Extraction of agar from locally grown *Gracilaria verrucosa* and development of gelatine free set-yoghurt product using agar

M.J. Paththuwe Arachchi[1], G.J. Ganegama Arachchi[*1], M.A.J. Wansapala[2] and M.P.K. Jayarathna[3]

[1]*Institute of Post-Harvest Technology, National Aquatic Resources Research and Development Agency (NARA), Crow Island, Colombo 15, Sri Lanka.*

[2] *Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka*

3. Research &Development Laboratory, Milco (Pvt) ltd, Narahenpita, Colombo 05.

Abstract

Seaweed agar is an important healthy food item. Currently seaweed agar is not extracted at commercial level in Sri Lanka. This study investigated an agar extraction method that render high agar yield and development of agar incorporated gelatine free yoghurt targeting the needs of vegetarian communities. Agar was extracted using *Gracilaria verrucosa* under optimum conditions: Dried *G. verrucosa* was soaked at pH 5 for 30 min; soaked *G. verrucosa* was pressure-cooked with 45 times volume of water for 20 min; the agar extract was allowed to set in trays for 6 h at 25 ± 2 °C; the resulted gel layers were frozen for 8 h; and frozen agar layers were thawed for 4 h at 2 5 ± 2 °C. The melted water was drained out from gel layers and then, gel layers were cut into strips. Gel strips were dried at 45 °C for 36 h and dried agar were ground to obtain fine agar powder. A gelatine free set-yoghurt product was developed using extracted agar as a texture stabilizer. The developed yoghurt (0.25% agar) which scored high for sensory quality attributes, showed similar sensory properties as in gelatine (0.61 %) containing yoghurt ($p > 0.5$).

The pH and titratable acidity of the seaweed yoghurt were 4.5 and 0.85 % (w/w) respectively on 15th day of storage at 4 ± 2 °C. Agar extracted from *G. verrucosa* contained 80.1 % (w/w) of dietary fibre. It was found that seaweed yoghurt contains 0.18 % (w/w) of dietary fiber content while it was not detected in gelatine yoghurt. Agar contained set yoghurt consists of 77.34, 3.40, 3.10, 0.75 and 22.66 % (w/w) of moisture, protein, fat, total ash and total solid content, respectively. The technologies developed, in this study, to extract of food grade agar from *G. verrucosa* and to produce agar incorporated yoghurt has potential to commercialize as an industry.

Keywords: *Glacilaria verrucosa*, agar extraction, agar based set yoghurt, technology, gelatine free

**Corresponding author:janadari1@yahoo.com*

Introduction

Gracilaria sp., *Gelidium* sp., *Saragassum* sp., *Turbinaria* sp., *Ulva* sp., *and Caulerpa* sp. have been identified as economically valuable seaweed species that are available in Sri Lanka. Currently *Gracilaria verrucosa* are collected mainly from naturally grown stocks near shore at Kinniya in Trincomalee. Agar is extracted from red seaweeds, predominantly, from *Gracilaria* species and used as a gelling agent in different industries such as bakery, confectionary, dairy, pharmaceutical, biomedical and other fields. Agar has a wide variety of uses. Food grade agar is an excellent source of dietary fiber and has a growing demand as a health food type.Presently, gelatine free set-yoghurt products are not commercially manufactured in Sri

Lanka and also not available in local markets. Dynamic rheological experiments showed that yoghurts with added gelatine exhibits more solid like behaviour than the yoghurts prepared without it (Fiszman *et al.* 1999). Gelatine containing products are not acceptable for some communities, especially, for vegetarians. The aim of this study was to develop a method to extract agar from *G. verrucosa* and to develop a seaweed agar incorporated set-yoghurt product that has similar sensory properties to gelatine containing set-yoghurt targeting the vegetarian community.

Materials and Methods

Collection and preparation of agar from *G. verrucosa*: *Gracilaria verrucosa* samples were collected from natural seaweed beds in Trincomalee and agar was extracted using an acid digestion method. Briefly, washing and sun bleaching of seaweed repeatedly for 3 times until obtain light yellow colour pure material; soaking seaweed in a pH 5 acetic acid solution for 30 min; neutralization of soaked seaweed by washing with running water; addition of water to soaked seaweeds (45 times based on initial dry weight of seaweeds); pressure cooking of seaweeds for 20 min under low flame; filtering the cooked seaweed mass through a cheese cloth using a screw press; allowing setting seaweed sols for 6 h to retain layers (2 cm thickness) in aluminium trays under room temperature; freezing the gel layers for 8 h (-18 ± 2°C); thawing of frozen gel layers for 4 - 5 h at room temperature; draining of melted water from gels; cutting of gel in to strips and drying in a drying cabinet until moisture content become less than 18% (50 ± 5°C for 36 h) and milling/grinding of dry agar sheets into a powder.

Characterization of agar powder: The colour of the powder was decided using the Munsell Colour Guide 2005 (Munsell Colour Science Laboratory, Rochester Institute of Technology). Moisture, dry matter, ash content, were analysed as described in the (AOAC, 1995). Total fibre content was analysed using enzymatic gravimetric method (Prosky *et al.* 1983). Gel strength of 1.5 % gel was measured using Instron texture analyzer 4465. Melting point of 1.5 % gel was measured using method as described by (Marshall and Newton, 1949). The sol- gel transition temperature of 1.5 % agar solution was measured as described by (Esquivel *et al.* 2008).

Development of agar incorporated set yoghurt: Trial and error method was used to formulate the set yoghurt product that has similar textural properties as gelatine containing set yoghurt. The method included following a standard method by substituting agar powder in place of gelatine. Other ingredients used were fresh milk, milk powder, egg yolk colour (E102 and E122), vanilla essence and activated starter culture containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Veterinary Research Institute, Peradeniya, Sri Lanka).

Results and Discussion

Quality of agar is measured by means of gel strength, setting temperature and melting temperature.The highest agar yield (37.25 ± 0.8%) was obtained by soaking dried moss of *G. verrucosa* in acidic solution of pH 5 compared to that of in 6 other different acid treatments in the range pH 3 - 12.7. Ratio of seaweed to water at 1:45 was selected as the best dilution factor

while other processing conditions at constant: Soaking of seaweed at pH 5, pressure cooking of seaweed for 20 min under low flame, freezing of gel layers for 8 h (-18 ± 2°C), thawing of frozen gel layers for 4 h at room temperature, and drying of thawed gels at 50±5°C for 36 h in a drying cabinet.

Pressure cooking under low flame for 20 min yielded high agar percentage (38.45%) compared to boiling for 20 min and pressure cooking for 10 min. Pressure cooking may have caused to rupture cell walls and expedite the release of more agars trapped in the cell walls of *G. verrucosa* than that of cooking in an open pan. Freeze-thaw method used to purify the agar by removing water soluble impurities and to accelerate the drying process. Among different freezing times (2, 4, 8,10,12,17 h), 8 hour freezing time that resulted in ash content comparable to ash contents of gels frozen for 8, 10, 12, 17 hr ($p>0.5$). Proximate and physical testing values of agar-agar powder are given in Table 1.

Table 1. Specifications of *Glacilaria verrucosa* agar-agar powder extracted from the developed method

Parameter	Value
Mesh size	≤200 µm
Colour	8/2.5 Y
Moisture content	17.45±0.1% (w/w)
Total ash	2.07±0.1 % (w/w)
Total fibre	80.10±0.7% (w/w)
Gel strength (1.5 % gel at 25 °C)	793.67±10 g/cm^2
Melting point of 1.5 % gel	86.3±0.82 °C
Setting temperature	38.6±0.56 °C
Solubility	2 .5 min under medium power of a microwave oven (1.5% agar solution)

Screening of most acceptable yoghurt formula: According to the spider web diagram and statistical analysis flavour, appearance and overall acceptability of both agar yoghurt and gelatine yoghurt were not significantly different ($p>0.5$). Texture of the agar yoghurt was better than the gelatine yoghurt and bit more preference was gained by the aroma of gelatine yoghurt than the aroma of agar yoghurt. Proximate composition of developed yoghurt was within the recommended range and additionally agar incorporated yoghurt contained 0.18% dietary fibre where it was zero in gelatine yoghurt.

Shelf life determination of agar incorporated yoghurt: According to the SLSI standards when consider about the hygienic quality it should be free from coliforms, less than 1000/g yeasts and less than 1/g moulds. Microbiological counts of both seaweed agar incorporated yoghurt and gelatine yoghurt were complied with SLSI standards ($P < 0.5$) during 15 days of storage time. The pH of yoghurt decreased from 4.8 to 4.2 during the refrigerated storage for 20

days. Titratable acidity of yoghurt during the refrigerated storage retained steady at 0.85% increased to 0.9% on 15th day. Based on these data, shelf life of the seaweed agar incorporated yoghurt was determined as 16 days.

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