



UNIVERSITI PUTRA MALAYSIA

**EXTRACTION, CHARACTERISATION AND APPLICATION OF
AGAR FROM *GRACILARIA* SP.**

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**MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA**

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OF AGAR FROM *GRACILARIA SP.***

By

BENCHAMAPORN WONGSUBAN

**Thesis Submitted in Fulfillment of the Requirements for the
Degree of Master of Science in the Faculty of
Food Science and Biotechnology
Universiti Putra Malaysia**

June 1999



Dedicated to

My family

and

my beloved younger brother

who has been called by God while in the preparation of this thesis.



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GLOSSARY

MT	Metric tonne
FRI	Fisheries Research Institute
kg	kilogram
g	gram
ml	millilitre
cm	centimeter
min	minute
hr	hour
ppm	part per million
M	molarity
<i>G. changii</i>	<i>Gracilaria changii</i>
<i>G. fisheri</i>	<i>Gracilaria fisheri</i>
<i>G. tenuistipitata</i>	<i>Gracilaria tenuistipitata</i>
R ²	Coefficient of Determination
N	Normality
NaOH	Sodium Hydroxide
HCl	Hydrochloric Acid
USP	United States Pharmacopeia
FCC	Food Chemical Codex
FDA	Food and Drug Authority (U.S.A)
GRAS	General Recognition as Safe
RSM	Response Surface Methodology
L	Lightness
a	Yellowness
b	Redness
T	Transmittance
LSD	Least Significant Difference
DMRT	Duncan's Multiple Range Test



Abstract of the Thesis Presented to the Senate of Universiti Putra Malaysia in
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**EXTRACTION, CHARACTERISATION AND APPLICATION OF AGAR
FROM *GRACILARIA SP.***

By

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Faculty :Food Science and Biotechnology

The methods for extraction of agar from seaweeds differed either in the type of extraction solution, its concentration, the heating temperature or heating time used. Alkali pre-treatment of the seaweeds has also been performed in order to improve the agar gel strength. Sodium hydroxide has been used and the conditions were varied in terms of sodium hydroxide concentration, soaking temperature and time.

In this study, the optimum conditions for extraction of agar from *Gracilaria sp.* were determined. The characteristics of the extracted agar were then compared with that of commercial agar and the behaviour of the agar in roselle jelly was also determined. The three species of *Gracilaria* used in this study were *Gracilaria changii*, *Gracilaria fisheri* and *Gracilaria tenuistipitata*. Agar was extracted from the seaweeds using different extraction solutions. They were distilled water, sulphuric acid, acetic acid and sodium hexametaphosphate. The concentrations of sulphuric acid and acetic acid were in the range of 0.005-0.03% while the concentrations of sodium hexametaphosphate were in the range of 0.0005-0.003 M.



The extraction temperature was 121°C while the extraction time was varied from 10 to 60 minutes. After the optimum extraction conditions were established, the seaweeds were pre-treated with sodium hydroxide and the parameters studied were sodium hydroxide concentration which ranged from 1 to 9%, soaking time of 1 to 3 hours, and soaking temperature of 80 to 100°C.

The utilisation of sulphuric acid, acetic acid and sodium hexametaphosphate increased the yield of agar from *G.changii*, *G.fisheri*, and *G.tenuistipitata* but have a negative effect on the agar gel strength. An extraction method which employed 0.0005M sodium hexametaphosphate, 121°C and 10 minutes resulted in a high yield of agar with a high gel strength. Agar with a gel strength of 661-838 g/cm² could be obtained from *G.changii* after pre-treatment with 3-5% sodium hydroxide at 80-90°C for 1-2 hours. For *G.fisheri*, a high gel strength agar (612-713 g/cm²) could be obtained after 5-7% sodium hydroxide treatment at 80-90°C for 2-3 hours. The pH, water absorption capacity, clarity, percent syneresis, gelling temperature and melting temperature were found to be in the range of that obtained for commercial agar. Their moisture, lead, arsenic, crude protein, sulphate, starch, gelatin and ash content were also in the range of that obtained for commercial agar and did not exceed the values specified by the Japanese and American standards except for the foreign insoluble matter content. The organoleptic quality was found to be lower than that of the reference agar and the best roselle jelly could be prepared using 15.0% sucrose and 11.0% roselle juice.

Abstrak Tesis yang Dikemukakan kepada Senat Universiti Putra Malaysia sebagai
Memenuhi Keperluan untuk Ijazah Master Sains

**PENGEKSTRAKAN, PENCIRIAN DAN APLIKASI BAGI AGAR DARI
*GRACILARIA SP.***

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Kaedah-kaedah pengekstrakan agar daripada rumpai laut berbeza dari segi jenis larutan ekstrak, kepekatan larutan ekstrak, suhu larutan dan masa pernanasan yang digunakan. Pra-rawatan rumpai laut dengan alkali juga dilakukan untuk memperbaiki kekuatan gel. Larutan natrium hidroksida dengan pelbagai kepekatan, suhu dan masa rendaman digunakan.

Didalam kajian ini keadaan optimum pengekstrakan agar dari spesies *Gracilaria* ditentukan. Ciri-ciri agar yang dihasilkan telah di bandingkan dengan agar komersil dan perlakuan jeli roselle daripada agar juga ditentukan. Tiga spesies *Gracilaria* yang digunakan dalam kajian ini ialah *Gracilaria changii*, *Gracilaria fisheri* dan *Gracilaria tenuistipitata*. Agar diekstrak dengan menggunakan berbagai larutan ekstrak iaitu air suling, asid sulfurik, asid asetik dan natrium heksametafosfat. Kepekatan asid sulfurik dan asid asetik adalah dalam julat 0.005-0.03% manakala kepekatan natrium heksametafosfat adalah dalam julat 0.0005-0.003M. Suhu pengekstrakan ialah 121°C sementara masa pengekstrakan ialah



antara 10 hingga 60 minit. Setelah keadaan optimum ditentukan, rumpai laut tersebut dipra-rawat dengan natrium hidroksida dan parameter yang dikaji ialah kepekatan natrium hidroksida pada julat 1 hingga 9%, masa rendaman dari 1 hingga 3 jam dan suhu rendaman sehingga 100°C.

Penggunaan asid sulfurik dan asetik dan natrium heksametafosfat didapati meninggikan kadar perolehan agar daripada *G.changii*, *G.fisheri* dan *G.tenuistipitata* tetapi mengurangkan kekuatan gel yang dihasilkan. Satu kaedah pengekstrakan yang menggunakan 0.0005M natrium heksametafosfate, 121°C dan masa 10 minit menghasilkan kadar perolehan yang tinggi dengan kekuatan gel yang tinggi. Agar yang mempunyai kekuatan gel antara 661-838 g/cm² boleh didapati dari *G.changii* setelah dipra-rawat dengan 3-5% natrium hidroksida pada 80-90°C dan 1-2 jam. Untuk *G.fisheri*, gel agar berkekuatan tinggi (612-713 g/cm²) boleh dihasilkan setelah dirawat oleh 5-7% natrium hidroksida pada suhu 80-90°C selama 2-3 jam. Nilai pH, keupayaan penyerapan air, kecerahan, peratus sineresis, suhu penjelan and suhu lebur agar didapati berada dalam julat yang sama dengan agar komersil. Kandungan lembapan, plumbum, arsenik, protein kasar, sulfat, kanji, gelatin dan abu juga adalah sama dengan agar komersil dan tidak melebihi nilai-nilai yang ditentukan oleh piawaian Jepun dan US. Kualiti organoleptik didapati adalah lebih rendah daripada agar rujukan. Jeli roselle yang terbaik boleh dihasilkan dengan menggunakan 15% sukros dan 11.0% jus roselle.

CHAPTER I

GENERAL INTRODUCTION

The agar industries today probably represented a market value well about US130 million (Ruiter and Rudolph, 1997). An estimated 10,000 MT of raw agar and 3500 MT of final product entered the world market each year. Japan was the main agar consuming country (about 2000 MT a year), almost all its consumption came from domestic production. The United States of America, another major consumer (1000 MT per year), obtained more than 80% of its supply from imports. Its main suppliers were Chile, Morocco, Spain and the Philipines. The demand for agar in the European Economic Community was approximately 1300 MT per year. Thailand, Indonesia, Singapore and Malaysia import about 200 MT each year. The main suppliers for the Southeast Asia region are The Republic of Korea, Japan and Chile (Singh, 1992).

In Malaysia, as reported in the import and export trade statistics, there has been an import of 511 tonnes of agar for food consumption valued at RM 11,195,100 in 1994, and the demand was increased to 792 tonnes valued at RM 19,209,600 in 1995. However, Malaysia exported 17 tonnes of agar valued at RM 759,000 in 1994 and 6 tonnes of agar valued at RM 106,800 in 1995. The demand of agar consumption has been increasing, therefore, efforts should be made to produce agar in Malaysia (Kementarian Pertanian Malaysia, 1995).



Agar is commercially produced from red seaweeds (Rhodophyceae) (Araki, 1962) mainly from the genus *Gelidium* and *Gracilaria*. Total world production of red seaweeds from aquaculture in 1996 was 1,680,733 MT. There were *Rhodophyceae* (108,000 MT), *Porphyra tenera* (847,588 MT), *Gelidium sp.* (500MT), *Euchema alvarezii* (17,300 MT), *Euchema cottoni* (544,500 MT), *Euchema spinosum* (30,550 MT), *Euchema sp.* (3,420 MT), *Gracilaria sp.* (128,867 MT) (FAO Fisheries Department, 1997).

Gelidium is considered a superior raw material as its agar extract is of high quality (Matsushashi, 1990). Its supply, however, is limited as seaweeds belonging to this particular genus are slow growing (Combonga, 1995) and its production was 500 MT in 1996 from Korea (FAO, 1997). *Gracilaria* is a more abundant wild supply and is now the source of the world's agar production (Combonga, 1995). Agar extracted from *Gracilaria sp.* usually has low gel strength. The Japanese, however, has developed methods for improving its gel strength through alkali-treatment of the seaweeds (Singh, 1992). The world production of *Gracilaria sp.* was 71,533 MT in 1995. It was from Chile (49183 MT), China and Taiwan (8254MT), Italy (5000 MT), Korea (250 MT), Namibia (799 MT), Peru (2 MT), Philipines (4 MT), Saint Lucia (1 MT), Venezuela (40 MT) and Vietnam (8000 MT).

In Malaysia, site visits and surveys around the coastal waters in Pulau Pinang made by the Fisheries Research Institute (FRI) staff revealed prolific growth of *Gracilaria sp* in the middle bank. These red seaweeds were found to consist of

two species. Samples of these *Gracilaria* were sent to Dr. M.S. Doty at the University of Hawaii and results of chemical analyses showed that a high quality gel can be extracted from one of the species identified as *Gracilaria cylindrica*, which is now, called *Gracilaria changii*. In 1982, a programme for *Gracilaria* farming was drawn by the Fisheries Department based on the consultancy report on seaweed potential by Dr. Doty under the South China Sea Programme. Experts from the Aquatic Resource Development Project, Hawaii, were recruited by the Bay of Bengal Small Scale Fisheries Development Programme and work on the experimental culture of *Gracilaria changii* was initiated at the Pulau Pinang FRI in February 1983. The institute is now growing *G.changii* in Kedah and an agar factory, which was first set up in Kuala Lumpur had to relocate to Kedah due to the better supply of raw material.

The agar from *Gracilaria sp.* yields very weak gel so efforts were made to develop methods for improving the gel strength of agar. Alkali treatment of seaweeds was first introduced by Kojima and Funaki (1951) at Tokyo Institute of Technology. Nevertheless, the quality of agar produced not only depends on the alkali treatment of seaweeds but also on the seaweed species, harvesting site and extraction methods.

The extraction of agar involves heating of sun-bleached and alkali treated seaweeds in extraction solution, filtration of the extract that is then followed by gelification, freezing, thawing and drying of the agar extract. The extraction

methods can thus vary in terms of the type and concentration of acid used and the extraction temperature and time employed.

Therefore, the objectives of this study were as follows:

- 1 To determine the optimum conditions for extraction of agar from *Gracilaria sp.*
- 2 To determine the appropriate method for alkali treatment of seaweeds
- 3 To compare the characteristics of extracted agar with those of commercially available agar
- 4 To determine the behavior of the agar in roselle jelly

CHAPTER II

LITERATURE REVIEW

History of Agar

Agar was the first hydrocolloid discovered and prepared as a purified extract. According to the Japanese legend, the original manufacturing method of agar was discovered in the middle part of the 17th century, presumably in 1685. A Japanese officer in the winter of that year arrived at a little inn. The innkeeper Minoya Tarozaemon ceremoniously received him and offered a traditional seaweed jelly dish as dinner, which was prepared by cooking *Gelidium sp.* with water. After the seaweed was frozen during the night, thawed and dried under the sun. It was then left for several days and became a white, porous and dried substance. Tarozaemon found this soft substance and boiled it in water. On setting, he obtained a whiter jelly than the original one, thus the method of agar manufacture was accidentally discovered (Santos, 1990). Commercial agars available are shown in Plate 1.

Definition of Agar

According to the US Pharmacopoeia (1980) agar is defined as a dried hydrophilic colloid extracted from certain seaweeds of the class *Rhodophyceae* (red seaweeds or agarophytes) such as *Gelidium sp.*, *Gracilaria sp.*, *Pterocladia sp.*, and *Ahnfeltia sp.* (Infofish, 1995). Agar is insoluble in cold water and soluble in boiling water, although certain agars do swell in water (Meer, 1989).