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Hydroxymethylfurfural: A Possible Emergent Cause of Honey Bee Mortality?

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ABSTRACT: Hydroxymethylfurfural (HMF), a common product of hexose degradation occurring during the Maillard reaction and caramelization, has been found toxic for rats and mice. It could cause a potential health risk for humans due to its presence in many foods, sometimes exceeding 1 g/kg (in certain dried fruits and caramel products), although the latter still is controversial. HMF can also be consumed by honey bees through bad production batches of sugar syrups that are offered as winter feeding. In Belgium, abnormal losses of honey bee colonies were observed in colonies that were fed with syrup of inverted beet sugar containing high concentrations of HMF (up to 475 mg/kg). These losses suggest that HMF could be implicated in bee mortality, a topic that so far has received only little attention. This paper reviews the current knowledge of the presence of HMF in honey bee environment and possible consequences on bee mortality. Some lines of inquiry for further toxicological analysis are likewise proposed.

KEYWORDS: *hydroxymethylfurfural (HMF), honey bee, mortality, syrup*

INTRODUCTION

Global pollinators have declined in abundance and diversity, which can affect natural ecosystems and agriculture.[1,2] Specifically, for several years, abnormal mortalities and weakening of honey bee colonies have been often observed in Europe and North America.[3] Bee populations in Europe have nonetheless been seriously affected by human activities. Between 1970 and 2007, the number of honey bee colonies in Europe gradually decreased from over 21 million to about 15.5 million.[4,5] Moreover, beekeepers in Europe and also in North America have repeatedly been confronted with elevated and sometimes unexplained winter losses.[6−8] A multitude of factors that may contribute to increased winter losses have been discussed comprehensively in the recent literature: invasive species, increased pathologies, climate, food resources, and low farmland biodiversity. Most prominently among them were the invasive mite *Varroa destructor* and pathologies caused by viruses and the microsporidian *Nosema* spp.[6,9−13] Hydroxymethylfurfural (HMF) present in syrups for bee feeding during winter could be a new factor implicated in bee mortality.

Indeed, in 2009−2010, abnormal losses of bee colonies were observed in Belgium. Later analyses showed that some of these colonies had been fed during winter with syrup of inverted beet sugar, which presented a concentration of HMF up to 475 mg/kg due to a bad production batch.[14] Several studies confirm a toxic effect of the HMF on the health of the bee.[15−17] However, the absence of toxicological data does not allow establishing a standard to guarantee no toxic effects for honey bees.

The objective of this review is multiple: understanding the mechanisms of formation of HMF, its presence in bee environment, its toxicity for honey bees, and its implication in bee mortality.

HMF MECHANISMS OF FORMATION

5-Hydroxymethyl-2-furaldehyde or, as it is more commonly referred to, 5-hydroxymethylfurfural consists of a furan ring, containing both aldehyde and alcohol functional groups (Figure 1).

HMF is a common Maillard reaction (the nonenzymatic browning) product formed through the reaction between reducing sugars and amino acids during heat treatment of food.[18−20] HMF can also be formed through acid-catalyzed

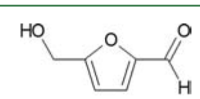

Figure 1. 5-Hydroxymethylfurfural.

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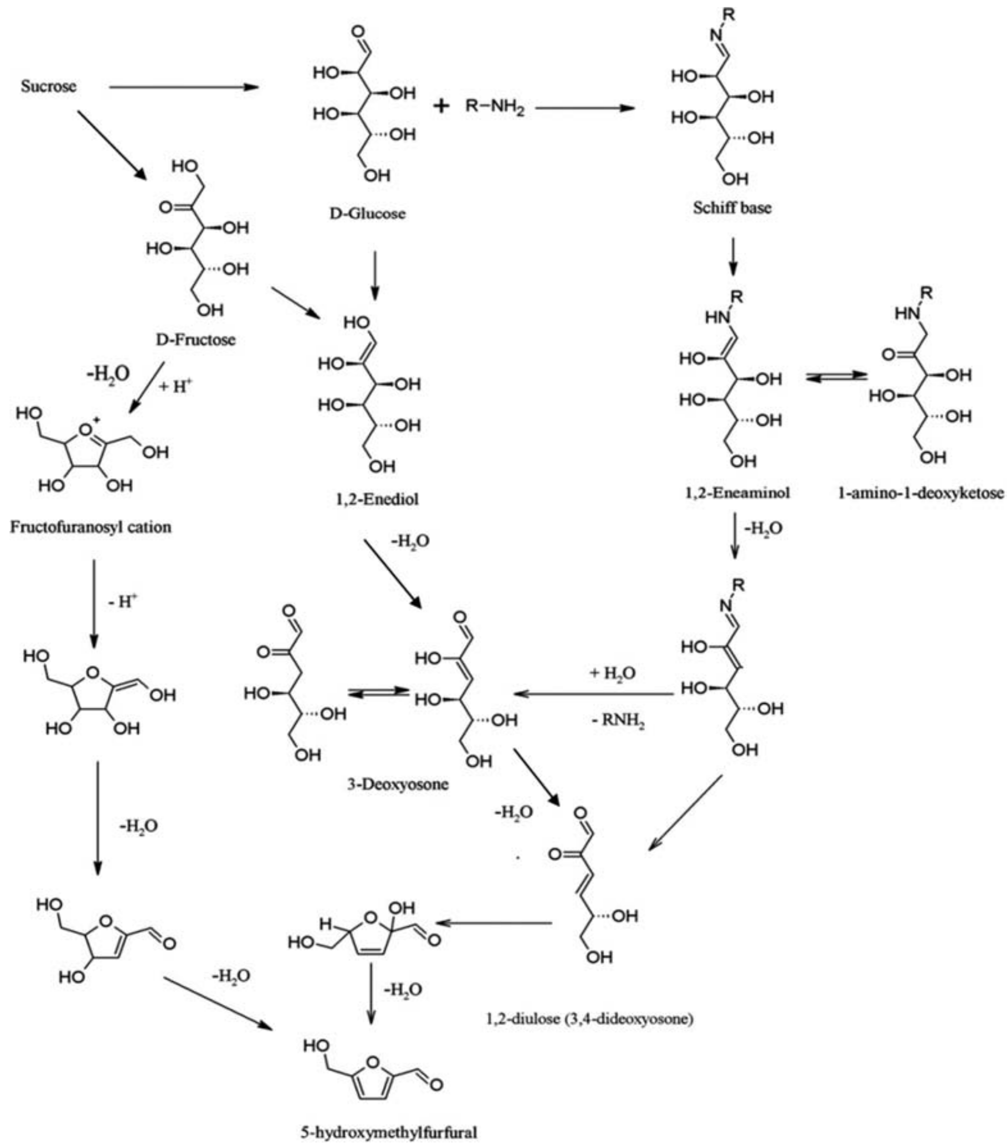

Figure 2. Proposed reaction scheme for the formation of 5-hydroxymethylfurfural in food (adapted from ref 54).

dehydration of hexoses, via 1,2-enolization followed by two consecutive dehydratation steps followed by a self-condensation and further dehydratation.[19,21,22] Figure 2 presents the main pathways to HMF formation in foods. HMF can be produced from all hexoses and also from those oligo- and polysaccharides that can yield hexoses upon hydrolysis. However, it appears to be more selectively produced from keto-hexose, notably from D-fructose.[23,24] HMF can also appear in products where water coexists with monosaccharides in acidic medium.[25] The activation energy for HMF formation is higher than that for HMF degradation, with the result that the maximum obtainable concentration increases with increasing temperature.[26] Apart from temperature, the rate of HMF formation in foods is dependent on the type of sugar,[27] pH,[28] water activity,[22,29] and concentration of divalent cations in the medium.[30]

The formation rate of HMF is increased by a higher enolization rate as well by a higher proportion of acyclic and furanose forms of fructose.[26] Detailed mechanisms of HMF formation were recently reviewed by Morales in 2009.[19]

HMF IN THE ENVIRONMENT AND IN BEE ENVIRONMENT

In overview, HMF is used in the synthesis of some fuel additives, organic compounds, and novolak type resins.[31,32] It is an intermediate in the synthesis of several crown ethers.[33] HMF is also utilized to produce polymers, surfactants, solvents, pharmaceuticals, and plant protection agents.[32,34] It is normally formed during thermal decomposition of sugars and carbohydrates. Moreover, glucose infusions are commonly used as vehicles for administering a variety of drugs. During its production, the solutions must be sterilized and HMF formation can occur.[19] Furthermore, HMF in foodstuffs has received special attention for years. Indeed, HMF is widely recognized as a marker of quality deterioration, resulting from excessive heating or inappropriate storage conditions in a wide range of foods containing carbohydrates.[19] In fact, the Codex Alimentarius of the World Health Organization and the European Union (EU Directive 110/2001) have defined a maximum HMF quality level in honey (40 mg/kg) and in apple juice (50 mg/kg) as a deterioration and heat treatment indicator. The HMF is also detected in spirits, wine, and other alcoholic beverages,[35−37] coffee,[38] milk,[39] fruit juices,[40−42] vinegars,[43] adult and baby cereals,[30,43,44] and breads.[45]

In bee environment, HMF is naturally present in honey in low quantity. It is produced by the action of the normal honey acidity on reducing sugars and sucrose at ambient temperature[19] and is also considered as a quality indicator for honey. As previously mentioned, to avoid heat treatment or long storage of honey, Directive 2001/110/CE[46] imposed HMF maximal concentrations in honey of 40 mg/kg for temperate regions and 80 mg/kg for tropical climates.

Different methods were applied to analyze HMF content in honey including gas chromatography coupled with mass spectrometry, a sensible method for minor constituents.[47] The International Honey Commission[48] recommends three methods for the analysis of HMF content in honey: two spectrophotometric methods (White and Winter methods)[49,50] and one RP-HPLC method.[36] These methods were recently compared[51] to show that White and Winter methods are fast but very few specific and sensitive, whereas the RP-HPLC method is slower but offers more precise results. In 1998, Ankalm[52] noted in a review that "... the suitability of the analytical methods for HMF is unsatisfactory and requires further investigation ...", and it is still true.

Bees can also be exposed to HMF through syrup that is offered as winter feeding. Indeed, it is a common beekeeping practice to replenish the food reserves of a bee colony after honey has been yielded in autumn. When bees feed on sugar syrup, they metabolize saccharose into glucose and fructose using invertase.[53] To facilitate the feeding of the bees, some beekeepers supply them with ready-made food before winter. This ready-made food is generally composed of inverted sugar syrup, in which fructose and glucose are directly available for honey bees. This ready-made food is therefore more susceptible to HMF formation. Moreover, beekeepers give homemade syrup based on sucrose and water and, sometimes, they add some ingredients such as vinegar or citrus juice that could enhance HMF formation. These compounds acidify syrups and increase HMF production. A long-term storage could bring an important evolution of the HMF content in syrups. Moreover, no study has focused on HMF metabolism in the digestive system of honey bees after oral ingestion, whereas pathways for HMF biotransformation were summarized in mammalians.[54]

All of these elements have to be considered to understand how HMF can contaminate honey bees and evaluate its impact on honey bee mortality.

■ TOXICITY OF HMF

There has been increased interest in HMF and furan derivates since data became available on the toxicity of these molecules. In fact, various animal experiments showed that HMF has a number of structural alerts that pose possible genotoxic and carcinogenic risks. Some studies have revealed that HMF may induce genotoxic and mutagenic effects in bacterial and human cells and promote colon and liver cancer in rats and mice.[55−59]

Human exposure to HMF can occur through pharmaceutical preparation, cigarette smoke, and consumption of a number of commonly available beverages and foods including breads, honeys, and fruit juices or jam. Humans can be exposed to HMF by inhalation, ingestion, or skin absorption. Although HMF is not yet considered a harmful substance for humans,[60] the subject is still a matter of debate. Some scientists have estimated the daily intake of HMF at 30−150 mg per person,[61] but no long-term cancer bioassays have been presented on HMF. However, few studies show cytotoxic effect on human blood cells[62] or DNA damages in several human cell lines after 3 h of exposure to 100 mM HMF.[63] Due to this potential risk for human health, some mitigation strategies of HMF in food were proposed, focusing on the most innovative and potentially exploitable at the industrial level.[64] Preventive and removal strategies were proposed at different levels of food processing including formulation, processing, and postprocessing.[64]

As explained previously, there has been an incident of honey bees that were exposed to HMF through syrup offered to bees as winter feeding. HMF seems to be toxic to honey bees: intestinal tract ulceration was suspected, which seemed to be lethal.[15] However, very few studies observed any toxicity of HMF to honey bees.[15−17] Jachimowicz et al.[16] found that the HMF content of 150 mg/kg in commercially acid-hydrolyzed invert sugar syrup caused a mortality of 50% within 16 days after the start of the feeding. HMF concentration of 30 mg/kg seems harmless to honey bees;[16] therefore, many specialists recommended that its concentration in inverted syrup may not exceed 20 mg/kg as it is in most honeys.[65] It was previously advised to control the HMF content of inverted sugar syrups before they were given to bees for feeding.[16] Several years later, during experimentations on the quality of syrups used for bee feeding, Ceksteryte and Racys[66] have suggested that HMF content of 48 mg/kg in sugar syrup from maize was harmless for wintering honey bees. Interestingly, they found that the content of HMF present in the initial syrups decreased in the syrups deposited by bees in the comb, suggesting that the bee organism is able to metabolize the HMF to some extent.[66] Later, Le Blanc et al.[17] used caged honey bees to evaluate the HMF dose−response effect on bee mortality with a high-fructose corn syrup (HFCS), a saccharose replacement for honey bees in the United States. They observed 50% of bee mortality after 19 days for HFCS with 150 mg/kg HMF. This is very close to the results of Jachimowicz et al.[16] After 26 days, they compared bee mortality for different HMF doses (57, 100, 150, 200, and 250 mg/kg) and found that only HFCS enriched with 250 mg/kg induced a significantly lower survival.

The toxicity of HMF to honey bees was increased by syrup crystallization. Indeed, during the crystallization process, a part of the syrup becomes solid and the HMF was concentrated in the liquor, being the unique phase accessible to honey bees.[67] These papers seemed to indicate that HMF participates in honey bee mortality, but standardization of experiments is necessary to define a HMF LD_{50} for bees.

Moreover, influence of HMF on bee mortality has to be associated with other mortality causes. For example, when the mite *Varroa destructor* parasitizes bees, it weakens their immune systems and makes the bees more susceptible to secondary infections, pesticides,[3,68,69] and probably others toxicants such as HMF. Another study has tested acaricides, fungicides, and drug interactions on honey bee mortality and found that approximately half the acaricide−acaricide and acaricide−fungicide combinations tested showed evidence of interactions, nearly all of which were agonistic and resulted in increased acaricide toxicity. On the other hand, two antimicrobial−acaricide combinations tested presented antagonistic interactions with the acaricide Fenpyroximate.[70] Antagonistic interaction is characterized by decreased toxicity of a drug or pesticide combination. Moreover, agonistic interactions between the model enzyme inhibitors piporonyl butoxide (PBO) and three acaricides (τ-fluvanilate, coumaphos, and Fenpyroximate) used in hives to eradicate the mite *V. destructor* were identified. These interactions imply that P450 enzymes play a role in detoxifying these acaricides in honey bees.[70] An agonistic interaction is defined by an elevated toxicity of a drug or pesticide combination. Some beekeepers give syrup to honey bees in combination with a treatment against *V. destructor* with acaricides, exposing honey bees to possible interactions between HMF and acaricides. Another study investigated the integrative effect of the microsporidian *Nosema* spp. and an insecticide (imidacloprid). Imidacloprid alone, in a concentration similar to that found in the natural environment, had no effect on honey bee mortality,[71] but a synergistic interaction was demonstrated on honey bee mortality when this pesticide was combined with *Nosema* spp.[72]

HMF, as some pesticides, could also be an indirect factor in bee mortality by modifying the natural behavior of honeybees. For example, recent research showed that a nonlethal exposure of honey bees to thiamethoxam (a neonicotinoid systemic pesticide) influences indirectly bee mortality due to homing failure at levels that could annihilate bee colonies.[73]

In conclusion, hydroxymethylfurfural is toxic for mice and rats, and some studies also suggest health risks for humans. It is not clear, though, what the impact of HMF and eventually other sugar degradation products is on honey bees, how they are metabolized in the bees, and what the impact is on bee behavior and mortality.

In this review we highlight that HMF can present a toxicological risk for honey bees giving rise directly or indirectly to bee mortality. We also noted the absence of toxicological data making it currently not possible to establish an action limit on the HMF content of sugar syrups used for bee feeding to manage the risk. Further experiments are necessary to evaluate the implication of HMF in honey bee mortality and to determine the maximal concentration in HMF authorized in winter feed syrups for honey bees.

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■ REFERENCES

(1) Biesmeijer, J. C.; Roberts, S. P. M.; Reemer, M.; Ohlemuller, R.; Edwards, M.; Peeters, T.; Schaffers, A. P.; Potts, S. G.; Kleulers, R.; Thomas, C. D.; Settele, J.; Kunin, W. E. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science* **2006**, *313*, 351−354.

(2) Gallai, N.; Salles, J. M.; Settele, J.; Vaissieres, B. E. Economic valuation of the vulnerability of world agriculture confroted with pollinator decline. *Ecol. Econ.* **2009**, *68*, 810−821.

(3) Haubruge, E.; Nguyen, B. K.; Widart, J.; Thomé, J.; Fickers, P.; Depauw, E. Le dépérissement de l'abeille domestique, *Apis mellifera* L., 1758 (Hymenoptera: Apidae): faits et causes probables. *Notes Fauniques Gembloux* **2006**, *59*, 3−21.

(4) Aizen, M. A.; Harder, L. D. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr. Biol.* **2009**, *19*, 915−918.

(5) FAO, Food and Agriculture Organization of the United Nations. *FAOSTAT*, 2009; http://faostat.fao.org.

(6) Higes, M.; Martin, R.; Meana, A. Nosema ceranae, a new microsporidian parasite in honey bees in Europe. *J. Invertebr. Pathol.* **2006**, *92*, 93−95.

(7) Oldroyd, B. P. What's killing honey bees? *PLoS Biol.* **2007**, *5*, e168.

(8) vanEngelsdorp, D.; Evans, J. D.; Saegerman, C.; Mullin, C.; Haubruge, E.; Nguyen, B. K.; Frazier, M.; Frazier, J.; Cox-Foster, D. L.; Chen, Y.; Underwood, R.; Tarpy, D. R.; Pettis, J. S. Colony collapse disorder: a descriptive study. *PLoS One* **2009**, *4*, e6481.

(9) Cox-Foster, D. L.; Conlan, S.; Holmes, E. C.; Palacios, G.; Evans, J. D.; Moran, N. A.; Quan, P.-L.; Briese, T.; Hornig, M.; Geiser, D. M.; Martinson, V.; vanEngelsdorp, D.; Kalkstein, A. L.; Drysdale, A.; Hui, J.; Zhai, J.; Cui, L.; Hutchison, S. K.; Simons, J. F.; Egholm, M.; Pettis, J. S.; Lipkin, W. I. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* **2007**, *318*, 283−287.

(10) de Miranda, J. R.; Cordoni, G.; Budge, G. The acute bee paralysis virus-Kashmir bee virus-Israeli acute paralysis virus complex. *J. Invertebr. Pathol.* **2010**, *103*, S30−S47.

(11) de Miranda, J. R.; Genersch, E. Deformed wing virus. *J. Invertebr. Pathol.* **2010**, *103*, S48−S61.

(12) Jonhson, R. M.; Evans, J. D.; Robinson, G. E.; Berenboum, M. R. Change in transcript abundance relating to colony collapse disorder in honey bees (*Apis mellifera*). *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 14790−14795.

(13) Rosenkranz, P.; Aumeier, P.; Ziegelmann, B. Biology and control of *Varroa destructor*. *J. Invertebr. Pathol.* **2009**, *103*, S96−S119.

(14) van der Zee, R.; Pisa, L. Bijensterfte 2009-10 en toxische invertsuikersiroop. *NCB Rapport 02/2010*; Nederlands Centrum Bijenonderzoek: Tersoal, The Netherlands, 2010; pp 1−15.

(15) Bailey, L. The effect of acid-hydrolysed sucrose on honeybees. *J. Apic. Res.* **1966**, *5*, 127−136.

(16) Jachimowicz, T.; El Sheribiny, G. Problematic der verwenung von invertzucker fur die bienenfutterung. *Apidology* **1975**, *6*, 121−143.

(17) LeBlanc, B. W.; Eggleston, G.; Sammataro, D.; Cornett, C.; Dufault, R.; Deeby, T.; St. Cyr, E. Formation of hydroxymethylfurfural in domestic high-frucose corn syrup and its toxicity to the honey bee (*Apis mellifera*). *J. Agric. Food Chem.* **2009**, *57*, 7369−7376.

(18) Friedman, M. Food browning and its prevention: an overview. *J. Agric. Food Chem.* **1996**, *44*, 631−653.

(19) Morales, F. J. Hydroxymethylfurfural (HMF) and related compounds. In *Process-Induced Food Toxicants: Occurence, Formation, Mitigation and Health Risks*; Stadler, R. H., Lineback, D. R., Eds.; Wiley-Blackwell: Hoboken, NJ, 2009.

(20) Tomlinson, A. J.; Landers, J. P.; Lewis, I. A. S.; Naylor, S. Buffer conditions affecting the separation of Maillard reaction products by capillary electrophoresis. *J. Chromatogr., A* **1993**, *652*, 171−177.

(21) Jun, M.; Shao, Y.; Ho, C. T.; Koetter, U.; Lech, S. Structural identification of nonvolatile dimerization products of glucosamine by gas chromatography−mass spectrometry, liquid chromatography−mass spectrometry, and nuclear magnetic resonance analysis. *J. Agric. Food Chem.* **2003**, *51*, 6340−6346.

(22) Kroh, L. W. Caramelisation in food and beverages. *Food Chem.* **1994**, *51*, 373−379.

(23) Rigal, L.; Gaset, A. Direct preparation of 5-hydroxymethyl-2-furancarboxaldehyde from polyholosides: a chemical valorisation of the Jerusalem artichoke (*Helianthus tuberosus* L.). *Biomass* **1983**, *3*, 151−163.

(24) Shalumova, T.; Tanski, J. M. 5-(Hydroxymethyl)furan-2-carbaldehyde. *Acta Crystallogr. E* **2010**, *E66*, o2266.

(25) Teixido, E.; Santos, F. J.; Puignou, L.; Galceran, M. T. Analysis of 5-hydroxymethylfurfural in foods by gas chrromatography-mass spectrometry. *J. Chromatogr., A* **2006**, *1135*, 85−90.

(26) Kuster, B. F. M. 5-Hydroxymethylfurfural (HMF). A review focussing on its manufacture. *Starch* **1990**, *42*, 314−321.

(27) Lee, H. S.; Nagy, S. Relative reactivities of sugars in the formation of 5-hydroxymethylfurfural in sugar-catalyst model systems. *J. Food Process. Preserv.* **1990**, *14*, 171−178.

(28) Gökmen, V.; Açar, Ö. C.; Kösksel, H.; Açar, J. Effect of dough formula and baking conditions on acrylamide and hydroxymethylfurfural formation in cookies. *Food Chem.* **2007**, *104*, 1136−1142.

(29) Gökmen, V.; Açar, Ö. C.; Serpen, A.; Morales, F. J. Effect of leaving agents and sugars on the formation of hydroxymethylfurfural in cookies during baking. *Eur. Food Res. Technol.* **2008**, *226*, 1031−1037.

(30) Gökmen, V.; Senyuva, H. Z. Improved method for the determination of hydroxymethylfurfural in baby foods using liquid chromatography-mass spectrometry. *J. Agric. Food Chem.* **2006**, *54*, 2845−2849.

(31) Brode, G. L.; Mark, H. F.; Othmer, D. F.; Overberger, C. G.; Seaborg, G. T.; Grayson, M. In *Encyclopedia of Chemical Technology*; Kirk, R. E., Othmer, D. F., Eds.; Wiley: New York, 1982; Vol. 17, pp 411−413.

(32) Zakrzewska, M. E.; Bogel-Lukasik, E.; Bogel-Lukasik, R. Ionic liquid-mediated fromation of 5-hydroxymethylfurfural − a promising biomass-derived building block. *Chem. Rev.* **2011**, *111*, 397−417.

(33) Larousse, C.; Rigal, L.; Gaset, A. Synthesis of 5,5′-oxydimethyl bis (2-furfural) by thermal dehydratation of 5-hydroxymethyl furfural in the presence of dimethylsulfoxide. *J. Chem. Technol. Biotechnol.* **1992**, *53*, 111−116.

(34) Kunz, M. Hydroxymethylfurfural, a possible basic chemical industrial intermediates. In *Inulin and Inulin-Containing Crops*; Fuchs, A., Ed.; Elsevier Science Publishers: Amsterdam, The Netherlands, 1993; Vol. 3, pp 149−160.

(35) Chatonnet, P.; Dubourdieu, D.; Boidron, J. N. Incidence des conditions de fermantation et d'élevage des vins blancs secs en barriques sur leur composition en substances cédées par le bois de chêne. *Sci. Aliment.* **1992**, *12*, 665−685.

(36) Jeuring, H. J.; Kuppers, F. J. E. M. High performance liquid chromatography of furfural and hydroxymethylfurfural in spirits and honey. *J. AOAC Int.* **1980**, *63*, 1215−1218.

(37) Laszlavik, M.; Gal, L.; Misik, S.; Erdei, L. Phenolic compounds in two Hungrian red wines matured in *Quercus robur* and *Quercus petrea* barrels: HPLC analyses and diode array detection. *Am. J. Enol. Vitic.* **1995**, *46*, 67−74.

(38) Arribas-Lorenzo, G.; Morales, F. J. Estimation of dietary intake of 5-hydroxymethylfurfural and related substances from coffee to Spanish population. *Food Chem. Toxicol.* **2010**, *48*, 644−649.

(39) Morales, F. J.; Romero, C.; Jimenez-Perez, S. An enhanced liquid chromatographic method for 5-hydroxymethylfurfural determination in UHT milk. *Chromatographia* **1992**, *33*, 45−48.

(40) Fukeli, T.; Pelayo, E. Sugars, alcohols, and hydroxymethylfurfural in aythentic varietal and commercial grape juices. *J. AOAC Int.* **1993**, *76*, 59−66.

(41) Gökmen, V.; Acar, J. Simultaneous determination of 5-hydroxymethylfurfural and patulin in apple juice by reversed-phase liquid chromatography. *J. Chromatogr., A* **1999**, *847*, 69−74.

(42) Lee, H. S.; Rouseff, R. L.; Nagy, S. HPLC determination of furfural and 5-hydroxymethylfurfural in citrus juices. *J. Food Sci.* **1986**, *51*, 1075−1076.

(43) Theobald, A.; Müller, A.; Anklam, E. Determination of 5-hydroxymethylfurfural in vinegar samples by HLPC. *J. Agric. Food Chem.* **1998**, *46*, 1850−1854.

(44) Fernandez-Artigas, P.; Guerra-Hernandez, E.; Garcia-Villanova, B. Browning indicators in model systems and baby cereals. *J. Agric. Food Chem.* **1999**, *47*, 2872−2878.

(45) Ramirez-Jimenez, A.; Guerra-Hernandez, E.; Garcia-Villanova, B. Browing indicators in bread. *J. Agric. Food Chem.* **2000**, *48*, 4176−4181.

(46) 2001/110/CE, D., Dec 20. *Off. J. Eur. Commun.* **2001**.

(47) Horvath, K.; Molnar-Perl, I. Silmultaneous GC-MS quantitation of o-phosphoric, aliphatic and aromatic carboxylic acids, proline, hydroxymethylfurfural and sugars as their TMS derivatives: in honeys. *Chromatographia* **1998**, *48*, 120−126.

(48) Bogdanov, S. Harmonized Methods of the International Honey Commission, IHC; http://www.bee-hexagon.net/files/file/fileE/IHCPapers/IHC-methods_2009.pdf, 2009.

(49) Whites, J. Spectrophotometric method for hydroxymethylfurfural in honey. *J. AOAC Int.* **1979**, *62*, 509−514.

(50) Winkler, O. Beitrag zum Nachwals und zur Bestimmung von Oxymethylfurfural in Honig und Kunsthonig. *Z. Lebensm. Unters. Forsch.* **1955**, *102*, 161−167.

(51) Zappala, M.; Fallico, B.; Arena, E.; Verzera, A. Methods for the determination of HMF in honey: a comparison. *Food Control* **2005**, *16*, 273−277.

(52) Anklam, E. A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chem.* **1998**, *63*, 549−562.

(53) Winston, M. L. *The Biology of the Honey Bees*; Harvard University Press: Cambridge, MA, 1987.

(54) Capuano, E.; Fogliano, V. Acrylamide and 5-hydroxymethylfurfural (HMF): a review on metabolism, toxicity, occurence in food and mitigation strategies. *LWT−Food Sci. Technol.* **2011**, *44*, 793−810.

(55) Glatt, H.; Sommer, Y. Health risk of 5-hydroxymethylfurfural (HMF) and related compounds. In *Acrylamide and Other Hazardous Compounds in Heat-Treated Foods*; Skog, K., Alexander, J., Eds.; Woodhead Publishing: Boca Raton, FL, 2006; pp 328−357.

(56) Monien, B. H.; Engst, W.; Barknowitz, G.; Seidel, A.; Glatt, H. Mutagenicity of 5-hydroxymethylfurfural in V79 cells expressing human SULT1A1: identification and mass spectrometry quantification of DNA adducts formed. *Chem. Res. Toxicol.* **2012**, *25*, 1484−1492.

(57) Severin, I.; Dumont, C.; Jondeau-Cabaton, A.; Graillot, V.; Chagnon, M.-C. Genotoxic activities of the food contaminant 5-hydroxymethylfurfural using different in vitro bioassays. *Toxicol. Lett.* **2010**, *192*, 189−194.

(58) Ulbricht, R. J.; Northup, S. J.; Thomas, J. A. A review of 5-hydroxymethylfurfural in parental solutions. *Fundam. Appl. Toxicol.* **1984**, *4*, 843−853.

(59) Zhang, X. M.; Chan, C. C.; Stamp, D.; Minkin, S.; Archer, M. C.; Bruce, W. R. Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethyl-2-furaldehyde in thermolyzed sucrose. *Carcinogenesis* **1993**, *14*, 773−775.

(60) Makawi, S. Z. A.; Taha, M. I.; Zakaria, B. A.; Siddig, B.; Mahmod, H.; Elhussein, A. R. M.; Kariem, A. G. Identification and quantification of 5-hydroxymethylfurfural HMF in some sugar-containing food products by HPLC. *Pakistan J. Nutr.* **2009**, *8*, 1391−1396.

(61) Janzowski, C.; Glaab, V.; Samimi, E.; Schlatter, J.; Eisenbrand, G. 5-hydroxymethylfurfural: assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food Chem. Toxicol.* **2000**, *38*, 801−809.

(62) Nassberger, L. Influence of 5-hydroxymethylfurfural (5-HMF) on the overall metabolism of human blood cells. *Hum. Exp. Toxicol.* **1990**, *9*, 211−214.

(63) Durling, L. J. K.; Busk, L.; Hellman, B. E. Evaluation of the DNA damaging of the heat-induced food toxicant 5-hydroxymethylfurfural (HMF) in various cells lines with different activities of sulfotransferase. *Food Chem. Toxicol.* **2009**, *47*, 880−884.

(64) Anese, M.; Suman, M. Mitigation strategies of furan and 5-hydroxymethylfurfural in food. *Food Res. Int.* **2013**, *51*, 257−264.

(65) Kammerer, F. X. Aktueller stand der erkenntnisse über die fütterung von bienen mit zucker. *Imkerfreund* **1989**, *1*, 12−14.

(66) Ceksteryte, V.; Racys, J. The quality of syrups used for bee feeding before winter and their suitability for bee wintering. *J. Apic. Sci.* **2006**, *50*, 5−14.

(67) Wilmart, O.; Reybroeck, W.; De Meulenaer, B.; de Graaf, D. C.; Nguyen, B. K.; Huyghebaert, A.; Saegerman, C. Analyse du risque posé en santé animale par la présence de l'hydroxyméthylfurfural dans les sirops de nourrissement des abeilles domestiques. *Ann. Med. Vet.* **2011**, *155*, 53−60.

(68) Gregory, P. G.; Evans, J. D.; Rinderer, T.; de Guzman, L. Conditional immune-gene suppression of honeybees parasitized by *Varroa* mites. *J. Insect Sci.* **2005**, *5*, 7.

(69) Yang, X.; Cox-Foster, D. L. Impact of an ectoparasite on the immunity and pathology of an invertebrate: evidence for host

immunosupression and viral amplification. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 7470−7475.

(70) Jonhson, R. M.; Dahlgren, L.; Siegfried, B. D.; Ellis, D. M. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). *PLoS One* **2013**, *8*, e54092.

(71) Nguyen, B. K.; Saegerman, C.; Pirard, C.; Mignon, J.; Widart, J.; Thirionet, B.; Verheggen, F.; Berkvens, D.; Depauw, E.; Haubruge, E. Does imidacloprid seed-treated maize have an impact on honey bee mortality? *J. Econ. Entomol.* **2009**, *102*, 616−623.

(72) Alaux, C.; Brunet, J.; Dussaubat, C.; Mondet, F.; Tchamitchian, S.; Cousin, M.; Brillard, J.; Baldy, A.; Belzunces, L.; Le Conte, Y. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). *Environ. Microbiol.* **2010**, *12*, 774−782.

(73) Henry, M.; Béguin, M.; Requier, F.; Rollin, O.; Odoux, J.; Aupinel, P.; Aptel, J.; Tchamitchian, S.; Decourtye, A. A common pesticide decrease foraging succes and survival in honey bees. *Science* **2012**, *336*, 348−350.