

## Extraction of Hydrocolloids from Plants, Fruits, Skins and Eggs

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### Abstract

Sodium alginate is natural polysaccharides that are extracted from brown seaweeds (*Macrocystis integrifolia*). The preparation of sodium alginate is divided into five steps such as acidification, alkaline extraction, solid/liquid separation, precipitation and drying. For the preparation of sodium alginate, the varying amount of hydrochloric acid concentration, weight of sodium carbonate and volume of hypochlorite were investigated. The pectin from pomelo (*Citrus Maxima*) rind was used in the manufacture of jams, jellies and marmalades. The inherent bitterness and colouring matter in pomelo rinds were reduced by extraction with ethanol rather than normally used technique of boiling with ammonium chloride and adding appropriate preservative to extend its shelf-life. The extraction of gelatin from fish skin (Nga Gin) (*Cirrhinus cirrhosis*) and it was used in the preparation of marshmallow. In this research, fish skin was pretreated with hydrochloric acid or lime water and then gelatin was extracted using pure water. The extracted gelatin and pectin were identified by the solubility test, precipitate formations, development of turbidity test and gel formation test. Lecithin was extracted from quail yolk by using solvent partition method. Ethanol and hexane were used to extract total lipids. After that, acetone precipitation was necessary to remove cholesterol. The physico-chemical properties of prepared sodium alginate, pectin, gelatin and lecithin such as trace elements, moisture content, ash content, pH, colour and viscosity were determined and compared with Food and Agricultural Organization specification and British Pharmacopoeia specifications. The resultant products of sodium alginate and pectin were identified by FT-IR spectroscopy.

**Keywords:** hydrocolloids precipitate formations, gel formation

### 1. Introduction

Hydrocolloids are the range of polysaccharides and proteins that are nowadays widely used in a variety of industrial sectors to perform a number of functions including thickening and gelling aqueous solutions, stabilising foams, emulsions and dispersions, inhibiting ice and sugar crystals formation and the controlled release of flavours, etc. It includes pectin, cellulose, alginate, gelatin, lecithin, gum, chitosan etc. (Phillips and Williams, 2000). Seaweeds are a group of large marine non flowering plant attached to the bottom in relatively shallow coastal water. Primarily seaweeds can be classified into three broad groups based on colour of Green (*Chlorophyceae*), Brown (*Phaeophyceae*) and Red (*Rhodophyceae*) (Chapman, 1980).

Alginates have a long history of use in foods and these uses are based mainly on their thickening, gelling and general colloidal properties. Thickening is useful in sauces, syrups and toppings for ice-cream, etc., pie fillings (Leigh, 1979). Pectin are derived from the breakdown of more complex protopectin which are present in plant tissue and also contain a range of neutral sugars, including rhamnose, galactose, arabinose and lesser amount of other sugars (Phillips and Williams (2000). The fruits ripe or mature, the pectin contained in them is split or separated by two enzymes-pectinase and pectin esterase. Commercially pectin is extracted from citrus peels or apple pomace. Dried citrus peel contains 20-30% pectin. Dried

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apple pomace yields 10-15% pectin. Pectin is used as a gelling agent in traditionally manufactured fruit-based products, especially jams and jellies (Baker, 1994). Gelatin is commercially derived from collagen by controlled acid or alkaline hydrolysis. Pure dry commercial gelatin is a tasteless, odorless, transparent, brittle, glasslike solid, very faint yellow color. Fish skins could be used as a source of gelatin, although it is well known that gelatin from aquatic sources is not as good as gelatin from land animal sources insofar as its gelling properties are concerned (Hollingworth, 2010).

## **2. Materials and Methods**

In this research, brown seaweeds were obtained from Kae village, Manaung Township, Rakhine state. Pomelo fruits obtained from Thahton Township, Mon State were used.

### **Extraction of sodium alginate from seaweeds**

Seaweed was washed with fresh water and soaked in 0.5%(vol%) hydrochloric acid for 1 hour. Then the sample was washed with fresh water again and cut into small pieces and digested with 8%(w/v) sodium carbonate solution for 30 minutes with occasional stirring. The resulting mixture was mixed with water before filtration. The filtrate was bleached with 5%(w/v) sodium hypochlorite solution for 30 min and precipitated with 0.9%(vol%) hydrochloric acid and then alginic acid was obtained. It was then filtered, washed with fresh water and 5%(w/v) of sodium carbonate was added drop wise until the pH reached 7. The water content of the spongy and viscous precipitate was diluted by adding 95% ethanol (about twice the volume of precipitate). The resulting sodium alginate was filtered and dried in an oven about 2 hr at 60°C. Finally, the dried sodium alginate was ground into powder form.

### **Extraction of pectin from pomelo**

The white portion of pomelo rind was prepared by removing green or yellowish green skin. They were cut into 2cm thickness and washed with water. About 50 g of pomelo rinds were boiled with 100 ml of 0.1% NH<sub>4</sub>Cl solution at 80°C for 20 min. Then, the solid and liquid portions were separated by using basket type centrifuge and the solid portion (pulp) was washed with distilled water twice to remove the bitter taste. The washed pulp was in turn boiled in a one liter beaker with 100 ml of 0.1% citric acid solution at 80°C for 10 min. The extract containing cellulose and liquid pectin was again separated by centrifugation. The filtered liquid pectin was poured in a thin stream with vigorous stirring into a 500 ml beaker containing 100 ml of 95% ethyl alcohol. After that, the gelatinous pectin precipitated and separated by using basket type centrifuge. The resultant semi-solid pectin precipitate was dried in a hot air oven at 60°C for about 3-4 hr. The dried solid pectin was ground.

### **Extraction of gelatin from fish skin**

#### **Acid treatment**

25g of fish skins were soaked in 0.02% hydrochloric acid solution for two hours. During this period, the fish skins were swelled to two fold. After soaking, they were thoroughly washed with water to remove excess acid and so that the skins were nearly neutral.

### **Alkali treatment**

25g of fish skins were soaked in 8% lime water solution (pH=12) for 8 days. During this period, the mixture was stirred gently. They were swelled to two fold. After soaking, they were neutralized with dilute acid solution until the external areas were acidic. After that, they were washed with water until the neutral.

### **Preparation of gelatin**

An acid or alkali treated fish skins were placed in a small pot and 75 ml of water was poured into it. This pot was covered and heated on a water bath at 80°C for 15 min. The liquor was separated from the residual skin fragment by using filter cloth. The extraction process was conducted on three times; each time lasted for 15 minutes. Then the extracted liquor was concentrated at 80°C for 30 min and it was cooled at the refrigerator at 20°C for 1 hr to form a solidified gel. These gels were cut into small pieces and dried in hot-air oven at 65°C for 10 hr. Finally, these gelatin sheets were ground and packaged in air tight plastic bag and stored in desiccators.

### **Extraction of lecithin from quail yolk**

#### **Separation of egg-yolk**

Egg-shell was carefully broken and the yolk was separated from the egg-white. Fresh yolk and dried yolk were used. Fresh yolk was dried in an oven controlled to 60°C for 5 hr.

#### **First ethanol extraction**

15g of raw dried yolk was placed in a 150ml beaker, 50 ml ethanol (95%) was added to it and stirred until the egg-yolk was completely dispersed. Then, the mixture was centrifuged for 5min. the supernatant containing water, some polar and natural lipids was transferred to a 150ml separating funnel.

#### **Hexane extraction**

Neutral lipids from the precipitate were extracted twice with 25ml hexane in the same manner of ethanol extraction. The hexane extracts were transferred to the same separating funnel.

#### **Second ethanol extraction**

The protein precipitate was extracted two times with 25ml hexane (87%) to remove any residual polar lipids. The ethanol extracts were combined with the previous ethanol and hexane extracts in the separating funnel and the residue was weighed.

#### **Phase separation**

The separating funnel was thoroughly mixed and left to equilibrate for one hour for phase separation. The ethanol phase was removed and the hexane phase was mixed with additional 25ml ethanol and left for phase separation. Hexane was removed by evaporation, leaving behind the natural lipids (NL-1) was weighed. The ethanol phase was combined with the previous ethanol phase and the solvent was evaporated.

#### **Acetone precipitation**

The remaining polar lipid material was dissolved in 17.5ml of hexane. The solution was mixed with 75ml of chilled acetone 4°C and carefully stirred to precipitate the phospholipids. Then, the beaker was placed in an ice water bath for 15min. The supernatant

was removed and solvent was evaporated. This fraction containing neutral oil and cholesterol (NL-2) was weighed. The precipitate was blown by air to drive off acetone and weighed. Lecithin was also isolated from fresh yolk in the same manner of the above procedure.

### 3. Results and Discussion

Sodium alginate was prepared from brown seaweed using different amounts of sodium carbonate, sodium hypochlorite and hydrochloric acid. The yield percent of sodium alginate from brown seaweed using different amounts sodium carbonate (160, 170, 180, 190 and 200g), sodium hypochlorite solution (500,600,700,800 and 900ml), and hydrochloric acid (0.5, 0.6, 0.7, 0.8, 0.9 and 1%(vol%). The most suitable the yield percent was found to be 23%(wt %). The possible functional groups for characteristics absorption peaks can be found in Figure(1). Table (1) shows the physical characteristics of the extracted sodium alginate such as moisture content and ash content were compared with literature value.

Pectin is mainly used in the preparation of food products such as jam, jelly, marmalade, etc. The quality of pectin used is considerably influenced on the quality of the products. The peels of citrus fruits are widely used as potential sources for pectin extraction. Gelatin was extracted from fish skin by using hot water. Before extraction, acid pretreatment and alkali pretreatment were carried out. The most suitable conditions for acid treated gelatin (Type I) and alkali treated gelatin (Type II) were 0.03 % hydrochloric acid for 2 hr soaking time and pretreated with lime water (pH 12) for 8 days respectively. Although the qualities of both types of gelatin were found to be same, the higher yield of gelatin was obtained by using Type II (alkali pretreatment method). So, Type II gelatin was used in the preparation of marshmallow.

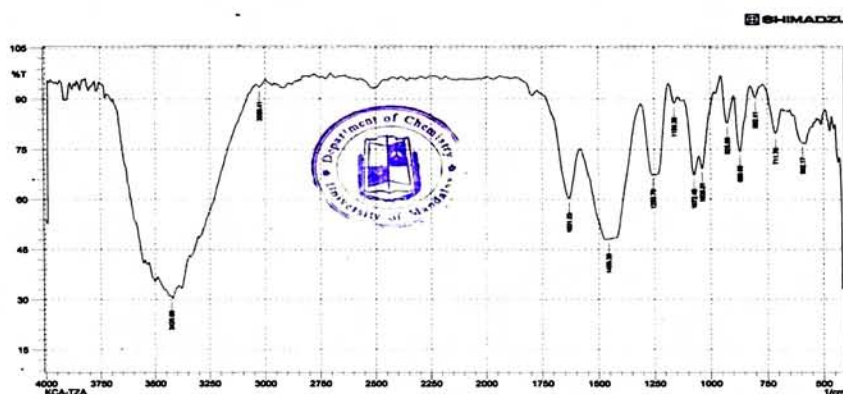


Figure 1. FT-IR Spectrum of sodium alginate sample from brown seaweed

Table 1. Analysis of sodium alginate sample from brown seaweed

| Characteristics          | Prepared Sodium Alginate | Literature Value* |
|--------------------------|--------------------------|-------------------|
| Moisture content (% w/w) | 14.2                     | 13                |
| Ash content (% w/w)      | 23.9                     | 23                |

\* Chapman, 1980.

Table 2. Identification test of pectin powders obtained from pomelo pectin

| Sample                                   | Solubility Test                       |            | Gel Formation Test               | Precipitate Formation Test                            |
|--|---------------------------------------|------------|----------------------------------|---|
|  | in water                              | in ethanol |                                  |   |
| Pomelo pectin extracted by citric acid   | formation of colloidal and opaque gel | no change  | formation of slightly opaque gel | colourless gelatinous precipitate was formed          |
| Pomelo pectin extracted by tartaric acid | formation of colloidal and opaque gel | no change  | formation of slightly opaque gel | colourless gelatinous precipitate was formed          |
| Commercial pectin                        | formation of translucent gel          | no change  | formation of translucent gel     | colourless slightly gelatinous precipitate was formed |

When the pomelo pectin was compared qualitatively based on identification tests as shown in Table (2), pectin obtained from citric acid, tartaric acid and commercial grade pectin exhibited solubility tests: colloidal and opaque gel, colloidal and opaque gel, translucent gel, gel formation test: slightly opaque gel and colorless gel solution, slightly opaque gel and colorless gel solution, translucent gel and colorless translucent gel solution, precipitate formation test: colourless gelatinous precipitate and slightly opaque gel, colorless gelatinous precipitate and slightly opaque gel, slightly gelatinous precipitate and transparent gel respectively.

Table 3. Physico-chemical properties of pectin

| Sample                          | Moisture Content (% w/w) | Ash Content (% w/w) | pH  | Trace Elements |           |
|---------------------------------|--------------------------|---------------------|-----|----------------|-----------|
|                                 |                          |                     |     | Cu (mg/l)      | Zn (mg/l) |
| Pectin extracted by citric acid | 1.65                     | 3.4                 | 4.5 | ND             | 0.228     |
| Commercial pectin               | 1.53                     | 3.6                 | 6.5 | ND             | 0.217     |

The various characteristics absorption bands of above spectra obviously show that the pectin obtained from this research was closely matched with the reference commercial pectin. Therefore, the extracted pectin obtained from this research is suitable for use as gelling agent in food processing. The physico-chemical properties of pectin such as pH, moisture, ash and trace elements: Cu, Zn is shown in Table (3).











