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Malabar Spinach (*Basella alba)* seed mucilage as a natural source of edible gum: Extraction, physiochemical characterization and functional properties

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Abstract
Spinach (*Basella alba*) is a popular green leafy vegetable its seeds contain non-exudate gum which may have gelling and stabilizing properties important in food industry applications. Presently, most of the commercially available food grade gums are imported, thus it is a necessity to look for locally available and inexpensive alternatives. *Basella alba* seed gum was scrutinized in the present study to get a better insight regarding this important polysaccharide. Mucilage of the sun-dried seeds were extracted with hot water. Extractable content of mucilage from seeds was 5.63% ± 0.63 in dry basis. Composition and functional properties (antioxidant capacity, water solubility, water holding capacity (WHC) and oil holding capacity (OHC) of the spinach seed gum (SSG) were analyzed and compared. FTIR spectroscopy analysis was conducted to identify organic functional groups present in extracted gum. The potential application of SSG as a stabilizer was evaluated by incorporating it into a yoghurt and the changes in pH and syneresis were observed. Results revealed that the SSG showed a radical scavenging ability that proves antioxidant capacity of isolated gum. Solubility of spinach seed mucilage was about 21.07±0.55% at room temperature and there was a positive relationship with the increment of temperature. Furthermore, isolated SSG showed 3.09 g water/g and 76.26 g oil/100 g WHC and OHC respectively. Incorporation of SSG into yoghurt have shown a significant effect at 0.5% levels. In conclusion, SSG has shown potentially applicable hydrocolloid for food industry.

Keywords: Edible gum, malabar spinach, mucilage, swelling index, syneresis

Introduction
Hydrocolloids are one of the widely applied functional carbohydrates in food systems for several approaches, such as texture modifiers, stabilizers, and gelling agents. There is a good potential to introduce plant seeds as a new source of hydrocolloids. There are some advantages such as availability, low processing cost and health safety in using plant seeds to isolate edible gums. Plant gums are one type of naturally available hydrocolloids classified as exudate and non-exudate gums. Non-exudate gums are seeds and mucilaginous compound which can be obtained from plant tissues applying a particular extraction procedure (Hamdani, Wani and Bhat, 2019) [8]. Application of gums into food system improves the palatability and nutraceutical potential of food stuffs. There are many studies are occurred related to such as durian, quince, tamarind, and chia seed gums (Jouki *et al.*, 2014) [12]. There may be many other hydrocolloids sources namely as gums which have not been revealed in previous studies. Gums are used in food industry for obtaining different benefits such as emulsifying, coating, stabilizing, thickening and bulking agents. There are many applications of these plant gums in the food sector in Sri Lanka. As an example using mucilage of *Neolitsea cassia* (Dawul Kurundu) leaves when preparing a sweet (Aasmi) in rural areas. The science behind using that is the characterization of gum properties. In addition to that many plant sources can be found around Sri Lanka. Some of them are tamarind, durian, and watermelon. Though there are many species are available in-country wise for gum extraction it should be considered many factors regarding the compatibility of the selected one for obtaining the expected outcomes. Spinach is a plant which has mucilaginous properties in seeds and stems. Spinach is a flowering plant with alternative leaves with variable sizes (from about 1 inch to over 12 inches long).

There maturing into small hard dry lumpy fruit cluster ¼ to ½ inch across containing several seeds. Spinach seeds contain lot of slime and it coming out when it is open. The behavior of slime is important as it gives thickening ability. Here it has been selected Malabar spinach for the study. It belongs to non-exudate gum type and required a relevant extraction process for spinach seeds regarding non-exudate gums. There are so many advantages over artificial gums in natural gums and mucilage. They are biodegradable, biocompatible and non-toxic, low cost, locally available and edible sources. When considering the availability of this spinach plant, it can be easily found in many areas all around Sri Lanka. It is worthy to study all the chemical, physicochemical, rheological characterization and functional properties regarding SSG.

Under the physical properties of seed mucilage's, turbidity measurement, viscosity, powder properties, emulsifying stability and foam stability are applicable in many industries due to specific functions by these properties. These properties are more special in the food industry to keep the quality attributes than other industries when using SSG. When studying the rheology of plant gums show differences in their rheological parameters according to variations in their chemical structure. Besides, the rheology of aqueous gum dispersions depends upon their concentration and magnitude of shear stress applied (Marcotte, Hoshahili and Ramaswamy, 2001) [14]. When considering the physiochemical properties of the seed mucilage including water absorption, swelling index, and the morphological structures which determined the chemical and physical behavior. Scanning electron microscopy (SEM) and FT-IR are the most common methods to study the structures and functional groups available in spinach seed. Different gums have different applications such as water retention and stabilization, stabilizers for meat products, ice-cream, instant pudding, dairy, confectionery, baked products and sauces (Bhosale, Osmani and Moin, 2014) [19]. Spinach seed may be a better alternative for gum as an additive in the industry near future gaining quality products and keep attributes and be saving the foreign exchanges and enhancing the exportation. There is a high potential to commercialize this gum as the physical, chemical, rheological, physiochemical, and functional properties are tallied with the commercial gum in the food industry.

2. Materials and methods

2.1 Material

Malabar Spinach (*Basella alba*) seeds were collected from Galle district in Sri Lanka. Iso propyl alcohol, Molisch's reagent, sulfuric acid, hydrochloride acid, methanol, gallic acid, Folin–Ciocalteu reagent, ascorbic acid were procured from Sigma (Sigma Co., St. Louis, MO, USA). All the purchased chemicals were analytical grade.

2.2 Extraction and purification of SSG

The spinach seeds were collected from the matured clusters of seeds. The seeds were sun dried and coarsely grounded using a grinder (Panasonic MX-AC400). The coarsely grounded seeds were hydrated using distilled water at 95 ^{0}C for 60 min using a heating magnetic stirrer (APEX). Then system was allowed to rest for 24h at 8˚C in the refrigerator. Supernatant solution was set aside after 24 hrs. This process was repeated four times continuously. Then respective supernatants were centrifuged at 6000 rpm for 1 hr using the centrifuge machine (EBA 20, Hettich ZENTRIFU). The clarified supernatant was separated and mixed with iso propyl alcohol as the ratio of 1:1.5 volumes in a beaker. Then it was allowed to precipitate the mucilaginous sediment for 2hrs. After that the precipitate was filtered through a Whatman no1 filter paper using a Buchner flask (ISO LAB). Then mucilaginous precipitate was separated from the filter paper and it was dried in a hot air oven (Memmert) at 35˚C for 48hrs. The dried gum was subjected to size reduction using a grinder (Amin *et al.*, 2007) [3]. The fine powder was sieved through 100 µm sieve and stored in air tight containers.

2.3 Proximate analysis

The moisture, ash, protein, fat and fiber content of the extracted gum were determined as described in AOAC (1984). Carbohydrate content was calculated by difference.

2.4 Physical properties

2.4.1 Bulk density, tapped density and compressibility determination

Bulk density and tap density were determined following the method describe by Arasi *et al.*, 2016 [4] with slight modifications. A sample of SSG powder (1 g) was transferred into 10 mL measuring cylinder. The measuring cylinder was tapped for 250 times until there was no observable volume reduction. The bulk and tapped densities were calculated using equations 01 and 02 respectively. Compressibility (Carr's) index is a method of measurement of free flow of powder.

$$\text{Bulk density} = \frac{\text{Weight of spinach seed powder}}{\text{Bulk volume of spinach seed powder}} \quad [01]$$

$$\text{Tapped density} = \frac{\text{Weight of spinach seed powder}}{\text{Tapped volume}} \quad [02]$$

Compressibility (Carr's) Index

$I = (\rho t - \rho b / \rho t) \times 100\%$

ρt indicates the tapped density; ρb indicates the bulk density.

2.4.2 Color determination

The color phase (L*, a*, b*) were determined using a CIELAB 1976 Chroma meter, where L* indicated the lightness, a* indicated redness to greenness, and b* indicated yellowness to blueness. The sample was evaluated in duplicate and mean values were recorded.

2.4.3 Viscosity determination

Intrinsic viscosity is to determine the contribution of a particular polysaccharide towards final viscosity of the dispersion. The SSG powder (2 g) was dispersed in a 2 mL of distilled water and prepared a viscous solution. Then it was measured using the ATAGO viscometer (El-Awad El-Daw, Sc and Honours, 1998) [7].

2.5 Purity and extraction yield

Purity of SSG will be tested for carbohydrates, alkaloids,

glycosides, flavonoids, amino acids, tannins and phenols. The yield of the extracted of SSG was evaluated considering the initial dry mass of seed sample used before extraction process and the final amount of dry powder mucilage obtained after the extraction process with an average yield (Amid and Mirhosseini, 2012) [3] using the equation [03].

$$\text{Extraction yield } \% = \frac{\text{Mass of the extracted gum}}{\text{Initial mass of the crude gum}} \times 100\% \quad [03]$$

2.6 Swelling index determination

SSG powder (1g) was added into a centrifuge tube and its volume was recorded. Then 10 mL of distilled water was poured into centrifuge tube and mixed for 2 minutes. Then mixture was kept aside for 10 minutes and followed by centrifuged at 1000 rpm for 10 min using centrifuge machine (EBA 20). The supernatant was cautiously removed and volume of sediment phase was measured. Swelling index of SSG was calculated using following equation [04] (Arasi *et al.*, 2016) [4].

$$SI = \frac{V2}{V1} \times 100\ \% \quad [04]$$

SI- swelling index, V1- volume occupied by the SSG powder before hydration, V2-volume occupied by the SSG after hydration.

The test was repeated using 0.1N hydrochloric acid (pH 2.2) and phosphate buffer solution (pH 4.4) instead of distilled water.

2.7 pH determination

pH determination was conducted following the method in Eddy *et al.*, 2013. 1% w/v dispersions of the SSG and xanthan gum sample in distilled water (pH = 6.98) were prepared and shaken for 5 minutes. pH was determined at room temperature using a pre calibrated Thermo scientific pH meter (EUTECH).

2.8 Fourier transform infrared spectroscopy (FT-IR)

Functional groups of SSG were determined using FTIR using FT-IR GX System. All the spectra were an average of 16 scans from 4000 to 400 $^{cm-1}$ at a resolution of 2 $^{cm-1}$. Triplicate spectra readings for each sample were obtained (Alpizar-Reyes *et al.*, 2017) [1].

2.9 Scanning electron microscopic (SEM) analysis

The dried sample was mounted under 250 magnifications at 5.0KV. This will be used to examine the characteristic crystalline morphology influencing hydration behavior of gum. Magnification ranges from 100 to 6000 (Alpizar-Reyes *et al.*, 2017) [1].

2.10 Functional properties determination

2.10.1 Solubility

The water solubility of SSG was evaluated following the method described in (Amid and Mirhosseini, 2012) [3] with slight modifications. SSG powder sample (0.5 g) was added to 50 mL of distilled water and the mixture was stirred for 30 min. The solubility was measured by stirring the mixture at different room temperatures (29 ± 1°C) and 80 °C in order to determine the effect of temperature on the solubility of the gum using a magnetic heating stirrer (AREX, Italy). The effect of temperature was determined at 45°C and 60°C. Then mixture was centrifuged (6,000 rpm for 30 min) to remove insoluble material. Supernatant solution was transferred to a petri dish and it was oven dried at 105 °C for 24 h in a hot air oven (Memmert, USA). The solubility was calculated by weight difference and expressed in dry basis.

Solubility (%) = (C1/C2) × 100 ..... [05]

C1 - supernatant concentration (mg), C2 - initial concentration (mg)

2.10.2 Water holding capacity and oil holding capacity

Water-holding capacity of SSG powder and xanthan gum was evaluated as described in Chiranthika *et al* (2022) [5] with minor modifications. Each sample (250 mg) was suspended in 100 mL of distilled water followed by stirred for 20 min and then centrifuged at 4500 rpm for 30 min. WHC of seed gum was calculated according to the equation [06].

WHC = (SSW – SW) / SW ..... [06]

SSW- swollen sample weight, SW - sample weight

Oil holding capacity (OHC) was evaluated by dispersing 250 mg of SSG powder and xanthan gum in separate 50 mL of refined vegetable oil. OHC was expressed as grams of oil absorbed per 100 g of seed gum. It was expressed as the following equation.

OHC = (OSW – SW)/SW ..... [07]

SW- sample weight, OSW - oil absorbed sample weight

2.10.3 Water absorption

Powdered SSG sample (1 g) was suspended in 10 mL of distilled water. It was mixed for 2 min using vortex mixture and then centrifuged in a refrigerated centrifuge at 2100 rpm for 10 minutes. After centrifugation, weight of the SSG sediment was recorded. Then water absorption was calculated as grams of water absorbed per 100 g of seed gum as following equation [08] (Poosarla and Muralikrishna, 2015) [18].

$$\text{Water absorption}\% = \frac{\text{Amount of absorbed water}}{\text{Weight of the initial sample}} \times 100\% \quad ..... \quad [08]$$

2.11 Determination of total phenolic content (TPC)

Total phenolic content of SSG was analyzed using Folin Ciocalteu reagent method with slight modifications (Janarny *et al.*, 2021) [11]. Supernatant of diluted methanolic extract (0.2 mL) was mixed with 1mL of 1:10 dilution of Folin Ciocalteu reagent. Then the mixture was kept aside for 10 minutes and followed by 0.8 mL of 7.5% sodium carbonate was added to the mixture. The mixture was kept for 30 min of incubation period in the dark at room temperature. Absorbance measurements were taken at 743 nm. TPC was expressed as mg of gallic acid equivalents (GAE) per g of product.

2.12 Determination of DPPH scavenging capacity

DPPH radical scavenging ability of the SSG was determined according to the method described in Hettiarachchi *et al*., 2022 [10]. Aliquots of 0.4 mL, 0.8 mL, 1.2 mL, 1.6 mL, 2.0 mL, 2.4 mL, 2.8 mL, 3.2 mL, 3.6 mL and 4.0 mL of 0.1 mg/mL solution of ascorbic acid standard (antioxidant) were added to 10 mL volumetric flasks. 1.0 mL of 0.2 mg/ mL DPPH solution was added to each volumetric flask. Volumetric flask was made up with methanol, and the flasks were shaken vigorously and absorbance values were measured immediately at 517 nm by UV/VIS spectrophotometer (V-1100, Thermo Scientific, USA). The percentage inhibition of the radicals due to the antioxidant activity of gum extracts was calculated using the following formula [09].

$$\% \text{ Inhibition} = \{(A_{control} - A_{sample}) / A_{control} \} \times 100\% \text{ .....[09]}$$

2.13 Yoghurt preparation adding edible gums

Standardized milk was preheated at 55°C-65°C and homogenized at 10000 rpm for 10 min at room temperature by using homogenizer. Milk was pasteurized at 90-95°C for 5 min. gelatin and xanthan gum were added as commercially available stabilizers at the rate of 0.4% and 0.2% respectively. Two treatments from SSG were done as the portions of 0.5% and 1.5%. After heat treatment, milk was allowed to cool upto inoculation temperature (42°C-45°C) which is the optimum growth temperature for thermophilic starter culture. Yogurts were put in the incubator (VELP SCIENTIFICA) for fermentation process at 42°C for 3-4 hrs. The point of yogurts took off from incubator was determined by measuring pH whether it was around 4.5-4.6. Then yogurts were kept in refrigerator at 4°C until further analyses. These samples were subjected to sensory evaluation.

2.14 pH determination of yogurt

pH was determined in yogurts which were added different portions of gums as stabilizers, using a pH meter (EUTECH, Thermo Scientific, USA) for four days with a one day interval.

2.15 Syneresis index determination of prepared yoghurts

Syneresis index of formulated yogurts was analyzed following the method proposed by Han *et al.*, 2016 [9] with slight modifications. Yogurt sample (15 g) was prepared in a centrifuge tube and it was centrifuged at 2000 ppm for 10 min at 25ºC. Clear supernatant solution was collected and weighed. Syneresis was calculated according to the following equation [10]. It was measured up to 4 days with one day interval and noted.

$$\text{Syneresis (\%)} = \frac{\text{Weight of supernatant (g)}}{\text{Weight of yogurt sample (g)}} \times 100\% \quad \text{..... [10]}$$

2.16 Statistical analysis

All the analyses were conducted in triplicate and the data were expressed as mean ±standard deviation. The sample means were compared at the 95% confidence level ($p < 0.05$) using Tukey's test in SPSS 16.0 software

3. Results and discussion

3.1 Proximate composition of extracted SSG

Proximate composition analysis is very important as it reflects about the percentage of different components contain in the SSG powder. It can be used to find out the quantitative and qualitative chemical reactions occur in product that on the overall quality and shelf life of the product. Proximate composition of extracted SSG was analyzed and results are shown in Table 1.

Table 1: Proximate composition of SSG

Component	SSG sample (%) Dry basis
Crude protein	11.15±0.62
Crude fat	15.2±1.19
Crude fiber	1.44±0.03
Moisture	0.9 ±0.3
Ash	2.30 ±0.05
Carbohydrate	69.01±0.03

Mean value from triplicate, Mean± standard deviation

Moisture content of SSG was 0.9%±0.3 and total carbohydrate content was found as 69.01%±0.03. The crude protein content has been determined as 11.15% ±0.62. This value is comparable to those reported but higher than those in guar gum (3.5%) (Monrroy *et al.*, 2017) [16]. The interactions which occur between certain hydrophilic functional groups of polysaccharides and proteins are vital regarding mucilage proteins form three-dimensional networks that can enhance the uniformity and stability of the matrix and show potential applications in food industry (Monrroy *et al.*, 2017) [16]. In the mucilage extracted from leaf tissue of spinach moisture content was 2.63% and total carbohydrate content was found to be as 84.05%. Further, the mucilage showed the presence of water insoluble ash (0.54%), acid insoluble ash (0.36%), sulphated ash (1.35%) (Deshmukh and Gaikwad, 2014) [6] According to previous studies.

3.2 Physical characteristics

Physical characteristics such as color, pH, viscosity, solubility, tap density, bulk density ad compressibility were analyzed and results are shown in Table 2.

Table 2: Physical characteristics of SSG

Property	Value
Color	
L^*	25.03 ±0.37
a^*	8.27 ±0.02
b^*	18.10 ±0.11
pH(25°C)	6.42 ±0.01
Viscosity(mPa)	211.6 ±0.46
Solubility (%)	21.07 ±0.55
Tap Density (g/cm^{-3})	0.88 ±0.04
Bulk Density (g/cm^{-3})	0.45 ±0.01
Compressibility (Carr's) Index	36.9±1.07

Mean value from triplicate, Mean± standard deviation.

According to the results obtained for physical properties, that mucilage is mainly a polysaccharide with pH ranging between 6.42 ±0.01. Density determination indicates the tapped density was 0.88 ±0.04 g/cm^{-3} and the bulk density was 0.45±0.01 g/cm^{-3}. The bulk and tapped densities be evidence of particle arrangement and the compaction behavior of material. The Carr's index was found as 36.9±1.07%, indicating the gum has somewhat less flow property and compressibility (Malsawmtluangi *et al.*, 2014) [13]. The Carr's index value which lower than 15% indicates

excellent flow characteristics of a powder, whereas more than 25% indicate poor flow ability. The granules were found to possess less flow property as indicated by the Carr's index (Bhosale, Osmani and Moin, 2014b) [19].

3.3 Phytochemical properties

Total phenolic content and DPPH radical scavenging activity of xanthan gum and SSG are shown in Figure 1 and 2 respectively.

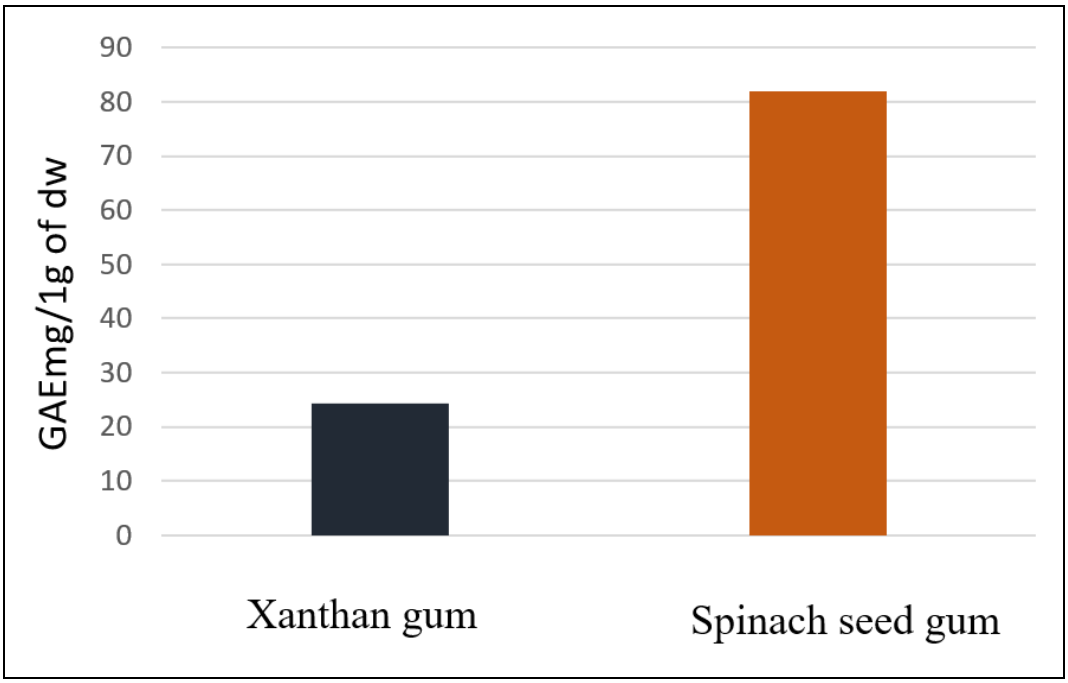

Fig 1: Total phenolic content of xanthan and SSG

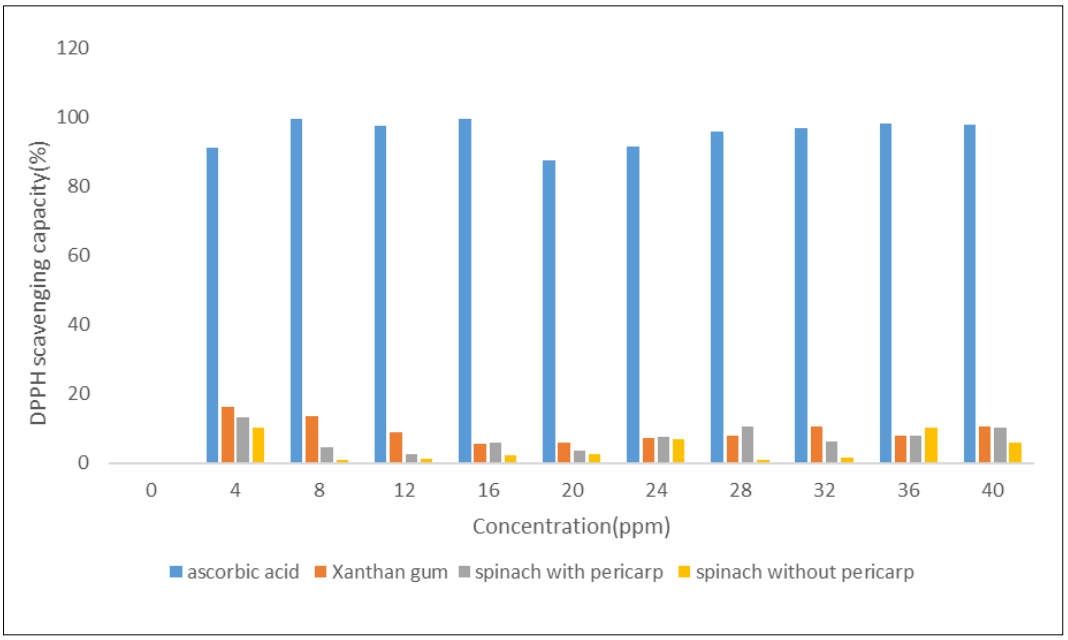

Fig 2: Total antioxidant capacity of different gums with ascorbic acid standard

Results showed significant difference ($p<0.05$) in total phenolic content and DPPH radical scavenging activity of SSG and xanthan gum. Results showed that the highest value of total phenolic content was in SSG. Further, low values of DPPH radical scavenging activity were observed in all gums.

DPPH is a free radical that can donate its hydrogen atom to make a stable DPPH molecule. DPPH molecules accept protons and undergo for oxidation. It has been widely employed to evaluate the free radical-scavenging capacity of several natural compounds. The DPPH radical scavenging ability of gums is presented in Figure 2 as a comparable illustration using ascorbic acid standard with xanthan gum and SSG from the seeds with and without outer pericarp. There was a relatively low inhibition activities/ mild antioxidant activity are available in all the tested gum samples. As plant parts in the origin of gums (seed, leaves, stems) are dried in different ways. Hence the antioxidants are deteriorated in high temperatures. Further, the elevated extraction temperatures cause reduction of antioxidant activity. Extraction time is also one of the major factors that effect on extraction of antioxidant compounds. Thus those compounds are readily degrading if exposed to ambient conditions for long time. As SSG are prepared using sun

dried seeds a considerable amount of antioxidants are diminished. It can be minimized using a different way of drying like freeze drying and may remain relatively high content of phytochemicals. Pericarp contain lot of antioxidants are available and dried seeds with pericarp showed a relatively high antioxidant activity compared to SSG without pericarp. Previous studies have been reported that long extraction time, increased the antioxidant reduction in spinach seeds (Jouki *et al.*, 2014) [12].

The total phenolic content determined by the Folin Ciocalteau method was significantly higher ($p<0.05$) in SSG compared to xanthan gum (Figure 2). In present study, the phenolic content of SSG gum samples was quite high 81.88 ±5.63 mg GAE/g) in methanolic extracts. However, it was significantly ($p < 0.05$) lower than xanthan gum 24.38±6.88 mg GAE/g in the samples where methanol was used extraction solvent.

3.4 Physicochemical properties

Swelling index, solubility, water holding capacity and oil holding capacity were determined as physicochemical properties and data are shown in Table 3, Table 4 and Figure 3.

Table 3: Swelling indexes of different gums in different pH conditions

pH	Xanthan gum	Spinach seed gum	Gelatin
2.2	2.55 ±0.06^{b}	1.42 ±0.06^{a}	4.91 ±0.07^{c}
4.4	3.05 ±0.30^{b}	1.62 ±0.07^{a}	1.48 ±0.05^{a}
7.1	4.91 ±0.07^{b}	1.16 ±0.04^{a}	1.07 ±0.06^{a}

Mean value from triplicate, Mean± standard deviation
Means within each raw superscripted by different letters are significantly different at $p<0.05$

Table 4: Solubility of SSG at different elevated temperatures

Temperature (°C)	Solubility (%)
30	21.07 ±0.55
45	27.60 ±0.79
60	31.20±1.32
80	40.45±0.99

Mean value from triplicate, Mean± standard deviation
Means within each raw superscripted by different letters are significantly different at $p<0.05$

3.5 Water holding capacity and oil holding capacity

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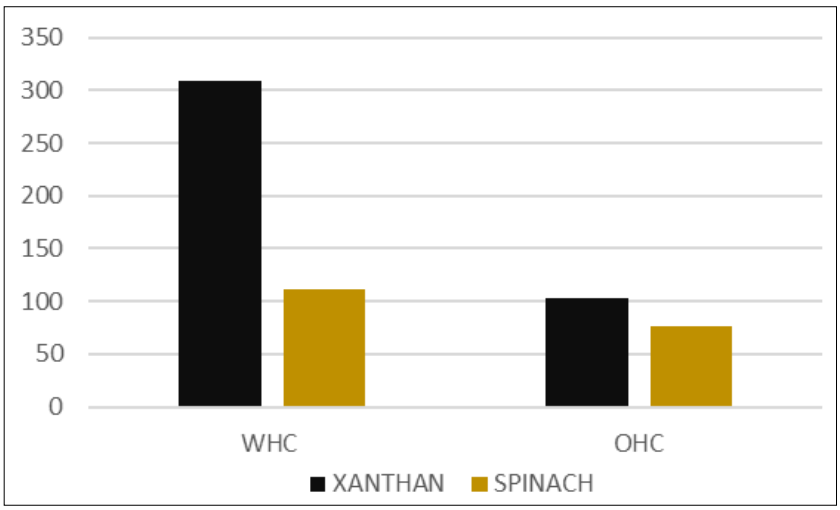

Fig 3: WHC and OHC of SSG and Xanthan gum

Plant gums have physicochemical properties endemic for the compounds themselves. SSG powder is less soluble in water and swells. The swelling index is low in water and 0.1N hydrochloric acid while the highest solubility in phosphate buffer (pH 4.2). Lower swelling properties in acidic pH conditions related to polymeric backbone. The swelling analysis results revealed that SSG is pH sensitive hydrocolloid (Arasi *et al.*, 2016) [4]. In xanthan gum the variation of swelling index is in the correct mechanism, thus swelling index tends to increase when pH increased. Swelling index denotes the degree of granule hydration. However, swelling index variation pattern of spinach has been slightly changed. High swelling capacity indicates weak binding forces in the particles. Moreover, the increasing of swelling index with increasing of pH may be due to the electrostatic repulsion in functional groups which cause higher volume available to retain water molecules (Alpizar-Reyes *et al.*, 2017) [1]. Since pH determines the solubility of gums, functional properties of a particular gum may be affected by change in hydrogen ion concentration (Poosarla and Muralikrishna, 2015) [18]. The pH measurements of SSG show which mucilage is slightly acidic (6.42±0.01). This acidic nature may be due to presence of uronic acids in mucilage structure. The resulted pH values were significantly ($p<0.05$) higher comparable to those reported previously for gum arabic (4.5–5.6). The pH is also a critical factor in coagulation processes, where in the optimal pH should be between 5 and 7.5 (Monrroy *et al.*, 2017) [16]. As SSG are having a slightly acidic nature it can be applied to dairy industry without interrupting quality of the product.

3.6 FTIR analysis

FTIR spectroscopy is mainly applied to qualitatively analyze organic functional groups presence in natural compounds. The FTIR spectra exhibit the typical bands and peak characteristic for mucilage. The FTIR spectra of SSG is shown in Figure 4.

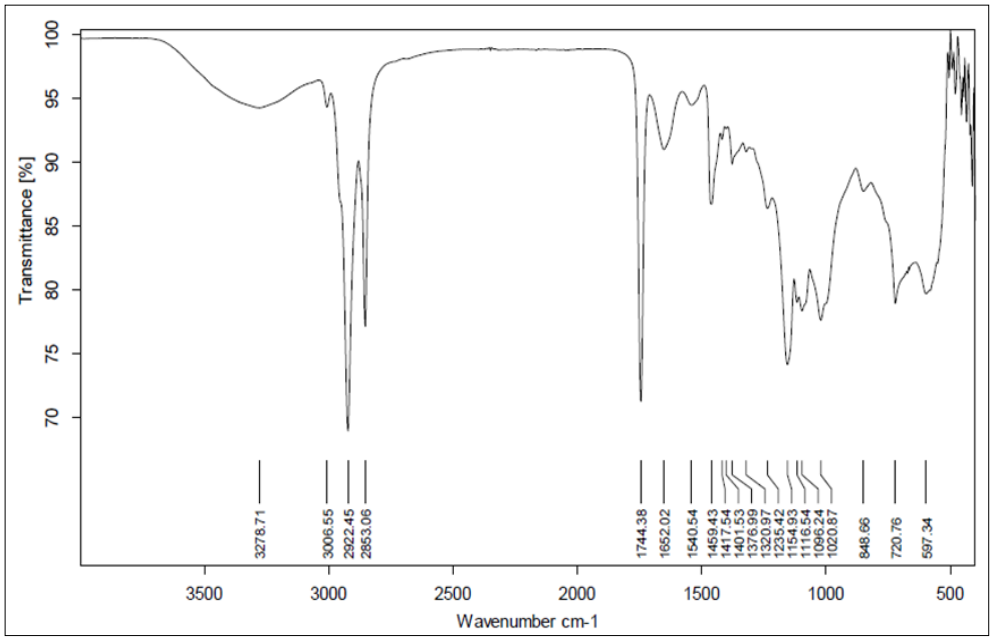

Fig 4: FTIR spectrum of spinach seed gum powder

Natural gums mainly consist with fractions of sugar acid units which would usually give a weakly anionic nature to gum macromolecules. A typical broad absorption peak at 3278.71 cm^{-1} might be attributed to the presence of hydroxyl groups within the molecule while a small band at 3006.55 cm^{-1} might be due to by –C═O overtone. The bands at 2922.45 cm^{-1} and 2853.06 cm^{-1} referred to vibrational symmetric stretching of sp^3 C–H and vibrational stretching of C–H, suggesting the existence of grafting. These bands, which serve as binding sites for ions, had an vital effect on the gel forming ability that can interact with water to obtain a proper gel (Jouki *et al.*, 2014) [12].

In current study, absorption peak at 1744.38 cm^{-1} is typical of acetyl groups. The region between 1500 and 1800 cm^{-1} is typically used to determine presence of carboxylic groups of uronic acid residues in the gum polysaccharide (Malsawmtluangi *et al.*, 2014b) [13]. In the FTIR spectra, 1652.02 cm^{-1} stands for the stretching vibrations of –COO– (asymmetric vibrations) groups, respectively, in carbohydrate and uronic acid molecules (Monrroy *et al.*, 2017) [16]. The absorptions at wavenumber 1417.54 cm^{-1} is caused by C–OO symmetric stretching (Jouki *et al.*, 2014) [12]. It has also been reported that this region is due to hydrogen bonding with the hydroxyl groups of gluco pyranose rings. The soft peak at 1154.93 cm^{-1} corresponds to C-O-H bonds, primary alcoholic compounds (Alpizar-Reyes *et al.*, 2017) [1] and characteristics bands between 900 to 1200 cm^{-1} are known as Polysaccharide's fingerprint region.

3.7 SEM analysis

Scanning electron microscopic image of extracted SSG is shown in Figure 5.

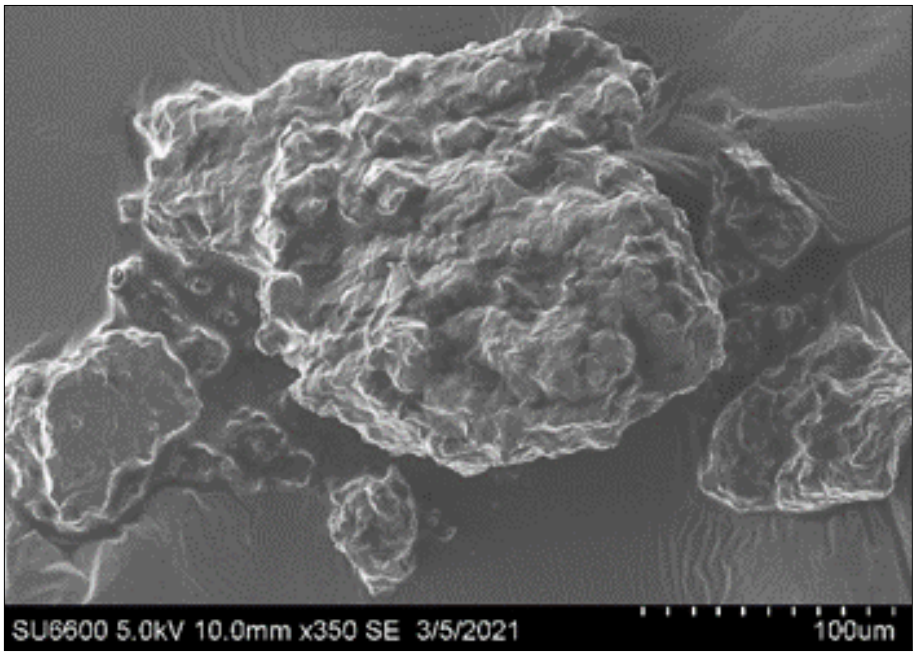

Fig 5: Scanning electron microscopic images of SSG mucilage at magnification of x 250 SE

The particles can be observed as aggregates of irregular shape and dimension which were fibrous in nature. The surface is not appeared fissures, cracks or interruptions. Semispherical particles with a skin-like structure or polymeric appearance are observed. The SEM results showed that, surface properties of mucilage effect on its hydration capacity. The gums had irregular shapes, smooth surface and in uniform sizes. As particle size and specific surface area influence the hydration behavior of gums, which in turn influence their intrinsic viscosity and molecular masses. The dissolution rate of polysaccharide powders generally elevates with reduction in particle size (Malsawmtluangi *et al.*, 2014b) [13].

Particle size and specific surface area influence the hydration behavior of gums, which in turn influence their intrinsic viscosity and molecular mass. It was reflect from the figure that the molecules of the gum are tiny granules and slightly elongated with rugged appearance (Ameh, Sani and Nwoye, 2015) [4]. The shape and structure or surface topography of the mucilage also can be changed by the extraction method, purification and preparation of the product (Singh and Bothara, 2014) [20].

3.8 Syneresis in yogurts

Syneresis of yogurts with different treatments was determined with time and results are shown in Table 5.

Table 5: Syneresis of yogurts under different treatments with time

Treatment	D_1	D_4	D_7	D_{10}
T_1	14.51±0.90 ^{a}	19.66±1.14^a	22.13±1.90^a	26.71±1.34^a
T_2	6.166±1.50^b	12.17±0.72^b	17.35±1.27^b	25.51±0.80^a
T_3	6.60±1.31^b	13.55±1.03^b	15.84±0.62^b	20.71±0.54^b
T_4	4.60±1.04^b	9.26±0.54^c	12.06±0.64^c	19.02±0.50^{bc}
T_5	5.91±0.87^b	12.17±0.71^b	14.97±1.57^{bc}	22.38±0.71^c

Means within a column with different superscripts were significantly different ($p<0.05$). T_1 (control group), T_2 (xanthan gum), T_3 (gelatin), T_4 (0.5% spinach) and T_5 (1.5% spinach). Values are means ± SD, $\alpha = 0.05$.

The stabilizers cause yogurt less susceptible to rearrangement within its network; the level of syneresis in the control was significantly higher ($P < 0.05$) than the level of syneresis in the treatments with SSG, xanthan gum and gelatin. SSG (0.5%) was less susceptible to syneresis and observed as significantly ($P < 0.05$) lower syneresis index compared to other treatments. Then gelatin can be considered as best followed by SSG as it has been controlled syneresis into some extent. This low syneresis in the spinach (0.5%) gum-stabilized yogurt can be attributed to the improved water holding capacity by the SSG. The main reasons for syneresis in fermented products include high temperature incubation, low solids content or inadequate storage temperatures. The mean for syneresis in the case of untreated samples were 14.51, 19.66, 22.13, and 26.71 at 25°C after one and ten days of storage with two day intervals. Yogurt samples contained spinach seed gum at a concentration of 0.5% during storage had less syneresis 4.60, 9.26, 12.06, 19.02 at 25°C compared to other samples. Syneresis increased with storage time, however syneresis was significantly lower ($p<0.05$) in treated samples with stabilizers over control samples. Incorporating dry matter and protein content is common practices of minimizing whey separation in yoghurts. It has been reported that higher degree of syneresis showed in low-fat yogurts than high-fat yogurts. Thus stabilizers are usually added to low-fat yogurts to reduce syneresis or whey separation. Yogurt is normally prepared using homogenized milk to enhance stability. Homogenizing coats increased surface of fat globules with casein, enabling the fat globules to participate as a copolymer with casein to strengthen the gel network and reduce syneresis. Therefore, it can be concluded that the SSG helped in forming Protein-coated SSG spheres, which reinforced the gel structure by their association with casein micelles of the protein network (Mugo *et al.*, 2020) [17]. Thus additions of SSG into yoghurts have a positive effect on the formation of syneresis than other commercially available edible gums. These findings suggest that spinach seed gum is better at reducing syneresis and modifying the texture of foods.

3.9 pH variations in yogurts

pH variations in different yogurt formulas with storage time was determined and data are shown in Table 6.

Table 7: Mean pH values of yogurts under different treatments

Treatment	D_1	D_2	D_3	D_4	D_5
T_1	4.69±0.01 ^{c}	4.62±0.01^a	4.61±0.01^a	4.60±0.02^a	4.54±0.03^a
T_2	4.73±0.015 ^{b}	4.49±0.03^b	4.43±0.25^c	4.46±0.02^c	4.43±0.02^{bc}
T_3	4.80±0.02 ^{a}	4.53±0.02^b	4.51±0.01^b	4.48±0.01^c	4.47±0.01^b
T_4	4.64±0.01 ^{d}	4.59±0.02^a	4.54±0.02^b	4.52±0.01^b	4.40±0.02^c
T_5	4.66±0.01^{cd}	4.60±0.02^a	4.50±0.25^b	4.48±0.15^c	4.42±0.25^{bc}

Means within a column with different superscripts were significantly different ($p<0.05$). T_1 (control group), T_2 (xanthan gum), T_3 (gelatin), T_4 (0.5% spinach) and T_5 (1.5% spinach). Values are means ± SD, $\alpha = 0.05$.

pH is a key factor to determine the quality of yogurt. Lactic acid formation leads to reduce the pH in yogurts with storage time. It was determined by while syneresis was increased with storage time which was due to the lactic acid formation with increase in storage time. However, pH was significantly lower in yogurt treated with SSG in each day than control yogurt while syneresis was significantly lower (Table 7). Adding stabilizer seems slower the acid development and syneresis of yogurt. pH was decreased from 4.69 to 4.54 in case of control and 4.64 to 4.40 in case of SSG treated yogurt from first to tenth day. Decrement of pH throughout the storage period might be due to the formation of lactic acid by certain bacteria of yogurt .less subsidence in pH was observed in case of sample treated with SSG than control. Based on observation of this study, decreased pH values are around the isoelectric point (4.6) of the yogurt where the casein micelles in milk flocculate to give a desirable gel structure .So it is not altered the gel structure of the yogurt. Many plant gums are consisted with acidic nature as galacturonic acid is a component of the mucilage. So it is not affected not to increase the pH of acidic nature of yoghurt's food matrix. Therefore, using SSG it can be managed all the attributes without altering the quality of yoghurts.

4. Conclusion

Crude fat, crude fiber, ash and carbohydrate in SSG were and, 15.2%, 1.44%, 2.30% and 69.01% respectively in dry basis. It is concluded that average total yield of mucilage was about 5.63%. Total phenolic content, in SSG shows significantly positive difference with xanthan ($p<0.05$) and they were 81.88 ±5.63 mg GAE/g. Total antioxidant

capacity of gums from spinach is relatively close to commercially available gums. Solubility of spinach seed mucilage has been revealed 21.07 ±0.55% at room temperature and there is a positive relationship with the increment of temperature. According to results SSG has been obtained WHC 3.09 g water/g and OHC 76.26 g oil/100 g gum. There is a significant interaction between swelling index and gum type shown considerable swelling index at every pH. Application of SSG in dairy industry for yoghurts has been revealed that facilitate a proper quality with a minimum syneresis compare to other gums as a stabilizer. Thus, SSG has a potential to be used as an alternative natural food gum in food industry for different functionalities.

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6. Competing Interests
Authors have declared that no competing interests exist.

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