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EFFECTS OF DIFFERENT DRYING METHODS ON QUALITY PARAMETERS OF SALEP (*Serapias vomeracea*)

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Signature

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LIST OF SYMBOLS

°C	Centigrade degree
g	Gram
mL	Milliliter
min	Minute
Pa	Pascal
%	Percent
rpm	Revolutions per minutes
s	Second
η	Viscosity
K	Consistency coefficient
n	Flow behaviour index
$\dot{\gamma}$	Shear rate
τ	Shear stress

LIST OF ABBREVIATIONS

SD	Sun Drying Method
HAD	Hot-air Drying Method
FD	Freeze-drying Meyhod

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ABSTRACT

Effects Of Different Drying Methods On Quality Parameters Of Salep (*Serapias vomeracea*)

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Master Of Science Thesis

Supervisor: Prof. Dr. Hasan SADIKOĞLU

The main objective of this work is to increase the quality parameters of salep by using different drying methods. In this study, *Serapias vomeracea*, the raw material for salep, was subjected to different drying processes (sun-, hot-air, and freeze-drying). Then, moisture content, glucomannan content, starch content, ash content, color change, solubility value, water absorption capacity, and rheological properties were determined and evaluated.

Experimental results indicate that, salep that obtained by the sun-drying method has 13,66% moisture content, 22,08% glucomannan content, 14,96% starch content and 4,90% ash content. While solubility value, water absorbency capacity, viscosity, consistency coefficient and fluid behavior index were determined to be 55,00%, 666,0%, 61,19 cp, 0,265 Pa.sn and 0,646, respectively. Salep that obtained by hot-air drying method has 7,48% moisture content, 16,19% glucomannan content, 10,05% starch content and 4,48% ash content. While solubility value, water absorbency capacity, viscosity, consistency coefficient and fluid behavior index were determined to be 68,87%, 715,00%, 22,70 cp, 0,058 Pa.sn and 0,776, respectively. Salep that obtained by freeze drying process has 11,34%

moisture content, 17,66% glucomannan content, 22,08% starch content and 4,48% ash content. Besides, solubility value, water absorbency capacity, viscosity, consistency coefficient and fluid behavior index were found to be 65,00%, 1350,00%, 302,48 cp, 8,327 Pa.sn and 0,195, respectively. Moisture content, glucomannan content, starch content, ash content, solubility value, water absorbency capacity, viscosity, consistency coefficient, and fluid behavior index of salep that obtained by freeze drying process after pretreatment, were determined to be 6,98%, 22,30%, 22,08%, %4,95, 55,00%, 1400,00%, 236,60 cp, 3,50 Pa.sn and 0,343, respectively.

The experimental results clearly indicate that the quality of the final product is affected by the drying method used to obtain salep. When different drying methods are compared for quality salep production, it can be easily seen that the freeze-drying method is the best. The water absorption capacity, viscosity, and consistency coefficient of salep produced by the freeze-drying method are quite high compared to other alternative drying methods. The high cost of freeze-drying can be easily compensated by the product quality. Thus, for the production of quality salep, the freeze-drying method can be commercialized for the pharmaceutical and food industry.

Keywords: *Serapias vomeracea*, Salep, Freeze-drying

ÖZET

FARKLI KURUTMA YÖNTEMLERİNİN SALEBİN *(Serapias vomeracea)* KALİTE PARAMETRELERİ ÜZERİNE ETKİLERİ

Buket BİLGİÇ

Kimya Mühendisliği Anabilim Dalı

Yüksek Lisans Tezi

Danışman: Prof. Dr. Hasan SADIKOĞLU

Bu çalışmanın amacı, farklı kurutma yöntemleri kullanılara salebin kalite parametrelerini arttırmaktır. Çalışma kapsamında; salebin hammaddesi olan *Serapias vomeracea* bitkisine farklı kurutma yöntemleri uygulamış (güneşte kurutma, sıcak hava ile kurutma ve dondurarak kurutma) ve ardından yapılan analizlerle nem içeriği, glukomannan miktarı, nişasta miktarı, renk değişimi, kül miktarı, çözüne bilirlik değeri, nem tutma kapasitesi ve reolojik özellikleri belirlenmiş ve karşılaştırılmıştır.

Çalışma sonucunda, güneşte kurutma yöntemi ile nem içeriği %13,66, glukomannan miktarı %22,08, nişasta miktarı %14,96, kül miktarı %4,90, çözünebilirlik değeri %55,00, nem tutma kapasitesi %666,00, viskozitesi 61,19 cp, konsistens katsayısı 0,265 Pa.sn ve akış davranış indeksi (n) 0,646 olan ürün elde edilmiştir. Sıcak hava ile kurutma yönteminde ise nem içeriği %7,48, glukomannan miktarı %16,19, nişasta miktarı %10,05, kül miktarı %4,48, çözünebilirlik değeri %68,87, nem tutma kapasitesi %715,00, viskozitesi 22,70 cp, konsistens katsayısı 0,058 Pa.sn ve n değeri 0,776 olarak bulunmuştur. Dondurarak kurutma yönteminde nem içeriği %11,34, glukomannan miktarı

%17,66, nişasta miktarı %21,20, kül miktarı %4,48, çözünebilirlik değeri %65,00, nem tutma kapasitesi %1350,00, viskozitesi 302,48 cp, konsistens katsayısı 8,327 Pa.sn ve n değeri 0,195 olan ürün elde edilmiştir. Ön işlemi takiben gerçekleştirilen dondurarak kurutma prosesi ile nem içeriği %6,98, glukomannan miktarı %22,30, nişasta miktarı %22,08, kül miktarı %4,95, çözünebilirlik değeri %55,00, nem tutma kapasitesi %1400,00, viskozitesi 236,60 cp, Konsistens katsayısı 3,50 Pa.sn ve n değeri 0,343 olan ürün elde edilmiştir.

Sonuç olarak, salebin kalite özellikleri arasında fark gözlemlenmiş olup kurutma işlemindeki farklılığın ürün kalitesini etkilediği belirlenmiştir. Dondurarak kurutma prosesi ile hem farmasötik hem de gıda alanında yaygın kullanım alanına sahip olan daha kaliteli salep üretimi gerçekleştirilmiştir. Katma değeri yüksek olan salep üretimi için geliştirilmiş olan bu prosesin yüksek ticarileşme potansiyeline sahip olacağı düşünülmektedir.

Anahtar Kelimeler: *Serapias vomeracea*, Salep, Dondurarak Kurutma

1
INTRODUCTION

Serapias vomeracea is one of the tuberous terrestrial orchid species that belongs to the Orchidaceae family, and it is widely used for producing Salep. It represents both powdered tubers of suitable orchid species and the traditional hot beverage obtained by boiled salep powder. (Turkish Official Gazette, 2013; Dictionary of Turkish Language Institution, 2013). Salep is used as a raw material of the drugs due to its therapeutic effects on the cough, headache, flu, intestine, and sexual problems. Additionally, Salep is used in the food industry, especially in making ice cream. It is also an indispensable stabilizer for Maras ice cream due to certain qualities like relative delay in melting and improved taste. Nowadays, Salep is obtained by drying orchid tubers growing widely under the sun in Turkey. However, some researches show that orchid species face the danger of extinction because of urbanization, industrialization, unconscious grazing, and expanding the area for tourism (Erzurumlu & Doran, 2011; Sandal & Söğüt, 2010; Tamer et al., 2006; K. K. Tekinşen & Güner, 2010).

Drying is the most fundamental and easiest food preservation method that has been used since ancient times. It has been observed that the nutrition value of food cannot preserve adequately in the sun drying method, which is used for drying salep and other plants traditionally. Additionally, the hot-air drying method cannot provide the high-level preservation of nutrition values though it can supply long shelf life (Ratti, 2001). However, in the freeze-drying method, some negative states like enzymatic reactions, non-enzymatic blackening, and degradation of proteins in the food experience in minimum since the drying process performs at low temperature and the local water loss occurs very rapidly (Sadıkoğlu & Özdemir, 2003). According to the research regarding the freeze-drying method, it is stated that the nutrition value, flavor, taste, and texture of freeze-dried products preserves the utmost (Acar et al., 2011; Ratti, 2001).

1.1 Literature Review

1.1.1. Salep

Salep obtained from tuberous terrestrial orchids containing different genera and species belonging to the Orchidaceae family that have completed their flowering is a raw food material (Dictionary of Turkish Language Institutions, 2013). Spreading worldwide, except in Antarctica, orchids can grow at altitudes up to 5,000 meters above sea level. While the whole world hosts more than 22.000 species of the Orchidaceae family belonging to approximately 880 genera, Turkey is one of the countries with the highest species diversity in the world, with 150 species. Turkey attracts attention with its diversity of species due to its geographical location and structure, as well as 28 endemic orchid species belonging to 5 genera (Arslankaya, 2015 ;Sandal & Söğüt, 2010; Şen, 2016).

From Orchids that can be grown in almost every region of Turkey, only 125 orchid species belonging to 16 genera are tuberous. However, not all tuberous orchids can be used to produce salep. Salep can be obtained from 117 species belonging to the genera *Aceras, Anacamptis, Barlia, Comperia, Dactylorhiza, Himantoglossum, Neotinea, Ophrys, Orchis,* and *Serapias* in Turkey (Sezik, 2002). It has been possible to obtain salep from *Plantanthera* genus orchid tubers in recent years. Orchid species and species commonly used for salep production in Turkey are given in Table 1.1. Orchids used in making salep are commonly found in Eastern Mediterranean, Southern Anatolia, Northern Anatolia, Eastern Anatolia, and Southeastern Anatolia regions (Erzurumlu & Doran, 2011).

Table 1.1 Orchid species and species commonly used for salep production in Turkey (Erzurumlu & Doran, 2011; Pridgeon, 1999)

Genus	Species
Aceras	*A. anthropophorum*
Anacamptis	*A. pyramidalis*
Barlia	*B. robertiana*
Comperia	*C.comperiana*
Dactylorhiza	*D. iberica, D. osmanica*
Himantoglossum	*H. afine*
Neotinea	*N. maculata*
Ophrys	*O. bombyliflora, O. ferrumequinum, O. fusca*
Orchis	*O.anatolica, O.coriophora, O.italica, O.laxiflora, O.morio, O.pallens, O.palustris, O.pinettorum, O.provincialis, O.purpurea, O.sancta, O.simia, O.spitzelii, O.tridentata*
Serapias	*S. vomeracea*

Salep has been used for various purposes in Anatolian geography for centuries. At the beginning of these areas, it is used as the raw material of a traditional hot drink with the same name. The culture of salep drink emerged as an alternative to drinks such as wine and kumiss, which were banned in the 8th century due to conversion to Islam. The salep drink, which still maintains its popularity today, is generally preferred in the winter months and is ready for consumption by mixing the salep powder with sugar and then boiling it with milk (Tamer et al., 2006). Salep drink has managed to attract the attention of industrial manufacturers

thanks to the intense consumer interest it has even today. It is possible to find powder (instant-fast dissolving) and liquid (produced by Ultra High-Temperature processing technique) forms in the market. Salep drink in powder form, which is offered for sale in the markets, contains salep powder, sugar, milk powder, salep flavor, various stabilizers, and cinnamon (Dogan & Kayacier, 2004; Tamer et al., 2006).

Salep is a raw pharmaceutical material that has been widely used in medicine for centuries due to its therapeutic effect against many diseases. Ibn-i Sina has extensively mentioned the aphrodisiac, appetizing, expectorant, paralytic, and mind-opening effects of salep in his book, which is titled "The Law". Besides these effects, it is used to treat summer diarrhea in infants and children, chronic diarrhea in adults, malaria, and dysentery due to its positive effects on intestinal problems, flu, and cough. Additionally, it has also had an important place in treating arthritis and wounds in Anatolia for many years (Erzurumlu & Doran, 2011; Tamer et al., 2006; Yılmaz, 2015).

Salep also acts as the primary stabilizer in the production of Maraş ice cream, one of the unique flavors of Turkey. The main reason for using the stabilizers in ice cream is to provide a smooth structure and texture to the ice cream. In addition, they use to prevent the formation of large ice crystals during the production and storage of the ice cream and increase the product's resistance to melting. Hence, they directly affect the quality of the ice cream. Quality ice creams are expected to be preserved at low temperatures (around -18 °C) without changing their structure. For these reasons, salep is used to provide Maraş ice cream with its unique flavor, concise and slightly chewy elastic-hard structure, flexible texture, resistance to melting, and maintainability at low temperatures, thanks to the glucomannan it contains. Starch, which is found in the construction of salep and has the swelling feature, contributes to forming this particular structure as an auxiliary component. Salep is used in ice cream production in powder form at the 7-8 g salep (approximately 0.5 - 1.0%) per 1 liter of milk (Şen, 2016; K. K. Tekinşen et al., 2011; K. K. Tekinşen & Güner, 2010; O. C. Tekinşen & Karacabey, 1984; Türkmen, 2019).

In addition to the widely known uses of salep, there are also studies in the literature on increasing emulsion stability, extending the shelf life of some dairy desserts, and using it in edible film production (Ayar et al., 2009; Georgiadis et al., 2012; Kurt & Kahyaoglu, 2014).

The main component that gives salep its unique flavor and structure is glucomannan. Glucomannan is a hydrocolloid polysaccharide consisting of glucose and mannose units linked by β-(1,4) glycosidic bonds. The basic structure of glucomannan is indicated in Figure 1.1. This compound, which has a high molecular weight and high water-binding (1 gram absorbs 200 ml of water), can be used in many areas, from the food industry to the pharmaceutical industry. In the food industry, due to its gelling, thickening, film-forming, and emulsifying properties, It is used in bakery, dessert, beverage, and flour products production sectors. Glucomannans are used as excipients in the pharmaceutical industry due to their gelling, solubility, and biodegradability properties. In addition to these properties, it has been the subject of many studies due to its functions as a bulking laxative, delaying gastric emptying, preventing the rise of typical post-meal blood sugar levels, and lowering cholesterol. Due to these known and studied properties, glucomannans in weight loss products are becoming more and more common (Alonso-Sande et al., 2009; Kurt & Kahyaoglu, 2017; Şen, 2016; Tamer et al., 2006; Yılmaz, 2015).

In spite of the fact that glucomannan is not yet qualified as a drug, there are studies regarding its use as a bioactive polymer. At the same time, studies on glucomannan-based hydrogel production in targeted drug delivery systems are available (Alonso-Sande et al., 2009; Chen et al., 2005). The konjac plant meets the need for glucomannan in industrial food and cosmetics. Although the Konjac plant is the most widely cultivated and known source of glucomannan globally, it has not been meeting the demand in these areas well due to its low-quality characteristics (fishy smell, slightly bitter taste, low viscosity, inability to be cleared) (Türkmen, 2019; Xu et al., 2014).

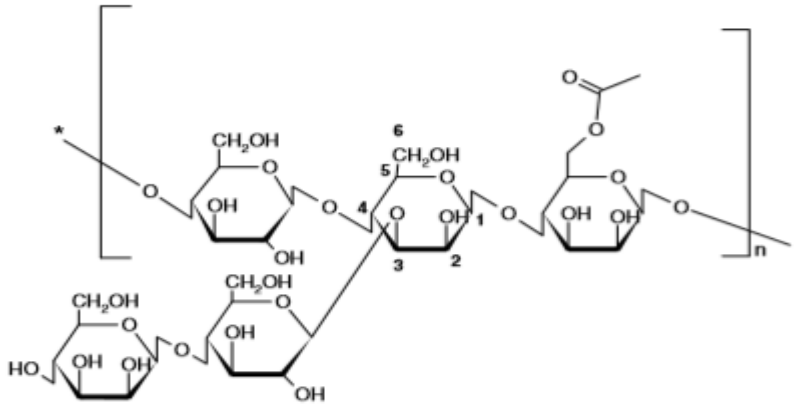


Figure 1.1 The chemical structure of Glucomannan (Alonso-Sande et al., 2009)

The content of salep varies according to the type of orchid tubers from which it is obtained, the harvest season, and the region where its collected. Therefore, standard data on salep content are not available in the literature. According to Sezik's (1967) study, salep; Although its content varies between species, generally contains 7-61% glucomannan, 1-36% starch, 0.5-1% nitrogenous substances, 0.2-6% ash, and 6-12% moisture (Kurt & Kahyaoglu, 2015; Sezik & Baykal, 1988; K. K. Tekinşen & Güner, 2010). The data obtained from the study conducted by Tekinşen and Güner (2010) on salep obtained from different orchid species, which reached similar results with this difference, are given in Table 1.2.

Table 1.2 Chemical compositions of salep samples from different species (g/100 g) (K. K. Tekinşen & Güner, 2010)

Species	Glucomannan	Starch	Moisture	Ash	Protein
O. italica	54,6	5,44	11,02	0,97	3,92
O. morio	51,2	7,99	10,38	1,91	4,95
D. osmanica var. osmanica	22,5	38,7	9,35	2,80	3,11
O. simia	38,3	10,4	11,78	1,95	3,53
S. vomeracea ssp. orientalis	44,8	13,4	10,25	1,96	4,83
O. anatolica	43,5	14,7	10,71	1,94	3,20
O. tridentata	42,4	15,2	12,40	1,92	4,94
O. coriophora	29,0	17,0	11,64	2,83	3,16
O. palustris	20,5	27,6	11,90	0,95	3,17
Oph. mammosa	17,7	33,8	11,36	1,76	3,25

Although the quality of products containing salep depends on the rheological properties, this property varies according to the chemical structure of salep. As with all other hydrocolloid solutions, salep solutions show pseudoplastic flow properties. Pseudoplastic behavior, one of the non-Newtonian flow behavior patterns, decreases as the shear rate increases; in other words, the substance's resistance to flow, which means the viscosity of the solution is decreasing (Arduzlar, 2003; Dogan & Kayacier, 2004; Kurt & Kahyaoglu, 2017).

$$\tau = K.\dot{\gamma}^{n} \qquad (2.1)$$

Where τ denotes the shear stress (Pa), $\dot{\gamma}$ demonstrates the shear rate (s^{-1}), K coefficient of consistency (Pa.sec), and n denotes flow behavior index (unitless).

The value of n in the equation indicates how much the flow behavior deviates from the Newtonian behavior, for example, and varies between 0 and 1 in

pseudoplastic flow. For example, when n moves away from 1, the flow type transforms from a Newtonian flow type to a non-Newtonian flow type. The consistency coefficient and flow behavior index that characterize pseudoplastic behavior are affected by the temperature and composition of food (Arduzlar, 2003; Türkmen, 2019).

The storage period of salep is directly related to the amount of moisture it contains. Less than 10% moisture content extends the shelf life of tubers. And also, the amount of ash contained in the tubers is of great importance for obtaining white and clean salep. White salep powder can be obtained from tubers with less than 5% ash (Şen, 2016; Sezik & Baykal, 1988). Because of these effects, the determination of glucomannan, starch, moisture, and ash amounts is of great importance in determining salep quality and adulteration. Although the nitrogen amounts of salep tubers do not vary significantly, these analyses are not required since they do not directly affect the quality (Sezik & Özer, 1983).

Serapias vomeracea (Figure 1.2) type orchid used in this study is a plant that usually contains 2, rarely 3 or 4 oval tubers, and its height varies between 20-40 cm. It is a plant that can grow up to 1000 meters above sea level, generally in clay soils, maquis, dry and wet meadows. Also known as 'deaf ear' among the people, this orchid species blooms between March and May (Bektaş, 2014; Şen, 2016).



Figure 1.2 *Serapias vomeracea* (Encylopedia of Life, 2021)

Orchid species that can be used for salep have two tubers usually. The first of these is the 'old, mature, large, main or midwife tuber' formed the previous year, with the nutrients the plant will spend the winter together. Another is the tuber known as the 'young, small, plump, sister or nurse tuber,' which begins to form towards the time of flowering and will start the following year's plant. In the process that the new tuber develops and prepares the following year's plant, the old tuber remains stuck to the new tuber in an emptied and shrunken form. When the plant starts to bloom, the tubers are collected for salep. This process will be sufficient to gather only the young tubers, and it will be possible to bury the remaining old tubers in the soil and germinate again by protecting them. The new tubers collected are 0.7-3.6 cm in diameter, 0.3-1.2 cm in width, and weighing between 0.2 and 1.6 grams, depending on the orchid species, and are translucent, yellowish, hard, odorless, and tasteless (Erzurumlu & Doran, 2011; Kreutz, 2009; Şen, 2016; Tamer et al., 2006).

Young tubers collected from nature are ready for consumption as salep powder after several processes. First, the tubers are washed in cold water to get rid of their soil. Afterward, the tubers are boiled in order to stop the enzymatic activities, loosen their outer shells and soften them. The boiling process is usually carried out in water or milk, and in some cases, it can also be done in ayran, whey, or cottage cheese. The boiling medium varies depending on the region where salep will be obtained. Immediately after the boiling process, which takes about 10-15 minutes, the tubers are washed with cold water and cooled to prevent their sticking. Following this, the tubers are left to dry in a sunny and airy place by stringing them on a string or laying them on a clean cloth until they reach a 'hardness that will not cut teeth.' Salep is obtained by grinding the dried tubers. Tubers lose about 70-90% of their weight during this process. The amount of water lost varies according to the harvest time of the tubers which have been used for producing salep (Bulut-Solak et al., 2017; Erzurumlu & Doran, 2011; Kaya & Tekin, 2001; Şen, 2016; Sezik, 2002; Tamer et al., 2006; Türkmen, 2019).

Salep has been known for its unique taste and consistency since it has been started using; it has attracted great interest in both domestic and foreign markets. The export data for the years 1955-1993 were examined, and it was found that the

average export amount until 1989 was approximately 5 tons per year, and after 1990, this amount reached up to 30 tons per year due to the salep sales in powder form and packaged fast drink form (Çalışkan & Kurt, 2019). The literature shows that the export of salep, which continued until 1996, was approximately 10 tons per year, and this figure reached an average of 20 tons per year with domestic use. 1000 – 4000 tubers are needed to produce 1 kilogram of salep, 20 – 80 million tubers are needed to meet those mentioned above substantial domestic and foreign demand (Erzurumlu & Doran, 2011; Sandal & Söğüt, 2010; Sezik, 2002).

This large amount of dismantling has been quite destructive for orchid plants, which require a long time to renew themselves in nature. For this reason, various arrangements have been made since 1974 in order to protect orchid species. Salep orchids, which are included in the category of products with export licenses as of this year, are included in the list of products whose export is completely prohibited with the communiqué published by the Ministry of Agriculture and Rural Affairs regarding the Regulation on the Removal, Production, and Export of Natural Flower Bulbs published in the Official Gazette dated 09 October 1991 and numbered 21016 has been added. With the Communiqué No. 96/31 on Export Prohibited and Pre-authorized Goods published by the Undersecretariat of Foreign Trade and published in the Official Gazette dated 19 July 1998 and numbered 23407, the export of salep (powder, tablet, and all kinds of forms) was completely prohibited. With the export communiqué, which was renewed in 2001, the export of any product with the indication of salep was prohibited. Turkey, which has been a party to the CITES (Convention on International Trade in Endangered Plant and Animal Species) since 1996, has 55 orchid species on the CITES Annex-II (Species that are threatened with absolute extinction but whose trade is subject to specific principles in order to prevent use incompatible with their survival) list (Sandal & Söğüt, 2010; Yaman, 2013).

According to today's data, it is assumed that an average of 45 tons of salep is produced annually. In order to meet this amount, 45 – 180 million orchid tubers are needed. In addition to this figure, according to unofficial information obtained from buyers, annual salep production in Turkey varies between 80-100 tons. Based on this information, it can be deduced that salep and salep-added products,

which are prohibited from being collected and sold illegally, are common. Salep orchids, which are not prohibited from being collected and sold in the domestic market, are under threat due to unconscious foraging, industrialization, urbanization, natural disasters, expansion of agricultural areas, and use of the natural environment orchids for livestock and tourism activities (Çalışkan & Kurt, 2019; Erzurumlu & Doran, 2011; Sandal & Söğüt, 2010).

In-vitro and in-vivo studies have been conducted for many years to protect orchid species, which are used for salep production and are therefore in danger of extinction (Bektaş et al., 2013; Ekinoğlu, 2017; Gümüş, 2009). In addition to these studies, studies on orchid production with high efficiency in field conditions and culture medium continue. (2012) in the study carried out in the Aegean Region; *Orchis sancta* L. and *Serapias vomeracea* (Burm. fil.) Brig. They have developed a production model that farmers can quickly implement under field conditions. However, this study was limited to only these two species and the Aegean Region. Although positive results have been obtained from such studies, there is not yet a method developed for commercial production to meet the existing consumer demand in Turkey.

In addition to the studies on cultivating the salep orchids, there are studies on the conversion of the produced salep powders into a higher quality product. In the study of Kurt and Kahyaoğlu (2015), they succeeded in increasing the glucomannan percentage by removing impurity components by ethanol treatment of salep powder and, in parallel, improving the rheological properties of the product.

1.1.2. Drying

Drying is expressed as the process of thermally removing the volatile components (usually water) contained in the substance in order to obtain a solid substance. The drying process has a vast area in the literature as the process that dates back to the oldest history among the fundamental processes of chemical engineering has the most used area and contains the most diversity. As a result of drying, a low level of microbial activity will occur in the material in parallel with the decrease in the water activity of the material; thus, drying is the most basic method preferred for the preservation of biological materials and foods. Since dried foods

are more resistant to all kinds of changes caused by bacteria, mold, or insects, which may result in losing their original properties, their shelf life is quite long. Dried food products, preserved with their high nutritional values and flavors, provide ease of packaging and transportation. The drying process is widely preferred in many fields such as chemistry, polymer, biotechnology, agriculture, ceramics, and pharmacy (Mujumdar, 2015; Özenir, 2009; Tekgül, 2019).

Drying is a complex separation process in which heat and mass transfer processes occur simultaneously. The heat energy required for drying; is transferred to matter by convection, conduction, radiation, or in some cases, a combination of these methods. As a result of this heat transfer, the water in the material evaporates and diffuses from the material's surface into the dryer air and leaves the environment. As a result of these transfers, insects and other organisms are eliminated from the dried material. For this reason, drying is a method that is frequently used, especially in making food products healthy. However, these transfers can cause physical changes in the form of shrinkage, precipitation, swelling, fracture, cracking, or crystallization, which negatively affect the quality of the end product. In addition, in some cases, desired or undesired chemical or biochemical reactions may occur during drying, and changes may occur in the odor, taste, color, or texture of the product. For this reason, the most essential criterion to be considered in the drying process is determining the drying method suitable for the material to be dried (Mujumdar, 2015; Özenir, 2009).

1.1.2.1. Sun Drying

The process in which solar heat is used to remove the volatile components in the substance and takes place in natural climatic conditions without affecting the drying parameters is called sun drying. This method, which is preferred locally in drying small amounts of agricultural products, is the oldest and most environmentally friendly drying method. The drying time can vary from a few days to a month depending on the nature of the product, the intensity of the sun's rays in the region, the relative humidity of the air, and the speed of the environmental wind. However, this drying method, which occurs uncontrollably in line with the variables it depends on, is not suitable for scientific design and technical

calculations. For this reason, the desired quality characteristics and moisture content in the final product cannot be standardized. The drying process in the sun is based entirely on the experience of the person performing the process (Belessiotis & Delyannis, 2011; Tekgül, 2019).

Sun-drying method, which is preferred intensively because it is economical and easy to apply in local production, has a considerable handicap because the dried product is open to all kinds of contamination that may come from the environment. In addition, adverse effects such as decay or destruction may occur in the final product since the product may be exposed to changes in natural weather (hail, storm, etc.) during the drying (Belessiotis & Delyannis, 2011).

Artificial drying methods are used in order to eliminate the adverse effects of the natural drying method on the quality of the final dried product. Unlike natural drying, the process in which the drying process is carried out in a closed environment with controllable conditions is called 'artificial drying'. Hot-air drying is one of these methods used in the industry.

1.1.2.2. Hot-air drying

The hot-air drying method is one of the most preferred methods for industrial-scale drying because of the advantages such as ease of application, short drying time, and low cost. In this method based on convection, heat is contacted with the wet material through heated air or gas. The moisture transferred to the surface from the inside of the material by convection is removed with drying air simultaneously. The drying air removed from the dryer is refed to the drying system after mixing with fresh air and heating. The air used a few times in the dryer and reached 60-70% relative humidity is removed from the drying system. Thanks to this recycling, the hot-air drying method has a low cost (Aktaş, 2015; Sarıkulak, 2017).

In the drying process, which is based on the principle of the hot air drying method, undesirable color changes and browning may occur due to working at high temperatures. The reason why these situations are undesirable is not only the appearance of the product. Since the colors are directly related to the antioxidant and vitamin contents in the substances, the preservation of the color of the product

during the drying process is of great importance for the quality of the final product (Ratti, 2001).

1.1.2.3. Freeze-drying

Freeze-drying which is also known as lyophilization is a dehydration process based on sublimation. It is widely preferred in the pharmaceutical and food industries for substances that are inconvenient to be dried with the traditional method, are very sensitive to temperature, and can quickly deteriorate (Sadıkoğlu, 2010; Sadıkoğlu & Özdemir, 2003).

Substances that are used for drying by freeze-drying are as follows;

- Blood plasma, serum, drugs, hormone solution
- Food ingredient
- Inanimate materials such as ceramics, superconductors, historical documents (archaeological finds)
- Inanimate materials such as transplantable organs, artificial skin, and bone
- Living cells need to live for long, such as yeast, viruses, and bacteria (Sadıkoğlu & Özdemir, 2003).

In the freeze-drying process, the water or any solvent contained in the frozen material to be dried is separated by sublimation at low pressure (in a high vacuum environment). The solvent, separated as vapor, is removed from the environment where the drying process takes place utilizing the condenser (Sadıkoğlu & Özdemir, 2003).

Lyophilization consists of 'freezing' and 'drying (primary drying step and secondary drying step) steps which occur consecutively. In the freezing step, which is the first step of the process, the substance is cooled to a temperature below freezing in a way that will allow all of the solvents it contains to freeze. At the end of the freezing stage, generally, 65-90% of the initial amount of water can be obtained as frozen water (frozen water in the form of ice crystals), while the remaining 10-35% water is contained in the substance as unfrozen water (bound water). The freezing stage is the most crucial step that affects the operation of the whole process and directly affects the quality of the final product. The structure of the ice crystals formed at this stage and their distribution in the substance

affects the drying rate. If the ice crystals formed have a large and homogeneous distribution, a rapid drying process will occur. However, if the ice crystals are tiny and have limited contact, a relatively slow drying process will occur. Besides these, it is the step that plays an active role in determining the dried product's morphological character, biological activity, and structural stability. Therefore, the freezing method, freezing speed, and content of the solution are of great importance for the whole process (Aydın, 2014; Sadıkoğlu & Özdemir, 2003).

In the primary drying step, which is the first stage of the drying process, the frozen solvent is removed from the substance by sublimation. For this process, the pressure of the drying chamber should be close to or less than the vapor pressure of the frozen solvent. While the solvent sublimes, the interface starting from the surface of the substance to be dried moves upwards and downwards (Figure 1.3). The heat required for sublimation is provided to the material by conduction, convection, or radiation. The sublimation heat transfer required for sublimation to the material is provided through the top dried or bottom layers. However, the temperature of the dried layer; the system must be heated in a controlled manner since it must be kept at temperature values that will not lose bioactivity, will not change the color of the product, will not cause chemical and biochemical reactions, and will not spoil the structure of the dried layer. The evaporated solvent is carried through the porous dried layer. A small amount of unfrozen water is removed from the substance during the first drying period. The effect of desorption (removal of bound water) in the dried layer on the heat conduction to the interface and the interface's speed is relatively high. It is called the end of the first drying period when all the frozen layer in the substance to be dried is finished (the moment when the sublimation interface disappears) (Aydın, 2014; Sadıkoğlu & Özdemir, 2003).

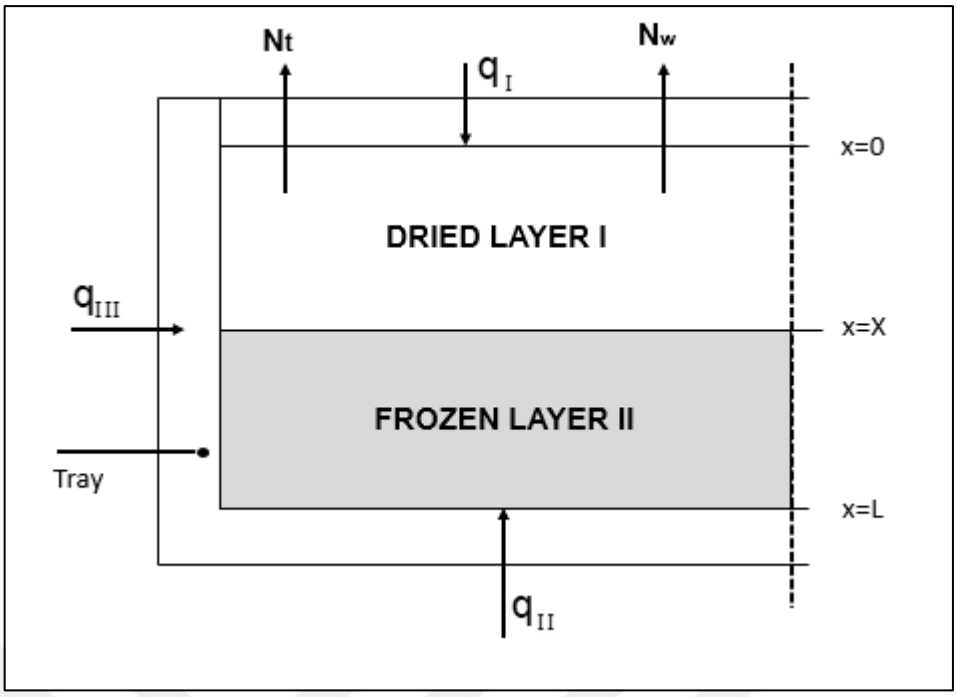


Figure 1.3 Freeze-drying of the product in a tray

The secondary drying stage starts when all the frozen water in the dried substance is exhausted. The second drying period is removing the bound water in the substance from the substance by desorption under a high vacuum. This step aims to reduce the final moisture content to improve the product's stability and shelf life in the first drying period. This process operates at temperatures between 10 and 35 °C for temperature-sensitive materials and 50 °C and above for temperature-resistant materials (Aydın, 2014; Sadıkoğlu, 2010; Sadıkoğlu & Özdemir, 2003).

Coffee, onions, soups, some seafood, and fruits, which are used daily and are very difficult to dry using traditional methods, are freeze-dried. The advantages of freeze-drying foods can be listed as follows;

- The moisture content of the dried materials is very low
- When water is added to dried substances, they quickly absorb water into their bodies and reach a very close to their pre-drying structure (fast rehydration)
- Dried substances lose very little taste and aroma during the drying process
- Dried substances retain their nutritional values at a high rate

- Due to the lower moisture content of freeze-dried items, their shelf life is longer
- Protein degradation and enzymatic reactions in the structure of the food are minimal (Sadıkoğlu & Özdemir, 2003)
- No cooler is required during the storage of freeze-dried products (Chakraborty et al., 2006)
- Substances with very low moisture content can be easily pulverized thanks to their porous structure (Özenir, 2009).

The freeze-drying method is a relatively expensive drying method due to its low drying rate and high energy consumption compared to other drying methods. However, the best quality product is obtained in freeze-drying compared to other drying methods. These advantages compensate for the economic problems of freeze-drying. Considering the advantages mentioned above and disadvantages together, freeze-drying is of great importance, especially in foods with high financial value (Acar et al., 2011; Sadıkoğlu et al., 2006; Sadıkoğlu & Özdemir, 2003).

In their study, Acar et al. (2011) used the freeze-drying process as an alternative to the traditional drying method in obtaining saffron, one of the most expensive herbal products globally. They discussed the effects of different drying processes on the quality of the final product. In this study, it has been observed that the amounts of crocin and safranal, which give saffron its unique color, smell, and aroma, are preserved at a higher rate in freeze-drying than in sun drying. It was stated that the crocin value was preserved five times and the safranal value 40% more with the freeze-drying method.

In the study examining the effects of drying methods on carrot slices' physical properties and aroma characteristics (D. carota L.), the material was exposed to hot air drying and freeze-drying processes. The operating results showed that drying with hot air caused a significant change in the physical properties of the carrot slices and completely spoiled the aroma characteristics. Despite that; It was revealed that freeze-drying did not change the physical properties of the carrot slices, and the aroma loss during drying was minimal (Rajkumar et al., 2017).

1.2 Objective of the Thesis

The objective of the study is to dry with different methods *Serapias vomeracea*, an orchid species, to produce high-quality salep. In this study, *Serapias vomeracea*, the raw material for salep, was subjected to sun drying, hot air, and freeze-drying processes. Then, moisture content, glucomannan content, starch content, ash content, color change, solubility value, water absorption capacity, and rheological properties were determined and evaluated.

1.3 Original Contribution

To contribute to nature, it is predicted that with today's knowledge and drying technologies, higher quality salep can be obtained with less orchid destruction. The detailed literature review determined that there is no study examining the effect of different drying methods on the quality of salep. The original contribution of the study is that it is the first study for improving the quality properties of salep by changing the drying process in producing it.

2
MATERIAL AND METHODS

2.1. Materials

Serapias vomeracea tubers used in this study were purchased from a local farmer in Kaş (Altitude:40 m), Antalya in Turkey. The young tubers which are harvested in May of the orchids were supplied directly as they were removed from the ground. The tubers were washed with cold water in order to remove the soil on their surface (Sezik, 2002; K. K. Tekinşen & Güner, 2010). The tubers which were used in the study are given in Figure 2.1.



(a) (b)

Figure 2.1 *Serapias vomeracea* tubers which were used in the study (a) Tubers covered with soil (b) Tubers after washing

2.2. Methods

Salep powders which were obtained by drying *Serapias vomeracea* tubers with different drying methods, which are sun-drying, hot-air drying, and freeze-drying, were compared in terms of their quality parameters such as moisture content, ash content, glucomannan content, starch content, solubility value, water absorption capacity, and rheological properties.

In order to define the moisture content of *Serapias vomeracea* tubers' before the drying, the oven drying method was used. The tubers, which will be dried with

sun-, and hot-air drying methods, were prepared for drying using traditional methods. Firstly, the tubers were boiled in hot water for 10 minutes to inhibit their enzymatic activity, and they were rinsed with cold water (Sezik, 2002; Sezik & Baykal, 1988). The operations are given in Figure 2.2. The tubers which will be dried with freeze-drying methods were divided into two groups. While the first group was not subjected to any pre-treatment (FD-1), freezing pre-treatment was applied under -18 °C to the second one (FD-2) and they both were dried under the same conditions. Before each drying process, all tubers were cut with a knife into small and equal size pieces.



Figure 2.2 Pre-treatments before sun and hot-air drying a) boiling b) rinsing.

2.2.1. Drying Methods

The initial moisture content of tubers was determined as 85%±1.02. As explained below, the prepared tubers for drying have been dried by different methods.

2.2.1.1. Sun-drying method

In the sun-drying method, the traditional procedure was followed. The tubers, prepared with required pre-treatments, were placed in a clean cloth and laid under the sun (Şen, 2016). The drying operation was under 24 – 29 °C and 70% humidity in Istanbul. The drying lasted for three days until the tubers reached the desired hardness, which was that the teeth could not cut them (Sezik & Baykal, 1988). The sun-drying of the *Serapias vomeracea* tubers is given in Figure 2.3.



Figure 2.3 The sun-drying of the *Serapias vomeracea* tubers

2.2.1.2. Hot-air drying method

The tubers prepared with required pre-treatments were dried in the cabinet dryer (APV&Pasilac, Cumbria, UK) shown in Figure 2.4. In the hot-air experiment, the dryer air temperature and airflow rates were 60 °C and 2 m/s, respectively (Doymaz, 2011). The drying operation lasted for 6 hours and 15 minutes.



Figure 2.4 APV&Pasilac (Cumbria, UK) cabinet dryer

2.2.1.3. Freeze-drying method

The freeze-drying experiments were conducted in Virtis Ultra 25 Super XL (New York, US) pilot-scale freeze-dryer (Figure 2.5). The dryer has four plates in the drying chamber, and it can operate at temperatures between -60 °C and 65 °C. The dryer is capable of both manual and automatic drying thanks to the Wizard control system, which also allows control throughout the process (Kayhan, 2008). In the study, the drying chamber pressure was kept at 10 Pa during the entire

process. During the process, the freezing and drying temperatures were set to -25 °C and 20 °C, respectively (Özenir, 2009). The drying process lasted 29 hours. *Serapias vomeracea* tubers which will be exposed to freeze-drying operation, are given in Figure 2.6.



Figure 2.5 The pilot scale freeze-dryer (Virtis Ultra 25 Super XL, New York,US)



Figure 2.6 *Serapias vomeracea* tubers before the freeze-drying operation

The drying methods and conditions are given in Table 2.1. In order to prepare dried samples for analysis, they were milled with an analytical grinder (IKA, A11 Basic Analytical Mill, Germany) and subsequently with a coffee grinder (Bosch, TSM6A011W, China).

Table 2.1 The drying methods and conditions used in the study

Drying method	Pre-treatment	Drying Condition	Drying time
Sun-drying	Boiling	Open air	72 hours
Hot-air drying	Boiling	Air temperature: 60 °C Air flow rate: 2 m/s	6 hours 15 minutes
Freeze-drying 1	No pre-treat-ment	Freezing temperature -25 °C Drying temperature: 20 °C Pressure: 10 Pa	29 hours
Freeze-drying 2	Freezing	Freezing temperature: -25 °C Drying temperature: 20 °C Pressure: 10 Pa	29 hours

2.2.2. Analyses Methods

2.2.2.1. Moisture Content (%)

The oven-drying method was used to determine of moisture content of the dried tubers (Sezik & Özer, 1983). In this method, approximately 1.5 – 2.0 g samples were taken into the petri dishes, which were at a constant weight. The samples were dried at 105 °C in an oven dryer until they reached constant weighing. After that, the samples which were cooled down to room temperature in the desiccator were weighed. The analyses were conducted as double repeatitaion. The moisture contents of the samples were determined by the formula (Equation 2.1) given below.

$$\text{Moisture } \% = \frac{m_2 - m_3}{m_2 - m_1} x\ 100 \qquad (2.1)$$

m_1: Tare of the petri dish (g)

m_2: initial weight of tubers + tare (g)

m_3: final weight of tubers + tare (g)

2.2.2.2. Glucomannan Content (%)

Glucomannan content of the samples was determined according to procedure (Catalog number: K-GLUM 12/19) which was specified in Glucomannan Assay Kit. Sample and blank solutions prepared according to the Glucomannan Assay Procedure were analyzed by measuring the absorbance values (A1, A2, A3) in UV-VIS Spectrophotometer (Shimadzu UV-1800, Japan) at 340 nm. Then, the glucomannan content of the samples was determined by using Equation 2.2 (Megazyme International Ireland Limited, 2019a).

$$\text{Glucomannan (g/100 g)} = [(A3 - A1)_{\text{Sample}} \times (A3 - A1)_{\text{Blank}}] \times 36{,}8 \qquad (2.2)$$

2.2.2.3. Starch Content (%)

The Total Starch Assay Kit (Megazyme International Ireland Limited, Catalog number: AA/AMG 08/19) was used. The samples were prepared pursuant to the procedure described in the Total Starch Assay Kit and compatible with the AOAC 996.11 method. The absorbance values of the solutions were measured by using the UV-VIS Spectrophotometer (Shimadzu UV-1800, Japan) at 510 nm. The starch content of the samples was calculated by using Equation 2.3 below (Megazyme International Ireland Limited, 2019b).

$$\text{Starch (g/100 g)} = \Delta A \times F \times EV \times (D/W) \times 0.90 \qquad (2.3)$$

Where ΔA the absorbance value of the samples is read against the reagent blank while F is the factor of converting the absorbance values to μg glucose, EV is the sample extraction volume (100), D is the dilution of sample solution, W is the weight of the samples (100 mg), and 0.90 represents the factor of converting from free glucose to anhydroglucose (Megazyme International Ireland Limited, 2019b).

2.2.2.4. Ash Content (%)

The ash content of samples were analyzed using the Official Method 923.03 gravimetric method (AOAC International, 2005).

2.2.2.5. Solubility value (%)

The solubility values of the samples were determined using the method of Betancur-Ancona et al. (2003) and Sciarini et al. (2009). In this method, 1% (v/v), 30 ml salep suspensions were prepared with distilled water. The suspensions were placed into the hot water bath for 30 minutes under continuous mixing. The suspensions were centrifuged under 5000 rpm for 20 minutes. Aliquots (10 ml) of supernatant were dried in an oven at 125 °C for 4 h. The samples which were cooled down to room temperature were weighed. The solubility values of the samples were determined by using the following formula (Equation 2.4) (Betancur-Ancona et al., 2003; Sciarini et al., 2009).

$$\text{Solubility \%} = (\text{Dried material at } 125°C(g) \times 300)/\text{Initial sample weight (g)} \quad (2.4)$$

2.2.2.6. Water absorption capacity (%)

Koroskenyi and McCharthy's (2001) procedure was followed to identify the water absorption capacity. In this method, 30 ml distilled water was added to 0.1 g of dried samples. The mixture was stirred with a vortex mixer for 30 seconds and was stood for 3 h. The mixture was centrifugated at 6000 rpm for 30 min, then the supernatant was decanted, and the pellet was weighed. The weight difference between initial and final measurements was specified as the absorbed water.

2.2.2.7. Rheological Properties

The aqueous solution of 1% (v/w) of the dried salep was prepared in 30 ml distilled water by stirring at 85 °C for 30 min. Rheological analysis was carried out using a rheometer (Modular Compact Rheometer MCR 302, Anton Paar, Australia) in cone configuration (diameter:50 mm, cone angle: 1°, gap:0.101 mm). The samples were kept for 2 min to equilibrate before each test for the steady-shear test. Samples were sheared continuously at a rate ranging from 0.1 to 300 s-1 for 3 minutes at 25 °C (Kurt & Kahyaoglu, 2015). Each measurement was repeated four times.

The Ostwald-de Waele model described the flow behavior of the salep solutions based on Equation 2.5 (Kurt & Kahyaoglu, 2015):

$$\tau = K.\dot{\gamma}^{n} \quad (2.5)$$

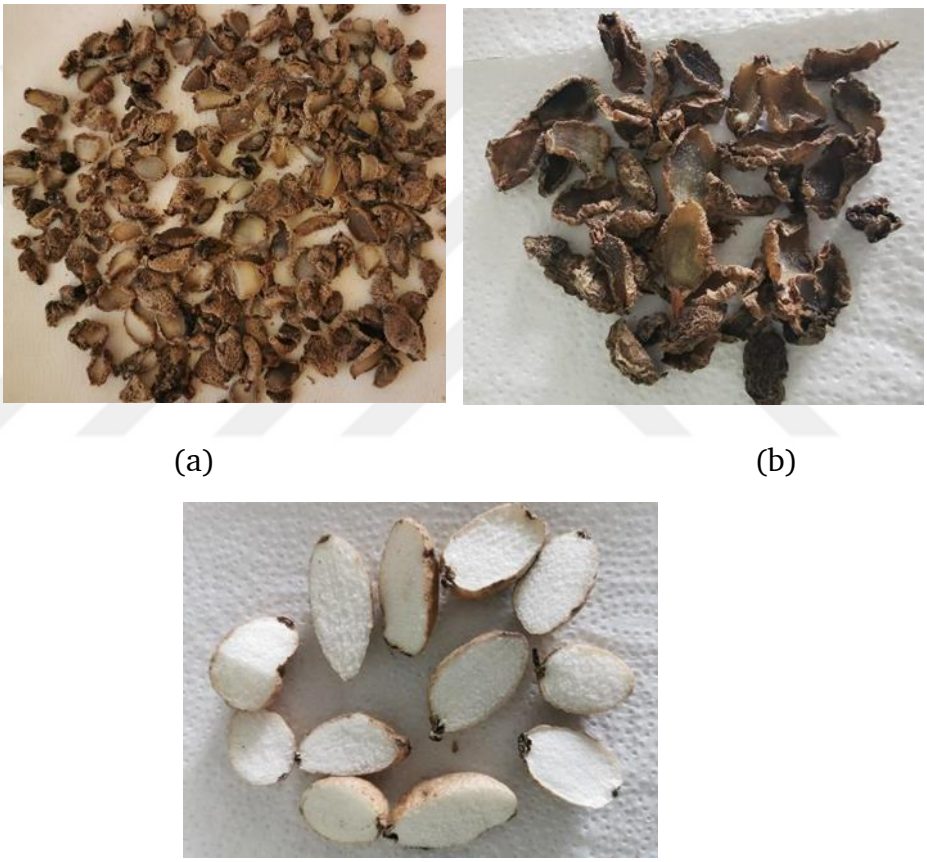
Where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency coefficient (Pa.sec), and n is the flow behavior index (dimensionless).

2.2.2.8. Statistical Analysis

Tukey's test determined analysis of variance and significant difference between means at 5% level of significance, were performed SPSS Statistical Package Program (Version 22.0, IBM, USA).

3
RESULTS AND DISCUSSIONS

The physical appearance of salep samples is highly important since it affects consumer preference. The completely dried *Serapias vomeracea* tubers using different methods are presented in Figure 3.1. It can be seen in Figure 1 that the undesired changes such as shrinkage and tarnishing of the salep samples have been observed during the sun- and hot-air drying of the samples.



(a) (b)

(c)

Figure 3.1 The dried Serapias vomeracea tubers a) Sun-dried tubers b) Hot-air dried tubers c) Freeze-dried tubers (1-2)

The salep powders obtained by grinding after the drying operations are given in Figure 3.2. It can be seen in Figure 3.2 that the original physical structure and color of the samples have been maintained utmost during the freeze-drying process. Consequently, it can be stated that the drying method affects the physical

appearance of salep powder and freeze-drying is the best method to protect their physical properties.

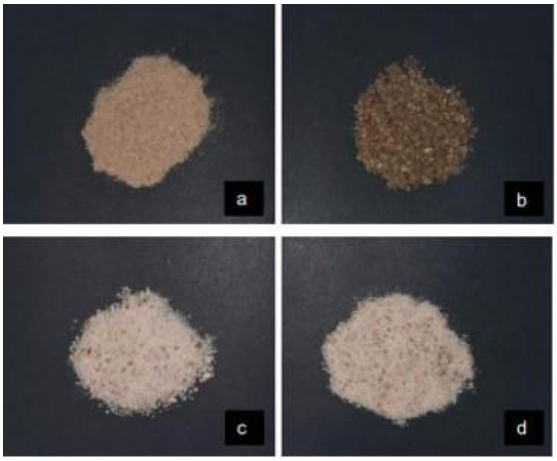


Figure 3.2 Salep powder was obtaining different drying methods a) Sun-dried b)Hot-air dried c)Freeze-dried 1 d)Freeze-dried 2.

3.1. Moisture content analyses results

In the present study, the moisture content of salep samples was obtained by different drying methods varied significantly ($p<0,05$) between 6,99% and 13,61% (Table 3.1). The effect of the drying methods on the moisture content of the salep samples is given in Figure 3.3.

Table 3.1 The final moisture content of the dried tubers

Drying Method	Moisture content (%)
Sun-drying	13,66 ± 0,075
Hot-air drying	7,48 ± 0,000
Freeze-drying-1	11,34 ± 1,005
Freeze-drying-2	6,98 ± 0,116

The moisture content is the essential factor in identifying the shelf life of the salep, like in most food ingredients. It has been stated that the moisture content of the salep samples has to be less than 10% for long-term storage (Sezik & Baykal, 1988). Sezik (1967) stated that the moisture content of the salep obtained from orchid tubers that belonged to the Muğla region ranged between 6.40% and

11.70%. Moreover, it has been detected the moisture content of the salep obtained from orchid tubers that harvested from the Kastamonu region varied from 7.77% to 13.09% (Sezik & Özer, 1983). The study, which examined the moisture content of the salep obtained from different orchid species tubers, represented that the moisture content of the salep samples varied between 6.74% and 10.85% (Şen, 2016). As is presented in Table 3.1, the results of the moisture content analyses of the salep obtained by different drying methods are compatible with the previous studies. Salep, which has been obtained with all other drying methods that have been planned to be performed instead of conventional sun-drying methods, has less moisture content. The salep with the lowest moisture content has been obtained among all the methods by the freeze drying-2 process (Table 3.1). Even FD processes (FD-1 and FD-2) have taken place under the same conditions, and it was determined to be different final moisture content in the dried materials. The freezing pre-treatment provided faster drying by forming larger and homogeneously distributed ice crystals in dried tubers (Sadıkoğlu & Özdemir, 2003). Hence, it can be stated that the most reasonable method to manufacture salep is Freeze drying with freezing pre-treatment regarding moisture content.

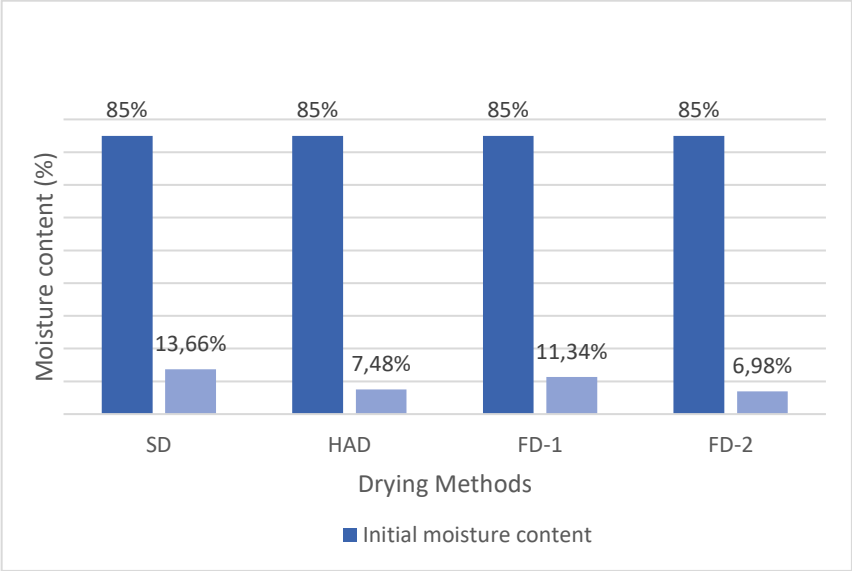


Figure 3.3 The effect of drying methods on the moisture content of the salep samples (SD:Sun-drying, HAD:Hot-air drying, FD-1:Freeze-drying, FD-2:Freeze-drying with pre-treatment)

3.2. Glucomannan assay results

The glucomannan contents of salep samples varied between 16,19% and 22,30% (Table 3.2).

Table 3.2 The glucomannan content of the salep samples

Drying methods	Glucomannan content (%)
Sun-drying	22.08
Hot-air drying	16.19
Freeze-drying-1	17.66
Freeze-drying-2	22.30

Glucomannan is the crucial component that characterizes salep's taste, aroma, and rheological properties. Moreover, glucomannan is responsible for bringing viscous features to Maras ice cream (İşler & Sezik, 2019; Kaya & Tekin, 2001). Tekinşen and Guner (2010) stated that the glucomannan content of the salep obtained from 10 different orchid species tubers varied from 17.70% to 54.60%. Şen (2016) has reported that the glucomannan content of salep ranged between 7.84% and 48.54%, and similarly, Turkmen (2019) stated that the glucomannan content of the salep varied from 7.55% to 26.39%. The study examining the salep obtained from the market indicated that the glucomannan content of salep has been equal to 18.92% (Vatansever, 2015). All previous work showed that the glucomannan content of salep could evaluate on a large scale since the glucomannan content is related to the orchid species, their harvesting period, and region (Şen, 2016).

In this work, the glucomannan content of the salep samples obtained by different drying methods varied between 16.19% and 22.30%. As seen in Table 3.2, the results of the glucomannan content analyses of the salep samples are compatible with the studies in the literature. Hot-air dried salep samples are the group with the lowest glucomannan content, with a rate of 16.19%. Therefore, it can be stated that the glucomannan content could not maintain sufficiently during the hot-air drying. Also, it has been seen that the Freeze-drying-1 process is not a successful

process as well as the sun-drying method in terms of the protection of the glucomannan content. However, the Freeze-dried-2 salep samples have closer glucomannan content (22.30%) to the sun-dried salep samples (22.08%).

Previous works showed that the most quality and the quality characteristics are closest to the original product can be obtained by freeze-drying (Sadıkoğlu & Özdemir, 2003). According to the knowledge regarding freeze-drying, it would have been expected that the high glucomannan content could have been obtained from freeze-dried salep samples. However, the literature information and experimental results are not compatible. This is because it has been thought that material loss can be caused due to insufficient separation during centrifugation, which is described in the experimental procedure (Megazyme International Ireland Limited, 2019a) of the freeze-dried samples. On the other hand, it has been presented that the Freeze-dried-2 process can be an alternative to sun-drying in this study.

3.3. Starch Assay Results

In this work, the starch content of salep samples was obtained by different drying methods varied significantly ($p<0,05$) between 10,05% and 22,08% (Table 3.3).

Table 3.3 The starch content of the salep samples

Drying methods	Starch content (%)
Sun-drying	14,96 ± 1,04
Hot-air drying	10,05 ± 0,09
Freeze-drying-1	21,20 ± 0,34
Freeze-drying-2	22,08 ± 0,34

Starch is the storage material for plants and is stored in the roots of tuberous plants (Demirci,2008). The starch enables salep to be consumed as a beverage and be used in making ice cream, thanks to its swelling feature (Öğretmen et. al., 2015). Sezik and Özer (1983) stated that the starch content of the salep samples

varied between 7,31% and 15,17%. The study reported that the starch content of the salep samples ranged from 5,44% to 38.7% (K. K. Tekinşen & Güner, 2010).

The results, given in Table 3.3, are coherent with the work stating that the salep's starch content varied from 4,58% to 43,98% (Şen, 2016). However, this finding contrast with the study on commercial salep, which reported that the starch content of salep was 41,60% (Bulut-Solak et al., 2017). According to the data, hot-air dried salep samples have the lowest starch content. Therefore, it can be stated that the least effective method for preserving the starch is the hot-air drying method. The present study observed the highest starch contents in FD-1 and FD-2 samples with 21,20% and 22,08%, respectively. Hence, it can be stated that freeze-drying processes (Freeze-drying-1, Freeze-drying-2), which preserve a relatively high level of starch compared to the sun-drying method, can be an alternative to traditional production.

3.4. Ash Content Analyses Result

In this work, the ash content of salep samples was obtained by different drying methods varied significantly ($p<0,05$) between 4,48% and 4,95% (Table 3.4).

Table 3.4 The ash content of the salep samples

Drying methods	Ash content (%)
Sun-drying	4,90 ± 0,09
Hot-air drying	4,48 ± 0,48
Freeze-drying-1	4,48 ± 0,48
Freeze-drying-2	4,95 ± 0,00

Ash content of the plant foods describes the mineral content of the material. The ash consists largely of potassium phosphate, calcium phosphate, potassium chloride, and calcium chloride. The quality and nutritious attributes of the food materials are described by their ash content. The ash content determination is a substantial factor in determining the level of the adulteration of salep and its quality (Sezik, 1967; Marshall, 2010; Öğretmen et.al, 2015).

Sezik (1967) stated that the ash content of the salep samples varied between 0,24% and 5,95%. And also, Şen (2016) showed that the ash content of the salep samples, which were obtained from different orchid species harvested from various locations in Turkey, ranged from 1,46% to 6,72%. According to the study which examined the commercial salep sample, its ash content was 2,07% (Kurt & Kahyaoglu, 2015). The results, shown in Table 3.4, are acceptable when compared with the results of previous studies. In the study, less ash content is seen from Freeze-dried-1 and hot-air dried salep samples. The salep samples produced with sun-drying and freeze-drying-1 have relatively high ash content. Nevertheless, the findings revealed that the drying method has no remarkable effect on the ash content of salep. This can be explained by the ash content of salep is depend on the soil's chemical composition where the orchid was collected and the precipitation regime of the region where the orchid grows (Şen et al., 2018).

3.5.Solubility value

The solubility values of salep samples are represented in Table 3.5. The results varied significantly ($p<0,05$) varied between 55,00% and 68,87%.

Table 3.5 The solubility value of the salep samples

Drying methods	Solubility value (%)
Sun-drying	55,00 ± 5,00
Hot-air drying	68,87 ± 1,13
Freeze-drying-1	65,00 ± 5,00
Freeze-drying-2	55,00 ± 5,00

There is limited study regarding the solubility value of salep in the literature. Şen et al. (2018) reported that the solubility value of the salep samples obtained from different orchid species harvested from various locations in Turkey is around 33,00%. The experimental results from Table 3.5 show that the solubility values of sun-dried and freeze-dried-2 salep samples were 55,00%. Moreover, the solubility values of freeze-dried-1 and hot-air dried salep samples were 65,00%

and 68,87%, respectively. In accordance with the results, it can be declared that the drying method has significant effects on the solubility value of salep. This study shows that the solubility value of salep, which has crucial effect preparation of salep beverages, can be improved with freeze-drying and hot-air drying methods.

3.6. Water absorption capacity

Table 3.6 represents the water absorption capacity of the salep samples obtained by different drying methods. The results varied between 666,00% and 1400,00%, no significant differences were detected ($p>0,05$) among groups.

Table 3.6 The water absorption capacity of the salep samples

Drying methods	Water absorption capacity (%)
Sun-drying	666,00 ± 94,00
Hot-air drying	715,00 ± 45,00
Freeze-drying-1	1350,00 ± 10,00
Freeze-drying-2	1400,00 ± 150,00

The highest water absorption capacity was observed from the Freeze-dried salep samples. The water absorption capacity of the samples dried in a freeze dryer is about 2 times higher than that of samples dried in the conventional method. This can be explained by freeze-dried products preserving the most quality glucomannan which is a substance that can hold a high level of water. Moreover, it showed that the drying process has a remarkable effect on water absorption capacity values.

3.7. Rheological Properties

The consistency coefficients and n values of salep solutions prepared salep samples obtained by different drying methods are determined in this work. Table 3.7 shows the consistency coefficients, n values, and experimental viscosity values of salep samples. To describe the variation in the flow attributes of all salep solutions

under continuous shear, the Ostwald-de Waele model (Equation 2.5) adequately describes the experimental data for the flow curves; R^2 values (Table 3.7) also prove that. The flow curves for the solutions of salep obtained by different drying methods are given in Figure 3.4. The viscosity values (η) of the solutions against shear rate ($\dot{\gamma}$) are given in Figure 3.5.

Table 3.7 The viscosity values and Ostwald-de Waele model parameters of salep solutions

Drying method	Viscosity,Pa.s (η_{60})	Ostwald-de Waele model parameters		R^2
		K(Pa.s^n)	n	
SD*	0,0612	0,265 ± 0,006	0,646 ± 0,004	0,999
HAD*	0,0227	0,058 ± 0,001	0,776 ± 0,005	1,000
FD-1*	0,3024	8,327 ± 0,242	0,195 ± 0,006	0,988
FD-2*	0,2366	3,500 ± 0,099	0,343 ± 0,050	0,997

K:Consistency coefficient (Pa.s^n), n:Flow behaviour index, R^2:Regression coefficient

*SD:Sun-drying, HAD:Hot-air drying, FD-1:Freeze-drying-1, FD-2:Freeze-drying-2

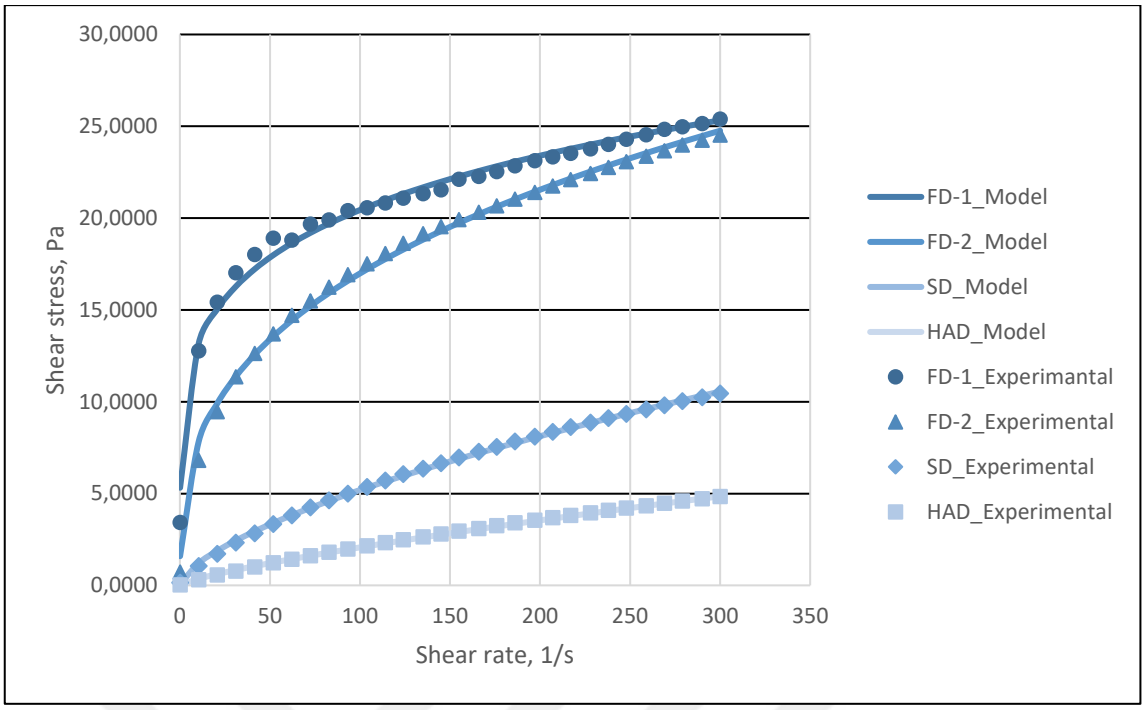


Figure 3.4 Effects of drying methods on shear stress of salep solutions. Symbols represent the experimental data, and the solid lines represent the Ostwald-de Waele model. (SD: Sun-drying, HAD: Hot-air drying, FD-1: Freeze-drying, FD-2: Freeze-drying with pre-treatment).

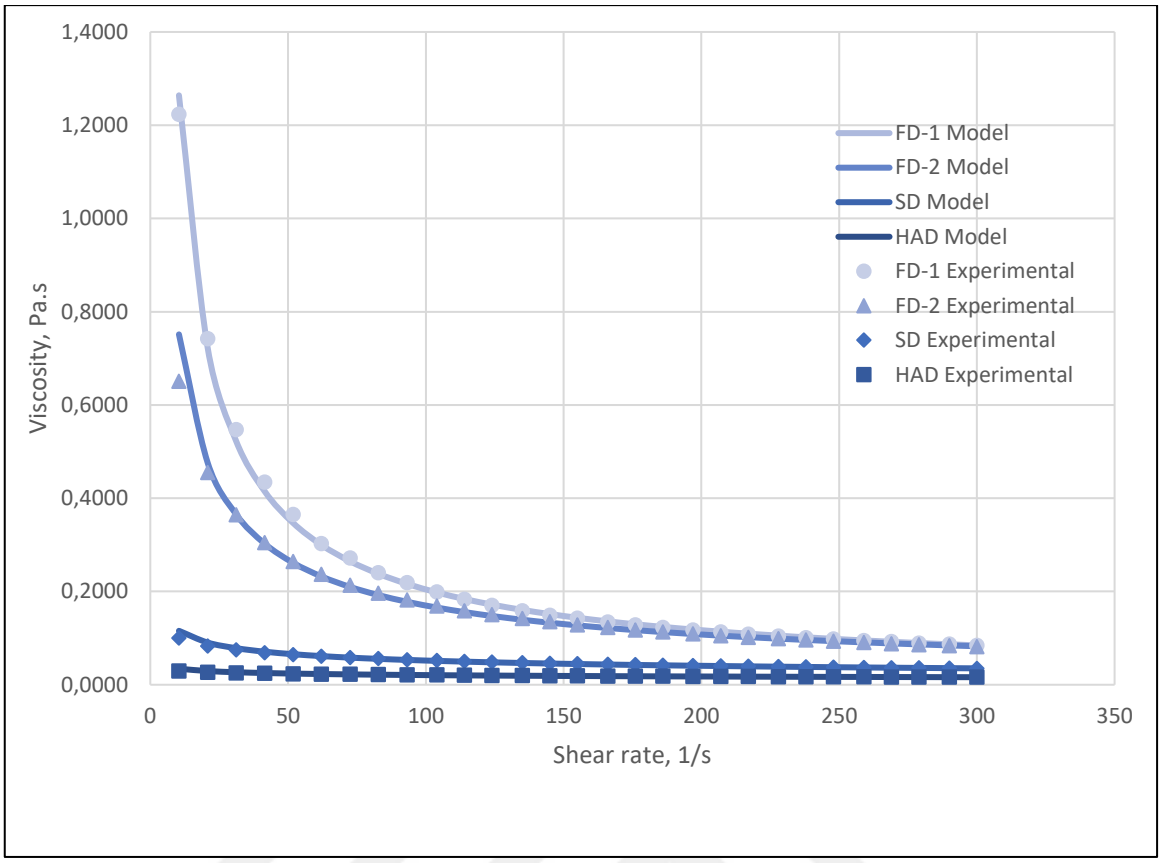


Figure 3.5 Effects of drying methods on viscosity of salep solutions. Symbols represent the experimental data, and the solid lines represent the Ostwald-de Waele model. (SD: Sun-drying, HAD: Hot-air drying, FD-1: Freeze-drying, FD-2: Freeze-drying with pre-treatment).

The viscosity of the salep beverage prepared by boiling the salep powders in water or milk affects consumer preference. Like other gum solutions, salep solution also shows pseudoplastic behavior. Szczesniak and Farkas (1962) stated that the gum solutions with high n value leave a sticky feeling in the human mouth. For this reason, it has been declared that both high viscosity and low n value are highly affected by consumer preference (Marcotte et al., 2001). In the Non-Newtonian fluid, the viscosity value of a single point is used to measure the sensation created in the human mouth. 60 s^{-1} shear rate is used for this purpose since the viscosity value is related to human perception (Steffe, 1966); the viscosity value at the 60 s^{-1} shear rate was evaluated in this study.

Tekinşen and Güner (2010) stated that the viscosity values of the samples varied between 6.40 cp and 148.00 cp. A similar study showed that the viscosity values of the samples were between 4.25 cp and 16.14 cp (Şen, 2016). According to the

results (Table 3.7), the viscosity values of the samples varied from 222.70 cp to 302.40 cp in this study. When the viscosity of the sun-dried salep samples was considered as a reference, hot-air dried samples had the lowest viscosity values. It can result from the hot-air dried samples' lower glucomannan and starch content. The viscosity values of Freeze-dried-1 and Freeze-dried -2 samples were five and four times higher than the viscosity values of sun-dried samples, respectively. Even if Freeze-dried -1 samples contained less glucomannan than sun-dried samples, they constituted more viscous salep solutions. This can be explained by the high-quality freeze-drying process of preserved glucomannan and starch.

Farhoosh and Riazi (2007) showed that K values of the samples varied between 7.251 Pa.s and 68.542 Pa.s, and n-values of the samples varied from 0.381 to 0.443. They also stated that the K and n-values depend on the temperature and concentration of the solution. Moreover, it was found that the flow behavior of the solutions in the study showed Pseudoplastic behavior (Farhoosh & Riazi, 2007). Kurt (2015) stated that the K and n-values of the solutions which showed Pseudoplastic flow behavior was found to be 0.332 Pa.s and 0.696, respectively. Additionally, Türkmen (2019) declared that the K and n-values of the solutions varied between 0.01 Pa.s and 269.6 Pa.s, 0.03 and 1.03, respectively.

According to Table 3.7, K values of the samples varied from 0.058 Pa.s to 8.327 Pa.s, and n-values ranged between 0.195 and 0.646. The K values have increased substantially in the freeze-dried samples regarding sun-dried samples. The lowest n-value has been obtained from the freeze-dried samples in parallel with the results. The FD-1 sample has the highest K value and the lowest n value, which means that the sample has the highest quality.

3.8. Conclusion

Salep powders were obtained by applying different drying methods (sun drying, hot air drying, freeze-drying) to the *Serapias vomeracea* plant, one of the raw materials of salep and one of the orchid species. The physical and chemical properties of salep powders were examined and compared with the analyses.

The experimental data obtained from the study showed that the physical and chemical properties of salep powders obtained by the freeze-drying method are

better preserved than salep powders obtained by other drying methods. Compared to other drying methods, the freeze-drying process, in which the highest quality salep powder is obtained, compensated for the high-cost disadvantage it brought. In this direction, a new production process has been developed for salep, which has a high added value for Turkey both in biotechnology and in the field of food, together with the production of 'quality salep' using the freeze-drying method. With this production process, since better quality products will be obtained with less salep consumption, the orchid slaughter in nature will be prevented.

In the future studies to be carried out based on this project, experiments with different temperature values and drying times for orchid tubers in the freeze-drying process can be performed, and optimum conditions for this method can be determined with the support of computer programs. Therefore, the high quality of salep production can be evaluated commercially. In addition, in future studies, the usability of salep powder obtained by the freeze-drying method in the production of Maraş ice cream can be examined by supporting it with sensory tests.

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A

GLUCOMANNAN ASSAY PROCEDURE

1. 0.1 g of ground sample was taken into a test tube, and 5 ml of ethanol solution (80%, v/v) was added to it and homogenized using a vortex mixer (Witeg, Wisemix VM-10, Germany).

2. The tube was incubated in a water bath at 85 °C for 5 minutes, and at the end of the period, 5 ml of ethanol solution (80%, v/v) was added and mixed with a vortex mixer.

3. The prepared tube was centrifuged at 5000 rpm for 10 minutes (Hettich, Universal 320R, Germany).

4. The upper phase (supernatant) was discarded, and the pellet was resuspended with 5 ml of ethanol solution (80%, v/v) and homogenized by a vortex mixer.

5. The tube, which was added to 5 ml of ethanol solution (80%, v/v) and mixed in a vortex mixer, was centrifuged again for 10 minutes at 5000 rpm.

6. The supernatant was discarded, and the pellet was resuspended in 8 ml of Solution-1 (Preparation of Solution-1: The content of Bottle-1 in the kit was completed to 450 ml with distilled water, then the pH was adjusted to 4.5 using 1 M HCl) and then mixed on a vortex mixer to ensure complete dispersion.

7. The prepared tube was placed immediately in a boiling water bath and incubated for 30 seconds, then homogenized with a vortex mixer.

8. In this step, the heating and mixing steps performed in Step 7 were applied to the prepared tube again.

9. The prepared tube was incubated in a boiling water bath for 4 minutes to ensure glucomannan hydration.

10. The incubated tube was mixed vigorously on a vortex mixer and then placed in a water bath at 40 °C.

11. After 5 minutes, 20 μl of β-mannanase (Bottle-4) was added to the tube and mixed vigorously in a vortex mixer for 30 seconds.

12. While the prepared tube was incubated in a water bath at 40 °C for 60 minutes, it was mixed vigorously in a vortex mixer intermittently (approximately three times).

13. The contents of the prepared tube were taken into the beaker by washing with distilled water, and the volume was completed to 70 ml.

14. The pH of the solution in the beaker was adjusted to approximately 12.5 using 1 M NaOH and left at room temperature for 10 minutes for complete deacetylation of glucomano-oligosaccharides.

15. 20 ml of 200 Mm sodium phosphate buffer solution (pH 6.5) was added to the beaker, and the pH of the solution was adjusted to 6.5 using 1 M HCl.

16. The solution was taken into a 250 ml volumetric bottle, and the volume was completed with distilled water.

17. 0.5 ml of the prepared sample solution was taken into spectrophotometer cuvettes. 0.05 ml of distilled water (~35 °C) was added to one of these cuvettes to perform a blank reading, and 0.02 ml of Bottle-5 (β-glucosidase + β-mannosidase) solution which is included in the kit was added to the other cuvette.

18. The prepared blank and sample cuvettes were gently shaken and incubated for 20 minutes in a 35 °C water bath with their mouths closed.

19. After that, 2.00 ml of distilled water (~35 °C), 0.20 ml of Bottle-2 (Buffer; 25 ml, pH:7.6 + Magnesium chloride and sodium azide, 0.02% w/w), and 0.10 ml of Solution-3 (Preparation of solution-3: Dissolving the contents of Bottle-3 in the kit in 12 ml of distilled water) was added to each cuvette.

20. The blank and sample cuvettes were incubated at 30 °C for 3 minutes after shaking gently. Then, absorbance values were read at 340 nm wavelength in a spectrophotometer (Shimadzu UV-1800, Japan) (A1).

21. 0.02 ml of Bottle-6 (HK/GP6-DH) solution was added to the blank and sample cuvettes and gently shaken. After the solutions were held for about 12 minutes,

new absorbance values at 340 nm wavelength were read in the spectrophotometer (A2).

22. After reading, 0.02 ml of Bottle-7 (PGI/PMI) was added to the cuvettes and shaken gently. The new absorbance values of the cuvettes, which were kept for 20 minutes, were read in the spectrophotometer (A3).

23. Absorbance values were checked by reading at 2-minute intervals until they reached a constant value. Within the scope of the study, the absorbance values of the sample and blank solutions were fixed after 26 minutes.

24. The amount of glucomannan contained in the sample (g/100 g) was calculated using Equation 2.2.

B

STARCH ASSAY PROCEDURE

1. 0.1 g of the ground sample was taken into a test tube and 0.2 ml of ethanol solution (80%, v/v) was added on it and homogenized using vortex mixer (Witeg, Wisemix VM-10, Germany).

2. 3 ml of thermostable α-amylase solution (Thermostable α-amylase solution: The solution prepared by 30-fold dilution of the contents of Bottle-1 which included 50 mM, pH: 7.0 MOPS solution) prepared as specified in the analysis kit, was added to the prepared tube and it was incubated for 6 minutes in a boiling water bath. At the 2^{nd}, 4^{th}, and 6^{th} minutes of the incubation, the tube was mixed vigorously using a vortex mixer.

3. The tube was placed in a water bath at 50 °C, and 4 ml of sodium acetate buffer (200 mM, pH: 4.5) and 0.1 ml of amyloglycosidase solution (Bottle-2) were added.

4. The tube was incubated in a water bath at 50 °C for 30 minutes.

5. Then, the contents of the tube were transferred to a 100 ml volumetric bottle by washing with distilled water and the volume was adjusted using sodium acetate buffer (200 mM, pH: 4.5).

6. 2.0 ml of the prepared solution was taken and centrifuged at 13,000 rpm for 5 minutes.

7. After centrifugation, 0.1 ml of solution from the supernatant was taken into the test tube. 3.0 ml of GOPOD Reagent was added to it.

GOPOD Reagent: The GOPOD Reagent Buffer (Bottle-3) included in the kit is diluted to 1 liter with distilled water. GOPOD Reactive Enzymes (Bottle-4) were dissolved in 20 ml of solution-3 and prepared by quantitatively transferring the solution-3 into the bottle.

8. Glucose Control solution was prepared by adding 3.0 ml GOPOD Reagent to 0.1 ml glucose standard solution (Bottle-5). A Blind Reagent solution was

prepared by adding 3.0 ml of GOPOD Reagent to 0.1 ml of sodium acetate buffer (100 mM, pH: 5.0).

9. The absorbance values of the prepared sample and glucose control solutions were read against the blank reagent solution at 510 nm wavelength with a spectrophotometer (Shimadzu UV-1800, Japan).

10. The starch amount of the samples (g/100 g) was calculated with the help of Equation 2.3.

C

ASH ASSAY PROCEDURE

AOAC Official Method 923.03

1. The powdered samples were weighed as approximately 1.0 g and put into constant weight crucibles.

2. The crucibles were placed in the ash oven (WiseThern, Daihan, Germany).

3. The samples were burned in the ash oven at 550 °C until they reached constant weight.

4. After the incineration process, the samples taken to the desiccator were weighed after they reached room temperature, and the ash content of the sample was calculated.

PUBLICATIONS FROM THE THESIS

Conference Papers

1. Bilgiç B., Baydar Atak G. , Sadıkoğlu H. Investigation of the impact of different drying methods on the physical properties and quality of Salep. 4th International Eurasian Conference on Biological and Chemical Sciences. Ankara, Turkey, 24 - 26 November 2021, ss.1156.
2. Bilgiç B., Baydar Atak G. , Sadıkoğlu H. Effects of different drying methods on the quality of dried *Serapias vomeracea*. International Eurasian Conference on Biotechnology and Biochemistry (BioTechBioChem 2020), Ankara, Turkey, 16 - 18 December 2020, ss.17.
3. Bilgiç B., Baydar Atak G. , Sadıkoğlu H. Theoretical Investigation of Freeze-Drying. 5th International Conference on Advances In Mechanical Engineering ,İstanbul, Turkey, 17 - 19 December 2019, ss.1362-1363.

Projects

1. B. Bilgic (Scholarship Student), H.Sadikoglu (Executive) *Serapias vomeracae*'nın Dondurarak Kurutulmasıyla Salep Üretimi: Ana Bileşen Olan Glukomannan İçeriği ve Fonksiyonunun (Nem Tutma) Artırılması Ve Sürecin Matematiksel Modellenmesi. (2019-2021), **TÜBİTAK**: 119O893.