MJoSHT
Malaysian Journal of Science, Health & Technology
[mjosht.usim.edu.my]

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Pectin from Duckweed (Lemnaceae) As Potential Commercial Pectin and Its Gelling Function in Food Production: A Review

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Article Info
Article history:
Received: 5 August 2021
Accepted: 28 October 2021
Published: 11 February 2022

Academic Editor:
Azira Khalil

Malaysian Journal of Science, Health & Technology

MJoSHT2022, Volume 8, Issue No. 1
eISSN: 2601-0003

https://doi.org/10.33102/2022236

Abstract*— Duckweed, a small flowering plant which comes from Lemnaceae family, has been rising in popularity to many researches and applications for its numerous valuable functional properties. One of its special features is fast growing plant and easily adapted to various types of regions which makes it attractive to be manipulated in many applications including in biofuel production, waste water treatment and also for pharmaceutical and medicinal purposes. Pectin is the common component found in plant cell wall and it has been used as food additive for its capability to gel, emulsify and stabilize food products. Several requirements for commercial pectin such as galacturonic acid content (GalA) and degree of amidation have been regulated. Studies have proven that duckweed have significant amount of pectin which make it as a potential source of gelling agent in food industry. However, the structural properties of pectin from duckweed need to be investigated, in order to ensure if they meet the regulated requirements for industrial production. It is also to determine the suitability of duckweed pectin utilization in different type of food products since pectin of different sources have different structural characteristics, thus exhibit different gelling capability. This present review discusses on the potential of pectin from duckweed species to be utilized as food additive with gelling function in food products. Several extraction methods also have been reviewed, which each of them showed different efficiency and affect the extracted pectin characteristics.

***Keywords*— duckweed; Lemnaceae; pectin; gelling agent; gelation**

I. INTRODUCTION

The increasing demand and expectation on food safety and quality in the past few decades had promoted the utilization of additional substances in food products. The incorporation of these substances, which are known as food additives, allow some alteration in food products that ought to improve the quality and safety to the desired level [1]. There are many kind of food additives with each of them serves different purposes including to improve the physicochemical and sensorial properties, to enhance the product shelf life and also to add some nutritional value into food products [1, 2]. Pectin, which is the common component found in plant cell wall, is one of the substances that has been widely used in food industry for its various beneficial function in improving the quality of food products. The commercialized pectin is usually extracted from apple fruits and citrus fruits such as lemon and orange [3]. In recent years, many researchers become more interested in studying the utilization of pectin obtained from wider range of sources including agricultural and industrial waste such as from sunflower by-product [4] and fig skin [5]. However, pectin taken from different sources would give different gelling properties. The differences are affected by the varying structural properties such as the neutral sugar content and the amount of methyl-esterified galacturonic acids (GalA). In food industry, pectin mainly play role as gelling agent, thickening agent and stabilizer. Other than that, pectin also has many other functional properties in food application, where it is widely used in food preservation, play role as potential probiotics and prebiotics.

The application of pectin not only limited in the food processing industry, instead it has wide application in other industries such as in cosmetic, personal care and pharmaceutical. It is reported that since 2000s, scientist become more aware that pectin can contribute to many health and nutritional benefits as dietary fibre and prebiotic. Pectin also started to be recognized for its potential as a nutritional supplement. Since then, the conventional pectin producers started the production of pectin for the use as health ingredient. Meanwhile, in food industry, the utilization of pectin had also arisen, not only meant to be used to enhance the quality and texture of food products, but also used as dairy substitute and as an active ingredient in functional food product. In consequences, scope of pectin application become widen and as a result, the demand for pectin production keeps increasing in order to meet the demand for pectin in each sector [3].

For all the above reason, finding new alternative pectin sources is a good effort in attempt to fulfil the increasing request for pectin in every demanding industry. It is also one of the ways to provide cheaper pectin in many developing countries, which will reduce the production cost of products of pectin indirectly. While exploring novel pectin sources, it is also crucial to ensure the suitability of the application of new pectin in different type of food products. In this review, we are going to discuss the potential of pectin from duckweed to be utilized as a gelling agent in food products and its production for commercial purposes. This review also studies the characteristics of duckweed itself and some of the pectin properties which affect the gelling capability of pectin. Several pectin extraction methods are compared to determine the method that yield pectin with desired characteristics and better quality.PAGE LAYOUT This document is a template. For questions on paper guidelines, please contact the managing editor as indicated on the journal website. Information about final paper submission is available from the journal website.

II. TYPES AND CHARACTERISTICS OF PECTIN

Pectin is one of the main groups of polysaccharides found in plant primary cell wall (abundantly found in the middle lamella) along with cellulose and hemicellulose [6]. Generally, pectin takes up to 35% of the cell wall components [7]. It is a carbohydrate polymer with a high molecular weight [8]. Pectin generally function as a structural support for the plant cell wall, protect the plant from surroundings and also crucial for the plant cell growth [9]. It is an acidic heteropolysaccharide [10], highly branched and is mainly made up of galatacturonic acid chain, may consist of xylogalacturonan, homogalacturonan and rhamnogalacturonan components covalently connected by α-1,4 glycosidic bonds. Several types of neutral sugars can be found in the branched side of galacturonic backbone structure, mainly arabinose and galactose, with rhamnose, glucose and xylose residues [8, 11]. It is basically generated through the partial depolymerisation of precursor of pectin known as protopectin [12].

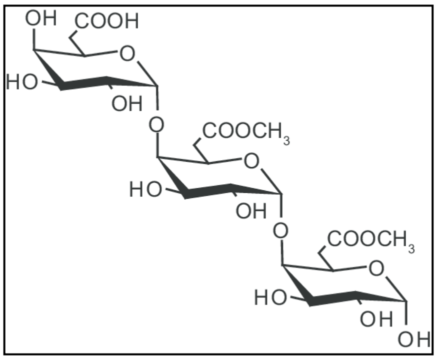


Figure 1. Structure of pectin [13]

Pectin can be categorized into two groups, which are high methoxyl pectin (HM) and low methoxyl pectin (LM) depending on their methyl ester content or degree of methylation (DM). DM is described as the amount of GalA units esterified by methyl group. HM pectin has degree of methylation above 50%, while LM pectin has degree of methylation below 50% [14]. HM pectin will form gel in high sugar concentration, therefore, they are often used in jam and preserved products. Meanwhile, LM pectin need the presence of calcium ions for gel formation and they can be applied in low or sugar free products since they able to form gel without presence of sugar [15]. Acetyl groups can also be present in the GalA residues located at O-2 and/or O-3, which become the measurement for the degree of acetylation (DA) of the pectin [16]. Those differences in pectin structural characteristics would affect its cohesiveness, viscosity, gelation and emulsifying properties [17, 18]. A recent study observed that methyl content and acetyl content in pectin structure play role in affecting the emulsifying function of pectin of sugar beet pulp [19].

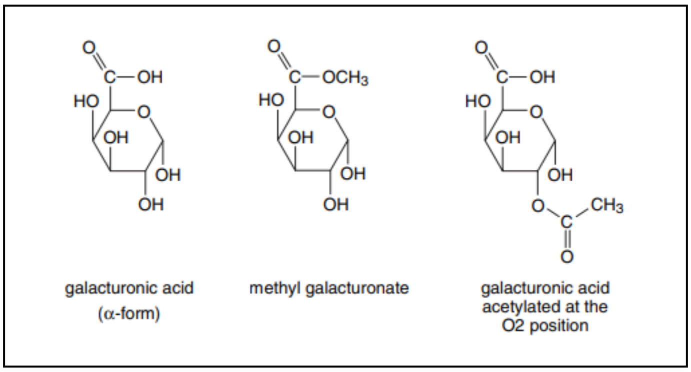


Figure 2. Structure of galacturonic acid, methylated and acetylated galacturonic acid. [20]

III. COMMERCIAL PECTIN

A. Source of Commercial Pectin

By-products of apple and citrus fruit juice industries such as apple pomace and citrus peels has become the main source of industrial pectin ever since 1908 [3]. Citrus fruits production is mainly dominated by the mainland of China, followed by Mediterranean region and USA countries as the top most citrus fruits producers. At the same time, many countries depend on imported citrus fruits, as well as pectin for the application in food processing and also other in industries such as cosmetic and pharmaceutical [21]. Argentina and Colombia are the countries that have most of their pectin being imported due to no significant production of pectin [10]. As of today, source of pectin production is widely available since other foods and agriculture waste including kiwifruit pomace [22] and sugar beet [23] have been studied and begin to be employed as new pectin sources.

B. Production of Commercial Pectin

The quality of raw materials is the most important factor to be considered for the production of pectin since it has significant effect on the production cost and the characteristics of the final product. The raw materials should be dried prior storage for further processing to prevent de-esterification activity by enzyme that would cause the pectin produced become more sensitive to calcium. In commercial production, the raw material is treated in diluted mineral acid (pH 2.0) at high temperature and is heated up to 10 hours. The heating time differ depending on the type of raw materials used and the type of pectin to be produced [15]. For industrial purpose, the amount of GalA of pectin should be at minimum of 65% on ash-free and dry-weight basis and degree of amidation of pectin should be less than 25% of the total carboxyl groups [8]. Pectin in the market is usually present in the form of powder. There are many types of pectin available, in which each of them serves different gelling capability for different food products. Pectin of high degree of esterification (DE) is modified through de-esterification process to produce pectin with lower DE. The process is done via hydrolysis by acid treatment. The hydrolysis also can be done using ammonia, which will produce pectin containing amide groups. Both treatments will result in production of LM pectin and amidated pectin, which is also a type of LM pectin. For commercial HM pectins, they are mainly classified into rapid-set pectin (DE more than 70%) and slow-to-medium-set pectin (DE 60-70%) [24].

IV. DUCKWEED

A. Background of Duckweed

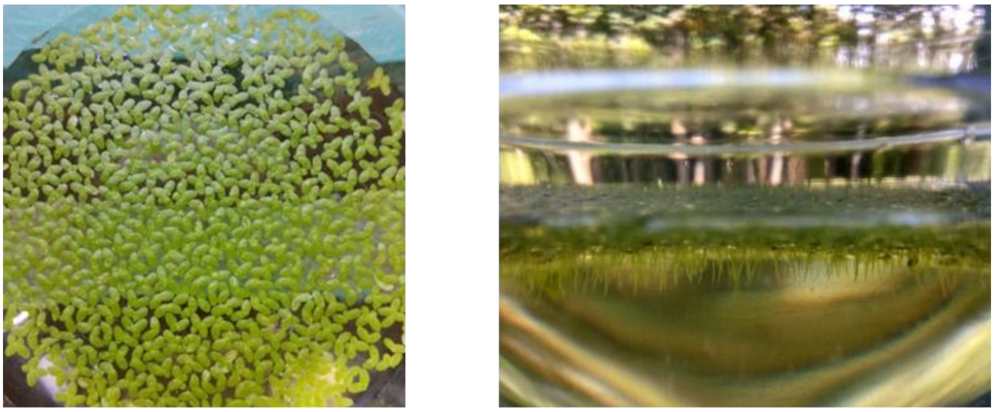
Duckweed comes from the botanical small flowering plants family; namely Lemnaceae. Basically, there are 38 species of duckweeds which are classified into five different genera namely; Landoltia, Lemna, Spirodela, Wolffia, and Wolffiella [25]. Landoltia, Spirodela and Lemna fall under category called lemnoids, while both Wolfiella and Wolffia are referred as wolffioids [26]. Duckweed is a plant with the simplest morphology. Lemnoids have one to two small leaves attached to a simple root as shown in Figure IV I. However, wolffioids are characterized by their very small frond-like structure without root attached [27]. They are usually found floating on the water surface in lakes or ponds. Its morphology allows duckweed to grow much faster than other bigger aquatic plants, since it takes short period of time; only two to four days to double its biomass depending on the species [28].

Duckweed is a plant that can easily be found in various types of regions since it can grow and adapt in a wide range of geographic areas and climatic zones. It can be found almost in any areas except in desert and polar region, where the climates are extremely dry or completely frozen. Tropical and temperate zones are the best areas they can develop; however, many species can also survive in area with extreme temperature [29].

Duckweed is popular as cheap source of protein and has been used in many applications in food, feed and biofuel production. This plant is commonly used in animal feed since it can provide amino acids as diet supplement to promote the growth of animal. Some duckweed species including Spirodela polyrhiza, Lemna gibba, and Lemna minor has been explored their application and use in pharmaceutical and environmental tests, genetic and biochemical research. Other than that, several researches have been done regarding the utilization of duckweed in wastewater treatment [30], feedstock for alternative energy sources [31] and nutrient supplement [32]. Many studies have been focused on the biofuels conversion due to the high biomass production and rich in starch content which make the bioprocessing much more cost-effective compared to the other plants [33–35].

In recent years, duckweed has become more popular to be consumed in supplemented food as additional protein in daily diets. *Wolffia microscopica sp.* is quite the most practical to be applied in human consumption due to its fast growing rate and very high yield compared to the other species of Genus *Wolffia* [36]. Reference [37] reported that duckweed species; *Wolffia arrhiza* has been traditionally consumed by poor people in Laos, Thailand and Myanmar. It is eaten as food known as "Khai-nam" or "Kai-pum" which means "eggs of the water" in Thailand and is considered as a good and cheap source of protein. Protein-based foods are important especially in these countries where their staple-foods are majorly rich in starch content such as noodles and rice [38].

Despite the fact that duckweed is a fast and easy-growing plant, optimization of the growth rates and nutrient-enriched biomass is also a vital component in large-scale practices to achieve a high-yield production and a significant cost reduction. Recent researches have suggested several methods to increase the duckweed biomass production while optimizing the starch content. The utilization of indigenous bacteria from factory wastewater [39] and also the combination of mixotrophy and heterotrophy growth condition [40] in duckweed cultivation may become one of the approach for an environmental and economic sustainable biomass production.



(a) (b)

Figure 3. (a) Upper view of duckweed Lemna minor sp. (b) Side view of duckweed Lemna minor sp. showing its tiny simple root.

B. *Duckweed Structural Components and Nutritional Value*

Many previous studies have indicated that many duckweed species exhibit great qualities to be used as nutrient supplements. However, focusing on our scope of discussion, not much of studies on pectin composition or pectin characteristics in duckweed species can be found. Previous analysis done by [11], there was about 20% pectin in Lemna minor sp. cell wall. This value, however, is lower than that of pectin in Lemna minor sp. studied by [41], which was 30.1%. These values, in average, are close to the amount of pectin from orange peel (28.0%) which is the main source for the production of commercial pectin [42]. However, a very small amount of pectin obtained from Lemna minor sp. in the investigation by [43] which was only 4.3%. This large difference might mainly due to pretreatment process of raw material duckweed, where [43] involve the usage of pectinase enzyme during the preparation of duckweed powder. The presence of pectinase might have reduced large part of polysaccharide pectin in the cell wall structure, which explain the small amount of pectin obtained. Study by [44] on polysaccharide of Lemna minor sp. showed the presence of rhamnose and apiose as sugar components of the pectic polysaccharide. Recent study on species Spirodela polyrhiza, Lemna gibba, and Wolffia australiana showed that pectin in these three species were mostly composed of apiogalacturonans and xylogalacturonan [45]. As of to date, there is still no research on the duckweed pectin characterisation of any duckweed species including on the methyl ester content and also the acetyl content which highly influence the gelling properties in food application.

Based on the study on five duckweed genera stated earlier, by [38], it is reported that protein is the major macronutrient found in whole duckweed, where about 20% to 35% per dry basis is protein, fat spanned from 4% to 7%, and starch from 4% to 10%. The polyunsaturated fatty acids content makes up from 48% to 71% [38]. Based on the analysis done by [11], the duckweed showed high starch and cellulose content, thus makes it suitable to be utilized in biofuel production. The analysis on duckweed cell wall demonstrated a high cellulose content which make up 43.7% of the cell wall, respectively, much higher than percentage reported by [43] that is 30.4%. Meanwhile, the cell wall also comprises of 3.5% hemicellulose, 0.03% phenolic compounds. Linoleic acid, α–linolenic acid and p-coumaric acid are the major fatty acids and phenolic compound found in entire duckweed [11]. Some form of antioxidants such as carotenoids and α-tocopherol are also found in duckweed plant. The amount of nutritional composition of duckweed vary depends on the plant species and growth condition [36], therefore, study on the nutritional content of different species is necessary to determine which species have the best fit for the requirements in each different applications such as high quality protein for use in supplements [46] or suitable characteristics of pectin for use in food processing [47], and also to prevent any possible rising issues in the future such as legal issues or anything that could affect human health.

V. COMPARISON OF PECTIN EXTRACTION METHODS

The growing demand for use of pectin in food industry has led to more research on various source of pectin with different gelling and stabilizing capability. At the same time, many new pectin extraction techniques have been developed with varying effectiveness, in attempt to enhance the pectin extraction yield and the quality of the extracted pectin with more environmental benefits [48]. A suitable extraction method needs to be determined in order to yield pectin product which closely meet the characteristics measurement of commercial pectin. Currently, there are three extraction techniques of pectin that are commonly employed in studies which are, acid extraction, microwave extraction and enzymatic extraction method [49].

A. *Acid Extraction*

This technique is the most commonly used for pectin extraction, where it involves boiling the pectin source in acidic solvent, and then is precipitated by using ethanol to get the pectin extracted [50]. This is the method that are used in the present manufacturing process of the pectin production [3]. There are several extracting agents have been used for pectin extraction and acid is the strongest chemical agent to extract pectin since it helps to release the tightly bound pectin to the cell wall matrix, thus increase the production of pectin extraction [51]. Various studies have shown that the strength of acid used would affect the amount of pectin extracted and its structural, physiochemical and functional characteristics. The strength of the acid also can influence the neutral sugars and uronic acid content in the extracted pectin. Reference [18] has reported that potato pectin extracted with acetic acid has higher neutral sugar content compared to other acid extractants, since it has the lowest hydrolysing activity, thus more neutral sugars could be retained in the GalA chain.

Acids that are usually used for pectin extraction are acetic, citric, lactic, hydrochloric, nitric, phosphoric and sulphuric acid. The effectiveness of different types of acids used vary depends on the sources of pectin such as from citrus fruits, apple, cocoa, potato, etc. It is reported that extractant with lower pH able to precipitate higher yield of pectin [52]. This is likely because of the acid solubility towards the protopectin from the albedo has improved with an enhanced of the acid strength, which makes it able to sustain pectin molecule more efficiently [53]. However, the use of strong acid like hydrochloric acid will produce pectin with smaller molecular weight (Mw) and smaller DM range since they tend to degrade rapidly in acid, despite the high pectin yield. Meanwhile, weak organic acid such as citric acid and acetic acid is reported to be able to produce pectin with wider range of DM; it can be HM or LM [54]. Citric acid also able to yield high amount of pectin due to its chelating activity which allow higher extraction of chelator-soluble pectin compared to other acids with no chelating action [18]. Many studies have suggested that the use of citric acid in pectin extraction have better result than other acids overall, since it has the least effect on pectin degradation, thus able to yield pectin with better quality and have better gelling function [18, 54, 55].

B. Microwave Assisted Extraction

This type of extraction is assisted with the use of microwave which involve dielectric heating of plant molecule through the penetration of microwave energy. The heat generated by microwave give a sharp increase in temperature in a short time and a very high pressure that cause a strong destruction to the plant cell wall. Microwave energy give a swelling effect on the plant cell that force the cell to disintegrate, thus cause the pectin to be released [56]. Water in the plant acts as polar solvent that helps to deliver heat into the plant matrix. Due to the dipolar rotation of water molecules, microwave energy that is absorbed into the plant tissue will generate molecular vibration, which leads to heating inside the plant tissue [57]. The rapid increase in temperature helps to break down the cell wall matrix and opened up the plant tissue, thus would efficiently release the pectic substances in a short time. In addition, the disruption of parenchyma cells has increased the surface area, which in turn improve the absorption of water into the plant cell and then helps to enhance the extraction yield of the pectin [58]. Previous study showed that the pectin yield, GalA content, and DE increase when microwave power and time increase. However, excessive heating can also cause pectin degradation. pH value also can affect the pectin yield where pectin yield increases in lower pH condition. In comparison, MAE technique is better compared to conventional extraction technique in terms of quantity and quality since the traditional method would consume relatively longer time for heating process and pectin extracted from conventional extraction tend to be exposed to prolonged heating that would cause pectin degradation. MAE consume only a few minutes or seconds while conventional method would consume hours to get relatively similar yield of pectin. MAE technique require less solvent consumption, has much shorter time consuming, and has higher extraction rate, thus able to extract pectin more efficiently compared to the commonly used conventional methods [59, 60].

C. Enzymatic Extraction

Pectin in the cell wall matrix is made up with other polysaccharides components. The use of enzyme for pectin extraction will help to degrade the non-pectin components in the plant cell wall with minimum pectinolytic activity to extract pectin with high amount of GalA [61]. Enzymes that are used for enzymatic extraction such as protopectinase, α-amylase and cellulase are usually obtained from microorganisms [10, 18, 62]. Other than that, commercial enzymes such as Celluclast, Alcalase, Cytolase and Cellulyve can also be used in the extraction of pectin [63, 64]. Enzymatic extraction technique contributes to more effective pectin yield and is more environmental-friendly compared to chemical extraction since enzymatic extraction does not require high temperature and or any acidic or alkaline solutions, therefore, there would be less pectin degradation and can reduce the disposal of hazardous waste to the environment [10]. Previous studies demonstrated the pectin extracted using enzyme have higher yield, higher DE, Mw, and GalA content, but consumed longer time compared to that of pectin obtained by acid [62, 65].

VI. PECTIN AS GELLING AGENT IN FOOD PRODUCTS

One of the significant features of pectin in the food industry has been its gelation properties. Pectin has been utilized as gelling agent since long time ago. The pectin was once found out its ability to form different kind of gel solution under different conditions. As consequence, pectin is widely exploited in food industry for jams, jellies and marmalades production. Usually, pectin is already naturally present in fruits and different fruits consist of different level of pectin. Therefore, the quantity of pectin added into food products with fruits as main ingredient, such as jam, varies depending on the type and quantity of fruits used to achieve a good level of gelation [15]. Pectin rheological properties vary depending on several parameters; pectin source, its degree of esterification, methyl content, degree of acetylation and the molecular weight of pectin. Extrinsic factors including temperature, pH, pectin concentration and divalent ions concentration also affect the pectin behaviors depend on the types of pectin [66]. Pectin can be categorized into rapid, medium and slow set pectin based on its gelling process [67]. Different types of pectin require different mechanisms to form gel stably. As example, HM pectin need pH range in 2.0-3.5 to form gel in the presence of high sugar concentration (>55% w/v). As HM pectin gelatinized, cross-linkage between two or more pectin molecules will form junction zones which are stabilized by weak molecular forces such as hydrogen and hydrophobic bonds between polar and nonpolar methylated groups [68].

On the other hand, presence of divalent cations such as $Ca2+$ is necessary for LM pectin able to gel [66]. LM pectin is also more resistant to pH, since it able to form gel in wider range of pH compared to HM pectin [69]. Recent study by [70] has reported that LM pectin from green grass jelly perform best gelation properties in pH 2.0 at 15-20°C [70]. Gel formed by

LM pectin is stabilized by the formation of electrostatic bonds between the positively charged calcium ions (Ca2+) and negatively charged carboxyl groups (COO-) on GalA blocks. Ca2+ ions are entrapped within the carboxyl groups of the pectin resulting in a zigzag network form, which looks like eggbox. Thus, LM pectin gelation is said to follow the eggbox model. The formation of calcium bridge within the gel structure helps the solution become more viscous [67]. Amidated pectin, a type of LM pectin, has amide groups which actually helps to enhance the tolerance towards larger range of Ca2+ ions concentration, thus contributes to stronger gel stability [15].

Pectin with high acetyl groups content is not suitable to be used as gelling agent since the acetyl groups will disturb the formation of bond between Ca2+ ions and GalA molecules, thus prevent the gel formation. Based on previous research, highly acetylated pectin structure can be modified through enzymatic modification (i.e. using acetyl esterases or a-arabinofuranosidase) to enhance its gelling performance [71], [72]. Other than that, acidic methanol also can be used to eliminate the acetyl groups and improve the ester content to form pectin with better gelling capability [15]. HM pectin have low capability to form gel in the presence of Ca2+. Previous study showed that poor stiffness of gel is formed from the pectin with higher percentage of DM (>60%) in presence of Ca2+ compared to pectin with relatively lower DM [73]. HM pectin is usually used in food products with high sugar content such as jams and jellies, while LM pectin is more preferable in the production of low-calorie or sugar-free foods such as milk desserts since it does not need sugar for gelation. LM pectin is also used in yogurt product as thickener to replace modified starch to prevent the masking of fruit flavour in yogurt that is caused by addition of the starches. Meanwhile, HM pectin also useful in dairy field such as stabiliser in UHT-treated drinkable yogurt and milk blends [15].

IV. CONCLUSIONS

In the attempt of finding novel sources of pectin to be utilized in food processing, other factors need to be considered including the suitability of its consumption into specific food products and also the consumer acceptance since pectin from different sources will give different effect on the appearance and texture of food products. Therefore, studies need to be done prior to the consumption of pectin from new sources in any food product to determine its suitability and how it affects the physicochemical and physical appearance of the products, in order to make sure the food products produced are of good quality. The widening scope of pectin utilization especially in food industry has led to the increasing demand of commercial pectin. Many researches on pectin from various sources had been carried out. The significant amount of pectin in duckweed makes it a possible source for industrial pectin production. However, there is still lack of research done on pectin from duckweed in food application. More detailed investigation on characterization of pectin from duckweed, which are related to the gelling function, need to be conducted, in order to explore its potential as a gelling agent. This review is expected to give some new understanding in discovering novel pectin source and also led to more findings in the potential of pectin from duckweed species to be utilized in food production or even in the pharmaceutical and cosmetic products.

ACKNOWLEDGEMENT

The authors would like to thank the Faculty of Science, Universiti Sains Islam Malaysia for providing funds under project PPP/GPI/FST/051014/60419.

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