Effect of Temperature, pH and Some Salts on Physicochemical and Rheological Properties of Nettle seed gum (NSG)

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To cite this article:
Arash Dara. Effect of Temperature, pH and Some Salts on Physicochemical and Rheological Properties of Nettle seed gum (NSG). International Journal of Food Engineering and Technology. Vol. 5, No. 1, 2021, pp. 1-8. doi: 10.11648/j.ijfet.20210501.11

Received: December 28, 2020; Accepted: January 11, 2021; Published: March 10, 2021

Abstract: Nettle seed gum (NSG) or Urtica pilulifera is a novel source of hydrocolloid, in this study the physicochemical, structural, functional and rheological properties of Urtica pilulifera seed mucilage were compared with gum arabic and pectin. Functional properties, water absorbance was significantly affected by temperature and with increasing temperature, water absorbance increased. In the study of urtica pilulifera oil absorbance, Urtica pilulifera had a very low oil absorbance, which can make this gum suitable for use in frying products. In the stability study, the emulsion of Urtica pilulifera mucilage was significantly higher compared to pectin and gum arabic. On the other hand, with increasing temperature, the stability of Urtica pilulifera emulsion decreased. The effect of pH on emulsion stability was also observed. Urtica pilulifera mucilage has the highest stability at pH 5. With increasing the concentration of gum, the amount of surface tension in all samples decreased and pectin gum had the highest surface tension in all concentrations. In investigating the effect of temperature and pH on particle size distribution at 70°C, pH changes had the greatest effect on particle size distribution and increased with decreasing pH, particle diameter. In the study of rheological behavior at all concentrations, with increasing shear rate, the amount of shear stress also increased and with increasing concentration, the flow behavior changed to non-Newtonian fluids or pseudoplastic. In the study of mucilage structure using FTIR, many peaks have been observed, the most important of which are related to the tensile vibrations of the hydroxyl group (fat, protein and carbohydrate), ketone groups (fat, pectin, aromatic ring with CH bond) Also to investigate the possibility of using Urtica pilulifera mucilage in foods that are exposed to low temperatures Freezing test and freezing removal were performed on it After freezing, this emulsion was unstable and completely broke.

Keywords: Urtica pilulifera Seed Gum, Surface Tension, Emulsion Stability, Rheology

1. Introduction

Gums and their derivatives are a large group of polysaccharides that can produce high viscosity products at low concentrations. [1] Gums are widely used in the food industry to make gels and as stabilizers and suspending agents. Hydrocolloids in the food system may play the role of emulsifiers, stabilizers, or both. Surface activity is a necessary feature of an emulsifier, and since polysaccharides are basically hydrophilic [2] most hydrocolloids cannot act as an emulsifier. In practice, Gum arabic is the only hydrocolloid commonly used as an emulsifier. [3] This hydrocolloid is a compound stabilizing agent that gives long-term stability to an emulsion. Plant gums and mucilages are highly hydrophilic and are water-soluble polysaccharides. [4] Plant mucilages are polysaccharides that form a colloidal solution in water and They can often be forced to sediment using ammonium sulfate, sodium chloride, and common protein precipitators. Plant mucilages are not able to form gels and they are different from pectin in this respect. Urtica is a genus of flowering plants in the family Urticaceae. They have a long history of use as an herbal remedy. The main varieties of the Urtica genus (Urtica dioica L., Urtica urens L., Urtica pilulifera L., Urtica cannabina L., Urtica membranacea Poiret, and Urtica kiovensis Rogoff) well known for their stinging hairs found on the leaves and stems of the plants Urtica pilulifera, also known as Roman nettle, as an annual plant is common in many parts of the world and
extensively cultivated in the Mediterranean region. It is widely used in folk remedy to treat hypertension, hyperglycemia and inflammation of some organs. [5] Seed mucilage of Urtica pilulifera can be added to the list of these hydrocolloids as a new compound and use this mucilage to stabilize the product or improve the rheological properties of all products that are in the form of an emulsion system and need stabilization, including sauces or products with special properties. From the root of Urtica pilulifera to the present steroids, phenylpropanoids, coumarins [6], terpenoids [7], polysaccharides [8], and lectins [9] have been extracted and from its flakes 7 flavonol glucosides extracted. lipid analysis of Urtica pilulifera seed shows high proportions of saturated fatty acids, especially palmitic acid, and small amounts of omega-3 unsaturated fatty acids [10]. The seeds of Urtica pilulifera, like the seeds of many plants, including kdomeh, have mucilages on their epidermis [11] that concentrate the aqueous medium. In this study, the physicochemical, structural, functional, and rheological properties of Urtica pilulifera seed mucilage as a new plant hydrocolloid were compared with gum arabic and pectin.

2. Materials and Methods

Materials The Nettle seeds (species Urtica pilulifera) were acquired from a regional medical plant market, Rasht, Iran. The seeds were manually cleaned to pick up all foreign material. It was then packaged and stored in 4°C for further experiments. D-glucose, D-galacturonic acid, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich, USA. Sodium azide (NaN3) and albumin were purchased from Applichem Inc. (Dram- Stadt, Germany).

2.1. Gum Extraction from Nettle Seeds & Samples Preparation

Nettle seed gum was provided under the optimized conditions [12] In summary, Nettle seeds were soaked in the distilled water at a water/seed ratio of 40:1 and a fixed temperature of 59±1.0°C for a period of 3.4 h. Then, Separation of mucilage from the swollen seeds was achieved through the scraping technique. The seeds were passed through an extractor (model 402, Pars-Khazar Com., Iran) equipped with a rotating plate that scraped the mucilage layer on the seed surface. Then the crude extract was passed through a vacuum filter to remove excess particles and dried at 36°C. The dried gum was milled, sieved (mesh 18 sifter). NSG powders were stored in a cool and dry place.

To prepare NSG dispersions with different concentrations, a certain weight of NSG powder was added slowly and gradually to a particular volume of deionized water at a temperature of 25°C. Moreover, sodium azide with a concentration of 0.02% (w/v) was added to all dispersions to prevent microbial activity. Dispersions were gently stirred on a magnetic stirrer for 4 h to complete dissolution. Dispersions were then stirred with a roller mixer for 24 h at 25°C to complete hydration and were finally refrigerated for 24 h at 4°C.

2.2. Chemical Compositions Analysis & FTIR Test

The amounts of moisture, total ash, lipid, and crude protein (the nitrogen to protein conversion factor of 6.25) of NSG powder were determined by the standard methods as described elsewhere (AOAC, 2005). The metal ions (Ca, Mg, K, Zn, and Na) were measured by applying a flame photometer (PPF7 model, JANEWAY, UK).

For the FTIR test, Spectrum RX 1 infrared Fourier transform spectroscopy device made by PerkinElmer USA was used. To obtain a spectrum in solid-state of 2 mg, a sample of Urtica pilulifera mucilage powder was mixed with about 4 mg KBr and after grinding in a press mortar, it was transformed into a 13 mm diameter tablet and irradiated with a light transmission spectrum in the range of 1400 cm\(^{-1}\) to 14000 cm\(^{-1}\). [13]

2.3. Thermal Stability of the Emulsion

This method was used to compare the mucilage of Urtica pilulifera, gum arabic, and pectin. First, colloidal dispersion of 0.5% gum was produced using deionized water and stirring for 30 minutes. This dispersion was then kept at room temperature for 24 hours to completely dehydrate the gum. Then an oil-in-water emulsion in the ratio of 20 to 80 (w / w) was prepared by adding soybean oil to the dispersion. During stirring, the dispersion was added dropwise by a mechanical stirring of soybean oil to form an emulsion. To determine the stability of the emulsion at high temperature, the produced emulsion was heated at 80°C for 30 minutes and finally centrifuged at 1200 g for 5 minutes and determined using the emulsion stability ratio [14].

2.4. Investigation of the Effect of pH and Temperature on the Stability of Urtica Pilulifera Mucilage Emulsion

To investigate the effect of pH and temperature on the stability of Urtica pilulifera mucilage emulsion, the first 0.5% colloidal solution of Urtica pilulifera mucilage was produced by using deionized water and stirring for 30 minutes. The pH of the product solution was then adjusted by using 1 normal citric acid buffer at 3, 5, and 7, and the oil-in-water emulsion in the ratio of 20 to 80 (w / w) was prepared by adding soybean oil to the solution. The emulsion was then divided into three parts and heated at three temperatures of 70, 80, and 90° C for half an hour. Finally, it was centrifuged for 12 minutes at 1200 g and by using the equation the emulsion stability was determined [15].

2.5. Effect of pH and Temperature on the Particle Size Distribution of Urtica Pilulifera Emulsion

To investigate the effect of pH and temperature on the particle size in Urtica pilulifera emulsion, the particle size distribution of the emulsion produced according to the previous step using a static Horiba laser beam scattering device (model LA-930, Japan) equipped with 5 mV helium
laser beam / Neon (635 nm) was measured at room temperature (20±2°C). Before injection into the device, all samples were homogeneous and injected into the cell to the extent that the amount of light passing through the deionized water is 70-85% [16].

2.6. Effect of Mucilage on Surface Tension Between Air/Water

Surface tension was measured using the Wilhelmy plate method based on force measurement. The measuring instrument was a platinum plate with dimensions of 10×19.9×0.2 mm and 25 ml of the aqueous solution containing Urtica pilulifera mucilage, gum arabic, and pectin with a concentration of 0.25, 0.5, and 1% was prepared and the reduction of surface tension was measured at 25°C [17].

2.7. Water and Oil Storage Capacity

The amount of oil and water absorption was measured based on the method of Alpizar-Rees at different temperatures (25, 45, and 65°C). [18]

2.8. Determining the Effect of Gum Concentration & pH and Salt Concentration on Rheological Behavior

To determine the effect of concentration on the rheological behavior of Urtica pilulifera mucilage, 0.25, 0.5, 0.75, and 1% solutions were prepared and kept at room temperature for 24 hours for complete hydration. Then, the apparent viscosity and changes in shear stress relative to the shear rate were measured using a Brookfield viscometer (RV model, made in the USA) and a UL spindle at 25°C in the range of 40 to 250 rpm [19]. To determine the effect of pH on rheological behavior after preparation of 0.25% solution of gum arabic, pectin, and Urtica pilulifera mucilage, their pH was adjusted using a citric acid buffer in 3, 5, and 7, and the apparent viscosity and shear stress changes relative to the shear rate was determined. [20] The effect of sodium chloride, potassium chloride, and calcium chloride salts on the rheological behavior of Urtica pilulifera mucilage was also investigated. Therefore, after preparing 0.25% mucilage solution, the concentration of each salt in mucilage solution as separate solutions in 20, 40, 60, 80, and 100 mM were adjusted and its rheological behavior was studied. The power-law equation was used to model the rheological behavior, which showed the highest matching with laboratory data [21].

\[ \tau = k \gamma^n \]

2.9. Stability to Freezing and Thawing Processes

To investigate the effect of freezing and thawing properties of Urtica pilulifera seed mucilage emulsion, 10 ml of oil-in-water emulsion in water ratio of 20 to 80 (w / w) was poured into a test tube and kept at -2°C for 24 hours and then solidified for 3 hours at 40°C and the stability of the emulsion after this freezing and thawing was investigated [22].

2.10. Statistical Analysis

The tests were performed in a completely randomized design with 3 replications. To statistically analyze the data obtained from the present study, SAS statistical software was used and the comparison of means was evaluated using the LSD test. Statistical differences of p <0.05 were considered as significant levels.

3. Results and Discussion

3.1. Physical and Chemical Properties

This mucilage has higher protein and ash compared to commonly used commercial gums such as gum arabic and pectin. According to reports, acacia gum contains (3.56%) ash and (2.31%) protein and high methoxyl pectin extracted from yellow apple has a small amount of protein and ash [23].

| Table 1. Percentage of compounds (grams per 100 grams of dry matter) Urtica pilulifera seed mucilage. |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Humidity | Fat | Protein | Ash |
| 6.51±0.71 | 1.88±0.68 | 21.42±0.64 | 14.31±0.48 |

Numbers are the mean±standard deviation (three repetitions).

3.2. Water and Oil Storage Capacity (Grams per Gram of Dry Matter)

| Table 2. Water and oil storage capacity (grams per gram of dry matter) |
|------------------------------------|-----------------|-----------------|
| Temperature (degrees Celsius) | Water absorption | Oil absorption |
| 25 | 24.1±88.13 | 0.0±827.109 |
| 45 | 27.1±36.71 | 0.0±940.91 |
| 65 | 32.1±9.08 | >0.0±940.091 |

Numbers, mean±standard deviation (three repetitions)
Different lowercase letters indicate a significant difference at the 95% level.

3.3. Comparison of Surface Tension of Urtica Pilulifera Mucilage, Gum Arabic and Pectin

According to the results of the analysis of variance of gum type, gum concentration and the interaction effect of gum type on concentration at the probability level of p <0.001 have shown a significant effect on the surface tension. The surface tension of pure water at 25°C is 72.5 mN/m [24].

| Table 3. The surface tension of Urtica pilulifera mucilage, gum arabic, and pectin (ml / m). |
|-----------------------------------------------|-----------------|-----------------|-----------------|
| Type of gum | Percentage | Pectin | Gum Arabic | Urtica pilulifera mucilage |
| 0.25 | 63.71±0.03 | 59.33±0.02 | 38.16±0.02 | |
| 0.5 | 60.11±0.03 | 52.68±0.02 | 59.43±0.03 | |
| 1 | 56.91±0.03 | 49.73±0.02 | 46.11±0.03 | |

3.4. Comparison of Thermal Stability of Urtica Pilulifera Mucilage Emulsion, Gum Arabic and Pectin

In this study, a comprehensive analysis of how to increase the stability of the emulsion obtained from Urtica pilulifera mucilage cannot be provided, but the effect of this mucilage on increasing the viscosity in the environment, which is
much more obvious than the other two hydrocolloids and can have a significant effect on emulsion stability [25].

Table 4. Emulsion stability (percentage).

|        | Pectin | Arabic gum | Urtica pilulifera mucilage |
|--------|--------|------------|---------------------------|
| 15±1b  | 13±2   | 27±1       |

3.5. Effect of Temperature and pH on the Stability of Urtica Pilulifera Mucilage Emulsion

The results of the pH and temperature effect on the stability of Urtica pilulifera mucilage emulsion are shown in the table. As can be seen, the stability of the emulsion decreases with increasing temperature. In the study of pH effect, it is observed that there is no significant difference between pHs 3 and 7, but at pH 5, the emulsion stability has increased significantly, and even despite temperature changes, the observed differences in emulsion stability for pH are not significant, indicating that the emulsion is more stable at this pH. Also, according to the analysis of the variance table, it is observed that temperature and pH at the level of 0.05 have a significant effect on emulsion stability, but their interaction has no significant effect. Thermal stresses cause hydrolysis and breakage of the mucilage structure and cause irreversible changes in its structure. As a result of these changes, the swelling and gelling capacity of the mucilage decrease, and the accumulation of the diffuse phase increases and this leads to a decrease in stability [26].

Table 5. Effect of pH and temperature on emulsion stability (percentage)

| Temperature (degrees Celsius) | 3   | 5   | 7   |
|-------------------------------|-----|-----|-----|
| PH 3                          | 33.6±0.5a | 41.2±1.1a | 33.3±0.6a |
| PH 5                          | 32.7±1.2bc | 40.1±1.7a | 33.7±0.6a |
| PH 7                          | 31.3±1.2c | 41.4±0.5a | 31.1±1.1b |

3.6. Effect of pH and Temperature on the Particle Size Distribution of Urtica Pilulifera Mucilage Emulsion

The results of the effect of pH and temperature on the particle size of Urtica pilulifera seed mucilage emulsion are shown in the figure. Examination of the shape of the particle size distribution curves shows that most of them are almost monomodal or monopodal or with a small peak, which indicates that the particle size distribution is normal, but in some of them two peaks were observed. In some studies, on particle size distribution in emulsions, a bipolar distribution has been observed due to the high viscosity of the emulsion and as a result of reduced performance of the homogenization process [27].

The diagram shows the results of changes in the apparent viscosity of Urtica pilulifera seed mucilage with concentrations of 0.25, 0.5, 0.75, and 1%. By examining these changes, it is observed that the solution containing 1% mucilage has the highest apparent viscosity at all shear velocities. Also, with increasing mucilage concentration, the slope decreases the apparent viscosity, and the dilution behavior with shear increases. According to the above, it can be concluded that the solution containing Urtica pilulifera seed mucilage of all concentrations except non-Newtonian fluids diluted by cutting or pseudoplastic is also more observed with increasing mucilage concentration. The shear dilution behavior occurs as a result of the orientation of the mucilage molecules in the direction of the shear force. As the shear force increases, the long polymer molecules are randomly placed in the flow direction, reducing the interaction between the chains, which reduces the viscosity. The pseudoplastic property of gums is a very useful property that makes liquids easy to pump and also creates a good texture and good oral sensation in the food. Medina Torso et al also reported that increasing the concentration of cactus mucilage increases the dilution behavior with cutting.

Figure 1. Effect of mucilage concentration on rheological properties.
3.7. Effect of Salt Concentration on the Rheological Behavior of Urtica Pilulifera Mucilage

The diagram below shows the effect of adding salt on the apparent viscosity of the 0.25% solution of Urtica pilulifera mucilage. As can be seen in the following table, with the addition of each of the salts, the consistency coefficient (k) decreases compared to the control sample, and with increasing salt concentration, it also shows a further decrease. Also, CaCl₂ salt has shown a greater decrease than KCl and NaCl and at a concentration of 20 mM salt has increased from 0.038 to 0.015. These results indicate that this mucilage has negatively charged polyelectrolyte molecules. In these molecules, in the absence of positive ionic attractions, negative charges cause the molecular structure to expand, resulting in higher viscosity in distilled water. On the other hand, the addition of positively charged ions reduces the expansion of the molecular structure and thus reduces the viscosity. Medina Torres et al also stated that the effect of divalent salts Ca²⁺ and Mg²⁺ on the viscosity of cactus mucilage is higher and the viscosity of this mucilage under calcium and magnesium salts is further reduced [28].

| Type of salt | Salt concentration (millimolar) | 20 | 40 | 60 | 80 | 100 |
|-------------|---------------------------------|----|----|----|----|-----|
| KCl         |                                | n  | 0.7917 | 0.8141 | 0.8019 | 0.8223 | 0.8188 |
|             | k                               |    | 0.022  | 0.018  | 0.016  | 0.016  |      |
| CaCl₂       |                                | n  | 0.7908 | 0.8055 | 0.8199 | 0.8185 | 0.8223 |
|             | k                               |    | 0.015  | 0.011  | 0.015  | 0.009  | 0.009  |
| NaCl        |                                | n  | 0.7939 | 0.8079 | 0.8143 | 0.8183 | 0.8179 |
|             | k                               |    | 0.023  | 0.021  | 0.019  | 0.017  | 0.016  |
| Control     |                                | n  | 0.7592 | ----- | ----- | ----- | ----- |
|             | k                               |    | 0.038  | ----- | ----- | ----- | ----- |

3.8. Effect of pH on the Rheological Behavior of Urtica Pilulifera Mucilage and Its Comparison with Gum Arabic and Pectin

In the study of the viscosity of biopolymers in different concentrations, it has been determined that their viscosity is affected by soluble conditions such as pH, salt, and temperature. The diagram shows the effect of pH changes on the rheological properties of gum arabic, pectin, and mucilage of Urtica pilulifera. Also, the model of the law of
power was well adapted to these changes. [29]

| Type of gum          | pH | n  | k  |
|----------------------|----|----|----|
| Urtica pilulifera    | 3  | 0.811 | 0.009 |
|                      | 5  | 0.7669 | 0.047 |
|                      | 7  | 0.747  | 0.041 |
| Arabic gum           | 3  | 0.8692 | 0.002 |
|                      | 5  | 0.8469 | 0.003 |
|                      | 7  | 0.9091 | 0.003 |
| Pectin               | 3  | 0.9017 | 0.003 |
|                      | 5  | 0.8959 | 0.004 |
|                      | 7  | 0.8915 | 0.003 |

3.9. FTIR Test

FTIR spectroscopy shows the sample of produced mucilage powder and in the following table the position of the bonds are shown and their relationship is expressed according to the articles. The peak shown in the wave number 13415 cm\(^{-1}\) is related to the tensile vibrations of the hydroxyl group (O-H) which its intensity decreases with decreasing hydroxyl groups. Observation of this bond, confirms the presence of polysaccharide as the main and dominant compound in the mucilage. Peak 1614 cm\(^{-1}\) belongs to ketone groups (C=O) which can confirm the presence of protein in mucilage. According to Sadeghifar and Mirshakari, the peak of 1422 cm\(^{-1}\) is related to the aromatic ring with a C-H bond. According to the study of Maskal et al., the peaks in the area of 1030 to 1058 are related to bending (C-O-H) and tension (C-O), in which a peak with the number 1044 cm\(^{-1}\) was observed.

3.10. Stability to Freezing and Thawing Processes

Lee et al investigated the effect of various gums, including gum Arabic, on the stability of starch gel during freezing and thawing and stated that only guar gum, alginate, and xanthan gum could stabilize the gel. In their study of the effect of pectin on emulsion stability, Moon reported that coating beta-lactoglobulin with 2% pectin could stabilize its emulsion in a water-oil-in-water emulsion during the freezing and thawing process. Comparing the cryopreservation stability of emulsion stabilized with pectin and gum Arabic, Wu et al observed higher stability for pectin at all concentrations (0.5, 1, and 2%) than gum Arabic. Also with increasing gum concentration stability of both emulsions increased.

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Table 8. Position of bonds identified in FTIR test of Urtica pilulifera mucilage and its comparison with articles

| Bond position in Urtica pilulifera (cm\(^{-1}\)) | According to articles (cm\(^{-1}\)) | Bond type | Type of material | reference          |
|------------------------------------------------|---------------------------------|-----------|-----------------|--------------------|
| 3415                                           | ≈3400                           | Tensile groups O-H and N-H | Fat, protein, carbohydrates | Moskal et al., 2018 |
| 1614                                           | 1600-1650                       | C=O Groups | Fats, pectin, protein | Coates, 2000       |
| 1422                                           | 1422                            | Aromatic ring with C-H bond | Cell wall polysaccharide | Mirshakarei, 2005  |
| 1044                                           | 1030-1058                       | C-O-H bonds | Cellulose | Moskal et al., 2018 |
| 667                                            | 657-697                         | Phosphate groups | ----- | Tiwari et al., 2017 |
4. Conclusion

Urtica pilulifera is a novel source of hydrocolloid, in this study the physicochemical, structural, functional and rheological properties of Urtica pilulifera seed mucilage were compared with gum arabic and pectin. functional properties, water absorbance was significantly affected by temperature and with increasing temperature, water absorbance increased. In the study of urtica pilulifera oil absorbance, Urtica pilulifera had a very low oil absorbance, which can make this gum suitable for use in frying products. In the stability study, the emulsion of Urtica pilulifera mucilage was significantly higher compared to pectin and gum arabic. On the other hand, with increasing temperature, the stability of Urtica pilulifera emulsion decreased. The effect of pH on emulsion stability was also observed. Urtica pilulifera mucilage has the highest stability at pH 5. With increasing the concentration of gum, the amount of surface tension in all samples decreased and pectin gum had the highest surface tension in all concentrations. In investigating the effect of temperature and pH on particle size distribution at 70°C, pH changes had the greatest effect on particle size distribution and increased with decreasing pH, particle diameter. In the study of rheological behavior at all concentrations, with increasing shear rate, the amount of shear stress also increased and with increasing concentration, the flow behavior changed to non-Newtonian fluids or pseudoplastic. In the study of mucilage structure using FTIR, many peaks have been observed, the most important of which are related to the tensile vibrations of the hydroxyl group (fat, protein and carbohydrate), ketone groups (fat, pectin, aromatic ring with CH bond). Also to investigate the possibility of using Urtica pilulifera mucilage in foods that are exposed to low temperatures Freezing test and freezing removal were performed on it. After freezing, this emulsion was unstable and completely broke.

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