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FREEZE DRYING HONEY: IMPACT OF CARRIER AGENTS ON PHYSICOCHEMICAL CHARACTERISTICS

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Abstract

This study examines the impact of various encapsulating materials on the phycial and chemical characteristics of honey powder produced via freeze drying under vacuum. The freeze drying technique was employed under controlled condition, (Different time and temperature) and the resulting powders were evaluated for key parameters including.. Raw honey, characterized by 82% Brix, 3.3% acidity, 15 mg/kg HMF,(*2 hydroxymethyl 2-furfuraldehide*) 34.8 DN diastase activity, 71% total sugars, and 67% reducing sugars, was combined with carriers at varying ratios and freeze-dried. Three formulations were evaluated: (H 50% with GA 50%, H 60% with MD 40%, and H 70% with DX 30%). The Gum Arabic sample exhibited a moisture content of 1.3%, total sugars of 53.8%, reducing sugars of 45.6%, HMF of 24.55 mg/kg, and diastase activity of (14.3 DN), indicating moderate preservation of enzymatic activity with minimal sugar degradation. The Maltodextrin formulation resulted in a lower moisture content (0.63%), higher total (66%) and reducing sugars (65.9%), but also an increased HMF (51.20 mg/kg) and reduced diastase activity (11.6 DN). In contrast, the Dextrin-based sample had a moisture content of 0.88%, total sugars of 61.4%, reducing sugars of 61%, yet showed a marked increase in HMF (148.5 mg/kg) alongside a significant decline in diastase activity (3 DN), suggesting pronounced degradation. These results underscore the critical role of carrier selection in optimizing the condition of sublimation-dried honey, with Gum Arabic providing the most balanced preservation of key quality attributes.

Keywords: Freeze drying, honey, carrier agents, Arabic gum, maltodextrin, dextrin.

1. Introduction

Honey is a natural substance produced by honeybees (*Apis mellifera*; Family: *Apidae*) from the nectar of flowers *(Saeed et al., 2017).* The bees collect nectar, plant exudates, and secretions from plant-sucking insects, which they then transform into honey *(Marta et al.,2023).* Nutritionally, honey represents a rich supply of both macro- and micronutrients, primarily composed of a saturated sugar solution, in which fructose and glucose constitute the major sugars. Additionally, honey contains a variety of minor components, notably phenolic compounds, which contribute to its unique properties *(Alvarez et al., 2014).* Physically, honey is characterized by its viscous and gelatinous texture, though it does not have a specific color *(Shahid et al., 2017).* The composition of honey is influenced by various factors, including its botanical and geographical origins, as well as the conditions under which it is processed and stored *(Siluana et al., 2021).* Honey is versatile and can be used in various food products such as biscuits, lassi, laddu, ice cream, muffins, and bread. Its inclusion in food items not only enhances flavor but also improves nutritional value (*Parth et al., 2023*). Several honey-derived products, including honey powder, granulated honey, and honey flakes, have emerged as effective alternatives to liquid honey in various applications (*Rastogi et al., 2008).* Despite its advantages, honey's high moisture content and propensity to crystallize make it difficult to store for extended periods of time or use in industrial settings. Honey is frequently put through drying procedures to solve these problems and increase its stability.

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Spray drying is the most efficient way to turn liquid honey into powder among the different drying methods (*Samborska et al., 2019).*
These methods, however, frequently subject honey to high temperatures, which can cause changes in its physicochemical characteristics, the loss of volatile components, and the degradation of heat-sensitive compounds. To mitigate these issues, freeze-drying has emerged as an alternative technique that effectively preserves the physicochemical properties of honey. One of the best ways to remove water from biological materials is to freeze-dry them under vacuum, producing the highest-quality end products. With minimal volume reduction, the solid state of water during the freeze-drying process preserves the products' primary structure and shape *(Sagar et al., 2020).* This method involves freezing the honey at low temperatures and subsequently removing the ice through sublimation under vacuum conditions, minimizing thermal damage while maintaining the product's structural integrity and functional attributes. Despite the advantages of vacuum freeze-drying, the process requires the addition of carrier agents to optimize the drying process and prevent stickiness caused by the rich sugar content of honey. Carrier agents help to reduce common problems during drying, like stickiness and drying challenges. The use of large amounts of drying carriers increases the production costs and can alter the original flavor of raw honeys and the resulting powders, with the risk of consumer disapproval *(Leire et al., 2023).* Carrier agents such as gum Arabic, Maltodextrin, and Dextrin are commonly used to facilitate drying and enhance the stability of the final product. These agents play crucial role in influencing the physico-chemical features of freeze dried honey, which includes moisture content, humidity level and structural properties. Nevertheless, the powders made with Arabic gum had worse physical characteristics, including greater cohesion and hygroscopicity as well as a longer wetting time (*Samborska et al., 2015*). Maltodextrin is a well-known carrier that improves the stability and flowability of honey powder due to its low viscosity and good solubility (*Sobulska et al., 2020).* Dextrin has been used to reduce stickiness and caking in honey powders, thereby enhancing storage stability *(Sahu et al., 2008*). While specific studies on dextrin's impact on freeze-dried honey are limited, its general properties suggest it aids in moisture control and improves powder handling characteristics. Despite its advantages, freeze-drying honey presents certain challenges:
Stickiness and Hygroscopicity: Due to its high sugar content, honey remains sticky even after freeze-drying, complicating handling and storage *(David et al., 2023)*
Extended Drying Time: Freeze-drying is a time-consuming process, often requiring 24 hours or more to complete, which can be inefficient for large-scale production.
High Energy Consumption: The process demands significant energy input to maintain low temperatures and vacuum conditions, leading to increased operational costs *(Aneta et al., 2022).*
Equipment Costs: The specialized equipment required for freeze-drying involves substantial initial investment and maintenance expenses.

2. Material and Methods

Sunflower honey; the color light golden yellow to amber, with a hint of sunflower seeds, sweet with a slightly tangy aftertaste, a rapid crystallization. It typically crystallizes into a fine or medium grain, resulting in a creamy texture. The total solid content was 82.0±0.1% d.b, with a water content 17.6±0.1% w/w. Maltodextrin, gum Arabic and Dextrin were used. The experimental design plan for freeze-drying honey with these carrier agents, is presented in Table 1.

Table.1 Experimental plan

Sample	Honey %	Gum Arabic	Maltodextrin	Dextrin
1	50%	50%		
2	60		40	
3	70			30

*Freeze Drying Honey under Vacuum (VFD***):** Honey was dried using Lab Freeze Drying under Vacuum system manufactured by Labconco-USA. Collector Temperature: -50°C, Ice Holding Capacity: 2.5l, Style: Benchtop, equipped with a vacuum pump capable of at least 98 L/min, and 0.02 mBar pressure. During drying, the temperature (-50°C) and pressure (0.100 mbar) are under control.

Total soluble solids (TSS): The TSS shows how much sugar is in honey and is in important sign of possible adulteration *(Kamal et al., 2019).* Total soluble solids were measured as total soluble sugars. Their amount, shown in °Brix (1°Brix means 1% sugar at 20°C) was read from a table linking the refractive index at 20 °C with °Brix *(Rusu et al., 2021).* A hand-held honey refractometer (50° Brix to 90° Brix) was standardized and calibrated with deionized water before taking measurements (brand: ATC; accuracy: 0.1% water).

Moisture content: The moisture level of honey is a key factor affecting its shelf stability (Obiegbuna et al., 2017). A high moisture content of honey is also a sign of possible adulteration *(Abioye et al., 2015).* Moisture significantly affects several physical properties of honey, including crystallization, viscosity and rheological behavior. Other important factors include appearance, color, taste, solubility, conservation, and even the commercial value of honey *(Milica et al., 2019).* The powder samples (approximately 2 g each) were analysed, with Moisture analyser DBS 60-3, Weighing capacity [Max]60g, Readability [d]0,001g, Temperature [Min]50°C, [Max]200°C, Stabilization time 3s.

Hydroxylmethylfurfural (HMF): is a compound that forms during the heating and storage of honey, serves as an indicator of honey quality *(Shapla et al., 2018).* Accurately determining HMF amount is essential, especially when evaluating processing methods like freeze-drying. The HMF amount was determined using (HPLC) High-Performance Liquid Chromatography method. This method involves solid-phase extraction cleanup followed by liquid chromatography with UV absorbance detection. It has been validated for various honey types, demonstrating suitability for accurate HMF quantification.

Honey samples were diluted to 5 g in 50 mL of distilled water, filtered through a 0.45 μm filter, and immediately injected into an HPLC system (Waters 1525 Binary HPLC Pump) with a Diode Array Detector (Waters 2487 Dual Absorbance Detector). The HPLC column used was a Merck Lichrospher, RP18, 5 μm, 125×4 mm, with a guard cartridge packed with the same stationary phase (Merck). The HPLC conditions were as follows: an isocratic mobile phase consisting of 90% water with 1% acetic acid and 10% methanol; a flow rate of 0.7 mL/min; and an injection volume of 20 μL. All solvents used were HPLC grade (Merck). The wavelength range was 220-660 nm, with chromatograms monitored at 285 nm. HMF was identified by comparing the chromatographic peak in honey samples with that of an HMF standard (Alfa Aser LOT 10183841) and by analyzing the absorbance spectrum of the standard in comparison with honey samples. The HMF content was determined using an external calibration curve by measuring the signal at λ = 285 nm *(Nebojsa et al., 2020).*

Diastase activity: One of the main enzymes in honey is diastese. Diastase activity and HMF content are useful metrics for evaluating product quality *(Ioannis N et al., 2017).* Using the standard AOAC method, the diastase activity was measured spectrophotometrically. The hydrolysis of the starch and iodine test served as the main foundation for this technique. Certain amounts of honey were added to the starch solution, and because honey contains the enzyme

diastase, the starch was gradually hydrolyzed. If there is still a lot of residual starch after adding the iodine solution, the entire mixture should turn deep blue; if the starch is completely broken down, the solution becomes clear, and if it is partially broken down, it turns brown-red. A spectrophotometer set to 660 nm would measure the absorbance of the entire mixture at various reaction times, and the specified absorbance determined time was required to compute DN. According to *(Huang et al., 2019),* one unit is the quantity of enzyme that, under test conditions, will convert 0.01 g of starch to the specified end-point in one hour at 40 °C.

Sugars in Honey: The determination of sugars in honey by HPLC was performed according to the method described by the authors previously. Briefly, standard solutions of fructose (2 g%), glucose (2 g%), and sucrose (0.5 g%) were prepared in distilled water. The working sugar mixture was prepared by transferring 1 ml of each standard solution into a 10 ml volumetric flask, and then the final volume was adjusted with distilled water. Sample preparation involved dissolving 2.5 g of the honey sample in 25 ml of deionized water in a beaker. The resulting solution was filtered through a 0.045 µm nylon filter into a 50 ml volumetric flask. The appropriate volume of the solution was then transferred to the HPLC vial. HPLC analysis was performed using liquid chromatography coupled with a refractive index detector (LC-RID), and the data were processed using OpenLab Software. Separation was carried out with a ZORBEX 150 × 4.6 mm carbohydrate column. Chromatographic analysis was performed with a 20:80 v/v mixture of distilled water and acetonitrile as the mobile phase. A 10 µl sample was injected at a flow rate of 1.5 ml/min. The column temperature was maintained at 27°C throughout *(Budour et al., 2020).*

Statistical Analysis: All measurements were carried out in triplicate and expressed as mean ± standard deviation (SD). We analyzed the results using ANOVA, by Tukey's test to see where the actual differences accurred among the samples ($p < 0.05$ was considered statistically significant).

The influence of:

Carrier agent (gum Arabic, maltodextrin, dextrin),
Drying time (28 h, 48 h, 72 h), and
Drying temperature (constant at −48 °C) was considered in evaluating the effects on each measured parameter.

While the temperature was constant across all samples, changes in drying time had a significant impact of on some sensitive components, including hydroxymethylfurfural (HMF) and diastase activity, indicating that vacuum exposure time is a critical factor in honey degradation and enzyme stability.

3. Results and discussion

The physico-chemical characteristics of freeze-dried honey powders with different carrier agents (Gum Arabic, Maltodextrin, and Dextrin) showed significant differences among the three samples in terms of total soluble solids (TSS), moisture content, hydroxymethylfurfural (HMF), diastase activity, and sugar composition.

The maltodextrin group had the highest TSS values (S2: 66.2 °Brix), followed by the dextrin group (S3: 61.9 °Brix) and the gum Arabic group (S1: 54.0 °Brix). Significant differences between the groups were revealed by ANOVA ($F > 150$, $p < 0.001$). Due to its lower solubility and absence of inherent sugar content, the gum Arabic sample had a lower TSS. Although gum arabic has good emulsifying properties, its limited sweetness and partial dissolution in water lead to a lower-than-expected Brix value *(Saha et al., 2010).* Because it is more soluble than gum arabic, maltodextrin, a partially hydrolyzed starch with a moderate dextrose equivalency

(DE), is commonly used in honey drying to improve powder flowability *(Cristhiane et al., 2012).*

Since dextrin is a high-molecular-weight carbohydrate, its contribution to dissolved solids in solution may be limited due to its limited solubility and lower dextrose equivalency (*Renata et al., 2008).* Nevertheless, despite the binding effect of dextrin that might have impeded solubility, the higher honey content (70%) raised the sugar concentration and helped maintain a relatively high Brix value (*Shrestha et al., 2007).*

The moisture content of freeze-dried honey formulations plays a crucial role in determining the stability, shelf-life, and physical characteristics of the final product. Honey is highly hygroscopic, and its moisture retention depends on the type and proportion of carrier agents used during freeze-drying. The results obtained for moisture content in the tested samples indicate significant differences due to variations in composition and drying time *(Nebojsa et al., 2020).* The moisture content of the freeze-dried honey powders varied significantly depending on the type of carrier agent used. Statistical analysis (ANOVA, $p < 0.01$) confirmed significant differences between the formulations. The maltodextrin sample (S2) exhibited the lowest moisture content (mean: 0.63%), followed by the dextrin sample (S3, 0.87%) and gum Arabic (S1, 1.27%). Gum Arabic's strong film-forming ability, which helps stabilize powder morphology despite higher residual water, is probably why the higher moisture in S1 did not affect the powder's crystalline structure. These results are consistent with earlier research that suggests gum Arabic improves powder stability while maltodextrin reduces stickiness due to its low viscosity *(Samborska et al., 2019; Sobulska et al., 2020).*

Hydroxy methyl furfural (HMF): The following conclusions about the effects of carrier agents and processing time on the formation of hydroxymethylfurfural (HMF) can be drawn from the HPLC measurements of HMF in honey taken both before and after freeze-drying. Prior to processing, fresh honey had a low HMF content of 15 mg/kg, indicating good quality. All samples showed a significant increase in HMF levels following freeze-drying, though the degree of this increase varied based on the samples' composition *(Fallico et al., 2004).*

As drying time increased, the HMF content increased sharply, reaching 24.1 mg/kg (S1), 50.5 mg/kg (S2), and 147.3 mg/kg (S3). The dextrin-based sample degraded the most, most likely as a result of its limited protective effect and longer exposure (72 hours). Extremely significant differences were revealed by ANOVA ($F > 200$, $p < 0.001$). This pattern confirms earlier research showing that the composition of carbohydrates and temperature have a significant impact on HMF formation *(Shapla et al., 2018).*

Diastase activity (DN): The results of the diastase activity (DN) measurements show a significant decrease in enzymatic activity after the freeze-drying process, in comparison to fresh honey (34.8 DN). This decrease is caused by heat stress and dehydration during processing (*Schade et al., 2006*). The gum Arabic formulation (S1: 14.0 DN) maintained the most enzymatic activity, followed by dextrin (S3: 3.0 DN) and maltodextrin (S2: 11.2 DN). There was a highly significant statistical difference ($F > 300$, $p < 0.001$). These findings suggest that gum Arabic might function as a more effective matrix for protecting enzymes.

It is clear that diastase degradation increases with the length of the freeze-drying process. The dextrin sample has the lowest activity, which could be explained by the 72-hour processing time.

Sugars in Honey: The results show that the amount of reducing and total sugars in honey samples before and after the freeze-drying process differed significantly. Both total and reducing sugars are noticeably lower in the freeze-dried samples than in the liquid honey (71% total sugars and 67% reducing sugars). This shift might result from nutrients being absorbed or lost during the freeze-drying process, which involves solidifying and eliminating water content. The maltodextrin sample had the highest total sugar content (S2: 65.1%), followed by gum Arabic (S1: 53.3%) and dextrin (S3: 61.2%). The trend for reducing sugars was similar, with

S2 having the highest values (65.4%) and S1 having the lowest (45.3%). For both parameters, statistical analysis revealed significant differences ($F \approx 25–40$, $p < 0.01$). Maltodextrin's stabilizing effect, which prevents sugar degradation by creating protective hydrogen bonds, is responsible for S2's superior sugar retention. Stronger interactions with gum Arabic during drying may be the cause of the sugar loss in S1, as also noted by (*Tonon et al. 2008*).

Table 2 Summary of Statistical Analysis

Parameter	F-value (approx.)	p-value (approx.)	Conclusion
Moisture (%)	18.24	<0.01	Significant
TSS (°Brix)	150+	<0.001	Highly significant
HMF (mg/kg)	200+	<0.001	Highly significant
Diastase Activity (DN)	300+	<0.001	Highly significant
Total Sugars (%)	25–30	<0.01	Significant
Reducing Sugars (%)	35–40	<0.01	Significant

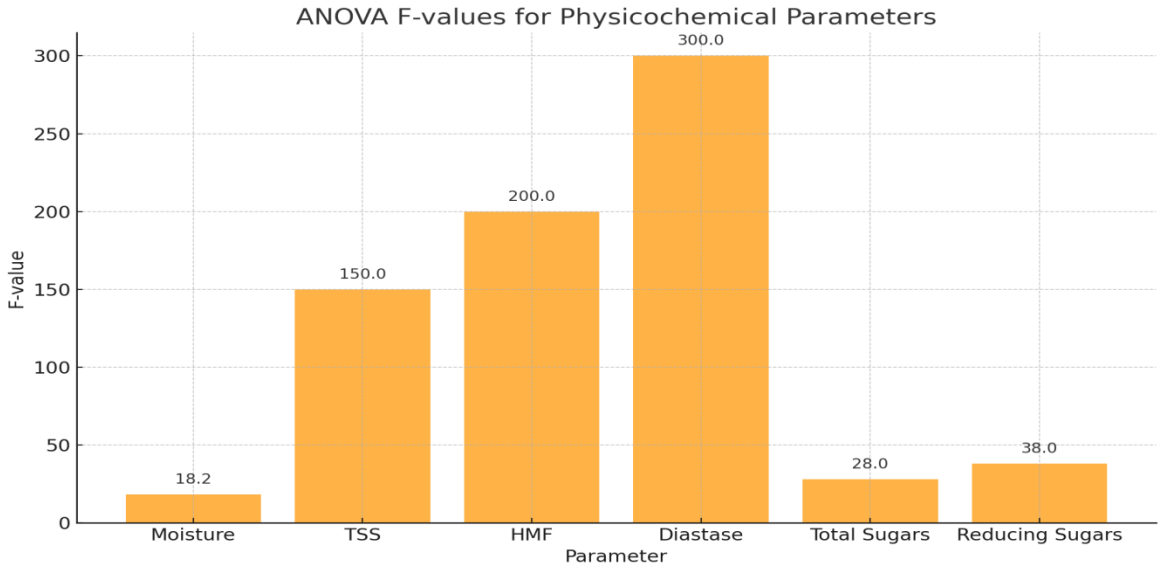

Figure 1 Anova F-values graphics

4. Conclusion

This study assessed how the physicochemical properties of vacuum-freeze-dried honey were affected by the various carrier agents gum arabic, maltodextrin, and dextrin. The findings demonstrate how important carrier selection is to maintaining the structural integrity and quality of honey powder. Interestingly, samples containing gum arabic took 28 hours to dry, samples containing maltodextrin took 48 hours, and samples based on dextrin took 72 hours. These variations imply that the drying effectiveness and the preservation of honey's natural constituents are directly impacted by the type of carrier. Gum Arabic outperformed the other tested carriers in terms of preserving the stability of freeze-dried honey, whereas Dextrin needed a longer drying period, which might have an effect on production efficiency. Maltodextrin provided stability and drying time balance, but also the color of the sample was more pronounced in yellow, while during the use of GA and Dx the color of the samples was paler. These results support the potential uses of honey powder in the food and pharmaceutical industries by offering insightful information for optimizing its production through vacuum

freeze-drying. To increase the final product's commercial viability, future studies should investigate its storage and functional qualities in more detail.

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