PREBIOTICS BEVERAGES BASED ON CASHEW NUT ALMOND AND GRAPE JUICE: PREFERENCE ANALYSIS BY MIXED MODELS

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ABSTRACT

In the Food and Technology Science is important to improve products and methods and this work aimed to present an analysis strategy for sensory data, using the ordinal nature of the response variables, that corresponds the hedonic scale. The data are from a study with prebiotic beverages made from cashew nut added to grape juice to evaluate its sensory characteristics and the experimental study followed a balanced incomplete block design, in which each panelist evaluated 4 of the 13 proposed beverage formulations. As statistical methods were used correspondence analysis techniques and proportional odds mixed models. Three better beverage formulations were selected: 8% sugar and 40% grape juice, 6% sugar and 44% grape juice and 9% sugar and 30% grape juice. It was verified that the correspondence analysis as well as the ordinal mixed models were useful for sensory data analysis, contributing to methods in the area.

Keywords: Sensory analysis; logit model; ordinal response; cumulative probabilities.

INTRODUCTION

The increasing tendency of functional benefits provided by fruits is influenced by changes in the dietary practices and consumers preferences, by the increase of population is age and by the pursuit of higher life quality (LEMOS, 2015). Therefore, food products with prebiotics are increasingly being marketed as they are healthier and help intestinal function. Another ally of good health is the cashew nut which is rich in monounsaturated and polyunsaturated fats, which lower bad cholesterol (LDL, Low Density Lipoproteins) and increases the good (HDL, High Density Lipoproteins). Moreover, the cashew apple is considered a good source of vitamin C, minerals (calcium, sodium and iron) and antioxidant substances (phenolics and carotenoids) (PEREIRA, 2013).

Demand for more research on consumer acceptability has been identified as a priority in the food industry. One area that studies the development of new beverage formulations is Food Science and Technology. It is worth mentioning that they are also responsible for evaluating products before they are released to the consumer. The evaluation of this product is carried out through sensory analysis. According to the Brazilian Association of Technical Standards (ABNT, 1993), the sensory analysis is a science used to analyze and interpret reactions to the characteristics of food and materials based on the senses of vision, smell, taste, touch, and hearing. In the evaluation of food and beverages, it is an important indicator of the acceptability of the product in the market, with the tasting experiment being properly planned by a technical team and the panelists, in turn, can be trained people or not (TEIXEIRA, 2009).

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n general, experiments for sensory analysis are incomplete blocks design, because this area may involve many treatments (types of products or brands) with heterogeneity or limitations that restrict the size of blocks, represented by the panelists (MONTEGOMERY, 2013). Moreover, in this experimental design, each panelist does not test all products, in order not to exhaust their sensory senses, which would compromise the final analysis.

Regarding the response variable for each sensory attribute, it appears that it is on a hedonic scale, from 1 to 9, where 1 is the most unfavorable category and 9 is the most favorable category. In the discrete data literature, the variables of interest are usually ordinal polytomous (AGRESTI, 2010). In this context, although there are parametric or non-parametric methods for analyzing these data, including models for response surfaces (KHURI & CORNELL, 2018), it is recommended to respect the nature of the variable, using models for categorical data (FATORETTO et al. 2018). Also, to consider the structure of the design, random effects must be considered in the linear predictor, since the evaluator enters as a block of random nature. Therefore, the methods of mixed models are necessary (MOLENBERGHS & VERBEKE, 2006).

Thus, the present work aims to present an analysis for ordinal data, in a balanced incomplete block design, by means of the correspondence analysis and the mixed cumulative logit models with proportional odds. These methods can be applied to sensory analysis and thus contribute to product selection strategies. As motivation we have an application to a set of real data of the sensory analysis with variable ordinal polytomous response, to check the association of beverage formulation (prebiotic based on almonds of cashew nuts flavored with grape juice) and sensory factors.

MATERIAL AND METHODS

The motivational study comes from research developed in the Department of Food Science and Technology of the Federal University of Ceará, in the year 2016 (REBOUÇAS, 2016). The aim was to develop prebiotic beverages based on almond cashew nuts added to grape juice, in order to evaluate the acceptance under the sensory attributes: overall impression, aroma, thickness, sweetness and flavor, using a hedonic scale, that is composed by 9 ordinal points. Moreover, in this work, the scale was reduced to 5 points, that is, considering “5 = I liked it extremely or very much”, “4 = I liked it moderately or slightly", “3 = neither liked nor disliked”, “2 = disliked slightly or moderately”', “1 = disliked very or extremely”', to facilitate the process of fitting categorical models.

Two prebiotic substances were used for the composition of the beverages: inulin (degree of polymerization ≥10, Orafti GR) and oligofructose (2 - 8 monomers, Orafti P95). Also, commercial crystal sugar was used. To obtain the water-soluble extract, cashew nuts were used and raw. Grape juice concentrate (pH = 2.99; 15.2º Brix) was used to flavor the beverages, which was defined by means of preliminary studies.

These formulations were made by means of a 2 × 2 factorial of a central rotational compound design, with five replicates at the central point (Figure 1), in which combinations of percentages of grape juice and sugar were used, totaling 13 beverage formulations (Table 1).

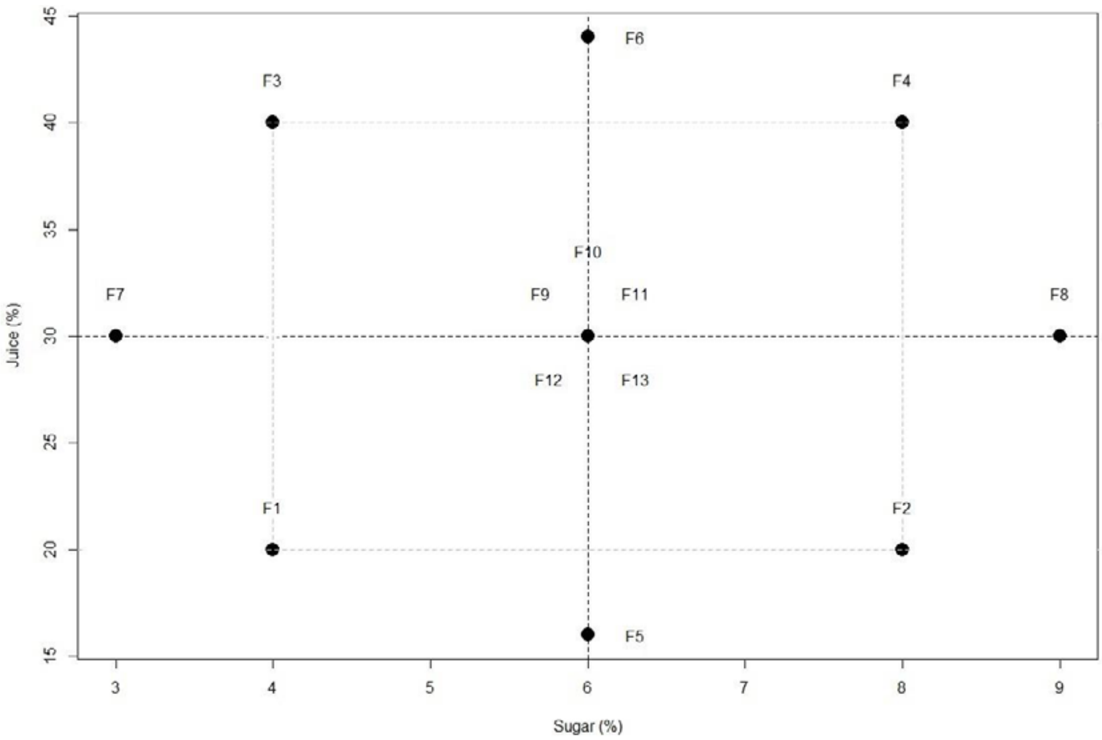

Figure 1.
Scheme 2 × 2 factorial of a central rotational compound design, with five replicates at the central point, in the study developed at the Federal University of Ceará by Rebouças (2016).

Table 1.
Beverage formulations developed at Federal University of Ceará, in 2016, with grape juice and prebiotic substances, where formulations 9-13 are defined as "Central Point".

Beverage Formulation	Actual Values		Coded Values	
	Juice(%)	Sugar (%)	Juice	Sugar
F_1	20	4	-1,00	-1,00
F_2	20	8	-1,00	+1,00
F_3	40	4	+1,00	-1,00
F_4	40	8	+1,00	+1,00
F_5	16	6	-1,41	0,00
F_6	44	6	+1,41	0,00
F_7	30	3	0,00	-1,41
F_8	30	9	0,00	+1,41
F_9	30	6	0,00	0,00
F_{10}	30	6	0,00	0,00
F_{11}	30	6	0,00	0,00
F_{12}	30	6	0,00	0,00
F_{13}	30	6	0,00	0,00

Source: Adapted from Rebouças (2016)

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The sensory acceptance evaluation of the proposed formulations (F_1 to F_{13}) was performed in different sessions, in which 130 untrained participated (panelists). Samples were served sequentially monadic, following a balanced incomplete block design, in which each panelist evaluated 4 of the 13 formulations of the proposed beverages, as showed in the Figure 2.

When studying categorical data, contingency tables and association tests between variables are useful techniques for exploratory data analysis. Therefore, in this work we have used these techniques to verify the association between beverage formulations and sensory attributes, using the Chi-square test. Also, multivariate techniques to categorical data by means correspondence analysis graphics (JOHNSON &WICHERN, 2008) were applied to complete data description. These methods, although exploratory, previously identify possible associations and clusters.

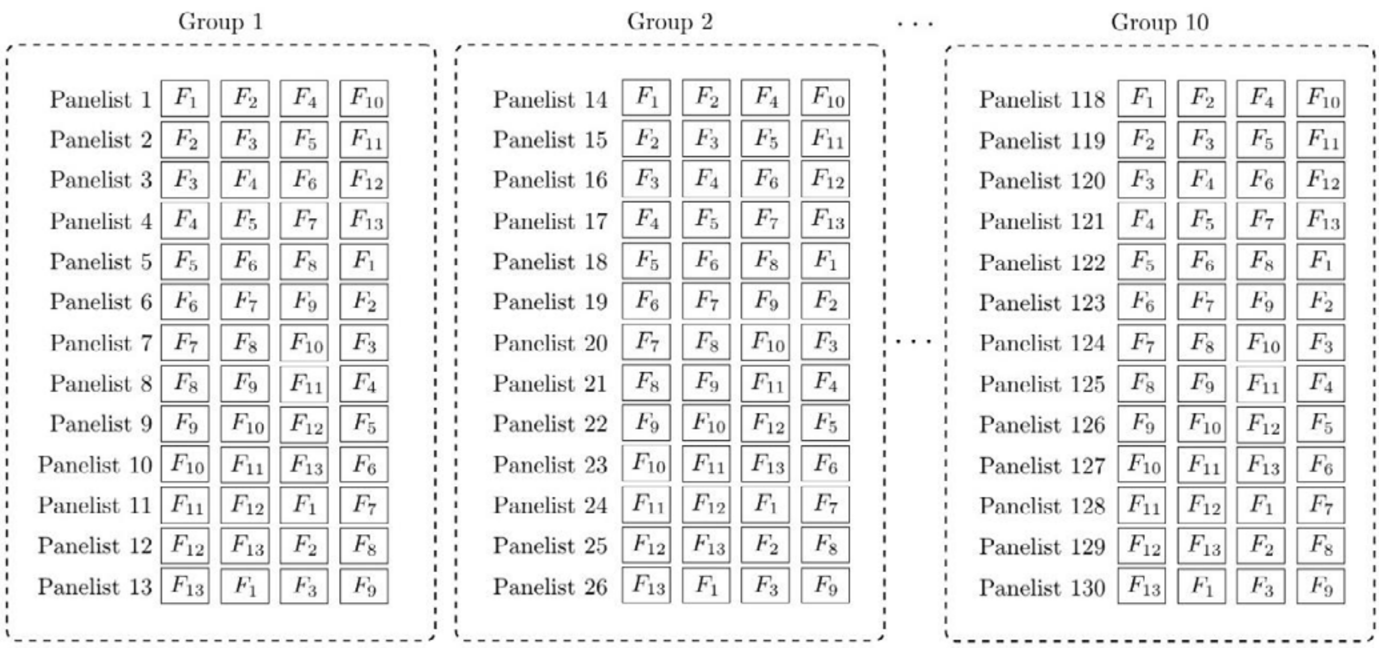
To relate the formulations to the response variables (overall impression, aroma, body, sweetness, and flavor) we use models for categorical data, called cumulative logits (AGRESTI, 2010). These models are multivariate extensions to the logistic regression model, specific to dichotomous data. In this class models, we can use the simplest structure, proportional odds, where formulation effects are constant for each category of the hedonic scale. Also, we include a random effect associated to panelist, due to design structure (incomplete blocks) as well as the subjective variability to the panelist. In this context, the mixed proportional odds model is given by

$$\eta_j = logit[P(Y \le j)] = ln\left[\frac{P(Y \le j \mid x,z)}{1-P(Y \le j \mid x,z)}\right] = \boldsymbol{\alpha}_j + \beta_j^T \boldsymbol{x} + b^T \boldsymbol{z} \qquad (1)$$

where, $\boldsymbol{\alpha}_j$ is the intercept, $\boldsymbol{\beta}_j=(\beta_1,\beta_2,\ldots,\beta_{13})^T$ is the regression parameters vector associated to beverage formulation, which vary according to each response category (j=1,2,3,4,5), and **b** is the random effect associated with the panelist, where we supposing $\boldsymbol{b} \sim N(0,\sigma_b^2)$. A more parsimonious model than the cumulative logit model (eq. 1) is the so-called proportional odds model (eq. 2), which assumes proportionality of the odds ratio in each logit, as follows:

$$\eta_j = logit[P(Y \le j)] = ln\left[\frac{P(Y \le j \mid x,z)}{1-P(Y \le j \mid x,z)}\right] = \boldsymbol{\alpha}_j + \beta^T \boldsymbol{x} + b^T \boldsymbol{z} \qquad (2)$$

Figure 2.
Structure for evaluating prebiotic drinks (incomplete blocks) in the study developed at the Federal University of Ceará by Rebouças (2016).



where, $\boldsymbol{\beta} = (\beta_1, \beta_2, \ldots, \beta_{13})^T$ is the same for each response category and the other parameters are defined as in model given the eq. 1. Additional details about the cumulative logit models can be seen in Paulino and Singer (2006), Agresti (2010), and Tutz (2012).

The estimates of these parameters are obtained by the maximum likelihood theory, by means the Newton-Raphson's interactive process (AZZALINI, 2017). The computational implementation was conducted in the R software (R CORE TEAM, 2022), using the packages: ordinal (CHRISTENSEN & BROCKHOFF, 2013) and VGAM (YEE, 2019). Moreover, as the models (1 and 2) are nested the verification of the proportionality condition is done by the Likelihood Ratio Test (LRT). The statistical significance of the formulation effect as well as is done by LRT. The LRT is defined by the expression:

$$\lambda = -2\log\left(\frac{L_0}{L_1}\right)$$

where L_0 is the likelihood function under the model with less parameters, that is, restricted model, and and L_1 represents the likelihood function under unrestricted model. For decision we consider that $\Lambda \sim \chi^2_{(m,95\%)}$, where m is the degree of freedom (difference of the parameters number). After model selection and with the estimated regression parameters in the in linear predictor (η_j), the accumulated probabilities associated with each formulation for each attribute are given by:

$$\hat{\theta}_j = \hat{P}(Y \leq j) = \frac{\exp(\hat{\eta}_j)}{1 + \exp(\hat{\eta}_j)}$$

Moreover, in this work, as a criterion for selecting the best formulations, we consider predictions greater than or equal to 4, $\hat{P}(Y \geq 4) = \hat{\theta}_{j=5} - \hat{\theta}_{j=3}$.

RESULTS AND DISCUSSIONS

Before applying correspondence analysis, we have verified the associations among beverage formulations (F_1 to F_{13}) and the sensory attributes (overall impression, aroma, thickness, sweetness and flavor). For this, the chi-square association test was applied to evaluate the possible dependence.

The five sensory attributes (Overall Impression, Aroma, Thickness, Sweetness and Flavor) have presented significant association with formulations (*values -p<0.05*). There is evidence that the sensory attributes and beverage formulations are dependent and we can apply the correspondence analysis. The graphics with exploratory analysis are shown in Figure 3 for all attributes.

The percentages of explained observations in two dimensions by each attributes (Overall Impression, Aroma, Thickness, Sweetness and Flavor) were: 86.9% (Figure 3a), 83.2% (Figure 3b), 80.0% (Figure 3c), 83.8% (Figure 3d) and 84.1% (Figure 3e), respectively.

It is also observed that the beverage formulations that came closer to category 5 ("liked extremely"), for overall impression attribute were F_3, F_4, F_6, F_{11} and F_{13} (Figure 1a). Considering aroma attribute, it was F_5 (Figure 1b) and for body attribute they were F_2, F_3, F_6, F_8 and F_{13} (Figure 1c). Furthermore, for sweetness attribute they were F_6, F_{11} and F_{13} (Figure 1d). Finally, the formulations F_{10}, F_{11}, F_{12} and F_{13} for flavor attribute (Figure 1e).

The proportional odds likelihood ratio test (2) results are shown in Table 2. For all sensory attributes the tests were not significant (*values -p>0.05*). Consequently, the mixed proportional odds models (1) were fitted considering a random panelist effect.

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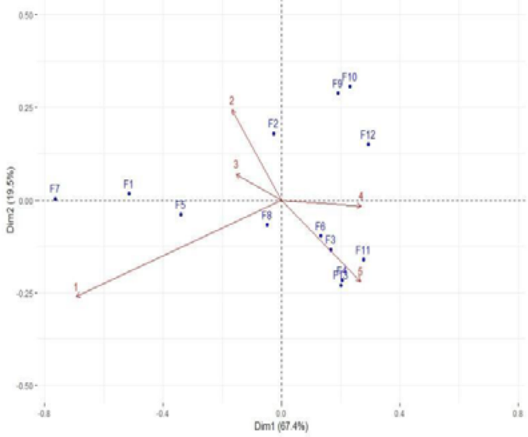
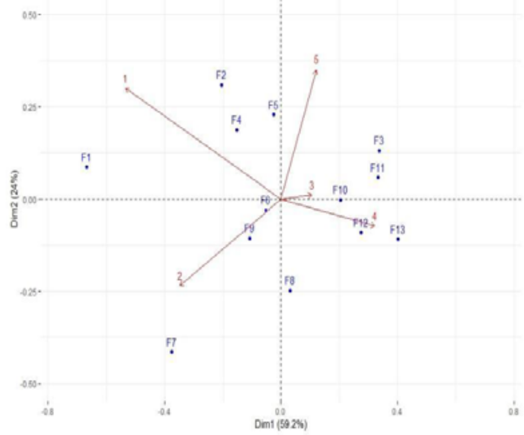
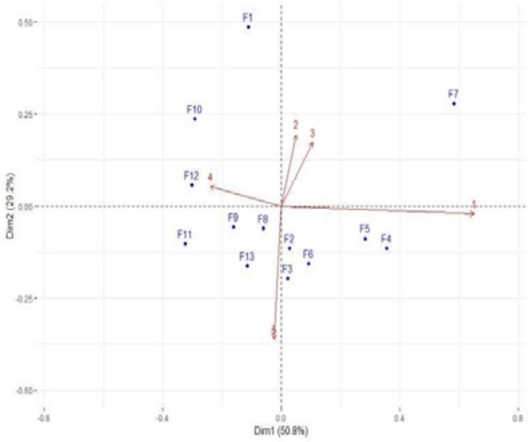
Figure 3. (a,b,c,d,e) Correspondence analysis of beverage formulation versus sensory attributes



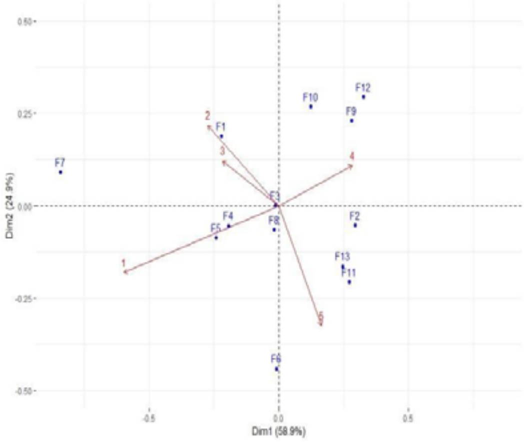
(a) Sensory attribute points cloud overall impression versus beverage formulation



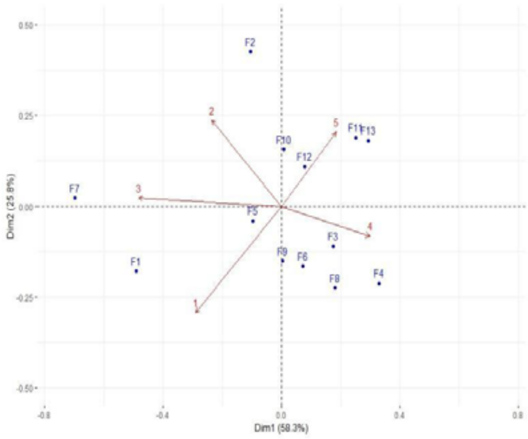
(b) Sensory attribute points cloud aroma versus beverage formulation



(c) Sensory attribute points cloud body versus be-beverage formulation



(d) Sensory attribute points cloud sweetness versus beverage formulation



(e) Sensory attribute points cloud flavor versus beverage formulation

It was verified that the standard deviation estimation of random effect was $\hat{\sigma}_b$=2.13, with an 95% confidence interval equal to (1.75; 2.58), that does not contain zero, indicating the existence of a random effect for the model (1) with overall impression as response.

existence of a random effect for the model (1) with overall impression as response. The estimated parameters, standard errors, Z statistic test and *p-values* for this model are presented in the Table 3. We can observe that β_5 and β_7 were non-significant, indicating that

$\beta_1=\beta_5=\beta_7$, meaning that F_1, F_5 and F_7 beverage formulations can be considered with equal effects. It is also verified that the estimates of β_4, β_6 and β_{13} obtained the highest values (in module) showing a greater acceptance of F_4, F_6 and F_{13} beverage formulations by the panelists, results previously indicated by the exploratory analysis (Figure 3).

The estimated probabilities for overall impression from this fitted model as well as the expected cumulative probability values for response categories 4 (liked slightly) and 5 (liked extremely) are shown in Table 4. Here we use the cumulative probability of these categories (which indicate better evaluation) as a criterion to select the best formulations, it is observed that the formulations F_4, F_6 and F_{13} obtained the highest accumulated probabilities with 0.80, 0.77 and 0.75, respectively.

We chose not to present the results for the other attributes, but the model used is the same, that is, mixed proportional odds. Also, a similar criterion was used to select the attributes. The categories "4 = I liked it moderately or slightly" and "5 = I liked it extremely or very much", for each attribute, indicates better formulation evaluations obtained the highest cumulative probability as following results: aroma - F_6: 0.49, F_{12}: 0.47, F_{13}: 0.48; thickness - F_6: 0.81, F_9: 0.77, F_{13}: 0.78; sweetness - F_6: 0.84, F_{11}: 0.79, F_{13}: 0.80 and flavor - F_4: 0.85, F_6: 0.75, F_{13}: 0.74. Due to these factors, we can observe that formulations F_6 and F_{13} are included in all attribute results. As an evaluation criterion of model (1) for overall impression, we present in Figure 4 the observed proportions and predicted probabilities, and the measures are quite close, indicating the model is satisfactory to explain the functional relationship.

Table 2. Proportional odds test, beverage formulations versus sensory attributes.

Sensory Attribute	p-values
Overall Impression (OI)	0.246
Aroma (AR)	0.054
Thickness (TH)	0.067
Sweetness (SW)	0.170
Flavor (FL)	0.157

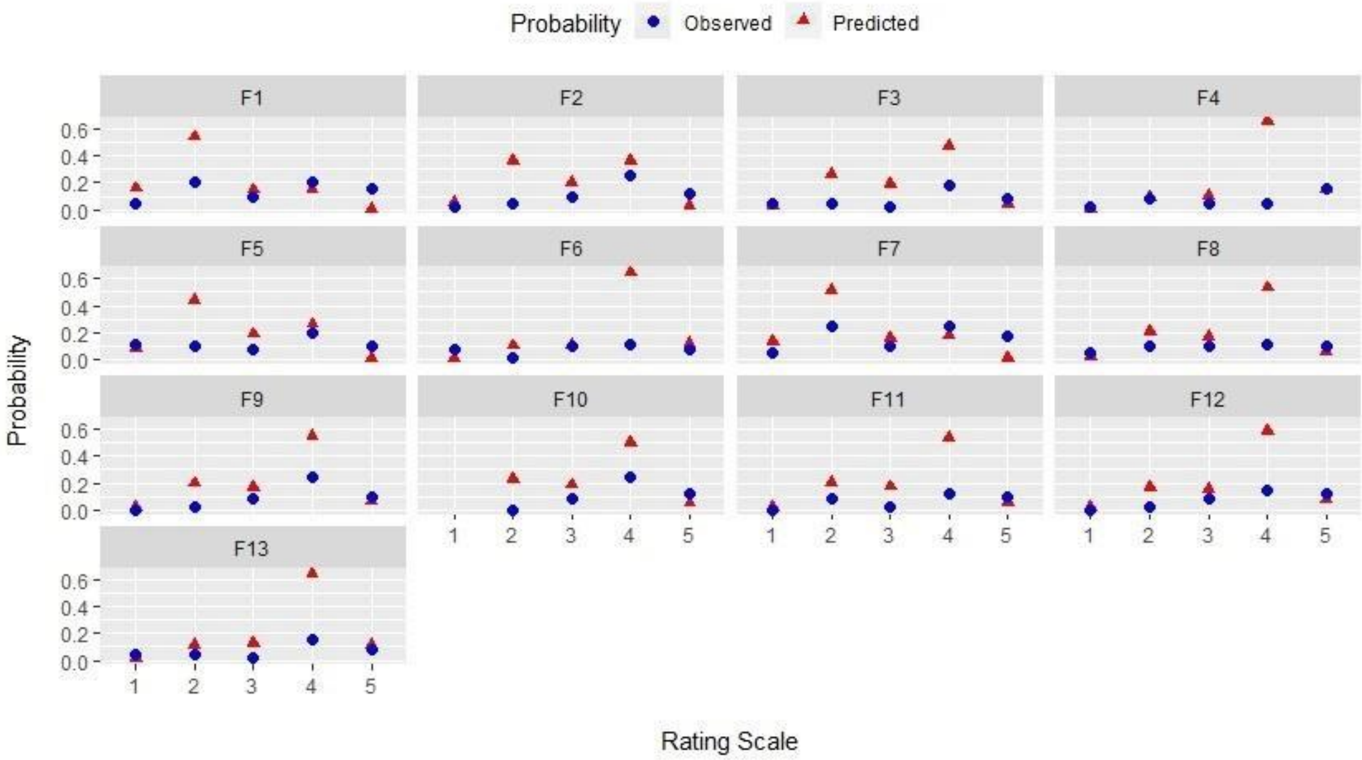
Table 3. Parameter estimates, standard errors and statistical tests for beverage formulation effects for overall impression attribute response

Parameter	Estimate	Standard error	z	*p-values*
α_4	-4.79	0.48	-10.04	<0.01
α_3	-1.65	0.41	-3.99	<0.01
α_2	-0.82	0.41	-2.02	0.04
α_1	1.67	0.41	4.03	<0.01
β_2	-1.18	0.49	-2.42	0.02
β_3	-1.71	0.50	-3.44	<0.01
β_4	-3.02	0.52	-5.77	<0.01
β_5	-0.70	0.49	-1.43	0.15
β_6	-2.85	0.52	-5.52	<0.01
β_7	-0.19	0.50	-0.38	0.70
β_8	-2.03	0.51	-4.01	<0.01
β_9	-2.09	0.49	-4.23	<0.01
β_{10}	-1.86	0.49	-3.80	<0.01
β_{11}	-2.04	0.50	-4.11	<0.01
β_{12}	-2.31	0.50	-4.61	<0.01
β_{13}	-2.76	0.52	-5.34	<0.01

Table 4.
Proportions of each classification of beverage formulation with sensory attribute overall impression.

Beverage Formulation	Overall Impression response category					P≥4
	1	2	3	4	5	
F_1	0.16	0.54	0.15	0.15	0.01	0.16
F_2	0.06	0.36	0.20	0.36	0.03	0.39
F_3	0.03	0.26	0.19	0.47	0.04	0.52
F_4	**0.01**	**0.09**	**0.10**	**0.65**	**0.15**	**0.80**
F_5	0.09	0.44	0.19	0.26	0.02	0.28
F_6	**0.01**	**0.11**	**0.12**	**0.64**	**0.13**	**0.77**
F_7	0.14	0.52	0.16	0.18	0.01	0.19
F_8	0.02	0.21	0.18	0.53	0.06	0.59
F_9	0.00	0.20	0.17	0.55	0.06	0.61
F_{10}	0.00	0.00	0.19	0.50	0.05	0.55
F_{11}	0.00	0.20	0.18	0.54	0.06	0.60
F_{12}	0.00	0.17	0.16	0.58	0.08	0.66
F_{13}	**0.01**	**0.11**	**0.12**	**0.64**	**0.12**	**0.75**

Figure 4.
Observed and predicted proportions for overall impression attribute by the beverage formulation.



CONCLUSIONS

The present work shows a statistical approach for data analysis in sensory area, with emphasis in mixed cumulative logit models with proportional odds. Also, we present the correspondence analysis as an important exploratory technique. Using these procedures, we have concluded that F_6 and F_{13} formulations were the best evaluated in the global impression criterion and the other attributes. Although in this work the orientation was by the proportional chances model, it is worth mentioning that this condition is not always accepted. In these cases, the mixed cumulative logit model can be used, which will have more parameters. One of the classic problems that these models present is the lack of convergence due to the excess of parameters. This is one of the reasons why we group response categories together, which is also not always advisable. It is always recommended common sense in the grouping in such a way as not to lose the practical sense of the sensory evaluation.

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