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Optimizing the thermal processing of honey by studying the physicochemical properties and its hydroxymethylfurfural content†

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Abstract: Hydroxymethylfurfural (HMF) is a naturally occurring compound that arises because of the thermal processing and storage of honey. The Codex Alimentarius Commission has established a threshold of 40 ppm as the upper limit for HMF content in honey. This research aimed to investigate the impact of varying heating temperatures (55, 65, and 75°C) and heating times (10, 20, and 30 min), as well as storage temperatures (25 and 40°C), over a period of three months. The study employed response surface methodology (RSM) to evaluate the outcomes. The impact of the variables mentioned above on the physicochemical properties, including the Lab color factors, pH, and moisture was determined. Additionally, the concentration of HMF in the samples was also analyzed. The prediction model of each treatment was computed. The analyses of the results obtained after the storage periods of 45 and 90 days were conducted. The findings indicated that pH, moisture content, and color were not significantly influenced by temperature, duration of heat treatment, and storage time. However, storage temperature considerably impacted both L* and a*. Furthermore, it was observed that all the variables significantly influenced HMF content, and its concentration increased with the escalation of thermal processing and storage duration. Within the examined samples, HMF content outpaced the standard limit in the model subjected to heating at 75°C for 20 min and maintained at 40°C for 90 days. In contrast, heating at 55°C for 10 minutes, followed by storage at 25°C for 45 days produced the optimal HMF level.

Keywords: Honey; Hydroxymethylfurfural; Response surface methodology; Storage; Thermal Process

1. Introduction

Honey is a natural sweet substance yielded by honeybees using plant nectar or living plant secretions, as defined by the Codex Alimentarius. It has been collected by the bees and transformed by combining with specific substances of their own, depositing, dehydrating, storing and leaving in the honeycomb to ripen and mature [1].

Honey comprises diverse components, including sugars, water, vitamins, enzymes, minerals, and other constituents. The presence of these compounds, along with enzymes and polyphenolic compounds, has made honey a valuable elixir and food used in traditional medicine for its restorative, antimicrobial, and anti-inflammatory properties. Although honey has numerous nutritional and medicinal properties, improper storage, and processing results in some undesirable non-nutritional compounds in this product. In recent years, HMF has gained much attention as a high-potential toxicological and carcinogenic contaminant in honey [2,3].

HMF is a furanic compound produced through the Maillard reaction or via the direct dehydration of sugar under acidic conditions [4,5]. Two primary metabolic pathways have been identified for 5-Hydroxymethylfurfural (5-HMF). The first one includes the synthesis of 5-hydroxymethyl-2-furoyl glycine (HMFG) involves the oxidation of the aldehyde functional group to yield 5-hydroxymethyl-2-furoic acid (HMFA), which is subsequently conjugated with glycine. Another metabolic pathway which holds greater significance from a toxicological perspective involves the sulfonation of the allylic hydroxyl function of 5-hydroxymethylfurfural (5-HMF) [6]. 5-sulfooxymethylfurfural (SMF) is a potent genotoxic with mutagenic effects [7]. The international standard commissions have established the maximum allowable level of HMF in honey at 40 mg/kg [8]. The HMF content in honey is subject to various factors, such as its sugar content, pH, acidity, water activity, diastase and invertase activity and divalent cations concentration [9]. The correlation between the initial pH and HMF content in honey has been observed. It has been predicted that honey samples with a pH less than 4 may contain approximately 40 mg/kg of HMF, while those with a pH greater than 4 may have HMF content ranging from 20 to 25 mg/kg. These findings shed light on the interplay between pH and HMF in honey [10,11].

Honey producers have been heating honey to prevent post-bottling crystallization and delay microbial spoilage. Selecting the best operational parameters, including time and temperature, is a major challenge in honey production and packaging. The amount of HMF present in honey depends on the duration and temperature of its thermal treatment; As such, its content serves as the primary criterion to predict the optimization of said processing. Modeling and finding a relation between different heat treatments and storage parameters could be a reasonable approach to optimize the process and increase the quality and safety of the end product. This study investigated the effect of process conditions (temperature and heating time) and storage conditions on HMF content and physicochemical properties (Lab color factors, pH, and moisture) of honey to determine the optimal processing and storage conditions.

2. Materials and methods

Honey samples with a moisture content of 14% and pH of 3.8 were obtained from a local honey packaging factory. For this purpose, honey samples were poured into special honey bottles, and to assess the influence of temperature and heating time, heat treatment was applied with indirect heating by the thermostatic bath at temperatures of 55, 65, and 75℃ and 10, 20 and 30 min. During the investigation, tests were conducted at intervals of 45 days (0, 45, and 90), and three repetitions were performed.

Moisture was determined by measuring the refractive indices at 20℃ with a refractometer and the corresponding moisture content (%) was calculated according to previous authors [1]. A pH meter was used to measure the pH of the samples [2]. The digital imaging method was used to analyze the samples' color [12]. Measurement of HMF concentration in honey was performed using a spectrophotometer according to the White method [3,13]. The data was analyzed with a Minitab16 using Response Surface Methodology (RSM) and Central Composite Design (CCD) (**Table 1**).

Table 1. Central composite design (by Minitab 16 software).

Run Order	Block	Heating pasteurization temperature (°C)	Time of heating (min)	Storage temperature (°C)
1	1	65	20	25
2	1	55	30	25
3	1	55	10	25
4	1	75	10	25
5	1	65	20	25
6	1	65	20	25
7	2	75	30	40
8	2	65	20	40
9	2	65	30	40
10	2	65	20	40
11	2	65	10	40
12	2	55	20	40
13	2	75	20	40
14	2	65	20	40

3. Results and discussion

The pH of honey samples during the 45 and 90 days storage period was 3.7-3.82 and 3.65-3.81, respectively. There was no significant relationship between pH and any of the variables studied. Regarding moisture, the effect of temperature and different times of thermal process and storage temperature did not significantly affect moisture values. During the storage period of 45 and 90 days, honey samples contained an average moisture content of 14.9 and 15, respectively. Only the storage temperature could affect the impacting variables on the *L index. Similar results were also observed regarding *a index of honey samples. However, on *b index, the effect of none of the variables was significant. All the studied factors showed a significant effect on HMF formation (p<0.05), indicating that this content was significantly increased with higher heating time, temperature, and storage conditions (**Figure 1** and **Figure 2**).

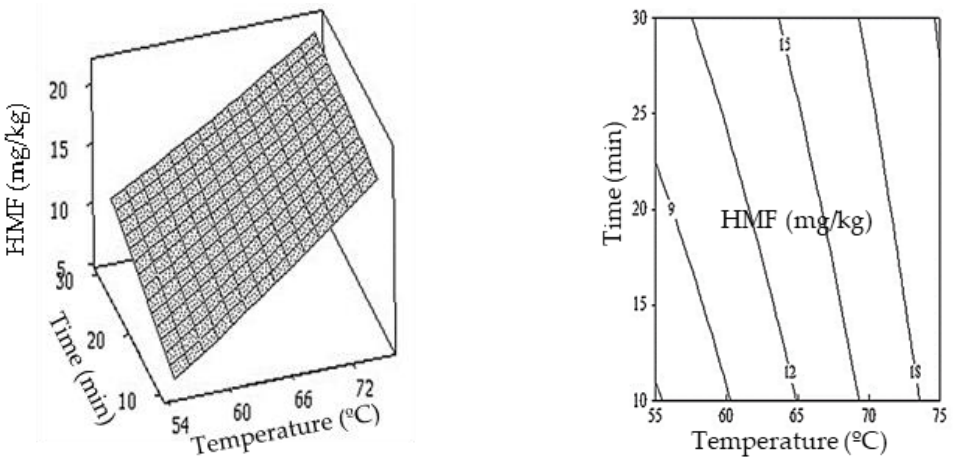

Figure 1. The effect of thermal process temperature on the amount of HMF for 45 days of storage.

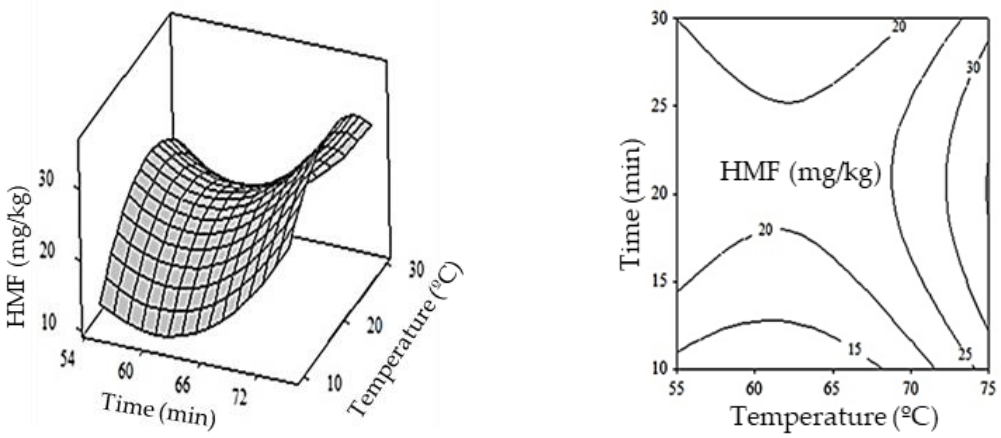

Figure 2. The effect of thermal process temperature on the amount of HMF for 90 days of storage**.**

4. Conclusions

From the optimization study, it can be concluded that the minimum content of HMF resulted from heating honey at 55°C for 10 min and keeping it at 25°C for 45 days. Therefore, these processing and storage conditions could help find the best operation conditions to preserve honey's quality and safety.

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Conflicts of Interest: The authors declare no conflict of interest.

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