Citation/Atıf: Ramírez-Miranda I, Moguel-Ordóñez Y, Betancur-Ancona D. Significance of Hydroxymethylfurfural and Melanoidins As Products Of Maillard Reactions In Honey. (Extended abstract in Turkish). U. Arı B. / U. Bee J. 2022, 22: DOI: 10.31467/uluaricilik.1091999

DERLEME / REVIEW

SIGNIFICANCE OF HYDROXYMETHYLFURFURAL AND MELANOIDINS AS PRODUCTS OF MAILLARD REACTIONS IN HONEY

Baldaki Maillard Reaksiyonlarinin Ürünleri Olarak Hidroksimetilfurfural ve Melanoidinlerin Önemi

Ina RAMÍREZ-MIRANDA[1], Yolanda MOGUEL-ORDÓÑEZ[2], David BETANCUR-ANCONA[3]*

[1]Posgrado Institucional de Ciencias Agropecuarias y Manejo de Recursos Naturales y Tropicales, UADY, Carretera Mérida-Xmatkuil km 15.5, Mérida, Yucatán, MÉXICO CP. 97315, E-posta: miranda120575yahoo.com.mx, ORCID No: 0000-0003-4255-0847

[2]Campo Experimental Mocochá, CIRSE, INIFAP, Antigua carretera Mérida-Motul km 25, Mocochá, Yucatán, MÉXICO CP.97454, E-posta: moguel.yolanda@inifap.gob.mx, ORCID No: 0000-0003-4805-4035

[3]* Facultad de Ingeniería Química, UADY, Lic. Manuel Berzunza 13615, Mérida, Yucatán, MÉXICO CP. 97203, Yazışma Yazarı/Corresponding author E-posta: bancona@correo.uady.mx, ORCID No: 0000-0002-9206-3222

Geliş Tarihi / Received: 23.03.2022 Kabul Tarihi / Accepted: 15.04.2022 DOI: 10.31467/uluaricilik.1091999

ABSTRACT

Honey presents exceptionally favorable conditions for a non-enzymatic glycation of proteins or Maillard reaction (MR), which is a complex network of chemical reactions which is favored during processing and storage and that often influence the quality and acceptability of honey. One of the organic compounds produced in the intermediate stages of MR that has been the subject of several investigations and controversies, due to its relationship with adverse effects on human health, is 5-hydroxymethylfurfural (HMF), which has become an indicator of honey quality. Conversely melanoidins, polymeric molecules responsible for non-enzymatic browning and which have been related to beneficial effects due to the antioxidant and antibacterial properties of honey, are produced in the final stages of MR. The aim of this article is to provide a review on the formation as well as the positive and negative effects associated with the formation of HMF and melanoidins as MR products in honey.

Keywords: honey; Maillard reaction; hydroxymethylfurfural; melanoidins.

ÖZ

Bal, proteinlerin enzimatik olmayan glikasyonu veya işleme ve depolama sırasında tercih edilen ve genellikle balın kalitesini ve kabul edilebilirliğini etkileyen karmaşık bir kimyasal reaksiyonlar ağı olan Maillard reaksiyonu (MR) için son derece uygun koşullar sunar. İnsan sağlığı üzerindeki olumsuz etkileri nedeniyle birçok araştırma ve tartışmaya konu olan MR'ın ara aşamalarında üretilen organik bileşiklerden biri de bal kalitesinin bir göstergesi haline gelen 5-hidroksimetilfurfural (5-HMF)'dir. Tersine, enzimatik olmayan esmerleşmeden sorumlu olan ve balın antioksidan ve antibakteriyel özelliklerinden dolayı faydalı etkileri ile ilişkilendirilen polimerik moleküller olan melanoidinler, MR'ın son aşamalarında üretilir. Bu makalenin amacı, balda MR ürünleri olarak HMF ve melanoidinlerin oluşumu ile ilgili olumlu ve olumsuz etkilerinin yanı sıra oluşumu hakkında bir inceleme sunmaktır.

Anahtar Kelimeler: bal; Maillard reaksiyonu; hidroksimetilfurfural; melanoidinler.

GENIŞLETILMIŞ ÖZET

Amaç: Bu makalenin amacı, balda MR ürünleri olarak HMF ve melanoidinlerin oluşumu ile ilgili olumlu ve olumsuz etkilerinin yanı sıra oluşumu hakkında bir inceleme sunmaktır.

Google Scholar, PubMed ve ScienceDirect gibi sunuculardaki tam erişim sağlanan bilimsel makalelerde bu bilgilere başvurulmuştur. Dahil edilen anahtar kelimeler şunlardır: tatlı, Maillard reaksiyonu; hidroksimetilfurfural; melanoidinler.

Giriş: Gıda endüstrisi, balın hidrasyon, viskozite, aroma, renk, higroskopiklik, karışabilirlik ve yayılabilirlik gibi bazı özellikleri desteklediği gözlemlendiğinden birçok farklı gıda ürününde bal kullanmaktadır (Otles, 2006). Ancak bal, Maillard reaksiyonu (MR) için son derece elverişli koşullar sunar. MR, genellikle gıda işleme veya depolama sırasında meydana gelen karmaşık bir kimyasal reaksiyonlar ağıdır (Martins ve diğerleri 2001). MR sırasında oluşan geniş ürün yelpazesi, gıdanın kalitesini ve tüketiciler tarafından kabulünü etkileyen bir öneme sahiptir (Martins ve diğerleri 2001, Bertrand ve diğerleri 2018). Organoleptik özelliklerde (aroma ve pigment oluşumu), protein işlevselliğinde ve sindirilebilirlikteki (Machiels & Istasse 2002, Lund & Ray 2017) değişikliklerin yanı sıra, MR ürünlerinin anti/pro-oksidan potansiyeli açısından sağlık üzerinde olumlu veya olumsuz etkileri olabilir. İmmünojenisite, alerjenite ve kanserojenite (Bertrand ve ark. 2018). Bu nedenle, bu reaksiyon gıda kimyasında en önemli olarak kabul edilir (Machiels & Istasse, 2002).

Tartışma: Balda yüksek konsantrasyonda indirgeyici şekerler, glukoz ve fruktoz ve proteinlerin ve serbest amino asitlerin (özellikle lizin) varlığı, depolama sırasında ve bazı bal işleme adımlarında meydana gelen MR (Türkmen ve ark. 2006) için uygun koşullardır (Türkmen ve ark. 2006). Isıya maruz kalmayı gerektiren ve nihai ürünün daha homojen bir sunumunu sağlamak (Blidi ve ark. 2017), viskoziteyi azaltmak ve paketleme sürecini kolaylaştırmak (Chua ve ark. 2014), kristalleşmeyi önlemek (Turhan ve ark. 2008) için tanıtılmıştır Escriche ve diğerleri 2009). Ayrıca ozmofilik mayaların yok edilmesi yoluyla fermantasyonun engellenmesi ve dolayısı ile (Subramanian ve diğerleri. 2007) raf ömrünü uzatılır (Guo ve diğerleri 2011). Ancak ısıtma, HMF ve diğer bileşiklerin konsantrasyonlarını artırarak bal kalitesini doğrudan etkileyebilecek (Chua ve ark. 2014) bir işlemdir (Annapoorani ve ark. 2010).

Sonuç: Çok sayıda reaksiyon yolu ve ürünü ile MR hala araştırma gerektirmektedir. Balın üretiminde ve sanayileşmesinde arılarda gözlemlenenin aksine balın insan sağlığına potansiyel olarak zararlı bir gıda haline getiren HMF konsantrasyonları hakkında bilgi bulunmamakla birlikte, balın kalitesini mümkün olduğu kadar uzun süre korumak amaçlanmaktadır. Toksik maddelerin üretimini önlemek ve balın antioksidan ve antibakteriyel özelliklerini güçlendiren arzu edilen bileşenleri oluşturmak yerine MR'nin oluşmasını önlemek veya yavaşlatmak. Melanoidinlerle ilgili olarak baldaki oluşumları hakkında çok az bilgi vardır. Bu nedenle hasat sonrası ısıl işlemlerin ve depolamanın bu bileşiklerin bileşimi üzerindeki etkilerini ve bunların balın besin değeri ve fonksiyonel özellikleri üzerindeki etkilerini belirlemek için daha fazla araştırmaya ihtiyaç vardır.

INTRODUCTION

Maillard reaction: The origin

MR is a non-enzymatic browning reaction involving proteins and reducing sugars (Bertrand et al. 2018); where temperature, reaction time, water activity (Aw), pH, concentration and nature of the reagents in the food are important factors to consider (van Boekel, 2001).

MR can be divided into three main stages. The first corresponds to the reversible formation of a glucosylamine and its Amadori or Heyns rearrangements. In the second, degradation of the Amadori and Heyns rearrangement products occurs, leading to the formation of aromatic heterocyclic compounds. In the third, polymerization of the reactive intermediates and production of melanoidins is observed (Machiels & Istasse 2002, Silvan et al. 2011). All stages are interrelated, can occur simultaneously and are affected by reaction conditions (Silvan et al., 2011).

Although MR was first described by Louis Maillard in 1912, a first coherent scheme was presented by Hodge in 1953 (Echavarría et al. 2012, De Oliveira et al. 2016). However, to facilitate its understanding, a modification of the scheme proposed by Zamora & Hidalgo (2005) is presented here. **Figure 1**

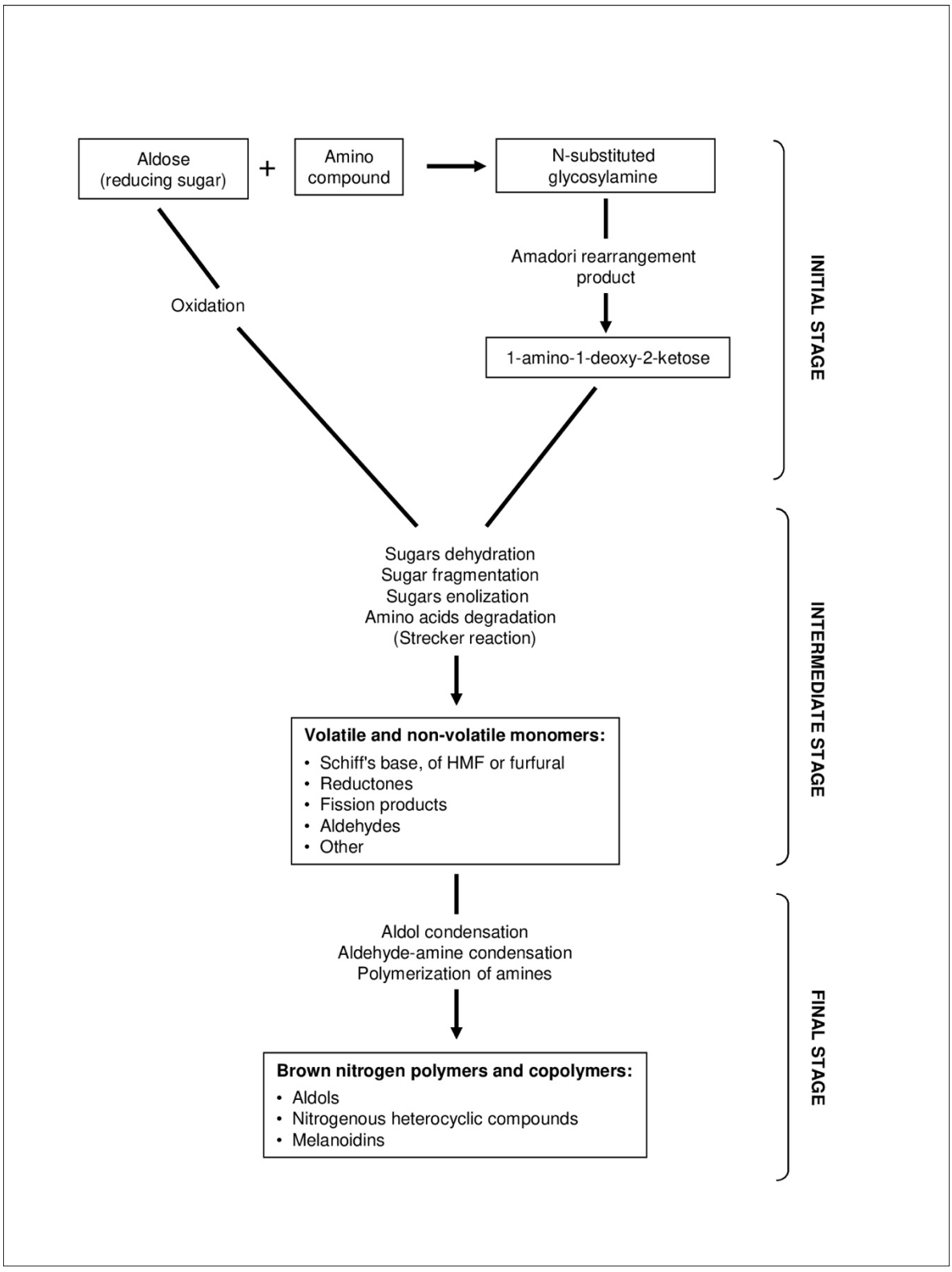

Figure. 1. General mechanism of Maillard reaction

First stage of MR is characterized by an initial glycosylation reaction (De Oliveira et al. 2016), a reaction that occurs when functional amino groups of free amino acids or amino groups of side chains of peptides and proteins condense with carbonyl groups of reducing sugars to form a Schiff base (Bertrand et al. 2018), with the release of a water molecule (De Oliveira et al. 2016). Schiff base obtained is in equilibrium with an N-glycosylamine, which will undergo a different rearrangement depending on the nature of the initial reducing sugar involved. If this is an aldose it will give rise to an aldosamine and then to the Amadori compound, a 1-amino-1-deoxyketose; whereas a ketose will give rise to a ketosamine and then to the Heyns compound, a 2-amino-2-deoxyketose (Echavarría et al. 2012, Bertrand et al. 2018). This second reaction is irreversible and obtaining the Amadori and Heyns intermediates marks the end of the first MR stage (Bertrand et al. 2018), where no color changes are observed (Martins et al. 2001, Echavarría et al. 2012;) and the nutritional value may be reduced due to decreased amino acid availability (De Oliveira et al. 2016).

Second MR or propagation stage depends on the reaction conditions, such as pH, temperature and Aw (Bertrand et al. 2018). With respect to a system such as honey, the Amadori product degrades producing mainly a 1, 2-enolysis with the formation of furfural (when dealing with pentoses) or hydroxymethylfurfural (HMF) (when dealing with hexoses) (Martins et al. 2001). All these compounds are highly reactive, resulting in the carbonyl groups being able to condense with free amino groups, leading to the incorporation of nitrogen into the reaction products, while the dicarbonyl compounds react with the amino acids to form aldehydes and aminoketones. This reaction is known as Strecker degradation (Martins et al. 2001). Bertrand et al. (2018) concluded that Strecker degradation plays an important role not only in the generation of aromatic properties of the product but also in the browning itself.

Finally, in the final stage of MR, condensation occurs between some of the formed products or with amino compounds to form brown pigments and polymers (Zamora & Hidalgo 2005). Meanwhile, amino acids could react with the unsaturated carbonyl molecules resulting in the formation of melanoidins (Bertrand et al. 2018).

Hydroxymethylfurfural, really harmful?

5-Hydroxymethyl-2-furaldehyde or, more commonly named, 5-hydroxymethylfurfural, consists of a furan ring containing functional groups: aldehyde and alcohol (Zirbes et al. 2013) and is formed at room temperature by dehydration of fructose in acidic media (Huidobro & Simal 1984).

Since the 1950s, the presence of HMF has been identified in a wide variety of thermally processed foods, and depending on the production technology and storage, levels in foods vary considerably (Abraham et al. 2011).

HMF formation in honey occurs naturally (Sanz & Sanz 1994) because the pH is set between 3.2 and 4.5 for *Apis mellifera* (Karabagias et al. 2014) and from 2.5 to 3.8 for stingless bee honeys (Nordin et al. 2018), which is accelerated if the honey has been heated or stored at high temperatures (Sanz & Sanz 1994). Thus, the concentration of HMF is in direct relation to the degree of heat or aging of honey (Khalil et al. 2010; Turkut et al. 2018).

From harvesting to packaging, honey can be exposed to a series of conditions that cause, to a greater or lesser extent, the deterioration of its intrinsic qualities (Visquert 2015). HMF concentration is one of the parameters that can be controlled with good practices mainly during storage, where several factors influence its formation such as: the use of metallic containers, the environmental humidity, thermal and/or photochemical stress (Spano et al. 2006).

European Union (EU 2002) and *Codex Alimentarius* have established that the HMF content of *Apis mellifera* honey after harvesting and/or blending should not exceed 40 mg/kg. However, in case of honey of declared origin from tropical countries or regions, as well as blends of these honeys, a maximum of 80 mg/kg is accepted (CXS 12 1981). Although stingless bee honeys are obtained in conditions of high humidity and temperature (Chuttong et al. 2016), Vit et al. (2004) suggested that for these honeys the maximum should be 40 mg/kg, while the Malaysian Standardization Department has established that the maximum HMF content should be 30 mg/kg (Suntiparapop et al. 2012). This is supported by researchers such as Sousa et al. (2016) and Biluca et al. (2016), who referred not to have detected quantifiable HMF concentrations in honeys of the *Melipona* genus from southern Brazil. De Almeida et al. (2013)

reported that low content of HMF in *M. subnitida* honey indicate that the samples were collected and stored under adequate conditions.

This possible resistance to HMF formation in stingless bee honey can be explained by several factors, for example, the type of carbohydrates, higher glucose content instead of fructose, and higher Aw and acidity which slows down MR (Biluca et al. 2014).

Considering the properties of honey, it is evident the need to prolong its shelf life without altering its nutritional and medicinal properties (Biluca et al. 2014). For this purpose, some applied techniques such as dehumidification, heat treatment and refrigeration (Turhan et al. 2008), have been used, being demonstrated that heat treatment has been the simplest and most effective (Biluca et al. 2014), and although the content of high concentrations of HMF in honey is more related to prolonged storage in inadequate conditions than to its heating (Turhan et al. 2008), it is important to establish time and temperature parameters to avoid loss of honey quality not only related to HMF production, but also to the loss of its nutritional (Chua et al. 2014) and bioactive properties such as antioxidant (Šarić et al. 2013) and antimicrobial (Libonnatti et al. 2014).

Souza et al. (2010) reported that *Melipona subnitida* honey subjected to heat treatment at 70°C for 4, 8, 16 and 24 hours caused a reduction in moisture content and total acidity, but increased the content of HMF and reducing sugars. Contrarily, Biluca et al. (2014), reported that HMF concentrations were not detected in 13 honey samples from stingless bees subjected to heat treatment of 75, 85 and 95° C for 20, 40 and 60 seconds, oppositely to what was observed in *A. mellifera* honey when subjected to the same conditions. These results suggest that high temperature associated with a short heat treatment could be an effective way to prolong shelf life without affecting the HMF content in stingless bee honey.

Effect of HMF on honey bee hives

Honey production depends to a large extent on the health, survival and quality of honey bees. Naturally, the main source of carbohydrates for honey bees is the nectar flower collected. However, according to the apibotanical cycle of each region, food and nutrients decrease at certain times of the year. Therefore, beekeepers must use alternative sources to replace the carbohydrate supply of bees, such as sucrose, high fructose corn syrup, fruit sugars or invert sugars (Neupane & Thapa 2005).

In any case, these sources are not safe due to the formation of HMF and its by-products, being a potential threat to bees (Neupane & Thapa 2005). As an example, in Belgium during 2009-2010 abnormal losses of bee colonies were observed and upon further analysis it was found that some of these colonies had been fed over winter with beet invert sugar syrup, which had a HMF concentration of up to 475 mg/kg (Zee & Pisa 2010). In fact, HMF present in bee feeding syrups during critical times could be a new factor involved in bee mortality, coupled with the invasive mite *Varroa destructor* and pathologies caused by the microsporidium *Nosema* spp and viruses (Zirbes et al. 2013).

In the case of *A. mellifera* honey used as prop food, mainly in stingless bee colonies, when this is fresh HMF may be absent or in very low concentrations (Bogdanov et al. 1999) However, temperatures inside a hive normally exceed 20°C and can reach up to more than 40°C, at which time HMF concentration can reach 10 mg/kg honey (Gregorc et al. 2020). And while HMF concentrations <10-15 mg/kg in honey pose little risk to bees, concentrations above 150 mg/kg can cause 50% colony mortality within 16 days to 19 days (Jachimowicz & El Sherbiny 1975) due to induction of lethal ulceration of the intestinal tract (Le Blanc et al., 2009).

And although several studies confirm a toxic effect of HMF on bee health, more research is needed to evaluate the involvement of HMF in their mortality to determine the maximum concentration of HMF in their sustaining food (Zirbes et al. 2013).

Negative effects of HMF on human health

The detection of HMF in several food products prompted the evaluation of potential health risks taking into account dietary intake (Abraham et al. 2011). *In vitro* studies indicate that HMF can be cytotoxic, mutagenic, carcinogenic and genotoxic (Capuano & Fogliano 2011) and thus the importance of controlling its concentrations in foods such as honey. However, most studies report this toxicity only at the preclinical level (Shapla et al. 2018), as presented in **Table 1.**

Table 1. Studies demonstrating the carcinogenic-mutagenic effect of HMF

Associated metabolite	Observed effect	Reference
5-hydroxymethylfurfural	Development of lipomatous renal tumors in rats.	Schoental et al. (1971)
5-hydroxymethylfurfural	Chromosomal aberrations in a Chinese hamster V79-derived cell line constitutively expressing human sulfotransferase SULT1A1 and CYP2E1.	Nishi, Miyakawa & Kato (1989)
5-hydroxymethylfurfural	Induction and promotion of foci of aberrant colon crypts (preneoplastic lesion) as a marker of colon cancer in rats.	Archer et al. (1992) Zhang et al. (1993) Bruce et al. (1993)
5-chloromethyfural	Induction of hepatocarcinoma in male B6C3F1 rats.	Surh et al. (1994)
5-sulfo-oximethylfurfural	Induction of cutaneous papillomas in mice.	Surh & Tannenbaum (1994)
5-sulfo-oximethylfurfural	Mutagenicidad en *Salmonella typhimurium* TA 104.	Lee et al. (1995)
5-hydroxymethylfurfural	DNA damage in five cell lines possessing different levels of SULT1A1 activity (mouse L5178Y, no activity; Chinese hamster: V79-Hp-PST, high activity; V79, negligible activity; human: HEK293, highest activity; and Caco-2, low activity).	Durling, Busk & Hellman (2009)
5-hydroxymethylfurfural 5-sulfo-oximethylfurfural	Increased number of small intestinal adenomas and flat dysplastic lesions (flat ACF) in the large intestine of mice.	Svendsen et al. (2009)
2,5 bishidroximethylfuran	Mutagenicity towards *S. typhimurium* TA100 expressing human SULT1C2	Glatt et al. (2011)
2,5 dimethylfuran	Genotoxicity in rat hematopoietic cells.	Fromowitz et al. (2012)
5- hydroxymethylfurfural	Genotoxicity in hepatic and renal cells of FVB/N (FVB) mice expressing hSULT1A1/1A2.	Høie et. al. (2015)

Although information obtained from bioassays have deduced the toxicity potential of HMF, epidemiological studies or case reports on the possible association of HMF and cancer in humans is insufficient, so in addition is required data to corroborate this activity also in vivo (Morales 2008).

As HMF is a product of the non-enzymatic reaction, there is no fixed concentration in foods, and due to the various factors involved, its content varies even among foods of the same type. Nevertheless, honey is a safe food with respect to HMF concentration when compared to other processed food products that require higher processing temperatures as well as longer times and different additives (Shapla et al. 2018).

As an example, Turhan et al. (2008) demonstrated that nectar honey processed at 95°C for 90 min and at 90°C for 75 min showed HMF levels below 40 mg/kg, while foods such as cookies baked at 300° C can contain up to 1100.1 mg/kg (Ameur et al. 2007); coffee up to 900 mg/kg roasted at 240°C (Murkovic

& Bornik 2007); bread between 3.4 to 176.1 mg/kg, depending on fermentation conditions, leavening agents added, crust and crumb thickness and type of bread (Ramírez et al. 2000).

In addition, it should be considered that, except for countries such as Turkey and Germany with a consumption 1.246 and 1.034 kg *per capita* of honey, the world consumption does not exceed one kilogram (Sanchez et al. 2018), so it is unlikely that honey is a high risk factor to be considered.

Positive effects of HMF on human health

Although the adverse effects of HMF have been studied, some research indicates that, as an antioxidant, the HMF exhibits free radical scavenging capacity as well as significant protective effects on erythrocytes (Zhao et al. 2013) and hepatocytes (Wang et al. 2010) against reactive oxygen species (ROS) induced damage.

Li et al. (2010) demonstrated that HMF could be a potent therapeutic agent against acute mountain sickness, high-altitude cerebral and pulmonary edema (HAPE) by observing that HMF increased mitochondrial membrane potential and decreased phosphorylated ERK levels in human umbilical cord cells. While in a murine model, pre-exposure to HMF significantly attenuated the extent of hypoxia-induced blood-brain barrier (BBB) permeability and decreased the extent of neuronal damage in the CA1 region of the hippocampus.

Yamada et al. (2011) demonstrated that HMF acts as an inhibitor of allergic reactions at different stages, to inhibit basophil and mast cell degranulation, interfering with antigen-antibody cross-linking, antibody-receptor binding and blocking calcium influx in IgE-sensitized cells.

Contrarily to studies demonstrating the carcinogenic-mutagenic effect of HMF, a study by Zhao et al. (2014) using the A375 cell line, indicated that HMF can induce apoptosis and G0/G1 arrest in DNA-damaged cells through the ROS mediated signal transduction pathway.

Regarding the use of HMF for other pathological conditions, it has been observed that HMF exerts an anti-inflammatory effect by decreasing nuclear factor kappa B NFκB activator (Kitts et al. 2012), inhibiting xanthine oxidase activity (Li et al. 2010) thereby decreasing purine catabolism and uric acid production, and thus the risk of hyperuricemia (Hayden & Tyagi 2004).

Tolerable daily intake (TDI) of HMF

HMF is found in many foods, with the estimated intake ranging from 4 to 30 mg per person per day (Abraham et al. 2011).

Many researches have revealed that the susceptibility of cells to HMF depends on the presence and expression levels of receptors, metabolism, structure and enzymatic activity (Shapla et al. 2018). At the preclinical level, no toxic effects have been observed at daily doses ranging from 80 to 100 mg/kg body weight (Zhao et al. 2014). Although the TDI for HMF has been established at 132 mg/day using a 40-fold safety margin and Janzowski et al. (2000) 30-150 mg per person, in a study carried out in Spain with 268 students, a statistically significant level of SMF in plasma was found although the daily intake of HMF of the students throughout the day was 10-70 mg (Pastoriza et al. 2016).

Currently, the European Food Safety Authority (EFSA) has set a threshold of 0.54 mg/day for the intake of furan derivatives used as flavoring agents in Europe (EFSA 2005). However, it should be noted that most of the experiments concerning the health effects of HMF have been conducted *in vitro* and in experimental animals. Therefore, it is not possible to determine a TDI based on the data available to date, which is why further research, especially at the clinical level, needs to be considered and assessed to update the TDI for HMF (Shapla et al. 2018).

HMF mitigation in the honey industry

There is no concrete strategy to mitigate HMF formation in honey due to numerous precursor and types of reaction orders involved. In addition to the fact that HMF is formed following a zero-order kinetic process in an exponential manner (Capuano et al. 2008). However, knowing the positive correlation between elevated temperature-time and acidic pH parameters for HMF formation is that several strategies can be adopted to mitigate its formation (Gökmen et al. 2007). Indeed, since long storage periods lead to elevated HMF formation in honey (Khalil et al. 2010) shortening the storage period as well as decreasing the processing temperature in case honey is subjected to heat treatment can significantly decrease HMF formation (Al-Diab & Jarkas 2015).

Even though more developed methods have been developed in recent years to mitigate HMF formation or to remove it from foods, including ultraviolet

irradiation, addition of phytochemicals, yeast fermentation, vacuum treatment microwave heating, non-thermal processing and formula adjustment (Lee et al. 2019), so far, there are few studies analyzing the effects of these technologies on physicochemical properties and sensory attributes in honey.

What about melanoidins?

Melanoidins are heterogeneous polymers of high molecular weight (5 kDa) that are formed in the final stage of MR (Tagliazucchi & Verzenolli 2014), absorb light at a wavelength of about 420 nm (Lindenmeier et al. 2002), and are largely responsible for the characteristic brown color of foods such as coffee, cocoa, bread or honey (Lindenmeier et al. 2002), which among other physical properties, make foods more palatable to consumers (Friedman 1996).

A critical step for the formation of melanoidins appears to be the degradation of Amadori products, in which several highly reactive intermediate propagators are produced (furans, pyrroles, pyrazines, dicarbonyl compounds) which, through reactions with each other and with free amino groups of amino acids or proteins, ultimately lead to the formation of melanoidin polymers (Van Boekel 1998).

Although at present, researchers have not been able to fully describe the structure of melanoidins (Liu et al. 2020), three main types have been described: the first consisting of furan or pyrrole repeat units (Tressl et al. 1998); the second based on cross-linking of proteins with low molecular weight color compounds (Hofmann 1998); and the third based on sugar degradation products forming polymers by aldol condensation and/or intact carbohydrates (Cämmerer et al. 2002).

Little information has been described on the occurrence and biological activities of melanoidins in honey; however, it appears to be an ideal natural system to study melanoidin formation and its influence on antioxidant activity by containing the main substrates for MR to occur (Brudzynski 2012).

Identification of melanoidins and methods for their quantification are usually based on the following criteria: the degree of browning and color formation after heat treatment, the molecular size of the pigments and their antioxidant activity (Manzocco et al. 2000), observing extremely significant correlations between these parameters in honey (Brudzinsky & Miotto 2011a, Brudzynski & Miotto 2011b, Brudzynski & Miotto 2011c).

Positive effects of melanoidins on human health

In the past, melanoidins were mainly perceived to cause a decrease in the nutritional value of foods, mainly due to the inactivation or destruction of amino acids or proteins or to the reduction of their absorption in the intestine following the inactivation of proteolytic enzymes (Martins et al. 2001, Silván et al. 2006), including trypsin (Ibarz et al. 2009).

Although the undesirable influence of MR end products on food quality, melanoidins show a number of beneficial effects, acting as antioxidant, antimicrobial, antihypertensive, antiallergic and prebiotic agents (Silván et al. 2006, Rufián-Henares & Morales 2007a). Melanoidins also demonstrate the ability to bind metal ions (Rufián-Henares & Pastoriza 2009, Tagliazucchi & Verzenolli 2014) and are considered antimutagenic compounds and tumor growth inhibitors (Langner & Rzeski 2013 as is presented in **Table 2.**

With respect to honey, in several of their investigations Brudzynski & Miotto (2011a, b, c) have demonstrated strong correlations between the concentrations of melanoidins and phenolic compounds and thus with their antioxidant and antibacterial capacity.

To date, the literature is contradictory on the levels of antibacterial activity of honey during storage, as some researchers have reported that exposure of honey to heat or prolonged storage resulted in a loss of antibacterial activity (Soliman et al. 2019), others found no correlation between time and antibacterial activity (Ríos et al. 2001). Therefore, Brudzynski (2012), concludes that the antibacterial and antioxidant activities of honey are influenced by the stages of MR (Brudzynski, 2012), since in the intermediate stage, where the generation of dicarbonyl compounds such as methylglyoxal, an important component in Manuka honey from *Leptospermum* spp. increases the antibacterial activity of honey (Adams et al. 2009, Mavric et al. 2008), in the advanced stage, a decrease in antibacterial activity occurs, associated with increased protein cross-linking, the formation of polyphenol-protein complexes and their incorporation into melanoidins (Brudzynski 2012).

Negative effects of melanoidins on human health

Even though most research has shown that

melanoidins play a crucial role in different biological activities, and have diverse functional properties with potential benefits on human health (Diaz et al. 2020), recent studies have shown that melanoidins may exert a pro-oxidant activity, which may be related to the formation of radicals by a Fenton mechanism due to the presence of iron or copper cations (Ibarz et al. 2009).

Wen *et al.* (2005) observed that the decrease of the antioxidant action based on the metal chelating activity of melanoidin is lost at high temperatures resulting in a gain of the cytotoxic pro-oxidant function.

Table 2. Studies demonstrating some functional properties of melanoidins

Effect	Melanoidins source	Results	Reference
Antimicrobial	Reaction between food protein or glycine and lactose or glucose.	Reduced adhesion and cell density of *H. pylori* in the gastric mucosa of mice.	Hiramoto et al. (2004)
	Red wine	Inhibition of in vitro growth of *L. monocytogenes, Salmonella* Enteritidis and *E. coli.*	Goulas et al. (2018)
Antioxidant	Bread crust	Activity as monofunctional glutathione-S-tranasferase inducers of "pronylated" proteins that are part of melanoidins.	Lindenmeier, Faist & Hofmann (2002)
	Malt barley	Radical scavenging capacity by metamyoglobin assay.	Carvalho et al. (2014)
Antihypertensive	Coffe	Inhibitory activity of angiotensin-I-converting enzyme (ACE) in vitro	Rufián-Henares & Morales (2007b)
	Red wine	Inhibitory activity of angiotensin-I-converting enzyme (ACE) in vitro.	Goulas et al. (2018)
Anti-allergenic	Pigments derived from a reaction between xylose and glycine	Induction of IFN-γ expression in mouse spleen cells and IL-12 in macrophages exposed with ovalbumin as allergen.	Hayase et al. (2005)
Antitumoral	Glucose and L-proline purified	Decreased organization and loss of microtubule integrity in MCF-7 human mammary carcinoma cells.	Marko et al. (2002)
	Soy sauce extract	Significant suppression of cell growth in human colon carcinoma-derived HCT-15 and human gastric carcinoma-derived AGS.	Kamei et al. (1997)
Prebiotic	Bread crust	Selective growth of bifidobacteria from bread crust.	Borrelli & Fogliano (2005)
	Malt barley	Significant divergence in gut microbiota profiles and sustained short-chain fatty acid production in barley malt-fed rats.	Aljahdali et al. (2020)

Also, it has been observed that together with the chelation of Fe and Mg metal ions interfering with bacterial growth and survival (Rufián-Henares & Pastoriza 2009), the antibacterial action of melanoidins may be the result of the inactivation of bacterial proteins due to their binding to semiquinones/quinones, leading to a permanent destruction of cell membranes (Rufián-Henares & Morales 2008). However, it should be noted that these effects have also been observed during plant and seed development affecting the viability of the latter during storage (Narayana Murthy & Sun 2000, Rawel & Rohn 2010). Thus, although this antibacterial effect could be considered beneficial during the therapeutic application of honey in the healing of wounds, the same cytotoxic characteristics towards human cells would pose a potential risk (Majtan 2011).

Therefore, although melanoidins have currently attracted much attention as a functional food ingredient, like polyphenols and other RM-derived products, they could also have dual (beneficial and potentially harmful) functions, depending on the balance (or lack thereof) between prooxidant and antioxidant activities (Brudzynski 2012).

CONCLUSION

MR with its multitude of reaction pathways and products still requires research. Although there is no information on HMF concentrations in honey that make it a potentially harmful food for human health, contrary to what has been observed in bees, in the production and industrialization of honey, the aim is to maintain its quality as long as possible, preventing MR from occurring or slowing it down to avoid the production of toxic substances and instead forming desirable components that potentiate the antioxidant and antibacterial properties of honey. With respect to melanoidins, there is little information on their formation in honey, so more research is needed to determine the effects of post-harvest heat treatments and storage on the composition of these compounds and their impact on the nutritional value and functional properties of honey.

Disclosure statement

The authors report no conflict of interest.

Acknowledgements

The autors acknowledge doctoral fellowship support from Consejo Nacional de Ciencia y Tecnología, CONACyT

REFERENCES

Abraham, K., Gürtler, R., Berg, K., Heinemeyer, G., Lampen, A., & Appel, K.E. 2011. Toxicology and risk assessment of 5-Hydroxymethylfurfural in food. Mol. Nutr. Food Res. 55(5): 667–678. doi: 10.1002/mnfr.201000564.

Adams, C.J., Manley Harris, M., & Molan, P.C. 2009. The origin of methylglyoxal in New Zealand manuka *(Leptospermum scoparium)* honey. Carbohydr. Res. 344(8): 1050–1053. doi: 10.1016/j.carres.2009.03.020.

Al-Diab, D., & Jarkas, B. 2015. Effect of storage and thermal treatment on the quality of some local brands of honey from Latakia markets. *J.* Entomol. Zool. 3(3): 328-334. (Retrieved by: 10.06.2021), https://www.entomoljournal.com/vol3Issue3/pdf/3-4-48.1.pdf.

Aljahdali, N., Gadonna-Widehem, P., Anton, P.M., & Carbonero, F. 2020. Gut microbiota modulation by dietary barley malt melanoidins. Nutrients. 12(1): 241. doi:10.3390/nu12010241.

Ameur, L., Mathieu, O., Lalanne, V., Trystram, G., & Birlouezaragon, I. 2007. Comparison of the effects of sucrose and hexose on furfural formation and browning in cookies baked at different temperatures. Food Chem. 101(4): 1407–1416. doi: 10.1016/j.foodchem.2006.03.049.

Annapoorani, A., Anilakumar, K.R., Khanum, F., Anjaneya, M.N., & Bawa, A.S. 2010. Studies on the physicochemical characteristics of heated honey, honey mixed with ghee and their food consumption pattern by rats. Aryuveda J. 31(2): 141-146. doi: 10.4103/0974-8520.72363.

Archer, M.C., Bruce, W.R., Chan, C.C., Corpet, D.E., Medline, A., Roncucci, L., Stamp, D., & Zhang, X.M. 1992. Aberrant crypt foci and microadenoma as markers for colon cancer. Environ. Health Perspect. 98: 195–197. doi: 10.1289/ehp.9298195.

Bertrand, E., El Boustany, P., Faulds, C., & Berdagué, J.L. 2018. The Maillard reaction in food: An introduction. In book: Reference Module in Food Science 1–10. doi: 10.1016/B978-0-08-100596-5.21459-5

Biluca, F.C., Braghini, F., Gonzaga, L.V., Costa, A.C.O., & Fett, R. 2016. Physicochemical profiles, minerals and bioactive compounds of stingless bee honey *(Meliponinae)*. J. Food Compos. Anal. 50: 61–69. doi: 10.1016/j.jfca.2016.05.007

Biluca, F.C., Della Betta, F., de Oliveira, G.P., Pereira, L. M., Gonzaga, L. V., Costa, A. C. O., & Fett, R. 2014. 5-HMF and carbohydrates content in stingless bee honey by CE before and after thermal treatment. Food Chem. 159: 244–249. doi: 10.1016/j.foodchem.2014.03.016.

Blidi, S., Gotsiou, P., Loupassaki, S., Grigorakis, S., & Calokerinos, A.C. 2017. Effect of thermal treatment on the quality of honey samples from Crete. Adv. Food Sci. Eng. 1(1): 1-8. doi: 10.22606/afse.2017.11001.

Bogdanov, S., Lüllmann, C., Martin, P., von der Ohe, W., Russmann, H., Vorwohl, G., et al. 1999. Honey quality and international regulatory standards: review by the International Honey Commission. Bee World. 80(2): 61–69. doi: 10.1080/0005772x.1999.11099428.

Borrelli, R.C., & Fogliano, V. 2005. Bread crust melanoidins as potential prebiotic ingredients. Mol. Nutr. Food Res. 49(7): 673–678. doi: 10.1002/mnfr.200500011.

Bruce, W.R., Archer, M.C., Corpet, D.E., Medline, A., Minkin, S., Stamp, D., Yin, Y., & Zhang, X.M. 1993. Diet, aberrant crypt foci and colorectal cancer. Mutat. Res. 290(1): 111–118. doi: 10.1016/0027-5107(93)90038-h.

Brudzynski, K. 2012. Honey melanoidins: Emerging novel understanding on the mechanism of antioxidant and antibacterial action of honey. In book: Honey: Current Research and Clinical Application. Chapter: II. Nova *Science Publishers, Inc.* 17-38. (Retrieved by: 27.07.2021), https://www.researchgate.net/publication/230899121_Honey_Melanoidins_Emerging_Novel_Understanding_on_the_Mechanism_of_Antioxidant_and_Antibacterial_Action_of_Honey.

Brudzynski, K., & Miotto, D. 2011a. Honey melanoidins: Analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. Food Chem. 127(3): 1023–1030. doi: 10.1016/j.foodchem.2011.01.075.

Brudzynski, K., & Miotto, D. 2011b. The recognition of high molecular weight melanoidins as the main components responsible for radical-scavenging capacity of unheated and heat-treated Canadian honeys. Food Chem. 125(2): 570–575. doi: 10.1016/j.foodchem.2010.09.049.

Brudzynski, K., & Miotto, D. 2011c. The relationship between the content of Maillard reaction-like products and bioactivity of Canadian honeys. Food Chem. 124(3): 869–874. doi: 10.1016/j.foodchem.2010.07.009.

Cämmerer, B., Jalyschko, W., & Kroh, L.W. 2002. Intact carbohydrate structures as part of the melanoidin skeleton. J. Agric. Food Chem. 50(7): 2083–2087. doi: 10.1021/jf011106w.

Capuano, E., & Fogliano, V. 2011. Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT - Food Sci. Technol. 44(4): 793–810. doi: 10.1016/j.lwt.2010.11.002.

Capuano, E., Ferrigno, A., Acampa, I., Ait-Ameur, L., & Fogliano, V. 2008. Characterization of the Maillard reaction in bread crisps. Eur. Food Res. Technol. 228(2): 311–319. doi: 10.1007/s00217-008-0936-5.

Carvalho, D.O., Correia, E., Lopes, L., & Guido, L.F. 2014. Further insights into the role of melanoidins on the antioxidant potential of barley malt. Food Chem. 160: 127–133. doi: 10.1016/j.foodchem.2014.03.074.

Chua, L.S., Adnan, N.A., Abdul-Rahaman, N.L., & Sarmidi, M.R. 2014. Effect of thermal treatment on the biochemical composition of tropical honey simples. Int. Food Res. J. 21(2): 773-778. doi: 10.18689/mjft-1000124.

Chuttong, B., Chanbang, Y., Sringarm, K., & Burgett, M. 2016. Physicochemical profiles of stingless bee (Apidae: Meliponini) honey from South East Asia (Thailand). Food Chem. 192: 149–155. doi: 10.1016/j.foodchem.2015.06.089.

CXS 12 1981. Adopted in 1981. Revised in 1987, 2001. Amended in 2019. Standard for Honey. Codex Alimentarius International Food Standards. (Access date: 05.09.2019)

https://www.fao.org/fao-who-codexalimentarius/sh-proxy/es/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B12-1981%252FCXS_012s.pdf.

De Almeida, M.L.B., Stramm, K.M., Horita, A., Barth, O.M., da Silva de Freitas, A., & Estevinho, L.M. 2013, Comparative study of the physicochemical and palynological characteristics of honey from *Melipona subnitida* and *Apis mellifera.* Int. J. Food Sci. Technol. 48(8): 1698–1706. doi: 10.1111/ijfs.12140.

De Oliveira, F.C., Coimbra, J.S. dos R., de Oliveira, E.B., Zuñiga, A.D.G., & Rojas, E.E.G. 2016. Food protein-polysaccharide conjugates obtained via the Maillard reaction: A Review. Crit. Rev. Food Sci. Nutr. 56(7): 1108–1125. doi: 10.1080/10408398.2012.755669.

Diaz, M.N., Cavia S.M., Salazar, G., Dolores R.P.M., & Muñiz, P. 2020. Cytotoxicity study of bakery product melanoidins on intestinal and endothelial cell lines. Food Chem. 343: 128405. doi: 10.1016/j.foodchem.2020.128405.

Durling, L.J.K., Busk, L., & Hellman, B.E. 2009. Evaluation of the DNA damaging effect of the heat-induced food toxicant 5-hydroxymethylfurfural (HMF) in various cell lines with different activities of sulfotransferases. Food Chem. Toxicol. 47(4): 880–884. doi; 10.1016/j.fct.2009.01.022.

Echavarría, A.P., Pagán, J., & Ibarz, A. 2012. Melanoidins Formed by Maillard Reaction in Food and Their Biological Activity. Food Eng. Rev. 4(4): 203–223. doi: 10.1007/s12393-012-9057-9.

EFSA 2005. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to flavouring group evaluation 13: furfuryl and furan derivatives with and without additional sidechain substituents and heteroatoms from chemical group 14. EFSA J. 215, 1–73 (Access 27.07.2019), https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2005.215.

Escriche, I., Visquert, M., Juan-Borrás, M., & Fito, P. 2009. Influence of simulated industrial thermal treatments on the volatile fractions of different varieties of honey. Food Chem. 112(2): 329–338. doi: 10.1016/j.foodchem.2008.05.068.

EU 2002. Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal, L 10, 47-52. (Access date: 18.08.2019), https://www.fao.org/faolex/results/details/es/c/LEX-FAOC037441/

Friedman, M. 1996. Food browning and its prevention: An overview. J. Agric. Food Chem. 44(3): 631–653. doi: 10.1021/jf950394r.

Fromowitz, M., Shuga, J., Wlassowsky, A.Y., Ji, Z., North, M., Vulpe, C.D., Smith, M.T., & Zhang, L. 2012. Bone marrow genotoxicity of 2, 5-dimethylfuran, a green biofuel candidate. Environ. Mol. Mutagen. 53(6): 488–491. doi: 10.1002/em.21707.

Glatt, H., Schneider, H., Murkovic, M., Monien, B.H., & Meinl, W. 2011. Hydroxymethyl-substituted furans: mutagenicity in *Salmonella typhimurium* strains engineered for expression of various human and rodent sulphotransferases. Mutagenesis. 27(1): 41–48. doi: 10.1093/mutage/ger054.

Gökmen, V., Çetinkaya Açar, Ö., Köksel, H., & Acar, J. 2007. Effects of dough formula and baking conditions on acrylamide and hydroxymethylfurfural formation in cookies. Food Chem. 104(3): 1136–1142. doi: 10.1016/j.foodchem.2007.01.008.

Goulas, V., Nicolaou, D., Botsaris, G., & Barbouti, A. 2018. Straw wine melanoidins as potential multifunctional agents: insight into antioxidant, antibacterial, and angiotensin-I-converting enzyme inhibition effects. Biomedicines. 6(3): 83. doi: 10.3390/biomedicines6030083.

Gregorc, A., Jurišić, S., & Sampson, B. 2020. Hydroxymethylfurfural affects caged honey bees *(Apis mellifera carnica)*. Diversity 2020, 12(1): 18. doi: 10.3390/d12010018.

Guo, W., Liu, Y. Zhu, X., & Wang, S. 2011. Temperature-dependent dielectric properties of honey associated with dielectric heating. J. Food Eng. 102(3): 209–216. doi: 10.1016/j.jfoodeng.2010.08.016.

Hayase, F., Usui, T., Nishiyama, K., Sasaki, S., Shirahashi, Y., Tsuchiya, N., et al. 2005. Chemistry and biological effects of melanoidins and glyceraldehyde-derived pyridinium as advanced glycation and products. Ann. N. Y. Acad. Sci. 1043(1); 104–110. doi: 10.1196/annals.1333.013.

Hayden, M.R., & Tyagi, S.C. 2004, Uric acid: A new look at an old risk marker for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus: The urate redox shuttle. Nutr. Metab. 1(1): 10. doi: 10.1186/1743-7075-1-10.

Hiramoto, S., Itoh, K., Shizuuchi, S., Kawachi, Y., Morishita, Y., Nagase, M., et al. 2004. Melanoidin, a food protein-derived advanced Maillard reaction product, suppresses *Helicobacter pylori in vitro* and *in vivo.* Helicobacter. 9(5): 429–435. doi: 10.1111/j.1083-4389.2004.00263.x.

Hofmann, T. 1998. Studies on melanoidin-type colorants generated from the Maillard reaction of protein-bound lysine and furan-2-carboxaldehyde - chemical characterisation of a red coloured domaine. Zeitschrift For Lebensmitteluntersuchung Und -Forschung A. 206 (4): 251–258. doi: 10.1007/s002170050253.

Høie, A.H., Svendsen, C., Brunborg, G., Glatt, H., Alexander, J., Meinl, W., & Husøy, T. 2015. Genotoxicity of three food processing contaminants in transgenic mice expressing human sulfotransferases 1A1 and 1A2 as assessed by the in vivo alkaline single cell gel electrophoresis assay. Environ. Mol. Mutagen. 56(8): 709–714. doi: 10.1002/em.21963.

Huidobro, J.F., & Simal, J. 1984. Parámetros de Calidad de la miel VI: hidroximetilfurfural. *Offarm.* 3 (12): 767-781. (Retrevied by: 24.06.2019), https://www.researchgate.net/publication/235698354_Parametros_de_calidad_de_la_miel_VI_Hidroximetilfurfural.

Ibarz, A., Garvín, A., Garza, S., & Pagán, J. 2009. Toxic effect of melanoidins from glucose–asparagine on trypsin activity. Food Chem. Toxicol. 47(8): 2071–2075. doi: 10.1016/j.fct.2009.05.025.

Jachimowicz, T., & El Sherbiny, G. 1975. Zur problematik der verwendung von invertzucker für die bienenfütterung. Apidologie, Springer Verlag, 6 (2): 121- 143. ffhal-00890379f (Retrevied by: 20.07.2019), https://hal.archives-ouvertes.fr/hal-00890379/document.

Janzowski, C., Glaab, V., Samimi, E., Schlatter, J., & Eisenbrand, G. 2000. 5-Hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. Food Chem. Toxicol. 38(9): 801–809. doi: 10.1016/s0278-6915(00)00070-3.

Kamei, H., Koide, T., Hashimoto, Y., Kojima, T., Umeda, T., & Hasegawa, M. 1997. Tumor cell growth-inhibiting effect of melanoidins extracted from miso and soy sauce. *Cancer* Biother. Radiopharm. 12(6): 405–409. doi: 10.1089/cbr.1997.12.405.

Karabagias, I.K., Badeka, A., Kontakos, S., Karabournioti, S., & Kontominas, M.G. 2014. Characterization and classification of *Thymus capitatus* (L.) honey according to geographical origin based on volatile compounds, physicochemical parameters and chemometrics. Food Res. Int. 55: 363–372. doi: 10.1016/j.foodres.2013.11.032.

Khalil, M.I., Sulaiman, S.A., & Gan, S.H. 2010. High 5-hydroxymethylfurfural concentrations are found in Malaysian honey samples stored for more than one year. Food Chem. Toxicol. 48(8-9): 2388–2392. doi: 10.1016/j.fct.2010.05.076.

Kitts, D.D., Chen, X.M., & Jing, H. 2012. Demonstration of antioxidant and anti-inflammatory bioactivities from sugar–amino acid Maillard reaction products. J. Agric. Food Chem. 60 (27): 6718–6727. doi: 10.1021/jf2044636.

Langner, E., & Rzeski, W. 2013. Biological properties of melanoidins: A review. Int. J. Food Prop. 17(2): 344–353. doi: 10.1080/10942912.2011.631253.

Le Blanc, B.W., Eggleston, G., Sammataro, D., Cornett, C., Dufault, R., Deeby, T., & St. Cyr, E. 2009. Formation of hydroxymethylfurfural in domestic high-fructose corn syrup and its toxicity to the honey bee *(Apis mellifera).* J.

Agric. Food Chem. 57 (16): 7369–7376. doi: 10.1021/jf9014526.

Lee, C.H., Chen, K.T., Lin, J.A., Chen, Y.T., Chen, Y.A., Wu, J.T., & Hsieh, C.W. 2019. Recent advances in processing technology to reduce 5-hydroxymethylfurfural in foods. Trends Food Sci. Technol. 93: 271-280. doi: 10.1016/j.tifs.2019.09.021.

Lee, Y.C., Shlyankevich, M., Jeong, H. K., Douglas, J. S., & Surh, Y.J. 1995. Bioactivation of 5-hydroxymethyl-2-furaldehyde to an electrophilic and mutagenic allylic sulfuric acid ester. Biochem. Biophys. Res. Commun. 209 (3): 996–1002. doi: 10.1006/bbrc.1995.1596.

Li, M.M., Wu, L.Y., Zhao, T., Xiong, L., Huang, X., Liu, Z.H., et al. 2010. The protective role of 5-HMF against hypoxic injury. Cell Stress Chaperones. 16(3): 267–273. doi: 10.1007/s12192-010-0238-2.

Libonnatti, C., Varela, S., & Basualdo, M. 2014. Antibacterial activity of honey: A review of honey around the world. J. Microbiol. Antimicrob. 6(3): 51–56. doi: 10.5897/jma2014.0308.

Lindenmeier, M., Faist, V., & Hofmann, T. 2002. Structural and functional characterization of pronyl-lysine, a novel protein modification in bread crust melanoidins showing in vitro antioxidative and phase I/II enzyme modulating activity. J. Agric. Food Chem. 50(24): 6997–7006. doi: 10.1021/jf020618n.

Liu, X., Xia, B., Hu, L., Ni, Z., Thakur, K., & Wei, Z. 2020; Maillard conjugates and their potential in food and nutritional industries: A review. Food Frontiers. 1(4): 382-397. doi: 10.1002/fft2.43.

Lund, M.N., & Ray, C.A. 2017. Control of Maillard Reactions in Foods: Strategies and Chemical Mechanisms. J. Agric. Food Chem. 65(23): 4537-4552. doi: 10.1021/acs.jafc.7b00882.

Machiels, D., & Istasse, L. 2002. La réaction de Maillard : importance et applications en chimie des aliments. Ann. Med. Vet. 146(6): 347-352. Available: http://www.facmv.ulg.ac.be/amv/articles/2002_146_6_04.pdf.

Majtan, J. 2011. Methylglyoxal—A potential risk factor of Manuka honey in healing of diabetic ulcers. Evid. Based Complement. Alternat. Med. 1–5. doi: 10.1093/ecam/neq013.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M.C., & Lerici, C.R. 2000. Review of non-enzymatic browning and antioxidant capacity in processed foods. Trends Food Sci. Technol. 11(9-10): 340–346. doi: 10.1016/s0924-2244(01)00014-0.

Marko, D., Kemény, M., Bernady, E., Habermeyer, M., Weyand, U., Meiers, S., Frank, O., & Hofmann, T. 2002. Studies on the inhibition of tumor cell growth and microtubule assembly by 3-hydroxy-4-[(E)-(2-furyl)methylidene]methyl-3-cyclopentene-1,2-dione, an intensively coloured Maillard reaction product. Food Chem. Toxicol. 40(1): 9–18. doi: 10.1016/s0278-6915(01)00093-x.

Martins, S.I.F.S., Jongen, W.M.F., & van Boekel, M.A.J.S. 2001. A review of Maillard reaction in food and implications to kenetic modelling. Trends Food Sci. Technol. 11: 364-373. doi: 10.1016/S0924-2244(01)00022-X.

Mavric, E., Wittmann, S., Barth, G., & Henle, T. 2008. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka *(Leptospermum scoparium)* honeys from New Zealand. Mol. Nutr. Food Res. 52(4): 483–489. doi: 10.1002/mnfr.200700282.

Morales, F.J. 2008. Hydroxymethylfurfural (HMF) and related compounds. Process-Induced Food Toxicants: Occurrence, Formation, Mitigation, and Health Risks. John Wiley & Sons, USA, 135–174. doi: 10.1002/9780470430101.ch2e.

Murkovic, M., & Bornik, M.A. 2007. Formation of 5-hydroxymethyl-2-furfural (HMF) and 5-hydroxymethyl-2-furoic acid during roasting of coffee. Mol. Nutr. Food Res. 51(4): 390–394. doi: 10.1002/mnfr.200600251.

Narayana Murthy, U.M., & Sun, W.Q. 2000. Protein modification by Amadori and Maillard reactions during seed storage: roles of sugar hydrolysis and lipid peroxidation. J. Exp. Bot. 51(348): 1221–1228. doi: 10.1093/jexbot/51.348.1221.

Neupane, K., & Thapa, R. 2005. Alternative to off-season sugar supplement feeding of honeybees. J Inst Agric Anim Sci. 26: 77–81.

doi: 10.3126/jiaas.v26i0.615.

Nishi, Y., Miyakawa, Y., & Kato, K. 1989. Chromosome aberrations induced by pyrolysates of carbohydrates in Chinese hamster V79 cells. Mutat. Res. Lett. 227(2): 117–123. doi: 10.1016/0165-7992(89)90007-9.

Nordin, A., Sainik, N.Q.A.V., Chowdhury, S.R., Saim, A.B., & Idrus, R.B.H. 2018. Physicochemical properties of stingless bee honey from around the globe: A comprehensive review. J. Food Compos. Anal. 73: 91-102. doi: 10.1016/j.jfca.2018.06.002.

Otles S. 2006. Antioxidative properties of honey in poultry meat. Science of honey. (Access date: 16.04.2019), http://eng.ege.edu.tr/~otles/honey/?hny=hnylnk27 [23 April 2019].

Pastoriza de la C.S., Álvarez, J., Végvári, Á., Montilla, G.J., Cruz, L.O., Delgado, A.C., & Rufián-Henares, J.A.. 2016. Relationship between HMF intake and SMF formation *in vivo:* An animal and human study. Mol. Nutr. Food Res. 61(3): 1600773. doi: 10.1002/mnfr.201600773.

Ramírez, J.A., Guerra, H.E, & García, V.B. 2000. Browning Indicators in bread. J. Agric. Food Chem. 48(9): 4176–4181. doi: 10.1021/jf9907687.

Rawel, H.M., & Rohn, S. 2010. Nature of hydroxycinnamate-protein interactions. Phytochem. Rev. 9 (1): 93–109. doi: 10.1007/s11101-009-9154-4 https://produccioncientificaluz.org/index.php/cientifica/article/view/14793.

Ríos, A.M., Novoa, M.L., & Vit, P. 2001. Effects of extraction, storage conditions and heating treatment on antibacterial activity of *Zanthoxylum fagara* honey from Cojedes, Venezuela. RevicyhLUZ. 11(5): 397-402. (Retrevied by: 21.07.2019), https://produccioncientificaluz.org/index.php/cientifica/article/view/14793.

Rufián-Henares, J.A., & Morales, F.J. 2007. Angiotensin-I converting enzyme inhibitory activity of coffee melanoidins. J. Agric. Food Chem. 55(4): 1480–1485. doi: 10.1021/jf062604d.

Rufián-Henares, J.A., & Morales, F.J. 2007. Functional properties of melanoidins: *In vitro* antioxidant, antimicrobial and antihypertensive activities. Food Res. Int. 40(8): 995–1002. doi: 10.1016/j.foodres.2007.05.002.

Rufián-Henares. J.A., & Morales, F.J. 2008. Antimicrobial activity of melanoidins against *Escherichia coli* is mediated by a membrane damage mechanism. J. Agric. Food Chem. 56: 2357–2362 doi: 10.1021/jf073300+.

Rufián-Henares, J.A., & Pastoriza, de la C.S. 2009. Antimicrobial activity of coffee melanoidins-A study of their metal-chelating properties. J. Agric. Food Chem. 57(2): 432–438. doi: 10.1021/jf8027842.

Sanchez, C., Castignani, H., & Rabaglio, M., 2018. El mercado apícola internacional. INTA. Argentina. (Access date: 11.06.2019) https://inta.gob.ar/sites/default/files/inta_cicpes_instdeeconomia_sanchez_mercado_apicola_internacional.pdf.

Sanz, C.S., & Sanz C.M.M. 1994. Índice de diastasas y contenido de hidroximetilfurfural en las mieles de La Rioja. Zubía 12:181-1991. (Retrieved by: 18.05.2019), https://dialnet.unirioja.es/servlet/articulo?codigo=110290.

Šarić, G., Marković, K., Vukičević, D., Lež, E., Hruškar, M., & Vahčić, N. 2013. Changes of antioxidant activity in honey after heat treatment. Czech J. Food Sci. 31(6): 601–606. doi: 10.17221/509/2012-cjfs.

Schoental, R., Hard, G., & Gibbard, S. 1971. Histopathology of renal lipomatous tumors in rats treated with the “natural” products, pyrrolizidine alkaloids and α, β-unsaturated aldehydes. *JNCI: J*. Natl. Cancer Inst. 47(5): 1037-1034. doi: 10.1093/jnci/47.5.1037.

Shapla, U.M., Solayman, M., Alam, N., Khalil, M.I., & Gan, S.H. 2018. 5-Hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chem. Cent. J. 12(35). doi: 10.1186/s13065-018-0408-3.

Silván, J.M., Assar, S.H., Srey, C., Dolores del Castillo, M., & Ames, J.M. 2011. Control of the Maillard reaction by ferulic acid. Food Chem. 128(1): 208–213.

doi: 10.1016/j.foodchem.2011.03.047.

Silván, J.M., van de Lagemaat, J., Olano, A., & Del Castillo, M.D. 2006. Analysis and biological properties of amino acid derivates formed by Maillard reaction in foods. J. Pharm. Biomed. Anal. 41(5): 1543–1551. doi: 10.1016/j.jpba.2006.04.004.

Soliman, W., El-Sharkawy, H., El-Santeel, F., & Khattaby, A. 2019. Effect of storage and heat on chemical-physical properties and antimicrobial activity of bee honey. J. Prod. Dev. 24(4): 773-786. doi: 10.21608/jpd.2019.81024.

Sousa, J.M.B. de, Souza, E.L. de, Marques, G., Benassi, M. de T., Gullón, B., Pintado, M.M., & Magnani, M. 2016. Sugar profile, physicochemical and sensory aspects of monofloral honeys produced by different stingless bee species in Brazilian semi-arid region. LWT - Food Sci. Technol. 65: 645–651. doi: 10.1016/j.lwt.2015.08.058.

Souza F.W.E., Mendes, A.E.M., Paiva, S., K. M., Barros, M.F.I., Reinaldo, O.V., Ribeiro L.C., Aroucha, S.M.C. 2010. Parâmetros físico-químicos do mel de abelha sem Ferrão (*Melipona subnitida*) após tratamento termico. Acta Vet. Bras. 4(3): 153-157. (Access date: 06.06.2019), https://www.researchgate.net/publication/277033378_PARAMETROS_FISICO-QUIMICOS_DO_MEL_DE_ABELHA_SEM_FERRAO_Melipona_subnitida_APOS_TRATAMENTO_TERMICO.

Spano, N., Casula, L., Panzanelli, A., Pilo, M., PIiu, P., Scanu, R., Tapparo, A., & Sanna, G. 2006. An RP-HPLC determination of 5-hydroxymethylfurfural in honeyThe case of strawberry tree honey. Talanta. 68(4): 1390–1395. doi: 10.1016/j.talanta.2005.08.003.

Subramanian, R., Umesh Hebbar, H., & Rastogi, N.K. 2007. Processing of Honey: A Review. Int. J. Food Prop. 10(1): 127–143. doi: 10.1080/10942910600981708.

Suntiparapop, K., Prapaipong, P., & Chantawannakul, P. 2012. Chemical and biological properties of honey from Thai stingless bee (*Tetragonula leaviceps).* J. Apic. Res. 51(1): 45–52. doi: 10.3896/ibra.1.51.1.06.

Surh, Y.J., & Tannenbaum, S.R. 1994. Activation of the Maillard reaction product 5-(hydroxymethyl) furfural to strong mutagens via allylic sulfonation and chlorination. Chem. Res. Toxicol. 7(3): 313–318. doi: 10.1021/tx00039a007.

Surh, Y. J., Liem, A., Miller, J.A., & Tannenbaum, S.R. 1994. 5-Sulfooxymethylfurfural as a possible ultimate mutagenic and carcinogenic metabolite of the Maillard reaction product, 5-hydroxymethylfurfural. Carcinogenesis. 15(10): 2375–2377. doi: 10.1093/carcin/15.10.2375.

Svendsen, C., Husøy, T., Glatt, H., Paulsen, J.E., & Alexander, J. 2009. 5-Hydroxymethylfurfural and 5-sulfooxymethylfurfural increase adenoma and flat ACF number in the intestine of Min/+ mice. Anticancer Res. 29: 1921–1926 (Retrieved by: 19.06.2019), https://ar.iiarjournals.org/content/anticanres/29/6/1921.full.pdf.

Tagliazucchi, D., & Verzelloni, E. 2014. Relationship between the chemical composition and the biological activities of food melanoidins. Food Sci. Biotechnol. 23(2): 561–568. doi: 10.1007/s10068-014-0077-5.

Tressl, R., Wondrak, G.T., Krüger, R.P., & Rewicki, D. 1998. New Melanoidin-like Maillard Polymers from 2-Deoxypentoses. J. Agric. Food Chem. 46(1): 104–110. doi: 10.1021/jf970657c.

Turhan, I., Tetik, N., Karhan, M., Gurel, F., & Reyhan Tavukcuoglu, H. 2008. Quality of honeys influenced by thermal treatment. LWT - Food Sci. Technol. 41(8): 1396–1399. doi: 10.1016/j.lwt.2007.09.008.

Turkmen, N., Sari, F., Poyrazoglu, E.S., & Velioglu, Y.S. 2006. Effects of prolonged heating on antioxidant activity and colour of honey. Food Chem. 95(4): 653–657. doi: 10.1016/j.foodchem.2005.02.004.

Turkut, G.M., Degirmenci1, A, Yildiz, O., Can, Z., Cavrar, S., Yaylaci K. F., Kolayli, S. 2018. Investigating 5-hydroxymethylfurfural formation kinetic and antioxidant activity in heat treated honey from different floral sources. J. Food Meas. Charact. 12:2358–2365.doi: https://doi.org/10.1007/s11694-018-9852-y.

Van Boekel, M.A.J.S. 1998. Effect of heating on Maillard reactions in milk. Food Chem. 62(4): 403–414. doi: 10.1016/s0308-8146(98)00075-2.

Van Boekel, M.A.J.S. 2001. Kinetic aspects of the Maillard reaction: a critical review. Nahrung. 45 (3): 150–159. doi: 10.1002/1521-3803(20010601)45:3<150::AID-FOOD150>3.0.CO;2-9.

Visquert, F.M. 2015. Influencia de las condiciones térmicas en la calidad de la miel. Universitat Politècnica de València. PhD diss., (Retrieved by: 13.04.2019), https://dialnet.unirioja.es/servlet/tesis?codigo=74887#:~:text=En%20la%20etapa%20de%20almacenamiento%2C%20los%20tiempos%20de%20residencia%20prolongados,condiciones%20de%20licuaci%C3%B3n%20y%20pasteurizaci%C3%B3n.

Vit, P., Medina, M. & Enríquez, M.E. 2004. Quality standards for medicinal uses of Meliponinae honey in Guatemala, Mexico and Venezuela. Bee World. 85(1): 2–5. doi: 10.1080/0005772x.2004.11099603.

Wang, M.Y., Zhao, F.M., Peng, H.Y., Lou, C.H., Li, Y., Ding, X., et al. 2010. Investigation on the morphological protective effect of 5-hydroxymethylfurfural extracted from wine-processed *Fructus corni* on human L02 hepatocytes. J. Ethnopharmacol. 130(2): 424–428. doi: 10.1016/j.jep.2010.05.024

Wen, X., Enokizo, A., Hattori, H., Kobayashi, S., Murata, M., & Homma, S. 2005. Effect of roasting on properties of the zinc-chelating substance in coffee brews. J. Agric. Food Chem. 53(7): 2684–2689. doi: 10.1021/jf048304i.

Yamada, P., Nemoto, M., Shigemori, H., Yokota, & Isoda, H. 2011. Isolation of 5-(Hydroxymethyl)furfural from *Lycium chinense* and its inhibitory effect on the chemical mediator release by basophilic cells. Planta Med. 77(05): 434–440. doi: 10.1055/s-0030-1250402.

Zamora, R., & Hidalgo, F.J. 2005. Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. Crit. Rev. Food Sci. Nutr. 45(1): 49–59. doi: 10.1080/10408690590900117.

Zee van der, R., & Pisa, L. 2010. Bijensterfte 2009-10 en toxische invertsuikersiroop Onderzoek naar de schadelijkheid voor bijen van Sint-Ambrosius (Fructo - Bee) Siroop. *Nederlands Centrum* Bijenonderzoek. 1-15. (Retrieved by: 27.06.2019), https://www.bijenonderzoek.nl/Downloads/Bijensterfte%202009-10_en%20toxische_%20invertsuikersiroop.pdf.

Zhang, X.M., Chan, C.C., Stamp, D., Minkin, S., C. Archer, M., & Bruce, W.R. 1993. Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethy1-2-furaldehyde in thermolyzed sucrose. Carcinogenesis. 14(4): 773–775. doi: 10.1093/carcin/14.4.773.

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z., Chen, T., 2013. *In vitro* antioxidant and antiproliferative activities of 5-Hydroxymethylfurfural. J. Agric. Food Chem. 61(44), 10604–10611 doi: 10.1021/jf403098y.

Zhao, L., Su, J., Li, L., Chen, J., Hu, S., Zhang, X., & Chen, T. 2014. Mechanistic elucidation of apoptosis and cell cycle arrest induced by 5-hydroxymethylfurfural, the important role of ROS-mediated signaling pathways. Food Res. Int. 66: 186–196. doi: 10.1016/j.foodres.2014.08.051.

Zirbes, L., Nguyen, B.K., de Graaf, D.C., De Meulenaer, B., Reybroeck, W., Haubruge, E., & Saegerman, C. 2013. Hydroxymethylfurfural: A possible emergent cause of honey bee mortality? J. Agric. Food Chem. 61(49): 11865–11870. doi: 10.1021/jf403280n.