FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food and Health 12(4), 387-398 (2026) • https://doi.org/10.3153/FH26030 Research Article

The risk of acrylamide formation in baby biscuits reformulated with natural sweeteners

Aytül HAMZALIOĞLU

Cite this article as:
Hamzalıoğlu, A., (2026). The risk of acrylamide formation in baby biscuits reformulated with natural sweeteners. *Food and Health*, 12(4), 387–398. https://doi.org/10.3153/FH26030

Hacettepe University, Faculty of Engineering, Department of Food Engineering, Food Quality and Safety (FoQuS) Research Group, 06800 Beytepe, Ankara, Türkiye

ORCID IDs of the authors:
A.H. 0000-0002-3659-2023

Submitted: 04.03.2026
Revision requested: 07.05.2026
Last revision received: 25.05.2026
Accepted: 05.07.2026
Published online: 02.10.2026

Correspondence:
Aytül HAMZALIOĞLU
E-mail: aytulhamzalioglu@hacettepe.edu.tr

© 2026 The Author(s)

Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

Baby biscuits are one of the snack foods widely consumed by toddlers and children. Health authorities recommend limiting added sugars in infant and toddler food until age two. For this aim, added sugar sucrose is substituted with different natural sweeteners; however, this might increase the acrylamide risk in baby biscuits, while acrylamide exposure levels of toddlers and children are high through consumption of baby biscuits. This study investigated how the substitution of sucrose with commonly used natural sweeteners such as apple juice concentrate, molasses, and syrups impacts acrylamide formation in baby biscuits. The replacement of sucrose in the baby biscuit formulation with natural sweeteners resulted in increased acrylamide formation in baby biscuits. Acrylamide levels in baby biscuits made with sucrose were 26±0.5 ng/g, while they ranged from 6 ng/g to 572.6 ng/g in the baby biscuits made with natural sweeteners. The highest acrylamide formation was observed in the baby biscuits made with mulberry molasses. Natural sweeteners like molasses, characterized by higher reducing sugar, dicarbonyl, and HMF contents, showed a greater acrylamide-forming potential than refined sugar. Thermal degradation markers, like dicarbonyl compounds and HMF, in natural sweeteners should be considered when reducing added sugar levels in baby biscuit formulations.

Keywords: Baby biscuits, Acrylamide, Natural sweeteners, Molasses, Sucrose substitution, Baby nutrition

Introduction

Biscuits are one of the widely consumed ready-to-eat dry foods that are commonly given to babies and children as snacks due to their convenience, palatability, and extended shelf life (Damen et al., 2019; Demonteil et al., 2019; Moore et al., 2019). Traditionally, added sugars such as sucrose or glucose syrups have been incorporated into baby biscuits to enhance their sweetness, texture, browning reactions during baking, and overall sensory acceptance. However, growing scientific evidence and public health concerns have highlighted the potential detrimental consequences of early-life exposure to added sugars, particularly the long-term implications for metabolic health (Herrick et al., 2020).

International health authorities are increasingly recommending the limitation or avoidance of added sugars in foods specifically formulated for infants and toddlers until the age of two (EU, 2021). The World Health Organization recommends that adults and children limit their daily intake of added sugars to less than 10% of their total daily energy intake (WHO, 2022). Furthermore, it is noted that reducing daily intake of added sugars below 5% provides additional health benefits. The findings suggest that limiting sugar exposure during pregnancy and the first two years of life could protect against the development of type 2 diabetes and hypertension (Gracner et al., 2024)

In response to these concerns, extensive research has been conducted to reformulate common snacks and foods consumed by infants and children, thereby reducing the amount of added sugars. As reported by Velázquez et al. (2020), the sugar content of vanilla milk desserts designed for children has been reduced by 40% compared to commercial products. Similarly, Velázquez et al. (2021) showed a 25% reduction in sugar content in chocolate milk, vanilla yogurt, and vanilla milk desserts without any adverse effects on the preferences of children. In addition to these, Lima et al. (2019) achieved a21.6% reduction in added sugar in grape nectars without affecting children's liking. However, studies dealing with the reformulation of baby biscuits are limited. A common strategy to minimize added sugar involves the use of natural sweeteners, including honey and molasses, both by the industry for commercial purposes and by mothers for the preparation of home-made baby biscuits. These natural sweeteners provide sweetness derived from naturally occurring sugars and are often perceived by consumers as more "natural" alternatives to added sugar. Natural sweeteners, in addition to their sweetness, may also contain minor components such as vitamins and minerals, potentially enhancing their nutritional value.

Fruit juice concentrates (e.g., grape juice concentrate or apple juice concentrate) are amongst the most common natural sweeteners added to baby biscuits (Cogswell et al., 2015; Hutchinson et al., 2021; Klerks et al., 2023). Honey is also commonly substituted for added sugars in numerous sweet food products. Molasses, also known as "pekmez" as a traditional Turkish food, is a boiled fruit juice commonly produced from grape and other fruit juices, including apple, carob, sugar beet, apricot, and fig (Alpaslan & Hayta, 2002; Sengül et al., 2005). These sweeteners, commonly referred to as "healthy" and "natural", are mostly subjected to extensive thermal treatments for extended periods.

The substitution of added sugars with natural sweeteners presents both advantages and challenges from a technological perspective. The variations in sugar profiles can influence rheology, Maillard reactions, baking, and textural characteristics of baby biscuits. Moreover, the darker color and characteristic flavor of molasses may also affect the final products' appearance and sensory characteristics.

However, the baking process of biscuits also triggers a series of chemical reactions within the food components due to high thermal conditions and low moisture levels applied. The formation of acrylamide, a potential carcinogenic compound, is one of the negative consequences of the baking process. Acrylamide is formed in baby biscuits during baking as a result of the Maillard reaction between carbonyl compounds and asparagine (Boyaci Gunduz et al., 2017). The rate of acrylamide formation is substantially influenced by the quantity and reactivity of precursors, such as carbonyl compounds, as well as the thermal conditions applied.

Dietary acrylamide exposure levels vary depending on the region and individual eating habits. Notably, the southern and western regions of Europe primarily derive acrylamide from bread, crisp bread, and rusks, with an average intake ranging from 24 to 50% (Freisling et al., 2013). In contrast, acrylamide intake primarily originates from coffee consumption in the northern regions of Europe, followed by bread, crisp bread, and rusks, with an average intake of 33 to 40% (Freisling et al., 2013). In the UK, potatoes were identified as a significant contributor to acrylamide intake, accounting for approximately 15% (Freisling et al., 2013). On the other hand, acrylamide exposure levels are significantly influenced by the consumer's age. Toddlers and children are exposed to higher levels of dietary acrylamide than adults because they consume more acrylamide-rich foods like bakery products and chips. Baby biscuits are considered a significant contributor to the total daily acrylamide intake of toddlers and children (Cengiz

& Gündüz, 2013). Given the potential health risks associated with acrylamide exposure for toddlers and children, mitigation of acrylamide in baby snacks, such as baby biscuits and crackers, is of importance.

In 2017, the European Commission presented a recent regulation proposal that outlined benchmark acrylamide levels (EU, 2017). The proposal suggested that benchmark levels could be used to evaluate the efficacy of mitigation measures implemented by the industry. Acrylamide benchmark levels for biscuits and rusks specifically designed for infants and young children are specified as 150 ng/g (EU, 2017).

Although reformulating baby biscuits with natural sweeteners is a viable strategy for reducing added sugar intake, the potential formation of acrylamide by these natural sweeteners must be carefully evaluated. Since most of the natural sweeteners have already been exposed to thermal treatment during their production, they might be involved in acrylamide formation during baking of baby biscuits. Although the existing literature extensively surveys acrylamide levels in commercially available baby snacks, there is still limited information regarding the specific role of natural sweeteners in acrylamide formation. Therefore, this study aimed to investigate the effect of replacing sucrose with commonly used natural sweeteners on acrylamide formation in baby biscuits.

Materials and Methods

Materials

Organic whole-meal flour was purchased from Beyorganik (İstanbul, Türkiye). Formic acid (99%), hydrochloric acid (37%), and acetonitrile (gradient grade) were obtained from Merck (Darmstadt, Germany). Acrylamide, glucose, fructose, and amino acid mix were purchased from Sigma-Aldrich (Deisenhofen, Germany). Nylon syringe filters (0.45 μm) were purchased from Isolab Laborgeräte GMBH (Wertheim, Germany). Food-grade ingredients for biscuits and crackers, including shortening, refined flour, invert sugar, soda, and salt, were supplied by a local bakery company in Turkey. Natural sweeteners, apple juice concentrate, mulberry molasses, molasses, molasses mix, date syrup, carob bean syrup, and honey were purchased from a local supermarket in Ankara. Oasis MCX cartridges were purchased from Waters Corp. (Massachusetts, USA). Hypercarb (5 × 100 mm, 3 μm) HPLC column and Dionex CarboPac PA1 (1 × 250 mm) ion chromatography column were purchased from Thermo Fisher Scientific Corp. (California, USA). Merck SeQuant ZIC-HILIC (150 × 4.6 mm i.d., 3.5 μm) column was obtained from Merck (Darmstadt, Germany).

Preparation of Baby Biscuits

Baby biscuits were prepared according to the recipe described by Manley (2001). The baby biscuits containing sucrose consisted of 100 g whole meal flour, 33 g sucrose, 11.4 g shortening, 2.5 g lecithin, 2.5 g skimmed milk powder, 1.2 g sodium bicarbonate, 0.4 g salt and 26 mL water. In the reformulated baby biscuits, sucrose was replaced with natural sweeteners including apple juice concentrate, mulberry molasses, grape molasses, molasses mix, date syrup, carob bean syrup and honey. The amounts of natural sweeteners and added water were adjusted based on their carbohydrate and moisture contents as given in Table 1, to ensure and standardize equivalent total sugar and moisture levels among all baby biscuit formulations. Accordingly, after adjustment of water addition according to the characteristics of each sweetener source, comparable dough consistency and initial moisture levels across formulations were obtained. Diameter and the thickness of the biscuits were carefully standardized and adjusted prior to baking to ensure consistency among formulations.

Table 1. The carbohydrate content of natural sweeteners used and the added amounts of sweetener and water for the preparation of baby biscuits

Natural sweeteners	Carbohydrate content (%)	Water added (mL)	Added sweetener, g
Sucrose	100	26	33
Apple Juice Concentrate	62	5.8	53.2
Mulberry Molasses	75	15	44
Grape Molasses	70	11.9	47.1
Molasses Mix	70	11.9	47.1
Date Syrup	82	18.8	40.2
Carob Syrup	67	9.8	49.2
Honey	81	18.3	40.7

The amount of water in all formulations was adjusted by taking the water content of the natural sweeteners into account

Biscuit doughs were prepared by first mixing the sugar and shortening in a stand mixer (KitchenAid, Whirlpool Co and Meyer Co, USA) for 3 min at low speed, with the bowl scraped using a spatula at the end of each minute to ensure uniform mixing. Subsequently, lecithin, skimmed milk powder, and the sodium bicarbonate and salt dissolved in water were added to the mixture. After mixing the ingredients for 1 min at low speed, whole meal flour was incorporated and mixed for an additional 1 min at high speed. The resulting dough was sheeted and shaped with a diameter of 15.3 mm and a thickness of 2.5 mm. Baking was carried out in a Memmert Oven (UNE 400, Germany) at 220 °C for 5 min. All biscuits were prepared and baked in duplicate.

Analysis of Sugars in Natural Sweeteners by Ion Chromatography

Extraction of sugars from natural sweeteners was done using deionized water at 80 °C. One mL of the sample was weighed into tubes, and 9 mL of deionized water, 0.5 mL of Carrez I, and 0.5 mL of Carrez II solutions were added; then the mixture was vortexed for 5 min. After vortexing, the mixture was centrifuged at 8000×g for 3 min. Supernatants were filtered using a 0.45 µm nylon filter and collected in autosampler vials after certain dilutions for sugar analysis.

The sugar analysis of the samples was carried out using a Dionex Ion Chromatography ICS-6000 system (Waldbronn, Germany) equipped with an ICS-6000 SP pump and an AS-AP autosampler, coupled with an ICS-6000 DC conductivity detector and a temperature-controlled column oven. The chromatographic separations were performed on a Dionex CarboPac PA1 (1 × 250 mm) (ThermoFisher Scientific, California, USA) conditioned at 30 °C. The mobile phase was 50 mM NaOH, and the flow rate was set at 0.1 mL/min. The chromatographic run was completed in 25 min. The concentrations of glucose, fructose, and sucrose were calculated from the calibration curves built for each compound in the range between 5 and 100 g/L (5, 10, 20, 50, and 100 g/L). All analyses were performed in duplicate.

Analysis of HMF in Natural Sweeteners by High-Performance Liquid Chromatography-Diode Array Detector

For the analysis of HMF content of alternative sweeteners, the samples were initially extracted. One gram of sample was extracted with water (10 mL) by vortexing for 3 min. The extract was clarified using Carrez clarification. The mixture was then centrifuged at 10,000 x *g* for 5 min, and two mL of supernatant was immediately filtered through a 0.45 µm syringe filter. The filtered extract was put into an autosampler vial after being diluted.

The filtered extract was injected onto an Agilent 1100 HPLC system consisting of a quaternary pump, an autosampler, a diode array detector, and a temperature-controlled column oven. The chromatographic separations were performed on an Atlantis dC18 column using an isocratic mixture of 10 mM aqueous formic acid solution and acetonitrile (90:10, v/v) at a flow rate of 1.0 mL/min at 25°C. Data acquisition was performed by recording chromatograms at 285 nm. Concentration of HMF in samples was calculated by means of a calibration curve built in the range between 0.5 and 50 µg/mL (0.5, 1, 2, 5, 10 and 50 µg/mL). All analyses were performed in duplicate.

Analysis of Acrylamide in Natural Sweeteners and Baby Biscuits using Liquid Chromatography with Tandem Mass Spectrometry

A multiple-stage extraction technique for the analysis of acrylamide in baby biscuit samples was performed as described before Kocadağlı et al. (2025). One gram of ground baby biscuit sample was extracted with 10 mM formic acid in water containing 20 µg/L acrylamide-d_3. The first extraction was performed with 9 mL of extraction solvent, 0.5 mL Carrez I, and 0.5 mL Carrez II. The tube was vortexed for 3 min and centrifuged at 5000 ×g for 3 min. The second and third extractions were performed with 5 mL of extraction solvent each. The clear supernatants were combined into a test tube. One milliliter of the combined extract was passed through a preconditioned 30 mg Oasis MCX cartridge (Waters, Milford, MA, USA). After the first eight drops of the eluent were discarded, the remaining drops were collected into an autosampler vial. Preconditioning was performed using 1 mL of methanol and 1 mL of water, subsequently.

Acrylamide was analyzed by an Agilent 6475 Triple Quadrupole MS coupled with an Agilent 1290 Infinity II UPLC (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was performed on a Hypercarb (100 mm ×5 mm id., 3 µm, Thermo Fisher Scientific, Waltham, MA, USA) column with a gradient mixture of 10 mM formic acid in water (A) and 0.1% formic acid in acetonitrile (B) with a flow rate of 0.8 mL/min at 40 ◦C. The gradient program was as follows: 100% A for 3 min, linear decrease to 70% A within 1 min, 70% A for 1 min, linear increase to 100% A within 0.5 min, and 100% A for 4.5 min. The chromatographic run was completed in 10 min. The injection volume was set at 5 µL. The electrospray source in positive mode had the following settings: capillary voltage of 2.0 kV; nozzle voltage of 0 V; gas flow of 13 L/min at 300 ◦C; sheath gas flow of 11 mL/min at 350 ◦C; and nebulizer pressure of 60 psi. Acrylamide was identified using the MRM mode at three channels. The precursor ion m/z 72 was fragmented, and product ions m/z 55 (quantifier, fragmentor voltage of 70 V, collision energy of 9 V), m/z 44 (qualifier, fragmentor voltage of 70 V, collision energy of 24 V) and m/z 27 (qualifier, fragmentor voltage of 70 V, collision energy of 24 V) were monitored. Acrylamide-d_3 was used as an internal standard with an MRM transition of m/z 75 to 58 (fragmentor voltage of 70 V, collision energy of 9 V). Dwell time was 100 ms for each MRM transition. The first quadrupole was set to unit resolution, and the second quadrupole was set to wide resolution for each MRM transition. Acrylamide was quantified by a linear calibration curve built in a range between 1 and 50 µg/L (containing 20 µg/L acrylamide-d_3). All analyses were performed in duplicate.

Statistical analysis

The statistical significance of the results was determined using one-way analysis of variance (ANOVA) with Tukey's test implemented in SPSS Statistics 26.0 (IBM, New York, USA). The p-value was set at 0.05 to indicate statistical differences. The mean and standard deviation were reported for each group.

Results and Discussion

Sucrose present in biscuit dough undergoes partial dissolution in water, which subsequently recrystallizes or transforms into a supercooled liquid state after baking. This phenomenon significantly influences the texture of the baked biscuit. The size distribution of sucrose crystals plays a crucial role in determining the dough's spread during baking, as well as its overall appearance and crispiness. For this reason, baby biscuits were initially prepared with sucrose. As presented in Figure 1, the color of baby biscuits prepared with natural sweeteners was darker. Baby biscuits made with whole-meal flour and sucrose contained 26±0.5 ng/g of acrylamide (Figure 2). In the study conducted by Cengiz and Gündüz (2013), baby rusks and baby biscuits from various brands available in the Turkish market were analyzed for their acrylamide content. It was reported that nine different baby rusks contained acrylamide levels ranging from less than the LOQ to 660 ng/g, while 33 different baby biscuits contained acrylamide levels between the LOQ and 588 ng/g. Furthermore, a survey conducted in Turkey analyzed 152 different baby biscuits to determine their acrylamide content. The results revealed that baby biscuits contained acrylamide levels ranging from 32 to 613 ng/g (Ölmez et al., 2008). Such variations in acrylamide content of baby biscuits were attributed to both ingredient composition and different processing parameters and formulations. Whole-meal flours exhibited higher levels of free asparagine, consequently leading to increased acrylamide formation in breads (Fink et al., 2006). On the other hand, baby biscuits undergo gentler heating conditions compared to industrial baking processes; therefore, baking at 220ºC for 5 min resulted in comparably lower acrylamide formation in baby biscuits in this study.

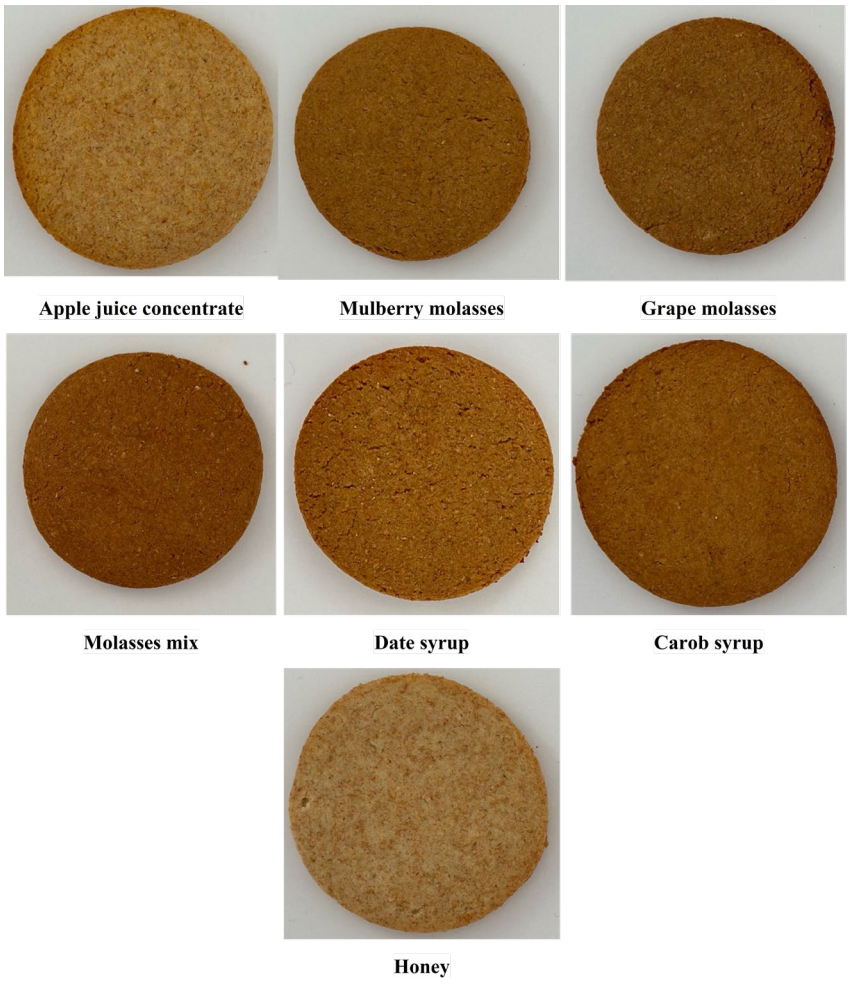
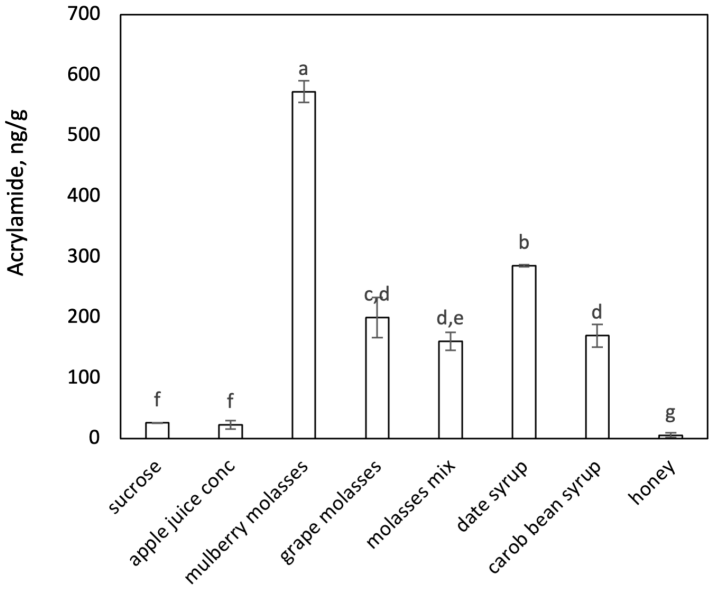


Figure 1. Baby biscuits prepared from natural sweeteners

Apple juice concentrate is a widely used natural sweetener in the industry, particularly for products labeled as "no added sugar". It is also used by mothers for the preparation of baby foods at home without added sugar at the household level. Notably, in baby biscuits made with apple juice concentrate, acrylamide concentration was not significantly different from that found in baby biscuits containing sucrose ($p>0.05$). This might be related to the filtration or settling processes applied to obtain clear apple juice concentrates. Similarly, honey is one of the popular natural sweeteners used in the preparation of various food products. In the case of baby biscuits, honey was replaced with sucrose, resulting in an acrylamide concentration of approximately 6 ± 3.8 ng/g (Figure 2). Comparably lower acrylamide levels observed in the biscuits made with honey may be attributed to the acidic pH of the honey, which is known to suppress acrylamide formation by reducing asparagine reactivity and altering Maillard reaction pathways.

In addition to honey and apple juice concentrate, the substitution of sucrose with natural sweeteners including mulberry molasses, molasses, molasses mix, date syrup, and carob bean syrup in the baby biscuit formulation resulted in a significantly elevated formation of acrylamide. The acrylamide concentration ranged from 160.5 ng/g to 572.6 ng/g in the baby biscuits produced using these natural sweeteners (Figure 2). These levels exceed the benchmark levels established for biscuits and rusks specifically formulated for infants and young children, which are set at 150 ng/g (EU, 2017). The substitution of sucrose with molasses and molasses mix resulted in acrylamide concentrations of 200 ±32.9 ng/g and 160 ±14.8 ng/g, respectively. The substitution of date syrup for sucrose in the formulation resulted in a notable increase (11-fold) in acrylamide formation. The acrylamide concentration in baby biscuits produced using carob bean syrup was determined to be 170 ± 18.8 ng/g, while those produced using date syrup exhibited a concentration of 285 ± 1.5 ng/g. Additionally, baby biscuits made with mulberry molasses instead of sucrose had an acrylamide concentration of 572 ± 17.8 ng/g.

Acrylamide formation has been documented in various sugar syrups, including molasses and date syrups (Henao Toro et al., 2022). For instance, molasses was found to contain 7.1 g/kg of acrylamide (Sung et al., 2020), while date syrups contained acrylamide levels ranging from not detected to 554 µg/kg (Phaeon et al., 2021). Accordingly, natural sweeteners were analyzed for their acrylamide contents. From mulberry molasses, grape molasses, molasses mix, date syrup, and carob bean syrup, it was found that grape molasses, carob bean syrup, and date syrup contained acrylamide. Carob bean syrup contained only 7.8 ng/g of acrylamide, whereas grape molasses contained 28.9 ng/g of acrylamide. However, it was found that the acrylamide concentration in date syrup was 214.7 ng/g. This concentration is comparable to the acrylamide levels reported in the literature for date syrups. The results of Bahrami et al. (2021) demonstrated that the date syrup samples contained acrylamide levels ranging from 141 to 554 ng/g; however, acrylamide was not detected in the date fruit samples. In a survey study, three different date syrups were reported to contain acrylamide concentrations of 199, 302, and 508 ng/g (Nasrolah et al., 2019). Despite some natural sweeteners in baby foods containing lower amounts, acrylamide levels still exceed the specified benchmark level of 40 ng/g. Considering that the tolerable daily intake for neurotoxicity is 40 ng/g per day and for cancer is 2.6 ng/g per day, even a few grams of molasses or date syrup consumption could potentially pose a risk to health.



* Different letters are statistically significantly different according to Tukey's test ($p<0.05$).

Figure 2. Acrylamide content in baby biscuits prepared with sucrose or natural sweeteners

Acrylamide concentrations in natural sweeteners did not exhibit a direct correlation with the acrylamide formation observed in baby biscuits prepared using natural sweeteners.

Spearman correlation analysis revealed a moderate positive association between the acrylamide content introduced through natural sweeteners and the acrylamide concentration measured in the final biscuits ($\rho = 0.69$). This finding suggests that sweeteners with higher intrinsic acrylamide levels may contribute to elevated acrylamide concentrations in the final product. However, the acrylamide concentrations detected in

the final baby biscuits were substantially higher than the initial amounts introduced by the natural sweeteners alone, indicating that most acrylamide was newly formed during baking through Maillard reactions. For instance, 19.9% of acrylamide in baby biscuits made with carob syrup was derived from carob syrup itself, while 80.1% of acrylamide was formed during baking od baby biscuits. Similarly, honey substitution with sucrose contributed to 10.4% of acrylamide formed in baby biscuits prepared with honey.

As is well known, carbonyl compounds and asparagine are the main precursors of acrylamide formation in biscuits. Although asparagine is considered the limiting factor in biscuits, the type and concentration of sugars also play an important role in acrylamide formation. Hamlet et al. (2007) investigated the relative reactivity of glucose, fructose, and sucrose in the generation of acrylamide in soft dough biscuits. The results showed that the addition of 1% fructose to the flour significantly increased acrylamide formation by approximately 20%. This increase was considerably higher than that observed for glucose and sucrose, indicating that fructose exhibited greater reactivity in the acrylamide formation reaction. A study conducted by Graf et al. (2006) investigated the impact of sugar type on acrylamide formation in biscuits. The researchers found that the use of sucrose resulted in a concentration of 46 mg/kg of acrylamide, whereas the use of inverted sugar led to a concentration of 170 mg/kg of acrylamide in biscuits. These findings suggest that substituting reducing sugars with sucrose might be a strategy for mitigating acrylamide in biscuits. Since sucrose is a non-reducing disaccharide, it must undergo hydrolysis (inversion) to yield glucose and fructose before participating in the Maillard reaction. This process might delay and restrict acrylamide formation under typical baking conditions. In this context, the sugar profile of natural sweeteners was also analyzed to elucidate the contribution of specific sugar types to acrylamide formation. According to the sugar profile of apple juice concentrate presented in Table 3, it predominantly comprises fructose (43.5%), accompanied by glucose (28.3%) and sucrose (10.5%). The reduced acrylamide content in baby biscuits made with apple juice concentrate might be attributed to the higher sucrose levels present in apple juice concentrate. In contrast, honey exhibited a high concentration of monosaccharides and did not contain sucrose. However, the lower acrylamide levels observed in honey-based biscuits may be attributed mainly to the acidic pH of the honey (pH 3.71), resulting in a lower pH in the biscuit dough (7.6), which is known to suppress acrylamide formation by reducing asparagine reactivity and altering Maillard reaction pathways. Despite its relatively high sucrose content and lower glucose/fructose levels, the biscuits made with carob syrup exhibited acrylamide concentrations above the benchmark level. This may be attributed to factors beyond sugar composition alone, including the presence of asparagine in carob, a key precursor of acrylamide formation, which may have promoted acrylamide generation during baking. On the other hand, baby biscuits formulated with molasses or syrups generally exhibited higher acrylamide formation compared to those prepared with sucrose, primarily due to variations in sugar compositions. Molasses and syrups are abundant in reducing sugars such as glucose and fructose, which readily participate in the Maillard reaction to form acrylamide. From the molasses, only the molasses mix sample contained 7.6% sucrose, while grape molasses and mulberry molasses predominantly contained glucose and fructose.

The highest acrylamide levels observed in baby biscuits made with mulberry molasses might be due to its higher fructose content. Similarly, the second-highest acrylamide formation was observed in baby biscuits made with date syrup, which contains 33% fructose. Nguyen et al. (2016) reported that glucose did not contribute to acrylamide formation in cookie models, whereas fructose was more effective in converting asparagine into acrylamide. This was attributed to the lower melting point of fructose compared to glucose.

However, Pearson correlation analysis showed weak negative relations between acrylamide formation and glucose ($r = -0.28$) and fructose ($r = -0.26$), indicating that monosaccharide levels were not strongly related to acrylamide formation in the studied biscuits. In contrast, sucrose content showed a strong negative correlation with acrylamide levels ($r = -0.80$), suggesting that formulations with higher sucrose content tended to exhibit lower acrylamide formation. However, this relationship likely reflects formulation-dependent matrix effects rather than a direct inhibitory effect of sucrose on acrylamide formation.

The pH values of the natural sweeteners (Table 3) and the biscuit doughs were measured and evaluated in relation to acrylamide formation. Pearson correlation analysis showed a weak negative relationship between pH and acrylamide levels ($r = -0.21$), indicating that pH of the dough alone had a limited effect on acrylamide formation in the present system.

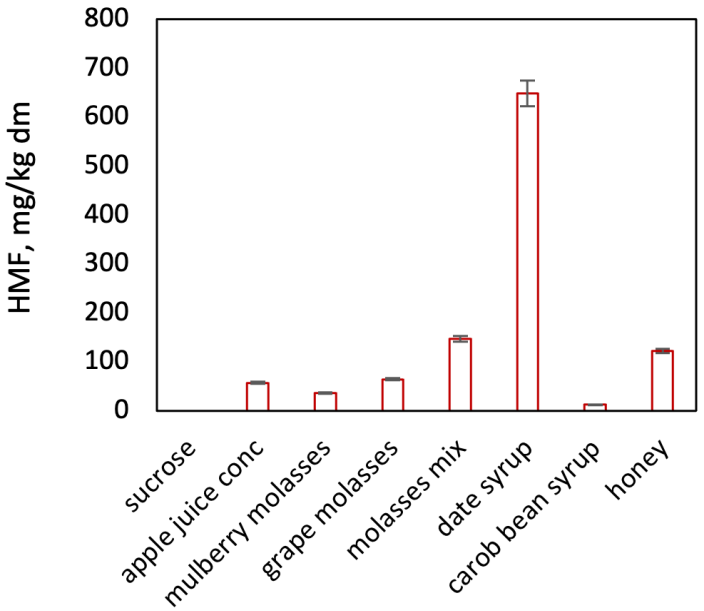
Natural sweeteners, such as molasses and syrups, undergo thermal processes that can lead to chemical alterations during these processes. Molasses is a concentrated product obtained by boiling sugar-rich fruits, including grapes, mulberries, and carobs, for extended periods (Erbil, 2020). During boiling, hydrolysis of molasses can produce various sugar decomposition products, including HMF, through the Maillard reaction or caramelization. HMF serves as an indicator of the intensity of

thermal processing and should be controlled. Turkish Standards specify that molasses should contain no more than 75 mg/L of HMF (Metin, 2014). As depicted in Figure 3, carob bean syrup exhibited the lowest HMF content among the natural sweeteners tested, while other sweeteners contained comparably higher levels of HMF. Carob bean syrup exhibited an HMF concentration of 8.7 mg/L, whereas apple juice concentrate, mulberry molasses, and grape molasses contained 37.6 mg/L, 27.2 mg/L, and 45.5 mg/L HMF, respectively. Among the natural sweeteners, honey, molasses mix, and date syrup, HMF levels exceeded the levels specified by Turkish Standards. While honey and molasses mix contained approximately 100 mg/L of HMF, date syrup contained 7-fold higher levels, reaching 532.3 mg/L.

Gökmen et al. (2012) reported that HMF, which also bears a carbonyl functional group, is capable of forming acrylamide in low-moisture model systems by reacting with asparagine. They also noted that HMF is highly reactive in converting asparagine into acrylamide compared to glucose, as it has a lower melting point. Hamzalıoğlu and Gökmen (2020) further demonstrated that HMF accumulation played a critical role in acrylamide formation in coffee during roasting. Based on these studies and the levels of HMF in natural sweeteners formed during their production, HMF in these natural sweeteners might contribute to acrylamide formation in baby biscuits.

Pearson correlation analysis was performed to evaluate the relationship between the HMF content of the natural sweeteners and the acrylamide levels detected in the final baby biscuits. The calculated correlation coefficient was low ($r = 0.18$), indicating a weak relationship between these two parameters under the experimental conditions applied in this study. In coffee roasting, HMF formation is generally associated with severe thermal treatment and has been reported as a more pronounced indicator of heat-induced reactions and browning development. However, the thermal processing conditions applied during the production of baby biscuits are considerably milder and involve different matrix characteristics compared to coffee roasting. Therefore, it might be difficult to attribute acrylamide formation directly to a single thermal marker alone.

In addition to HMF, sugar decomposition during thermal processes of natural sweeteners may also generate reactive sugar decomposition products such as dicarbonyl compounds. As these dicarbonyl intermediates are reported to be more reactive than glucose, they could potentially contribute to acrylamide formation; however, their role could not be evaluated in the present study, as dicarbonyl compounds were not analyzed (Zyzak et al., 2003). Honey is a well-known source of sugar decomposition products, including 1,2-dicarbonyls. A survey study indicated that honeys contained 3-deoxyglucosone ranging from 271 to 1641 mg/L, while 3-deoxygalactosone ranged from 14 to 46 mg/L, and methylglyoxal ranged from not detectable levels to 463 mg/L (Degen et al., 2012). In another survey study, sweet bread condiments, such as bee honey, apple molasses, and sugar syrup, were examined for their sugar decomposition compounds, which exceeded levels of 200 mg/kg (Marceau & Yaylayan, 2009). Methylglyoxal ranged from 0 to 736 mg/kg in sweet bread condiments, with the highest concentrations observed in apple molasses. Similarly, glyoxal levels ranged from 0 to 37 mg/kg in sweet bread condiments, with the highest level reported in apple molasses. The accumulation of HMF during thermal processing of mulberry molasses, grape molasses, and molasses mix, together with the possible formation of dicarbonyl compounds, might be among the factors contributing to elevated acrylamide levels in baby biscuits made with these ingredients.



* Different letters are statistically significantly different according to Tukey's test ($p<0.05$).

Figure 3. 5-hydroxymethylfurfural (HMF) content of natural sweeteners

Table 3. Sugar profile of sucrose and natural sweeteners

Sweetener	Glucose (%)	Fructose (%)	Sucrose (%)	pH	Acrylamide, ng/g
Sucrose	<LOQ	<LOQ	100.0±1.48	-	<LOQ
Apple Juice Concentrate	28.3±5.13	43.5±0.18	10.5±1.52	3.87	<LOQ
Mulberry Molasses	26.9±0.25	29.6±0.71	<LOQ	4.97	<LOQ
Grape Molasses	35.2±0.25	34.9±0.25	<LOQ	4.89	28.87 ±0.25
Molasses Mix	27.6±0.10	25.0±0.53	7.6±0.37	4.84	<LOQ
Date Syrup	33.4±3.38	33.0±3.38	<LOQ	4.13	214.73 ±2.51
Carob Bean Syrup	6.0±0.45	5.5±0.45	45.6±1.52	5.12	7.85 ±0.17
Honey	40.8±0.42	48.0±4.95	<LOQ	3.71	<LOQ

Cereal-based snacks, such as baby biscuits and baby rusks, are one of the main sources of acrylamide for toddlers aged between 1-3 years. As reported by EFSA, infants, toddlers, and other children are the most susceptible groups to acrylamide exposure (EFSA, 2011). A study conducted in Turkey to estimate the acrylamide exposure of toddlers revealed that the mean consumption of baby biscuits was 12 g/day, while the average daily consumption of baby crackers was 7 g/day (Cengiz & Gündüz, 2013). Exposure levels were determined for toddlers by considering mean consumption levels and the acrylamide concentrations found in baby biscuits. The average body weights were estimated as 6 kg for the toddlers aged 6 months, 8 kg for toddlers aged 1 year, and 10 kg for toddlers aged 2 years. Accordingly, estimated daily exposure to acrylamide from baby biscuits formulated with natural sweeteners ranged from 0.05 to 1.15 µg/kg bw/day for toddlers aged 6 months in this study. Notably, the estimated exposure to acrylamide from baby biscuits decreased as toddlers aged 1 year due to their increased body weight. The estimated maximum level of acrylamide exposure for toddlers aged 1 year consuming baby biscuits prepared with the mulberry molasses as a natural sweetener is 0.86 µg/kg bw/day. These levels are in agreement with the reported dietary exposure levels for toddlers (≤2 years) in the US (Abt et al., 2019). Compared to adults, toddlers and infants generally exhibit higher acrylamide exposure levels due to their lower body weight and higher dietary intake per kilogram of body weight. However, acrylamide poses a significant concern for infants and toddlers. In contrast to baby biscuits prepared with refined sugar (sucrose), those prepared with natural sweeteners such as date syrup or molasses increase the risk of acrylamide, consequently resulting in elevated exposure levels.

Conclusion

The results demonstrated that the addition of natural sweeteners influenced acrylamide levels in baby biscuits; however, the magnitude and direction of this effect varied depending on the type of sweetener used. This variability may be attributed to variations in sugar composition, reactive carbonyl profiles, and processing histories of the sweeteners. Sweeteners containing reducing sugars, particularly glucose and fructose, exhibited a tendency for elevated acrylamide formation. This phenomenon is attributed to their increased reactivity within the Maillard reaction pathways, in comparison to formulations predominantly composed of sucrose. In addition, the presence of reactive dicarbonyl compounds and elevated HMF levels in some thermally processed sweeteners indicates prior sugar degradation, which may contribute to acrylamide formation potential. Notably, thermally processed syrups and molasses exhibited relatively higher acrylamide-forming potential compared to refined sugar; however, not all natural sweeteners followed this pattern, as some formulations showed lower acrylamide levels despite containing comparable sugar contents. This suggests that acrylamide formation is governed by multiple interacting factors rather than sugar composition alone.

Given the high exposure to acrylamide, particularly through cereal-based snacks, toddlers aged 1–3 years may be at increased risk of dietary acrylamide intake. In this context, consumption of baby biscuits formulated with certain natural sweeteners such as date syrup or molasses may lead to higher acrylamide exposure compared to biscuits prepared with refined sugar, depending on the sweetener's compositional and processing characteristics.

These findings highlight the importance of carefully selecting the type of natural sweetener used in baby foods, as the term "natural" does not inherently guarantee lower process contaminant formation in thermally processed foods. Therefore, selecting sweeteners with lower reducing sugar contents, along with monitoring thermal degradation markers such as dicarbonyl compounds and HMF, is important to minimize acrylamide formation in bakery products specifically developed for toddlers.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare that they have no actual, potential, or perceived conflicts of interest related to this article.

Ethics committee approval: The authors declare that this study does not include experiments with human or animal subjects, so ethics committee approval is not required.

Data availability: The data will be made available upon request by the author(s).

Funding: -

Acknowledgments: -

Disclosure: -

References

Abt, E., Robin, L. P., McGrath, S., Srinivasan, J., DiNovi, M., Adachi, Y., Chirtel, S. (2019). Acrylamide levels and dietary exposure from foods in the United States, an update based on 2011-2015 data. *Food Additives and Contaminants Part A: Chemical Analysis and Control of Exposure Risk Assessment*, 36(10), 1475–1490. https://doi.org/10.1080/19440049.2019.1637548

Alpaslan, M., & Hayta, M. (2002). Rheological and sensory properties of pekmez (grape molasses)/tahin (sesame paste) blends. *Journal of Food Engineering*, 54(1), 89–93. https://doi.org/10.1016/S0260-8774(01)00197-2

Bahrami, M.E., Honarvar, M., Nasrolah, M. (2021). Potential for acrylamide formation in Iranian dates and date syrups: Influence of amino acids and processing conditions. *Journal of Food Measurement and Characterization*, 15(5), 4073–4082. https://doi.org/10.1007/s11694-021-00977-9

Boyaci Gunduz, C., Bilgin, A., Cengiz, M.F. (2017). Acrylamide Contents of Some Commercial Crackers, Biscuits and Baby Biscuits. *Akademik Gıda*, 1–1. https://doi.org/10.24323/akademik-gida.305277

Cengiz, M.F., & Gündüz, C.P.B. (2013). Acrylamide exposure among Turkish toddlers from selected cereal-based baby food samples. *Food and Chemical Toxicology*, 60, 514–519. https://doi.org/10.1016/j.fct.2013.08.018

Cogswell, M.E., Gunn, J.P., Yuan, K., Park, S., Merritt, R. (2015). Sodium and Sugar in Complementary Infant and Toddler Foods Sold in the United States. *Pediatrics*, 135(3), 416–423. https://doi.org/10.1542/peds.2014-3251

Damen, F.W.M., Luning, P.A., Fogliano, V., Steenbekkers, B.L.P.A. (2019). What influences mothers' snack choices for their children aged 2–7? *Food Quality and Preference*, 74, 10–20. https://doi.org/10.1016/j.foodqual.2018.12.012

Degen, J., Hellwig, M., Henle, T. (2012). 1,2-Dicarbonyl Compounds in Commonly Consumed Foods. *Journal of Agricultural and Food Chemistry*, 60(28), 7071–7079. https://doi.org/10.1021/jf301306g

Demonteil, L., Tournier, C., Marduel, A., Dusoulier, M., Weenen, H., Nicklaus, S. (2019). Longitudinal study on acceptance of food textures between 6 and 18 months. *Food Quality and Preference*, 71, 54–65. https://doi.org/10.1016/j.foodqual.2018.05.010

European Food Safety Authority (EFSA) (2011). Results on acrylamide levels in food from monitoring years 2007–2009 and exposure assessment. *EFSA Journal*, 9(4), 2133. https://doi.org/10.2903/j.efsa.2011.2133

Erbil, D. (2020). Endüstriyel ve geleneksel yöntemlerle üretilmiş farklı pekmez çeşitlerinin bazı fizikokimyasal ve kalite özelliklerinin belirlenmesi, İstanbul Teknik Üniversitesi. İstanbul, Türkiye. Yüksek Lisans Tezi

European Commission (2017). Commission Regulation (EU) 2017/2158 of 20 November 2017 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. *Official Journal of the European Union,* L 304, 24–44

EU Pledge. (2023). EU Pledge Nutrition White Paper – Updated March 2023. EU Pledge. https://eu-pledge.eu/wp-content/uploads/EU_Pledge_Nutrition_White_Paper.pdf?bot_detected=1&utm_source=chatgpt.com

Fink, M., Andersson, R., Rosén, J., Åman, P. (2006). Effect of Added Asparagine and Glycine on Acrylamide Content in Yeast-Leavened Bread. *Cereal Chemistry*, 83(2), 218–222. https://doi.org/10.1094/CC-83-0218

Freisling, H., Moskal, A., Ferrari, P., Nicolas, G., Knaze, V., Clavel-Chapelon, F., Boutron-Ruault, et al. (2013). Dietary acrylamide intake of adults in the European

prospective investigation into cancer and nutrition differs greatly according to geographical region. *European Journal of Nutrition*, 52(4), 1369–1380. https://doi.org/10.1007/s00394-012-0446-x

Gökmen, V., Kocadağlı, T., Göncüoğlu, N., Mogol, B. A. (2012). Model studies on the role of 5-hydroxymethyl-2-furfural in acrylamide formation from asparagine. *Food Chemistry*, 132(1), 168–174. https://doi.org/10.1016/j.foodchem.2011.10.048

Gracner, T., Boone, C., Gertler, P.J. (2024). Exposure to sugar rationing in the first 1000 days of life protected against chronic disease. *Science*, 386(6725), 1043–1048. https://doi.org/10.1126/science.adn5421

Graf, M., Amrein, T. M., Graf, S., Szalay, R., Escher, F., Amadò, R. (2006). Reducing the acrylamide content of a semi-finished biscuit on industrial scale. *LWT - Food Science and Technology*, 39(7), 724–728. https://doi.org/10.1016/j.lwt.2005.05.010

Hamlet, C.G., Sadd, P.A., Liang, L., Jayaratne, S.M., & Skingle, M. (2007). *Exploiting processing conditions to reduce acrylamide in cereal-based foods* (Report No. C021; Project No. C03052). RHM Technology, High Wycombe, UK. Prepared on behalf of the UK Food Standards Agency.

Hamzalıoğlu, A., & Gökmen, V. (2020). 5 Hydroxymethylfurfural accumulation plays a critical role on acrylamide formation in coffee during roasting as confirmed by multiresponse kinetic modelling. *Food Chemistry*, 318, 126467. https://doi.org/10.1016/j.foodchem.2020.126467

Henao Toro, S.J., Gómez-Narváez, F., Contreras-Calderón, J., Arisseto, A.P. (2022). Acrylamide in sugar products. *Current Opinion in Food Science*, 45, 100841. https://doi.org/10.1016/j.cofs.2022.100841

Herrick, K.A., Fryar, C.D., Hamner, H.C., Park, S., Ogden, C.L. (2020). Added Sugars Intake among US Infants and Toddlers. *Journal of the Academy of Nutrition and Dietetics*, 120(1), 23–32. https://doi.org/10.1016/j.jand.2019.09.007

Hutchinson, J., Rippin, H., Threapleton, D., Jewell, J., Kanamäe, H., Salupuu, K., Caroli, M., et al. (2021). High sugar content of European commercial baby foods and proposed updates to existing recommendations. *Maternal & Child Nutrition*, 17(1), e13020. https://doi.org/10.1111/mcn.13020

Klerks, M., Román, S., Sánchez-Siles, L. (2023). Baby, children, and adult biscuits. Differences in nutritional quality and naturalness. *Food Science and Nutrition*, 11(12), 7946–7956. https://doi.org/10.1002/fsn3.3711

Kocadağlı, T., Göncüoğlu Taş, N., Hamzalıoğlu, A., Gökmen, V. (2025). Formation of acrylamide and aroma compounds in biscuits and crackers prepared with sprouted wheat wholemeal. *Food Chemistry*, 489, 145032. https://doi.org/10.1016/j.foodchem.2025.145032

Lima, M., Ares, G., Deliza, R. (2019). Comparison of two sugar reduction strategies with children: Case study with grape nectars. *Food Quality and Preference,* 71, 163–167. https://doi.org/10.1016/j.foodqual.2018.07.002

Manley, D. (2001). Chapter 11 - Recipes for dietetic biscuits. In D. Manley (Ed.), *Biscuit, Cracker and Cookie Recipes for the Food Industry* (pp. 155–173). Woodhead Publishing. https://doi.org/10.1533/9781855736269.155

Marceau, E., & Yaylayan, V. (2009). Profiling of α-Dicarbonyl Content of Commercial Honeys from Different Botanical Origins: Identification of 3,4-Dideoxyglucosone-3-ene (3,4-DGE) and Related Compounds. *Journal of Agricultural and Food Chemistry*, 57, 10837–10844. https://doi.org/10.1021/jf903341t

Metin, Z. (2014). Ankara piyasasında satışa sunulan nar ekşisi, nar ekşisi sosu ve üzüm pekmezlerinin hidroksimetilfurfural düzeyinin saptanması. Hacettepe Üniversitesi, Ankara, Türkiye, Yüksek Lisans Tezi

Moore, A.M., Vadiveloo, M., Tovar, A., McCurdy, K., Østbye, T., & Benjamin-Neelon, S.E. (2019). Associations of less healthy snack food consumption with infant weight-for-length Z-score trajectories: Findings from the Nurture Cohort Study. *Nutrients*, 11(11), 2752. https://doi.org/10.3390/nu11112752

Nasrolah, M., Honarvar, M., Bahrami, M. E., Zulfiqar, A., Bayat, M., Bahrami, F., Khanlarloo, H. (2019). The Occurrence of Acrylamide in Iranian Date Syrup. *Journal of Food Biosciences and Technology*, 9(1), 65–68.

Nguyen, H.T., Van der Fels-Klerx, H.J., Peters, R.J.B., van Boekel, M.A.J.S. (2016). Acrylamide and 5-hydroxymethylfurfural formation during baking of biscuits: Part I: Effects of sugar type. *Food Chemistry, 192,* 575–585. https://doi.org/10.1016/j.foodchem.2015.07.016

Ölmez, H., Tuncay, F., Özcan, N., Demirel, S. (2008). A survey of acrylamide levels in foods from the Turkish market. *Journal of Food Composition and Analysis*, 21(7), 564–568. https://doi.org/10.1016/j.jfca.2008.04.011

Phaeon, N., Chapanya, P., Mueangmontri, R., Pattamasuwan, A., Lipan, L., Carbonell-Barrachina, Á. A., Sriroth, K., Nitayapat, N. (2021). Acrylamide in non-centrifugal sugars and syrups. *Journal of the Science of Food and Agriculture*, 101(11), 4561–4569. https://doi.org/10.1002/jsfa.11098

Sengül, M., Fatih Ertugay, M., Sengül, M. (2005). Rheological, physical, and chemical characteristics of mulberry pekmez. *Food Control*, 16(1), 73-76. https://doi.org/10.1016/j.foodcont.2003.11.010

Sung, W.-C., Chi, M.-H., Chiou, T.-Y., Lin, S.-H., Lee, W.-J. (2020). Influence of caramel and molasses addition on acrylamide and 5-hydroxymethylfurfural formation and sensory characteristics of non-centrifugal cane sugar during manufacturing. *Journal of the Science of Food and Agriculture*, 100(12), 4512-4520. https://doi.org/10.1002/jsfa.10492

Velázquez, A.L., Vidal, L., Alcaire, F., Varela, P., Ares, G. (2021). Significant sugar reduction in dairy products targeted at children is possible without affecting hedonic perception. *International Dairy Journal*, 114, 104937. https://doi.org/10.1016/j.idairyj.2020.104937

Velázquez, A.L., Vidal, L., Varela, P., Ares, G. (2020). Cross-modal interactions as a strategy for sugar reduction in products targeted at children: Case study with vanilla milk desserts. *Food Research International*, 130, 108920. https://doi.org/10.1016/j.foodres.2019.108920

World Health Organization (WHO) Regional Office for Europe (2022). Use of nutrient profile models for nutrition and health policies: Meeting report on the use of nutrient profile models in the WHO European Region, September 2021. Copenhagen: WHO Regional Office for Europe. WHO Reference No. WHO/EURO:2022-6201-45966-66383

Zyzak, D.V., Sanders, R.A., Stojanovic, M., Tallmadge, D.H., Eberhart, B.L., Ewald, D.K., Gruber, D.C., et al. (2003). Acrylamide Formation Mechanism in Heated Foods. *Journal of Agricultural and Food Chemistry,* 51(16), 4782–4787. https://doi.org/10.1021/jf034180i