The use of protein binders and sorghum crisps as potential ingredients in a cereal bar for dogs

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Abstract
This study aimed to evaluate the inclusion of different protein binders and sorghum crisps in cereal bars for dogs and their effect on sensory properties, product texture, and dog preference. Fifteen cereal bars were developed in which three crisp sources (rice crisp, white and red sorghum crisp) and five sources of binders (corn syrup, spray dried plasma, gelatin, albumin, and egg product) were evaluated. An interaction effect between binder and crisp sources was found for textural properties ($p < .05$). A total of 103 volatile compounds were identified and semi-quantified in the cereal bar samples, with aldehydes being the most represented. Unlike crisp source, protein binders played a major role on sensory properties and impacted the dog's preference. This study suggests that sorghum crisps and protein binders may be used in cereal bars for dogs; however, considerations regarding sensory attributes and dog's preference should be taken to maximize product acceptance.

Practical applications
This is the first study to report information regarding the use of novel ingredients in a cereal bar application for dogs. The findings observed wherein provide a comprehensive understanding about product development and the impact of ingredients on final product quality, sensory properties, and animal preference. The methodologies and outcomes of our work can be directly translated to the pet food industry to aid in the development of dog treats.

1 | INTRODUCTION

The pet food industry was worth over $69 billion in 2017 with a market growth projected to be over $72 billion for the following year (APPA, 2018). This rapidly growing market is driven by the development of new products, which involve the addition of new ingredients and food forms. The pet food segment can be nutritionally segregated into two major categories: complete & balanced, and snacks & treats. Although complete & balanced products compose the greatest share of the market sales, snacks & treats represent an important part of the industry. More than ever, pets are considered part of the family, and these products are offered as a demonstration of affection and love.

The humanization of pets has greatly impacted the pet food industry trends. Human food trends are being translated to the pet food industry as pet owners demand pet foods that reflect their own dietary choice. Trends toward more healthy and natural food have gained popularity in the pet food industry (Sprinkle, 2018). Cereal bars were introduced in the human food industry several decades ago as a healthy snack alternative (Bower & Whitten, 2000). These products remain popular, and they may represent a potential alternative to healthy treats for dogs. However, a previous study revealed that the...
chemical composition of some cereal bars in the market was similar to confectionary (Boustani & Mitchell, 1990). This may not be perceived as healthy by pet owners, and it could exclude the product from this claim.

Most cereal bars are composed of several ingredients that are bound by the addition of sugar syrups. This amplifies the levels of soluble carbohydrates in the final product. Although carbohydrates are well utilized by dogs, many pet owners perceive sugars as unhealthy and seek protein-rich food forms for their pets. Proteinaceous ingredients such as egg white and bovine plasma have been used in the gluten-free industry as potential binders (Crockett, Le, & Vodovoz, 2011; Furlan, Padilla, & Campderrós, 2015; Han et al., 2019), and may be a replacement for sugar syrup for manufacturing cereal bars. Rice crisps are also commonly used in cereal bar recipes. The replacement of rice crisps for nutraceutical ingredients such as sorghum may add value to the product. Sorghum is rich in phytochemicals that are known to have antioxidant and antiradical activities (Hagerman et al., 1998). The replacement of sugar syrup and rice crisps for soluble animal protein binders and sorghum crisps, respectively, may improve the nutritional quality of cereal bars and add value to the product for the pet market. However, to validate the use of these new ingredients in cereal bars, it is crucial to evaluate how these replacements (rice crisp vs. sorghum crisp and sugar syrup vs. protein binders) are accepted not only by the dog, but also by the owners as they make the purchase decision. To date, there are no studies evaluating acceptability/preference of cereal bars by dogs, and the use of protein binders and sorghum crisps as potential ingredients for this application. Thus, the objectives of this study were to (1) evaluate the effect of different proteinaceous binders and sorghum crisps on the texture and volatile compounds profile of cereal bars for dogs, (2) describe the sensory profile of the cereal bars using a highly trained human panel, and (3) to evaluate the effect of these ingredients on dog preference.

2 | MATERIALS AND METHODS

This research was approved by Kansas State University Institutional Review Board protocol #5930 and Institutional Animal Care and Use Committee under protocol #3722.

2.1 | Cereal bar production

This experiment was conducted as a $3 \times 5$ factorial arrangement for simultaneous evaluation of three sources of crisp (rice crisp, RC; white sorghum crisp, WSC; and red sorghum crisp, RSC) and five sources of binder (corn syrup, CS; spray dried plasma, SDP; gelatin, GL; albumin, AL; and egg product, EP). The RC and the CS were used as positive control from the crisp and binder effect, respectively. The WSC and RSC used in this study were produced using a single screw extruder (model E525, ExtruTech, Inc., Sabetha, KS) from a previous experiment at Kansas State University (Pezzali, Suprabha-Rah, Siliveru, & Aldrich, 2020). The RC was sourced from local manufacture (Cereal Ingredients Inc., Leavenworth, KS). The SDP (Innomax Porcine Plasma), GL (Pro-Bind Plus 50), and AL (Innomax MPI) were acquired from Sonac® (Maukokota, IA), and the EP (Oxabind-RSD 80) was acquired from IsoNova® (Springfield, MO). Last, CS (Light Corn Syrup, Kroger®) was acquired from a local grocery market.

Fifteen dietary treatments were developed (Table 1) and produced in 800 g batches. Dry ingredients (crisp (RC, RSC, or WSC), oatmeal (Quick 1-minute Oats, Quaker® Oats), coconut flakes (Unsweetened Shredded Coconut, Great Value®), dried blueberry (Its Delish®, flaxseed (Organic Ground Flaxseed, Great Value®), pepitas seeds (Organic Pepitas, Food to Live®), wheat germ (Kretschmer®), salt (Morton®), and palatant (Palasurence, Kemin®) were manually mixed in a stainless-steel bowl. Before mixing, the WSC and RSC were manually ground to decrease particle size. The agglomerating syrup was added to the dry ingredients, and the mixture was stirred until the crisps were uniformly coated. The syrup was composed of corn syrup or one of the protein sources. Protein sources required hydration prior addition into the mix and were prepared as follows: SDP (1:1.76 ingredient/water, wt/wt), GL (1:2.5 ingredient/water, wt/wt), AL (1:1.18 ingredient/water, wt/wt), and EG (1:1.47 ingredient/water, wt/wt). Protein sources required different processing conditions to create a consistent agglomeration syrup. The GL and AL were hydrated under heat. An attempt to hydrate SDP and EP under heat was performed, but these protein sources started to cook; therefore, this step was not implemented. Corn starch was added to all proteinaceous agglutination syrups with exception of the SDP. The final mixture was transferred to a cookie sheet covered with parchment paper and baked for 20 min at 163°C in a convection oven (MEA 21-93-E; Garland Commercial Industries, PA). After baking, the dough was cut into approximately 4 x 4 cm square pieces. Treatments that contained protein ingredients in the agglomerating syrup were dried overnight at 55°C in a convection oven (212041, HotPack, PA) to achieve a moisture content below 10%. Experimental treatments were produced as described above over three replicate-days.

2.2 | Nutritional analysis

For each dietary treatment, a sample of 50 g from each day of production was composited and ground through a 1-mm screen in a fixed blade laboratory mill (Retsch, type ZM200, Haan, Germany). Composite samples were analyzed for dry matter (DM; AOAC 930.15), crude protein (CP; AOAC 990.03), fat by acid hydrolysis (AOAC 954.02), crude fiber (CF; AOCS Ba 6a-05), and ash (AOAC 942.05) in a commercial laboratory (Midwest Laboratories, Omaha, NE). Nitrogen-free extract (NFE) was calculated by difference.

2.3 | Textural properties

Hardness and toughness of cereal bars were assessed using a Shimadzu EZ-SX Texture Analyzer (Shimadzu Corporation, Kyoto,
Five cereal bar square pieces were randomly selected for each replicate, totaling 15 samples per treatment. A compression test was performed using a toothed pushrod B probe at a speed of 1.67 mm/s. Hardness was defined as the highest peak fracture force. Toughness was defined as the total energy required to break the sample as it was calculated as the total area under the fracture curve.

### 2.4 Preference ranking test

The preference test took place at the Large Animal Research Center (LARC) at Kansas State University where 12 castrate Beagle dogs were used. Dog's preference was evaluated under a preference ranking procedure according to Li et al. (2018). Four preference ranking tests were performed, and each one consisted of 10 days (5 days of acclimation and 5 days of data collection). Each dog was presented simultaneously with five cereal bar treats in a rubber puzzle toy (Kong®). Dogs were allowed to smell the Kongs then each was placed randomly in the left corner of the 1.5 m x 1.5 m test room. The order in which the cereal bar was extracted and consumed by the dog was considered as the preference ranking order. This ranged from 1 to 5, where 1 was considered the most preferred, and 5 the least preferred.

First, the effect of protein source on dog's preference was evaluated. Three ranking tests were performed in which the treatments having the same crisp source and differing in the binder source were compared. Second, the effect of crisp source on dog's preference was assessed using a modified preference ranking test (Li et al., 2017). The SDP protein source was selected for evaluation. Three cereal bar treats produced with the SDP but differing in crisp source were presented to the dogs. Preference was assessed as described above.

### 2.5 Descriptive analysis

The 15 cereal bars were evaluated by five highly trained panelists from the Center for Sensory Analysis and Consumer Behavior at Kansas State University (Manhattan, KS). The panelists had received a minimum of

**Table 1 Ingredient and chemical composition of cereal bars**

| Item                        | Corn syrup | Spray-dried plasma | Gelatin | Albumin | Egg product |
|-----------------------------|------------|--------------------|---------|---------|-------------|
| Dry ingredients, %          | RC         | WSC                | RSC     | RC      | WSC         | RSC       | RC      | WSC        | RSC       | RC          | WSC        | RSC       | RC          | WSC       | RSC       |
| Oatmeal                     | 24.8       | 24.8               | 30.6    | 30.6    | 30.6       | 31.2      | 31.2    | 31.2       | 30.1      | 30.1        | 30.1       | 30.1      | 30.1        | 30.1      |
| Rice crisp                  | 14.9       | –                  | 18.4    | –       | –          | 18.7      | –       | –          | 18.0      | –           | –          | 18.0      | –           | –         |
| White sorghum crisp         | –          | 14.9               | –       | 18.4    | –          | –         | 18.7    | –          | –         | 18.0        | –           | 18.0      | –           | –         |
| Red sorghum crisp           | –          | –                  | 14.9    | –       | 18.4       | –         | 18.7    | –          | –         | 18.0        | –           | 18.0      | –           | –         |
| Coconut flakes              | 4.95       | 4.95               | 4.95    | 6.13    | 6.13       | 6.13      | 6.24    | 6.24       | 6.01      | 6.01        | 6.01       | 6.01      | 6.01        | 6.01      |
| Corn starch                 | 4.95       | 4.95               | 4.95    | 6.13    | 6.13       | 6.13      | 6.24    | 6.24       | 6.01      | 6.01        | 6.01       | 6.01      | 6.01        | 6.01      |
| Dried blueberry             | 3.54       | 3.54               | 3.54    | 4.38    | 4.38       | 4.38      | 4.46    | 4.46       | 4.29      | 4.29        | 4.29       | 4.29      | 4.29        | 4.29      |
| Flaxseed                    | 3.54       | 3.54               | 3.54    | 4.38    | 4.38       | 4.38      | 4.46    | 4.46       | 4.29      | 4.29        | 4.29       | 4.29      | 4.29        | 4.29      |
| Pepita seed                 | 3.54       | 3.54               | 3.54    | 4.38    | 4.38       | 4.38      | 4.46    | 4.46       | 4.29      | 4.29        | 4.29       | 4.29      | 4.29        | 4.29      |
| Wheat germ                  | 2.83       | 2.83               | 2.83    | 3.50    | 3.50       | 3.50      | 3.57    | 3.57       | 3.44      | 3.44        | 3.44       | 3.44      | 3.44        | 3.44      |
| Salt                        | 0.35       | 0.35               | 0.35    | 0.44    | 0.44       | 0.44      | 0.45    | 0.45       | 0.43      | 0.43        | 0.43       | 0.43      | 0.43        | 0.43      |
| Palatant                    | 0.35       | 0.35               | 0.35    | 0.44    | 0.44       | 0.44      | 0.45    | 0.45       | 0.43      | 0.43        | 0.43       | 0.43      | 0.43        | 0.43      |

| Agglutination syrup, %      |            |                    |         |         |            |                  |         |         |            |                  |         |
| Corn syrup                  | 36.4       | 36.4               | 36.4    | –       | –          | –                 | –       | –         | –          | –                | –         |
| Spray-dried plasma          | –          | –                  | –       | 21.3    | 21.3       | 21.3              | –       | –         | –          | –                | –         |
| Gelatin                     | –          | –                  | –       | 17.8    | 17.8       | 17.8              | –       | –         | –          | –                | –         |
| Albumin                     | –          | –                  | –       | –       | –          | –                 | –       | 20.9      | 20.9       | 20.9             | –         |
| Egg white                   | –          | –                  | –       | –       | –          | –                 | –       | 20.9      | 20.9       | 20.9             | –         |
| Corn starch                 | –          | –                  | –       | –       | –          | –                 | –       | 20.9      | 20.9       | 20.9             | –         |

| Chemical composition, %     |            |                    |         |         |            |                  |         |         |            |                  |         |
| Dry matter                  | 92.0       | 92.5               | 92.5    | 90.8    | 91.7       | 91.9              | 92.5    | 90.3      | 89.2       | 93.2             | 92.9      | 92.3        | 90.4        | 92.6        | 92.0       |
| Crude protein               | 9.79       | 9.88               | 9.34    | 29.1    | 29.0       | 28.3              | 27.8    | 28.0      | 28.6       | 31.3             | 32.0      | 32.3        | 28.6        | 29.2        | 28.5       |
| Fat                         | 11.2       | 9.9                | 11.2    | 12.0    | 12.0       | 11.8              | 12.1    | 12.1      | 12.6       | 11.6             | 11.4      | 11.4        | 12.4        | 11.9        | 11.5       |
| Nitrogen-free extract       | 68.6       | 70.5               | 69.7    | 44.8    | 45.9       | 46.7              | 49.0    | 46.9      | 44.5       | 47.1             | 46.0      | 44.5        | 44.8        | 47.2        | 47.6       |
| Crude fiber                 | 0.53       | 0.28               | 0.33    | 0.72    | 0.68       | 1.13              | 0.83    | 0.80      | 1.23       | 0.20             | 0.82      | 1.14        | 1.42        | 1.39        | 1.59       |

Abbreviations: RC, rice crisp; RSC, red sorghum crisp; WSC, white sorghum crisp.
120 hr of general descriptive sensory analysis training and had experience in testing food and pet food sensory properties. The cereal bar samples were evaluated in eight sessions (1.5 hr per session), including two sessions for orientation and six sessions for evaluation.

During the orientation sessions, the panelists generated a list of attributes for appearance, aroma, flavor, and texture perceived in the cereal bar samples (appearance: color brown, roughness of surface; aroma: vitamin, grain complex, coconut, cardboard, oil—heated, toasted, barnyard, butyric, nutty, sweet aromatics, stale; flavor: meaty, dry fruit complex, nutty, vitamin, grain, cardboard, eggy, coconut, barnyard, butyric, metallic, sweet, sour, salt, bitter; texture: firmness, cohesiveness of mass, roughness of mass, particles amount, particles size). The reference materials for the attributes were provided, similar to Di Donfrancesco, Koppel, and Chambers IV (2012) and Di Donfrancesco and Koppel (2017), and discussed during the orientation sessions. Then, the panel evaluated the cereal bars using a modified flavor profile method during the evaluation. The appearance, aroma, flavor, and texture of the samples were first evaluated individually by the panelists, and then the panel came to a consensus intensity score for each attribute per sample. A 0–15 scale with 0.5 increments was used for scoring, in which 0 means no intensity and 15 means extremely high intensity.

The cereal bar samples were stored at –18°C and thawed overnight at 4°C a day before the sensory evaluation. For aroma evaluation, 5 g of each ground sample was served in medium snifters, covered with watch glasses. Three pieces of 2 cm × 2 cm square cereal bars were served in Ziploc bags (S.C. Johnson & Son, Inc. Racine, WI) for appearance, flavor, and texture evaluation. All of the samples were labeled with random, three-digit blinding codes.

2.6 | Volatile compounds profile

The isolation, tentative identification, and semi-quantification of the volatile compounds were performed on a gas chromatograph (Shimadzu GC-2010 Plus, Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (GCMS-QP2020, Kyoto, Japan). First, volatile compounds were extracted from samples using headspace solid phase micro extraction (HS-SPME) according to a modified version of Koppel, Adhikari, and Di Donfrancesco (2013). The samples were ground in a coffee grinder for 10 s, and a 0.5 g of ground sample was weighed into a 10 ml screw-cap vial equipped with a polytetrafluoroethylene/silicone septum (Supelco, Bellefonte, PA). Exactly 0.99 ml distilled water was added to the ground sample in the vial, and 10 μl of 100 ppm 1,3-dichlorobenzene (Sigma Aldrich, St. Louis, MO) was added as the internal standard. Each sample was incubated at 50°C for 1 min before extraction. A 50/30 μm divinylbenzene/carbonex/polydimethylsiloxane fiber (Supelco, Bellefonte, PA) was exposed to the sample headspace for 20 min at 50°C for volatile compounds extraction. The volatiles were desorbed from the SPME fiber coating in the injection port at 240°C for 3 min in splitless mode. The GC-MS system was equipped with an SH-Rxi-5Sil MS Crossbond® column (Shimadzu, Tokyo, Japan; 30 m × 0.25 mm × 0.25 μm film thickness), which was heated from 40 to 240°C. The identification of the compounds was done using NIST library version 14. All analyses were run in two replicates.

2.7 | Statistical analysis

Textural properties were analyzed by analysis of variance using GLIMMIX procedure in statistical software (9.4 SAS Inst. I., Cary, NC). The effects of crisp and binder source and their interaction were analyzed. Day of production was considered as a random effect. Preference ranking data was analyzed by Friedman’s analysis of variance using the XLStat version 2017.4.46756 (Addinsoft, New York, NY). Descriptive analysis of the cereal bars and volatile compounds was analyzed by Clustering Analysis and Principal Component Analysis (PCA) using XLSTAT version 2017.4.46756 (Addinsoft, New York, NY). Partial least square regression (PLSR) analysis was performed to investigate the relationship between descriptive analysis data (Y-matrix) and instrumental GC-MS data (X-matrix), using XLSTAT version 2017.4.46756 (Addinsoft, New York, NY). The PCA was run using the correlation matrices for sensory attributes and all samples combined.

3 | RESULTS

3.1 | Chemical composition

The chemical composition of cereal bars is reported in Table 1. Cereal bars produced with proteinaceous agglutination syrups had CP content an average 19.72% greater than those produced with corn syrup, while NFE content was reduced by 23.38%. Minor differences were observed for fat, CF, and DM.

The hardiness and toughness of the cereal bars was affected by an interaction between the binder and crisp source (Table 2). The CS-RC presented the highest toughness value followed by CS-RSC (p < .05). Compared to these, the EP-RC, EP-WSC, EP-RSC, AL-RC, AL, RSC, SDP-WSC, and SDP-RSC treatments exhibited lower toughness (p < .05). The highest numerical value was observed for GL-WSC while the lowest hardness was observed for CS-RSC.

3.2 | Descriptive sensory analysis

All the cereal samples had low to medium levels of brown color (score range: 4–7) and medium to a high level of roughness of surface (score range: 7–10; Table 3). Samples made with RC + AL and RSC + AL exhibited the darkest brown color among the 15 treatments, while samples made with EP had the lightest brown color. The WSC + AL sample was found to have the roughest surface, on the other hand, samples produced with GL appeared to have a smoother surface than other samples. The 15 samples exhibited a high level of roughness of mass, particles amount, and had medium particle size (Table 3). Furthermore, all treatments had extremely high firmness (Table 3), except for the two
treatments made with CS. The cohesiveness of mass was low in all samples (score range: 0–3).

The aroma intensities were low for the attributes found among the samples (score range: 0–5). Vitamin, grain complex, cardboard, heated oil, toasted, nutty, and sweet aromatics were found in all the samples (Table 4). Cereal bars produced with CS did not exhibit butyric nor stale aromas, which were commonly found in the other samples. Samples made with AL had a relatively stronger butyric aroma than other treatments.

Similar to aroma intensities, the flavor intensities were relatively low for the attributes found in all the samples (score range: 0–6.5). Only five flavor attributes were scored for the 15 treatments including dry fruit complex, nutty, vitamin, grain, and cardboard (Table 5). Grain was the most dominant flavor among all samples, followed by nutty and cardboard. Samples made with the same binder had similar flavor profiles. Cereal bars made with CS did not exhibit metallic and butyric flavors. On the other hand, samples made with AL were characterized with relatively high intensity of butyric flavor with no coconut flavor or sweetness detected.

### 3.3 Preference ranking test

No signs of refusal were observed throughout the four different preference tests performed (Table 6). Dogs preferred WSC cereal bars produced with SDP over those produced with EP ($p < .05$). When RSC cereal bars were presented to dogs, a preference for SDP compared to GL was observed ($p < .05$). In the last preference ranking test where

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### TABLE 2 Effect of binder and crisp source on textural properties of cereal bar for dogs

| Treatment | Textural properties |
|-----------|---------------------|
|           | Hardness (kg) | Toughness (kg * mm) |
| **Binder** | **Crisp** | | |
| Corn syrup | RC | 23.63ab | 118a |
| | WSC | 12.69cde | 28.98bc |
| | RSC | 11.61e | 48.77h |
| Spray-dried plasma | RC | 20.26abcd | 18.54bc |
| | WSC | 15.59bcd | 13.19c |
| | RSC | 14.66cde | 15.59c |
| Gelatin | RC | 17.42bcde | 20.68bc |
| | WSC | 26.14a | 42.51bc |
| | RSC | 20.45abc | 29.99h |
| Albumin | RC | 13.78bcde | 16.96c |
| | WSC | 16.74bcde | 23.06bc |
| | RSC | 11.93c | 15.04c |
| Egg product | RC | 21.07abc | 23.34b |
| | WSC | 16.07bcde | 16.39c |
| | RSC | 17.75abcde | 16.27c |

Note: Different letters following the means in the same column indicate a significant difference ($p < .05$).

Abbreviations: RC, rice crisp; RSC, red sorghum crisp; SEM, standard error of the mean; WSC, white sorghum crisp.

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### TABLE 3 Appearance and texture profiles of the 15 samples

| Sample | Color brown (ap) | Roughness of surface (ap) | Firmness (t) | Cohesiveness of mass (t) | Roughness of mass (t) | Particles amount (t) | Particles size (t) |
|--------|------------------|---------------------------|--------------|--------------------------|----------------------|---------------------|-------------------|
| RC + CS | 5.5 | 9 | 14 | 2 | 12 | 11 | 7 |
| WSC + CS | 5.5 | 9 | 10 | 2 | 12 | 11 | 7 |
| RSC + CS | 5.5 | 10 | 10 | 2 | 12.5 | 11 | 7 |
| RC + SDP | 5 | 9 | 14 | 3 | 9 | 11 | 7 |
| WSC + SDP | 6 | 8 | 14 | 2.5 | 10.5 | 11 | 7 |
| RSC + SDP | 6 | 10 | 14 | 2.5 | 10.5 | 12 | 7 |
| RC + GL | 5 | 7 | 15 | 2 | 12 | 11 | 7.5 |
| WSC + GL | 5 | 7 | 15 | 0 | 12 | 12 | 9 |
| RSC + GL | 5 | 7.5 | 15 | 2 | 11 | 12 | 8 |
| RC + AL | 7 | 8.5 | 13 | 2 | 12 | 11 | 8 |
| WSC + AL | 6 | 10.5 | 14 | 2 | 12 | 11 | 7 |
| RSC + AL | 7 | 8.5 | 14 | 2 | 13 | 12 | 8 |
| RC + EP | 4 | 9 | 14 | 2.5 | 12 | 11 | 7 |
| WSC + EP | 4 | 10 | 14.5 | 2 | 12.5 | 12 | 6.5 |
| RSC + EP | 4 | 8.5 | 15 | 0 | 13 | 11 | 8 |

Note: Scores are given based on a 0–15 scales with 0.5 increments.

Abbreviations: AL, albumin; ap, appearance; CS, corn syrup; EP, egg product; GL, gelatin; RC, rice crisp; RSC, red sorghum crisp; SDP, spray-dried plasma; t, texture; WSC, white sorghum crisp.
the crisp source was evaluated, one dog was unable to perform the test and was removed from the study.

3.4 Drivers of preference—sensory attributes

Based on principal components analysis, the samples were separated by butyric flavor, aromas of toasted, cardboard, and barnyard on the positive axis; coconut aroma, sweet aromatics, sweetness, flavors of nutty, coconut, eggy on the negative axis of Factor 1 (Figure 1). Factor 2 differentiated the samples by grain complex aroma, meaty flavor, cohesiveness of mass, and nutty aroma on the positive axis; particle size, particles amount, and grain flavor on the negative axis. The two first principal components explained 65.43% (F1: 35.82%; F2: 29.61%) of variability in sensory attributes among the WSC samples (Figure 1).

WSC bars made with AL and SDP were highly correlated to aromas of toasted, cardboard, and dry fruit complex flavor. F2 was loaded in the positive axis with roughness of surface, flavors of meaty, barnyard, and vitamin; and in the negative axis with aromas of coconut, cardboard, and butyric. The two first principal components explained 69.06% (F1: 41.21%; F2: 27.85%) of variability in sensory attributes among the RSC samples.

The RSC samples were separated into different quadrants according to their sensory profile. RSC + CS and RSC + SDP, were strongly associated with flavors of nutty, coconut, and cohesiveness of mass. RSC + GL was highly correlated to cardboard and vitamin aromas. Dogs preference for the RSC cereal bars was toward the positive direction of F2. This result was in line with the preference ranking test, where RSC samples using SDP were preferred by dogs compared to those produced using GL as the binder source. The preference was found to be driven by meaty, barnyard, and vitamin flavors.

3.5 Volatile compounds and drivers of preference

A total of 103 compounds were identified and semi-quantified within the cereal bars produced, 12 of these compounds were found in all treatments. These 12 compounds were 2-Methyl-propanal, 3-Methyl-butanal, Hexanal, Nonanal, 1-Pentanol, 1-Hexanol, Dodecane, Tetradecane, Acetic acid, Ethyl ester, Toluene, Methoxy-phenyl-oxime, and α-Limonene. Hexanal was the most abundant volatile in the cereal bars (Tables S1–S3). Aldehydes were the major volatile class followed by alcohols and alkanes.

| Sample      | Vitamin | Grain complex | Coconut | Cardboard | Oil, heated | Toasted | Barnyard | Butyric | Nutty | Sweet aromatics | Stale |
|-------------|---------|---------------|---------|-----------|-------------|---------|----------|---------|------|-----------------|-------|
| RC + CS     | 2       | 5             | 3       | 2.5       | 3           | 3       | 0        | 0       | 2.5  | 2.5             | 0     |
| WSC + CS    | 2       | 4.5           | 3       | 2.5       | 3           | 2.5     | 0        | 0       | 2.5  | 2.5             | 0     |
| RSC + CS    | 2       | 4             | 2       | 3.5       | 3           | 3       | 2        | 0       | 2.5  | 2.5             | 0     |
| RC + SDP    | 2.5     | 4             | 0       | 3.5       | 3.5         | 3       | 2.5      | 2       | 2    | 2               | 2.5   |
| WSC + SDP   | 2.5     | 4             | 0       | 4         | 3.5         | 3       | 4        | 2       | 2.5  | 2               | 2     |
| RSC + SDP   | 2       | 4             | 2       | 3.5       | 3           | 2.5     | 2        | 0       | 2    | 2               | 2.5   |
| RC + GL     | 2       | 4             | 0       | 4         | 3.5         | 3       | 2.5      | 2       | 2    | 2               | 2.5   |
| WSC + GL    | 2       | 3.5           | 0       | 3         | 2           | 2.5     | 2        | 2       | 2    | 2               | 2     |
| RSC + GL    | 2.5     | 4             | 2.5     | 4         | 3.5         | 2.5     | 0        | 2       | 2.5  | 2               | 2.5   |
| RC + AL     | 2.5     | 4             | 0       | 3.5       | 3.5         | 3       | 2        | 2       | 2    | 2               | 2.5   |
| WSC + AL    | 2       | 4             | 0       | 4.5       | 3.5         | 3.5     | 3        | 3       | 2.5  | 2               | 2.5   |
| RSC + AL    | 2       | 4             | 0       | 3         | 3.5         | 3       | 2        | 2       | 2    | 2               | 2.5   |
| RC + EP     | 2       | 4             | 0       | 4         | 3.5         | 3       | 2.5      | 0       | 2    | 2               | 2.5   |
| WSC + EP    | 2.5     | 4             | 0       | 4         | 3.5         | 3       | 2.5      | 0       | 2    | 2               | 2     |
| RSC + EP    | 2       | 4             | 0       | 3.5       | 3.5         | 3       | 2        | 2       | 2.5  | 2               | 2     |

Note: Scores are given based on a 0–15 scales with 0.5 increments.

Abbreviations: AL, albumin; CS, corn syrup; EP, egg product; GL, gelatin; RC, rice crisp; RSC, red sorghum crisp; SDP, spray-dried plasma; WSC, white sorghum crisp.
| Sample   | Meaty | Dry fruit complex | Nutty | Vitamin | Grain | Cardboard | Eggy | Coconut | Barnyard | Metallic | Sour | Salt | Bitter | Sweet | Butyric |
|----------|-------|-------------------|-------|---------|-------|-----------|------|---------|----------|----------|------|------|--------|-------|---------|
| RC + CS  | 3     | 2                 | 5     | 3       | 6     | 3.5       | 2.5  | 3       | 3.5      | 0        | 2    | 1.5  | 3      | 2     | 0       |
| WSC + CS | 3     | 4                 | 5     | 3       | 5     | 3.5       | 3    | 3       | 3        | 0        | 2    | 1.5  | 2.5    | 2     | 0       |
| RSC + CS | 2.5   | 3.5               | 5.5   | 3       | 6.5   | 3         | 3    | 5       | 3        | 0        | 2    | 2    | 2.5    | 1.5   | 0       |
| RC + SDP | 2     | 2                 | 5     | 2.5     | 6.5   | 3         | 0    | 4       | 2.5      | 1.5      | 3    | 2.5  | 0.5    | 2.5   | 0       |
| WSC + SDP| 2     | 3                 | 4     | 2       | 5     | 3         | 0    | 2.5     | 3        | 0        | 1.5  | 1.5  | 2.5    | 0     | 3.5     |
| RSC + SDP| 3     | 4                 | 4     | 2.5     | 5.5   | 3         | 0    | 2       | 3.5      | 0        | 1.5  | 1.5  | 2.5    | 0     | 0       |
| RC + GL  | 0     | 6                 | 4     | 2       | 5     | 2.5       | 2    | 2       | 2        | 0        | 0    | 2    | 0      | 0     | 0       |
| WSC + GL | 0     | 2                 | 4     | 2       | 6     | 4         | 3    | 2       | 3        | 0        | 1.5  | 1.5  | 2      | 0     | 2       |
| RSC + GL | 0     | 4                 | 3.5   | 2       | 6.5   | 3         | 2    | 2       | 2        | 0        | 1.5  | 1.5  | 2      | 1.5   | 0       |
| RC + AL  | 3     | 2.5               | 3     | 3       | 4.5   | 3         | 2    | 0       | 4.5       | 0        | 2    | 2    | 4      | 0     | 4       |
| WSC + AL | 2     | 2                 | 3     | 3       | 5     | 3.5       | 0    | 0       | 4.5       | 2        | 2    | 2    | 4      | 0     | 3.5     |
| RSC + AL | 3     | 2                 | 3     | 3       | 4.5   | 3.5       | 2    | 0       | 4         | 2        | 3    | 2    | 3.5    | 0     | 3.5     |
| RC + EP  | 2     | 4                 | 4     | 2       | 6.5   | 3         | 0    | 2       | 2         | 2        | 2    | 2    | 3      | 0     | 0       |
| WSC + EP | 0     | 3                 | 5.5   | 2.5     | 6.5   | 3         | 2    | 2       | 3         | 1.5      | 2    | 1.5  | 3      | 1.5   | 0       |
| RSC + EP | 0     | 2                 | 4     | 2       | 6     | 3.5       | 2    | 2.5     | 0         | 2        | 2    | 2    | 3      | 0     | 2       |

Note: Scores are given based on a 0–15 scales with 0.5 increments.
Abbreviations: AL, albumin; CS, corn syrup; EP, egg product; GL, gelatin; RC, rice crisp; RSC, red sorghum crisp; SDP, spray-dried plasma; WSC, white sorghum crisp.
Cereal bars made with RSC + CS, RC + CS, and RSC + GL had the most versatile volatile compositions, in which 62, 60, and 58 volatiles were identified in the samples, respectively. On the contrary, the RC + EP, WSC + EP, and WSC + AL had the least volatiles being found in the cereal bars (32, 34, and 36 volatiles, respectively). Although RC + EP contained a smaller number of volatile compounds than the other treatments, its concentration of the total volatiles was the highest among all the treatments followed by cereal bars made with WSC + EP and WSC + AL.

Principal components analysis was conducted to understand how volatile composition in the WSC (Figure 3) and RSC (Figure 4) bars differentiated among samples, and to further investigate key volatiles that drove preference. We did not observe a strong correlation between dog preference and volatile composition of cereal bars when either WC or RC were used. The WSC + SDP, had higher amounts of 3,5-Octadien-2-one, Tridecane, 6-Methyl-2-Heptanone, 3-Octen-2-one, 2,3-Octanedione, Octanoic acid methyl ester, Hexanoic acid methyl ester, Heptanal, and 2-Pentanone compared to the other samples. Both WSC + GL and WSC + CS were distinguished by their volatile profiles. The principal components (factor 1—F1; factor 2—F2) explained 67.28% (F1: 34.83%; F2: 32.45%) of variability in volatiles among the WSC samples (Figure 3).

The RCS samples were separated on the PCA bi-plot, except RSC + SDP and RSC + GL. Those samples were clustered on the positive end of F1, close to 1-Dodecanol and Undecane. However, (Z)-2-Heptenal, Hexanoic acid, methyl ester, 3-Octen-2-one, 2,3-Octanedione, 2-Hexenal, (E), 2-Heptenal, (Z), 2-methyl-1-butanol, 2,4-dimethyl-cyclohexanol (SPD), Hexanoic acid methyl ester, 2,3-Octanedione, 3,5-Octadien-2-one were found in relatively higher concentrations in RSC + SDP, while 4-Methyl-dodecane, Furfural, 3-methyl-undecane, 4-methyl-dodecane, 5-methyl-undecane, Dodecane, 5-butyl-nonane, Decyl heptyl ether were found in high concentrations in RSC + GL. Principal components (factor 1—F1; factor 2—F2) explained 60.99% (F1: 33.21%; F2: 28.78%) of variability in volatiles among the RSC samples (Figure 4).
FIGURE 2  Principal component analyses of sensory attributes: aroma (a), flavor (f), and texture (t) of red sorghum crisp cereal bars. Dog’s preference (mean of rank) was included as a supplementary variable.

FIGURE 3  Principal component analyses of volatiles of white sorghum crisp cereal bars. Dog's preference (mean of rank) was included as a supplementary variable. Volatile compounds are listed ID numbers (Table A2).
3.6 | Relationship between sensory attributes and volatile compounds of cereal bars

Possible associations between sensory attributes identified in the cereal bars and volatile compounds were found by PLSR analysis (Figure 5). Some sensory attributes were highly correlated with the volatiles. For example, sweet aromatics and coconut aroma were associated to 6-Methyl-tridecane, 3-Ethyl-3-methylheptane, Dimethyl disulfide, and ethanol. The bitter flavor was associated to 3-Methyl-butanolic acid, 3-Ethyl-2,5-dimethyl-pyrazine, 3-Methyl-butanal, Benzeneacetaldehyde, and 2-Methyl-butanolic acid. The barnyard aroma was associated with Acetic acid methyl ester. Other sensory attributes had relatively lower correlation with the volatiles.

The 15 cereal bars were clustered into three groups in the PLSR plot (Figure 5). Cereal bars made with AL were positively correlated with volatile of 3-Methyl-butanolic acid, 3-Ethyl-2,5-dimethyl-pyrazine, 3-Methyl-butanal, Benzeneacetaldehyde, 2-Methyl-butanolic acid, 2-Butanethiol, and 2-Methyl-propanolic acid. These samples were characterized with toasted aroma, butyric aroma, flavors of barnyard, butyric, tastes of bitter and salt. Cereal bars produced with CS were associated with volatiles of 6-Methyl-tridecane, 3-Ethyl-3-methylheptane, Dimethyl disulfide, ethanol, Pentyl-cyclopetane, Decyl-cyclopetane, (Z)-2-methyl-3-Octen-2-ol, Hexylmethyl ether, and 3-Methyl-decane. The samples are featured with stronger notes of eggy, nutty, sweet aromatics, coconut, grain complex, and sweetness. The rest of the nine cereal bars were positively correlated with flavors of dry fruit complex and grain, and vitamin aroma; however, those samples had a low correlation with most volatiles (Figure 6).

4 | DISCUSSION

The objective of our study was to evaluate the effect of protein binders, and white and red sorghum crisps in cereal bars on sensory properties, textural properties, and dog preference. There is a lack of research regarding processing conditions and animal preference of snacks and treats. The use of alternative ingredients in cereal bars to enhance their protein content has been investigated. Specifically, peanut flour, soy flour, and mesquite cotyledon (Escobar, Estevez, Tepper, & Aguayo, 1998; Estevez, Escobar, & Ugarte, 2000) and black and red beans (Maurer et al., 2005) were included in cereal bars to increase their protein content. However, this is the first study to investigate the replacement of corn syrup by a proteinaceous ingredient in a novel dog treat application. The results reported herein suggest that it is possible to enhance the CP content of cereal bars by using either AL, GL, SDP, or EP as a replacement for CS without compromising product integrity and animal acceptance. To form an agglomeration syrup that effectively binds the dry ingredients, hydration of protein sources was performed differently according to their protein type. Each protein source has a unique amino acid composition, sequence, and molecular weight, which have a direct impact on the protein functionally. This corroborates with the fact that different hydration conditions were necessary to develop each of the proteinaceous agglutination syrups. Gelatin has been used in a wide range of applications such as food, cosmetics, and pharmaceutical due to its gel-forming properties (Gómez-Guilén, Giménez, López-Caballero, & Montero, 2011). Spray-dried plasma (Furlan et al., 2015) and egg white (Crockett et al., 2011) are commonly used in gluten-free
FIGURE 5  Plot of Partial Least Square Regression (PLSR) analysis of the volatile compounds (X-matrix) and sensory attributes (Y-matrix), 15 cereal bars were shown in green. Volatile compounds are listed as ID numbers (Table A2).

FIGURE 6  Cereal bars were successfully produced with three different crisp sources (RC, rice crisp; RSC, red sorghum crisp; and WSC, white sorghum crisp) and five sources of binders (corn syrup; spray dried plasma; gelatin; albumin; and egg product).
applications due to their foam-stabilizing activity. This was the first attempt to use SDP, GL, AL, and EP as binder sources in cereal bar applications. Our study indicated that these protein sources can effectively work as binding agents for cereal bars. It was not our intention to investigate the best inclusion level of each protein source, but rather to evaluate the potential of each protein as binders. The inclusion level of the protein source used in our study may serve as a starting point for future research aiming to investigate the adequate inclusion level of the protein of interest to maximize product quality and animal acceptance. Processing conditions such as baking time and temperature should also be investigated as each protein source may have different processing requirements to perform at its best.

The use of WSC and RSC were also evaluated as replacements for RC. The sorghum crisps used in this research were produced from a previous study (Pezzali et al., 2020) while the rice crisp was acquired from a commercial source. The rice crisps were denser (348 g/L) compared to the white and red sorghum crisps (107 and 92 g/L, respectively). This compromised aggregation of ingredients due to a lower surface area for contact. To include the same mass of rice and sorghum crisps in the recipe, they had to be manually ground (crushed) to decrease particle size, and improve aggregation. Thus, extrusion conditions for production of sorghum crisps may be adjusted in future (e.g., decrease specific mechanical energy) to decrease expansion and increase the density of the product to improve their use as an ingredient for cereal bars. A recent study showed that extruded sorghum flour can promote health benefits in obese rats (Arbex et al., 2018). Extruded sorghum crisps might provide similar results in obese rats (Arbex et al., 2018). This compromised aggregation of ingredients due to a lower surface area for contact. To include the same mass of rice and sorghum crisps in the recipe, they had to be manually ground (crushed) to decrease particle size, and improve aggregation. Thus, extrusion conditions for production of sorghum crisps may be adjusted in future (e.g., decrease specific mechanical energy) to decrease expansion and increase the density of the product to improve their use as an ingredient for cereal bars. A recent study showed that extruded sorghum flour can promote health benefits in obese rats (Arbex et al., 2018). Extruded sorghum crisps might provide similar results in obese rats (Arbex et al., 2018).

The preference ranking test was used to assess the dog's preference. In the protein source evaluation, dogs showed a preference for SDP compared to EP and GL in cereal bars produced with WSC and RSC, respectively. In this test, the aroma is the first factor driving the animal's choice. However, dogs have the chance to associate the aroma profile of the sample with the texture and flavor after the treats are presented for 5 days. The PCA revealed a low correlation between dog's preference for cereal bars produced with either WSC or RSC and the volatiles within the samples. Nevertheless, the lack of a strong correlation does not imply that the volatile profile of the cereal bars did not play a role as a driver of dog preference. Although the preference ranking test has been previously validated (Li, Smith, Aldrich, & Koppel, 2018), it may lack sensitivity to discriminate samples that are overall well accepted by the animals. The fact that the dogs preferred cereal bars produced with SDP over those produced with EP and GL, when WSC and RSC were used as crisps sources, respectively, and the high correlation between cereal bar samples and volatile compounds suggest that the volatile profile of those samples may be playing a role on dog's preference. The WSC + SDP and the RSC + SDP samples both had higher concentrations of aldehydes compared to WSC + EP and RSC + GL. Low molecular weight aldehydes are considered important contributors to the aroma due to their low odor thresholds; thus, the higher concentration of this class of volatiles may help explain the dog's preference toward WSC + SDP and RSC + SDP. Sorghum has been associated with bitter and astrin-gent notes (Kobue-Lekalake, Taylor, & de Kock, 2007), but the extru-sion process performed to produce sorghum crisps probably reduced these characteristics (Di Donfrancesco & Koppel, 2017), resulting in no differences between RC, WSC, and RSC.

The use of sensory analysis methodologies can also provide additional information to better understand the dogs' preference toward a specific product. Thus, the second objective of this study was to use analytical (identification of aroma compounds) and sensory descriptive methods to aid in understanding the sensory profile of the cereal bars and its impact on the dog's preference. The cereal bar samples were grouped based on the similarity of their sensory characteristics. Samples manufactured with the same binder were found to roughly group together in the same cluster. This indicates that the source of the binder was the major factor impacting the sensory properties rather than the crisp source. There is a dearth of data regarding the impact of sorghum on the sensory properties of pet foods, and this is the first
study assessing how it impacts the sensory profile of dog treats. Some studies evaluated the nutritional impact (Alvarenga, Ou, Thiele, Alavi, & Aldrich, 2018), sensory properties (Di Donfrancesco & Koppel, 2017), and consumer acceptance (Di Donfrancesco, Koppel, & Gregory Aldrich, 2018) of sorghum into pet food. Our results agree with Di Donfrancesco and Koppel (2017) who found a similar aroma and flavor profile of dry dog food produced with red sorghum or with rice, corn, and wheat as grain sources. Furthermore, the similar sensory profile of cereal bars produced with different crisp sources corroborates with the results obtained from the preference ranking test—where dogs showed no preference for any crisp sources. Thus, the results wherein indicate that the replacement of RC for WSC or RSC is of high potential because it impairs neither the sensory profile of cereal bars nor the dog’s preference.

Cereal bars made with protein binders exhibited different sensory characteristics compared to those produced with CS. The cereal bars produced with CS brought out characteristics related to the raw materials, such as grain complex and coconut. To the contrary, samples made with protein binders had lower intensities for these attributes. The use of GL, EP, and SDP as binders resulted in a milder sensory profile. However, AL promoted higher cooked or processed attributes, such as toasted, butyric, and barnyard. Because all binders were well accepted by dogs, one should investigate the preference of these sensory attributes by dog owners as they have the purchase decision.

The high number of volatile compounds identified in the cereal bars indicated the complex food matrix of the products. This was expected as more than 15 ingredients were used to produce each treatment. The high variation observed between and within the same treatment for some volatile compounds may be due to variation between processing days and/or to high volatility of the compound. With regards to the volatile groups identified, aldehydes, alcohols, and alkanes were the most abundant ones. This result is consistent with a previous study (Di Donfrancesco & Koppel, 2017), in which aldehydes represented 50% of the total volatiles in extruded pet foods made with different sorghum fractions. In another study, Koppel et al. (2013) also observed aldehydes as the major volatiles in commercial dry dog food. Within the aldehyde volatile group, hexanal, 2-methyl-propanal, and furfural were the most prominent ones in the cereal bars. Although hexanal and benzaldehyde were found to be the most abundant compounds in grain-based pet food (Koppel et al., 2013), benzaldehyde was not the second abundant compound in the cereal bars even though the oatmeal and grain crisps were the major ingredients in the formula. Heenan et al. (2012) investigated the effects of sugar concentration on flavor release of cereal bars, and they identified 17 flavor compounds which is much lower than we identified in the present study (108 volatile compounds). This can be explained by the higher number of ingredients used in our study, and by the use of different proteinaceous ingredients which have strong flavor and aroma characteristics. Overall, cereal bars produced with proteinaceous ingredients had a 62% higher concentration of volatile compounds compared to those produced with CS. The interaction between the volatile compounds and the food components and the solubility and hydrophobicity of the volatiles will determine if the compound will stay bound in the food matrix or released in the head-space (Heenan et al., 2012). The higher concentration of volatile compounds produced with proteinaceous ingredients may be due to increased release rates of volatiles as a result of changes in the food matrix (e.g., viscosity) leading to decrease nutrient-volatile interaction. Furthermore, simply replacing CS with one of the proteinaceous ingredients probably contributed to the higher content of volatile compounds. The proteinaceous ingredients used in this study are produced under high temperatures, and it is well established that heating increases the concentration of almost all volatile compounds and also results in the appearance of novel ones (Qvist & von Sydow, 1974).

The presence of aldehydes and alcohols in the cereal bar samples may indicate lipid oxidation/degradation and Maillard reaction (Bak, Petersen, Lametsch, Hansen, & Ruiz-Carrascal, 2018). Oxidation of n-6 polyunsaturated fatty acids can lead to the production of hexanal (Pignoli, Bou, Rodriguez-Estrada, & Decker, 2009). Branched chain aldehydes, such as 2-methyl-propanal and 3-methyl-butanal, are products of Strecker degradation reactions in which an alpha-amino group from an amino acid is converted into an aldehyde under heat (Smit, Engels, & Smit, 2009). These chemical reactions probably occurred when cereal bars were baked at 163°C for 20 min. Due to their lower thresholds, these compounds are potent flavors and are considered to be important contributors to the aroma. Understanding the aroma profile of a dog food/treat is of extreme importance as it affects the human purchase decision. A consumer study is needed to better understand pet owners’ acceptance of the cereal bars, which can be further associated with the sensory attributes to find a possible indicator for liking.

5 | CONCLUSION

The replacement of rice crisps for white and red sorghum crisps did not lead to differences in animal preferences and sensory properties, but the size and density of sorghum crisps should be reconsidered in order to improve product quality. On the other hand, dogs preferred cereal bars produced with spray dried plasma over gelatin and egg product when red and white sorghum crisps were used as the crisp source, respectively. Combinations of different protein binders and crisp sources impacted product texture, thereby the interaction of ingredients should be examined more closely during product development. With regards to the aroma profile, more than 108 volatile compounds were identified in the cereal bars, and their concentration was mostly affected by the proteinaceous ingredients. Thus, proteinaceous binders and sorghum crisps are potential ingredients to replace sugar syrup and rice crisps, respectively, in cereal bar treat application for dogs.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.
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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of this article.

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### TABLE A1
Attributes used in the descriptive sensory analysis of cereal bars

| Modality         | Attribute         | Definition                                                                 | Reference with intensity                         |
|------------------|-------------------|---------------------------------------------------------------------------|--------------------------------------------------|
| Appearance       | Color brown       | Light to dark evaluation of brown color.                                   | Pantone Coated Plus Series 465C = 6.0             |
|                  | Roughness of Surface | A visual evaluation of the uneven texture of the top surface of the product. Smooth → Rough. | Kellogg’s Rice Krispies Treat = 8.0              |
| Aroma            | Vitamin           | The aromatics associated with a just opened bottle of vitamin pills.       | Nature Made Super B-Complex Capsule = 4.0         |
|                  | Grain Complex     | A general term used to describe the light, dusty/musty aromatics associated with grains such as corn, wheat, bran and rice. | Cereal Mix (dry) = 7.5                           |
|                  | Coconut           | Slightly sweet, nutty, somewhat woody aromatics associated with coconut.   | McCormick Imitation Coconut Extract = 7.5        |
|                  | Cardboard         | The aromatic associated with cardboard or paper packaging.                 | 2’ Cardboard Square in Water = 7.5               |
|                  | Oil, heated       | The aromatics commonly associated with heated oil.                        | Wesson Vegetable Oil = 7.0                       |
|                  | Toasted           | A moderately browned/baked impression.                                    | Post Shredded Wheat = 3.0                        |
|                  | Barnyard          | Pungent, slightly soured hay-like and/or animalic aromatics.               | White Pepper in water = 5.0                      |
| Butyric          |                   | Aromatics reminiscent of baby vomit; is sour and cheesy.                   | Kraft Shredded Parmesan Cheese = 5.5             |
| Nutty            |                   | A combination of slightly sweet, brown, woody, oily, musty, astringent, and bitter aromatics commonly associated with nuts, seeds, beans, and grains. | Le nez du café n. 29 “roasted hazelnuts” = 7.5    |
| Sweet aromatics  |                   | Aromatics associated with the impression of sweet substances.              | Lorna Doone Cookies = 3.0                        |
| Stale            |                   | The aromatics associated with wet cardboard that is characterized by a lack of freshness. | Mama Mary’s Pizza Crust = 4.5                    |
| Flavor           | Meaty             | A measure of how much a sample is recognized as distinctly animal muscle tissue. | Canned Swanson Beef Broth = 6.0                   |
|                  | Dry fruit complex | Aromatics associated with dried brown fruit.                               | Mix of Chopped Prunes, Sun-Maid Figs, and Sun-Maid Raisins = 8.5 |
|                  | Nutty             | A light, brown, slightly musty aromatic associated with nuts, wheat germ, and certain whole grains. | Quake quick oats (uncooked) = 4.0                 |
|                  | Vitamin           | The aromatics associated with a just opened bottle of vitamin pills.       | General Mills Wheaties = 2.5                      |
|                  | Grain             | The light dusty/musty aromatics associated with grains such as corn, wheat, bran, rice, and oats. | Cereal Mix (dry) = 8.0                           |
|                  | Cardboard         | A flat flavor note associated with cardboard or paper packaging that may be associated with a stale characteristic. | Mission Tortilla White Flour = 4.0               |
| Eggy             |                   | Aromatics/flavors associated with cooked whole chicken eggs, with savory, earthy, salty, buttery, and sulfur overtones. May also include sweet, metallic, and cardboard notes. | Sara Lee Pound Cake = 6.0                        |
|                  | Coconut           | Slightly sweet, nutty, somewhat woody aromatics associated with coconut.   | Bob’s Red Mill Unsweetened Flaked Coconut = 6.0   |
|                  | Barnyard          | Pungent, slightly soured hay-like and/or animalic aromatics.               | White Pepper in water = 7.0                      |
|                  | Butyric           | Aromatics reminiscent of baby vomit; is sour and cheesy.                   | Kraft Shredded Parmesan Cheese = 7.0             |
|                  | Metallic          | An aromatic and mouth feel associated with tin cans or aluminum foil.      | 0.10% Potassium Chloride Solution = 1.5           |

(Continues)
### Table A1 (Continued)

| Modality | Attribute | Definition | Reference with intensity |
|----------|-----------|------------|--------------------------|
| Sweet    | A fundamental taste factor of which sucrose is typical. | 1% Sucrose Solution = 2.0 |
| Sour     | The fundamental taste factor associated with a citric acid solution. | 0.015% Citric Acid Solution = 1.5, 0.050% Citric Acid Solution = 3.5 |
| Salt     | A fundamental taste factor of which sodium chloride is typical. | 0.15% NaCl Solution = 1.5, 0.25% NaCl Solution = 3.5 |
| Bitter   | The fundamental taste factor associated with a caffeine solution. | 0.01% Caffeine Solution = 2.0, 0.02% Caffeine Solution = 3.5 |

| Texture | Firmness | The force required to bite completely through the sample with the molar teeth. Evaluate on first bite down with the molars. | Nature Valley Crunchy Bars = 9.0, Gingersnap Cookies = 13.5 |
|---------|----------|---------------------------------------------------------------|-------------------------------------------------------------|
|         | Cohesiveness of mass | Degree to which mass holds together during mastication after 5–7 chews. | Nabisco Triscuit = 2.5, General Mills Cheerios = 7.0 |
|         | Roughness of mass | The degree of abrasiveness perceived when gently manipulating the mass against the palate after 5–7 chews. | General Mills Wheaties = 8.0, Nabisco Triscuit = 11.0 |
|         | Particles amount | Perception of pieces within the sample which do not break down during mastication. Evaluated by chewing 8–10 times, then manipulating the sample with the tongue 3–5 times. | General Mills Wheaties = 10 |
|         | Particles size | Perception of pieces within the sample which do not easily break down during mastication. Evaluated by chewing 8–10 times, then manipulating the sample with the tongue 3–5 times. | General Mills Wheaties = 5.0 |

### Table A2

| Identification number | Volatile compound             |
|-----------------------|-------------------------------|
| 1                     | 2-methyl-1-Butanol            |
| 2                     | 3-methyl-1-Butanol            |
| 3                     | 1-Dodecanol                   |
| 4                     | 1-Hexanol                     |
| 5                     | 2-ethyl-1-Hexanol             |
| 6                     | 1-Octen-3-ol                  |
| 7                     | 1-Pentanol                    |
| 8                     | 2-Butanethiol                 |
| 9                     | 2-Butanone                    |
| 10                    | 6-methyl-2-Heptanone          |
| 11                    | 2-Heptenal, (Z)               |
| 12                    | 2-Hexanol, methyl ether       |
| 13                    | 2-Pentanone                   |
| 14                    | 2,3-Octanedione               |
| 15                    | Tetrahydro-6-propyl-2H-Pyran-2-one |
| 16                    | 3-Ethyl-3-methylheptane       |
| 17                    | 3-Octen-2-one                 |
| 18                    | 3,5-Octadien-2-one            |
| 19                    | 6-methyl-5-Hepten-2-one       |
| 20                    | Acetic acid                   |
| 21                    | Acetic acid, methyl ester     |
| 22                    | Benzaldehyde                  |
| 23                    | 1,3-dimethyl-Benzene          |
| 24                    | Benzeneacetaldehyde           |
| 25                    | 3-methyl-Butanal              |
| 26                    | Butanoic acid                 |
| 27                    | 2-methyl-Butanoic acid        |
| 28                    | Butanoic acid 2-methyl- methyl ester |
| 29                    | Butanoic acid methyl ester    |
| 30                    | Carbonic acid dimethyl ester  |
| 31                    | D-Limonene                    |
| 32                    | Decanal                       |
| 33                    | Disulfide dimethyl            |
| 34                    | Dodecane                      |
| 35                    | 2,5-dimethyl-dodecane         |
| 36                    | 2,6,11-trimethyl-dodecane     |
| 37                    | 2,7,10-trimethyl-dodecane, 2,7,10-trimethyl dodecane |
| 38                    | 4-methyl-dodecane             |
| 39                    | Ethanol                       |
| 40                    | 2-butoxy-ethanol              |
| 41                    | Ethylbenzene                  |
| 42                    | Furfural                      |
| 43                    | Hexanal                       |
| 44                    | Hexanoic acid methyl ester    |
| Identification number | Volatile compound                  |
|------------------------|-----------------------------------|
| 45                     | Methional                         |
| 46                     | Methyl methacrylate               |
| 47                     | Methyl propionate                 |
| 48                     | Nonanal                           |
| 49                     | Nonane                            |
| 50                     | 2-methyl-5-propyl-nonane          |
| 51                     | 2,5-dimethyl-nonane               |
| 52                     | 3-methyl-nonane                   |
| 53                     | 3-methyl-5-propyl-nonane          |
| 54                     | 5-(1-methylpropyl)-nonane         |
| 55                     | 5-(2-methylpropyl)-nonane         |
| 56                     | 5-butyl-nonane                    |
| 57                     | 5-methyl-nonane                   |
| 58                     | o-Xylene                          |
| 59                     | Octanal                           |
| 60                     | 2,5-dimethyl-octane               |
| 61                     | Octanoic acid, methyl ester       |
| 62                     | Oxime-, methoxy-phenyl-           |
| 63                     | Pentanal                          |
| 64                     | 2-methyl-propanal                 |
| 65                     | 2-methyl-propanoic acid           |
| 66                     | 2,5-dimethyl-pyrazine             |
| 67                     | 3-ethyl-2,5-dimethyl-pyrazine     |
| 68                     | Trimethyl-pyrazine                |
| 69                     | Sulfurous acid, hexyl octyl ester |
| 70                     | Tetradecane                       |
| 71                     | Toluene                           |
| 72                     | Tridecane                         |
| 73                     | 6-methyl-tridecane                |
| 74                     | Undecane                          |
| 75                     | 3-methyl-undecane                 |
| 76                     | 3,6-dimethyl-undecane             |
| 77                     | 4,4-dimethyl-undecane             |
| 78                     | 5-methyl-undecane                 |
| 79                     | Undecane2                         |
| 80                     | 2-Furanmethanol                   |
| 81                     | 2-Hexenal, (E)-                   |
| 82                     | 2,4-Dodecadienal, (E,E)-         |
| 83                     | 3-methyl-butanoic acid            |
| 84                     | Cyclobutanol                      |
| 85                     | 2,4-dimethyl-cyclohexanol         |
| 86                     | 3-methyl-decane                   |
| 87                     | Heptanal                          |
| 88                     | Hexyl methyl ether                |
| 89                     | p-Xylene                          |
| 90                     | Pentadecane                       |
| 91                     | 2-methyl-pentanal                 |
| 92                     | 2,6-dimethyl-pyrazine             |
| 93                     | 3-Octen-2-ol, 2-methyl-, (Z)-     |
| 94                     | Decyl-cyclopentane                |
| 95                     | Pentyl-cyclopentane               |
| 96                     | Decyl heptyl ether                |

(Continues)