INFLUENCE OF THE TIME/TEMPERATURE BINOMIAL ON THE HYDROXYMETHYLFURFURAL CONTENT OF FLORAL HONEYS SUBJECTED TO HEAT TREATMENT

Influência do binômio tempo e temperatura nos teores de hidroximetilfurfural em méis florais submetidos ao aquecimento

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ABSTRACT

Hydroxymethylfurfural (HMF) content is internationally recognized for its ability to indicate the freshness or lack of freshness of honey and can be used to judge the processing and storage condition of honey. The objective of the present study was to evaluate the evolution of HMF levels in fresh extracted honeys submitted to different temperatures ranging from 30 to 100º C, during pre-established time intervals (30, 45, 60, 180 and 720 minutes).The test was conducted in floral honey with an initial HMF content of 2.2 mg/Kg. The maximum value recommended by the Brazilian law (60 mg/Kg) was not exceeded in the samples subjected to heating for 30, 45 and 60 minutes regardless of the temperatures used. When the samples were heat treated during 180 minutes at 90º C the official value was surpassed. The highest values were observed in samples subjected to heating over 720 minutes, and the limit was exceeded at 70º C. The results obtained indicate that the HMF content gradually increases when the honey is heated at high temperatures for long periods. Therefore, we suggest a process optimization, considering the initial HMF content of the product and standardizing times and temperatures to ensure a good quality of the final product.

Index terms: Honey quality, HMF, heating.

RESUMO

O teor de hidroximetilfurfural (HMF) é reconhecido internacionalmente pela capacidade de indicar o frescor do mel, podendo ser usado para avaliar o processamento e condições de armazenamento do mel. Neste trabalho objetivou-se avaliar a evolução dos níveis de HMF em mel recém-obtido, submetidas a diferentes temperaturas que variaram de 30 a 100º C, por intervalos de tempo pré-definidos (30, 45, 60, 180 e 720 minutos). O ensaio foi realizado em mel floral, cujo valor inicial de HMF foi de 2,2 mg/Kg. O valor máximo preconizado pela legislação brasileira não foi ultrapassado nas amostras submetidas ao aquecimento por 30, 45 e 60 minutos independente das temperaturas. Por um período de 180 minutos, o limite legal de 60 mg/Kg foi extrapolado nas amostras tratadas a 90º C. Os valores mais elevados foram observados nas amostras submetidas ao aquecimento por 720 minutos, tendo o limite legal ultrapassado em temperatura mais branda (70º C). Com base nos resultados obtidos, pôde-se concluir que o conteúdo de HMF aumenta gradativamente quando o mel é exposto a altas temperaturas, por tempo prolongado. Sendo assim, sugere-se uma otimização nos processos de beneficiamento, considerando a quantidade inicial de HMF no produto, com processamento padronizado em temperaturas e tempo controlados para garantia da qualidade do produto final.

Termos para indexação: Qualidade do mel, HMF, aquecimento.

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INTRODUCTION

Honey is a natural product that must be delivered to the consumer with its essential composition and its quality minimally altered (CODEX, 1987). However, honey suffers a natural ageing process which starts on the field when it is harvested and placed in barrels by the beekeepers; it goes on with transport and storage previous to the industrial treatment and continues during the industrial process and even after it (SANCHO et al., 1992).

5-hydroxyimethylfurfural (HMF) is a furanic compound produced by sugar degradation (RAMIREZ et al., 2000), naturally formed as an intermediate in the Maillard Reaction (AMES, 1992) or from dehydration of hexoses in acid medium (BELITZ; GROSCH, 1999), mainly products with pH value up to 5.0 (DAMODARAN et al., 2010). The presence of simple sugars and water in acid medium favors the formation of this furanic compound (NOZAL et al., 2001).

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HMF formation process can be accelerated during the heat treatments applied to honey (KROH, 1994). However, in acid media, HMF can be formed even at low temperatures (LEE; NAGY, 1990), which explains the gradual increase of HMF levels during storage. Some factors, such as the use of metallic containers (WHITE, 1979), honey physico-chemical properties (pH, total acidity, mineral content), the floral origin (AANAM; DART, 1995) and the thermal stress to which the product is subjected in the hive (SPANO et al., 2006), directly influence HMF formation during storage. Due to the factors above mentioned, HMF is considered one of the degradation products more frequently used as honey quality indicator (TOSI et al., 2002; FALLICO et al., 2004), once this product is almost absent in fresh extracted honeys and its concentration increases with time (SPANO et al., 2006), inadequate exposure in retail outlets, aging and improper use of heat to make honey more liquid.

In recent decades, HMF has drawn the attention of the scientific community for its carcinogenic potential for humans. Some studies have shown that this metabolite can be converted *in vivo* to 5-sulfooxymethylfurfural (SMF), a genotoxic compound (SURH et al., 1994). In addition, at high concentrations, HMF is cytotoxic, causing irritation to eyes, upper respiratory tract, skin and mucous membranes (ULBRICHT et al., 1984; BRUCE et al., 1993). For this reason, the *Codex Alimentarius* and the European Commission have set a maximum HMF level for honey of 40 mg/Kg, except for honeys coming from tropical countries and honeys with low enzyme levels, the HMF limit of which was set in 80 and 15 mg/Kg respectively (CODEX, 1987; EUROPEAN COMMISSION, 2001). The Brazilian legislation recommends a maximum limit of 60 mg/Kg for all kinds of honey (BRASIL, 2000). The amount of HMF detectable in honey is directly related to the intensity of the heat and the exposure time applied during processing.

The objective of the present study was to evaluate the evolution of HMF content in fresh extracted honeys, subjected to different temperatures ranging from 30 to 100ºC, over pre-established time intervals (30, 45, 60, 180 and 720 minutes).

MATERIALS AND METHODS

Honey samples

Honey samples were directly obtained from an apiary located in the mountain region of Rio de Janeiro, in the municipality of Teresopolis, a region characterized by diverse vegetation with altitude tropical climate and average annual temperature of 16±2ºC. Approximately 6 Kg of multifloral honey were fractionated in 120 glass bottles with 20 mL capacity and stored at 20±1° C.

Treatments

In order to evaluate the effects of time and temperature on the variation of HMF levels in the samples of floral honey, heat treatments were performed, in triplicate, by immersion in a thermostatic bath with periodic shaking, set at the following temperatures: 30, 40, 50, 60, 70, 80, 90 and 100º C. During this stage, the samples were kept at those temperatures during 30, 45, 60, 180 and 720 minutes. Temperature was monitored with a thermocouple introduced at the geometrical center of the bottles. The time was measured from the moment the temperature reached the value established in the methodology. Then, the samples were cooled by immersion in cold water at 4±2º C and immediately analyzed.

Physico-chemical analysis

The samples of honey were submitted to physico-chemical analysis in order to verify their quality before undergoing heat treatment. The following techniques were used: determination of pH, fixed mineral residue, insoluble matter, reducing and non-reducing sugars, Lund reaction, acidity analysis, Fiehe reaction.

HMF content determination

The content of HMF in the samples was determined by Winkler spectrophotometry method. Five grams of honey were weighted, dissolved in distilled water and transferred to a 25mL volumetric flask and make up to the mark. Immediately after preparation a 2 mL aliquot was transferred to a test tube and 5mL of 10% p-toluidin solution in isopropanol and 1mL of 5% barbituric acid aqueous solution were added. In another test tube (reference) was added 2mL of honey solution, 5mL of p-toluidin solution and 1mL distilled water. The reagents were used in up to 2 minutes after preparation in order to ensure the stability of the solutions. The absorbance at 550 nm was determined using a spectrophotometer. The honey used contained an initial HMF value of 2.2 mg/Kg before heating.

Statistical Analysis

Data were statistically analyzed using the two-way analysis of variance (ANOVA), aiming at identifying differences in HMF content in honey caused by the interaction of time/temperature variables. Multiple

regression was applied with HMF content as dependent variable and the sample immersion time in the hydrostatic bath and the temperature of the bath as independent variables. All the analyses were performed using a statistic package available in the market (GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, California, USA).

RESULTS AND DISCUSSION

The values obtained during the experiment are presented in tables and figures that summarize the influence of the heat treatments on HMF content in the samples of floral honey analyzed. HMF content significantly varied ($P<0.001$) with the increase of temperature and immersion time to which the samples were subjected. The regression coefficient ($r=0.98$), indicated that only 2% of the regression variances do not depend on the variables studied. It was observed that HMF content increases slowly up to 70º C and, above this temperature, its production rate increases in a more significant way, mainly at temperatures above 80º C. The HMF content increase is more evident when the immersion time in the hydrostatic bath increases, reaching its highest value in the samples exposed to 100º C for 720 minutes, which presented an average value of 101.24 mg/Kg (Figure 1).

The HMF content variation was small in the treatments at 30, 40 and 50º C for up to 180 minutes, varying from 4.46 mg/Kg at 30º C during 30 minutes to 15.28 mg/Kg at 50º C for 180 minutes, indicating that the use of those temperatures in honeys with low initial HMF content does not cause a significant increase of this compound. In general, it can be observed that honeys heat treated at 40 and 50º C for up to 180 minutes does not present a significant difference of HMF formation ($P<0.05$). Karabournioti and Zervalaki (2001) reported a slightly significant increase of HMF content in orange honeys heat treated at 35º C, 45º C, 55º C and 65º C for 24 hours, from 2.25 mg/Kg (without heating) to 3.45, 3.75, 4.35 and 19.00 mg/Kg, respectively and concluded that mild temperatures up to 55º C, do not influence the increase of HMF regardless of the exposure time.

The effects of heating on HMF increase can be observed when the exposure time is longer, the time variable being the most significant factor in increasing HMF content, when temperatures between 30 and 90º C are considered. The results show that there was no significant difference ($p>0.05$) on HMF levels in samples submitted to temperatures of 30, 40, 50, 60, 70 and 80ºC for 30 minutes. The values increased from 2.2 mg/Kg (fresh extracted honey) to 4.46, 5.68, 6.96, 7.12, 8.88 and 10.68 mg/Kg, respectively. Samples heated at 80, 90 and 100º C for 30 minutes, significantly differed ($P<0.05$) from samples kept in thermostatic bath for 45 minutes confirming the trend of increased levels of HMF when the heating time increases (Table 1).

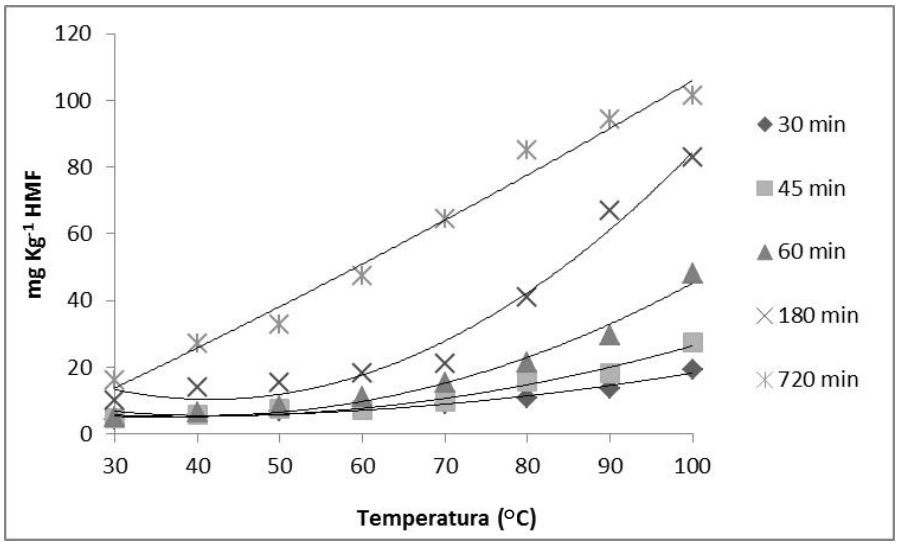

Figure 1 – Results of the determinations of HMF content according to temperature and time of immersion of honey samples.

Table 1 – Variation of the concentration of Hydroxyimethylfurfural (mg/Kg) in honey samples as a function of temperature and time of immersion in a thermostatic bath.

Temperature	Immersion time				
	30 min	45 min	60 min	180 min	720 min
30ºC	4.46±0.15^{Aa}	4.77±0.12^{Aa}	5.04±0.90^{Aa}	9.97±0.43^{Ba}	16.18±0.37^{Ca}
40ºC	5.68±0.83^{Aa}	5.82±0.32^{Aa}	6.58±0.53^{Aa}	14.05±0.68^{Bb}	27.15±0.18^{Cb}
50ºC	6.96±0.13^{Aa}	7.39±0.22^{Aa}	8.18±0.14^{Aa}	15.28±0.80^{Bb}	32.63±0.37^{Cc}
60ºC	7.12±0.22^{Aa}	7.33±0.16^{Aa}	10.97±0.10^{Aa}	18.32±0.29^{Bb}	47.19±3.19^{Cd}
70ºC	8.88±0.37^{Aa}	9.61±0.43^{Aa}	15.24±0.19^{Bb}	20.96±0.11^{Cb}	64.37±0.11^{De}
80ºC	10.68±0.28^{Ab}	15.62±0.24^{Bb}	21.35±0.30^{Cc}	41.03±0.08^{Dc}	85.10±0.26^{Ef}
90ºC	13.54±1.10^{Ac}	18.36±0.24^{Bc}	29.55±0.30^{Cd}	66.96±0.28^{Dd}	94.23±0.56^{Eg}
100ºC	19.31±2.40^{Ad}	27.48±0.32^{Bd}	48.19±1.35^{Ce}	82.79±3.23^{De}	101.24±0.35^{Eh}

The appearance of honey plays an important role on its commercial acceptance, once consumers demand a fluid, non-crystallized product. Fresh extracted honey is liquid. However, it may crystallize during storage at higher or lower speed depending on several factors such as origin (botanical and geographical), temperature, moisture content and sugar content (PIRO et al. 1996; BARTH; SINGH,1999). In order to delay the natural crystallization process and ensure stability during its shelf-life, fresh honey is usually submitted to heating before being packed, with the purpose of dissolving sugars and destroying yeasts (BATH; SINGH, 1999; TOSI et al., 2002). The results of the present study indicate that honey should be heated at mild temperatures, between 40º C and 50º C, heat treatments above 90º C are not recommended.

According to Jeanne (1985) de-crystallization of finely crystallized honeys requires different times and temperatures, for example, it is recommended that a 20 Kg recipient is heated at 40º C during 24 hours, and the author suggests a time/temperature binomial of 72 h at 50º C for a 300 Kg recipient. However, the present study showed that high contents of HMF (2.15 and 32.63 mg/Kg, for samples exposed to 40 and 50º C, for 12 hours, respectively), can be obtained when heat treatments are applied during long periods. Although the maximum level of HMF was not exceeded at temperatures up to 50º C, the value was close to that recommended by international legislation, which could limit the international trade of samples submitted to heating. An effective temperature control should be kept during honey de-crystallization stages, because a considerable HMF increase may occur when heating at 70º C as shown in the present study. After 720 minutes heating, the HMF content was 64.37 mg/Kg, a value that exceeds the value allowed by the Brazilian law (60mg/Kg) (BRASIL, 2000). Fallico et al. (2004), observed a significant increase of HMF content in eucalyptus honey subjected to 70º C, for 96 hours, from an initial value below detection level to 513 mg/Kg, in agreement with data obtained in the present study.

At high temperatures, such as 80 and 90º C, a gradual increase was observed that significantly varied ($P<0.01$) with heating time, which was the factor that mostly influenced the increase of HMF content at these temperatures of samples that when heated at 90º C presented HMF levels of 29.55 and 66.96 mg/Kg, after 60 and 180 minutes heat treatment, respectively. However, Turhan et al. (2008) presented different results. They reported low HMF contents in floral honeys subjected to 75 and 90º C heat treatments for up to 90 minutes. Those authors concluded that there was no significant increase of HMF in floral honeys heated at 90º C for up to 90 minutes, obtaining a maximum HMF content of only 11.24 mg/Kg.

It should be enhanced that, at 100º C, the formation of HMF presented a different behavior than at other temperatures, and, in this case, temperature was the factor that most significantly ($P<0.01$) influenced the increase of HMF, since there was an increase at all the times studied. In the same way, Turhan et al. (2008), described an increase of HMF content in floral honeys heated at 100º C for 30, 45, 60, 75 and 90 minutes, from 0.62 mg/Kg in samples without heating to 13.99, 26.4, 37.98, 55.41 and 73.78 mg/Kg, respectively. These authors observed a low HMF content in honeys subjected to heat treatments up to 90º C, demonstrating that extreme heating significantly accelerates HMF formation.

Tosi et al. (2002) studied the effects of applying temperatures between 100 and 160º C in short time intervals, varying from 14 to 60 s. The authors concluded that

temperatures above 140º C, even when applied during short time intervals, considerably increased HMF contents, which reached values above those recommended by international standards, demonstrating the influence of temperature on the increase of HMF content even in short time intervals. In the present study, we observed an increase of HMF content in samples submitted to heat treatment at 100º C, regardless the heating time, significantly differing ($P<0.05$) at all levels, reinforcing the relevance of the study and in accordance with the above mentioned authors. After 60 minutes heating, HMF concentration was 48.19 mg/Kg, exceeding the limits recommended by the European legislation. Such increasing trend was maintained up to 720 minutes, when HMF concentration reached the highest levels, above100 mg/Kg.

The maximum value recommended by the Brazilian legislation of 60 mg/Kg (BRASIL, 2000), was not exceeded in the samples subjected to heating for 30, 45 and 60 minutes, regardless the temperature used. However, increasing the time that the samples were kept in the thermostatic bath to 180 minutes, the samples exceeded the legal values at temperatures above 80º C. It was also observed that samples heated for 180 minutes significantly differed ($P<0.05$) of samples heated for 720 minutes regardless the temperature used.

The samples heated during a 720- minute period did not exceed the acceptable limit of 60 mg/Kg, when the temperature was 70º C and, the samples submitted to 40º C, presented an average HMF content of 27.15 mg/Kg. Some authors observed that the increase of HMF is not related only to heating, and can occur during long storage periods, regardless the temperature. Khalil et al. (2010) studied HMF concentration in Malasian honeys stored during more than a year. They concluded that honey samples when stored during 12 to 24 months presented HMF concentrations that exceeded the recommended levels, reaching values of 118.47 and 1139.95 mg/Kg, respectively. Kalábová et al. (2003) demonstrated that the content of HMF gradually increases during storage, eventually reaching values above those recommended by current legislation. Therefore, the time/temperature binomial used for honey processing should be as low as possible, considering that HMF increase may be influenced by factors other than exposure to adverse temperatures.

However, it is important to emphasize, that, in this study, honey was collected at Teresopolis municipality, a region characterized by altitude tropical climate, with cold, dry winters and template, humid summers, with annual average temperature of 16±2º C and relative humidity of the air of 84%. Although the European legislation is more flexible with products from tropical regions, allowing contents of up to 80 mg/Kg, the floral honey acquired for this study was not exposed to high temperatures in the hive, which explains the initial low content of HMF (2.2mg/Kg). Thus, we used 40 mg/Kg and 60 mg/Kg, as references, the first is the value recommended by the Codex Alimentarius (CODEX, 1987) and the European Union Council (EUROPEAN COMMISSION, 2001) for honeys of non-tropical regions and the second the value recommended by the Brazilian legislation (BRASIL, 2000). These values are represented in figure 1, by grey and black horizontal lines.

CONCLUSION

HMF content significantly increases with the exposure to high temperatures during a long time. Considering the positive correlation between the thermal treatment and the increase of HMF content in fresh extracted honeys, it is suggested that HMF content is used as an indicator of honey quality loss caused by heating. Honeys with low initial HMF content may be submitted to temperatures up to 90ºC for periods up to 60 minutes, for de-crystallization. However, for longer periods of time, up to 180 minutes, the temperature should not exceed 70º C. Since honey overheating is not necessary for the filling process, mild temperatures should be used during limited times to reduce viscosity and prevent crystallization, without causing a significant increase of HMF content.

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