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

3

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20 **Abstract**

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

27 To this end, a mixture design was used. First, we obtained three fractions (the pellet,
28 permeate, and retentate) from two pea protein isolates, resulting in a total of six fractions.
29 Second, we used various combinations of the six fractions to create a set of 46 pea-protein-
30 based solutions via various processes (solubilization, centrifugation, filtration, and mixing).
31 Each fraction was specifically representative of the following constituent groups: insoluble
32 proteins (the pellet); soluble compounds, such as volatiles, peptides, and phenolics (the
33 permeate); and soluble proteins interacting with volatiles (the retentate). Factor levels were

34 chosen with two aims: to explore the widest possible range of combinations and to
35 realistically represent protein concentrations so as to build optimal mixture models. Third, 17
36 trained panelists were asked to score the attributes of the solutions using sensory profiling.
37 Model performance was assessed using analysis of variance; results were significant for 18/18
38 attributes, and there was no significant lack-of-fit for 17/18 attributes. It was also assessed
39 using the results of trials conducted with six supplementary solutions. These results clarified
40 the origin of the perceived beany, bitter, and astringent notes. Beaniness was mainly
41 influenced by the retentate and permeate fractions and was strongly affected by hexanal
42 levels. Bitterness was mainly influenced by the retentate fraction, whereas astringency was
43 influenced by the retentate and pellet fractions. Additionally, perception of these latter two
44 attributes was affected by caffeic acid levels.

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

49

50 **Keywords**

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

52

53 **Main text**

54 **1. Introduction**

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

63 Industrial pea-protein ingredients are traditionally generated via a several-step wet process.

64 Pea seeds are solubilized in an alkaline solution, which is then centrifuged to remove
65 insoluble compounds; the precipitate is obtained at the isoelectric point using acidification
66 and centrifugation. The resulting isolate has a protein content of 80–90% (mainly globulins),
67 but also contains lipids, sugars, salts, and other small compounds (e.g., phenolics), which are

68 the products of seed metabolism (Schutyser et al., 2015). The isolate can also serve as an
69 ingredient in the formulation of many food products, including dietary supplements, bakery
70 and confectionery products, beverages, yogurts, ice creams, meat products, and meat and
71 dairy alternatives.

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

79 Research in this area has often focused on the perception of beaniness, which is a complex
80 flavor associated with bean products (Bott & Chambers, 2006). It results from the intricate
81 composition of the volatile aroma compounds found in pulses; present at the highest
82 concentrations is hexanal, whose occurrence is linked to the green notes of peas (Murat et al.,
83 2013). Bitterness arises from the interaction of bitter compounds (e.g., amino acids, peptides,
84 phenolics, complex heterosides) with the TAS2R family of receptors, which are found on the
85 apical membranes of taste receptor cells (Maehashi et al., 2008; Meyerhof et al., 2010). For
86 example, the caffeic acid in coffee and other plant products generates an intense sensation of
87 bitterness (Frank et al., 2007; Streit et al., 2007). Astringency is produced by “the complex
88 sensations due to shrinking, drawing, or puckering of the epithelium” and results from
89 interactions between phenolics and saliva proteins (ASTM, 1991; Gibbins & Carpenter,
90 2013). From an industrial and scientific point of view, it has proven extremely challenging to
91 clarify how pea-based ingredients give rise to these sensory attributes.

92 Research on the perception of pea-based products has largely focused on the role of volatile
93 aroma compounds in creating sensations of beaniness (Azarnia et al., 2011; Ben-Harb et al.,
94 2020; Bi et al., 2020; Bott & Chambers, 2006; El Youssef et al., 2020; Murat et al., 2013;
95 Mutarutwa et al., 2018; Schindler et al., 2012; Trikusuma et al., 2020; Wang & Arntfield,
96 2015; Xu et al., 2019; Xu et al., 2020). A few studies have exclusively examined the peptides
97 that could be related to bitterness (Akin & Ozcan, 2017; Jakubczyk et al., 2013; Pownall et
98 al., 2010; Sirtori et al., 2012); the phenolics related to bitterness and astringency (Bucalossi et
99 al., 2020; Guo et al., 2019; Padhi et al., 2017); and the saponins related to bitterness (Daveby
100 et al., 1998; Heng et al., 2006; Heng et al., 2006; Price & Fenwick, 1984). However, to our
101 knowledge, no study to date has used a more global approach to examine how the complex

102 perception of pea protein isolates arises from both volatile and non-volatile compounds and
103 their potential interactions.

104 Several research strategies have been used to understand how complex products are perceived
105 and to account for the interactions between matrix molecules. Omission testing is commonly
106 used to estimate the effect of specific compounds on the sensory characteristics of products
107 (Engel et al., 2002; Stevens, 1997). Thanks to this technique, aroma models have been built
108 that reconstitute complex odors—such as those of different types of wine, olive oil, cheese,
109 boiled beef, coffee, and whey protein—using only a small fraction of the great number of
110 volatiles occurring in these foods (Czerny et al., 1999; Dinnella et al., 2012; Ferreira et al.,
111 2002; Grosch, 2001; Whitson et al., 2010). However, in addition to being very time
112 consuming, these experiments are less effective when volatile compounds are included in
113 mixtures because the volatiles interact with other ingredients. Indeed, the ability of volatile
114 compounds to modify how something tastes depends on both their relative concentrations and
115 their interactions within the food matrix (Guichard, 2002). Mixing congruous volatiles and
116 taste stimuli can enhance taste intensity, while mixing incongruous stimuli can suppress taste
117 intensity (Caporale et al., 2004; Pfeiffer et al., 2006; Stevenson, 1999). Omission testing has
118 also been used in tandem with gel permeation chromatography to study the water-soluble
119 fraction of peptides found in cheese (Andersen et al., 2010; E. Engel et al., 2000; Engel et al.,
120 2002; Gómez-Ruiz et al., 2007; Molina et al., 1999; Salles et al., 1995; Toelstede & Hofmann,
121 2008). The compounds in pea protein isolates that are potentially responsible for sensory
122 attributes (e.g., peptides, phenolics, salts) are very complex and challenging to purify and
123 identify. Moreover, most analytical techniques require the use of non-food-grade solvents or
124 buffers that are difficult to handle and that can pose problems if the extracts are to be used in
125 sensory evaluations.

126 Studies have shown that attribute perception may be similar for a complex product and a
127 fraction-based reconstruction of the product. For example, artificial ikura (Japanese salmon
128 caviar) was prepared using vegetable oil and a low-calorie natural gel (e.g., one made with
129 alginic acid) (Hayashi et al., 1990); each component of the food was then analyzed using
130 chemical and sensory methods. Based on the analytical data, a synthetic ikura was
131 reconstituted using pure reagents. There were very few sensory differences in the taste
132 profiles between the reference food and the reconstructed food (Hayashi et al., 1990). In
133 another study (Niimi et al., 2014), a cheese solution was reconstituted using a mixture of
134 sucrose, NaCl, monosodium glutamate, lactic acid, and caffeine that was then adjusted using a
135 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.

157

158 **2. Materials and methods**

159 **2.1. Production of pea protein isolate fractions**

160 Two pea protein isolates (protein content Nx6.25, 83% dry matter) were used; they were
161 called isolate a and b, respectively. The isolates were dispersed in tap water in a tank to obtain
162 a final suspension containing 4% (w/w) dry matter content. This suspension was maintained
163 under agitation for 12 h at 3°C with an external agitator (U-shaped stirrer shafts); it was then
164 centrifuged with two centrifuges (Jouan Kr4i and a Sorvall Lynx 4000 [Thermo Scientific,
165 Waltham, US]; 6000 g, 10 min, 4°C). The supernatant was manually separated from the
166 pellet. The pellet was subsequently diluted with tap water to arrive at a dry matter content of
167 12.35%, which facilitated solution creation. A tangential filtration module (TIA, Bollene,
168 France) was used for the ultrafiltration process. The module employed two ST-3B-1812 PES
169 Synder membranes (46-mil spacer; 10-kDa MWCO). Total membrane surface was 0.67 m².

170 The filtration pilot was equipped with a high-pressure diaphragm pump (Wanner Hydra-Cell
171 G10, Wanner International Ltd, Church Crookham, UK)). The retentate was maintained at
172 13°C throughout filtration. The inlet pressure (P1) was 1.5 bar, the outlet retentate pressure
173 (P2) was 1 bar, and the mean transmembrane pressure ($[P1 + P2]/2$) was 1.25 bar. First,
174 ultrafiltration was used to obtain around 10 L of permeate; then, diafiltration was performed
175 employing the same parameters to partially wash the retentate (one diavolume was used). Six
176 fractions were obtained: permeates a and b, retentates a and b, and pellets a and b.

177

178 **2.2 Characterization of the pea protein isolate fractions**

179 Each fraction was characterized to determine the key pea protein compounds it contained
180 (Figure 1). Nitrogen content was determined via the Kjeldahl method (nitrogen content \times
181 6.25), and dry matter content was determined by a certified external laboratory (SAS
182 IMPROVE, Amien, France) via drying (prepASH@219 analysis system). Sodium content was
183 also determined by a certified external laboratory (SAS QUALTECH, Vandoeuvre-les-Nancy,
184 France) using inductively coupled plasma mass spectrometry. Caffeic acid content was
185 determined using gas chromatography–mass spectrometry (GC-MS) and comparison with an
186 external standard (CAS 331-39-5, grade $\geq 98.0\%$ HPLC, MW 180.16, Sigma Aldrich, Saint-
187 Louis, US). Hexanal levels were determined using GC-MS as per El Youssef et al. (2020).

188

189 **2.3 Mixture design**

190 An optimal mixture design was used to create a wide range of reference and experimental
191 solutions from the fractions (permeates a and b, retentates a and b, and pellets a and b).
192 Response surface models were created and included quadratic terms and first-order
193 interactions. The experimental design was such that there was orthogonality among all the
194 terms, which allowed variable effects to be differentiated from one another. A blocking factor
195 was used to control for the effect of the day on which sensory evaluation took place. The
196 order of solution evaluation within the blocks was fully balanced. Overall, the mixture design
197 had eight independent variables (see Table 1 for the levels), and 10 solutions were replicated.
198 The total number of trials was 40. Variable levels were chosen so as to represent a wide range
199 of variation while remaining realistic in terms of the protein concentrations actually
200 experienced when pea protein isolates are used to create foods.
201 This experiment was designed with a view to minimizing solution number (final solution
202 count: 40), which facilitated solution evaluation. In contrast, a central composite design or a
203 Box-Behnken design would have required ~ 60 and ~ 80 solutions, respectively. Furthermore,

204 we used an optimal design because it is the only design that allows the addition of a blocking
205 factor. This experiment displayed better or equivalent efficiency—with a D-optimal value of
206 13.25% and a G-optimal value of 50.85%—compared to experiments based on other designs.
207 These metrics reflect goodness of fit relative to a hypothetical orthogonal design: the D-
208 optimal value indicates whether the design minimizes the volume of the joint confidence
209 region for the vector of regression coefficients, and the G-optimal value indicates whether the
210 design minimizes the maximum prediction variance over the design region.
211 To validate the model's predictive capacity, six solutions that were not initially included in
212 the design were added to the sensory evaluations (for more details, see Table 1—sensory
213 session ID 9).

214

215 **2.4. Solution creation**

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

230

231 **2.5. Sensory evaluation conditions**

232 We recruited 17 panelists (13 women and 4 men; mean age = 23 years old) based on their
233 interest in participating in a long-term study that required their presence at two evaluation
234 sessions per week for three months. They had already been trained to carry out sensory
235 evaluations of pea products or to use sensory evaluation methods, but they all received
236 additional training for this study. They were not informed of the precise aim of the
237 experiment. They gave their free and informed consent to participate and received

238 compensation for their participation. They were asked to not eat, drink, or smoke for at least 1
239 h prior to any of the sessions (training or experimental). Sensory profiling was carried out in
240 individual booths under white light (the solutions were similar in color) in an air-conditioned
241 room (20°C). To reduce sensation build-up, the following palate-cleansing protocol was used
242 between solutions during the experimental sessions: panelists had to consume an apple slice,
243 drink water, and wait 40 seconds before consuming the subsequent solution (as described in
244 Cosson et al., 2020).

245

246 **2.6. Sensory profiling method**

247 Panelists were asked to assess solutions using the sensory profiling method (with a block
248 protocol) described by Cosson et al. (2020). The objective was to score the intensity of a
249 solution's sensory attributes along an unstructured scale ranging from 0 to 10. To select the
250 attributes, panelists were asked to fill out a check-all-that-apply (CATA) survey. It contained
251 30 attributes, and it was possible for panelists to add more. For our final list, we selected
252 attributes that were cited more than 20% of the time and that allowed significant
253 discrimination among solution types. We also wished to limit total attribute number to avoid
254 panelist fatigue. Panelists were trained to assess the attributes along the unstructured scale
255 using external references. Training took place over 8 sessions that each lasted 45 min.
256 Afterward, panelist performance was evaluated.

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

271

272 **2.7. Statistical analysis of the sensory data**

273 Analyses were performed using XLStat (Addinsoft, 2017, Paris, France) and R (R Core Team,
274 2017). For analyses of an inferential nature, $\alpha = 0.05$ was the threshold for statistical
275 significance. To analyze the sensory profiling results, we carried out a three-way ANOVA.
276 Solution identity (ID), replicate ID, and panelist ID were the fixed factors, and all the first-
277 order interactions were included. To visually explore differences in the results obtained using
278 the classical versus block profiling protocol, we carried out principal component analysis
279 (PCA) on a correlation matrix; the data were averaged across replicates and panelists. To
280 study the possible drift between sessions, we carried out a two-way ANOVA on the data for
281 the reference solution. Panelist ID, sensory session ID, and their interaction were the fixed
282 factors.

283

284 **2.8. Statistical analysis of the mixture design**

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

293

294 **3. Results**

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

305

306 **3.1. Construction of surface response models from the sensory data**

307 **3.1.1. Assessment of panelist performance over the 3-month experiment**

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

313 Reproducibility and homogeneity were examined first. Solution ID was significant for all 18
314 attributes, which indicates that panelists distinguished among solutions. Panelist ID and the
315 interaction between panelist ID and solution ID were also significant for all the attributes.
316 Such interactions are common when sensory attributes are evaluated using unstructured scales
317 and are difficult to control even when panelists have undergone extensive training (Jourjon et
318 al., 2005; Lawless & Malone, 1986). The interaction between replicate ID and solution ID
319 was not significant for 10/18 attributes. Replicate ID was not significant for 11/18 attributes,
320 but the interaction between panelist ID and replicate ID was significant for all 18 attributes.
321 However, the F-values for these interactions were low compared to the F-values for the main
322 effect of solution ID. For example, for the broth-M attribute, $F(39,624) = 54.09$ for solution
323 ID; $F(1,624) = 5.39$ for replicate ID; $F(16,624) = 3.08$ for the panelist-by-replicate interaction;
324 and $F(16,39) = 1.43$ for the solution-by-replicate interaction (model degrees of freedom [DF]
325 = 735, residual DF = 624).

326 The presence of between-session drift was examined by looking at the scores for the reference
327 solution across the entire experiment. To this end, a two-way ANOVA (fixed factors: panelist
328 ID and sensory session ID) was performed using scores for each attribute given to the
329 reference solution (Table 3). Sensory session ID was not significant for any of the attributes
330 except broth-M and granularity-NC: these attributes were assigned slightly higher and slightly
331 lower scores, respectively, during a single session. Although using the reference can make
332 solution preparation more cumbersome, it was important in helping to validate panelist
333 performance. In addition, panelists found the reference useful as they scored the other
334 solutions. In past research, monadic presentation has been found to be faster and less tiring
335 than comparative presentation (Mazzucchelli & Guinard, 1999). However, comparative
336 presentation allows panelists to detect smaller differences among food products and to make
337 more accurate decisions about these relative differences (Mcbride, 2007; A. Saint-Eve et al.,
338 2006). Here, via its use of blocks, the presentation method combined monadic and
339 comparative elements. Consequently, the panelists could base their attribute scoring on both

340 their memories from the training period as well as on the reference, which was always the first
341 solution in the sequence (Hastie & Park, 1986).

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

346

347 **3.1.2. Attribute choice**

348 Plant-protein-based ingredients are often said to be “beany,” a multidimensional and complex
349 descriptor (Bott & Chambers, 2006). Here, the decision was made not to use the term
350 “beany.” Instead, its multiple components were parsed out and expressed via other terms (see
351 Cosson et al., 2020). Thus, six aroma attributes were selected: potato, pea, cereals, broth,
352 almond, and nuts. Plant-protein-based ingredients are also often described as being
353 persistently bitter and astringent (Roland et al., 2017); consequently, bitterness and
354 astringency were included as well. Finally, two taste attributes—salty and sugar—and two
355 texture attributes—mouthfeel and granularity—were also chosen because they have been
356 found to be important in descriptions of food quality and preference (van Vliet et al., 2009).
357 Attribute intensities for the different solutions were investigated using a three-way ANOVA
358 (Table 2). Solution ID was significant for the 18 attributes (model DF: 735; residual DF: 624),
359 which means the solutions had distinct sensory profiles. There were pronounced differences in
360 perceived texture ($F = 261.12$ for granularity and $F = 116.94$ for mouthfeel) and smaller
361 differences in perceived sweetness ($F = 9.20$ for sugar). These results are not surprising. It is
362 easier to describe food products based on texture and taste than on aroma (Kora et al., 2003;
363 Lundgren et al., 1986; Anne Saint-Eve et al., 2011). Furthermore, temporally, they are the
364 first attributes to become dominant in the mouth (Le Calvé et al., 2019; Pineau et al., 2009;
365 Anne Saint-Eve et al., 2011). Additionally, when describing overall preferences and sensory
366 satisfaction, consumers appear to primarily focus on taste and then on texture, paying the least
367 attention to aroma (van Vliet et al., 2009). Finally, since the solutions had very low levels of
368 natural sugar content (and no sugar was added), it was not surprising that sweetness did not
369 greatly contribute to the perceived differences among the solutions. Consequently, this
370 attribute was not included in the statistical model.

371 To build upon these results, PCA was used to visually depict the relationships among solution
372 types and attributes (Figure 2). The solutions were well distributed along axes F1 and F2,
373 which accounted for 82.68% of the variance. Thus, maps based on the first two axes seemed

374 to provide a good-quality projection of the initial multidimensional table, even though some
375 information might have remained hidden in the subsequent axes. The 12 aroma attributes
376 were clustered within one quarter of the correlation circles and thus clearly interacted in
377 multiple ways. Aroma attributes assessed in the mouth and nose were strongly correlated (R^2
378 = 0.86 for pea, R^2 = 0.88 for broth, R^2 = 0.83 for cereals, R^2 = 0.95 for nuts, R^2 = 0.87 for
379 almond, R^2 = 0.89 for potato). These results suggest that panelists assigned similar scores to
380 aroma attributes perceived orthonasally and retronasally and that food processing in the
381 mouth had a minor effect on olfactory perception. Orthonasal odors result from volatile
382 compounds traveling from the external environment and through the nares to the olfactory
383 mucosa, whereas retronasal odors result when volatile compounds travel to the olfactory
384 mucosa after they have been released during food destructurement in the oral cavity (Sun &
385 Halpern, 2005). That said, orthonasal and retronasal responses are often similar, except in
386 cases where there are physicochemical or sensory interactions induced by texture, taste, or in-
387 mouth food destructurement (Goldberg et al., 2018). Furthermore, results for the attributes pea-
388 M and salty-NC were also correlated (R^2 = 0.87), which suggests possible congruency
389 (Oladokun et al., 2017). Consequently, our results indicate that there may have been limited
390 interactions between texture, taste, and flavor (except in the case of the attributes pea and
391 salty) and that food oral processing had a minimal impact on these attributes. Therefore, the
392 aroma attributes evaluated via the nose were not included in the statistical model.

393 The aroma attributes potato, almond, cereals, and nuts as well as the attributes astringent and
394 mouthfeel were significantly correlated (R^2 range = 0.72–0.98). They were also correlated
395 with the dry matter content (%) of the solutions (R^2 = 0.97 for mouthfeel, R^2 = 0.88 for
396 cereals-M, R^2 = 0.85 for almond-M, R^2 = 0.82 for potato-M, R^2 = 0.81 for nuts-M, and R^2 =
397 0.73 for astringent). These results suggest that the perception of these attributes was mainly
398 driven by dry matter content and, thus, protein concentration. However, dry matter content
399 was not correlated with the perception of the attributes pea and bitter. It is therefore necessary
400 to build a more complex model to understand the origin of these attributes.

401

402 **3.1.3. Validation of the study methodology—fraction-based formulation of solutions**

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

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

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

425

426 **3.1.4. Construction of the optimal mixture models**

427 In past studies, various experimental and statistical methods have been used to explore the
428 sensory perception of food, and the choice of techniques depends on the research question,
429 variable type and number, and food product number (Chapman et al., 2019; Seisonen et al.,
430 2016; P. Yu et al., 2018; Zielinski et al., 2014). While classical approaches such as fractional
431 factorial design and simple regression have been widely used, they may be inadequate for
432 fully describing a complex food. Thus, this study employed optimal mixture models. This
433 approach made it possible to limit solution number, while also minimizing the degree of
434 aliasing to ensure less collinearity among the independent variables (P. Yu et al., 2018).

435 The attribute scoring data were used to develop the optimal mixture models. Model
436 performance was tested using ANOVAs (global model; F-test for significance), lack-of-fit
437 tests (which calculate a pure-error negative log-likelihood by constructing categories for every
438 combination of model effect values in the data), and the coefficients of determination (R^2)
439 (Table 4). The results of the ANOVAs were significant: the F-ratios ranged from 23 to 520,
440 and the p-values were below 0.01. The lack-of-fit tests were not significant for the 10
441 attributes examined, which means that the error for each model was smaller than the pure

442 error associated with replication. Thus, the models developed for each sensory attribute have
443 relevance. Since the R^2 values were between 82 and 96%, a large amount of the variation in
444 the attribute scores was explained, so the models' results could be interpreted with
445 confidence. These results showed a good-quality fit. The model with the best fit was the one
446 for the attribute mouthfeel. This finding is not surprising because past research has found that
447 models relating food product composition and perceived texture often have the greatest
448 explanatory value (Burseg et al., 2009; Cook et al., 2005).

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

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

471 To validate the model's predictive capacity, panelists were also asked to evaluate six
472 supplementary solutions (created with the same fractions as the main experimental solutions
473 but using different fraction concentrations) (Table 1). Although the data for these solutions
474 were located towards the range limits of our main data set, there was overlap between the
475 95% confidence intervals for the observations and predictions in most cases (Table 6).

476 Predictions were least accurate for the solutions P43 and P44 (8% pellet), notably for the
477 attributes salty, bitter, astringent, and pea. The model generated good predictions when
478 interpolating (i.e., predicting data points that would fall within the range of our observed
479 data). However, its predictions were of lower quality in the case of extrapolation (i.e.,
480 predicting data points outside the range of the observed data).
481 However, despite the low degree of collinearity among the independent variables and the
482 incomplete orthogonality of the design, this model has helped clarify the perception of plant-
483 protein-based foods.

484

485 **3.2. Use of the models to better understand sensory perceptions**

486 **3.2.1. Identification of the fractions underlying beaniness**

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

489 The results show that the perception of the attributes cereals and nuts was largely influenced
490 by retentates a and b (respectively: $F[14,65] = 512$ and $F[14,65] = 613$ for cereals; $F[14,65] =$
491 225 and $F[14,65] = 209$ for nuts). The same was true for the attributes almond, potato, and
492 broth (retentates a and b respectively: $F[17,62] = 255$ and $F[17,62] = 271$ for almond;
493 $F[13,66] = 331$ and $F[13,66] = 220$ for potato; $F[15,64] = 929$ and $F[15,64] = 144$ for broth),
494 which were also affected by permeates a and b (respectively: $F[17,62] = 94$ and $F[17,62] =$
495 148 for almond; $F[13,66] = 135$ and $F[13,66] = 70$ for potato; $F[15,64] = 253$ and $F[15,64] =$
496 95 for broth). Finally, the perception of the attribute pea was simultaneously affected by
497 pellets a and b (respectively: $F[9,76] = 423$ and $F[9,76] = 441$); retentates a and b
498 (respectively: $F[9,76] = 370$ and $F[9,76] = 225$); and permeates a and b (respectively: $F[9,76]$
499 $= 264$ and $F[9,76] = 419$). Retentate a, which had higher hexanal levels, led to more intense
500 potato, broth, and pea attributes.

501 Dry matter content was similar among the fractions. However, pellets and retentates differed
502 in their main protein type: insoluble proteins versus soluble proteins, respectively. In contrast,
503 permeates were mainly composed of non-proteins, such as sodium, caffeic acid, and hexanal
504 (Figure 1). Thus, unsurprisingly, the volatile-rich permeates contributed to the perception of
505 the aroma attributes, as observed in previous studies. Indeed, the beaniness of pulses has been
506 found to be strongly related to volatile composition and, notably, hexanal levels (Bott &
507 Chambers, 2006; Vara-Ubol et al., 2004). More recently, Murat et al. (2013) examined the
508 volatile composition of pea isolates and pea flour and suggested that certain aldehydes,
509 alcohols, and ketones were responsible for beaniness (Murat et al., 2013). These results were

510 confirmed recently by Bi et al., who demonstrated that six aroma compounds (including 3-
511 methylbutanoic acid and hexanal) significantly contributed to the characteristic aroma of peas
512 and that fifteen aroma compounds (including pyrazines and pyranones) significantly
513 contributed to the characteristic aroma of roasted peas (Bi et al., 2020).

514 Initially, the influence of the retentates and pellets on beaniness was quite surprising.
515 However, hexanal levels in these fractions were rather high, especially in retentate a (Figure
516 1). Interactions between volatiles and proteins may be playing a role (Houde et al., 2018;
517 Wang & Arntfield, 2015). Indeed, in pea protein isolates, most volatiles are bound to proteins
518 (Kuhn, 2004); for example, 88% of the octanal present may be bound to pea vicilin. These
519 interactions might also be related to protein solubility (Suppavorasatit et al., 2013). As
520 proteins were present at higher concentrations in the retentates and the pellets, interactions
521 between proteins and volatiles could explain the hexanal levels in these fractions and their
522 effect on perceived aroma intensities. In addition, the perception of the attributes almond,
523 broth, and pea may also have been influenced by the composition of peptides and amino
524 acids, which were richer in the retentates. Indeed, Henriksen showed that the bouillon note of
525 dried sausage was related to a mixture of different amino acids and peptides and that the
526 intensity of the potato note was positively correlated with levels of tyrosine (in both its free
527 and peptide residue forms) (Henriksen, 1997).

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

532

533 **3.2.2. Identification of the fractions underlying mouthfeel, bitterness, and astringency**

534 The mixture models were also a useful tool for gaining insight into the origin of the taste and
535 texture attributes. The results show that perceived mouthfeel intensity mainly depended on
536 pellets a and b (respectively: $F[8,72] = 1923$ and $F[8,72] = 1072$). Past work found that
537 texture was relatively balanced in hydrocolloid solutions due to the high number of factors at
538 play (e.g., hydrocolloid type, viscosity range, food matrix, choice of sensory evaluation
539 technique) (van Vliet et al., 2009). In this study, the ratio of dry matter content to protein
540 content was 0.83 for pellets, 0.88 for retentates, and 0.2 for permeates. The difference in
541 texture perception among the fractions was therefore not due to protein concentration but
542 rather to protein type. Pea protein isolates mainly consist of globulins, which represent 65–
543 80% of total protein concentration and belong to three major groups (legumin 11S, vicilin 7S,

544 and convicilin 7S); some albumins are also present (Kimura et al., 2008; Sirtori et al., 2012).
545 In addition, in commercially produced isolates, proteins tend to be highly denatured and form
546 large aggregates primarily structured by hydrophobic interactions (Chihi et al., 2016; Oliete et
547 al., 2018; Peng et al., 2017; Ryan et al., 2012). Consequently, the process of creating new
548 food products from isolate fractions can induce changes in this protein network. Our results
549 suggest that different types of proteins are present in different concentrations in the pellet and
550 retentate and that the specific pattern likely depends on protein size, solubility, and
551 hydrophobicity. These compositional differences are probably responsible for the differences
552 in perceived texture.

553 Perceived astringency mainly depended on retentates a and b (respectively: $F[14,65] = 721$
554 and $F[14,65] = 1001$) but also on pellet b ($F[14,65] = 776$). Past research has indicated that
555 the perceived astringency of foods and beverages is mainly due to the composition of
556 phenolics, namely monomeric and polymeric phenols, such as flavan-3-ols, as has been
557 described in wine (Damodaran & Arora, 2013; Hufnagel & Hofmann, 2008; Peleg et al.,
558 1999). Here, perceived bitterness was influenced by retentates a and b (respectively: $F[13,66]$
559 $= 582$ and $F[13,66] = 693$). Like astringency, bitterness has been found to be influenced by
560 the composition of phenolics, but, additionally, there is an influence of saponins (Heng et al.,
561 2006) and peptides (Aubes-Dufau et al., 1995). We expected phenolics, saponins, and
562 peptides to mainly be present in the permeates (i.e., they are small, soluble molecules).
563 However, it was the retentates and pellets (especially from isolate b) that had higher
564 concentrations of caffeic acid, which is considered to be a marker of phenolic levels (Figure
565 1). In plant-protein-based foods, phenols can bind to proteins via hydrophobic and hydrophilic
566 interactions (Bucalossi et al., 2020; Morton & Murray, 2001; Potter et al., 1993; Zhang et al.,
567 2016). In these interactions, important roles are played by phenol chemical structure, phenol
568 size and composition (including the number of OH groups), and food environment (e.g., pH)
569 (de Freitas & Nuno Mateus, 2012). In this study, the fractions had different pH values (~7.5
570 for the retentates and pellets vs. ~9 for the permeates), which suggests that phenol-protein
571 interactions may have been different as well. Thus, our results suggest that the proteins in the
572 pellets and the retentates interacted with phenolics, leading to differences in perceived
573 astringency and bitterness.

574 Finally, perceived saltiness depended simultaneously on permeates a and b (respectively:
575 $F[14,65] = 584$ and $F[14,65] = 481$) and retentates a and b (respectively: $F[14,65] = 641$ and
576 $F[14,65] = 273$). Solutions made from isolate a had higher sodium contents and were
577 perceived as more salty. Relative to their dry matter content, the permeates and retentates had

578 higher levels of sodium (Figure 1). Previous work has found that both sodium and chloride
579 ions are required to activate the salt receptor (Van Der Klaauw & Smith, 1995). However,
580 when Frankowski et al. (2014) studied the sensory characteristics and composition of
581 permeate obtained from whey ultrafiltration, they showed that, in addition to sodium, both
582 lactic acid and potassium chloride can heighten the intensity of perceived saltiness. Based on
583 past research, we can assume that, compared to the pellets, the permeates and retentates were
584 richer in minerals.

585 Thus, the mixture models provided insight into the origin of the taste and texture attributes.
586 Our results suggest that the protein composition of the pellets and retentates influenced
587 perceived texture. Interactions between proteins and phenolics in the pellets and retentates
588 may have affected perceived astringency. Retentates may also be richer in phenolics,
589 saponins, and peptides, whose presence may have impacted perceived bitterness. Finally, the
590 permeates and retentates may have been richer in salts, heightening perceived saltiness.

591

592 **3.2.3. Optimizing ingredient choice and product formulation**

593 Based on these results, recommendations can be developed to improve the flavor of the pea
594 protein isolates used in plant-protein-based food products. First, attention should be paid to
595 ingredient optimization. Our results suggest that the filtration step was not especially effective
596 in removing the compounds responsible for off-notes. In this regard, the centrifugation step
597 seemed more useful: the pellets were described as less beany, bitter, and astringent than the
598 retentates. Consequently, it could be useful to formulate plant-protein-based products using
599 pellets. However, because pellets consist mainly of insoluble compounds, there might be a
600 loss of functionality. Thus, employing a pellet/retentate mixture could help limit off-notes
601 while retaining functionality. The results for the retentates also highlight the importance of
602 protein conformation and the interactions between both proteins and aromatics as well as
603 proteins and phenolics. These mechanisms appear to play an important role in the sensory
604 perception of pea protein isolates and must be studied further.

605 The specific nature of these recommendations will depend on food type, which will, in turn,
606 determine protein concentration and functionality, matrix type, and ingredient choice. Indeed,
607 pea protein isolates are used in different applications, for which protein concentrations vary
608 widely (from < 1% to > 50%, with a median of 5%). For example, they are used in sports
609 nutrition and to replace casein and whey proteins in fermented and unfermented dairy
610 products (Akin & Ozcan, 2017; Ben-Harb et al., 2020; Korhonen & Pihlanto, 2006; Panesar,
611 2011; Schindler et al., 2012); they can serve as substitutes for egg proteins (Hoang, 2012);

612 they can help enrich protein levels in baked foods, cereals, and snacks (Philipp et al., 2017);
613 and they can improve the cooking yield, water/fat binding, and sliceability of meat, fish,
614 processed foods, soups, and sauces (Baugreet et al., 2016; Tahmasebi et al., 2016). They are
615 also emerging as an alternative ingredient in specialized foods, such as gluten-free products
616 (Mariotti et al., 2009; Miñarro et al., 2012) and infant formula (Le Roux et al., 2020). The
617 results of this study can help inform product formulation. For example, to improve the aroma
618 of a product containing 3% pea protein, a mixture of pellet b (25%) and water (75%) would
619 seem to be ideal (Figure 3). In such a product, undesirable aromas would be relatively less
620 intense (broth score of 1.4/10, pea score of 2.4/10, and potato score of 1.2), while desirable
621 aromas would be relatively more intense (almond score of 4.2/10, cereals score of 3.9/10, and
622 nuts score of 2.9/10). To provide another example, it might be helpful to decrease the
623 bitterness and astringency of a flavored product containing 3% pea protein; in this context, a
624 mixture containing 74% permeate b and 26% pellet a could be useful (Figure 3). This
625 formulation should result in less intense bitterness (score of 1.6/10) and astringency (score of
626 1.7/10).

627 Here, we discuss using customized combinations of isolate fractions as a strategy for reducing
628 the off-notes of pea-protein-based products. Past research has identified several other
629 strategies (see the review Roland et al., 2017). First, some approaches attempt to prevent the
630 formation of certain contributing precursors (e.g., LOX, isoflavones) via cultivar selection
631 (Stephany et al., 2015) or heat treatments (which limit oxidation; Azarnia et al., 2011). Other
632 approaches try to remove or modify off-notes via soaking or heat treatments (Curti et al.,
633 2018; Peng et al., 2017), by influencing germination (Simons & Hall, 2018), or by solvent-
634 based extraction (Heng, 2005). However, such strategies often lead to a loss in functionality,
635 which is a major drawback. Other approaches more selectively target off-notes using
636 ultrasound technology (Miano et al., 2019), radio frequency treatments (Jiang et al., 2018), or
637 enzyme treatments (Liu et al., 2017). In particular, fermentation can change the volatile
638 profiles of foods (Ben-Harb et al., 2020; El Youssef et al., 2020; Meinlschmidt et al., 2016;
639 Schindler et al., 2012). Another strategy focuses on protein-bound precursors and aims to
640 form inclusion complexes with β -cyclodextrin (Damodaran & Arora, 2013). Filtration can
641 also limit the presence of compounds responsible for off-notes (Roozen & Pilnik, 1979; H. Yu
642 et al., 2017). The last strategy involves masking off-notes by adding sugars, salts, acids, or
643 flavoring (Bertelsen et al., 2018; Heng, 2005; Zha et al., 2019). The new strategy described in
644 this study can serve as a complement to these other techniques for improving the flavor of
645 pea-protein-based foods.

646

647 **4. Conclusions**

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

674

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681

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685

686 **Credit authorship contribution statement**

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

691

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1108 **Captions to Figures**

1109

1110 **Figure 1:** Key characteristics of the six pea protein isolate fractions used in the study (pellet a
1111 and b, permeate a and b, retentate a and b): dry matter content (%), protein content (%
1112 N_x6.25), caffeic acid content (ng/g), sodium content (mg/kg), and hexanal levels (GCMS
1113 area).

1114

1115 **Figure 2:** Results of the principal component analysis (PCA) examining the solutions' sensory
1116 profiles. On the left is a loading plot showing the correlational relationships between PCA
1117 axes 1 and 2 and the sensory attribute values in the original dataset. On the right is a PCA plot
1118 with the same two axes that shows the relative similarity of the solutions' sensory profiles. In
1119 green are the active observations corresponding to the raw product (Refa and Refb), in blue
1120 are the others active observations, in red are the supplementary observations corresponding to
1121 the experimental solutions with the same composition as the reference solutions (Refa-R and
1122 Refb-R).

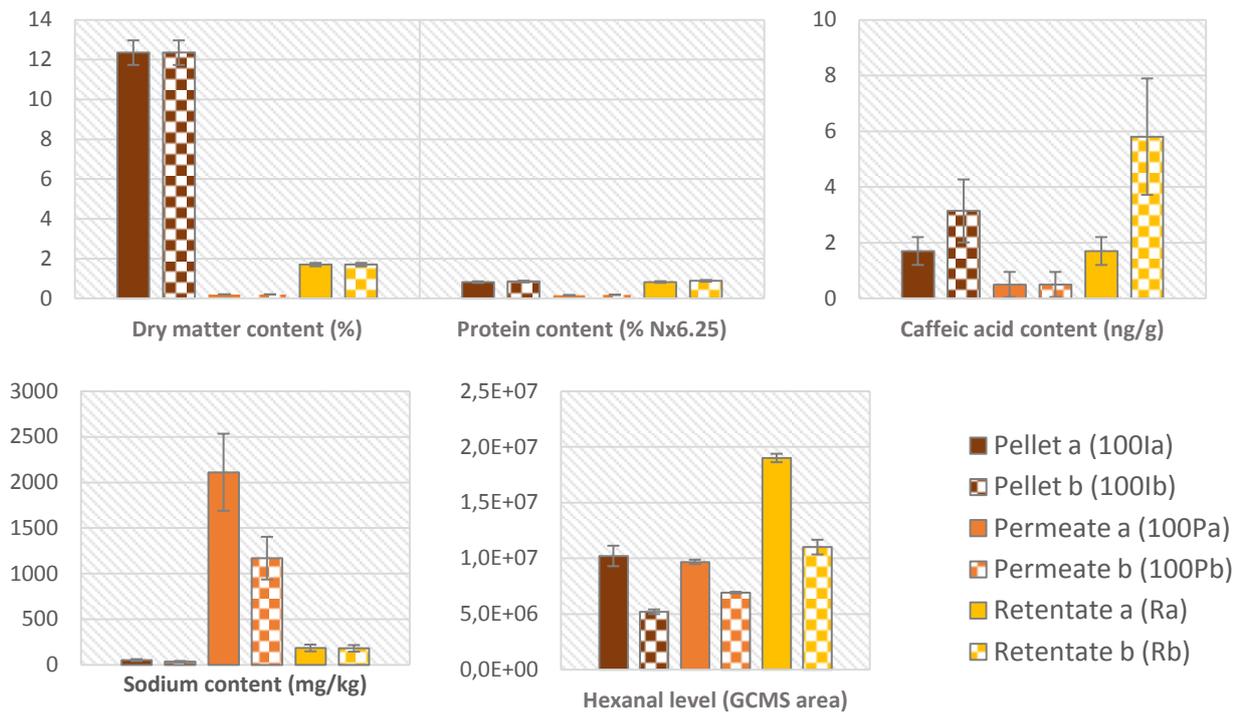
1123

1124 **Figure 3:** Cross-sectional view of the predicted attribute scores as a function of a solution's
1125 fractional composition for a target dry matter content of 3%: (a) solution formulation in which
1126 astringency and bitterness are minimized; (b) solution formulation in which undesirable
1127 attributes (potato, pea, and broth) are minimized, whereas desirable attributes (almonds, nuts,
1128 and cereals) are maximized. The vertical red lines correspond to the current values of the
1129 factors (also indicated in red below the x-axes). The horizontal red lines correspond to the
1130 mean predicted scores based on the current factor values (also indicated to the left of the y-
1131 axes [95% confidence intervals in blue]). The confidence intervals are represented in gray on
1132 the plots. Overall solution desirability is shown in the last plot row and column. It was defined
1133 as the geometric mean of the desirability functions for the individual responses.

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1135

1136 **Figure 1**

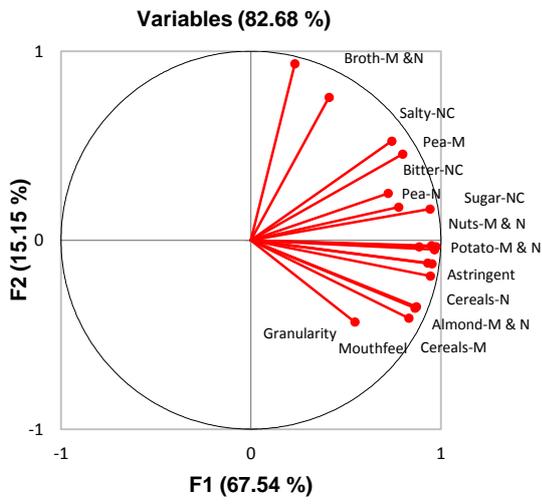


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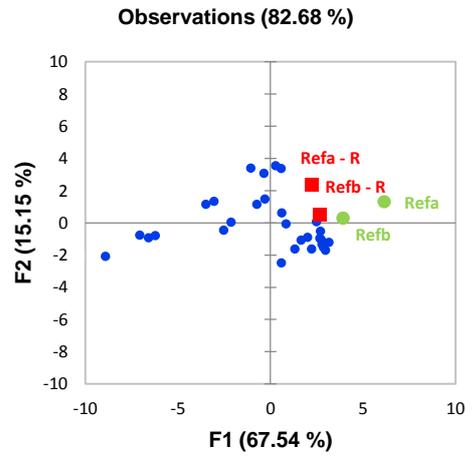
1139 **Figure 2**

1140

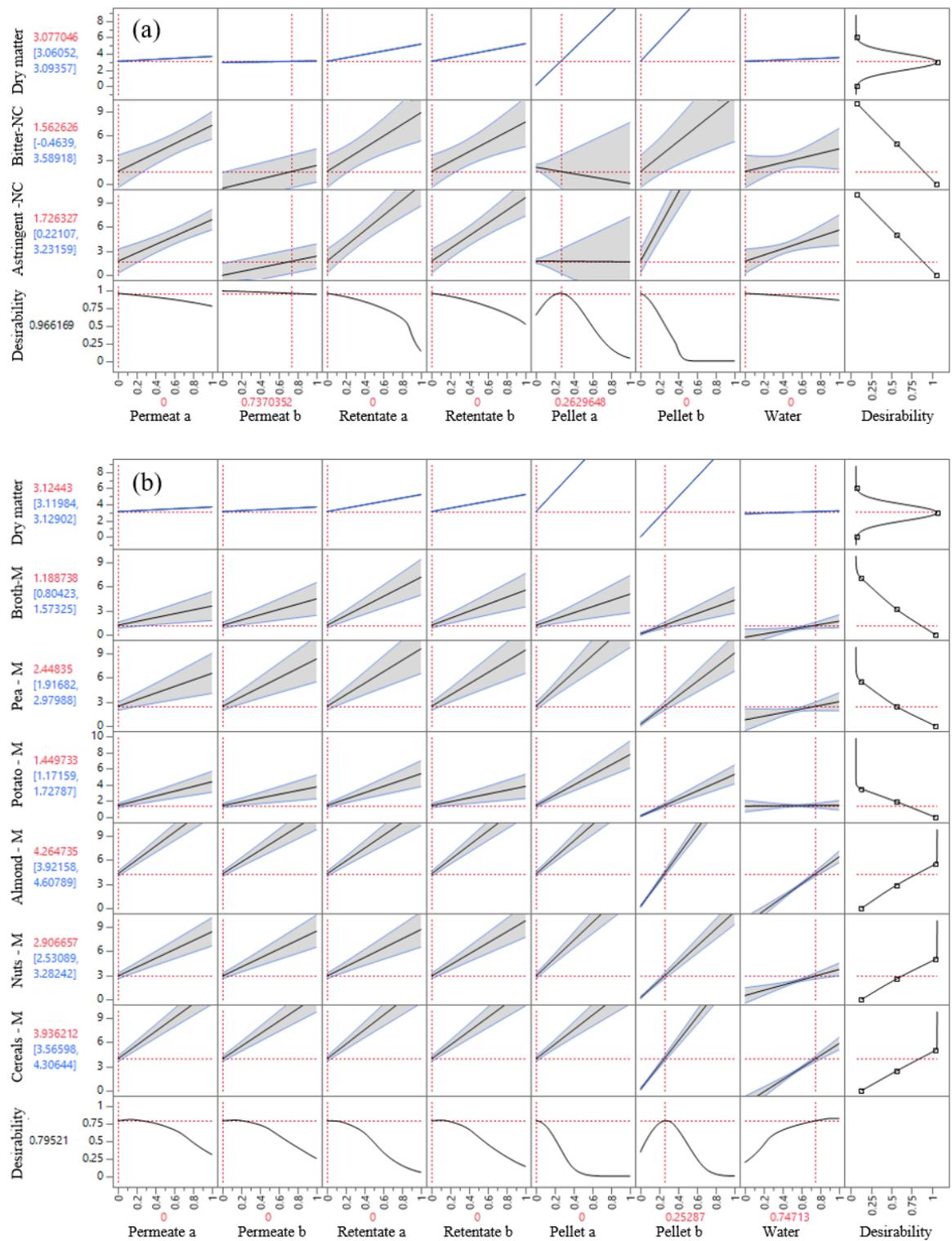


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1143 **Figure 3**



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1147 **Captions to Tables**

1148

1149 **Table 1:** Composition of the different solutions used in this study, which were created by
1150 mixing permeates a and b, retentates a and b, and pellets a and b. In bold are the solutions that
1151 were replicated. In italics are the supplementary solutions used for validation purposes.

1152

1153 **Table 2:** Assessment of panelist performance in scoring the intensities of the six aroma
1154 attributes evaluated by nose (N), the six taste attributes evaluated in mouth with the nose clip
1155 (NC), and the six aroma attributes evaluated in mouth (M) for the range of solutions used in
1156 the study; solution evaluation employed a block protocol. In the three-way ANOVA, the fixed
1157 factors were solution ID, panelist ID, replicate ID, and their first-order interactions. F: Fisher
1158 statistic for the fixed effects. Pvalue: p-value for the Fisher test. Significant p-values
1159 (threshold of 0.05) are in bold. Model degrees of freedom (DF): 735; residual DF: 624.

1160

1161 **Table 3:** Assessment of panelist performance in scoring the intensities of the six aroma
1162 attributes evaluated by nose (N), the six taste attributes evaluated in mouth with the nose clip
1163 (NC), and the six aroma attributes evaluated in mouth (M) for the main reference solution. In
1164 the two-way ANOVA, the fixed factors were panelist ID, sensory session ID, and their
1165 interaction. F value: Fisher statistic for the fixed effects. Pvalue: p-value for the Fisher test.
1166 Significant p-values (threshold of 0.05) are in bold. Model DF: 35; residual DF: 304.

1167

1168 **Table 4:** Performance of the optimal mixture models as assessed via ANOVAs; lack-of-fit
1169 tests; and the coefficients of determination (R²). F: Fisher statistic for the fixed effects.
1170 Pvalue: p-value for the Fisher test. DF: degrees of freedom. Significant p-values (threshold of
1171 0.05) are in bold.

1172

1173 **Table 5:** Significant effects identified using a backward elimination procedure (p-value
1174 <0.05; DF for the effects = 1): F = Fisher statistic for the fixed effects; Est = estimated
1175 coefficient.

1176

1177 **Table 6:** Observed and predicted attribute scores with the 95% confidence intervals (CIs) for
1178 the six validation solutions (two replicates performed).

1179

1180 **Table 1**

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

1181

1182

1183 **Table 2**

	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

1184

1185

1186 **Table 3**

	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

1187

1188

1189 **Table 4**

	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

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1191

1192 **Table 5**

	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 *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												

1193

1194

1195 **Table 6**

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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