Journal of Natural Compounds Chemistry (2023) 2: 75-81
https://doi.org/10.22092/jncc.2023.129603

Original Article: The Effect of Temperature on Amount of Mucilage and Dry matter in Two Medicinal Plant, *Plantago psyllium* and *Plantago major*

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Received: 21 November 2021
Accepted: 10 September 2022

Abstract

The most of medicinal plant seeds contain of polysaccharides with specific functional properties and used as a hydrocolloids source. Mucilages are polysaccharides with long chain molecules. In order to study the effect of different temperatures on the amount of mucilage and dry matter extraction (DME) in two medicinal plants of *Plantago psyllium* L. and *Plantag omajor* L. an experiment was carried out in a randomized complete block design with three replications at five temperature levels: 30, 40, 50, 60 and 70 °C. The results showed that in *P. psyllium*, the highest amount of mucilage (24.55 ml) and DME were obtained from 40 °C treatments and the lowest amounts of mucilage and DME were recorded at 70 °C. It found that when the temperature reached from 60 to 70 °C, the amount of DME in *P. psyllium* was decreased significantly, so that at 70 °C the amount of DME decreased was 56% in compared of 60 °C. In *P. major*: The highest amount of mucilage (28.6 ml) was obtained at 40°C and the lowest of it was observed at 70 °C. The highest (0.897 gram) and the lowest (0.523 gram) amount of DME was obtained at 30 °C and 70 °C in *P. major*, respectively. In general, the amount of extracted mucilage seems to depend on the water-to-grain ratio, and as the ratio decreases, the amount of mucilage decreases at high temperatures.

Keywords: Dry matter, Extraction, Secondary metabolite

Introduction

Plants have adapted to challenging environmental conditions during their evolution by activating physiological, biochemical pathways and producing specific substance. Biochemical responses have led to the synthesis of secondary metabolites in plants (Zhang *et al*., 2015). Plant metabolites consist of a wide range of low-weight macromolecules (less than 2000 daltons) that contain substances such as carbohydrates, acids, amino acids, phenolics, polyols, polyamines, lipids and etc., each of which perform different functions in the plant. Plant metabolites are classified into primary and secondary metabolites according to sources of production and the target of the reaction. Metabolites such as carbohydrates, are direct result of the process of photosynthesis, that it is the raw material for respiration process in order to produce energy and metabolites during of tricarboxylic acid cycle (TCA) such as citric acid and oxaloacetic acid (OAA), amino acids and etc., which are all among the primary metabolites and are essential for plant life. Under environment stress conditions, pathways such as photo respiration, glucogenesis and the TCA are activated, leading to the formation of compounds such as glucose, malate, and proline (Cramer *et al*., 2007). Derivatives of phenyl propanoids, coumarins, lignins, flavonoids, anthocyanins and tannins are all semi-polar compounds that are produced and activated in defense systems for plant survival and adaptation to changing environmental conditions (Arbona *et al*., 2010).

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There are several methods for extracting the active ingredients of plants. The choice of extraction process depends on the origin of the materials and the compounds containing them. Before choosing the extraction method, it is necessary to determine the purpose of extracting and necessary in gredients in the extract. Therefore, knowledge of the constituent compounds of the extract and their role in the development of their functional properties is essential. Although water is a traditional component in many extraction protocols, modern methods use organic and polar solvents to extract plant components. Extraction processes of plant materials can be done by diffusion, solvent extraction methods (extraction by penetration, extraction of solvent under pressure, extraction of solvent by ultrasound, reflex extraction and steam distillation). In the process of hot extraction, it requires dissolving substances that are directly soluble in water and their enzymatic hydrolysis after dissolving the substances continuously, which determines the type and quality of the extract (Taheri *et al*., 2017).In the heat extraction process, there is an equilibrium temperature between the temperature required for the hydrolytic activity of enzymes such as amylase, proteases, peptidases, transglucosidases, phosphorylase and the degree of thermal inactivation of these enzymes (Montanari *et al*., 2005). Researchers believe that the temperature and time of extraction have an effect on improving the quantity and quality of extract. For example, Briggs and Wadeson (1986), in the first step was used at 50 °C for extracting malt for one hour and used of at65 °C for the second stage of extraction for one hour. Jones (2005) stated that when the temperature starts from 45 °C during the extraction process and gradually continues to 70 °C, it is increased the conversion of starch to sugar, in this study, it was also showed that Endo-radiation activity is increased at 38 °C for 58 minutes. Uquiche *et al*. (2004) stated that the synthetic extraction was done by used of supercritical dioxideat 40 °C in red pepper plant. They also observed that plateleting of plant raw material (particle size 0.2 width and length 3.9 mm) was increased process efficiency and it was found that the performance of pigmented grains and carotenoid pigments have increased due to increased extract pressure and decline of temperature.

Aqueous extraction is the most common method of extraction in *P. major*, in which the substance is extracted from hot water and the results showed that the temperature of the extract, pH, water to grain ratio has a significant effect on the amount of polysaccharides in *P. major*. (Alizadeh Behbahani *et al*., 2017). Most grains contain various polysaccharides with special functional properties and are used as a hydrocolloid source. Mucilages are polysaccharides with long chain molecules (Campos *et al*., 2016). *P. psylliumis* known as an important source of natural mucilage production and the amount of mucilage is about 25% of grain weight yield (Hansol *et al*., 1992). Due to the presence of mucilage in the seeds of this plant, it has properties such as: anti-cough, anti-inflammatory, anti-dermatological, moray laxative and immune stimulants. *P. psylliumis* also used in the preparation of jellies, condiments, beverages and in the cosmetics industry to make various creams and cosmetic lotions (Fakhr Tabatabai, 1990).

At present, the use of medicinal plants is increasing due to the production of effective substances that are beneficial in curing of diseases, so that 35% of drugs contain natural compounds (Nouri *et al*., 2016).Due to the high demand for plant extracts, the use of new technologies such as ultrasound, microwave, extraction with supercritical fluid has improved effectively the performance of the extract (Marr and Games, 2000).On the other hand, process optimization through changes in environmental conditions can be effective in increasing the quantity and quality of the extracted substance. Therefore, this study was conducted to investigate the effect of different extraction temperatures on the amount of extract in two medicinal plants, *P. psyllium* and *P. major*.

P. psylliumis

known as an important source of natural mucilage production and its amount is about 25% of grain weight yield (Hansol *et al.*, 1992). Due to the presence of mucilage in the seeds of this plant,it has properties such as: anti-cough, anti-inflammatory, anti-dermatological, moray laxative and immune stimulants. *P. psyllium*is also used in the preparation of jellies, condiments, beverages and in the cosmetics industry to make various creams and cosmetic lotions (Fakhr Tabatabai, 1990).

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Materials and Methods

In order to investigate the effect of temperature on the amount of extract substance in *P. psyllium* and *P. major* plants (Fig. 1), an experiment was conducted as a completely randomized block design with three replications in the Development Technology Center affiliated to Khorasan Razavi Agricultural Research and Education Center (KRAREC) in 2019. Experimental treatments were temperature at five level (30, 40, 50, 60 and 70 °C). For this purposed, the seeds of *P. psyllium* and *P. major* was prepared from natural resources department of KRAREC. At the first, the seeds were cleaned for foreign substances and weeds. To extract the seeds of *P. major* plant, three small laboratory humans were selected for each temperature treatment and then 5 grams of seeds with 50ml of distilled water was added to them (Ghorbani *et al*., 2017) then they were subjected to temperature treatments for two hours. After that time, the seeds were poured into a cotton cloth and separated by mucilage pressure, and the volume of mucilage was measured (Fig. 2). Then the seeds that had been removed from their mucilage were poured on filter paper and placed in a dryer to calculate their dry weight and then weight the amount of extract substance (Fig. 3). For extraction of *P. psyllium* seeds the same methodsused. After drying the extract, the extract became viscous, so the freezer dryer method was used to produce powder.

Data were analyzed by using Mstat C software and the means were compared using Duncan test at 5% probability level.

Fig. 1 Images of two plants was extracted from their seeds in this experiment, A: *P. major* and B: *P. psyllium*

Fig 2. Extracts taken from the plant

Fig 3. Methods of drying for calculating of dry matter.

Results and Discussion

P. psyllium

The results of analysis of variance showed that there was a significant effect (P≤0.01) of temperature on the amount of mucilage in *P. psyllium* (Table 1). The highest amount of mucilage (24.65 ml) was obtained at40 °C and the lowest amount of it was recorded at 70 °C. However, the amount of mucilage obtained at 60 °C was not statistically significant at 70 °C. At 50 and 30 °C, the amount of mucilage was decreased by 16.6% and 26.4%, respectively, in compared of 40 °C (Fig. 4, A). It seems that the reason for increasing the amount of mucilage up to 40 °C was attributed to the amount of water available, which increased the solubility of grain macromolecules and their release at this temperature. Ascan be seen, with increasing temperature, the amount of mucilage decreased, which can be attributed to factor changes in the pH of the experimental medium. On the other hand, increasing the temperature to 40 °C leads to reducing the radius of viscosity of the extract and increasing the flexibility of long-chain macromolecules and ultimately reducing the compression of molecules, which reduces the amount of viscosity and increases the amount of mucilage due to water available in the environment (Launay *et al*., 1986).

The results Alizadeh Behbahani *et al*., (2017) showed that among of different water to grain ratios, including 20 to 1, 40 to 1 and 60 to 1 in *P. psyllium* plant, the maximum amount of mucilage was obtained from 60 to 1 ratio of water to grain at 70 °C and pH= 6.8. In the present experiment, the water to grain ratio was 10 to 1. On the other hand, the cause of fluctuations in the amount of mucilage produced can be attributed to the presence of natural hydroxyl groups and the expansion of hydrodynamic interactions between molecules at the tested temperatures. So that the amount of water absorbed in the mucilage can be attributed to the effect of temperature and pH interactions in the formation of polar bonds between water molecules with other macromolecules in the environment (Koocheki *et al*., 2012).Therefore, in the adjustment ratio of the experiment (water: grain equal to 10 to 1) with increasing temperature from 30 to 40 °C caused an increase in solubility of molecules and increased lubrication and separation of materials from grain and at higher temperatures the amount of water required decreased in solution and naturally reduced the amount of mucilage. In an experiment, it was shown that temperatures between 20 and 50 °C has no effect on the viscosity of psyllium. Psyllium mucilage has many special properties (Verma and Mogra, 2013). Temperature treatment had a significant effect (P≤0.05) on the amount of extracted dry matter in *P. psyllium* (Table 1). The highest and lowest amounts of extracted dry matter were obtained from temperature treatment at 40 °C and 70 °C, respectively. The amount of dry matter extracted at 30 °C was 0.14 g, which was not statistically significant with 40 °C treatment.

The results showed that when the temperature was increased from 60 °C to 70 °C, the amount of extracted material decreased sharply, so that the amount of extracted dry matter at 70 °C declined about 56% in comparison to60 °C (Fig. 5, A).

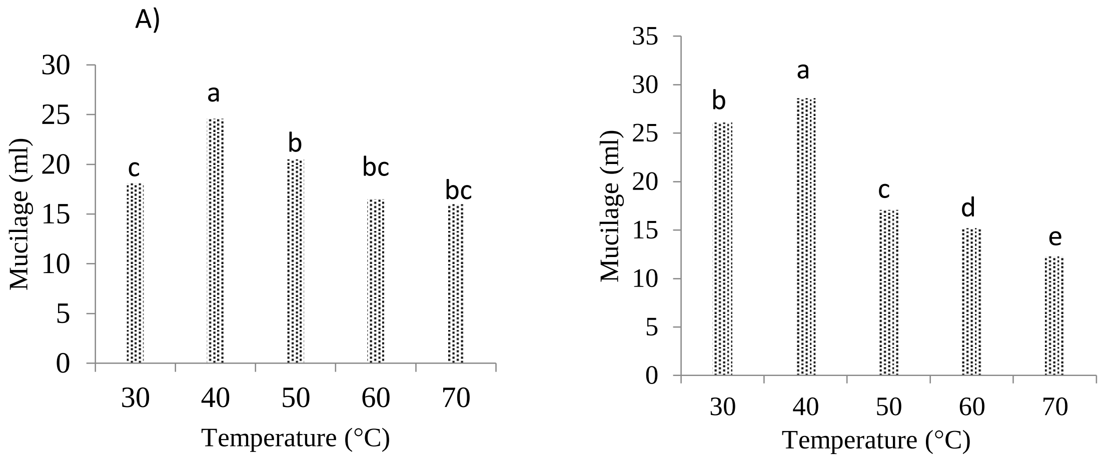

Fig. 4. Effect of different temperature on amount of mucilage in A) *P. psyllium* and B) *P. major* plant.

Table 1. Results of analysis of variance (mean squares) effect of temperature treatments on the amount of mucilage and extracted dry matter in *P. psyllium* and *P. major* plants.

		P. psyllium		*P. major*	
S.O.V	df	Amount of mucilage	Extract substance	Amount of mucilage	Extract substance
Block	2	2.45ns	0.001ns	1.40ns	0.012ns
temperature	4	28.6**	0.021*	175.5**	0.086*
Error	8	0.70	0.0001	0.090	0.008
CV		2.43	13.2	10.3	12.5

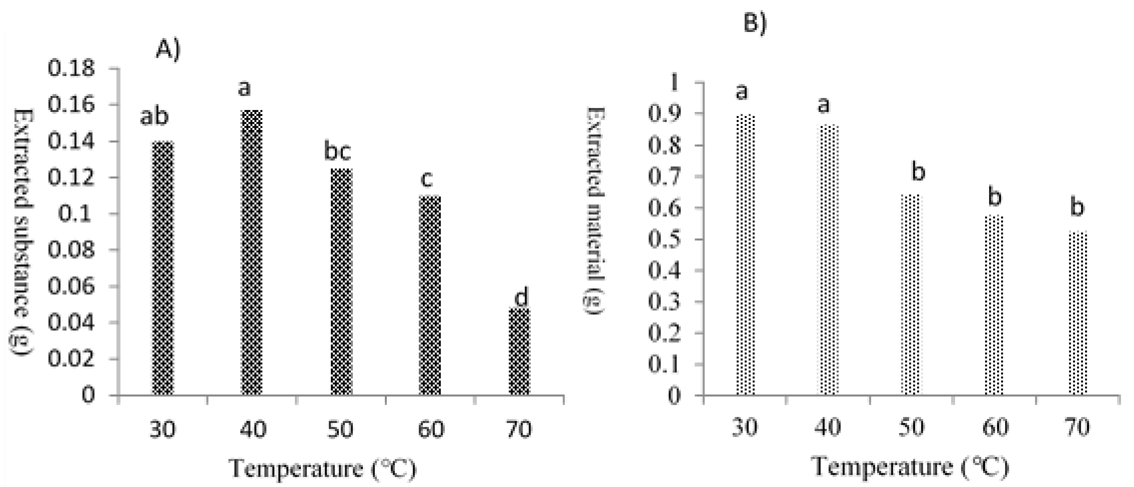

Fig 5. The amount of substance extracted in A) *P. psyllium* and B) *P. major* plant under the influence of different temperature treatments

P. major

The results of analysis of variance table showed that temperature treatment had significant effect (P≤0.01) on the amount of mucilage in *P. major* (Table 1). The highest amount of mucilage (28.6 ml) was obtained from 40 °C and the lowest amount was obtained at 70 °C. Application temperatures at 30, 50 and 60 °C were obtained 26.1, 17.1 and 15.2 ml of extracted substance, respectively (Fig. 4, B). There was a significant difference (P≤0.05) between temperature treatments in terms of dry matter production in *P. major* (Table 1). The highest and the lowest amounts of extracted dry matter were obtained at 30 °C and 70 °C with 0.897 and 0.523 g, respectively. There were no statistically significant differences between the three temperature treatments of 50, 60 and 70 °C with the production of dry matter of 0.643, 0.577 and 0.523 g respectively. At 40 °C, the amount of extracted dry matter was 0.863 g, which produced only 4% less dry matter at 30 °C and no statistically significant difference was observed between them (Fig. 5, B).

P. major plant produces a large amount of seeds, the seed coat of which contains polysaccharide components that, by absorbing water, form mucilage with a high concentration. These polysaccharides contain oxylose, arabinose, galacturonic acid, glucuronic acid, rhamnose, galactose and glucose (Samuelsen, 2000). Aqueous extract is the most important method for extracting the substance from the seeds of this plant. Extraction is traditionally based on hot water. The amount of extract depends on time and temperature (Koocheki *et al*., 2009). Therefore, changes in environmental factors such as temperature, pH, and water to grain ratio have a significant effect on the properties of raw grain polysaccharides. The results of Alizadeh Behbahani *et al*., (2017) showed that at a constant ratio of water to grain of 40: 1 in *P. major* plant, with increasing temperature, the yield of extracted materials was increased. They attributed the increase in mucilage along with the increase in temperature to the decrease in mucilage viscosity and the facilitation of the release of mucilage from the grain, as well as the lubrication and greater solubility of the extract carriers. In the present study, in the ratio of water to grain 10: 1, the amount of extract increased with increasing temperature to 40 °C and the amount of mucilage decreased with increasing temperature in the next three temperature ranges. It seems that the reason for the decrease in the extract with increasing temperature in this ratio was attributed to the decrease in the amount of water required to increase the solubility of material in grain. Sepúlveda *et al*., (2007) reported that increasing the water in the solution increased the amount of extract substance in Opuntiaspp plant. Koocheki *et al* (2012) also stated that temperature had an effect on increasing the extracts of Yannang leaves and the amount of Erucasativa seed jelly. In the same study, the results showed that the highest amount of mucilage was obtained in Euricasative plant at 65.5 °C, pH= 4 and water to grain ratio of 60: 1. The findings of Sciarini *et al* (2009) showed that the amount of water in the extract is due to the experimental conditions, which can disrupt the three-dimensional structure of polysaccharide components and increase the branching ratios in them, which causes improved and lubricated extract.

In general, the results showed that the most effective in the extraction process, depending on the sources, the ratio of water to grain and then temperature. In the present experiment, the water to seed ratio in both plants was 10: 1 and the application of 40 °C treatment had greater amount of extracted substance and dry matter than other temperature treatments.

Acknowledgment

This research has been done as a special project. However, we are appreciated and thanked from support of the management Growth Center of the Khorasan Razavi Agricultural Research and Education Center and all of the colleagues in plant production group of the center.

References

Alizadeh Behbahani, B., Tabatabaei Yazdi, F., Shahidi, F., Hesarinejad, M.A., Mortazavi, S.A, and Mohebbi, M. 2017. *Plantago major* seed mucilage: Optimization of extraction and some physicochemical and rheological aspects. Carbohydrate Polymers, 155: 68–77.

Arbona, V., Argamasilla, R, and Gómez-Cadenas, A. 2010. Common and divergent physiological, hormonal and metabolic responses of Arabidopsis thaliana and Thellungiellahalophila to water and salt stress. Journal Plant Physiology, 167: 1342–1350.

Briggs, D.E, and Wadeson, A. 1986.The use of extruded barley, wheat and maize as adjuncts in mashind. The institute of brewing (JIB), 92: 468-474.

Campos, B.E., Ruivo, T.D., da Silva Scapim, M.R., Madrona, G.S, and Bergamasco, R.D.C. 2016. Optimization of the mucilage extraction process from chia seeds and application in ice cream as a stabilizer and emulsifier. LWT-Food Science and Technology, 65: 874–883.

Cramer, G.R., Ergül, A., Grimplet, J., Tillett, R.L., Tattersall, E.A.R, and Bohlman M.C., et al. 2007. Water and salinity stress in grapevines: early and late changes in transcript and metabolite profiles. Function Integration Genomics, 7: 111–134.

Fakhr Tabatabai, M. 1990. Research on Iranian Asfarzeh, 4th Iranian Medicinal Plants Seminar, Faculty of Pharmacy, Tehran University of Medical Sciences.

Ghorbani, M., Aboonajmi, M., Ghorbani Javid, M., Arabhosseini, A.2017. Effect of ultrasound extraction conditions on yield and antioxidant properties of the fennel seed (Foeniculumvulgare) extract. Journal of Food Science and Technology, 67(14): 63-73.

Hansol, C.V.E.A., Oelke, D., Putnam, H, and Oplinger, E.S. 1992.Psyllium. Alternative Field Crops Manual. Horticulture. Purdue University, Indiana.

Jones, b.L. 2005. Endoproteasea of barley malt. Journal of cereal Science, 42: 139-156.

Koocheki, A., Taherian, A. R., Razavi, S. M, and Bostan, A. 2009. Response surface methodology for optimization of extraction yield, viscosity, hue and emulsionstability of mucilage extracted from Lepidiumperfoliatum seeds. Food Hydrocolloids, 23(8), 2369–2379.

Koocheki, A., Razavi, S.M.A, and Hesarinejad, M.A. 2012. Effect of Extraction Procedures on Functional Properties of Eruca sativa Seed Mucilage. Food Biophysics, 7:84–92.

Launay, B., Doublier, J.L, and Cuvelier, G. 1986.In J. R. Mitchell, & D. A. Ledward (Eds.), Functional properties of food macromolecules (pp. 1–78). London: Elsevier Applied Science.

Marr, R, and Gamse, T. 2000. Use of supercritical fluids for different processes including new developments—a review. Chemical Engineer Process, 39: 19-28.

Montanari, L., Floridi, S., Marconi, O., Tironzeli, M, and Fantozzi, P. 2005. Effect of mashing procedures on brewing. European Food Research and Technology, 221: 175-179.

Nouri, S., Kiast, A.R, Kolahif, M., Mirzakhani, R, and Seyedzad, S.M. 2016. Phytochemical study, antioxidants and optimization methods of various extraction methods to determine the best method of extracting curcumin from ethanolic extracts of the plant (Curcuma longa L.). Ecophytochemical Quarterly of Medicinal Plants, 15 (4): 1-11.

Samuelsen, A.B. 2000. The traditional uses, chemical constituents and biologicalactivities of *Plantago major* L. A review. Journal of Ethnopharmacology, 71(1): 1–21.

Sciarini, L.S., Malsonado, F., Ribotta, P.D., Perez, G.T, and Leon, A.E. 2009. Chemical composition and functional properties of Gleditsia Triacanthos Gum Food Hydrocoll, 23: 306-313.

Sepúlveda, E., Sáenz, C., Aliaga, E, and Aceituno, C. 2007.Extraction and characterization of mucilage in Opuntia spp. Journal of Arid Environments,68(4): 534–545.

Taheri, M., Nateghi, L., Maleki Kahaki, AR, Rahmani, A, and Soleimani, F. 2017. Investigation of effective variables in the industrial process of hot extraction of barley malt by thermal programming method. Journal of Food Science and Technology Innovation 9 (1)

Uquiche, E., del Valle, J. M, and Ortiz, J. 2004.Supercritical carbon dioxide extraction of red pepper (*Capsicum annuum* L) oleoresin. Journal Food Engineer, 65:55–66.

Verma, A, and Mogra, R. 2013. Psyllium (*Plantago movata*) Husk: A Wonder Food for Good Health. International Journal of Science and Research (IJSR), 4(9): 1581-1585.

Zhang, Y.P., Jiang, E Z.G., Wang, H., Zhou, L, and Zhu, D.F. 2015. A comparative study of stress-related gene expression under single stress and intercross stress in rice. Genetics Molecule Research, 14: 3702–3717.