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Research Article

Effect of Encapsulation on the Amount of HMF in Honey

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Abstract

Hydroxymethylfurfural (HMF) is recognized as an undesirable component in honey and is usually formed as a result of heat treatment or prolonged storage of honey. HMF is formed as a result of chemical processes such as the Maillard reaction. Low levels of HMF are often associated with the freshness of honey. To prevent the formation of HMF in honey, it is thought that the application of the encapsulation process can increase the stability of this component and reduce its direct interaction. This method can help to extend the shelf life of honey and preserve its nutritional value. The protection provided by encapsulation may improve the overall quality of honey by reducing HMF formation depending on factors such as temperature and storage time. The aim of this study was to demonstrate that undesirable HMF formation can be prevented by encapsulating honey, which is rich in nutrients, with a biocompatible material. Accordingly, unencapsulated honey and encapsulated honey were kept in an oven at 80 °C for 2 hours and HMF formation was analyzed. In addition, the HMF content of unexposed honey, exposed honey and encapsulated honey samples were analyzed by high performance liquid chromatography (HPLC). As a result of the analysis, it was observed that the highest to the lowest amount of HMF was observed in honey exposed to temperature, encapsulated honey and honey samples not exposed to temperature, respectively. The calculated HMF amounts were 17.21 mg L^{-1}, 14.80 mg L^{-1} and 14.70 mg L^{-1}, respectively. Furthermore, these results were supported by thermal gravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR). In conclusion, this study showed that the encapsulation method was effective in reducing HMF formation. The findings suggest that encapsulation can increase the stability of honey and therefore may be an effective method to extend its shelf life.

Keywords: Honey, hydroxymethylfurfural, encapsulation

1.Introduction

1.1 Hydroxymethylfurfural (HMF)

Hydroxymethylfurfural (HMF) is recognized as an undesirable component in food products, especially honey and milk. HMF is formed as a result of thermal degradation of monosaccharides and the Maillard reaction, usually during excessive heat treatment or prolonged storage of honey (Yurt, 2020; Jaya et al., 2016). It raises significant concerns for food safety by increasing the negative effects of HMF on food quality. High concentrations of HMF can negatively affect the nutritional value and sensory properties of food products (Urgu et al., 2017).

Scientific studies, especially on the toxic effects of HMF, reveal the negative effects of this compound on human health (Fang et al., 2018; Qu et al., 2022). High levels of HMF in honey increase health risks and its harmful effects become more pronounced, especially in case of excessive consumption. HMF can promote the formation of reactive oxygen species (ROS), leading to cellular damage and potentially carcinogenic effects (Saikia et al., 2021; Alamillo et al., 2012). In addition, metabolism of HMF in the body can result in the formation of various toxic compounds, posing additional health risks (Özdemir, 2023).

The potential adverse effects of HMF on human health may be more pronounced, particularly among vulnerable groups such as children and the elderly, who are more susceptible to the effects of toxic compounds (Akkemik et al., 2024). It is imperative to maintain HMF concentrations below the permissible limits to ensure public health and safety. In this regard, the Turkish Food Codex has stipulated maximum permissible levels of HMF in honey, with the understanding that exceeding these limits can pose significant health risks (Gündüz et al., 2023).The effects of HMF on food safety extend beyond mere toxicity, also impacting the quality parameters of honey.Storage conditions of honey have been identified as a critical factor in HMF formation. Increased HMF levels have been observed in honey stored at elevated temperatures or for extended periods (Torun & Bekar, 2024; Bilici, 2025). Consequently, it is imperative for both producers and consumers to prioritize proper storage conditions to mitigate HMF formation.

1.2 Encapsulation

Encapsulation is defined as the process of enveloping bioactive components within a protective matrix or coating. This technique has gained significant popularity in the food, pharmaceutical, and cosmetic industries due to its ability to enhance the stability of bioactive ingredients, facilitate controlled release, and mask undesirable tastes or odors (Okutan & Boran, 2023; Çeliközlü, 2023). In the food industry, it is regarded as a pivotal strategy for preserving and enhancing the effectiveness of vitamins, minerals, flavors, and other nutritional components (Gündoğdu et al., 2021).

In the food industry, encapsulation has been shown to extend shelf life by protecting bioactive components against environmental factors such as oxidation, temperature changes, and light (Okutan and Boran, 2023). For instance, the encapsulation of fish oil with emulsion systems enables the oil to retain its nutritional value without undergoing oxidation (Okutan and Boran, 2023). This method has been shown to enhance the bioavailability of bioactive components by facilitating controlled release within the digestive tract, making it a valuable technique for the development of nutritional supplements and drug formulations (Çeliközlü, 2023). The encapsulation of bioactive components in honey is a significant technology that plays a crucial role in maintaining the stability and enhancing the bioavailability of these components. This method protects the vitamins, minerals, flavors, and other bioactive components present in honey from environmental factors such as oxidation, heat, and light. Furthermore, the encapsulation process enhances the functional properties of honey by prolonging the shelf life of these components (Okutan and Boran, 2023).

2. Material and Methods

2.1 Experiment design

The objective of the present study was to investigate the effect of heat-induced 5-hydroxymethylfurfural (HMF) compound on HMF formation when added to honey by encapsulation method. To this end, honey was exposed to different temperature conditions, and the effect of encapsulation on HMF levels in this process was determined.In the experimental analysis phase, honey samples were prepared at different temperatures and times. In this process, traditional unprocessed honey samples will be compared with honey samples subjected to heat treatment under certain temperature and time conditions.In addition, honey samples under a protective coating by encapsulation method will be exposed to the same thermal conditions, and the changes in HMF formation will be observed.

Among the analytical methods employed, high performance liquid chromatography (HPLC) will be utilized to quantitatively determine the amount of HMF in the samples.HPLC analysis will allow for the determination of the HMF level in honey with high sensitivity and will allow for the comparison of the amount of HMF formed in relation to the heat exposure time of the samples.In addition, the thermal stability and degradation behavior of the samples will be investigated by thermal gravimetric analysis (TGA). TGA will provide comprehensive information on how the encapsulation treatment affects the thermal stability by determining the mass loss trend of honey against temperature.In addition, Fourier Transform Infrared Spectroscopy (FTIR) will be used to analyze the changes in the chemical structure of encapsulated and non-encapsulated samples. The utilization of FTIR analysis is expected to furnish a substantial dataset, thereby facilitating a comprehensive understanding of the impact of the encapsulation process on the functional groups of honey and enabling the identification of chemical alterations potentially associated with HMF formation.

2.2 Encapsulation process

Controlled dripping technique was used to obtain hydrogel beads through encapsulation process. 2% w v^{-1} sodium alginate and ~80% w v^{-1} honey solution were homogeneously mixed and dripped at a constant (1 ml min^{-1}) flow rate into a 0.2 M salt solution bath from a height of 8 cm using a syringe pump. At room temperature, the salt solution was continuously stirred at 300 rpm to obtain capsules of equal size. The capsules were allowed to mature for 30 min before washing in distilled water (Lee et al., 2013).

Table 1. Treatments applied to the samples

Description of experimental studies	
BL-80	It was kept in an oven at 80 °C for 2 hours
Enkp	Honey was encapsulated and placed in an oven at 80 °C for 2 hours
BL-1	No treatment was applied to the honey

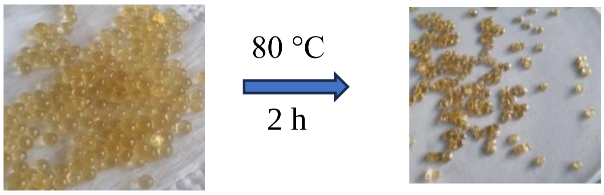

Figure 1. View of the encapsules after heating

2.3 HPLC Method

Each sample was extracted with distilled water at a 1:5 ratio.The samples were filtered through a 0.45 µm membrane filter and injected into a Shimadzu LC-20A model HPLC-UV system.In the HPLC analysis, a mixture of ultrapure water and methanol (90:10) was used as the mobile phase. The stationary phase column utilized was a Svea Gold C18 (250 x 4.6 mm) column with a particle size of 5 µm. The flow rate was set to 1.0 ml/min, and the injection volume was 20 µL of sample or standard solution.The measurements were performed at a wavelength of 285 nm (J. Jeuring and F. Kuppers, 1980).

3. Results and Discussions

At 5 different points, a calibration graph was drawn with the HMF standard and the samples were read.

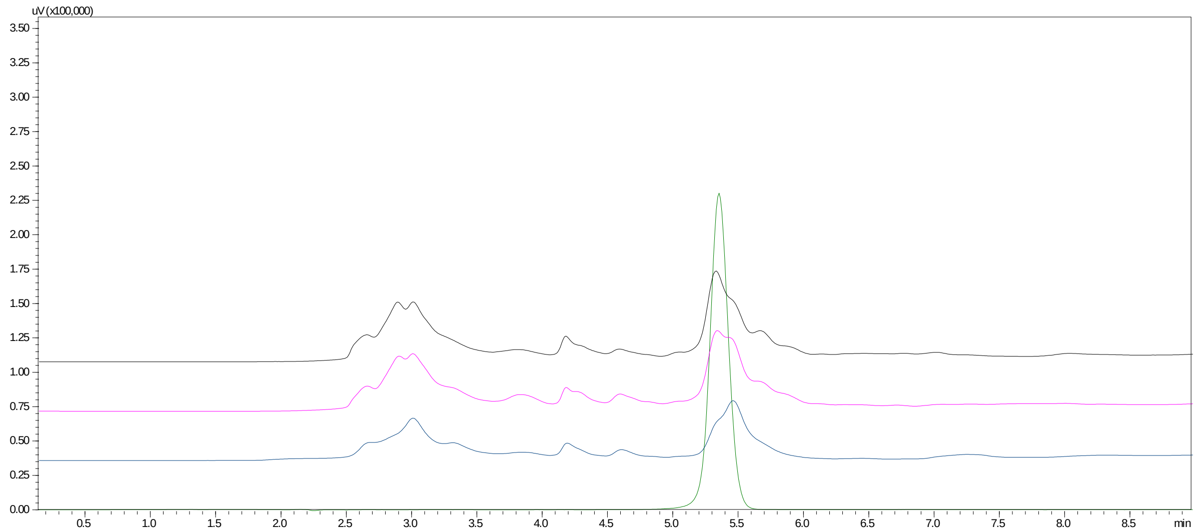

Figure 2. Chromatograms of BL-1 (black), BL-80 (pink) and ENKP (blue) samples.

Table 2. Amoont of HMF

Data obtained from the device	
BL-1	14.70 mg L^{-1}
Enkp	14.80 mg L^{-1}
BL-80	17.21 mg L^{-1}

The TGA (Shimadzu-DTG 60H) method was configured at a heating rate of 20 °C/min, with a temperature range of 1000 °C, and the alterations of the samples obtained with temperature were examined.BL-1 (Normal Honey): In comparison to the other samples, mass loss commenced at low temperatures (in the range of approximately 40-100°C), and water loss and the decomposition of volatile components were observed. In contrast, BL-80 (80 °C Treated Honey) exhibited early mass loss, suggesting potential water loss at 80 °C.ENKP (Encapsulated Honey) demonstrated a protective effect, with encapsulation resulting in reduced mass loss compared to the other samples.

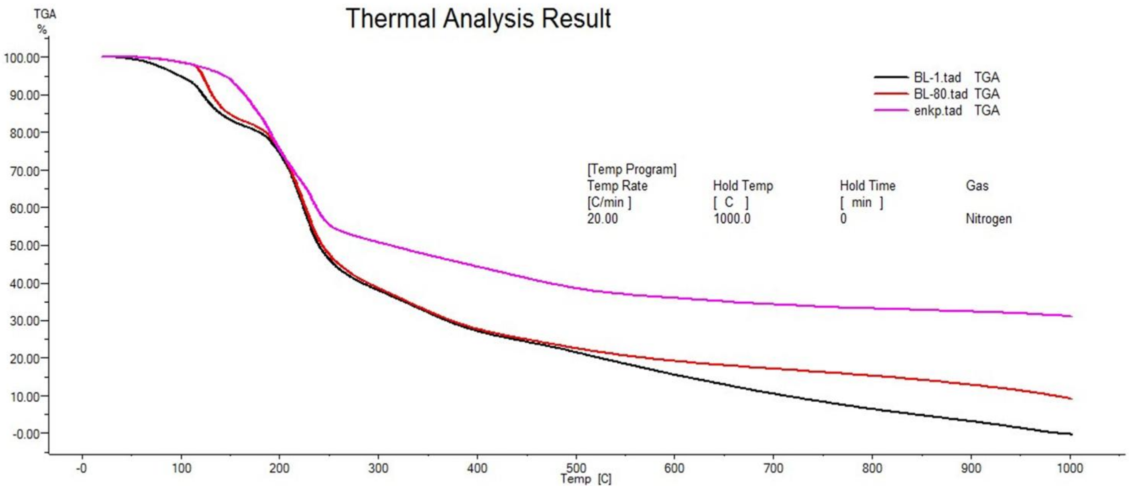

Figure 3. Multiple display of TGA data

FTIR (Shimadzu IRPrestige21) analysis was performed in the range of 4500-550 cm-¹ with a scan rate of 20 and a resolution of 0.4 using the Happ-Genzel method and compared for structural differences between the samples.

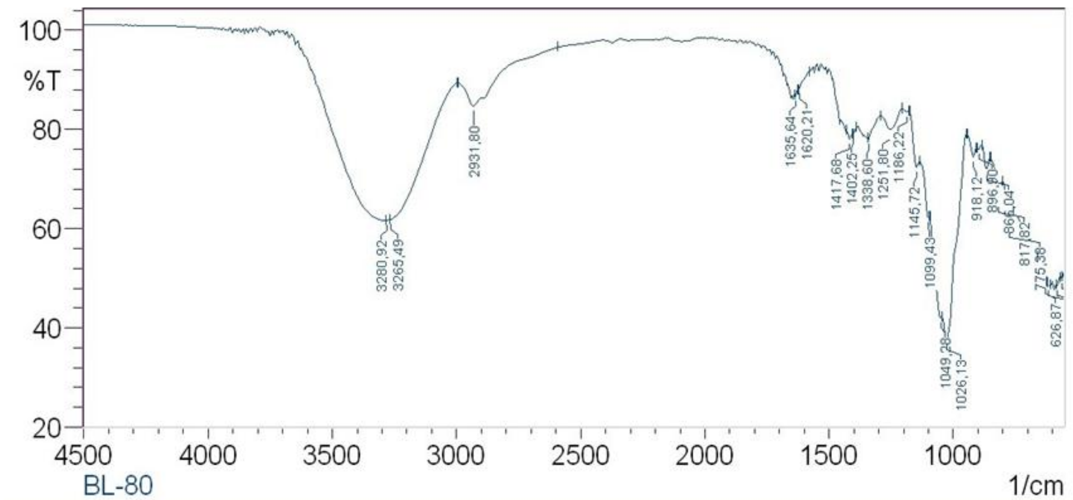

Figure 4. FTIR spectrum of BL-80

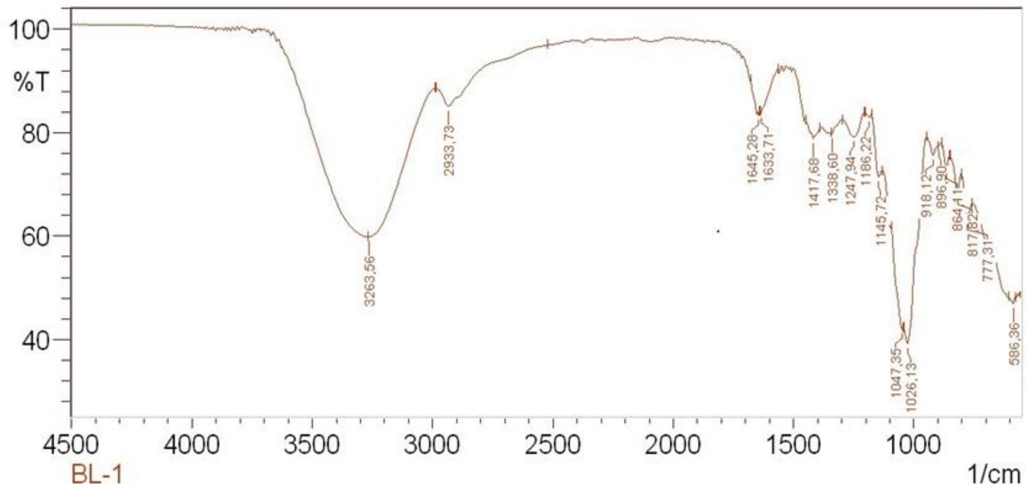

Figure 5. FTIR spectrum of BL-1

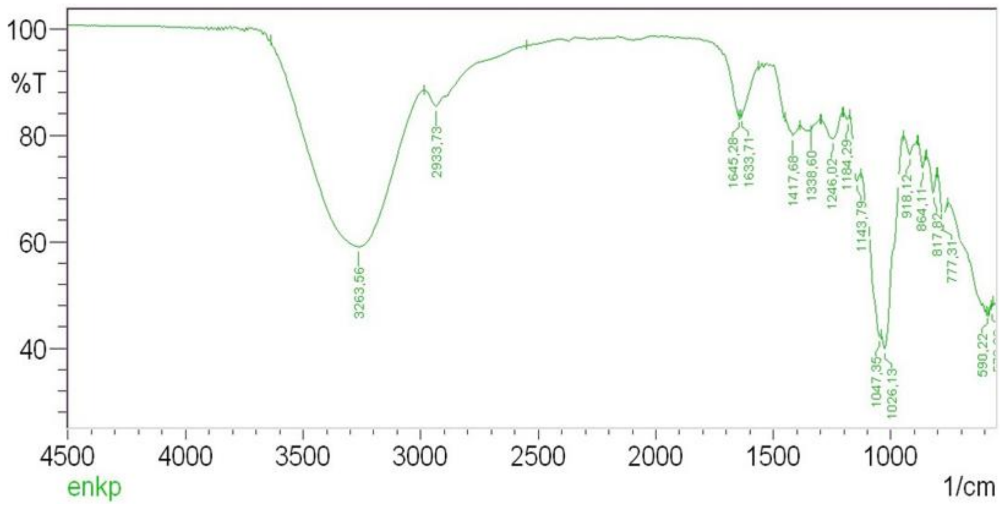

Figure 6. FTIR spectrum of enkp

Small changes were observed in the FTIR spectra, although not very pronounced. These changes occurred especially in BL-80, that is, in honey directly exposed to heat. Encapsulated honey and unexposed honey gave largely similar peaks. C-H Stress Region (~2900 cm-^{1}): In BL-80, this peak shifted to a lower wave number than the other two samples. Carbonyl and C=C Stress Region (~1600-1650 cm^{-1}): ENKP: 1645.28 cm^{-1}, 1633.71 cm^{-1}; BL^{-1}: 1645.28 cm^{-1}, 1633.71 cm^{-1}; BL-80: 1656.64 cm^{-1}, 1632.21 cm^{-1}`; the carbonyl peaks are slightly shifted in BL-80. Heating may have caused rearrangement or fragmentation of the carbonyl groups. In the other two samples, the peaks are located in exactly the same places. C-O, C-O-C and C-H Bending Regions (1000-1500 cm^{-1}): In BL-80, a new peak was formed especially at 1251.86 cm^{-1}, while the 1143-1145 cm^{-1} region was different. This can be interpreted as heat-induced fragmentation or rearrangement of the carbohydrate structure. The findings of this study show very positive and promising results when compared with some important studies in the literature. For example, a study by Önür et al. (2018) showed that HMF formation can be reduced by using high hydrostatic pressure (HHP) and ultrasonic treatment (US) instead of conventional heat treatment. In this study, it was found that the amount of HMF increased significantly in honey heated at 60 °C, but HHP and US treatments provided lower HMF levels (Önür et al., 2018). In our study, encapsulation showed a similar effect in suppressing HMF formation. Therefore, it can be said that encapsulation is a worthy option among the alternative methods used to maintain quality in honey. In addition, in a study conducted by Ünübol Aypak et al. (2019), it was found that the HMF levels of honey taken directly from hives were significantly lower than market honey. While HMF levels measured in market honeys were 56.70 mg kg^{-1}, this value was found to be 26.94 mg kg^{-1} in honey from hives (Ünübol et al., 2019). This finding indicates that honey can accumulate significant levels of HMF under inappropriate storage and processing conditions. The lower level of HMF in honey encapsulated in our study suggests that this method may also have positive effects in long-term storage conditions. In a study conducted by Zappalà et al. (2005), the amount of HMF in honey was measured using different analysis methods and it was reported that exposure to high temperatures increased HMF levels. In this respect, the encapsulation method used in our study seems to help honey become more resistant to heat treatment (Zappala et al., 2005).

4. Conclusion

Thanks to the encapsulation process of honey, HMF formation was prevented when exposed to heat. This method can be applied to other foods that cause HMF formation. In addition, the encapsulation materials used are biocompatible, so there is no harm in consumption as food. This makes the method attractive for preventing heat-induced harmful substances. This study has made an important contribution to the field of food science by investigating in detail the effects of encapsulation method on the formation of hydroxymethylfurfural (HMF), an undesirable component in honey. As a result of the experimental analyses, it was shown that HMF formation can be prevented or significantly reduced when honey is exposed to heat through the encapsulation process. This indicates that the encapsulation method can be used as an effective preservative method not only in honey but also in other foods prone to HMF formation. As a result of the HPLC analyses, the lowest HMF levels were detected in unencapsulated and non-heat-exposed honey samples, while the amount of HMF was significantly increased in honey exposed to heat but not encapsulated. In contrast, the HMF level in the encapsulated and heat-exposed honey was lower than in the sample directly exposed to heat. These findings suggest that encapsulation is an effective technology to prevent heat-induced degradation by maintaining the chemical stability of honey. TGA and FTIR analyses also supported the protective effect of encapsulation and revealed that heat-induced chemical changes occurred less in encapsulated honey samples. This proves that encapsulation method can extend the shelf life and preserve the nutritional value of honey by increasing both physical and chemical stability. The results of this study showed that the encapsulation method can be used to maintain the quality of honey and other foods. Further studies should be conducted to investigate whether the encapsulation method is effective in reducing HMF formation in other heat-treated foods besides honey. It is important to raise consumer awareness of the dangers of undesirable food components such as HMF. Educational and promotional activities should be carried out to explain the benefits of innovative methods such as encapsulation. Apart from the biocompatible encapsulation material used in this study, similar tests should be conducted using different encapsulation agents and techniques and alternative methods that may provide more effective protection should be investigated. This study demonstrated that encapsulation can play an important role in preserving the quality and nutritional value of honey and provided valuable data that can guide similar studies in the future. In this context, we would like to emphasize that our study has created a great added value in the field of food science. As our research team, we hope that this study will lead to the development of new approaches in the industry.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article, that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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