Sumerianz Journal of Medical and Healthcare, 2021, Vol. 4, No. 2, pp. 109-113
ISSN(e): 2663-421X, ISSN(p): 2706-8404
Website: https://www.sumerianz.com
DOI: https://doi.org/10.47752/sjmh.42.109.113

Original Article Open Access

Manufacture of Hard Gelatin Capsules From a Lyophilisate of the *Morus Nigra* Fruit

Sandra Isabel Hernández-González
Laboratorio B de Investigación, Facultad de Ciencias Químicas Campus Gómez Palacio, Universidad Juárez del Estado de Durango, Mexico

Jesús Iván García-Castañeda
Laboratorio B de Investigación, Facultad de Ciencias Químicas Campus Gómez Palacio, Universidad Juárez del Estado de Durango, Mexico

José de Jesús Alba-Romero
Laboratorio B de Investigación, Facultad de Ciencias Químicas Campus Gómez Palacio, Universidad Juárez del Estado de Durango, Mexico

Aurora Martínez-Romero*
Laboratorio B de Investigación, Facultad de Ciencias Químicas Campus Gómez Palacio, Universidad Juárez del Estado de Durango, Mexico
Email: aurora.martinez@ujed.mx

Rodolfo Gerardo Chew-Madinaveitia
Instituto Tecnológico de Torreón. Coahuila, Mexico

José Luis Ortega-Sanchez
Unidad Regional Universitaria de Zonas Áridas. Universidad Autónoma Chapingo, Mexico

Article History
Received: April 22, 2021
Revised: May 27, 2021
Accepted: June 4, 2021
Published: June 7, 2021

Abstract

Morus nigra L. (*M. nigra* L.) belongs to the Moraceae family. Traditional medicine for its physicochemical properties. To evaluate the physicochemical composition and pharmaceutical stability of hard gelatin capsules of the freeze-dried fruit of *M. nigra* L. The content of total phenols (CFT) and antioxidant capacity (CA) were evaluated by spectrophotometry, the content of ash, moisture, protein, fat and fiber were determined according to their own standard. The CFT and CA were 25.4 mg gallic acid equivalents/g of sample, and 74.1% inhibition, respectively. On the other hand, the values of ash, moisture, protein, fat and fiber were 4.2%, 7.9%, 3.9%, 0.5% and 3.6%, respectively. Lyophilized *M. nigra* fruit may be a novel candidate for the development of gelatin hard capsules and other new pharmaceutical products.

Keywords: Physicochemical composition; Pharmaceutical stability; *M. nigra* L.

1. Introduction

Morus nigra L. (Moraceae family), also known as blackberry, is one of the most important species of the genus *Morus* [1].This is native to Iran and belongs to both cultivated and wild mulberry varieties [2].The fruit is in blackberry form and typically black, but sometimes dark blue with a sweet taste [3], and has been used as part of traditional Chinese medicine for a long time [4], because the fruit of *M. nigra* is rich in compounds phenolics [5], anthocyanins, proteins, and vitamins for urinary incontinence, depression, fever, cancer and diabetes [6]. Among the phenolic compounds it has are gallic acid, ascorbic acid, p-coumaric acid, caffeic acid, to mention some have a great nutritional and organoleptic significance and give the commercial characteristics of the fruits [7].

However, it is difficult to preserve fresh blackberry fruit due to its low acidity. In addition, phenolic compounds are unstable in various solutions and, therefore, it is necessary to coat them to stabilize them, which can be achieved by an encapsulation process [8]. For this reason, there is a real need to apply technologies to improve their useful life, qualities nutritional, organoleptic and health benefits of blackberry fruit to boost commercial production with economic and health benefits [9]. Encapsulation provides a means to control stability, solubility and bioavailability, as well as for the release of bioactive components [10].

On the other hand, stability is the ability to verify the quality of a drug within its specifications. Stability tests must be conducted under conditions that allow information on the stability of the product to be provided in the shortest possible time, for which, the samples must be stored in conditions that accelerate the possible changes that may occur during the validity period; Accelerated stability studies are carried out under exaggerated storage conditions, to increase the rate of chemical, biological degradation or physical changes of a drug [11]. However, there are not yet any studies that show the pharmaceutical stability of freeze-dried capsules of the fruit. *M. nigra* from the Comarca Lagunera region. Therefore, the purpose of this study was to evaluate and investigate for the first

*Corresponding Author

time the physicochemical composition and pharmaceutical stability of the hard gelatin capsules of a lyophilisate of the *M. nigra* fruit.

2. Material and Methods

2.1. Harvesting the Plant and Preparing Capsules

The fruits of *M. nigra* L. were collected in the mature stage from some blackberry trees from the city of Bermejillo, Durango, Mexico. The samples were preserved in fresh bags to be transported to the laboratory. The fruits were lyophilized at -40°C and once lyophilized, they were powdered using mortar and pestle. The hard gelatin capsules were then filled manually.

2.2. Capsule Stability

The study of pilot tests of hard gelatin capsules with freeze-dried fruit of *M. nigra* was carried out by means of a non-probabilistic sampling for convenience for its analysis, considering them as a new drug [12]. It should be mentioned that the microbial limit was not carried out, because only physicochemical tests were considered.

The hard gelatin capsules were subjected to an accelerated stability study in compliance with NOM-073-SSA1-2005 (Table 1), in a Victor® stability chamber.

Table-1. Stability specifications according to NOM-073-SSA1-2005

Type of study	Storage conditions	Minimum period	Analysis frequency
Accelerated stability	40°C±2/75%±5% *RH	6 months	0, 3 y 6 months

*RH- Relative humidity

3. Determination of Physical Parameters

3.1. Appearance Color and Odor

The capsule appearance, color and odor tests were determined as organoleptic tests.

3.2. Average weight

10 units taken at random were individually separated and the average weight of each capsule with contents was determined, then the capsule was opened without losing any part of the cover and the contents were removed as completely as possible. The empty capsule was weighed, where the weight of the content is the difference between said weights (Table 2).

Table-2. Specifications and results of the accelerated stability pilot test study in a period of 6 months with an analysis frequency of 0.3 and 6 months under conditions of 40°C ± 2/75% ± 5% according to FEUM and NOM-073-SSA1-2005

Determination	Condition:	40°C±2/75%±5%		
	Analysis frequency:	Result per month 0*	Result per month 3*	Result per month 6*
	Specification			
Appearance	According	Conformal*	Conformal*	Conformal*
Color	Conformal	Conformal*	Conformal*	Conformal*
Odor	Conformal	Conformal*	Conformal*	Conformal*
Dose uniformity	238-250 mg	249 mg	247 mg	239 mg
CFT	100-90%	98.6%*	98%*	97.4%*
CA	100-90%	89.1%**	87.9%**	88%**
Humidity	<10%	7.9%**	7.93%**	8.6%**
Ash	<5%	4.4%	4.4%	3.8%
Protein	<10%	4.1%	4%	3.6%
Fat	<1%	0.7%	0.7%	0.1%
Fiber	<10%	3.85%	3.8%	3.15%

* Once these results were obtained, the frequency of analysis was averaged during the 6 months of each assessment. The average was reported in the discussion.

3.3. Determination of Chemical Parameters

3.3.1. Quantification of total Phenolic Compounds

First, the fruit powder (0.5 g) was extracted with 0.1N HCl (aqueous) and Ethanol (conc), EtOH: 0.1N aqueous HCl (80:20). The extraction was assisted with ultrasound (Bransonic Ultrasonic Cleaner 3510R-MTH 40 KHz) at 40 to 50°C for 30 minutes. The extracts obtained were filtered and centrifuged at 3500 rpm for 10 minutes. The *M. nigra* extract was filtered with Whatmann No. 1 filter paper. The total phenol content was established by the spectrophotometric method of Slinkard and Singleton, 1977 [13]. Briefly, 100 µl of the *M. nigra* extract was mixed with 250 µl of the reagent. of Folin-Ciocalteu freshly prepared and with 1,250 µl of 20% sodium carbonate, and finally, 400 µl was added. This was incubated at room temperature for 30 minutes, then it was measured at absorbance at 760 nm.

3.3.2. Determination of Antioxidant Activity

The antioxidant activity of the M. nigra extract was determined by the capacity of the donor H^+ for the stable radical 2,2-diphenyl-1-picrylhydracil (DPPH), according to the spectrophotometric method of Fukumoto and Mazza, 2000 [14]. Then, 200 µl extract of *M. nigra* was mixed with 2,400 µl of the freshly prepared DPPH reagent. This was incubated at room temperature for 10 minutes and then it was determined at an absorbance of 520 nm.

3.3.3. Determination of Humidity

The percentage of moisture of *M. nigra* was determined in compliance with MGA 0671. 5g of the sample was weighed into a previously tared capsule. Then, the porcelain capsule with a lid was placed in the oven at 105°C for one hour. This was transferred to a desiccator until room temperature was reached. Finally, each tray with the sample was weighed.

3.4. Ash determination

The ash percentage was determined using MGA 0751. Then, in a crucible at constant mass, 3 g of sample was placed. Then the sample was pre-incinerated in a burner. The crucible was placed in the muffle at 600°C, and the sample was incinerated for 2 hours, then it was moved to a desiccator until reaching room temperature. The crucible was weighed and calculations were made and reported as dry ash percentage.

$$\%\ ash = \frac{Ash\ weight\ (g)}{Sample\ weight\ (g)} \times 100$$

3.5. Protein Determination

The protein content was established according to MGA 0611. First, 1 g of the sample was weighed and placed in a Kjeldahl flask. Then, 2 g of copper sulfate, 10 g of anhydrous sulfate, 25 cm^3 of sulfuric acid and some glass beads were added to this. Briefly, the flask was placed in a digester and carefully heated until all material was charred, gradually increasing the temperature until the solution was completely clear. This was incubated at room temperature for 30 minutes. Then 450 cm^3 was added, which was mixed with zinc granules and 50 cm^3 of 1:1 NaOH. It was immediately placed in a distillation system, to which an Erlenmeyer flask was previously placed with 50 cm^3 of boric acid and a few drops of Shiro Tashiro's reagent as indicator. Finally, the Kjeldahl flask was removed and the distillate was titrated with 0.1N HCl.

$$\%\ protein\ = \frac{V\ x\ N\ x\ 0.014}{Sample\ weight\ (g)} \times 100$$

3.6. Fat Determination

The fat content was obtained by NMX-F-427-1982. 2 g of sample was weighed into a 50 cm^3 beaker, which was mixed with 2 cm^3 of alcohol, 10 cm^3 of HCl. This was placed in a water bath at 80°C and stirred for 40 minutes with 5 minute intervals. Then, 10 cm^3 alcohol was added and incubated at room temperature. Once this was done, it was transferred to a Mojonnier flask, washed with 25 cm^3 of ethyl ether in 3 portions, which was stirred for 60 seconds. Briefly, it was incubated at room temperature and the clear solution was decanted onto filter paper. The ether-fat layer was decanted, and filtered in a funnel, the ether was obtained in a 125 cm^3 beaker which was previously weighed. Finally, the excess liquid was extracted 2 more times with 15 cm^3 of ether. It was dried in an oven for 90 minutes at 100°C and the containers were weighed when they reached room temperature.

$$\%\ fat\ = \frac{Fat\ weight\ (g)}{Sample\ weight\ (g)} \times 100$$

3.7. Fiber Determination

The fiber content was determined by NMX-F-090-S-1978. 2 g of sample was weighed into a 600 cm^3 beaker, which was mixed with 1 g of prepared asbestos and 200 cm^3 of boiling 1.25% sulfuric acid. Briefly, the beaker was placed in a digester on the plate and boiled for 30 minutes. During those 30 minutes, the glass was periodically shaken. Then, the contents of the beaker were filtered with filter paper. The residue was washed with distilled water at boiling temperature until the water obtained a pH equal to that of the distilled water. The residue was placed and mixed with 200 cm^3 of 1.25% sodium hydroxide at boiling temperature for 30 minutes. The solution obtained was filtered with Buchner with filter paper. Again, the residue was washed with boiling water until the water obtained a pH equal to that of distilled water. Finally, the residue was placed in a crucible at constant mass, dried at 130°C for 2 hours, and its mass was determined. Then, it was calcined at 600°C for 30 minutes and its mass was determined.

$$\%\ fiber\ = \frac{Fiber\ weight\ (g)}{Sample\ weight\ (g)} \times 100$$

3.8. Statistic Analysis

All the corresponding studies were carried out in triplicate, and the results obtained were expressed as the mean of the sample. Compatibility with the t distribution was determined by Student's t test. Student's t test was used to compare the two groups. $p < 0.05$ was considered significant.

4. Results

4.1. Determination of Chemical Parameters

For the quantification of total phenolic compounds, the results were calculated based on a calibration curve with gallic acid as standard and were reported in percentage (Table 2). The results of the physical and chemical parameters determined such as antioxidant activity, moisture, ash, proteins, fat and fiber, the results were expressed as a percentage according to the specifications shown in Table 2.

5. Discusion

Capsule shells are typically made of gelatin or hypromellose (hard gelatin capsules) and consist of two parts: the body and the cap. After filling the body, the cap is automatically placed on the body, in addition, they are frequently used for powders and in dry powder inhalers for the administration of a medicine [15]. Recently on the market, a larger number of pharmaceutical or cosmetic products with the combination of many herbal extracts [16]. The *M. nigra* L. fruit has been used as part of traditional Chinese medicine for a long time [4]. Therefore, it is due to the widespread use of the blackberry fruit for various purposes, that the study of the composition of blackberry fruit has been taken into greater consideration [2]. Since several studies have been found in which the physicochemical characteristics of blackberry fruit have been investigated in recent years [17, 18], however, there is no recent study that shows the physicochemical composition and pharmaceutical stability of hard gelatin capsules of a lyophilisate of the *M. nigra* L. fruit. Therefore, it is rea This study was conducted on the physicochemical composition of the pharmaceutical stability of capsules from the lyophilisate of *M. nigra*.

Then, the appearance, color and odor of the capsule content of the *M. nigra* lyophilisate were found to be in agreement in the analyzed samples. The appearance, color and odor indicate the preservation of the product over time, a change in the appearance, color and odor is indicative of impurities, chemical reactions between the excipients and the active principle, among others [11]. Therefore, based on the aforementioned by Rojas and his collaborators, our results were excellent.

In addition, in determining the average weight of the capsules, the result was 245 mg. Its determination is important in the uniformity of the dosage of the sample if a correct filling of the capsules has been carried out. Then, Arellano stated that a variation in temperature and humidity generates softening, as well as degeneration and rupture of the capsule [19]. In accordance with the above, our results express that there was no degradation or alteration of the capsule with the active principle, therefore, our result indicates a correct conservation of the capsule. On the other hand, the results of the chemical evaluations regarding the average content of the analysis frequency during the 6 months of the total phenolic compounds and the antioxidant activity present in the content of the capsules were 98% and 88%, respectively. Although the value of the total phenolic compounds is within the specifications mentioned in the FEUM and NOM-073-SSA1-2005, while the value of the antioxidant activity was slightly lower than the specifications for said assessment [20, 21].

On the other hand, the value of the average percentage of the frequency of analysis during the 6 months of humidity of the lyophilisate of the *M. nigra* fruit contained in the capsule was 7.9%. According to what was mentioned by Kuklinski, lyophilization is a method of enzymatic inhibition that removes water from the vegetable to values lower than 10%, since the main responsible for the alteration of the chemical components of the vegetable is the high presence of water (up to 70% in the meatiest parts and to a lesser amount in drier parts; when the amount of water decreases, the enzymes stop their activity, being inhibited and the vegetable is preserved [22]. While Prieto et al. reported on the percentage of leaf moisture of *M. alba* from La Habana, Cuba a value of 75.9% [23]. According to Kuklinski and in comparison with Prieto et al., our result was extremely satisfactory.

In this study, the percentage of ash from the freeze-dried fruit of *M. nigra* was 4.2%, however, the percentage of ash from the fruit of *M. alba* from a region of Costa Rica has been reported at 2.5% [24]. Compared with the results of these studies our results are approximately double. The protein percentage of the freeze-dried fruit of *M. nigra* obtained was a value of 3.9%, while the percentage of protein of *M. alba* leaves from Havana, Cuba with a value of 20.1% was reported [23].Therefore, It is worth mentioning that the amount of moisture and proteins can vary from a region due to the climatic conditions of the place to which the plant is exposed, the physical state (fresh, dry, or lyophilized) of the organ and even the organ (root, stem, leaf, fruit, etc.) of the plant used to investigate, as well as the soil and the type of fertilizer used in the plant. Our result compared to that study was much lower.

The value of the fat percentage of the fruit of *M. nigra* reported was 0.5%. However, the value obtained from the fruit of *M. nigra* in another region of Turkey was 0.95% [25]. In contrast to that research, our results show a lower amount of fat. In another study, Boschini and Vargas reported a fiber percentage of *M. alba* a value of 6% [24], while the reported value was 3.6%. Our result was inferior compared to that study.

6. Conclusion

In this study, regarding the physicochemical composition and stability of the hard gelatin capsules of the lyophilized *M. nigra* L. fruit, it provides the necessary bases for the development of both these hard gelatin capsules and new pharmaceutical products. However, additional studies on bioavailability, on other routes of administration, as well as further studies on the shelf life of this pharmaceutical formulation are required.

References

[1] Mascarello, A., Orbem, M. A. C., Calcaterra, A., Alves, M. P. G., Chiaradia, D. l., Torre, L. D., and D´Acquarica, I., 2018. "Naturally occurring diels-alder-type adducts from Morus nigra as potent inhibitors

of Mycobacterium tuberculosis protein tyrosine phosphatase B." *Eur. J. Med. Chem.* , vol. 144, pp. 277-288.

[2] Bao, T., Xu, Y., Gowd, V., Zhao, J., Xie, J., Liang, W., and Chen, W., 2016. "Systematic study on phytochemicals and antioxidant activity of some new and common mulberry cultivars in China." *J. Funct Foods*, vol. 25, pp. 537-547.

[3] Zoofishan, Z., Hohmann, J., and Hunyadi, A., 2018. "Phenolic antioxidants of Morus nigra roots, and antitumor potential of morusin." *Phytochem Rev.*, pp. 1-15. Available: http://DOI:10.1007/s11101-018-9565-1

[4] Liu, H.-Y., Wang, J., Ma, J., and Zhang, Y.-Q., 2016. "Interference effect of oral administration of mulberry branch bark powder on the incidence of type II diabetes in mice induced by streptozotocin." *Food Nutr. Res.*, vol. 60, p. 31606.

[5] Qiu, D., Guo, J., Yu, H., Yan, J., Yang, S., and Li, X., 2018. "Antioxidant phenolic compounds isolated from wild Pyrus ussuriensis Maxim. fruit peels and leaves." *Food Chem.*, vol. 241, pp. 182-187.

[6] Turan, I., Demir, S., Kilinc, K., Burnaz, N. A., and Yaman, S. O., 2017. "Antiproliferative and apoptotic effect of Morus nigra extract on human prostate cancer cells." *Saudi. Pharm. J.*, vol. 25, pp. 241-248.

[7] Russo, M., Fanali, C., Tripodo, G., Dugo, P., Muleo, R., and Dugo, L., 2018. "Analysis of phenolic compounds in different parts of pomegranate (*Punica granatum*) fruit by HPLC PDA ESI/MS and evaluation of their antioxidant activity: application to different Italian varieties." *Anal. Bioanal. Chem.*, vol. 410, pp. 3507-20.

[8] Tumbas, Š. V., Cetkovic, G., Canadanovic-Brunet, J., Dilas, S., Pajin, B., Petrovic, J., Stajčić, S., and Vulić, J., 2017. "Encapsulation of sour cherry pomace extract by freeze drying: characterization and storage stability." *Acta. Chim. Slov.*, vol. 64, pp. 283-289.

[9] Kwaw, E., Ma, Y., Tchabo, W., Apaliya, M. T., Wu, M., Sackey, A. S., Xiao, L., and Tahir, H. E., 2018. "Effect of lactobacillus strains on phenolic profile, color attributes and antioxidants activities of lactic acid fermented mulberry juice." *Food Chem.*, vol. 250, pp. 148-154.

[10] Matouskova, P., Marova, I., Bokrova, J., and Benesova, P., 2016. "Effect of encapsulation on antimicrobial activity of herbal extracts with lysozyme." *Food Technol Biotech*, vol. 54, pp. 304-316.

[11] Rojas-Anhuaman, C. P., Castillo-Saavedra, E. F., and Rodrigo-Villanueva, M., 2016. "Estudio de estabilidad acelerada de cápsulas de fluoxetina 20 mg." *Revista Cientifica Tzhoecoen*, vol. 8, pp. 125-140.

[12] Tapia-Tenorio, A. E., 2002. *Muestreo y tamaño de muestra. Una guía práctica para personal de salud que realiza investigación*. 1st ed. Argentina: Buenos Aires. p. 172.

[13] Slinkard, K. and Singleton, V. L., 1977. "Total phenol analysis: automation and comparison with manual methods." *Am. J. Enol. Viticult.*, vol. 28, pp. 49-55.

[14] Fukumoto, L. R. and Mazza, G., 2000. "Assessing antioxidant and prooxidant activities of phenolic compounds." *J. Agric. Food Chem.*, vol. 48, pp. 3597-3604.

[15] Madlmeir, S., Loidolt, P., and Khinast, J. G., 2019. "Study of the capsule- filling dosator process via calibrated DEM simulations." *Int. J. Pharmaceut.*, vol. 567, pp. 1-12.

[16] Ghimeray, A. K., Jung, U. S., Lee, H. Y., Kim, Y. H., Ryu, E. K., and Chang, M. S., 2015. "In vitro antioxidant, collagenase inhibition, and in vivo anti-wrinkle effects of combined formulation containing Punica granatum, Ginkgo biloba, Ficus carica, and Morus alba fruits extract." *Clin Cosmet Investig Dermatol*, vol. 8, pp. 389-396.

[17] Montenote, M. C., Wajsman, V. Z., Konno, Y. T., Ferreira, P. C., Silva, R. M. G., Therezo, A. L. S., Silva, L. P., and Martins, L. P. A., 2017. "Antioxidant effect of Morus nigra on Chagas disease progression." *Rev. Inst. Med. Trop. SP.*, vol. 59, pp. 1-11.

[18] Popescu, G. S., Velciov, A.-B., Costescu, C., Gogoasa, I., Gravila, C., and Petolescu, C., 2014. "Chemical characterization of white (Morus alba), and black (*Morus nigra*) mulberry fruits." *J. Hortic Forest Biotech.*, vol. 18, pp. 133-135.

[19] Arellano-Yáñez, P. A., 2004. "Estudio de estabilidad en estantería de dos series de Grisux día y noche capsulas blandas." Available: http://repositorio.uchile.cl/tesis/uchile/2004/arellano_p/sources/arellano_p.pdf

[20] FEUM, 2014. *Farmacopea de los estados unidos Mexicanos*. 11th ed. México: Secretaria de Salud, Comisión Permanente de la Farmacopea de los Estados Unidos Mexicanos. Primer volumen.

[21] NOM, 2005. "Norma Oficial Mexicana. Estabilidad de Fármacos y Medicamentos NOM-073-SSA1-2005." Available: http://www.salud.gob.mx/unidades/cdi/nom/073ssa105.html

[22] Kuklinski, F. C., 2003. *Estudio de las drogas y sustancias medicamentosas de origen animal*. Barcelona, España: Primera reimpresión, ediciones Omega. pp. 14-15.

[23] Prieto-Abreu, M., Díaz-Solares, M., Pérez-Hernández, M. C., and Martín-Prieto, D., 2016. "Influencia de tres variedades de *Morus alba* L. en el crecimiento de *Bombyx mori* L." *Pastos Y Forrajes*, vol. 39, pp. 132-137.

[24] Boschini-Figueroa, C. and Vargas, R. C. F., 2009. "Rendimiento y calidad de la morera (*Morus alba*) fertilizada con nitrógeno, fósforo y potasio." *Agronomía Mesoamericana*, vol. 20, pp. 285-296.

[25] Ercisli, S. and Orhan, E., 2007. "Chemical composition of white (*Morus alba*), red (*Morus rubra*) and black (*Morus nigra*) mulberry fruits." *Food Chem.*, vol. 103, pp. 1380-1384.