Using a mixture design and fraction-based formulation to better understand perceptions of plant-protein-based solutions

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ABSTRACT

The food industry is focused on developing plant-based foods that incorporate pea protein isolates. However, these ingredients are often described as having persistent beany, bitter, and astringent notes, which can decrease the desirability of the resulting foods. These perceptions are rooted in the complex composition of volatile and non-volatile compounds in foods. The aim of our study was to better understand how the volatile and non-volatile fractions of pea protein isolates influence the perception of pea-protein-based foods.

To this end, a mixture design was used. First, we obtained three fractions (the pellet, permeate, and retentate) from two pea protein isolates, resulting in a total of six fractions. Second, we used various combinations of the six fractions to create a set of 46 pea-protein-based solutions via various processes (solubilization, centrifugation, filtration, and mixing). Each fraction was specifically representative of the following constituent groups: insoluble proteins (the pellet); soluble compounds, such as volatiles, peptides, and phenolics (the permeate); and soluble proteins interacting with volatiles (the retentate). Factor levels were chosen with two aims: to explore the widest possible range of combinations and to realistically represent protein concentrations so as to build optimal mixture models. Third, 17 trained panelists were asked to score the attributes of the solutions using sensory profiling.

Model performance was assessed using analysis of variance; results were significant for 18/18 attributes, and there was no significant lack-of-fit for 17/18 attributes. It was also assessed using the results of trials conducted with six supplementary solutions. These results clarified the origin of the perceived beany, bitter, and astringent notes. Beaniness was mainly influenced by the retentate and permeate fractions and was strongly affected by hexanal levels. Bitterness was mainly influenced by the retentate fraction, whereas astringency was influenced by the retentate and pellet fractions. Additionally, perception of these latter two attributes was affected by caffeic acid levels.

This study has increased understanding of the relationship between pea protein fractions and the undesirable sensory attributes of pea protein isolates. It has also revealed how fraction-based formulation could be used to reduce the beaniness, bitterness, and astringency of pea-protein-based foods.

KEYWORDS

Legume; Pea protein; Experimental design; Surface response methodology; Beany; Bitter

TITLE

Using a mixture design and fraction-based formulation to better understand perceptions of plant-protein-based solutions

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1. INTRODUCTION

Over the last few years, plant-based protein ingredients have received much attention from the food industry and consumers because of their environmental sustainability, attractive prices, nutritional values, and protein content (Davis et al., 2010). In particular, yellow field pea (Pisum sativum L.) is an increasingly common ingredient in plant-based foods (Siddique et al., 2012). Its proteins exhibit low allergenicity; have a high nutritional value; and can restore the amino acid balance of grain-based diets. They also display functional properties that are useful in food formulation: they promote emulsification, foaming, gelation, and whipping (Adebiyi & Aluko, 2011; Gharsallaoui et al., 2009).

Industrial pea-protein ingredients are traditionally generated via a several-step wet process. Pea seeds are solubilized in an alkaline solution, which is then centrifuged to remove insoluble compounds; the precipitate is obtained at the isoelectric point using acidification and centrifugation. The resulting isolate has a protein content of 80–90% (mainly globulins), but also contains lipids, sugars, salts, and other small compounds (e.g., phenolics), which are the products of seed metabolism (Schutyser et al., 2015). The isolate can also serve as an ingredient in the formulation of many food products, including dietary supplements, bakery and confectionery products, beverages, yogurts, ice creams, meat products, and meat and dairy alternatives.

However, a challenge remains: pea-protein-based products are usually described as having strong beany, bitter, and astringent notes, which makes them less desirable to consumers. The mechanisms and chemical compounds underlying the perception of food are partly understood and may be multifarious (Owusu- Ansah & McCurdy, 1991). Indeed, the composition of pea protein isolates is complex: they have a high protein content but also contain various peptides, volatile compounds, phenolics, complex heterosides, sugars, fibers, and salts. All these constituents could influence the perception of pea-based ingredients.

Research in this area has often focused on the perception of beaniness, which is a complex flavor associated with bean products (Bott & Chambers, 2006). It results from the intricate composition of the volatile aroma compounds found in pulses; present at the highest concentrations is hexanal, whose occurrence is linked to the green notes of peas (Murat et al., 2013). Bitterness arises from the interaction of bitter compounds (e.g., amino acids, peptides, phenolics, complex heterosides) with the TAS2R family of receptors, which are found on the apical membranes of taste receptor cells

(Maehashi et al., 2008; Meyerhof et al., 2010). For example, the caffeic acid in coffee and other plant products generates an intense sensation of bitterness (Frank et al., 2007; Streit et al., 2007). Astringency is produced by "the complex sensations due to shrinking, drawing, or puckering of the epithelium" and results from interactions between phenolics and saliva proteins (ASTM, 1991; Gibbins & Carpenter, 2013). From an industrial and scientific point of view, it has proven extremely challenging to clarify how pea-based ingredients give rise to these sensory attributes.

Research on the perception of pea-based products has largely focused on the role of volatile aroma compounds in creating sensations of beaniness (Azarnia et al., 2011; Ben-Harb et al., 2020; Bi et al., 2020; Bott & Chambers, 2006; El Youssef et al., 2020; Murat et al., 2013; Mutarutwa et al., 2018; Schindler et al., 2012; Trikusuma et al., 2020; Wang & Arntfield, 2015; Xu et al., 2019; Xu et al., 2020). A few studies have exclusively examined the peptides that could be related to bitterness (Akin & Ozcan, 2017; Jakubczyk et al., 2013; Pownall et al., 2010; Sirtori et al., 2012); the phenolics related to bitterness and astringency (Bucalossi et al., 2020; Guo et al., 2019; Padhi et al., 2017); and the saponins related to bitterness (Daveby et al., 1998; Heng et al., 2006; Heng et al., 2006; Price & Fenwick, 1984). However, to our knowledge, no study to date has used a more global approach to examine how the complex perception of pea protein isolates arises from both volatile and non-volatile compounds and their potential interactions.

Several research strategies have been used to understand how complex products are perceived and to account for the interactions between matrix molecules. Omission testing is commonly used to estimate the effect of specific compounds on the sensory characteristics of products (Engel et al., 2002; Stevens, 1997). Thanks to this technique, aroma models have been built that reconstitute complex odors—such as those of different types of wine, olive oil, cheese, boiled beef, coffee, and whey protein—using only a small fraction of the great number of volatiles occurring in these foods (Czerny et al., 1999; Dinnella et al., 2012; Ferreira et al., 2002; Grosch, 2001; Whitson et al., 2010). However, in addition to being very time consuming, these experiments are less effective when volatile compounds are included in mixtures because the volatiles interact with other ingredients. Indeed, the ability of volatile compounds to modify how something tastes depends on both their relative concentrations and their interactions within the food matrix (Guichard, 2002). Mixing congruous volatiles and taste stimuli can enhance taste intensity, while mixing incongruous stimuli can suppress taste intensity (Caporale et al., 2004; Pfeiffer et al., 2006; Stevenson, 1999). Omission testing has also been used in tandem with gel permeation chromatography to study the water-soluble fraction of peptides found in cheese (Andersen et al., 2010; Engel et al., 2000; Engel et al., 2002; Gómez-Ruiz et al., 2007; Molina et al., 1999; Salles et al., 1995; Toelstede & Hofmann, 2008). The compounds in pea protein isolates that are potentially responsible for sensory attributes (e.g., peptides, phenolics, salts) are very complex and challenging to purify and identify. Moreover, most analytical techniques require the use of non-food-grade solvents or buffers that are difficult to handle and that can pose problems if the extracts are to be used in sensory evaluations.

Studies have shown that attribute perception may be similar for a complex product and a fraction-based reconstruction of the product. For example, artificial ikura (Japanese salmon caviar) was prepared using vegetable oil and a low-calorie natural gel (e.g., one made with alginic acid) (Hayashi et al., 1990); each component of the food was then analyzed using chemical and sensory methods. Based on the analytical data, a synthetic ikura was reconstituted using pure reagents. There were very few sensory differences in the taste profiles between the reference food and the reconstructed food (Hayashi et al., 1990). In another study (Niimi et al., 2014), a cheese solution was reconstituted using a mixture of sucrose, NaCl, monosodium glutamate, lactic acid, and caffeine that was then adjusted using a fractional factorial design. The reconstructed products did not significantly differ from the cheddar cheese reference in overall intensity, saltiness, sourness, umami, and bitterness (Niimi et al., 2014).

Thus, the aim of this study was to examine how the main fractions of pea protein isolates individually affected the perception of sensory attributes, namely undesirable attributes such as beaniness, bitterness, and astringency. To this end, an original approach was employed, in which different fractions were combined in various ways to create a range of pea-protein-based solutions. The focus was thus on different groups of compound types instead of on a single compound type. Three fractions were obtained from commercial pea protein isolates: an insoluble fraction (called the pellet), a soluble fraction (called the retentate), and a soluble fraction with a molecular weight of less than 10 kDa (called the permeate). Each fraction was associated with a main compound type: insoluble proteins in the case of the pellet; soluble compounds (e.g., volatiles, peptides, and phenolics) in the case of the permeate; and soluble proteins interacting with volatiles in the case of the retentate. Using a mixture design, a large number of diverse pea-protein-based solutions (> 40) were formulated by combining the different fractions in order to obtain continuous response curves and to build reliable statistical models. Trained panelists scored the attributes of the solutions using sensory profiling. Response surface models were generated, and their predictions were compared with the observed results. The results have improved our insight into the relationship between the different pea protein isolate fractions and perceptions of beaniness, bitterness, and astringency. Furthermore, the results may help optimize the formulation of plant-protein-based foods.

2. MATERIALS AND METHODS

2.1. Production of pea protein isolate fractions

Two pea protein isolates (protein content Nx6.25, 83% dry matter) were used; they were called isolate a and b, respectively. The isolates were dispersed in tap water in a tank to obtain a final suspension containing 4% (w/w) dry matter content. This suspension was maintained under agitation for 12 h at 3°C with an external agitator (U-shaped stirrer shafts); it was then centrifuged with two centrifuges (Jouan Kr4i and a Sorvall Lynx 4000 [Thermo Scientific, Waltham, US]; 6000 g, 10 min, 4°C). The supernatant was manually separated from the pellet. The pellet was subsequently diluted with tap

water to arrive at a dry matter content of 12.35%, which facilitated solution creation. A tangential filtration module (TIA, Bollene, France) was used for the ultrafiltration process. The module employed two ST-3B-1812 PES Synder membranes (46-mil spacer; 10-kDa MWCO). Total membrane surface was 0.67 m². The filtration pilot was equipped with a high-pressure diaphragm pump (Wanner Hydra-Cell G10, Wanner International Ltd, Church Crookham, UK)). The retentate was maintained at 13°C throughout filtration. The inlet pressure (P1) was 1.5 bar, the outlet retentate pressure (P2) was 1 bar, and the mean transmembrane pressure ([P1 + P2]/2) was 1.25 bar. First, ultrafiltration was used to obtain around 10 L of permeate; then, diafiltration was performed employing the same parameters to partially wash the retentate (one diavolume was used). Six fractions were obtained: permeates a and b, retentates a and b, and pellets a and b.

2.2 Characterization of the pea protein isolate fractions

Each fraction was characterized to determine the key pea protein compounds it contained (Figure 1). Nitrogen content was determined via the Kjeldahl method (nitrogen content x 6.25), and dry matter content was determined by a certified external laboratory (SAS IMPROVE, Amien, France) via drying (prepASH®219 analysis system). Sodium content was also determined by a certified external laboratory (SAS QUALTECH, Vandoeuvre-les-Nancy, France) using inductively coupled plasma mass spectrometry. Caffeic acid content was determined using gas chromatography–mass spectrometry (GC-MS) and comparison with an external standard (CAS 331-39-5, grade ≥ 98.0% HPLC, MW 180.16, Sigma Aldricht, Saint-Louis, US). Hexanal levels were determined using GC-MS as per El Youssef et al. (2020).

2.3 Mixture design

An optimal mixture design was used to create a wide range of reference and experimental solutions from the fractions (permeates a and b, retentates a and b, and pellets a and b). Response surface models were created and included quadratic terms and first-order interactions. The experimental design was such that there was orthogonality among all the terms, which allowed variable effects to be differentiated from one another. A blocking factor was used to control for the effect of the day on which sensory evaluation took place. The order of solution evaluation within the blocks was fully balanced. Overall, the mixture design had eight independent variables (see Table 1 for the levels), and 10 solutions were replicated. The total number of trials was 40. Variable levels were chosen so as to represent a wide range of variation while remaining realistic in terms of the protein concentrations actually experienced when pea protein isolates are used to create foods.

This experiment was designed with a view to minimizing solution number (final solution count: 40), which facilitated solution evaluation. In contrast, a central composite design or a Box-Behnken design would have required ~60 and ~80 solutions, respectively. Furthermore, we used an optimal design because it is the only design that allows the addition of a blocking factor. This experiment displayed

better or equivalent efficiency—with a D-optimal value of 13.25% and a G-optimal value of 50.85%—compared to experiments based on other designs. These metrics reflect goodness of fit relative to a hypothetical orthogonal design: the D-optimal value indicates whether the design minimizes the volume of the joint confidence region for the vector of regression coefficients, and the G-optimal value indicates whether the design minimizes the maximum prediction variance over the design region.

To validate the model's predictive capacity, six solutions that were not initially included in the design were added to the sensory evaluations (for more details, see Table 1—sensory session ID 9).

2.4. Solution creation

The six different fractions were combined in various ways to formulate the 46 solutions of the mixture design. This process was carried out at 4°C in 500 mL and 100 mL glass flasks, which were stored at -20°C. During fractionation and recombination, good hygiene practices were used to limit microbial contamination (usage of coat, gloves, and hygienic cap; cleaning and disinfection of hands and all equipment with pure ethanol, followed by air drying; work carried out in a 4°C chamber). In addition, the microbial safety of the solutions was tested by a certified external laboratory (Eurofins Scientific, France). However, for microbiological reasons, the solutions containing pellet b had to be heat treated (autoclaved at 110°C for 10 min) before oral sensory evaluation, so the supplemental effect of the autoclave procedure on perception was also evaluated. It was slightly significant for the attributes nuts, cereals, and almond and strongly significant for the attribute granularity (mean difference between autoclaved and unautoclaved solutions: 0.89/10 for nuts; 0.94/10 for cereals; 1.44/10 for almond, and 5.49/10 for granularity). Because this effect was minor (except in the case of granularity) and collinear with pellet b, it will not be discussed further.

2.5. Sensory evaluation conditions

We recruited 17 panelists (13 women and 4 men; mean age = 23 years old) based on their interest in participating in a long-term study that required their presence at two evaluation sessions per week for three months. They had already been trained to carry out sensory evaluations of pea products or to use sensory evaluation methods, but they all received additional training for this study. They were not informed of the precise aim of the experiment. They gave their free and informed consent to participate and received compensation for their participation. They were asked to not eat, drink, or smoke for at least 1 h prior to any of the sessions (training or experimental). Sensory profiling was carried out in individual booths under white light (the solutions were similar in color) in an air-conditioned room (20°C). To reduce sensation build-up, the following palate-cleansing protocol was used between solutions during the experimental sessions: panelists had to consume an apple slice, drink water, and wait 40 seconds before consuming the subsequent solution (as described in Cosson et al., 2020).

2.6. Sensory profiling method

Panelists were asked to assess solutions using the sensory profiling method (with a block protocol) described by Cosson et al. (2020). The objective was to score the intensity of a solution's sensory attributes along an unstructured scale ranging from 0 to 10. To select the attributes, panelists were asked to fill out a check-all-that-apply (CATA) survey. It contained 30 attributes, and it was possible for panelists to add more. For our final list, we selected attributes that were cited more than 20% of the time and that allowed significant discrimination among solution types. We also wished to limit total attribute number to avoid panelist fatigue. Panelists were trained to assess the attributes along the unstructured scale using external references. Training took place over 8 sessions that each lasted 45 min. Afterward, panelist performance was evaluated.

Attributes were evaluated in blocks. The first attribute block (pea, broth, nuts, almond, potato, and cereals) focused on aroma perception (i.e., evaluated by nose). The second attribute block (salty, sugar, bitter, astringent, mouthfeel, and granularity) focused on taste perception and mouthfeel, and the panelists wore nose clips. The third attribute block (pea, broth, nuts, almond, potato, and cereals) again focused on aroma perception, but the solutions were evaluated in mouth; the panelists did not wear nose clips. For each block, the solutions were presented monadically: for each solution, the panelists evaluated all the attributes within the block, which were printed on the same survey page. Solution order was the same for all three blocks for a given panelist; however, it differed among panelists. In addition, for the three blocks, the first solution in each session was always the reference solution (Refa), which limited and controlled drift between sessions. This reference was available in large quantities and was stored under highly stable conditions for the entire study period. To account for order and carry-over effects, solution order was balanced across panelists using a Latin square (Williams design). Each solution was evaluated in duplicate by the 17 panelists.

2.7. Statistical analysis of the sensory data

Analyses were performed using XLStat (Addinsoft, 2017, Paris, France) and R (R Core Team, 2017). For analyses of an inferential nature, $\alpha = 0.05$ was the threshold for statistical significance. To analyze the sensory profiling results, we carried out a three-way ANOVA. Solution identity (ID), replicate ID, and panelist ID were the fixed factors, and all the first-order interactions were included. To visually explore differences in the results obtained using the classical versus block profiling protocol, we carried out principal component analysis (PCA) on a correlation matrix; the data were averaged across replicates and panelists. To study the possible drift between sessions, we carried out a two-way ANOVA on the data for the reference solution. Panelist ID, sensory session ID, and their interaction were the fixed factors.

2.8. Statistical analysis of the mixture design

JMP (v. 13.1.0; SAS Institute Inc., Cary, SC, USA) was used to generate and analyze the optimal mixture design. Multiple regression analysis was performed to evaluate the effects of all the independent variables on each response variable (i.e., via the regression coefficients). The most influential independent variables ($p \leq 0.05$) were identified using backward elimination. The regression coefficients were calculated for each final model. Model performance was assessed via ANOVAs (F-test for significance), lack-of-fit tests, and coefficients of determination (R2). For the six validation solutions, the predicted and observed responses (with 95% confidence intervals) were calculated.

3. RESULTS

The aim of this study was to understand how the sensory perception of pea protein isolates is affected by the isolates' main fractions. To this end, we used a mixture design. The first part of the results/discussion section examines how the design model was built: it provides an assessment of panelist performance over the 3-month experiment, an explanation of how attributes were chosen, a validation of the study methodology (i.e., creating solutions by combining isolate fractions), and a statistical representation of the model. The second part of the results/discussion section focuses on how different sensory attributes (primarily beaniness, bitterness, and astringency) are affected by pea protein isolate composition (i.e., the main constituents—insoluble proteins, volatiles, and soluble compounds [proteins, peptides, phenolics, and salts]).

3.1. Construction of surface response models from the sensory data

3.1.1. Assessment of panelist performance over the 3-month experiment

Panelists used sensory profiling to assess the 46 solutions (reference and experimental; in duplicate) during two weekly sessions over the course of three months. Because solution number was high and study duration was long, it was important to examine panelist performance over time (i.e., reproducibility, homogeneity, and between-session drift). To do so, a three-way ANOVA was used to analyze the attribute scoring data (Table 2).

Reproducibility and homogeneity were examined first. Solution ID was significant for all 18 attributes, which indicates that panelists distinguished among solutions. Panelist ID and the interaction between panelist ID and solution ID were also significant for all the attributes. Such interactions are common when sensory attributes are evaluated using unstructured scales and are difficult to control even when panelists have undergone extensive training (Jourjon et al., 2005; Lawless & Malone, 1986). The interaction between replicate ID and solution ID was not significant for 10/18 attributes. Replicate ID was not significant for 11/18 attributes, but the interaction between panelist ID and replicate ID was significant for all 18 attributes. However, the F-values for these interactions were low compared to the F-values for the main effect of solution ID. For example, for the broth-M attribute, $F(39,624) = 54.09$ for solution ID; $F(1,624) = 5.39$ for replicate ID; $F(16,624) = 3.08$ for the panelist-

by-replicate interaction; and F(16,39) = 1.43 for the solution-by-replicate interaction (model degrees of freedom [DF] = 735, residual DF = 624).

The presence of between-session drift was examined by looking at the scores for the reference solution across the entire experiment. To this end, a two-way ANOVA (fixed factors: panelist ID and sensory session ID) was performed using scores for each attribute given to the reference solution (Table 3). Sensory session ID was not significant for any of the attributes except broth-M and granularity-NC: these attributes were assigned slightly higher and slightly lower scores, respectively, during a single session. Although using the reference can make solution preparation more cumbersome, it was important in helping to validate panelist performance. In addition, panelists found the reference useful as they scored the other solutions. In past research, monadic presentation has been found to be faster and less tiring than comparative presentation (Mazzucchelli & Guinard, 1999). However, comparative presentation allows panelists to detect smaller differences among food products and to make more accurate decisions about these relative differences (Mcbride, 2007; A. Saint-Eve et al., 2006). Here, via its use of blocks, the presentation method combined monadic and comparative elements. Consequently, the panelists could base their attribute scoring on both their memories from the training period as well as on the reference, which was always the first solution in the sequence (Hastie & Park, 1986).

Taken together, these results suggest that the panelists generally came up with repeatable and homogeneous scores and that there was no between-session drift in scoring. There was some disagreement in the case of certain attributes (e.g., sugar-NC), which was taken into account when the results were analyzed.

3.1.2. Attribute choice

Plant-protein-based ingredients are often said to be "beany," a multidimensional and complex descriptor (Bott & Chambers, 2006). Here, the decision was made not to use the term "beany." Instead, its multiple components were parsed out and expressed via other terms (see Cosson et al., 2020). Thus, six aroma attributes were selected: potato, pea, cereals, broth, almond, and nuts. Plant-protein-based ingredients are also often described as being persistently bitter and astringent (Roland et al., 2017); consequently, bitterness and astringency were included as well. Finally, two taste attributes—salty and sugar—and two texture attributes— mouthfeel and granularity—were also chosen because they have been found to be important in descriptions of food quality and preference (van Vliet et al., 2009).

Attribute intensities for the different solutions were investigated using a three-way ANOVA (Table 2). Solution ID was significant for the 18 attributes (model DF: 735; residual DF: 624), which means the solutions had distinct sensory profiles. There were pronounced differences in perceived texture (F = 261.12 for granularity and F = 116.94 for mouthfeel) and smaller differences in perceived sweetness (F = 9.20 for sugar). These results are not surprising. It is easier to describe food products based on

texture and taste than on aroma (Kora et al., 2003; Lundgren et al., 1986; Anne Saint-Eve et al., 2011). Furthermore, temporally, they are the first attributes to become dominant in the mouth (Le Calvé et al., 2019; Pineau et al., 2009; Anne Saint-Eve et al., 2011). Additionally, when describing overall preferences and sensory satisfaction, consumers appear to primarily focus on taste and then on texture, paying the least attention to aroma (van Vliet et al., 2009). Finally, since the solutions had very low levels of natural sugar content (and no sugar was added), it was not surprising that sweetness did not greatly contribute to the perceived differences among the solutions. Consequently, this attribute was not included in the statistical model.

To build upon these results, PCA was used to visually depict the relationships among solution types and attributes (Figure 2). The solutions were well distributed along axes F1 and F2, which accounted for 82.68% of the variance. Thus, maps based on the first two axes seemed to provide a good¬ quality projection of the initial multidimensional table, even though some information might have remained hidden in the subsequent axes. The 12 aroma attributes were clustered within one quarter of the correlation circles and thus clearly interacted in multiple ways. Aroma attributes assessed in the mouth and nose were strongly correlated (R2 = 0.86 for pea, R2 = 0.88 for broth, R2 = 0.83 for cereals, R2 = 0.95 for nuts, R2 = 0.87 for almond, R2 = 0.89 for potato). These results suggest that panelists assigned similar scores to aroma attributes perceived orthonasally and retronasally and that food processing in the mouth had a minor effect on olfactory perception. Orthonasal odors result from volatile compounds traveling from the external environment and through the nares to the olfactory mucosa, whereas retronasal odors result when volatile compounds travel to the olfactory mucosa after they have been released during food destructuration in the oral cavity (Sun & Halpern, 2005). That said, orthonasal and retronasal responses are often similar, except in cases where there are physicochemical or sensory interactions induced by texture, taste, or in-mouth food destructuration (Goldberg et al., 2018). Furthermore, results for the attributes pea-M and salty-NC were also correlated ($R^2 = 0.87$), which suggests possible congruency (Oladokun et al., 2017). Consequently, our results indicate that there may have been limited interactions between texture, taste, and flavor (except in the case of the attributes pea and salty) and that food oral processing had a minimal impact on these attributes. Therefore, the aroma attributes evaluated via the nose were not included in the statistical model.

The aroma attributes potato, almond, cereals, and nuts as well as the attributes astringent and mouthfeel were significantly correlated (R^2 range = 0.72–0.98). They were also correlated with the dry matter content (%) of the solutions ($R^2 = 0.97$ for mouthfeel, $R^2 = 0.88$ for cereals-M, $R^2 = 0.85$ for almond-M, $R^2 = 0.82$ for potato-M, $R^2 = 0.81$ for nuts-M, and R2 = 0.73 for astringent). These results suggest that the perception of these attributes was mainly driven by dry matter content and, thus, protein concentration. However, dry matter content was not correlated with the perception of the attributes pea and bitter. It is therefore necessary to build a more complex model to understand the origin of these attributes.

3.1.3. Validation of the study methodology—fraction-based formulation of solutions

In this study, a mixture design was used to create a large number of solutions by combining pea protein isolate fractions. To validate this methodology, the sensory properties of the two reference solutions, created directly from the pea protein isolates, were compared with the sensory properties of two experimental solutions that were created using the isolate fractions to have the exact same compositions as the reference solutions.

PCA was used to visually depict the main differences between the reference solutions and these experimental solutions (Figure 2). The results show that the two reference solutions (Refa and Refb) and the two experimental solutions (Refa-R and Refb-R) occur in relatively close proximity compared to the other solutions on the map. The distance is greater between Refa and Refa-R than between Refb and Refb-R. For the panelists, Refa was the "sensory reference". As a result, there may be a bias in its sensory properties that is directly due to the study's methodology.

The main difference between the reference solutions and the experimental solutions was in their perceived granularity. The experimental solutions were perceived as less granular than the reference solutions. In commercially produced isolates, proteins are highly denatured due to the extraction process (pH changes, high temperatures) and form large aggregates that are primarily structured by hydrophobic interactions (Chihi et al., 2016; Oliete et al., 2018; W. Peng et al., 2016; Ryan et al., 2012). It is likely that these aggregates are fairly insoluble, which could be responsible for the perceived granularity of the reference solutions. When the experimental solutions were created by combining the isolate fractions, the processes that they underwent (centrifugation and filtration) might have broken up these aggregates and induced structural changes, resulting in smaller, more soluble clusters.

3.1.4. Construction of the optimal mixture models

In past studies, various experimental and statistical methods have been used to explore the sensory perception of food, and the choice of techniques depends on the research question, variable type and number, and food product number (Chapman et al., 2019; Seisonen et al., 2016; P. Yu et al., 2018; Zielinski et al., 2014). While classical approaches such as fractional factorial design and simple regression have been widely used, they may be inadequate for fully describing a complex food. Thus, this study employed optimal mixture models. This approach made it possible to limit solution number, while also minimizing the degree of aliasing to ensure less collinearity among the independent variables (P. Yu et al., 2018).

The attribute scoring data were used to develop the optimal mixture models. Model performance was tested using ANOVAs (global model; F-test for significance), lack-of-fit tests (which calculate a pure-error negative log-likelihood by constructing categories for every combination of model effect values in the data), and the coefficients of determination (R^2) (Table 4). The results of the ANOVAs were

significant: the F-ratios ranged from 23 to 520, and the p-values were below 0.01. The lack-of-fit tests were not significant for the 10 attributes examined, which means that the error for each model was smaller than the pure error associated with replication. Thus, the models developed for each sensory attribute have relevance. Since the R^2 values were between 82 and 96%, a large amount of the variation in the attribute scores was explained, so the models' results could be interpreted with confidence. These results showed a good-quality fit. The model with the best fit was the one for the attribute mouthfeel. This finding is not surprising because past research has found that models relating food product composition and perceived texture often have the greatest explanatory value (Burseg et al., 2009; Cook et al., 2005).

When the backward elimination procedure was used (p-value < 0.05 for the F-statistic), the number of significant variables in the models ranged from 8 to 18 (main effects, first-order interactions) (Table 5). Consequently, attribute perception depended on several variables (permeate type, retentate type, and pellet type) as well as on their interactions. However, scores for different attributes were explained by different sets of variables. In other words, the perceptions of different attributes (e.g., pea, nuts, almonds, bitter) could be explained by differences in solution composition. Overall, retentate type and pellet type, but not permeate type, had strong effects on attribute perception. In addition, although the experiment was designed to incorporate orthogonality among the fixed factors, some interactions were significant. The interactions with the greatest effect on solution perception were permeate a*pellet b and pellet b*water. That said, the relative importance of the interactions was minimal compared to that of the main effects. This finding clearly suggests that the perception of pea-protein-based food products is influenced by the types of compounds present as opposed to the interactions among compound types.

Solutions created from isolate-b fractions were perceived as more bitter and astringent, with greater mouthfeel, and stronger notes of almond, cereals, nuts, and potato. In contrast, solutions created from isolate-a fractions were perceived as more salty with stronger notes of pea and broth. These results suggest that isolate identity does matter, even when isolates are reduced to their fractions. Furthermore, for almost all the significant effects, the coefficients were positive. This finding means that there was a positive relationship between fraction concentration and perceived attribute intensity and thus that the perception of pea-protein-based food products is driven by compound presence rather than compound absence.

To validate the model's predictive capacity, panelists were also asked to evaluate six supplementary solutions (created with the same fractions as the main experimental solutions but using different fraction concentrations) (Table 1). Although the data for these solutions were located towards the range limits of our main data set, there was overlap between the 95% confidence intervals for the observations and predictions in most cases (Table 6). Predictions were least accurate for the solutions P43 and P44 (8% pellet), notably for the attributes salty, bitter, astringent, and pea. The model generated good predictions when interpolating (i.e., predicting data points that would fall within the

range of our observed data). However, its predictions were of lower quality in the case of extrapolation (i.e., predicting data points outside the range of the observed data).

However, despite the low degree of collinearity among the independent variables and the incomplete orthogonality of the design, this model has helped clarify the perception of plant-protein-based foods.

3.2. Use of the models to better understand sensory perceptions

3.2.1. Identification of the fractions underlying beaniness

The mixture models helped clarify the origin of perceived beaniness and the respective contributions of the different isolate fractions.

The results show that the perception of the attributes cereals and nuts was largely influenced by retentates a and b (respectively: $F[14,65] = 512$ and $F[14,65] = 613$ for cereals; $F[14,65] = 225$ and $F[14,65] = 209$ for nuts). The same was true for the attributes almond, potato, and broth (retentates a and b respectively: $F[17,62] = 255$ and $F[17,62] = 271$ for almond; $F[13,66] = 331$ and $F[13,66] = 220$ for potato; $F[15,64] = 929$ and $F[15,64] = 144$ for broth), which were also affected by permeates a and b (respectively: $F[17,62] = 94$ and $F[17,62] = 148$ for almond; $F[13,66] = 135$ and $F[13,66] = 70$ for potato; $F[15,64] = 253$ and $F[15,64] = 95$ for broth). Finally, the perception of the attribute pea was simultaneously affected by pellets a and b (respectively: $F[9,76] = 423$ and $F[9,76] = 441$); retentates a and b (respectively: $F[9,76] = 370$ and $F[9,76] = 225$); and permeates a and b (respectively: $F[9,76] = 264$ and $F[9,76] = 419$). Retentate a, which had higher hexanal levels, led to more intense potato, broth, and pea attributes.

Dry matter content was similar among the fractions. However, pellets and retentates differed in their main protein type: insoluble proteins versus soluble proteins, respectively. In contrast, permeates were mainly composed of non-proteins, such as sodium, caffeic acid, and hexanal (Figure 1). Thus, unsurprisingly, the volatile-rich permeates contributed to the perception of the aroma attributes, as observed in previous studies. Indeed, the beaniness of pulses has been found to be strongly related to volatile composition and, notably, hexanal levels (Bott & Chambers, 2006; Vara-Ubol et al., 2004). More recently, Murat et al. (2013) examined the volatile composition of pea isolates and pea flour and suggested that certain aldehydes, alcohols, and ketones were responsible for beaniness (Murat et al., 2013). These results were confirmed recently by Bi et al., who demonstrated that six aroma compounds (including 3-methylbutanoic acid and hexanal) significantly contributed to the characteristic aroma of peas and that fifteen aroma compounds (including pyrazines and pyranones) significantly contributed to the characteristic aroma of roasted peas (Bi et al., 2020).

Initially, the influence of the retentates and pellets on beaniness was quite surprising. However, hexanal levels in these fractions were rather high, especially in retentate a (Figure 1). Interactions between volatiles and proteins may be playing a role (Houde et al., 2018; Wang & Arntfield, 2015). Indeed, in pea protein isolates, most volatiles are bound to proteins (Kuhn, 2004); for example, 88% of the octanal present may be bound to pea vicilin. These interactions might also be related to protein

solubility (Suppavorasatit et al., 2013). As proteins were present at higher concentrations in the retentates and the pellets, interactions between proteins and volatiles could explain the hexanal levels in these fractions and their effect on perceived aroma intensities. In addition, the perception of the attributes almond, broth, and pea may also have been influenced by the composition of peptides and amino acids, which were richer in the retentates. Indeed, Henriksen showed that the bouillon note of dried sausage was related to a mixture of different amino acids and peptides and that the intensity of the potato note was positively correlated with levels of tyrosine (in both its free and peptide residue forms) (Henriksen, 1997).

Thus, the mixture models helped reveal the factors that contribute to the perception of beaniness. The results suggest that beany notes are strongly related to volatile composition. However, there may also be an influence of protein-volatile interactions as well as peptide composition.

3.2.2. Identification of the fractions underlying mouthfeel, bitterness, and astringency

The mixture models were also a useful tool for gaining insight into the origin of the taste and texture attributes. The results show that perceived mouthfeel intensity mainly depended on pellets a and b (respectively: $F[8,72] = 1923$ and $F[8,72] = 1072$). Past work found that texture was relatively balanced in hydrocolloid solutions due to the high number of factors at play (e.g., hydrocolloid type, viscosity range, food matrix, choice of sensory evaluation technique) (van Vliet et al., 2009). In this study, the ratio of dry matter content to protein content was 0.83 for pellets, 0.88 for retentates, and 0.2 for permeates. The difference in texture perception among the fractions was therefore not due to protein concentration but rather to protein type. Pea protein isolates mainly consist of globulins, which represent 65–80% of total protein concentration and belong to three major groups (legumin 11S, vicilin 7S, and convicilin 7S); some albumins are also present (Kimura et al., 2008; Sirtori et al., 2012). In addition, in commercially produced isolates, proteins tend to be highly denatured and form large aggregates primarily structured by hydrophobic interactions (Chihi et al., 2016; Oliete et al., 2018; X. Peng et al., 2017; Ryan et al., 2012). Consequently, the process of creating new food products from isolate fractions can induce changes in this protein network. Our results suggest that different types of proteins are present in different concentrations in the pellet and retentate and that the specific pattern likely depends on protein size, solubility, and hydrophobicity. These compositional differences are probably responsible for the differences in perceived texture.

Perceived astringency mainly depended on retentates a and b (respectively: $F[14,65] = 721$ and $F[14,65] = 1001$) but also on pellet b ($F[14,65] = 776$). Past research has indicated that the perceived astringency of foods and beverages is mainly due to the composition of phenolics, namely monomeric and polymeric phenols, such as flavan-3-ols, as has been described in wine (Damodaran & Arora, 2013; Hufnagel & Hofmann, 2008; Peleg et al., 1999). Here, perceived bitterness was influenced by retentates a and b (respectively: $F[13,66] = 582$ and $F[13,66] = 693$). Like astringency, bitterness has been found to be influenced by the composition of phenolics, but, additionally, there is an influence of

519 saponins (Heng et al., 2006) and peptides (Aubes-Dufau et al., 1995). We expected phenolics,
520 saponins, and peptides to mainly be present in the permeates (i.e., they are small, soluble molecules).
521 However, it was the retentates and pellets (especially from isolate b) that had higher concentrations of
522 caffeic acid, which is considered to be a marker of phenolic levels (Figure 1). In plant-protein-based
523 foods, phenols can bind to proteins via hydrophobic and hydrophilic interactions (Bucalossi et al.,
524 2020; Morton & Murray, 2001; Potter et al., 1993; Zhang et al., 2016). In these interactions, important
525 roles are played by phenol chemical structure, phenol size and composition (including the number of
526 OH groups), and food environment (e.g., pH) (de Freitas & Nuno Mateus, 2012). In this study, the
527 fractions had different pH values (~7.5 for the retentates and pellets vs. ~9 for the permeates), which
528 suggests that phenol-protein interactions may have been different as well. Thus, our results suggest
529 that the proteins in the pellets and the retentates interacted with phenolics, leading to differences in
530 perceived astringency and bitterness.
531 Finally, perceived saltiness depended simultaneously on permeates a and b (respectively: $F[14,65] = 584$ and $F[14,65] = 481$) and retentates a and b (respectively: $F[14,65] = 641$ and $F[14,65] = 273$).
533 Solutions made from isolate a had higher sodium contents and were perceived as more salty. Relative
534 to their dry matter content, the permeates and retentates had higher levels of sodium (Figure 1).
535 Previous work has found that both sodium and chloride ions are required to activate the salt receptor
536 (Van Der Klaauw & Smith, 1995). However, when Frankowski et al. (2014) studied the sensory
537 characteristics and composition of permeate obtained from whey ultrafiltration, they showed that, in
538 addition to sodium, both lactic acid and potassium chloride can heighten the intensity of perceived
539 saltiness. Based on past research, we can assume that, compared to the pellets, the permeates and
540 retentates were richer in minerals.
541 Thus, the mixture models provided insight into the origin of the taste and texture attributes. Our results
542 suggest that the protein composition of the pellets and retentates influenced perceived texture.
543 Interactions between proteins and phenolics in the pellets and retentates may have affected perceived
544 astringency. Retentates may also be richer in phenolics, saponins, and peptides, whose presence may
545 have impacted perceived bitterness. Finally, the permeates and retentates may have been richer in
546 salts, heightening perceived saltiness.
547
548 3.2.3. Optimizing ingredient choice and product formulation
549 Based on these results, recommendations can be developed to improve the flavor of the pea protein
550 isolates used in plant-protein-based food products. First, attention should be paid to ingredient
551 optimization. Our results suggest that the filtration step was not especially effective in removing the
552 compounds responsible for off-notes. In this regard, the centrifugation step seemed more useful: the
553 pellets were described as less beany, bitter, and astringent than the retentates. Consequently, it could
554 be useful to formulate plant-protein-based products using pellets. However, because pellets consist
555 mainly of insoluble compounds, there might be a loss of functionality. Thus, employing a

pellet/retentate mixture could help limit off-notes while retaining functionality. The results for the retentates also highlight the importance of protein conformation and the interactions between both proteins and aromatics as well as proteins and phenolics. These mechanisms appear to play an important role in the sensory perception of pea protein isolates and must be studied further.

The specific nature of these recommendations will depend on food type, which will, in turn, determine protein concentration and functionality, matrix type, and ingredient choice. Indeed, pea protein isolates are used in different applications, for which protein concentrations vary widely (from < 1% to > 50%, with a median of 5%). For example, they are used in sports nutrition and to replace casein and whey proteins in fermented and unfermented dairy products (Akin & Ozcan, 2017; Ben-Harb et al., 2020; Korhonen & Pihlanto, 2006; Panesar, 2011; Schindler et al., 2012); they can serve as substitutes for egg proteins (Hoang, 2012); they can help enrich protein levels in baked foods, cereals, and snacks (Philipp et al., 2017); and they can improve the cooking yield, water/fat binding, and sliceability of meat, fish, processed foods, soups, and sauces (Baugreet et al., 2016; Tahmasebi et al., 2016). They are also emerging as an alternative ingredient in specialized foods, such as gluten-free products (Mariotti et al., 2009; Miñarro et al., 2012) and infant formula (Le Roux et al., 2020). The results of this study can help inform product formulation. For example, to improve the aroma of a product containing 3% pea protein, a mixture of pellet b (25%) and water (75%) would seem to be ideal (Figure 3). In such a product, undesirable aromas would be relatively less intense (broth score of 1.4/10, pea score of 2.4/10, and potato score of 1.2), while desirable aromas would be relatively more intense (almond score of 4.2/10, cereals score of 3.9/10, and nuts score of 2.9/10). To provide another example, it might be helpful to decrease the bitterness and astringency of a flavored product containing 3% pea protein; in this context, a mixture containing 74% permeate b and 26% pellet a could be useful (Figure 3). This formulation should result in less intense bitterness (score of 1.6/10) and astringency (score of 1.7/10).

Here, we discuss using customized combinations of isolate fractions as a strategy for reducing the off-notes of pea-protein-based products. Past research has identified several other strategies (see the review Roland et al., 2017). First, some approaches attempt to prevent the formation of certain contributing precursors (e.g., LOX, isoflavones) via cultivar selection (Stephany et al., 2015) or heat treatments (which limit oxidation; Azarnia et al., 2011). Other approaches try to remove or modify off-notes via soaking or heat treatments (Curti et al., 2018; Peng et al., 2017), by influencing germination (Simons & Hall, 2018), or by solvent-based extraction (Heng, 2005). However, such strategies often lead to a loss in functionality, which is a major drawback. Other approaches more selectively target off-notes using ultrasound technology (Miano et al., 2019), radio frequency treatments (Jiang et al., 2018), or enzyme treatments (Liu et al., 2017). In particular, fermentation can change the volatile profiles of foods (Ben-Harb et al., 2020; El Youssef et al., 2020; Meinlschmidt et al., 2016; Schindler et al., 2012). Another strategy focuses on protein-bound precursors and aims to form inclusion complexes with β-cyclodextrin (Damodaran & Arora, 2013). Filtration can also limit the presence of

compounds responsible for off-notes (Roozen & Pilnik, 1979; H. Yu et al., 2017). The last strategy involves masking off-notes by adding sugars, salts, acids, or flavoring (Bertelsen et al., 2018; Heng, 2005; Zha et al., 2019). The new strategy described in this study can serve as a complement to these other techniques for improving the flavor of pea-protein-based foods.

4. CONCLUSION AND PERSPECTIVES

This study adopted an original approach: to work with fractions instead of compounds to explore how combinations of volatiles and non-volatiles affect the sensory characteristics of pea-protein-based solutions. We broke down pea protein isolates into three fractions (pellet, retentate, and permeate), which were then recombined to form different experimental solutions using a mixture design. The study yielded several key results. First, we found that panelists generally came up with repeatable and homogeneous scores for the 46 solutions during the 3-month experiment. Second, attribute intensity did not significantly differ between the reference solutions and the experimental solutions. Third, among the 18 sensory attributes initially evaluated, 10 were identified as useful for building the optimal mixture models, whose performance was validated using ANOVA and data from six supplementary solutions. The results suggest that the models effectively predicted the perception of sensory attributes based on solution composition. Fourth, these models were also used to obtain greater insight into the origin of perceived beaniness, bitterness, and astringency. Our results suggest that beaniness is a multidimensional and complex descriptor that can be expressed via other attributes: almond, broth, cereals, nuts, pea, and potato. They also indicate that attributes contributing to perceived beaniness were mainly influenced by the retentate and permeate fractions, likely because of their levels of volatiles, which were indirectly reflected by the hexanal levels here. Perceived astringency was mainly influenced by the retentate and pellet fractions, while perceived bitterness was largely driven by the retentate fraction. Bitterness and astringency were associated with levels of phenolics, which were indirectly reflected by the caffeic acid content here. The results of this study will thus improve understanding of how different pea protein fractions contribute to the undesirable sensory characteristics of pea-protein-based ingredients. They have also revealed that fraction-based food formulation could help reduce beaniness, bitterness, and astringency. However, it is also clearly necessary to more precisely analyze food product composition (i.e., look beyond the levels of hexanal and caffeic acid) to clarify the deeper origins of the sensory perception of foods.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Audrey Cosson: Methodology, Investigation, Formal analysis, Writing - Original Draft. David Blumenthal: Methodology, Writing - review & editing. Nicolas Descamps: Funding acquisition, Conceptualization. Isabelle Souchon: Conceptualization, Supervision, Writing - review & editing. Anne Saint-Eve: Methodology, Supervision, Writing - review & editing.

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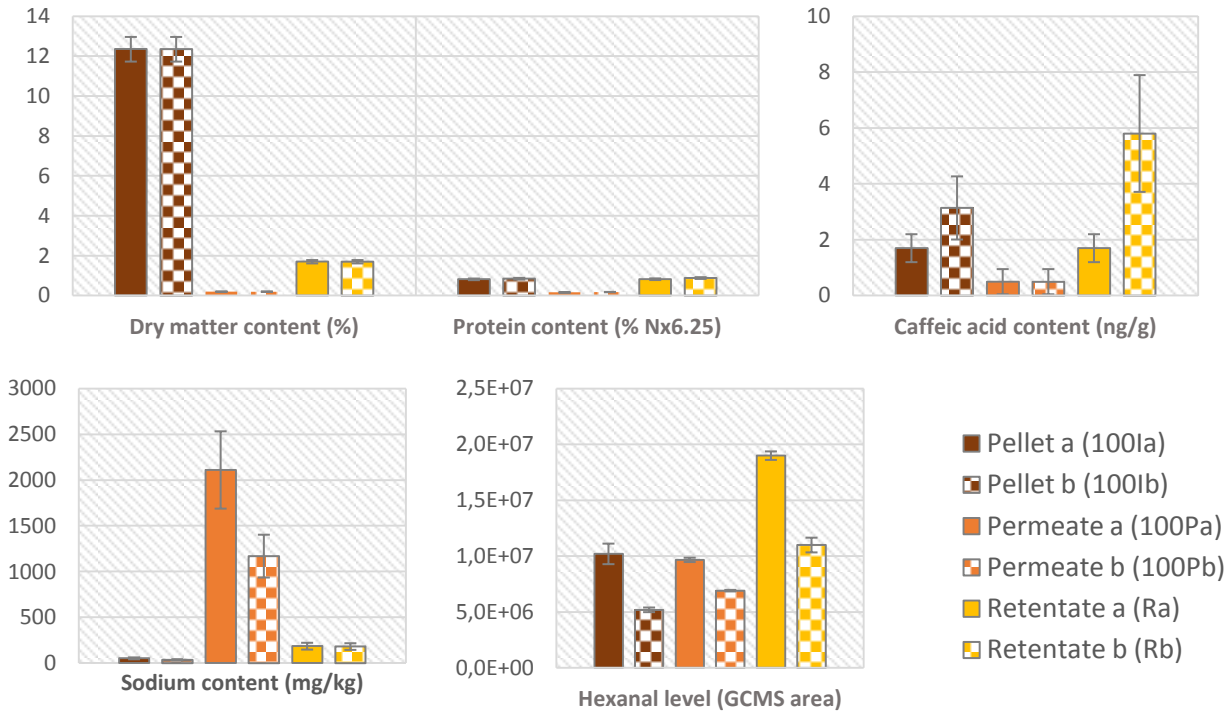
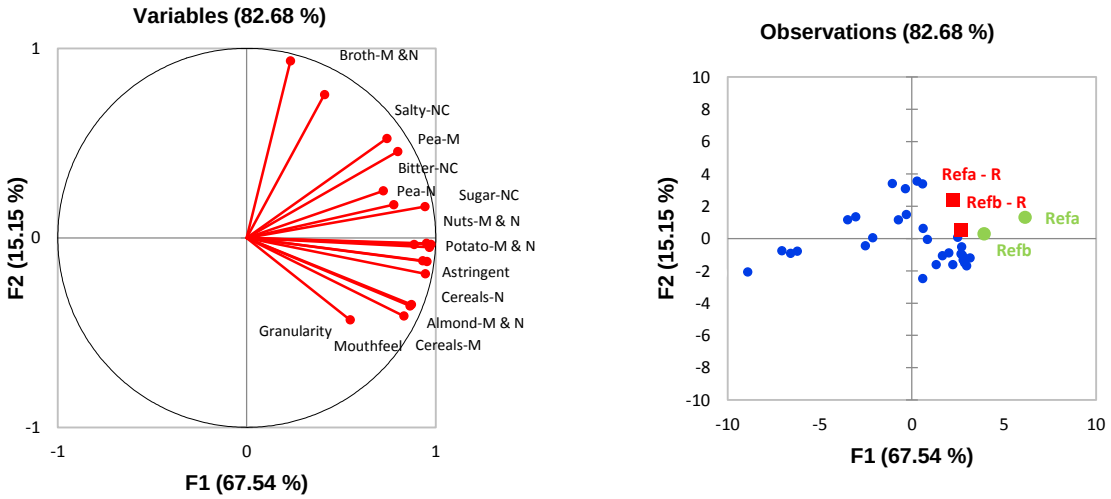
FIGURE



1014 Figure 1: Key characteristics of the six pea protein isolate fractions used in the study (pellet a and b,
1015 permeate a and b, retentate a and b): dry matter content (%), protein content (% Nx6.25), caffeic acid
1016 content (ng/g), sodium content (mg/kg), and hexanal levels (GCMS area).
1017



1018
1019 Figure 2: Results of the principal component analysis (PCA) examining the solutions' sensory profiles.
1020 On the left is a loading plot showing the correlational relationships between PCA axes 1 and 2 and the
1021 sensory attribute values in the original dataset. On the right is a PCA plot with the same two axes that
1022 shows the relative similarity of the solutions' sensory profiles. In green are the active observations
1023 corresponding to the raw product (Refa and Refb), in blue are the others active observations, in red are
1024 the supplementary observations corresponding to the experimental solutions with the same
1025 composition as the reference solutions (Refa-R and Refb-R).
1026

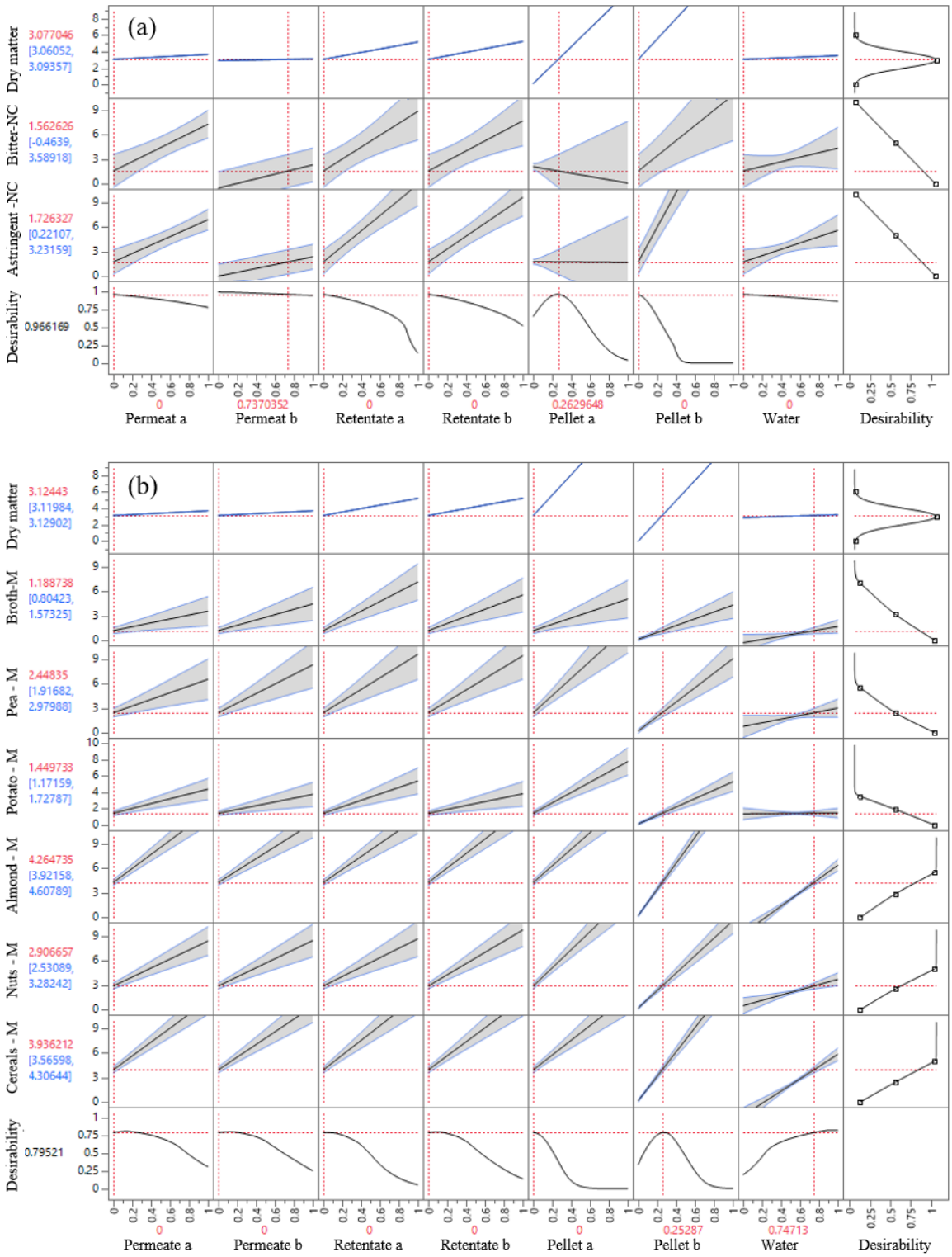


Figure 3: Cross-sectional view of the predicted attribute scores as a function of a solution's fractional composition for a target dry matter content of 3%: (a) solution formulation in which astringency and bitterness are minimized; (b) solution formulation in which undesirable attributes (potato, pea, and broth) are minimized, whereas desirable attributes (almonds, nuts, and cereals) are maximized. The vertical red lines correspond to the current values of the factors (also indicated in red below the x-

axes). The horizontal red lines correspond to the mean predicted scores based on the current factor values (also indicated to the left of the y-axes [95% confidence intervals in blue]). The confidence intervals are represented in gray on the plots. Overall solution desirability is shown in the last plot row and column. It was defined as the geometric mean of the desirability functions for the individual responses.

TABLE

Table 1: Composition of the different solutions used in this study, which were created by mixing permeates a and b, retentates a and b, and pellets a and b. In bold are the solutions that were replicated. In italics are the supplementary solutions used for validation purposes.

Solution ID	Permeate a (%)	Permeate b (%)	Retentate a (%)	Retentate b (%)	Pellet a (%)	Pellet b (%)	Water (%)	MS (%)	Sensory session ID
P12	100	0	0	0	0	0	0	0.20	3
P29	100	0	0	0	0	0	0	0.20	6
P9	0	100	0	0	0	0	0	0.20	2
P38	0	100	0	0	0	0	0	0.20	8
P4	0	0	100	0	0	0	0	1.70	1
P8	0	0	100	0	0	0	0	1.70	2
P13	0	0	100	0	0	0	0	1.70	3
P14	0	0	0	100	0	0	0	1.70	3
P31	0	0	0	100	0	0	0	1.70	7
P40	0	0	0	100	0	0	0	1.70	8
P3	0	0	0	0	0	0	100	0.00	1
P19	0	0	0	0	0	0	100	0.00	4
P25	0	0	0	0	0	0	100	0.00	5
P34	0	0	0	0	0	0	100	0.00	7
P6	0	0	0	0	0	25	75	3.09	2
P24	0	0	0	0	0	25	75	3.09	5
P11	25	0	25	0	12.5	0	37.5	2.02	3
P28	0	0	0	0	30	0	70	3.71	6
P1	40	0	0	0	0	0	60	0.08	1
P35	40	0	0	0	0	0	60	0.08	7
P18	0	40	0	0	0	0	60	0.08	4
P17	0	0	40	0	0	30	30	4.39	4
P37	0	0	0	40	30	0	30	4.39	8
P7	0	0	0	40	0	30	30	4.39	2
P2	0	0	0	0	50	0	50	6.00	1
P20	0	0	0	0	50	0	50	6.00	4
P30	0	0	0	0	0	50	50	6.00	6
P39	0	0	0	0	0	50	50	6.00	8
P33	50	0	0	0	0	25	25	3.19	7
P36	50	0	50	0	0	0	0	0.95	8
P10	50	0	0	0	25	0	25	3.19	2
P5	0	50	0	0	0	25	25	3.19	1
P23	0	50	0	50	0	0	0	0.95	5
P26	0	50	0	0	0	0	50	0.10	6
P22	0	0	50	0	25	0	25	3.94	5
P16	0	0	0	50	0	0	50	0.85	4
P21	0	0	0	50	0	0	50	0.85	5
P32	0	0	60	0	0	0	40	1.02	7
P15	0	70	30	0	0	0	0	0.65	3
P27	40	0	0	60	0	0	0	1.10	6
P41	*0*	*0*	*0*	*0*	*67*	*0*	*33*	*8.27*	*9*
P42	*0*	*0*	*0*	*0*	*0*	*50*	*50*	*6.00*	*9*
P43	*0*	*0*	*0*	*0*	*8*	*0*	*92*	*0.99*	*9*
P44	*0*	*0*	*0*	*0*	*0*	*8*	*92*	*0.99*	*9*
P45 (Refa-R)	*38*	*0*	*34*	*0*	*28*	*0*	*0*	*4.10*	*9*

P46 (Refb-R)	*0*	*40*	*0*	*36*	*0*	*24*	*0*	*3.70*	*9*

Table 2: Assessment of panelist performance in scoring the intensities of the six aroma attributes evaluated by nose (N), the six taste attributes evaluated in mouth with the nose clip (NC), and the six aroma attributes evaluated in mouth (M) for the range of solutions used in the study; solution evaluation employed a block protocol. In the three-way ANOVA, the fixed factors were solution ID, panelist ID, replicate ID, and their first-order interactions. F: Fisher statistic for the fixed effects. Pvalue: p-value for the Fisher test. Significant p-values (threshold of 0.05) are in bold. Model degrees of freedom (DF): 735; residual DF: 624.

	Solution ID		**Panelist ID**		**Replicate ID**		**Panelist*Solution**		**Replicate*Panelist**		**Replicate*Solution**	
	F	**Pvalue**	**F**	**Pvalue**	**F**	**Pvalue**	**F**	**Pvalue**	**F**	**Pvalue**	**F**	**Pvalue**
Almond-M	45.78	**<0.01**	86.43	**<0.01**	19.15	**<0.01**	2.17	**<0.01**	3.02	**<0.01**	1.22	0.18
Almond-N	22.20	**<0.01**	100.15	**<0.01**	0.22	0.64	2.03	**<0.01**	4.62	**<0.01**	0.99	0.49
Astringent-NC	33.94	**<0.01**	58.61	**<0.01**	0.17	0.68	1.75	**<0.01**	4.85	**<0.01**	1.42	0.05
Bitter-NC	14.83	**<0.01**	44.76	**<0.01**	11.48	**<0.01**	1.23	**<0.01**	2.72	**<0.01**	1.20	0.19
Broth-M	54.09	**<0.01**	49.60	**<0.01**	5.39	**0.02**	1.98	**<0.01**	3.08	**<0.01**	1.43	**0.04**
Broth-N	27.26	**<0.01**	48.37	**<0.01**	10.46	**<0.01**	1.24	**<0.01**	2.30	**<0.01**	1.66	**0.01**
Cereals-M	47.01	**<0.01**	48.42	**<0.01**	1.58	0.21	2.34	**<0.01**	2.72	**<0.01**	1.86	**<0.01**
Cereals-N	23.43	**<0.01**	53.96	**<0.01**	0.91	0.34	1.46	**<0.01**	3.43	**<0.01**	0.82	0.78
Granularity-NC	261.12	**<0.01**	24.39	**<0.01**	0.05	0.82	2.29	**<0.01**	1.77	**0.03**	1.11	0.30
Mouthfeel-NC	116.94	**<0.01**	42.39	**<0.01**	35.61	**<0.01**	2.07	**<0.01**	3.53	**<0.01**	2.16	**<0.01**
Nuts-M	29.50	**<0.01**	89.91	**<0.01**	0.93	0.34	1.80	**<0.01**	3.62	**<0.01**	1.10	0.31
Nuts-N	25.95	**<0.01**	99.72	**<0.01**	2.13	0.15	1.60	**<0.01**	3.39	**<0.01**	1.62	**0.01**
Pea-M	27.57	**<0.01**	41.31	**<0.01**	55.09	**<0.01**	1.88	**<0.01**	6.76	**<0.01**	1.54	**0.02**
Pea-N	28.12	**<0.01**	45.61	**<0.01**	28.95	**<0.01**	1.52	**<0.01**	2.92	**<0.01**	1.94	**<0.01**
Potato-M	23.79	**<0.01**	61.59	**<0.01**	1.52	0.22	1.91	**<0.01**	3.83	**<0.01**	1.34	0.08
Potato-N	19.63	**<0.01**	40.01	**<0.01**	2.18	0.14	1.16	**0.03**	3.18	**<0.01**	0.94	0.57
Salty-NC	15.74	**<0.01**	70.85	**<0.01**	0.51	0.48	1.33	**<0.01**	4.24	**<0.01**	0.83	0.76
Sugar-NC	9.20	**<0.01**	94.31	**<0.01**	17.01	**<0.01**	1.96	**<0.01**	5.53	**<0.01**	1.37	0.07

Table 3: Assessment of panelist performance in scoring the intensities of the six aroma attributes evaluated by nose (N), the six taste attributes evaluated in mouth with the nose clip (NC), and the six aroma attributes evaluated in mouth (M) for the main reference solution. In the two-way ANOVA, the fixed factors were panelist ID, sensory session ID, and their interaction. F value: Fisher statistic for the fixed effects. Pvalue: p-value for the Fisher test. Significant p-values (threshold of 0.05) are in bold. Model DF: 35; residual DF: 304.

	Sensory session ID		**Panelist ID**	
	F	**Pvalue**	**F**	**Pvalue**
Almond-M	1.17	0.28	89.99	**<0.01**
Astringent-NC	0.50	0.96	30.02	**<0.01**
Bitter-NC	1.49	0.09	32.90	**<0.01**
Broth-M	2.48	**<.01**	30.36	**<0.01**
Cereals-M	0.88	0.61	40.19	**<0.01**
Granularity-NC	2.88	**<.01**	19.64	**<0.01**
Mouthfeel-NC	1.51	0.08	24.90	**<0.01**
Nuts-M	0.69	0.83	35.17	**<0.01**
Pea-M	1.37	0.14	24.88	**<0.01**
Potato-M	0.99	0.47	36.41	**<0.01**
Salty-NC	1.29	0.18	22.97	**<0.01**
Sugar-NC	1.08	0.37	63.31	**<0.01**

Table 4: Performance of the optimal mixture models as assessed via ANOVAs; lack-of-fit tests; and the coefficients of determination (R2). F: Fisher statistic for the fixed effects. Pvalue: p-value for the Fisher test. DF: degrees of freedom. Significant p-values (threshold of 0.05) are in bold.

	ANOVA		**Lack-of-fit test**		**Coefficient of determination**
	F (model DF, residual DF)	**Pvalue**	**F** (model DF, residual DF)	**Pvalue**	R^2
Almond-M	62.97 (17,62)	**<0.01**	0.50 (8,54)	0.85	0.95
Astringent-NC	62.42 (14,65)	**<0.01**	0.94 (11,54)	0.51	0.93
Bitter-NC	23.12 (13,66)	**<0.01**	0.43 (12,54)	0.94	0.82
Broth-M	90.44 (15,64)	**<0.01**	0.94 (10,54)	0.50	0.95
Cereals-M	70.29 (14,65)	**<0.01**	0.94 (11,54)	0.51	0.94
Mouthfeel-NC	519.98 (8,72)	**<0.01**	1.04 (18,54)	0.44	0.96
Nuts-M	57.98 (14, 65)	**<0.01**	1.21 (11,54)	0.30	0.93
Pea-M	50.92 (9,70)	**<0.01**	0.88 (16,54)	0.59	0.87
Potato-M	53.68 (13,66)	**<0.01**	0.49 (12,54)	0.91	0.92
Salty-NC	33.54 (14,65)	**<0.01**	1.33 (11,54)	0.23	0.88

Table 5: Significant effects identified using a backward elimination procedure (p-value <0.05; DF for the effects = 1): F = Fisher statistic for the fixed effects; Est = estimated coefficient.

	Almond		**Astringent-NC**		**Bitter-NC**		**Broth-M**		**Cereals-M**		**Mouthfeel-NC**		**Nuts-M**		**Pea-M**		**Potato-M**		**Salty-NC**	
	F	**Est**	**F**	**Est**	**F**	**Est**	**F**	**Est**	**F**	**Est**	**F**	**Est**	**F**	**Est**	**F**	**Est**	**F**	**Est**	**F**	**Est**
Retentate a	*255*	2	*721*	4	*582*	5	*929*	5	*225*	2	*166*	2	*512*	3	*423*	4	*331*	2	*584*	3
Retentate b	*291*	2	*1001*	5	*696*	5	*144*	2	*209*	2	*214*	2	*613*	4	*441*	4	*220*	2	*481*	3
Pellet a	2	-3	4	3	0	0	*89*	3	10	5	*1923*	14	3	3	*370*	9	6	3	1	1
Pellet b	21	-8	*776*	10	1	-1	34	2	11	-6	*1072*	11	22	6	*225*	7	*504*	5	2	1
Permeate a	*94*	1	*177*	2	*94*	2	*253*	3	*58*	1	16	1	*151*	2	*264*	3	*135*	1	*641*	4
Permeate b	*148*	2	*159*	3	*162*	3	*95*	3	*46*	1	9	1	*115*	2	*419*	4	*70*	1	*273*	3
Permeate a*Pellet b	*107*	29	22	12	6	9	12	-8	*82*	28	9	8	13	9			14	6		
Pellet b*Water	*106*	32			20	15			*64*	28			5	5					15	8
Water	8	0	*132*	1	*121*	2	0	0	7	0			8	0	9	0	3	0	36	1
Permeate b*Pellet b	*72*	23	32	15	14	14			*79*	28	5	6	20	12			18	7	11	8
Retentate b*Pellet b	*52*	21							*53*	24										
Retentate a*Pellet b	29	16					*51*	-19	*51*	23					13	-13				
Permeat a*Pellet a	28	16	7	7	9	11			8	8			16	11			11	6		
Pellet a*Water	18	18	9	9	6	10			1	4			9	10			8	5	13	8
Retentate a*Pellet a	11	11					26	-13	7	7			10	9						
Retentate b*Water	7	2	5	2	6	3	5	2					7	2	7	3	4	1	8	2
Retentate b*Pellet a	6	8					5	-7												
Retentate a*Water	6	2	6	3			15	5							16	6	12	3	8	3
Permeate b*Water			5	-2	10	-4													4	-2
Blocking factor							6	0												
Permeate b*Retentate b																			9	3
Permeate b*Retentate a			10	5			26	7					7	4			8	3	6	3
Permeate a*Retentate a							8	3												

Table 6: Observed and predicted attribute scores with the 95% confidence intervals (CIs) for the six validation solutions (two replicates performed)

Result ± 95% CI	**Salty-NC**	**Bitter-NC**	**Astringent-NC**	**Mouthfeel-NC**	**Broth-M**	**Pea-M**	**Potato-M**	**Almond-M**	**Nuts-M**	**Cereals-M**
P41 Observed	2.96 ± 0.64	3.35 ± 0.61	4.73 ± 0.62	7.58 ± 0.75	2.31 ± 0.67	4.67 ± 0.55	2.83 ± 0.63	3.30 ± 0.79	4.00 ± 0.69	3.54 ± 0.68
P41 Predicted	2.93 ± 0.57	2.81 ± 0.98	4.40 ± 0.78	9.58 ± 0.44	2.88 ± 0.65	6.03 ± 0.58	2.91 ± 0.50	2.02 ± 1.19	4.37 ± 0.88	4.28 ± 0.84
P42 Observed	2.86 ± 0.71	4.18 ± 0.64	5.01 ± 0.73	5.01 ± 0.70	2.10 ± 0.69	4.61 ± 0.76	2.53 ± 0.58	3.09 ± 0.77	3.09 ± 0.72	2.79 ± 0.64
P42 Predicted	2.98 ± 0.28	3.94 ± 0.43	5.88 ± 0.34	5.36 ± 0.33	1.72 ± 0.40	3.65 ± 0.42	2.73 ± 0.22	4.20 ± 0.33	4.29 ± 0.31	4.02 ± 0.38
P43 Observed	2.39 ± 0.62	4.02 ± 0.75	3.31 ± 0.74	1.41 ± 0.47	1.70 ± 0.68	2.47 ± 0.79	1.34 ± 0.48	2.47 ± 0.59	1.85 ± 0.70	1.44 ± 0.43
P43 Predicted	1.36 ± 0.23	2.46 ± 0.36	2.26 ± 0.28	1.14 ± 0.05	0.88 ± 0.38	1.12 ± 0.27	0.74 ± 0.18	1.32 ± 0.29	1.28 ± 0.27	0.94 ± 0.27
P44 Observed	2.87 ± 0.63	4.43 ± 0.67	3.31 ± 0.69	1.42 ± 0.40	1.6 ± 0.55	2.68 ± 0.66	1.04 ± 0.35	2.11 ± 0.58	2.16 ± 0.65	1.29 ± 0.50
P44 Predicted	1.33 ± 0.21	2.72 ± 0.32	2.19 ± 0.22	0.86 ± 0.05	0.79 ± 0.36	0.96 ± 0.26	0.56 ± 0.14	1.99 ± 0.22	1.15 ± 0.22	1.83 ± 0.24

P45 Observed	3.91 ± 0.80	3.79 ± 0.66	4.19 ± 0.67	4.01 ± 0.68	5.22 ± 0.72	5.03 ± 0.71	2.51 ± 0.53	2.55 ± 0.65	3.42 ± 0.81	2.6 ± 0.57
P45 Predicted	2.94 ± 0.61	3.65 ± 0.90	4.03 ± 0.72	4.9 ± 0.22	3.78 ± 0.58	5.22 ± 0.32	2.51 ± 0.47	3.13 ± 0.65	4.77 ± 0.66	4.12 ± 0.65
P46 Observed	3.82 ± 0.85	4.05 ± 0.64	4.9 ± 0.71	3.68 ± 0.6	3.16 ± 0.86	5.06 ± 0.80	2.47 ± 0.51	3.02 ± 0.68	3.47 ± 0.80	2.5 ± 0.61
P46 Predicted	3.86 ± 0.59	4.15 ± 0.80	6.7 ± 0.50	4.08 ± 0.46	2.95 ± 0.31	4.85 ± 0.30	2.9 ± 0.31	3.59 ± 0.54	4.49 ± 0.54	4.31 ± 0.59

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