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Phenolic compounds and antioxidant activity of a functional honey-added marshmallow

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ABSTRACT

Objective: To evaluate the effect of adding honey and the gelatin degrees Bloom in the phenolic content and antioxidant capacity of a marshmallow product.

Design/methodology/approach: A 3^2 factorial design was carried out adding 0, 50 and 75% honey concentrations and 265, 300 and 315 degrees Bloom of gelatin.

Results: Adding of honey increased the phenolic content and antioxidant activity by 45% compared to samples without honey. Sensory analysis showed no differences in flavor, aroma and mouthfeel between samples with and without honey, however, higher degrees bloom improved the texture and appearance of the marshmallows.

Limitations of the study/implications: The sweetness of honey-added marshmallows increased with the honey concentration, being excessive for consumers.

Findings/conclusions: Functional honey-added marshmallow is a viable alternative that can be feasibly introduced to the confectionery market.

Keywords: Marshmallow, DPPH, Folin-Ciocalteu, texture, degrees bloom, gelatin.

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INTRODUCTION

The confectionery industry is one of the most profitable sectors in Mexico. According to data reported by the Secretariat of Agriculture and Social Development and the Secretariat of Agrifood and Fisheries Information (SADER and SIAP, 2019), a surplus of 293 million dollars was obtained in 2019 from the sale of confectionery products. However, these products have been related to an increase in cardiovascular diseases, diabetes and obesity, as a consequence of their high sugar content (Cabezas-Zabala *et al.*, 2015). Therefore, it is necessary to innovate traditional confectionery by turning products into vehicles that delivers compounds that contribute to the improvement of the health of the Mexican population.

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On the one hand, marshmallows are aerated confectionery products, with a characteristic foamy structure that is created when air and moisture are incorporated into a syrup mixture. They are composed of a stabilizing agent, a foaming agent, sweeteners, flavor enhancers and air. Gelatin is the most used stabilizer in marshmallows, because it produces an elastic texture that can be moderated by choosing the degrees bloom (°Bloom) strength.

On the other hand, honey is composed of minerals, vitamins, organic acids, proteins, enzymes, and phenolic compounds. The main responsible for the functional properties of honey are phenols, which provide to honey its antioxidant capacity, preventing from cardiovascular diseases and cancer (Viada *et al.*, 2017). Therefore, the objective of this study was to evaluate the effect of the addition of honey and gelatin bloom grades on the total phenolic content (TPC) and total antioxidant activity (TAA) of a marshmallow.

MATERIALS AND METHODS

The marshmallows were made with refined sugar (Great Value), glucose syrup (Deiman), 265, 300, and 315 °Bloom gelatin (Progel), egg albumin, and multifloral honey harvested in 2019. The reagents used were: gallic acid (Fermont, Mexico), Na_2CO_3 (Merck, Germany), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma-Aldrich, Russia), 2,2-Diphenyl-1-picryl hydrazyl (DPPH) (Sigma-Aldrich, Germany), and the Folin-Ciocalteu reagent (Sigma-Aldrich, USA).

Determination of honey quality

Moisture (AOAC 962.19), ash (AOAC 920.181), hydroxymethylfurfural (HMF) content (AOAC 980.23), total acidity (AOAC 926.19), reducing sugars (NMX-F-274-1984), electrical conductivity (NOM 004-SAG/GAN 2018) and color (Ferreira *et al.*, 2009) were determined using the Pfund scale.

Experimental design

A completely randomized design with a 3^2 factorial structure was used, with honey concentration (0, 50, 75) and gelatin bloom grades (265, 300, and 315) as factors, resulting in a total of nine treatment combinations. The experiment was performed in triplicate and an analysis of variance was performed using the GLIMMIX procedure of SAS.

Marshmallows making

To prepare 100 g of marshmallows, 5 mL of egg albumin were beaten in a KSM150PSER mixer (KitchenAid, USA), until stiff peaks were formed; subsequently, caramel (previously prepared with sugar, glucose, and water) was added when it reached a temperature of 121 °C. When the temperature of the mixture dropped to 80 °C, the liquid gelatin was added. In the case of honey-added marshmallows, honey was included after the gelatin had been added, when the mixture had a temperature of 40 °C. All ingredients were beaten at the highest speed (10) of the equipment used. The final mixture was poured into plastic trays with a 3.0-cm-high layer of cornstarch. The cylindrical molds were 2.5 cm wide and 2.0 cm tall. Finally, the marshmallows were covered with a cornstarch layer and left in the mold for 24 h, packed in cellophane bags and stored at room temperature.

Obtaining extracts

To prepare the marshmallow extracts, they were lyophilized with a 9.5×10^{-1} mm Hg vacuum pressure, at −49 °C for 24 h (Periche *et al.*, 2015). Per each treatment, 2.0 g of lyophilized sample were dissolved with acidified water in a 1:2 ratio (adjusted to pH 2 with 2 N HCl). The solutions were homogenized in a VM-300P vortex mixer (Gemmy, Taiwan) and then they were placed in an ultrasonic bath (Auto-science, Serial Ultrasonic Cleaner, USA) for 30 min. They were allowed to settle for 24 h in the dark, at room temperature. The next day the extracts were centrifuged at 4000 rpm for 20 min, using a Centrifuge 5810 R (Eppendorf, Germany). Each extract was separated from the solid phase by decantation and filtered through cotton. Meanwhile, for honey the extraction procedure was the same used for marshmallows; however, 1 g of honey was weighed to quantify the TPC and 2 g were weighed to determine the TAA. Both samples were dissolved with 10 mL of acidified water.

Total Phenolic Content Quantification

The TPC in honey and marshmallows was determined using the Folin-Ciocalteu method, as described by Cedeño-Pinos *et al.* (2020). The calibration curve was determined using gallic acid as standard. The results were expressed as mg of gallic acid equivalents (EAG)/100 g of sample.

Determination of Total Antioxidant Activity

The TAA of marshmallows and honey was determined with the 2,2-Diphenyl-1-picryl hydrazyl (DPPH) synthetic free radical, using the technique implemented by Dżugan *et al.* (2018). The Trolox compound was used as a standard for the calibration curve. The results were expressed in equivalent mg of Trolox/100 g of sample.

Sensory analysis of marshmallows

An acceptance test was performed using a nine-point hedonic scale, ranging from 1) Dislike extremely to 9) Like extremely. The attributes evaluated were appearance, texture, mouthfeel, flavor, sweetness and aroma (Periche *et al.*, 2015). This analysis was applied to 30 people between 15 and 60 years old, belonging to the community of San José del Corral, Yanga, Veracruz, Mexico.

The data obtained were analyzed with the Statgraphics Centurion software, through a multifactorial ANOVA ($p \leq 0.05$).

RESULTS AND DISCUSSION

Honey quality

The values of the physicochemical analysis of honey were 226.7 μs cm^{-1} (electrical conductivity), 17% (humidity), 0.1% (ash), 19.8 meq kg^{-1} (total acidity), 75.1 (reducing sugars), and 5.6 mg kg^{-1} (hydroxymethylfurfural content). These parameters fell within the permissible values of the NOM-004-SAG/GAN 2018 standard indicating; honey was suitable for making marshmallows. Regarding color, the honey used in this study had a value of 9.0 mm, which matches the water white color according to the Pfund scale.

Total Phenolic Content and Total Antioxidant Activity in honey

The honey used had a TPC of 22.7 mg EAG/100 g of honey and an TAA equivalent to 11.8 mg of trolox/100 g of honey. Several researches correlate the TPC with the color of honey. Sant'Ana *et al.* (2012) showed that the lowest TPC belongs to white honeys with 63.64 mg EAG/100 g of sample. Pontis *et al.* (2014) obtained similar data: honeys with lower mm of Pfund had the lowest TPC amount (25 mg EAG/100 g of sample).

The honey was stored for four months at room temperature before it was used. Kamal-Eldin and Appelqvist (1996) determined that higher temperatures result in a higher rate of hydroperoxide decomposition, causing an increase in general redox reactions, which probably influences the stability of the antioxidant agents. Šarić *et al.* (2012) showed that the TPC in acacia and floral honeys decreased 91.8 and 88.6%, respectively, after one year of storage at room temperature. Meanwhile, during processing, the crystallized honey was heated from 38 °C to 42 °C, which could have contributed to the decrease in TPC, since these compounds are susceptible to thermal degradation.

Total Phenolic Content in marshmallows

During the storage time, the concentration of the honey and the gelatin bloom grades significantly influenced the TPC of the marshmallows ($p<0.05$), while the treatments without honey (T1-T3) presented a TPC of 5.85-9.07 mg EAG/100 g of marshmallow (Figure 1). This could be caused by the interference of ingredients in the Folin-Ciocalteu assay and not by the presence of phenols.

Consequently, gelatin and egg albumin were analyzed and in the latter 1.30 mg EAG/100 mL of albumin was determined. Albumin is a protein that includes amino acid residues such as tryptophan, cysteine, tyrosine and methionine, which can reduce the Folin-Ciocalteu reagent through electron transfer (Elias *et al.*, 2008). Singleton *et al.* (1999) and other authors showed that reducing sugars, such as glucose and fructose, are involved in the Folin-Ciocalteu assay.

During storage time, honey-added marshmallows provided 45% more TPC (10.37-12.82 mg EAG/100 g of marshmallow) than those without honey. There was no significant difference in the TPC between T4-T9 treatments (50 and 75% honey). In addition, these treatments increased the TPC over time. On average, from day 1 to 6 the TPC increased 3.16% and from day 1 to 12 it rose 15.30%.

Mandura *et al.* (2020), Dżugan *et al.* (2018), and other authors showed that the TPC in candies decreases over time. Therefore, the TPC increase in marshmallows may be the result of the interference of gelatin (made up of 85-92% protein) in the Folin-Ciocalteu reaction: the structure of the gelatin begins to destabilize and fragment into amino acid chains, increasing the number of molecules that interfere with the reaction (Carbajal, 2018).

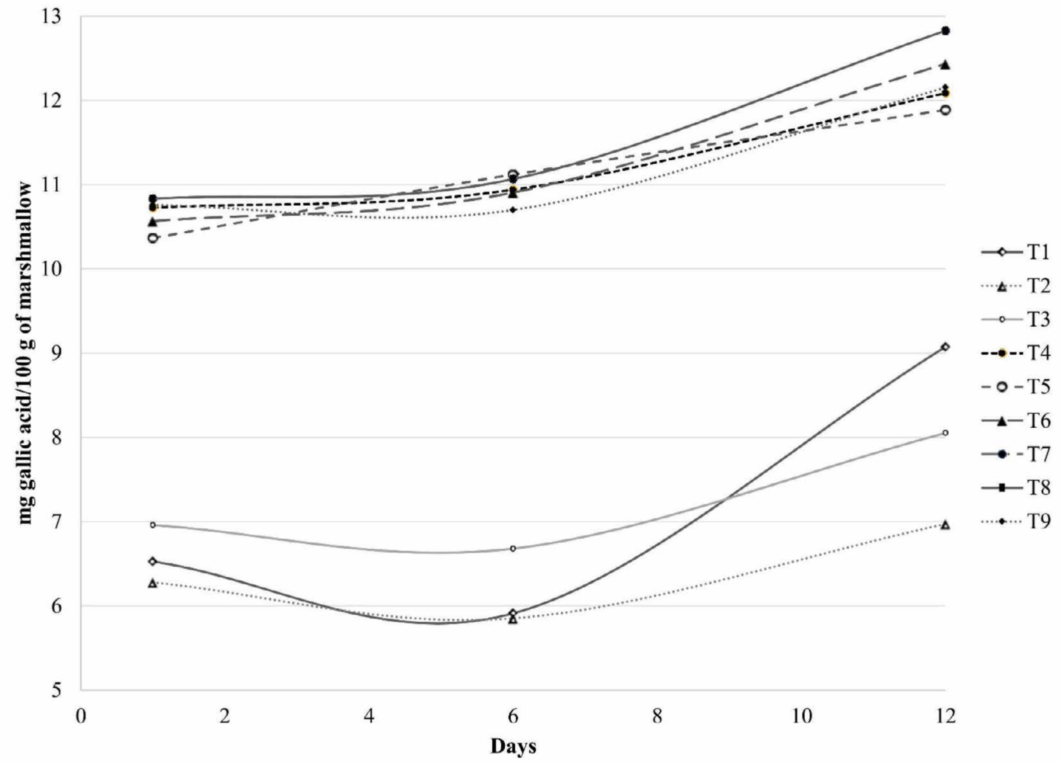

Figure 1. Total phenolic content means of the 9 marshmallow formulations during storage time. T1: 0%, 265; T2: 0%, 300; T3: 0%, 315; T4: 50%, 265; T5: 50%, 300; T6: 50%, 315; T7: 75%, 265; T8: 75%, 300; T9: 75%, 315. In each treatment the first value represents the honey concentration and the second the degrees bloom.

Total Antioxidant Activity in marshmallows

During storage, blooms and honey concentration had a statistically significant effect ($p<0.05$) on the TAA of marshmallows. The samples without honey had an TAA between 4.9-11.31 mg trolox/100 g of sample during storage time. The presence of TAA in marshmallows without honey could be caused by egg albumin (2.4 mg trolox/100 mL albumin) (Figure 2). This protein can contain amino acid residues (such as tryptophan, tyrosine, and cysteine) that have the ability to capture free radicals; however, this does not prove that it is an antioxidant (Elias *et al.*, 2008).

There were no significant differences between the formulations with 50 and 75% honey (T4-T9), which presented 8.73-10.03 mg trolox/100 g of sample (day 1), 9.78-11.84 mg trolox/100 g of sample (day 6), and 7.96-9.3 mg trolox/100 g of sample (day 12). In most treatments, TAA increased from day 1 to 6 and decreased on day 12. The polymerization of the stored phenols which formed a more stable polyphenol radical through conjugation may be the cause of those changes. This causes an increase in TAA; however, when the polymerization degree reaches a critical value, molecular complexity increases and steric impediment occurs, which leads to a reduction in the availability of hydroxyl groups in reactions with radicals, decreasing TAA (Pinelo *et al.*, 2004).

There are not many researches about confectionery candies supplemented with honey. On the one hand, Periche *et al.* (2015) evaluated the TAA of marshmallows made with

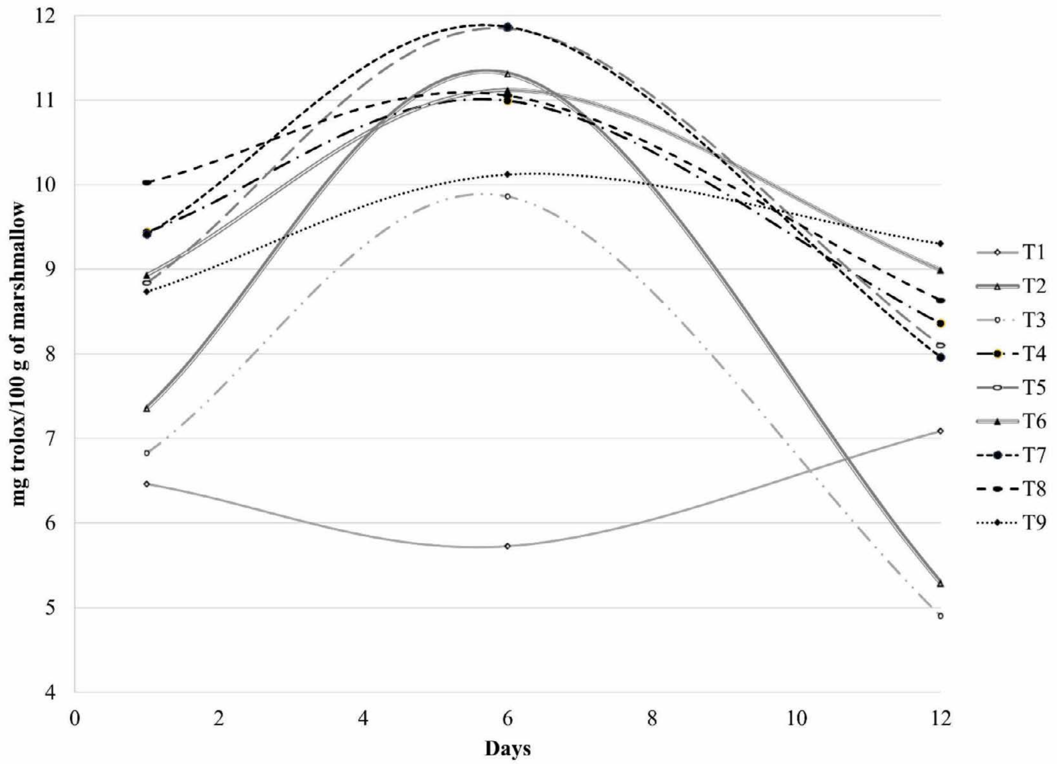

Figure 2. Antioxidant activity means of the 9 marshmallow formulations during storage time. T1: 0%, 265; T2: 0%, 300; T3: 0%, 315; T4: 50%, 265; T5: 50%, 300; T6: 50%, 315; T7: 75%, 265; T8: 75%, 300; T9: 75%, 315. In each treatment the first value represents the honey concentration and the second the degrees bloom.

oligofructose and isomaltose during one month of storage; the values obtained ranged from 40-22 mg Trolox/100 g of sample, showing a significant reduction after 10 days of storage. On the other hand, Mandura *et al.* (2020) added white tea extracts to gummies, providing them with an antioxidant capacity equal to 121 g of Trolox/100 g of sample. Compared to these researches, the TAA of the honey-added marshmallows were very low, as the honey had a low TAA value.

Sensory analysis

The following parameters were evaluated in the sensory analysis: appearance, texture, flavor, mouthfeel, sweetness and aroma. The results were represented in spiderweb chart (Figure 3).

The degrees bloom had a significant effect on the appearance of the marshmallows. Consumers found the appearance of the samples with higher degrees bloom more pleasing. The same trend was found with regard to the texture, since the marshmallows with 315 °Bloom had the highest liking.

Meanwhile, the honey concentration and the gelatin bloom degrade did not affect the liking of flavor, mouthfeel, and aroma. Sweetness increased proportionally to the concentration of honey; consequently, consumers found it excessively sweet for their taste. Treatments without honey were evaluated with better sweetness, categorizing them

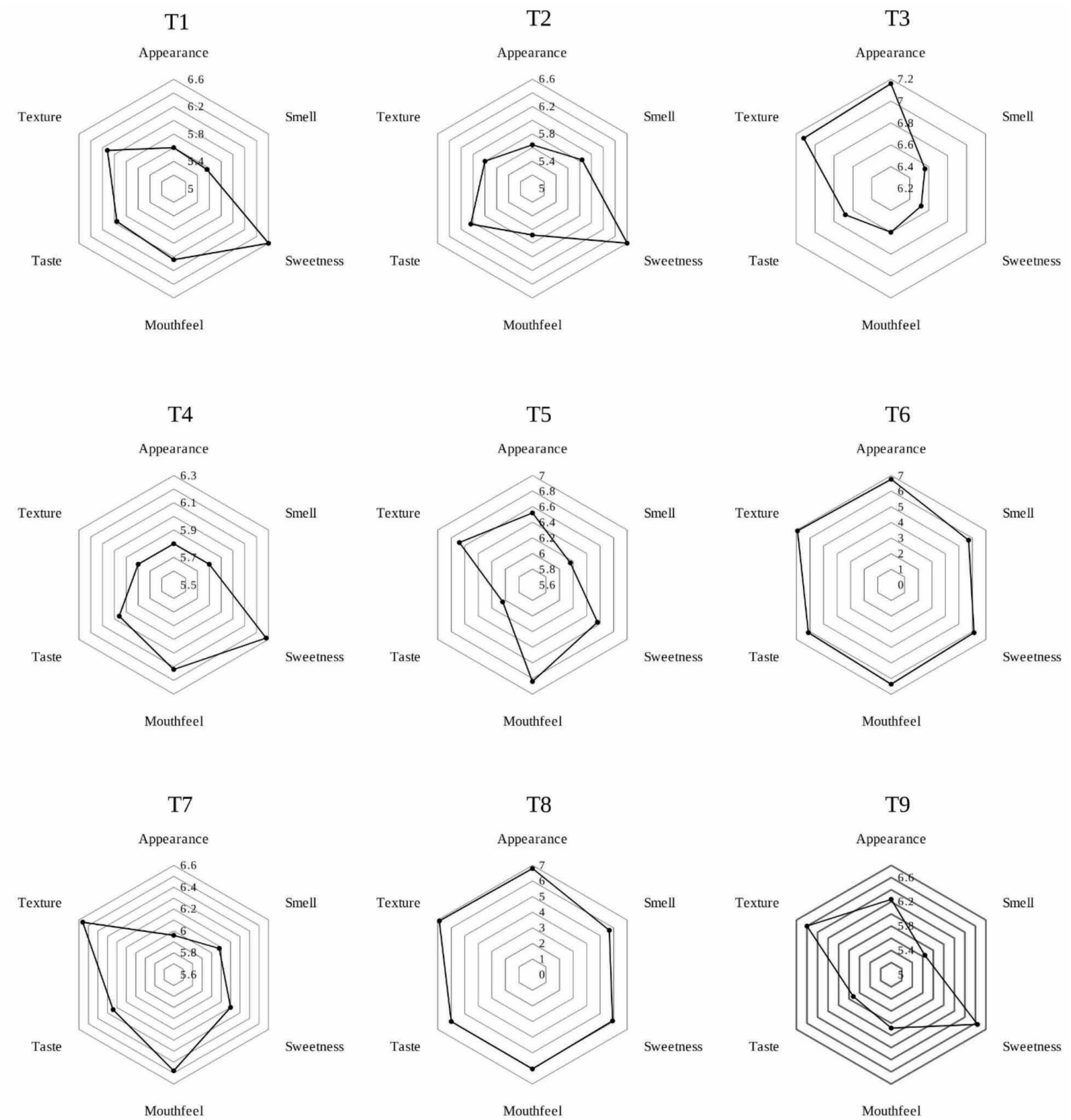

Figure 3. Spiderweb graphs representing the mean scores of the sensory liking for the different formulations of marshmallows. T1: 0%, 265; T2: 0%, 300; T3: 0%, 315; T4: 50%, 265; T5: 50%, 300; T6: 50%, 315; T7: 75%, 265; T8: 75%, 300; T9: 75%, 315. In each treatment the first value represents the honey concentration and the second the degrees bloom.

as “like slightly” (value=6). Nevertheless, the most popular treatments regarding most of the parameters evaluated were marshmallows with 50% honey-315 °Bloom and 75% honey-300 °Bloom.

CONCLUSIONS

Adding honey to the marshmallows incorporated phenolic compounds into the samples, providing them with antioxidant capacity. There was no difference in phenol content and antioxidant activity between treatments with honey (50 and 75%). Consumers found no difference between marshmallows with and without honey, with the exception of sweetness which was excessive for their flavor in honey-added marshmallows. Additionally, the highest degrees bloom improved the texture and appearance of the samples. Nevertheless, the treatments with honey were generally better qualified and the treatments with 50% honey-315 °Blooms and 75% honey-300 °Blooms had greater acceptance. Therefore, the innovated marshmallows could be well received in the confectionery market.

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