A MANUAL FOR THE PROCESSING OF AGAR FROM GRACILARIA

by

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1. INTRODUCTION

1.1 Agar - historical background

The name agar originated from the Malay word “Agar-agar”, the local name in the Dutch East Indies for Eucheuma muricatum (spinosum) (Tseng, 1944) which was exported to China for more than a century. For the sake of simplicity agar-agar was shortened to just agar and is now accepted universally whether in the food and other industries or as culture media. The introduction of agar in bacteriology was achieved by a German housewife, Frau Hesse, who prepared the bouillon from agar-agar for her husband's bacterial cultures. The agar-agar came from Frau Hesse's mother who lived in America which was given to her by some friends who lived in Java. Dr. Walther Hesse was so excited about the efficiency of the new medium which his wife prepared, so he relayed his findings immediately to Dr. Robert Koch who was working at the time on the tubercle bacilli. In 1882, Koch reported on the tubercle bacilli and mentioned the new culture medium (Hitchens and Leikind, 1939). Hitchens and Leikind even suggested agar should be called Frau Hesse's medium in honor of the woman who discovered it. According to Tseng (1944) the agar-agar of Frau Hesse from Java could have been carrageenan from Eucheuma.

1.2 Definition and description

Tseng (1944) defines agar as the dried amorphous, gelatin-like, non-nitrogenous extract from Gelidium and other red algae, a linear galactan sulfate, insoluble in cold but soluble in hot water, a 1 to 2 percent solution of which upon cooking solidifies to a firm gel at 35° to 50° and melting at 90° to 100°.

The USP XVIII defines and describes agar as the dried hydrophilic colloidal substance extracted from Gelidium cartilagenium (Linne) Gaillon (Fam. Gelidiaceae), Gracilaria confervoides (Linne) Greville (Fam. Sphaerococcaceae) and related red algae. Unground agar usually occurs in bundles consisting of thin, membranous, agglutinated strips or in cut, flaked or granulated forms. It may be weak yellowish orange, yellowish gray to pale yellow, or colorless. It is tough when damp, brittle when dry. It is colorless or has a slight odor and has a mucilaginous taste. Powdered agar is a white to a yellowish-white or pale yellow, insoluble in cold water, but soluble in boiling water. When boiled with 65 times its weight of water for 10 minutes, with constant stirring, and adjusted to a concentration of 1.5 percent, by weight, with hot water, agar forms a clear liquid which congeals at 32° to 39° to form a firm resilient gel, which does not melt below 85°. Armisen and Galatas (1987) reported a wider range of 34° to 43° for the gelling temperarature. Actually according to our observation, the gelling temperature as well as the melting temperature of a 1,5 percent concentration of agar vary according to the seaweed source, the method of preparation and the purity of the sample. The gelling temperature of the agar sols ranges from 30° to 50° and the melting temperature from 82° to 92°.

1.3 Chemistry

The chemical nature of agar varies according to the seaweed source, the environment where the seaweeds grow and on the method of preparation of the agar. Meer (1980) recognized two types of agar, the Gelidium and Gracilaria agars. Bacteriological agar is prepared mostly from Gelidium and Pterocladia. Gelidium cartilagenium collected along the West Coast of North America (Mexico) (Durrant & Sanford, 1970) is the common source of bacteriological agar in the U.S.A.

Araki (1965) reported that agar of Gelidium amansii is a mixture of two different polysaccharides, one a neutral agarose which consists of alternating 1,3-linked 3-D-galactopyranose and a 1,4-linked 3,6-anhydro-α-L-galactopyranose and the other a charged agaropectin. Agaropectin contains galactopyranose residues with sulfate and other charged groups present in varying degrees in the molecule. Hirase (1957) reported the presence of pyruvic acid in the agar of Gelidium amansii as a ketal attached to the C_4 and C_6 of the l,3-linked-$-D-galactopyranose residues (Fig, 1). Araki (1965) also proved that the D-galactose residues are 6-0-methylated to certain degrees.

Duckworth and Yaphe (1971) fractionated Difco Bacto agar using DEAE-Sephadex A-50 and showed that agar consists of a complex mixture of polysaccharides having the backbone structure of alternating 1,3-linked β-D-galactopyranose and 1,4-linked 3,6-anhydro-α-L-galactopyranose charged in varying degrees by sulfate and pyruvate and a galactan sulfate. Agarose is then the mixture of agar molecules having the lowest charge content and the greatest gelling ability. The gel strength of agar decreases with an increase in sulfate and a decrease in 3,6-anhydro-α-L-galactose concentration (Yaphe, 1984).

1.4 Properties of agar gels

Gelation occurs when a chain of macromolecules forms a network capable of entrapping the dispersing medium. Such a gel has that characteristic of having a composition approaching a pure liquid but may resemble a solid. It is an elastic colloid which actually retains the shape of the containing vessel even when removed from it. Gelation can be characterized (Whitney, 1977) by the time of gelation, the gelation temperature and the minimum concentration of the dispersed phase required for gelation. Agar gels (Rees, 1969) may contain as much as 99.9% water. Such gels exhibit strong syneresis (“weeping”) and behave like free water which can easily be separated by freezing and thawing. The stiffness of agarose gels may be due to the aggregation of the double helices forming a network phase which may contain as much as 100 parts of water for each part of agarose. Such a structural network would have relatively large voids through which large molecules and particles could diffuse. The aggregate in agarose gels may actually contain 10 to 10^4 double helices rather than what is shown in Fig. 2 (Arnott, et al, 1974). Formation of such a gel network is the property which makes agarose very useful in immunology, biotechnology and genetic engineering.

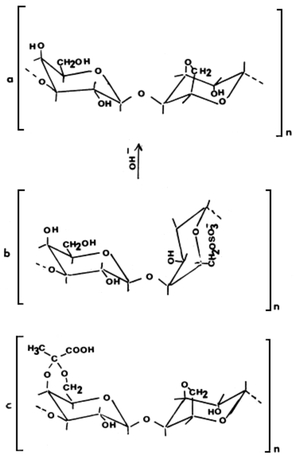


Fig. 1. a) Agarose, b) 6-galactan sulfate, and c) pyruvated agarose

Fig. 2. A schematic representation of the agarose gel network

Guiseley (1970) studied the relationship between methoxyl content and the gelling temperature of agarose using 50 agarose samples. Majority of the samples were prepared by Blethen's method (1966) from Chilean agar which could have been manufactured from Gracilaria lemanaeformis, the predominant Gracilaria species in Chile. The gelling temperature increases with an increase in methoxyl content of the agarose. Optical rotation studies on the solgel transition of agarose (Rees, 1972; Rees, et al, 1970; Rees and Scott, 1971; Arnott, et al, 1974) showed that agarose has a specific rotation of-44° at 589 nm with a left-handed double-helical conformation in contrast to the right-handed carrageenan. The temperature dependence of the optical rotation was determined for several agarose derivatives (Arnott, et al, 1974). The cooling curve for agarose which is almost free from any substituent is centered at almost 25° and has a transition width of 4°. Upon reheating the reverse transition occurred at 80° with very marked hysteresis but with no change in width (4°). The presence of a few 6-0-methyl substituents closed the hysteresis loop slightly, the cooking tansition moving to 30°. More 6-0-methyl groups displaced the cooking transition at a higher temperature and a substantial broadening of the hysteresis look (Fig. 3).

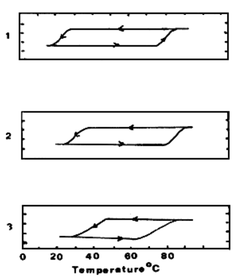


Fig. 3. Changes in optical rotation with the solgel transition for agarose samples. 1. Agarose, 2. Agarose with a few 6-0-methyl-D-galactose residues, and 3. Agarose with more 6-0-methyl-D-galactose

1.5 Properties of Gracilaria agars

Santelices and Doty (1989) reported that close to 5 000 tons of agar are processed annually from 25 000 to 30 000 tons of agar Gracilaria and the volume of the farmed production is not known but perhaps about 15 000 tons of dried seaweeds. In the manufacture of agar from Gracilaria not only one species is utilized so it is but appropriate to have an idea of the properties and chemical nature of the agars of the different species which have already been investigated.

The chemical studies on the majority of the Gracilaria agars were done by the Yaphe group. Polysaccharides from Gracilaria debilis, G. compressa, G. foliifera, G. domingensis, G. damaecornis and G. ferox were evaluated as sources of agar (Duckworth, et al, 1971). The agars obtained were different from one another as shown by the chemical and enzymatic hydrolysis and fractionation on DEAE-Sephadex A-50. Only G. debilis gave an agar of high gel strength. Agars containing 4,6-0-(l-carboxyethylidene)-D-galactose are always found in regions of the molecule that are low in sulfate. Replacement of 3,6-anhydro-L-galactose sulfate causes kinks in the helix thus forming an agar of lower gel strength. If the sulfate groups are in C_6 of the L-galactose molecules, alkali treatment converts L-galactose 6-sulfate into 3,6-anhydro-L-galactose which causes an increase in gel strength of the agar. The acid hydrolyzates of G. foliifera, G. damaecornis, G. domingensis and G. ferox agars contain 6-0-methyl-D-galactose and 4-0-methyl-L-galactose. The amount of 3,6-anhydro-L-galactose increased (except that of G. domingensis) after alkali treatment and no significant change in the 6-0-methyl-D-galactose content. The pyruvic acid values which vary in the agars of the six species are not affected by alkali treatment. Low pyruvic acid concentration favors higher 6-0-methyl-D-galactose values. Before alkali treatment only G. debilis agar (sulfate, 3.4%) has high gel strength. The sulfate content of G. compressa agar decreased after alkali treatment but it did not gel, may be because of the high pyruvic acid value. The sulfate groups of G. ferox, G. damaecornis and G. demingensis were alkali-stable indicating that such groups are not located at the C_6 of the L-galactose residues. The agars of the above six species discussed have gel strengths not comparable to the agars of Gelidium cartilagenium and G. sesquipedale which are known commercial sources of agar and so such Gracilaria agars can only be of application in the food industry. Agars of Gracilaria cf. verrucosa and Pterocladia capillacea from the Mediterranean were compared (Friendlander, et al, 1981). Fractionation on DEAE-Sephadex A-50 gave three fractions, namely, neutral agarose, sulfated agarose and a galactan sulfate. Alkali treatment increased the neutral agarose content for both agars. Agar from P. capillacea had higher agarose content and lower sulfate than the agar of G. cf. verrucosa and the former agar after alkali treatment had a structure approaching that of theoretical agarose.

Agars from Gracilaria verrucosa, G. tenuistipitata, G. blodgettii and G. eucheumioides from China were studied (Ji Minghou, et al, 1985) by fractionation on DEAE-Sephadex A-50 and ^{13} CNMR spectroscopy. The latter analysis showed L-galactose 6-sulfate as a minor constituent of the agars of G. verrucosa, G. tenuistipitata and G. blodgettii but not sensitive enough to detect the other sugars contributing to the charge density of the agarose molecules. 6-0-methyl-D-galactose was found as a minor sugar of the agarose from G. verrucosa and G. tenuistipitata and 2-0-methyl-3,6-anhydro-L-galactose as the major component of that of G. eucheumioides. The yield and quality of agar obtained from Gracilaria spp. collected from Taiwan and Micronesia were studied (Nelson, et al, 1983). G. edulis from Taiwan gave the highest agar yield while G. lichenoides from Micronesia gave the highest gel strength. Young algal tissues of G. tikvahiae (Craigie and Wen, 1984) synthesize agarose polymers with low methoxyl and high L-galactose-6-sulfate while the methylated agars are formed more in the older tissues and at higher temperature. Genetic improvement in clones of G. tikvahiae produced a mutant (MP-40) which gives an agar of high gel strength (over 1 000 g/cm^2 , conc. 1%) will be of value as raw material in the agar industry. G. lemanaeformis and G. verrucosa the two most important seaweeds of Chile (Kim, 1970) needed only 3-5% NaOH, heating at 95° for 60-90 minutes to yield an agar of high gel strength (600 g/cm^2) which is in great demand in the food industry especially in the U.S.A. and commands a price of $12 500/ton in 1986 (Santelices and Doty, 1989). Enzymatic hydrolysis showed the presence of 6-0-methyl-D-galactose in the hydrolytic product. Another methoxylated agar is obtained from G. secundata (Brasch, et al, 1983) of New Zealand which gives a gel strength of 495 g/cm^2 . Seven species of the genus Gracilaria from the Philippines have been studied (Hurtado-Ponce & Umezaki, 1988; Santos & Doty, 1978), G. arcuata, G. coronopifolia, G. edulis, G. eucheumioides, G. salicornia, G. verrucosa and G. sp. G. arcuata, G. salicornia and G. eucheumioides agars were investigated for possible raw material in the manufacture of agar in the country (Santos and Doty, 1978). The gel strength of the agar G. arcuata and G. salicornia were relatively high but although the G. eucheumioides agar has low gel strength it has a good consistency and mouth-feel that it could be quite applicable for fruit jellies and other jellied desserts.

The genus Gracilaria is widely distributed and can be found in temperate as well as in tropical countries. It is interesting to note that Gracilaria species in temperate countries yield agars of higher gel strength than the agars of the tropical species. The plausible explanation is that the Gracilaria species in cold countries grow very much slower giving the sugar molecules time to polymerize and form bigger molecules than the tropical species. Some tropical species yield agars of high gel strength after being subjected to the proper treatment. The Gracilaria species of Thailand were surveyed and the agar analyzed (Edward, et al, 1982; Tarn and Edwards, 1982; Edwards and Tam, 1984). In 1986, 1987 and 1988 another team made a renewed survey and screening of the Thai Gracilaria species. The Gracilaria samples were not treated with alkali before extraction but was done on the agar at room temperature (Chinadit and Chandrkrachang, 1986). The gel strength obtained for the agars of Polycavernosa changii, P. fastigiata and P. fisherii were 714, 1 100 and 947 g/cm^2 , respectively and the sulfate values were 0.07, 0.08 and 0.94%. Such agars can be considered of bacterial grade (Chandrkrachang, 1989).

The agar from Gracilaria cylindrica (now identified as Polycavernosa chang ii) has high gel strength, low gelling temperature and the melting temperature approaching those of Gelidium agars (Doty, et al, 1983) so the agar was fractionated using the method of Blethen (1966) to obtain agarose (Santos and Doty, 1983). The separation of agarose directly from the seaweed gave a better yield than when agar was used for fractionation. The gel strength of the agarose obtained range from 747–950 g/cm^2 of a 1% gel concentration and sulfate content of from 0.17 to 0.42% comparable to the sulfate values of commercial agarose. The study showed that there are some tropical Gracilaria species which could be possible raw material for the manufacture of agar not only for the food industry but also for the preparation of bacteriological grade agar and even agarose.

2. PROCESSING GRACILARIA FOR THE EXTRACTION OF AGAR

2.1 Sample collection

2.1.1 Have a notebook ready for recording the collection data. Record the place and date of collection, who collected the sample and how. Include the depth and the type of substratum whether sandy, coralline or muddy. The method of collection is usually by hand and then dumped in boats or on the beach.

2.1.2 Mounting of a herbarium specimen. Assign a voucher number for each collection. With the help of a botanist who knows Gracilaria, separate the collection into species and assign a number for each species. With the help of a magnifying glass it might be possible to recognize the male and tetrasporic thalli. The female thalli are easy to recognise. Maybe with a simple desk microscope the male thalli which are important in the identification of the species can be separated. The mounting of the specimen can be done temporarily on old newspaper if there is no mounting paper and presser on hand.

2.1.3 Cleaning of the sample is better done in the field when still fresh using seawater because it will be easier to remove attached shells, sand and other algae. If possible separate the other algae from the Gracilaria. Allow enough time for the cleaning because according to experience cleaning the sample at night is not advisable.

2.1.4 Drying of the sample. Spread the seaweed samples on bamboo mats or bamboo tables so that there will be air circulation on top and under the drying seaweeds which will facilitate faster drying. During very sunny days spreading on hot concrete pavement will be alright. Such techniques as practiced by villagers are shown in the following figures (Figs. 4–7). During rainy days the seaweed samples can be dried in an improvized drying oven using coconut or wood charcoal for heating (Fig, 8). It is not wise to keep the sea weed wet when it is already out of its natural habitat because of the presence of hydrolyzing enzymes in the Gracilaria. Dry the seaweed collection as soon as possible to prevent deterioration. Before collecting the Gracilaria whether wild or farmed it is always advisable to have the facilities ready for mounting specimens, sorting, cleaning, drying and weighing of the bulk samples. A triple beam balance will be enough for weighing small amount of samples. Top loading balances will be more convenient where electricity is available. The moisture content of the dry sample should be at least 15 to 20% because very moist samples may deteriorate faster and might produce inferior quality agar. Calculate the dry to wet ratio of the sample.

2.1.5 Bulk dry samples can be transported in gunny sacks to the plant or laboratory (Figs. 9–10). If the samples are quite dry, then plastic bags will do. Small amount of samples can be packed in durable small plastic bags. The samples should be provided with labels or tags showing the following information.

Fig. 4. Drying on coconut leaves close to the pier where the small boats used for hauling wet seaweed are docked.

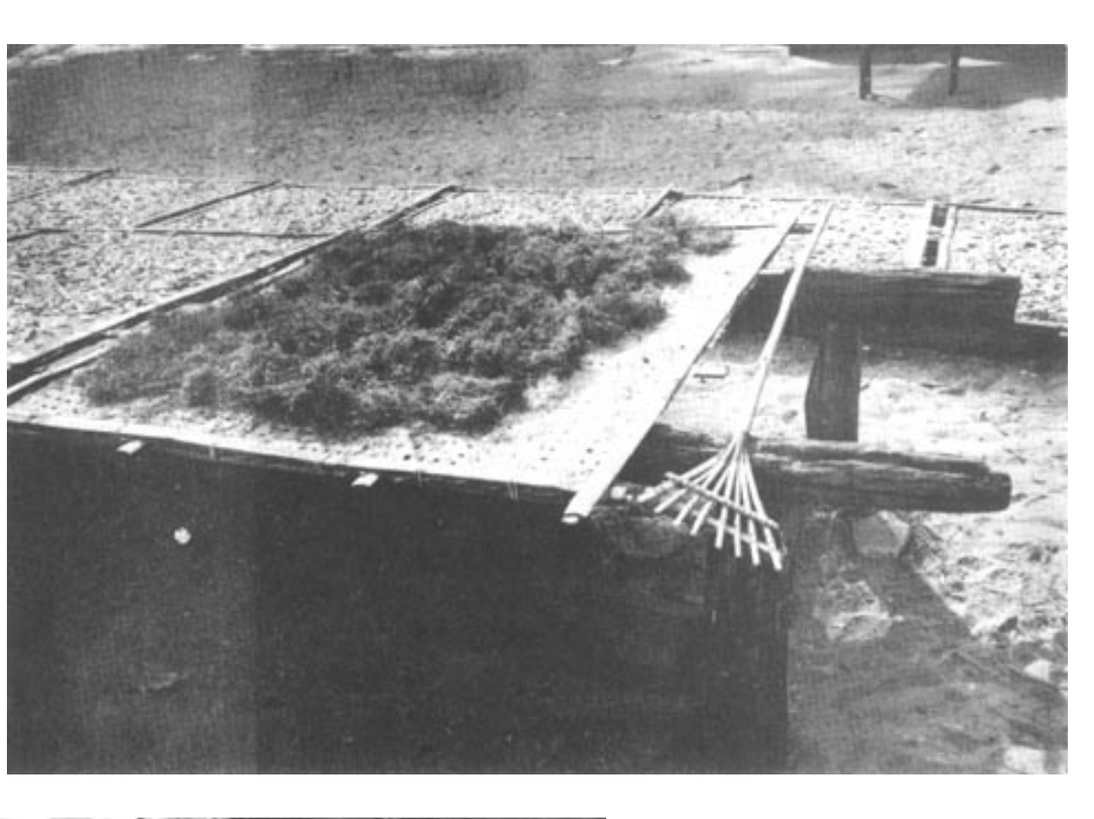


Fig. 5. Drying on bamboo mats with shrimp and fish

Fig. 6. Drying on bamboo mats close to the dock

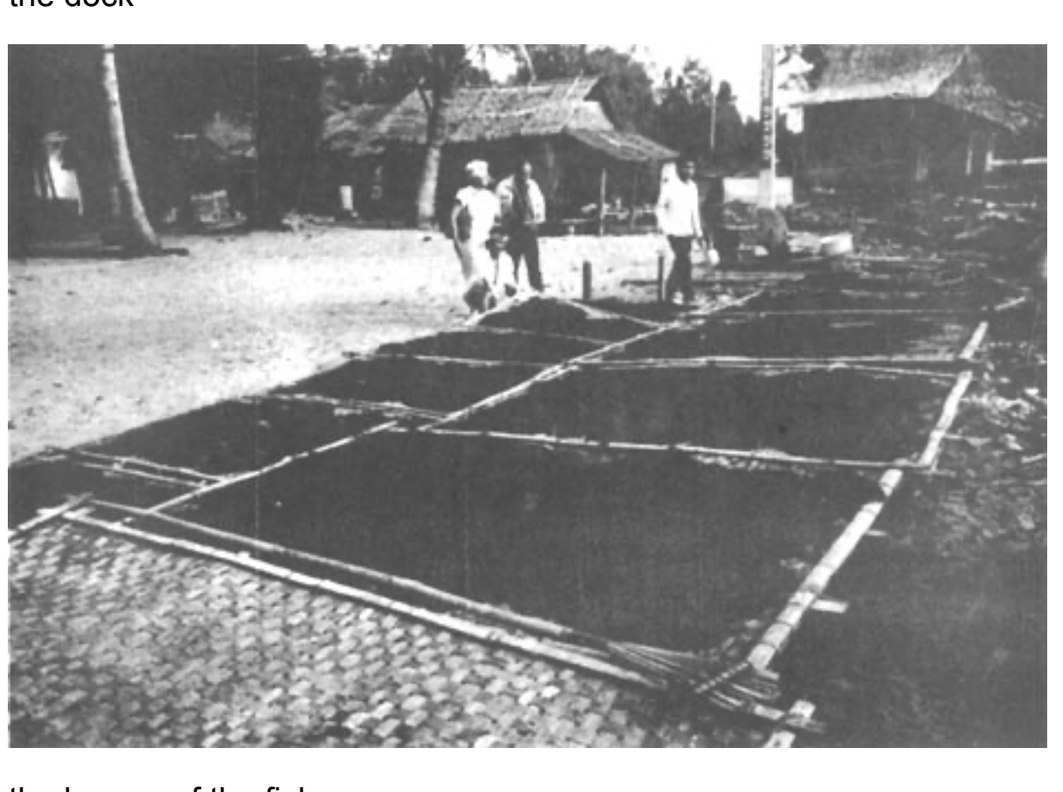


Fig. 7. Drying on bamboo mats close to the homes of the fishermen

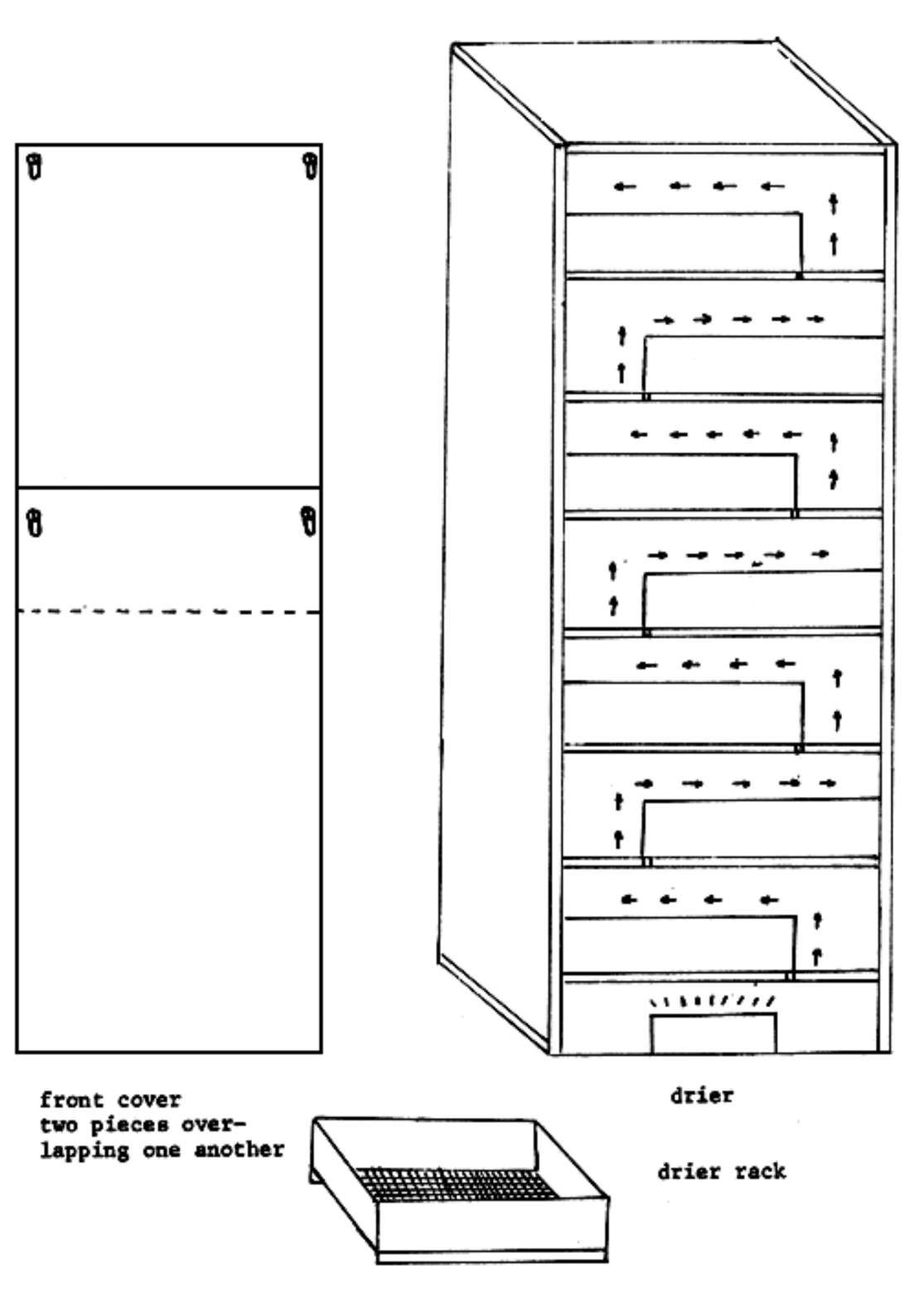


Fig. 8. Improvized drying oven

Fig. 9. Transporting of dried seaweeds in gunny sacks

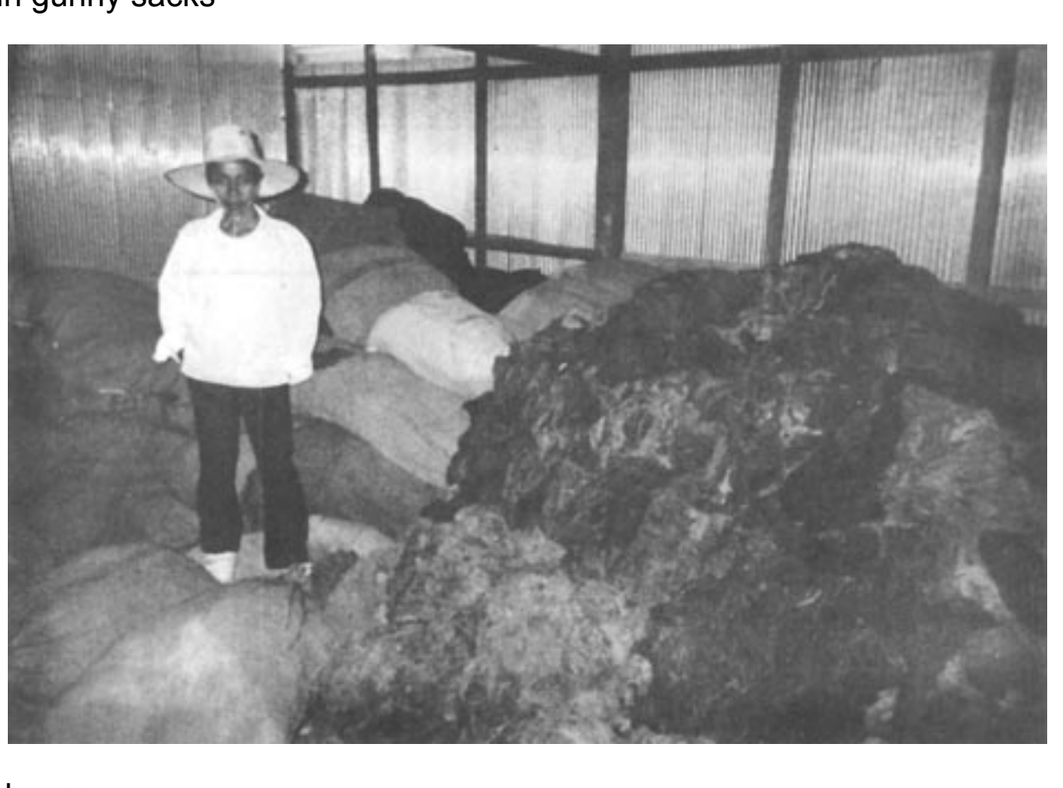


Fig. 10. The dried Gracilaria in the warehouse

Voucher or sample number	Place of collection
Species (if known)	weight
Date of collection	Name of collector

2.2 Determination of quality of seaweed sample

Although the samples have been cleaned and sorted fresh, yet there are still lots of impurities unnoticed or sometimes due to lack of time and facilities in the field samples are immediately dried after collection. So it will be necessary to determine the quality of the dry sample received in the laboratory or in the factory.

Percent of sand, rocks, shells, dry shrimp, small crabs, unwanted seaweeds, etc. Most samples received are just a kilogram or more so weigh out two 100 g samples for determination of impurities. Sort the samples separating the rocks, other seaweeds, shells and other impurities. Put each sample in a tared wire basket. Shake off the sand and fine impurities which could be separated. Collect the sand and other impurities and weigh.

$$\frac{\text{Weight of impurities}}{100\text{ g}} \times 100 = \%\text{ of impurities}$$

Weigh the wire baskets with the clean seaweed samples and then put them in an oven 100° and heat for 12 hours. Turn the oven off. Weigh the dried sample with the basket.

$$\begin{array}{rl} & \text{Weight of basket + seaweed sample before drying} \\ - & \text{Weight of basket + seaweed sample after drying} \\ \hline & \text{Weight lost (weight of moisture)} \end{array}$$

$$\begin{array}{rl} & \text{Weight of basket + seaweed sample before drying} \\ - & \text{Weight of basket} \\ \hline & \text{Weight of seaweed sample B.D.} \end{array}$$

$$\frac{\text{Weight of moisture}}{\text{Weight of seaweed sample B.D.}} \times 100 = \%\text{ moisture}$$

Weigh two 100 g sample cleaned of impurities. Soak the sample in 2 liters of tap water for 10 minutes. Repeat the soaking 2 more times. Drain the water and squeeze the remaining water off. Put the samples in aluminum pans and dry in a 70° oven for 12 hours. Weigh the samples after drying.

$$\frac{\text{Weight of dry sample}}{100\text{ g}} \times 100 \times \%\text{ clean anhydrous weed (CAW)}$$

The clean anhydrous weed value is going to be used for the calculation of the agar yield. In the factories large bulk of samples are cleaned and sorted to remove rocks, sand and other algae. The rocks and sand may cause problems during the extraction process. Other seaweeds may produce products other than agar.

2.3 Pre-extraction treatment

The pre-extraction treatment involves bleaching and alkali treatment in the processing of Gracilaria samples,

2.3.1 Bleaching. In tropical countries sun bleaching is very practical. The dry field sample is soaked in tap water or even in seawater if the bleaching is done in the field. The soaked sample is spread on bamboo mats, allowed to dry and then wet again by sprinkling water. The process is repeated until the Gracilaria sample is completely bleached. The bleached seaweed remains brittle and dry.

2.3.2 Bleaching with lime. Make a lime suspension and sprinkle this on the drying seaweed. Lime even helps in increasing the gel strength of the agar. Repeat the sprinkling of lime water on the seaweed sample until bleached.

2.3.3 Alkali treatment. The polysaccharides of Gracilaria have been reported as agaroids because of the high viscosity and low gel strength. To improve the gelling ability of Gracilaria verrucosa agar, Yanagawa (1938) treated the seaweed with aqueous sodium hydroxide solution. Since then the Japanese developed different methods of alkali treatment of Gracilaria for the industrial production of agar and thus making the seaweed one of the most important raw materials for the agar industry in Japan (Tagawa, 1968; Tagawa and Kojima, 1972). According to Guiseley and Renn (1975) Gracilaria became an important source of agar since the 1950s when different methods of alkali treatment to remove the primary sulfate group were developed. The authors claimed that as a result of such treatment Gracilaria agar gels are frequently stronger than Gelidium agars and in general the agarose obtained from such agars contains less sulfate. Shi and Tang (1982) used very strong alkali up to 45% sodium hydroxide, soaking the seaweed in the cold up to 40 days and found that the gel strength of the agar increased 10-fold. Depending upon the Gracilaria species it will be wise to use different concentrations of alkali, in the hot and in the cold with different duration of treatment. In the case of G. limanaeformis from Chile (Kim, 1970), treatment with 3–5% sodium hydroxide aqueous solution at 95° for 60–90 minutes was enough to get an agar gel strength of 910 g/cm^2 (1.5% conc.) by the Nikansui Shiki method. The concentration of alkali needed will depend upon how much sulfate is present in the original agar. As discussed earlier, the sulfate groups in some Gracilaria agars are alkali stable and cannot be removed by such treatment.

In a 5-gallon plastic tank which is provided with a drainage valve, put 14 liters of 20% sodium hydroxide solution. Put one kilo of dry sample into the sodium hydroxide solution and allow the seaweed to soak for 3 days. Drain the caustic alkali solution into another tank (this can be used for another 1 kilo batch of sample by just making up the volume to 14 liters by adding more 20% NaOH). Wash the treated seaweed with tap water until it is almost free of alkali. The first washing could be of future use. Dry the treated seaweed by spreading the sample on concrete or on bamboo mats under the sun. After drying calculate the percent yield of alkali-treated seaweed. In Taiwan such alkali-treated seaweed is being exported to Japan.

2.4 Extraction of agar

2.4.1 Weigh two 50-g samples from each of the resulting samples in the pre-extraction treatment process (such as 2.3.1, 2.3.2 and 2.3.3). Get six 2-1 capacity beakers and pour into each beaker 1 000 ml of water. Put each sample into each beaker and add enough acetic acid with stirring to pH 5.6-6.0. After soaking the seaweed samples in the acidic water for 30 minutes, drain the water and then add 1 000 ml of fresh water to each beaker. Better use stainless steel instead of glass beakers for the extraction of the agar for safety measures. Heat the samples on a water bath at 95° for one hour. While heating the seaweed samples, have some hot water ready in a wash bottle. All the six samples should be treated as follows: Transfer the hot seaweed to a blender. After blending return the gooey extract to the pot, wash the blender with hot water and combine the washing with the rest of the extract. Heat the extract for 3 hours, stir in 75 g filter aid for 30 minutes. Simultaneously heat the pressure filter (filter bomb, Fig. 11). Pour the extract into the pressure filter, add ca. 100 ml of hot water into the pot, scrape the sides of the pot with a rubber spatula and combine this washing with the rest of the extract to be filtered. Close the pressure filter and connect it to an air compressor and filter under 50–80 psi pressure. Wash the filter cake with 200 ml hot water and mix the washing with the rest of the filtrate and pour this into 2 – 9" × 13" aluminum pans. Allow the agar sol to gel at room temperature. Cut the gel into small squares with a thin blade spatula and put the pan in the freezer. Remove the pan from the freezer the next day and allow the gel to thaw. The thawed gel is transferred to a strainer which is lined with a very fine polyester piece of cloth. Drain the thawed water, wash the gel with tap water until almost colorless, wash with distilled water, squeezing the gel to remove washing and then finally with isopropanol to facilitate drying. Squeeze the isopropanol off. Shred the flaky agar, put in a tared aluminum pan or tray and dry at 55–60°C. Weigh the agar after drying and grind to a 40-mesh powder in a Wiley intermediate mill. Calculate the clean anhydrous yield by finding out how much of the 50 g treated sample is equivalent to the clean anhydrous weed.

$$\frac{\text{Agar yield}}{\%\text{ CAW}} \times 100 = \%\text{ yield of agar}$$

$$50 \times \frac{\%\text{ CAW}}{100}$$

2.4.2 Treatment of agar yield from (2.3.1 & 2.3.2) samples. Weigh l0 g of each of the powdered agar. Prepare 200 ml batches of 20% NaOH in 2 plastic beakers (glass beakers will be etched by the very strong alkali). Suspend the powdered agar in the strong alkali for 3 days at room temperature stirring the suspension every now and then. Drain the alkali by straining the agar through fine cloth. Wash the agar until free of alkali and then treat with 0,1% hydrocholoric acid to neutralize the residual alkali. Wash the iso-propanol and squeeze off the alcohol. Shred the agar flakes and dry in a 55–60°C oven. Weigh and calculate the percent yield.

A prototype agar extraction set-up is shown in Fig. 12.

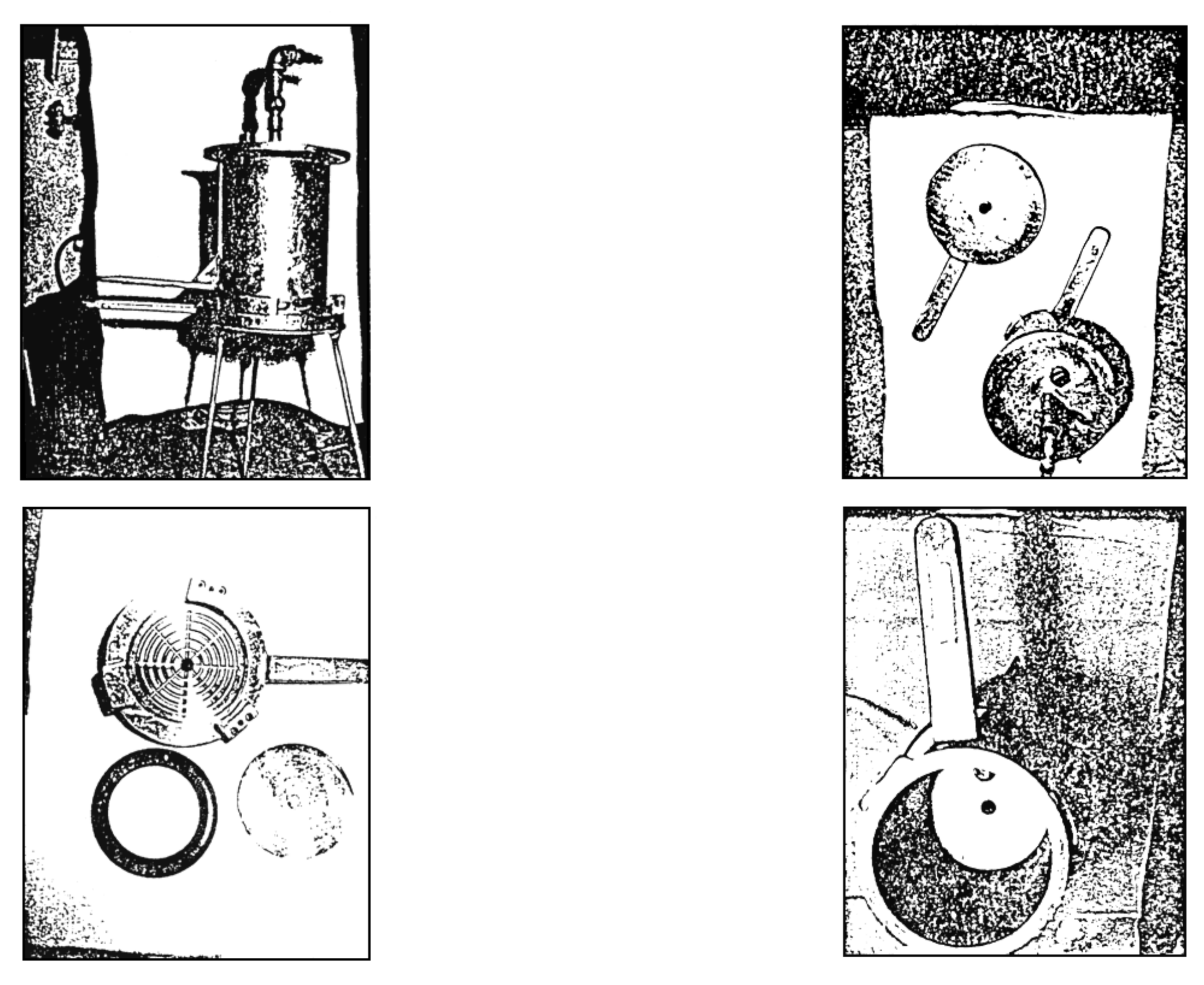


Fig. 11. Pressure filter

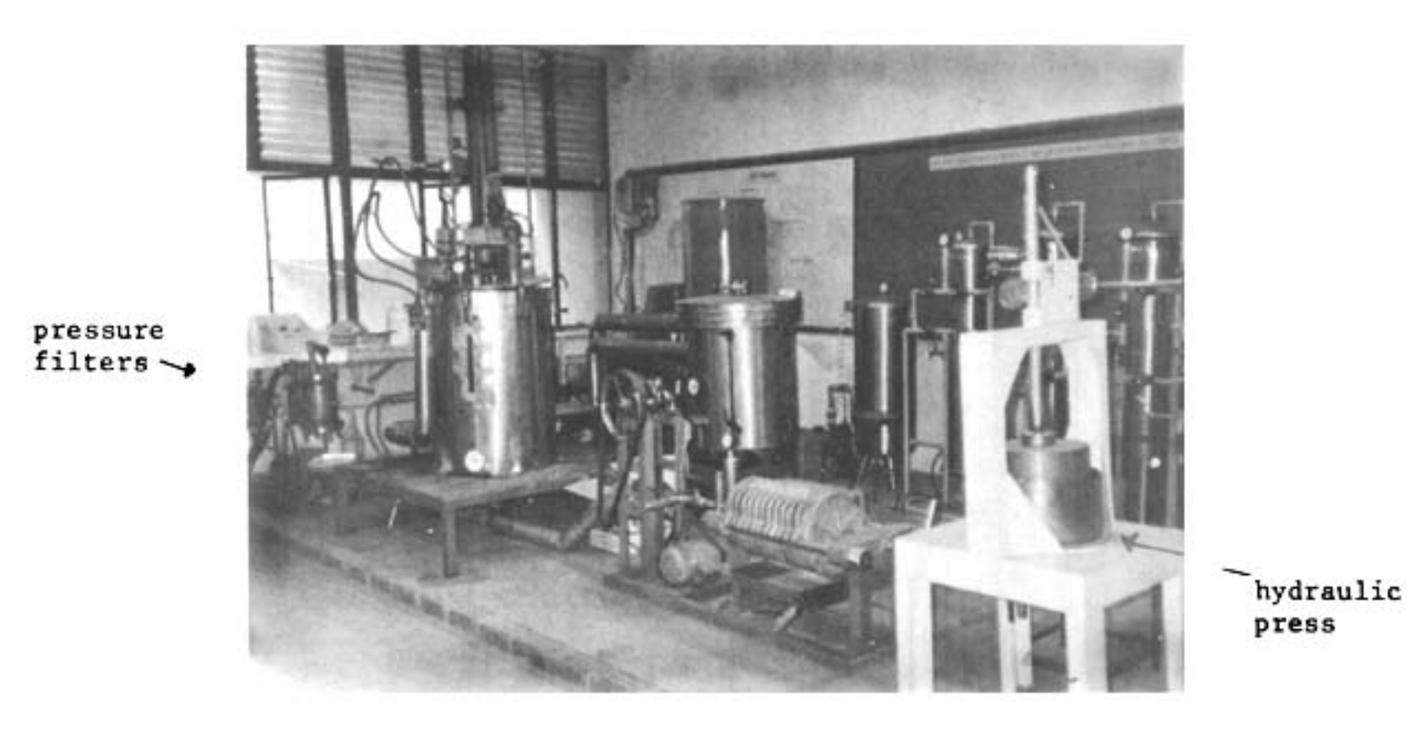


Fig. 12. Prototype agar extraction set-up (capacity - 200 liters)

2.5 Extraction of agarose

Agarose has been fractionated from the charged polysaccharides of agar by the acetylation method of Araki (1937) and by the Sephadex chromatography of Duckworth and Yaphe (1971). Allan, et al (1971) used chitosan Sviridov, et al (1971) rivanol, Fuse and Goto (1971) acrinol, Hjerten (1962) cetylpyridinium chloride, Barteling (1969) ammonium hydroxide and Egorov, et al (1970 ammonium sulfate for the precipitation of agaropectin. Russel, et al (1964) devised a method of precipitating agarose with polyethylene glycol.

2.5.1 Extraction of agarose from Gracilaria agar (Blethen, 1966).

Blend a 12 g agar sample with 2.4 g k-carrageenan and 50 g filter aid. Add the mixture to 800 ml water with stirring and boil for 5 minutes to dissolve the agar and carrageenan completely. Then add 200 ml of 5% benzothonium chloride rapidly, dropwise along the sides of the beaker, a bulky curd-like precipitate forms immediately. Filter the mixture while hot under low pressure (10–20 psi) using 2 layers of Whatman No. 5 filter paper laid on top of the filter felt. Rinse the filter cake with hot water (100–200 ml), pour the combined filtrate and washings into a 9" × 13" rectangular aluminum pan, allow the agarose solution to gel at room temperature, cut the gel into cubes with a spatula and then freeze overnight. Thaw the agarose gel the next day, wash with tap water, then with distilled water until almost colorless and finally twice with 85% isopropanol. Shred the agarose mass, dry at 55–60°C and powder to 40 mesh in an intermediate Wiley mill. Calculate the yield.

2.5.2 Extraction of agarose from the seaweed

Weigh 100 g of the dried Gracilaria sample, put this in a 2-1 stainless steel beaker, heat at 85-90°C for 1 hour with a solution of 30 g NaOH in 1 000 ml water. After heating, drain the alkaline solution and wash the seaweed 3 times with tap water until free of alkali. Soak the seaweed in 1 liter of 0.01% hydrochloric acid for 10 minutes then wash 3 times with water. Heat the sample with 700 ml water on a boiling water bath for 1 hour, blend, return the paste and washing into the pot and heat further for 3 hours at 95°C. Stir 75 g filter aid into the sample for 30 minutes and then filter hot under 50–80 psi pressure. Combine the filtrate and washings and return this to a clean 2-1 stainless steel pot or beaker, heat the extract in a water bath, stir with 2.4 g k-carrageenan and 50 g filter aid. After 5 minute heating, add 200 ml 5% benzothonium chloride solution, allowing it to flow along the sides of the pot, boil for 3 minutes, filter hot under 10-20 psi pressure in a preheated pressure filter through two layers of Whatman No. 5 filter paper. Wash the filter cake with 200 ml hot water. Combine the filtrate and washings and pour this into a 9" × 13" rectangular shallow pan. Allow the agarose sol to gel at room temperature, cut the gel into cubes with a narrow blade spatula and freeze overnight. Thaw the frozen gel the next day and transfer the thawed gel into a strainer lined with a very fine polyester cloth. Squeeze the thaw water off, wash the gel with tap water until colorless, next with distilled water, then with 60% and then 85% isopropanol. Squeeze the agarose to remove the washing liquid as much as possible. Shred the agarose flakes and dry at 55-60°C. Weigh the dry agarose and calculate the yield.

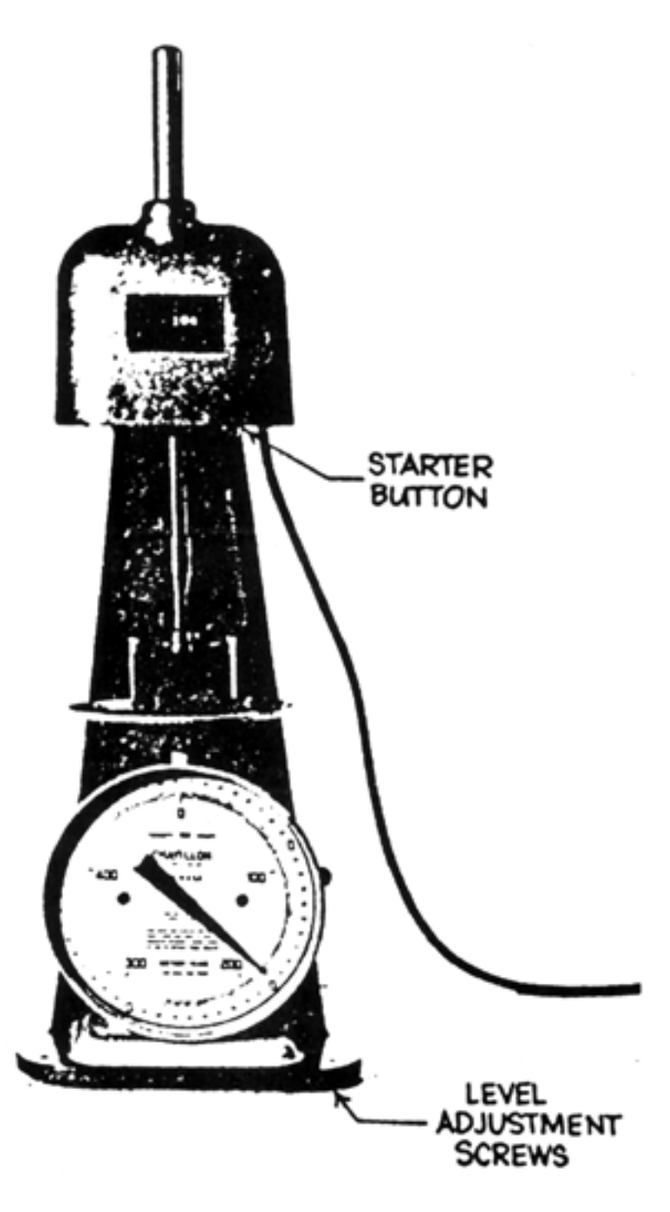
2.5.3 Extraction of agarose from the seaweed using powdered Eucheuma striatum instead of k-carrageenan

Use the same method as in 2.5.2. above except that 5 g of powdered Eucheuma striatum is added to the Gracilaria paster, heat for 3 hours, add the filter aid, stir for 30 minutes and filter hot.

2.6 Determination of extract properties

2.6.1 Gel strength determination

Weigh 2.7 g of powdered agar on a tared weighing paper. Pour about 100 ml hot water into a tared electrolytic beaker and put the weighed sample into it. Allow the agar to soak. Break the lumps with a narrow spatula and be sure to wash the spatula well with hot water and allowing the washing to drip into the beaker with the sample. Heat the beaker with the sample using a beaker holder and clamp on a water bath at 95°C with continuous stirring. A mechanical stirrer with a short paddle to fit into the bottom of the beaker is necessary for this purpose. Stir until the agar is dissolved. It will be necessary to replenish the water lost due to evaporation every now and then. Wash the stirrer with hot water allowing the washing to drip into the beaker. Weigh the beaker and sample on a top loading balance and add enough hot water from a wash bottle to make the weight of the agar solution to 180 g. Return the beaker with the agar solution into the water bath and stir for about a minute to mix the agar and water well. Pour the hot agar sol into a crystallizing dish (50 × 70 mm) up to the rim. Allow the agar to gel at 20°C and keep in a circulating water bath for 2 hours for the FMC gel tester (Fig. 13). and 24 hours using the Nikansui gel tester (Fig. 14). Cover the dish to prevent loss of water from the gel. Before determining the gel strength invert the gel so that the bottom surface is on top. This can be done by allowing the thin blade of a stainless steel spatula (20 cm long with the flat blade about 5 cm long) slide between the gel and the side of the dish and holding the dish in an inverted position until there is an air space between the dish and the gel so the gel will just slide and come off from the container. Put the gel back into the dish so that the bottom surface is on top. Then measure the gel strength. It has been observed that the top surface has always a stronger break force than the bottom surface. Determine the gel strength by FMC Gel Tester or by the Nikansui method.



An automatic, versatile gel testing instrument. Measures the “break force” required to rupture both milk and water gels, thereby determining the gel strength. May also be used for measuring curd tension of dairy products and strength of certain foams.

Fig. 13. Gel tester

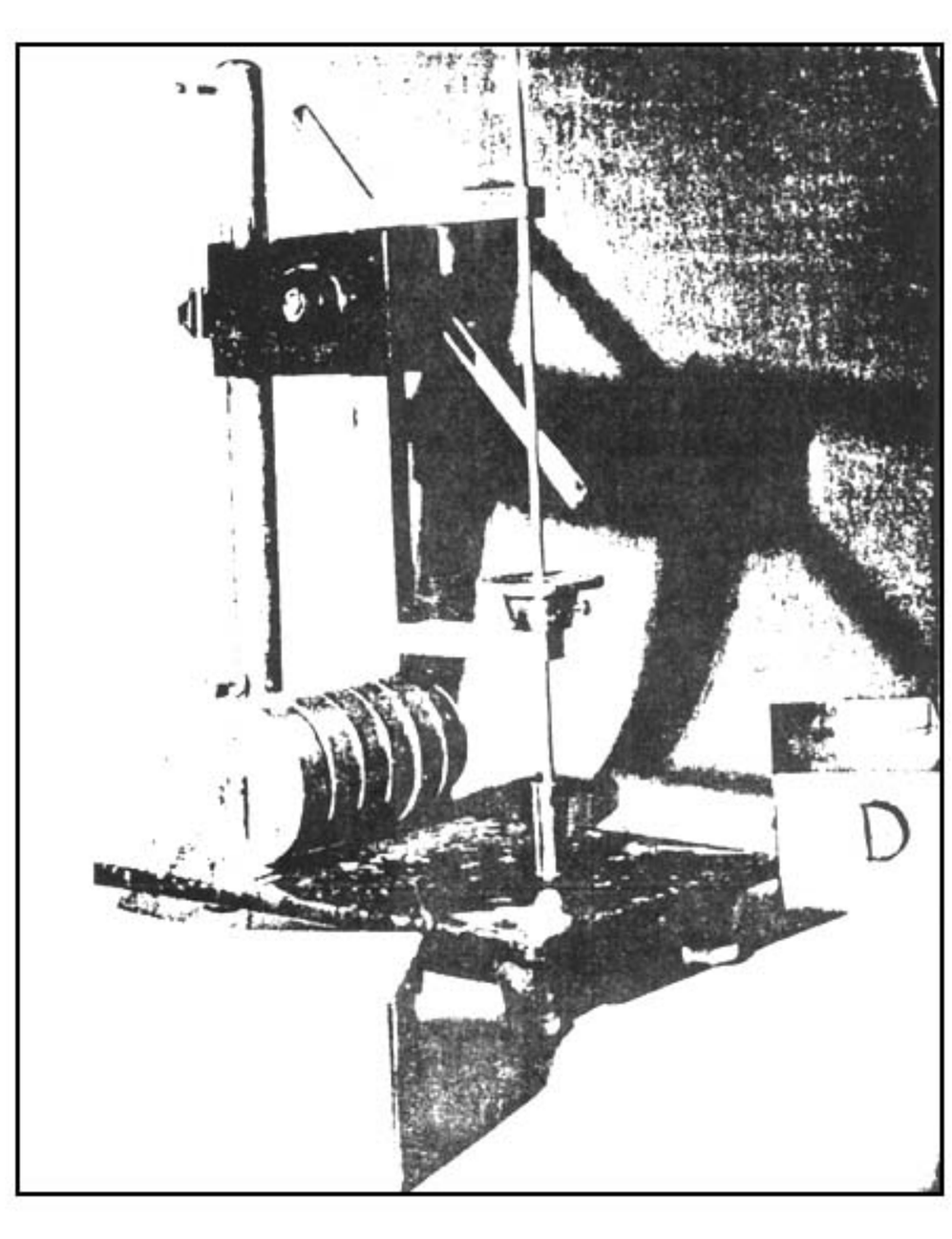


Fig. 14. Gel strength measurement. Nikansui method.

Gel strength determination by the Rowerbal method (Meer, 1980) (Fig, 15). Weigh out 3.00 g agar analytically. Add the water (hand stirring using a glass rod) and disperse the agar uniformly. Heat the beaker rapidly on an asbestos wire gauze with a gas flame with constant stirring until the agar solution boils. Heating should be regulated to 10 minutes total time. After boiling starts, make the flame smaller and boil 1 minute. Remove the beaker from the flame and break the foam by stirring and swirling for a few minutes. Make sure that the weight of the hot solution is at least 220 g immediately after boiling by adding water if necessary. Allow it to stand at room temperature for 2 hours away from air current. Keep the beaker with the gel in a 25°C water bath for 22 hours. The gel should weigh at least 195–205 g. Record the weight of the gel. Separate the gel that adheres to the side of the beaker by sliding a thin blade narrow spatula. Place the beaker containing the gel on the right pan of a two-pan balance. Balance the beaker and gel weight with a 1-liter beaker and conventional weights. Press down the right pan and lower the plunger (diameter, 1.13 cm; cross-sectional area 1 cm^2) against the top gel surface at a position approximately 10–14 mm from the outer edge of the gel and at the same time keep the beaker centered on the balance pan. Allow the water to run into the beaker from a graduated 1 000 ml separatory funnel at a rate of approximately 200 ml/minute. The flow rate should be adjusted with the stopcock fully open. Close the stopcock when the gel breaks. Record the amount of water used. Pour the water back in the funnel. Repeat the last six steps until there are four holes, placed symmetrically about a circle in the order of 0°C, 180°C, 90°C and 360°C. Get the average of the readings and calculate the gel strength according to the following formula:

$$G = x\,\frac{W}{200}$$

where G = gel strength in grams per square centimeter
W = millimeters of water necessary to break the gel
X = weight of gel in grams (to ± 0.lg) after 24 hours

2.6.2 Gelling temperature determination (Guiseley, 1970)

Dissolve 750 mg of agar in 50 ml water with stirring for 5 min in a 957deg;C water bath. Pour enough of the agar sol into 18 × 150 mm test tubes just enough to reach the immersion line of the thermometer placed inside the tube. Put the tubes in a rack (about 7 can be accommodated at a time) in a 60°C water bath. When the temperature of the tubes is the same as the bath, pass cold water through a copper coil in the bath. When the temperature in the tubes reaches 50°C, adjust the flow rate of the cold water so that the temperature drops about 0.3–0.5 per minute. Carefully withdraw the thermometers from the tubes. Gelation occurs when a neat hole remains and the agar sol does not flow back to form a normal miniscus. Record the gelling temperature to the nearest 0.5°C.

2.6.3 Melting temperature determination (Fuse and Goto, 1971)

Prepare 1.5% agar sol as above (2.6.2) and pour 5 ml of the sol into 18 × 150 mm test tubes and allow the sol to gel for one hour at 20°C. Put the tubes in a rack in a 60°C water bath. On the surface of each gel sample drop a lead shot (diameter ca. 1 mm, 25 mg). Gradually raise the temperature of the bath at 1°C per minute. When the gel melts the lead shots drop to the bottom of the tube. Record this as the melting temperature. Sometimes it is necessary to break the surface of the gel when dropping the lead shot.

2.6.4 Sulfate determination (AOAC Gravimetric method)

Determine the moisture content of the agar by weighing 100 mg samples and drying in vacuum dessicator at 70°C for 12 hours. Determine the sulfate by the normal gravimetric method. Weigh 0.5 g samples to the nearest mgm into 125 ml flasks. Add 10 ml conc. nitric adid to each sample. Insert the air condenser and boil (123°C). Do not allow the sample to dry up. Add more acid to insure complete oxidation which takes about 30 minutes. The final volume of digest is about 2–3 ml. Cool the sample and add 2–3 drops of 40% formaldehyde solution through the air condenser to reduce the excess HNO_3 . There will be evolution of N_2O_4 (brown gas) with a rise in temperature (the reaction should be performed in a fume hood). The reaction is complete when further addition of HCHO does not produce any brown gas. After completion of the reduction, wash the air condenser with distilled water allowing the water to flow into the flask. Filter the reaction mixture into a 400 ml beaker.

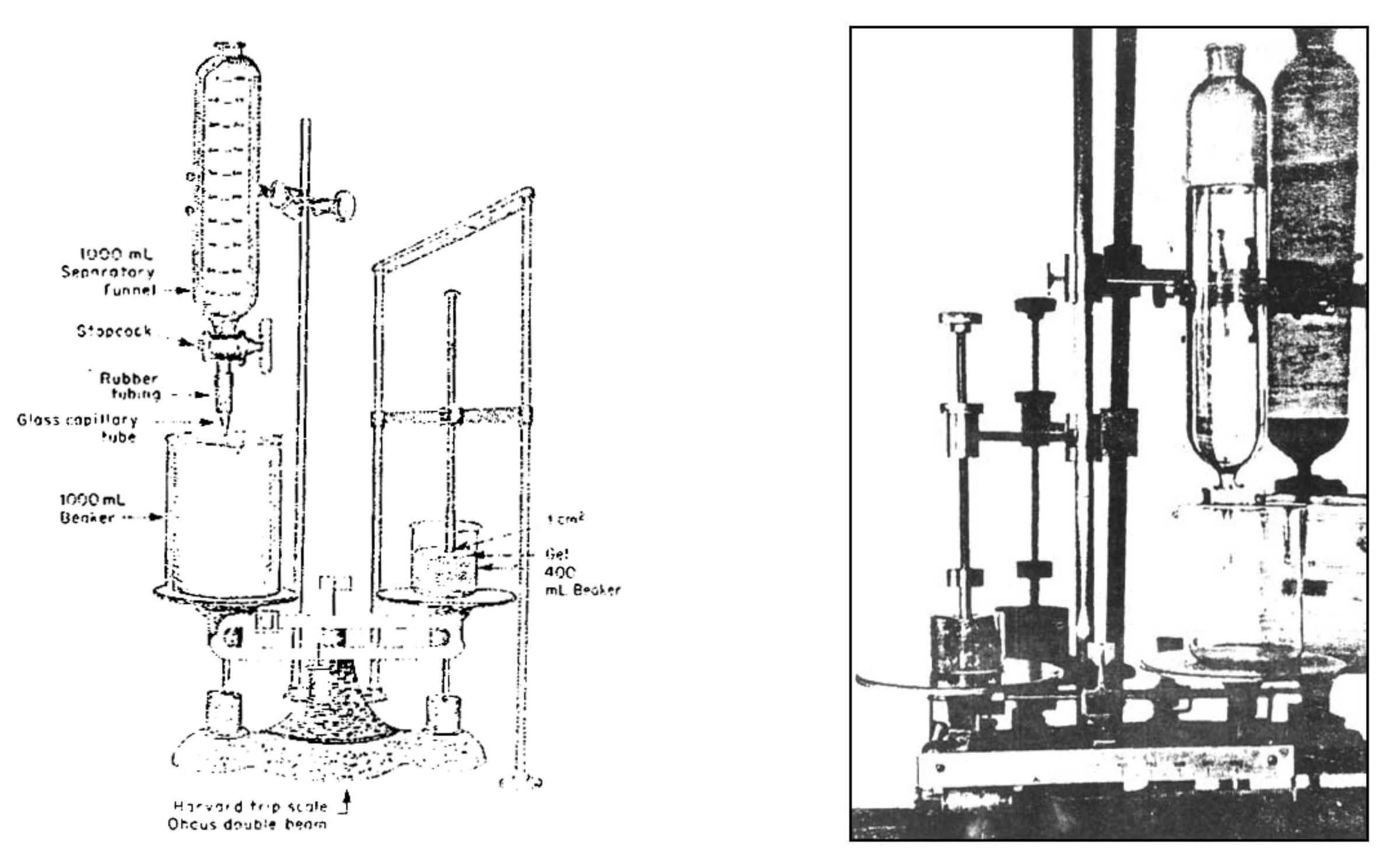


Fig. 15. Rowerbal method

Add 0.5 ml conc. hydrochloric acid to the filtrate. Add distilled water to bring the volume to 200 ml, heat to boiling and add 10 ml M/4 $BaCl_2$ solution dropwise with constant stirring (hot plate-stirrer is advised). Continue boiling for 5 minutes and then set aside in a warm place for 5 hours.

Ignite 30 ml fine porosity (filtering) crucibles for 30 minutes at 700°C in a muffle furnace. Cool in a desiccator for 15 minutes and weigh. Filter the $BaSO_4$ precipitate through the tared crucibles with suction. Wash the precipitate off from the beaker with hot water from a wash bottle using a rubber policeman to scrape off the precipitate from the sides of the beaker. Wash the precipitate five times with hot water or until the precipitate is free of chlorides.

Wash the outer surface of the crucibles with hot water before drying them in an oven. Transfer the crucible with the $BaSO_4$ to a muffle furnace preheated to 700°C and ignite the $BaSO_4$ for 1 hour or until the weight is constant. Remove the crucibles from the muffle and transfer to a desiccator to cool. Weigh the crucibles with the $BaSO_4$. Calculate the weight of the $BaSO_4$. Calculate the % sulfate in the samples.

$$\%SO_4 = \frac{41.16 \times \text{wt. } BaSO_4}{\text{Weight sample}} \times 100$$

The sulfate and methoxyl content of agarose can also be determined by ion-exchange chromatography. Another method of determining the sulfate is by the Jones and Letham method (1954). For the determination of sulfate in agarose by the gravimetric method 2 g samples are used. The ash content can be determined by igniting the sample at 550°C to constant weight. Agar clarity can be determined by measuring the transmittance of 1% agar gel at 500 nm wavelength using distilled water as standard.

3. AGAR AND AGAROSE APPLICATIONS

The great bulk of agar produced worldwide is used in the food industry. Only 4–5% is used as bacteriological media. According to Santelices and Doty (1989) close to 5 000 tons of agar are processed annually from 25 000–30 000 tons of Gracilaria. Agar has that unique property of holding so much water in its gels (99.9%) so it is used widely for such gelling and stabilizer qualities not only in food but also in other industries.

3.1 Applications in the food industry

Agar finds its way as stabilizer in pie fillings, piping gels, meringues, icings, cookies, cream shells and other food products.

For icings the concentration of agar ranges from 0.2 to 0.5%. Agar stabilizes the icing and prevents the adhesion of the sugar coating to the wrapper. An improved stabilizer for icings is prepared by addition of 0.4 to 0.6% surface active agent to an agar level of 1.2 to 2.4% agar.

For doughnut glaze, 0.5 to 1.0% agar is used depending upon the amount of sugar needed. Agar increases the viscosity of the glaze, increases its adhesion to the doughnut, its quicker setting and flexibility and reduces chipping and cracking. Agar prevents the glaze from melting.

Agar is used as bulking agent in low calorie breads and at a concentration of 0.1 to 1.0% as antistaling agent in breads and cakes.

As an additive in jellied candies and confectionary specialities as marshmallows and sugared fruit slices, filler in candy bars and preparation of rigid gels agar is used in a concentration of 0.3 to 1.8%.

Agar gives smoothness to sherbets and ices in combination with other gums like locust bean gum at such combination of 0.12% agar, 0.07% LBG and 0.2% gelatin. Used at 0.05 to 0.85% in cream cheeses and yoghurt. Reduces wheying off and improves body and slicing qualities in cheeses.

Thickening and gelling agent in poultry, fish and meat canning at level of 0.5 to 2.0% in the broth weight. Helps preserve color in meat products.

For sausage processing agar keeps the moisture and prevents evaporation and drying up of the sausage and prevents weight loss and preserves the sausage, too. Make a 1.5% solution of agar by heating water to a boil then adding powdered agar and heat until the agar dissolves. Remove the agar solution from the heat while still warm and liquid and add 2.5 to 5 liters of agar sol to 50 kg sausage mass gradually kneading the mass while the agar sol is being added. The process should be done in strictly sanitary conditions.

Biscuits are usually coated with chocolate or other coatings to improve taste. To prevent the glaze or coating (flat icing) to break during packing or transport agar is added to the chocolate to give elasticity and better adherence. The chocolate is melted and the agar added, 35 ml of 1.5% agar solution for every 100 g of chocolate or 350 ml of warm agar solution to 1 kg of molten chocolate, stirred continuously until the mixture is well mixed and homogenized.

The following are some formulations and recipes for application of agar in foods:

Icing Stabilizers

Agar is used in the formulation of water-based, quick-set and slow-set bakery icings needed for wrapped and unwrapped baked goods. The flat or sweet-roll icings are prepared by boiling the agar stabilizer in a sucrose-water solution, then adding this hot mixture to the icing sugar. The icing is then applied hot at 50–60°C and the iced item can be wrapped as soon as 60 seconds later. Shorter or longer drying time for the icing can be regulated by increasing or decreasing the amount of agar in the following formulation:

	Per cent
Agar	0.35
Salt	0.3
Emulsifier	0.4
Starch	1.0
Sucrose, granulated	15.0
Icing sugar	73.0
Water q.s.	100.0

A cake icing with a slightly slower setting time may be prepared by first dry-blending agar with salt, emulsifier, dextrose, starch and sucrose. This dry mix is metered into well-creamed Dex-Kreme (a Corn Products product); the entire mixture is heated to 48°C while mixing, and is then ready for application. The suggested basic formulation for the icing is:

	Per cent
Agar	0.5
Salt	0.2
Emulsifier	0.6
Dextrose	0.7
Starch, pregratinized	1.0
Sucrose	12.0
Dex-Kreme q.s	100.0

Condensed Sweet Agar Jelly

Highly sweetened, mild-flavored candy of low-acidity, a modernized version of Yokan. This is usually wrapped in oblate. Agar is dissolved in boiling water to which sugar and invert sugar are added. The thick solution is cooked to 1C6°C for further evaporation. Cool briefly, then add the fruit juice and/or synthetic organic acid, flavors, color additives are added, mixed and allowed to gel overnight. The content of solids is about 70 to 75%. Further drying is done by placing the gel in a 55°C oven for 15–30 hours to reduce the moisture content to 16 to 18%. The gel is cut into small pieces and wrapped in oblate and then in cellophane. The ingredients are:

	Weight (g)
Agar	50
Water	2000–3000
Sugar	1000–2000
Invert sugar	1500–3000
Citric acid	2
Color and flavor as desired	

Oblate or Edible Paper

"Oblate" is a registered trade name for edible paper, or edible film, which is made from starch and agar. Its thickness is 10–15 µm. The product is generally sold as 93 mm diameter circles at drug stores in Japan.

Oblate is prepared by adding 100 parts of a 5% aqueous starch solution to 200 parts of 2.5% aqueous agar solution and brushing the hot mixed solution onto the glossy surface of a metal or glass in order to produce a dry, rigid film which is then removed and cut into the desired shape. It is a convenient and useful material for wrapping the condensed, sticky jelly and for doses of powdered medicines.

Yokan is the largest single application of agar in Japan. Fruits can be substituted for beans. Yokans are molded into ingot-shaped bars and packed and sealed in laminated film containers. The recipe is as follows:

	Percent	
Water	26.5	
Sugar (total)	54.3	
(reducing sugar, 1.3%)		
Salt	0.2	
Water-insolubles	12.4	18% total dry weight, mashed beans
(unrecovered)	5.6	
Agar	1.0	

Ref. T. Matsuhashi and T. Shimada. 1971. J. Jap. Soc. Food Sci. Technol. 18:360

Mitsumame is a sweet dessert especially popular among women (Japan). It is colorful and attractive and served in a glass bowl. It is prepared by soaking 1 cup of 1% agar gel cubes (1 cm^2) in syrup, together with pieces of sweetened mandarin, pineapple, cherries and peas (Pisum sativum var. arvense; mame means peas). Agar is able to keep the corners of the cubes which permits sterilization of the canned product. The agar gel used here has a high melting point (minimum, 85°C).

Canned Fruits

Fruit pulp	20 kg
Water	18 l
Sugar	28 kg
Agar	450 g

Mix the agar with water and stir continuously for about 20–30 minutes so the agar swells and moistens. Meantime, mix the pulp and the sugar, heat and then add the agar suspension. Mix the ingredients and boil for a few minutes with continuous stirring. Before pouring the mixture into the molds, add the appropriate flavor, color and preservative.

Fruit Jellies and Pastes

Apple Paste

Agar	200 g
Apple pulp	10 kg
Sugar	10 kg

Suspend the agar in cold water, add the rest of the ingredients and boil for 10–15 minutes. Pour into molds.

Sweet Potato Paste

Agar	160 g
Sweet potato pulp	10 kg
Sugar	10 kg

Suspend the agar in cold water, mix the other ingredients thoroughly and boil 15–20 minutes. Pour into molds.

For jellies reduce the amount of agar by 50%.

Jellies and Sweets

Agar	3 g
Sugar	100/150 g
Ground coconut	10 g
Tartaric acid	0.5 g
Water	120 ml
Coconut oil	3 drops

Using the same quantities above, but substituting cooked chestnut puree, jellies with glazed chestnut flavor can be made.

Glucose can be used as substitute for the sugar. Jellies should be dried at room temperature. Jellies are covered with icing sugar to prevent them from adhering to the container or to one another.

Agar	2.5 g
Sugar	100 g
Water	120 ml
Tartaric acid	0.5 g
Flavor (vanilla)	3 drops

Dissolve half the sugar in water before boiling, gradually add the agar and stir until the mixture boils. Add the remaining sugar until the agar is completely dissolved which will take from 7 to 10 minutes heating. Boil moderately and continue stirring. Pour the solution into molds or cylindrical tubes which can be cut to convenient sizes. The jellies are coated with icing sugar.

Jams and Candied Fruit

Agar	15–20 g
Fruit pulp	10 kg
Sugar	10 kg
Water	1.5 l

Mix the pulp thoroughly with the sugar, heat and boil over a water bath. Add the agar which has been dissolved in water and continue heating for 3–4 minutes with continuous stirring. Pour into molds.

Vanilla Ice Cream

Agar	2.6 g
Vanilla	0.2 g
Sodium carbonate	0.1 g
Food color	0.05 g
Milk	500 ml

Mix the agar with a small quantity of milk, add the rest of the ingredients then boil from 5–10 minutes. Beat after cooking.

Lemon Ice Cream

Agar	2.25 g
Sugar	100 g
Color	0.05 g
Water	150 ml
White wine	150 ml
Juices of 2 lemons	
Grated lemon peel	

Dissolve the agar in water, mix all the ingredients and filter while still warm. Allow to cool and beat.

Peach Ice Cream

Agar	2.5 g
Peach pulp	250 g
Syrup (30% sugar)	250 g

The peach pulp can be prepared from fresh fruit or by straining the contents of a tin. Dissolve the agar in the syrup and mix it with the peach pulp. Allow to cool and beat.

Custard Pies and Custards

Agar	1.6–1.8 g
Starch or flour	0.8 g
Sodium carbonate	0.1 g
Coloring	0.05 g
Vanilla	0.2 g
Sugar	70 g

A mix of the above ingredients is dissolved and boiled 5–10 minutes with half a liter of milk. For custards, use twice the quantity of milk.

Household Uses of Agar

Agar can be added to milk, coffee, fruit, wine, soda, etc. Add agar to the drink, heat and stir continuously until the mixture starts to boil. Turn off the heat and add a little sugar. Add more coffee, fruit, milk, wine, etc. according to taste. Mix well and pour into a mold and cool.

Coffee Table Jelly

Agar	2 tsp
Instant coffee	4 g
Water	300 ml
Sugar to taste	

Orange Table Jelly

Agar	2 tsp
Orange juice	300 ml
Sugar to taste	

Fruit Table Jelly

Agar	2 tsp
Soda water	100 ml
Water	200 ml
Sugar as required	

Prepare the same way as coffee jelly just substituting fruit for the coffee.

Soda Water Table Jelly

Agar	2 tsp
Instant coffee	100 g
Water	200 ml
Sugar as required	

Dissolve the agar in water and heat with stirring until dissolved. Remove from heat and allow to cool a little, then add soda water and then allow to cool.

Wine Table Jelly

The preparation is the same as soda water table jelly using 50 ml wine and 250 ml water.

Milk Table Jelly

Dissolve the agar in water and boil. Mix thoroughly 2 tbsp. milk powder, enough sugar to taste, half an egg. Add the warm agar solution and heat. Pour into molds and cool.

Liqueur Table Jelly

Agar	1 tsp
Liqueur (peppermint)	50 ml
Water	100 ml
Sugar to taste	

3.2 Applications as culture media

In microbiology or solid culture media agar is used at a concentration of 1 to 2%. It is possible to culture anaerobes in air-exposed broths because at low concentration agar prevents the entry of oxygen into the liquid media and the usual range used is 0.007 to 0.03% agar. A very important application of agar is in tissue culture. With the growing interest in tissue culture as a standard method of propagation of selected strains of orchids from the meristem to grow virus-free clones and other ornamental plants, vegetable, fruit crops and other agricultural products the demand for agar as culture media is becoming so competitive so that the price has gone up immensely. Some tissues which are being cultured can tolerate food grade agar which offsets slightly the high cost of crop propagation. Propagation from seed of some forest trees involves the long wait for the trees to bear fruit and seeds to mature but with tissue culture the young tissues are taken and grown on nutrient media which thus facilitates reforestation within a shorter period of time.

Of course agar has been known for more than a hundred years as culture media for the separation of bacterial cultures in bacteriology.

3.3 Agar in impression materials (Meer, 1980)

As impression materials agar is used in prosthetic dentistry, in tool making and in criminology for making accurate casts. The formulation for dental impression material is as follows:

Ingredients	Per cent
Agar	13–17
Borates (borax)	0.2–0.5
Sulfates (K_2SO_4, $MgSO_4$)	1.0–2.0
Wax, hard	0.5–1.0
Thixotropic materials	0.3–0.5
(Emulsifying agent is triethanolamine)	
Water q.s. to make	100

The commercial agar dental impression material can be managed by changing the gel into a sol with heat. The material is placed in an impression tray in the sol state and impressed against the mouth tissues which is to be reproduced as a dental stone. The tray is held rigidly in place and water is circulated through the cooling tubes attached to the surface of the tray. When the impression material has gelled the tray is removed and the impression is prepared for the pouring of the dental stone.

The gelation temperature of agar is approximately 36–38°C, the temperature at which the gel changes to the sol is 60–70°C. Such property makes agar useful for such application.

3.4 Agar in medicine and pharmacy

Agar has been used as laxative for decades because when hydrated it provides smooth nonirritant bulk in the digestive tract. Such agar is in the form of flakes which absorb from 12–15 times its weight of fluids. Agar is used as an ingredient in the preparation of capsules and suppositories, in surgical lubricants, in the preparation of emulsions, as suspending agent for barium sulfate in radiology. Agar is also used as a disintegrating and excipient in tablets. Sulfated agar has antilipemic activity similar to heparin. It is believed to inhibit the aerobic oxidation of ascorbic acid,

3.5 Other applications of agar

Agar is used for clarifying beer, wine or Japanese "sake". It finds applications in photographic stripping films and paper. It is used in solidified alcohol fuel, dyed coatings for paper, textiles and metals and in pressure sensitive tapes, as flash inhibitor in sulfurmining explosives, as an ingredient in cosmetic creams and lotions, as corrosion inhibitor for aluminum, for the action of nicotine as insecticide in plant sprays. Agar is mixed with shellac and wax for shoe and leather polishes. It is used as setting inhibitor for deep well cement. Also as an adhesive in gloss finishing in paper products, agar is quite useful.

3.6 Application of agarose

The modern application of agarose is in biotechnology which includes molecular biology and genetic engineering; plant and animal cell and tissue culture; cell separations; immobilization supports; metabolic product recovery and purifications; clinical diagnostics in immunology and controlled release reagent delivery systems (Renn, 1984).

In electrophoresis agarose when containing appropriate buffer is an excellent medium in the separation of proteins and nucleic acids and their derivatives by applying an electric potential to the gel medium. Such separations depend upon the differential rates of migration of charged particles toward the oppositely charged electrodes when an electric potential is applied. Separation by molecular size depends upon the ability of particles to migrate through pores of the gel matrix. The smaller the molecule, the faster is the movement through the gel matrix and this is termed as molecular sieving electrophoresis. Such gel electrophoresis is used in chemical laboratories to identify proteins, enzymes, some abnormalities in serum and plasma and in biological fluids. Agarose is necessary in genetic engineering techniques in the separation of the desired gene DNA fragments and in gene mapping. Phages, viruses and capsides can be separated in low concentrations of agarose (0.035%). Recovery of fragments from electrophoretic separations could be accomplished by using the low-gelling, low-melting temperature hydroxyethylagarose.

In immunology agarose is used in the detection and study of disease indicators or antigenic materials and their specific antibodies. Antibodies (immunoglobulines) are specific proteins formed by animal cells in response to definite antigenic stimulus. The antigen will combine with the antibody to neutralize the threat of the disease to the animal. Methods have been devised using agarose to detect this antigen-antibody complexes, which appear as a cloudy or white precipitin band such as radial immunodiffusion, immunoelectro-phoresis, electroimmunodiffusion, counterelectrophoresis and so many other methods.

In chromatography agarose beads are used in columns where molecular size separations can be effected especially for molecules greater than 250 000 daltons. The almost neutral agarose effects only minimal nonspecific binding to the medium and thus retaining the biological activity of the molecule which are being separated. In affinity chromatography the enzymes, antigen, antibody, coenzyme, substrate, etc. are bound chemically or physically to the agarose gel particles. These are then eluted by chaning the pH or ionic strength of the eluant or solvent used in the fractionation.

Agarose finds application in immobilized cells and enzymes which attach to the agarose films, beads or particles and can transform one chemical to another. A very popular use of immobilized cells and enzymes is in the conversion of starch and sugar into alcohol by the use of immobilized yeast cells. Agarose is gaining popularity in fine highly technological applications.

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