the need to reach boiling temperatures of 100°C. It would enable the preparation of foods with ingredients that do not resist temperatures above 85°C. In parallel, industrial agars have increased in gel strength to be formulated in lesser proportions saving costs, as agar is one of the most expensive ingredients. In general there has been a tendency to increase the gel strength by increasing the average molecular weights and reducing the sulfate contents in industrial agars. It is necessary to spend longer periods at boiling temperature to dissolve agars of this type. Practically, when dealing with high sugar content products (saccharose), solution always occurs above 100°C due to the boiling phenomena that sugar produces.

New agars intend to improve the solution by enabling the agar to dissolve at lower temperatures and in shorter periods. They have been developed lately to achieve these seemingly opposed targets of a lower dissolving temperature with a minimum sacrifice in gel strength. One of the methods employed for some of these agars is based on the addition by means of mechanical pressure of substances that unite strongly to the agar in a dry state and upon dissolution accelerate the binding of the agar and water molecules. Agars obtained in this way present the inconvenience of lower gel strength (caused by the increased proportion of a non-gelling agent) as a trade-off for lowering the temperature of
solution. Hence, it loses some yield when used as an ingredient and has limitations for biotechnology uses such as microbiology culture media due to the foreign substance added that can interfere with the growth of organisms or even inhibit them.

In other cases, production processes have been refined to optimise the binding of agar and water molecules at lower temperatures without additives and without affecting the gel strength that is kept at normal agar levels. Therefore its performance as a gelling ingredient has not changed and its uses in biotechnology has not been disturbed. The utilisation of these agars in industry is dependent on the balance of its higher price and the convenience it can bring to foodstuff manufacturers. Being in the market only since the late 1990s, the novelty has not yet been fully introduced into each of the traditional areas of application. In Table 2.5 we can observe a comparison of the analysis performed on two of these types of agars that are easy to solubilise, samples which were available to us in sufficient quantities and have been tested in our laboratories. As can be seen in all cases, gel strengths of agars that have been dissolved at 80°C are specified.

Ina Food Industry and Setexam also produce agars which are easily soluble. However, at the time of writing we have not been able to obtain samples to carry out comparative analysis.

### Table 2.5 Some easily soluble agars: comparative analysis

<table>
<thead>
<tr>
<th>Product</th>
<th>Grand agar</th>
<th>Speed agar-80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Produced by</td>
<td>Hispanagar (Spain)</td>
<td>Taito (Japan)</td>
</tr>
<tr>
<td>Moisture</td>
<td>7.16%</td>
<td>7.08%</td>
</tr>
<tr>
<td>Ash</td>
<td>1.53%</td>
<td>1.47%</td>
</tr>
<tr>
<td>Solution clarity at 1.5%</td>
<td>26 Nephelos</td>
<td>510 Nephelos</td>
</tr>
<tr>
<td>pH in 1.5% solution</td>
<td>6.80</td>
<td>6.18</td>
</tr>
<tr>
<td>pH in 1.5% gel</td>
<td>6.48</td>
<td>6.56</td>
</tr>
<tr>
<td>Viscosity 1.5% 60°C</td>
<td>6.5 cps</td>
<td>4 cps</td>
</tr>
<tr>
<td>Gel temperature at 1.0%</td>
<td>31.9°C</td>
<td>34.9°C</td>
</tr>
<tr>
<td>Melting temperature at 1.0%</td>
<td>87.5°C</td>
<td>75.6°C</td>
</tr>
<tr>
<td>Gel strength (Nikan) at 1.5%</td>
<td>1,270 gr/cm²</td>
<td>590 gr/cm²</td>
</tr>
<tr>
<td>Gel strength at 1.5% (dissolved 5 minutes at 85°C)</td>
<td>680 gr/cm²</td>
<td>430 gr/cm²</td>
</tr>
<tr>
<td>Gel strength at 1.5% (dissolved 5 minutes at 90°C)</td>
<td>820 gr/cm²</td>
<td>440 gr/cm²</td>
</tr>
</tbody>
</table>

2.7 References and further reading

ARMSÍN, R. (1993) ‘Agar-Agar’. Lecture in Training Course T004 ‘Gels Thickeners and Stabilizing Agents’ held in Leatherhead, Surrey, UK on 25–8 May 1993 organised by Leatherhead Food Research Association (a copy of the paper was delivered to the thirty delegates present).
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