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

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1 ABSTRACT

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

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

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

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

28

29 KEYWORDS

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

31

32 TITLE

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

35

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45

## 46 1. INTRODUCTION

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

54 Industrial pea-protein ingredients are traditionally generated via a several-step wet process. Pea seeds  
55 are solubilized in an alkaline solution, which is then centrifuged to remove insoluble compounds; the  
56 precipitate is obtained at the isoelectric point using acidification and centrifugation. The resulting  
57 isolate has a protein content of 80–90% (mainly globulins), but also contains lipids, sugars, salts, and  
58 other small compounds (e.g., phenolics), which are the products of seed metabolism (Schutyser et al.,  
59 2015). The isolate can also serve as an ingredient in the formulation of many food products, including  
60 dietary supplements, bakery and confectionery products, beverages, yogurts, ice creams, meat  
61 products, and meat and dairy alternatives.

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

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

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

81 Research on the perception of pea-based products has largely focused on the role of volatile aroma  
82 compounds in creating sensations of beaniness (Azarnia et al., 2011; Ben-Harb et al., 2020; Bi et al.,  
83 2020; Bott & Chambers, 2006; El Youssef et al., 2020; Murat et al., 2013; Mutarutwa et al., 2018;  
84 Schindler et al., 2012; Trikusuma et al., 2020; Wang & Arntfield, 2015; Xu et al., 2019; Xu et al.,  
85 2020). A few studies have exclusively examined the peptides that could be related to bitterness (Akin  
86 & Ozcan, 2017; Jakubczyk et al., 2013; Pownall et al., 2010; Sirtori et al., 2012); the phenolics related  
87 to bitterness and astringency (Bucalossi et al., 2020; Guo et al., 2019; Padhi et al., 2017); and the  
88 saponins related to bitterness (Daveby et al., 1998; Heng et al., 2006; Heng et al., 2006; Price &  
89 Fenwick, 1984). However, to our knowledge, no study to date has used a more global approach to  
90 examine how the complex perception of pea protein isolates arises from both volatile and non-volatile  
91 compounds and their potential interactions.

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

112 Studies have shown that attribute perception may be similar for a complex product and a fraction-  
113 based reconstruction of the product. For example, artificial ikura (Japanese salmon caviar) was  
114 prepared using vegetable oil and a low-calorie natural gel (e.g., one made with alginic acid) (Hayashi  
115 et al., 1990); each component of the food was then analyzed using chemical and sensory methods.  
116 Based on the analytical data, a synthetic ikura was reconstituted using pure reagents. There were very  
117 few sensory differences in the taste profiles between the reference food and the reconstructed food  
118 (Hayashi et al., 1990). In another study (Niimi et al., 2014), a cheese solution was reconstituted using  
119 a mixture of sucrose, NaCl, monosodium glutamate, lactic acid, and caffeine that was then adjusted  
120 using a fractional factorial design. The reconstructed products did not significantly differ from the  
121 cheddar cheese reference in overall intensity, saltiness, sourness, umami, and bitterness (Niimi et al.,  
122 2014).

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

140

## 141 2. MATERIALS AND METHODS

### 142 2.1. Production of pea protein isolate fractions

143 Two pea protein isolates (protein content Nx6.25, 83% dry matter) were used; they were called isolate  
144 a and b, respectively. The isolates were dispersed in tap water in a tank to obtain a final suspension  
145 containing 4% (w/w) dry matter content. This suspension was maintained under agitation for 12 h at  
146 3°C with an external agitator (U-shaped stirrer shafts); it was then centrifuged with two centrifuges  
147 (Jouan Kr4i and a Sorvall Lynx 4000 [Thermo Scientific, Waltham, US]; 6000 g, 10 min, 4°C). The  
148 supernatant was manually separated from the pellet. The pellet was subsequently diluted with tap

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

159

## 160 2.2 Characterization of the pea protein isolate fractions

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

170

## 171 2.3 Mixture design

172 An optimal mixture design was used to create a wide range of reference and experimental solutions  
173 from the fractions (permeates a and b, retentates a and b, and pellets a and b). Response surface  
174 models were created and included quadratic terms and first-order interactions. The experimental  
175 design was such that there was orthogonality among all the terms, which allowed variable effects to be  
176 differentiated from one another. A blocking factor was used to control for the effect of the day on  
177 which sensory evaluation took place. The order of solution evaluation within the blocks was fully  
178 balanced. Overall, the mixture design had eight independent variables (see Table 1 for the levels), and  
179 10 solutions were replicated. The total number of trials was 40. Variable levels were chosen so as to  
180 represent a wide range of variation while remaining realistic in terms of the protein concentrations  
181 actually experienced when pea protein isolates are used to create foods.

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

186 better or equivalent efficiency—with a D-optimal value of 13.25% and a G-optimal value of  
187 50.85%—compared to experiments based on other designs. These metrics reflect goodness of fit  
188 relative to a hypothetical orthogonal design: the D-optimal value indicates whether the design  
189 minimizes the volume of the joint confidence region for the vector of regression coefficients, and the  
190 G-optimal value indicates whether the design minimizes the maximum prediction variance over the  
191 design region.

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

194

#### 195 2.4. Solution creation

196 The six different fractions were combined in various ways to formulate the 46 solutions of the mixture  
197 design. This process was carried out at 4°C in 500 mL and 100 mL glass flasks, which were stored at -  
198 20°C. During fractionation and recombination, good hygiene practices were used to limit microbial  
199 contamination (usage of coat, gloves, and hygienic cap; cleaning and disinfection of hands and all  
200 equipment with pure ethanol, followed by air drying; work carried out in a 4°C chamber). In addition,  
201 the microbial safety of the solutions was tested by a certified external laboratory (Eurofins Scientific,  
202 France). However, for microbiological reasons, the solutions containing pellet b had to be heat treated  
203 (autoclaved at 110°C for 10 min) before oral sensory evaluation, so the supplemental effect of the  
204 autoclave procedure on perception was also evaluated. It was slightly significant for the attributes nuts,  
205 cereals, and almond and strongly significant for the attribute granularity (mean difference between  
206 autoclaved and unautoclaved solutions: 0.89/10 for nuts; 0.94/10 for cereals; 1.44/10 for almond, and  
207 5.49/10 for granularity). Because this effect was minor (except in the case of granularity) and collinear  
208 with pellet b, it will not be discussed further.

209

#### 210 2.5. Sensory evaluation conditions

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

223

## 224 2.6. Sensory profiling method

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

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

247

## 248 2.7. Statistical analysis of the sensory data

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

258

## 259 2.8. Statistical analysis of the mixture design



260 JMP (v. 13.1.0; SAS Institute Inc., Cary, SC, USA) was used to generate and analyze the optimal  
261 mixture design. Multiple regression analysis was performed to evaluate the effects of all the  
262 independent variables on each response variable (i.e., via the regression coefficients). The most  
263 influential independent variables ( $p \leq 0.05$ ) were identified using backward elimination. The  
264 regression coefficients were calculated for each final model. Model performance was assessed via  
265 ANOVAs (F-test for significance), lack-of-fit tests, and coefficients of determination ( $R^2$ ). For the six  
266 validation solutions, the predicted and observed responses (with 95% confidence intervals) were  
267 calculated.

268

### 269 3. RESULTS

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

279

#### 280 3.1. Construction of surface response models from the sensory data

##### 281 3.1.1. Assessment of panelist performance over the 3-month experiment

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

287 Reproducibility and homogeneity were examined first. Solution ID was significant for all 18  
288 attributes, which indicates that panelists distinguished among solutions. Panelist ID and the interaction  
289 between panelist ID and solution ID were also significant for all the attributes. Such interactions are  
290 common when sensory attributes are evaluated using unstructured scales and are difficult to control  
291 even when panelists have undergone extensive training (Jourjon et al., 2005; Lawless & Malone,  
292 1986). The interaction between replicate ID and solution ID was not significant for 10/18 attributes.  
293 Replicate ID was not significant for 11/18 attributes, but the interaction between panelist ID and  
294 replicate ID was significant for all 18 attributes. However, the F-values for these interactions were low  
295 compared to the F-values for the main effect of solution ID. For example, for the broth-M attribute,  
296  $F(39,624) = 54.09$  for solution ID;  $F(1,624) = 5.39$  for replicate ID;  $F(16,624) = 3.08$  for the panelist-

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

299 The presence of between-session drift was examined by looking at the scores for the reference solution  
300 across the entire experiment. To this end, a two-way ANOVA (fixed factors: panelist ID and sensory  
301 session ID) was performed using scores for each attribute given to the reference solution (Table 3).  
302 Sensory session ID was not significant for any of the attributes except broth-M and granularity-NC:  
303 these attributes were assigned slightly higher and slightly lower scores, respectively, during a single  
304 session. Although using the reference can make solution preparation more cumbersome, it was  
305 important in helping to validate panelist performance. In addition, panelists found the reference useful  
306 as they scored the other solutions. In past research, monadic presentation has been found to be faster  
307 and less tiring than comparative presentation (Mazzucchelli & Guinard, 1999). However, comparative  
308 presentation allows panelists to detect smaller differences among food products and to make more  
309 accurate decisions about these relative differences (Mcbride, 2007; A. Saint-Eve et al., 2006). Here,  
310 via its use of blocks, the presentation method combined monadic and comparative elements.  
311 Consequently, the panelists could base their attribute scoring on both their memories from the training  
312 period as well as on the reference, which was always the first solution in the sequence (Hastie & Park,  
313 1986).

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

318

### 319 3.1.2. Attribute choice

320 Plant-protein-based ingredients are often said to be “beany,” a multidimensional and complex  
321 descriptor (Bott & Chambers, 2006). Here, the decision was made not to use the term “beany.”  
322 Instead, its multiple components were parsed out and expressed via other terms (see Cosson et al.,  
323 2020). Thus, six aroma attributes were selected: potato, pea, cereals, broth, almond, and nuts. Plant-  
324 protein-based ingredients are also often described as being persistently bitter and astringent (Roland et  
325 al., 2017); consequently, bitterness and astringency were included as well. Finally, two taste  
326 attributes—salty and sugar—and two texture attributes—mouthfeel and granularity—were also  
327 chosen because they have been found to be important in descriptions of food quality and preference  
328 (van Vliet et al., 2009).

329 Attribute intensities for the different solutions were investigated using a three-way ANOVA (Table 2).  
330 Solution ID was significant for the 18 attributes (model DF: 735; residual DF: 624), which means the  
331 solutions had distinct sensory profiles. There were pronounced differences in perceived texture ( $F =$   
332  $261.12$  for granularity and  $F = 116.94$  for mouthfeel) and smaller differences in perceived sweetness  
333 ( $F = 9.20$  for sugar). These results are not surprising. It is easier to describe food products based on

334 texture and taste than on aroma (Kora et al., 2003; Lundgren et al., 1986; Anne Saint-Eve et al., 2011).  
335 Furthermore, temporally, they are the first attributes to become dominant in the mouth (Le Calvé et al.,  
336 2019; Pineau et al., 2009; Anne Saint-Eve et al., 2011). Additionally, when describing overall  
337 preferences and sensory satisfaction, consumers appear to primarily focus on taste and then on texture,  
338 paying the least attention to aroma (van Vliet et al., 2009). Finally, since the solutions had very low  
339 levels of natural sugar content (and no sugar was added), it was not surprising that sweetness did not  
340 greatly contribute to the perceived differences among the solutions. Consequently, this attribute was  
341 not included in the statistical model.

342 To build upon these results, PCA was used to visually depict the relationships among solution types  
343 and attributes (Figure 2). The solutions were well distributed along axes F1 and F2, which accounted  
344 for 82.68% of the variance. Thus, maps based on the first two axes seemed to provide a good quality  
345 projection of the initial multidimensional table, even though some information might have remained  
346 hidden in the subsequent axes. The 12 aroma attributes were clustered within one quarter of the  
347 correlation circles and thus clearly interacted in multiple ways. Aroma attributes assessed in the mouth  
348 and nose were strongly correlated ( $R^2 = 0.86$  for pea,  $R^2 = 0.88$  for broth,  $R^2 = 0.83$  for cereals,  $R^2 =$   
349  $0.95$  for nuts,  $R^2 = 0.87$  for almond,  $R^2 = 0.89$  for potato). These results suggest that panelists  
350 assigned similar scores to aroma attributes perceived orthonasally and retronasally and that food  
351 processing in the mouth had a minor effect on olfactory perception. Orthonasal odors result from  
352 volatile compounds traveling from the external environment and through the nares to the olfactory  
353 mucosa, whereas retronasal odors result when volatile compounds travel to the olfactory mucosa after  
354 they have been released during food destructurement in the oral cavity (Sun & Halpern, 2005). That  
355 said, orthonasal and retronasal responses are often similar, except in cases where there are  
356 physicochemical or sensory interactions induced by texture, taste, or in-mouth food destructurement  
357 (Goldberg et al., 2018). Furthermore, results for the attributes pea-M and salty-NC were also  
358 correlated ( $R^2 = 0.87$ ), which suggests possible congruency (Oladokun et al., 2017). Consequently, our  
359 results indicate that there may have been limited interactions between texture, taste, and flavor (except  
360 in the case of the attributes pea and salty) and that food oral processing had a minimal impact on these  
361 attributes. Therefore, the aroma attributes evaluated via the nose were not included in the statistical  
362 model.

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

371

### 372 3.1.3. Validation of the study methodology—fraction-based formulation of solutions

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

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

384 The main difference between the reference solutions and the experimental solutions was in their  
385 perceived granularity. The experimental solutions were perceived as less granular than the reference  
386 solutions. In commercially produced isolates, proteins are highly denatured due to the extraction  
387 process (pH changes, high temperatures) and form large aggregates that are primarily structured by  
388 hydrophobic interactions (Chihi et al., 2016; Oliete et al., 2018; W. Peng et al., 2016; Ryan et al.,  
389 2012). It is likely that these aggregates are fairly insoluble, which could be responsible for the  
390 perceived granularity of the reference solutions. When the experimental solutions were created by  
391 combining the isolate fractions, the processes that they underwent (centrifugation and filtration) might  
392 have broken up these aggregates and induced structural changes, resulting in smaller, more soluble  
393 clusters.

394

### 395 3.1.4. Construction of the optimal mixture models

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

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

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

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

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

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

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

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

449

### 450 3.2. Use of the models to better understand sensory perceptions

#### 451 3.2.1. Identification of the fractions underlying beaniness

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

454 The results show that the perception of the attributes cereals and nuts was largely influenced by  
455 retentates a and b (respectively:  $F[14,65] = 512$  and  $F[14,65] = 613$  for cereals;  $F[14,65] = 225$  and  
456  $F[14,65] = 209$  for nuts). The same was true for the attributes almond, potato, and broth (retentates a  
457 and b respectively:  $F[17,62] = 255$  and  $F[17,62] = 271$  for almond;  $F[13,66] = 331$  and  $F[13,66] = 220$   
458 for potato;  $F[15,64] = 929$  and  $F[15,64] = 144$  for broth), which were also affected by permeates a and  
459 b (respectively:  $F[17,62] = 94$  and  $F[17,62] = 148$  for almond;  $F[13,66] = 135$  and  $F[13,66] = 70$  for  
460 potato;  $F[15,64] = 253$  and  $F[15,64] = 95$  for broth). Finally, the perception of the attribute pea was  
461 simultaneously affected by pellets a and b (respectively:  $F[9,76] = 423$  and  $F[9,76] = 441$ ); retentates a  
462 and b (respectively:  $F[9,76] = 370$  and  $F[9,76] = 225$ ); and permeates a and b (respectively:  $F[9,76] =$   
463  $264$  and  $F[9,76] = 419$ ). Retentate a, which had higher hexanal levels, led to more intense potato,  
464 broth, and pea attributes.

465 Dry matter content was similar among the fractions. However, pellets and retentates differed in their  
466 main protein type: insoluble proteins versus soluble proteins, respectively. In contrast, permeates were  
467 mainly composed of non-proteins, such as sodium, caffeic acid, and hexanal (Figure 1). Thus,  
468 unsurprisingly, the volatile-rich permeates contributed to the perception of the aroma attributes, as  
469 observed in previous studies. Indeed, the beaniness of pulses has been found to be strongly related to  
470 volatile composition and, notably, hexanal levels (Bott & Chambers, 2006; Vara-Ubol et al., 2004).  
471 More recently, Murat et al. (2013) examined the volatile composition of pea isolates and pea flour and  
472 suggested that certain aldehydes, alcohols, and ketones were responsible for beaniness (Murat et al.,  
473 2013). These results were confirmed recently by Bi et al., who demonstrated that six aroma  
474 compounds (including 3-methylbutanoic acid and hexanal) significantly contributed to the  
475 characteristic aroma of peas and that fifteen aroma compounds (including pyrazines and pyranones)  
476 significantly contributed to the characteristic aroma of roasted peas (Bi et al., 2020).

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

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

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

493

### 494 3.2.2. Identification of the fractions underlying mouthfeel, bitterness, and astringency

495 The mixture models were also a useful tool for gaining insight into the origin of the taste and texture  
496 attributes. The results show that perceived mouthfeel intensity mainly depended on pellets a and b  
497 (respectively:  $F[8,72] = 1923$  and  $F[8,72] = 1072$ ). Past work found that texture was relatively  
498 balanced in hydrocolloid solutions due to the high number of factors at play (e.g., hydrocolloid type,  
499 viscosity range, food matrix, choice of sensory evaluation technique) (van Vliet et al., 2009). In this  
500 study, the ratio of dry matter content to protein content was 0.83 for pellets, 0.88 for retentates, and 0.2  
501 for permeates. The difference in texture perception among the fractions was therefore not due to  
502 protein concentration but rather to protein type. Pea protein isolates mainly consist of globulins, which  
503 represent 65–80% of total protein concentration and belong to three major groups (legumin 11S,  
504 vicilin 7S, and convicilin 7S); some albumins are also present (Kimura et al., 2008; Sirtori et al.,  
505 2012). In addition, in commercially produced isolates, proteins tend to be highly denatured and form  
506 large aggregates primarily structured by hydrophobic interactions (Chihi et al., 2016; Oliete et al.,  
507 2018; X. Peng et al., 2017; Ryan et al., 2012). Consequently, the process of creating new food  
508 products from isolate fractions can induce changes in this protein network. Our results suggest that  
509 different types of proteins are present in different concentrations in the pellet and retentate and that the  
510 specific pattern likely depends on protein size, solubility, and hydrophobicity. These compositional  
511 differences are probably responsible for the differences in perceived texture.

512 Perceived astringency mainly depended on retentates a and b (respectively:  $F[14,65] = 721$  and  
513  $F[14,65] = 1001$ ) but also on pellet b ( $F[14,65] = 776$ ). Past research has indicated that the perceived  
514 astringency of foods and beverages is mainly due to the composition of phenolics, namely monomeric  
515 and polymeric phenols, such as flavan-3-ols, as has been described in wine (Damodaran & Arora,  
516 2013; Hufnagel & Hofmann, 2008; Peleg et al., 1999). Here, perceived bitterness was influenced by  
517 retentates a and b (respectively:  $F[13,66] = 582$  and  $F[13,66] = 693$ ). Like astringency, bitterness has  
518 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:  $F[14,65] =$   
532  $584$  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



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

560 The specific nature of these recommendations will depend on food type, which will, in turn, determine  
561 protein concentration and functionality, matrix type, and ingredient choice. Indeed, pea protein isolates  
562 are used in different applications, for which protein concentrations vary widely (from < 1% to > 50%,  
563 with a median of 5%). For example, they are used in sports nutrition and to replace casein and whey  
564 proteins in fermented and unfermented dairy products (Akin & Ozcan, 2017; Ben-Harb et al., 2020;  
565 Korhonen & Pihlanto, 2006; Panesar, 2011; Schindler et al., 2012); they can serve as substitutes for  
566 egg proteins (Hoang, 2012); they can help enrich protein levels in baked foods, cereals, and snacks  
567 (Philipp et al., 2017); and they can improve the cooking yield, water/fat binding, and sliceability of  
568 meat, fish, processed foods, soups, and sauces (Baugreet et al., 2016; Tahmasebi et al., 2016). They  
569 are also emerging as an alternative ingredient in specialized foods, such as gluten-free products  
570 (Mariotti et al., 2009; Miñarro et al., 2012) and infant formula (Le Roux et al., 2020). The results of  
571 this study can help inform product formulation. For example, to improve the aroma of a product  
572 containing 3% pea protein, a mixture of pellet b (25%) and water (75%) would seem to be ideal  
573 (Figure 3). In such a product, undesirable aromas would be relatively less intense (broth score of  
574 1.4/10, pea score of 2.4/10, and potato score of 1.2), while desirable aromas would be relatively more  
575 intense (almond score of 4.2/10, cereals score of 3.9/10, and nuts score of 2.9/10). To provide another  
576 example, it might be helpful to decrease the bitterness and astringency of a flavored product  
577 containing 3% pea protein; in this context, a mixture containing 74% permeate b and 26% pellet a  
578 could be useful (Figure 3). This formulation should result in less intense bitterness (score of 1.6/10)  
579 and astringency (score of 1.7/10).

580 Here, we discuss using customized combinations of isolate fractions as a strategy for reducing the off-  
581 notes of pea-protein-based products. Past research has identified several other strategies (see the  
582 review Roland et al., 2017). First, some approaches attempt to prevent the formation of certain  
583 contributing precursors (e.g., LOX, isoflavones) via cultivar selection (Stephany et al., 2015) or heat  
584 treatments (which limit oxidation; Azarnia et al., 2011). Other approaches try to remove or modify off-  
585 notes via soaking or heat treatments (Curti et al., 2018; Peng et al., 2017), by influencing germination  
586 (Simons & Hall, 2018), or by solvent-based extraction (Heng, 2005). However, such strategies often  
587 lead to a loss in functionality, which is a major drawback. Other approaches more selectively target  
588 off-notes using ultrasound technology (Miano et al., 2019), radio frequency treatments (Jiang et al.,  
589 2018), or enzyme treatments (Liu et al., 2017). In particular, fermentation can change the volatile  
590 profiles of foods (Ben-Harb et al., 2020; El Youssef et al., 2020; Meinschmidt et al., 2016; Schindler  
591 et al., 2012). Another strategy focuses on protein-bound precursors and aims to form inclusion  
592 complexes with  $\beta$ -cyclodextrin (Damodaran & Arora, 2013). Filtration can also limit the presence of

593 compounds responsible for off-notes (Roozen & Pilnik, 1979; H. Yu et al., 2017). The last strategy  
594 involves masking off-notes by adding sugars, salts, acids, or flavoring (Bertelsen et al., 2018; Heng,  
595 2005; Zha et al., 2019). The new strategy described in this study can serve as a complement to these  
596 other techniques for improving the flavor of pea-protein-based foods.

597

#### 598 4. CONCLUSION AND PERSPECTIVES

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

623

#### 624 CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

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

629

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635

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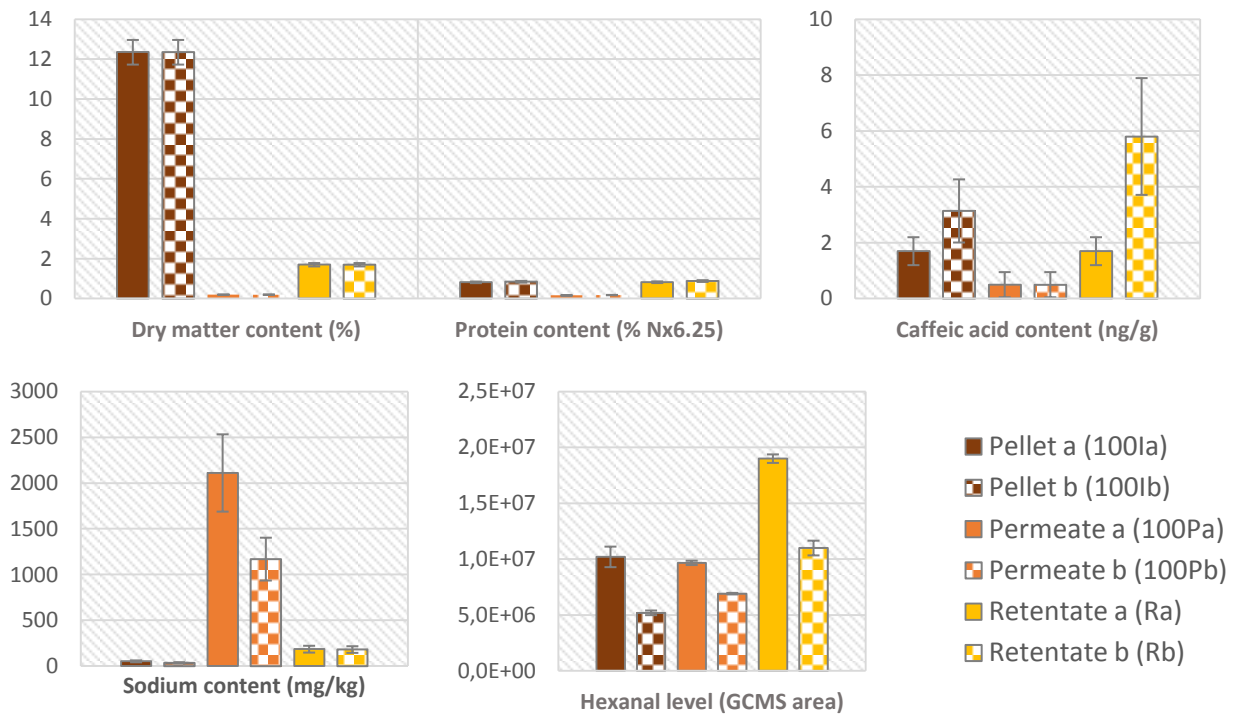
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1011

1012 FIGURE

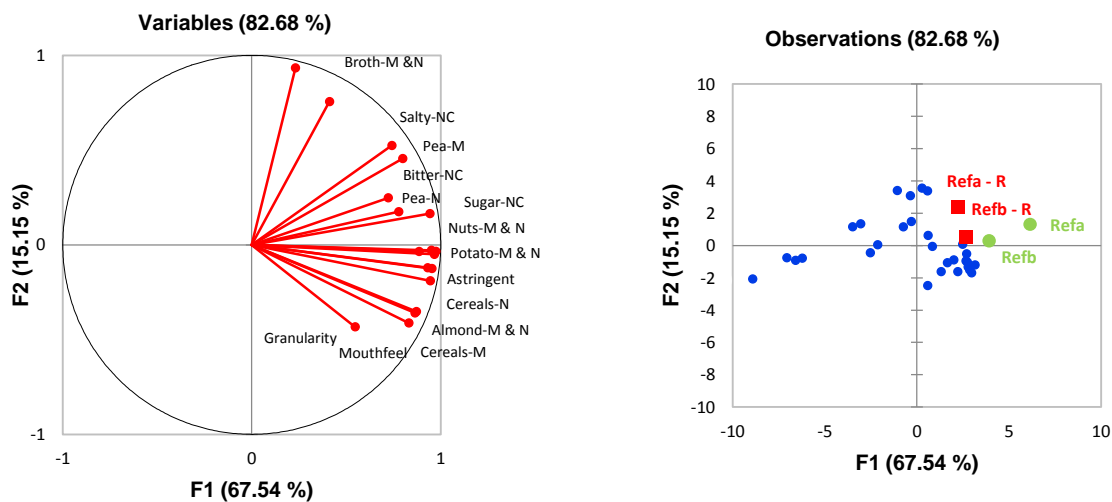
1013





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).

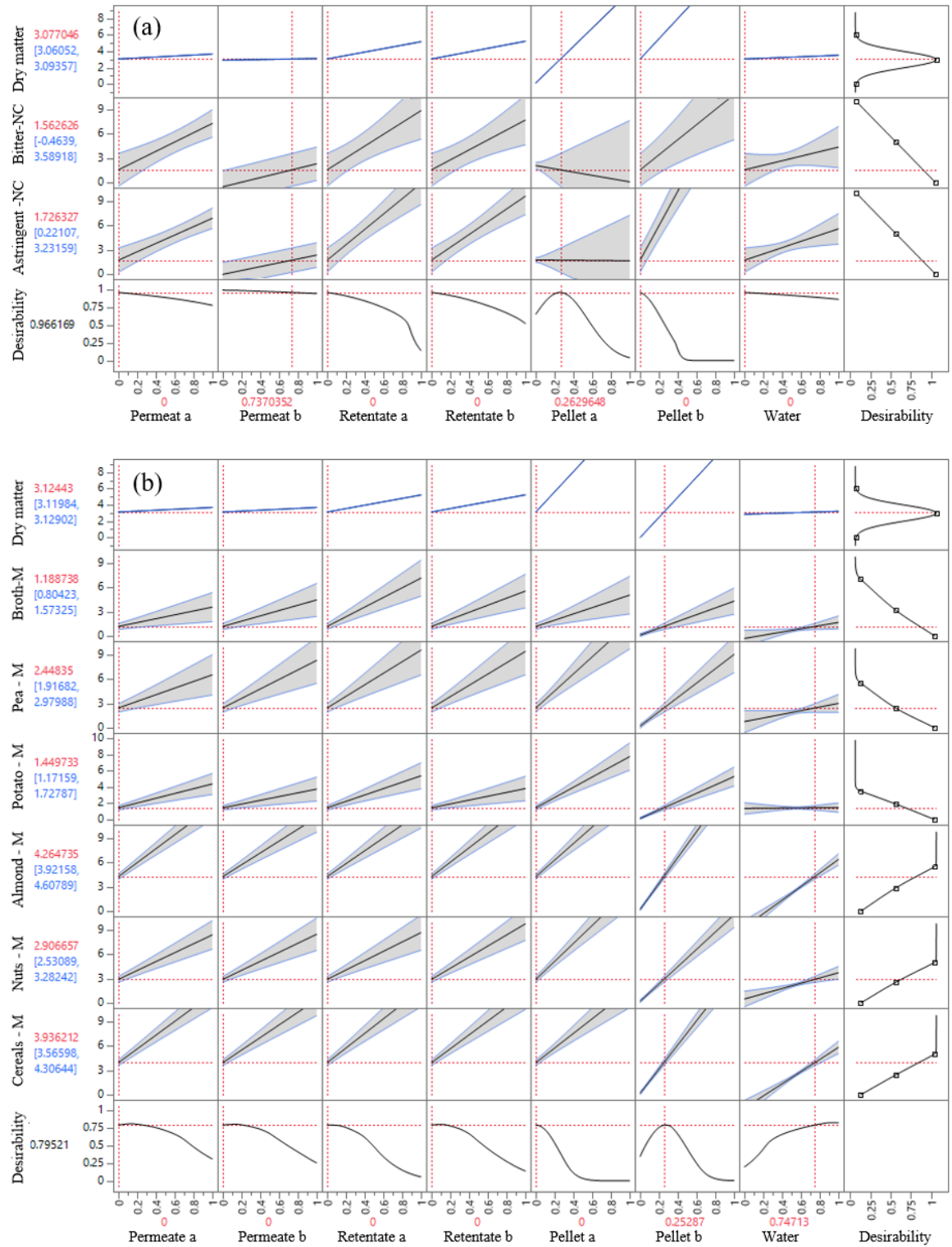
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1019 Figure 2: Results of the principal component analysis (PCA) examining the solutions' sensory profiles.  
 1020 On the left is a loading plot showing the correlational relationships between PCA axes 1 and 2 and the  
 1021 sensory attribute values 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).

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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-

1033 axes). The horizontal red lines correspond to the mean predicted scores based on the current factor  
 1034 values (also indicated to the left of the y-axes [95% confidence intervals in blue]). The confidence  
 1035 intervals are represented in gray on the plots. Overall solution desirability is shown in the last plot row  
 1036 and column. It was defined as the geometric mean of the desirability functions for the individual  
 1037 responses.

1038

1039 TABLE

1040 Table 1: Composition of the different solutions used in this study, which were created by mixing  
 1041 permeates a and b, retentates a and b, and pellets a and b. In bold are the solutions that were replicated.

1042 In italics are the supplementary solutions used for validation purposes.

1043

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

<i>P</i> <sub>46</sub> ( <i>Refb-R</i> )	0	40	0	36	0	24	0	3.70	9
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1044  
1045 Table 2: Assessment of panelist performance in scoring the intensities of the six aroma attributes  
1046 evaluated by nose (N), the six taste attributes evaluated in mouth with the nose clip (NC), and the six  
1047 aroma attributes evaluated in mouth (M) for the range of solutions used in the study; solution  
1048 evaluation employed a block protocol. In the three-way ANOVA, the fixed factors were solution ID,  
1049 panelist ID, replicate ID, and their first-order interactions. F: Fisher statistic for the fixed effects.  
1050 Pvalue: p-value for the Fisher test. Significant p-values (threshold of 0.05) are in bold. Model degrees  
1051 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

1052  
1053 Table 3: Assessment of panelist performance in scoring the intensities of the six aroma attributes  
1054 evaluated by nose (N), the six taste attributes evaluated in mouth with the nose clip (NC), and the six  
1055 aroma attributes evaluated in mouth (M) for the main reference solution. In the two-way ANOVA, the  
1056 fixed factors were panelist ID, sensory session ID, and their interaction. F value: Fisher statistic for the  
1057 fixed effects. Pvalue: p-value for the Fisher test. Significant p-values (threshold of 0.05) are in bold.  
1058 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

1059

1060 Table 4: Performance of the optimal mixture models as assessed via ANOVAs; lack-of-fit tests; and  
 1061 the coefficients of determination (R<sup>2</sup>). F: Fisher statistic for the fixed effects. Pvalue: p-value for the  
 1062 Fisher test. DF: degrees of freedom. Significant p-values (threshold of 0.05) are in bold.

1063

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

1064

1065 Table 5: Significant effects identified using a backward elimination procedure (p-value <0.05; DF for  
 1066 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				
Permeate 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
Blocking factor							6	0												-2
Permeate b*Retentate b																				9
Permeate b*Retentate a			10	5			26	7					7	4			8	3	6	3
Permeate a*Retentate a							8	3												

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1068 Table 6: Observed and predicted attribute scores with the 95% confidence intervals (CIs) for the six  
 1069 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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