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1 **Design of microbial consortia for the fermentation of pea-protein-enriched emulsions**

2

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8 **Abstract**

9 In order to encourage Western populations to increase their consumption of vegetables, we suggest turning
10 legumes into novel, healthy foods by applying an old, previously widespread method of food preservation:
11 fermentation. In the present study, a total of 55 strains from different microbial species (isolated from cheese or
12 plants) were investigated for their ability to:(i) grow on a suspension containing 100% pea proteins and no
13 carbohydrates or on a 50:50 pea: milk protein emulsion containing lactose, (ii) increase aroma quality and reduce
14 sensory off-flavors; and (iii) compete against endogenous micro-organisms. The presence of carbohydrates in the
15 mixed pea: milk emulsion markedly influenced the fermentation by strongly reducing the pH through lactic
16 fermentation, whereas the absence of carbohydrates in the pea emulsion promoted alkaline or neutral fermentation.
17 Lactic acid bacteria assigned to *Lactobacillus plantarum, Lactobacillus rhamnosus, Lactococcus lactis* and
18 *Lactobacillus casei* species grew well in both the pea and pea: milk emulsions. Most of the fungal strains tested
19 (particularly those belonging to the *Mucor* and *Geotrichum* genera) were also able to grow in both emulsions.
20 Although most *Actinobacteria* and *Proteobacteria* did not compete with endogenous microbiota (*Bacillus*), some
21 species such as *Hafnia alvei*, *Acinetobacter johnsonii* and *Glutamicibacter arilaitensis* grew strongly and appeared to
22 restrict the development of the endogenous microbiota when the pea emulsion was inoculated with a combination of
23 three to nine strains. In the mixed emulsions, lactic fermentation inhibited *Actinobacteria* and *Proteobacteria* (e.g.
24 *Brevibacterium casei, Corynebacterium casei, Staphylococcus lentus*) to the greatest extent but also inhibited
25 *Bacillus* (e.g. *Bacillus subtilis* and *Bacillus licheniformis*). Overall, this procedure enabled us to select two microbial
26 consortia able to colonize pea-based products and positively influence the release of volatile compounds by
27 generating a roasted/grilled aroma for the 100% pea emulsion, and a fruity, lactic aroma for the 50:50 pea: milk
28 emulsion. Moreover, the fermentation in the pea-based emulsions reduced the level of hexanal, which otherwise leads
29 to an undesired green pea aroma. Our present results show how the assembly of multiple microbial cultures can help
30 to develop an innovative food product.

31 **Keywords:** legume; aroma profile; bacteria; fungi; microbial assembly.

32 **1. Introduction**

33 Changing diets and demographic growth worldwide are challenging our ability to maintain a sustainable food
34 system. Global demand for meat, dairy and fish products continues to rise, as do the latter's environmental impacts
35 (Aiking, 2011; Pimentel and Pimentel, 2003). It is therefore essential to develop alternative sources of food protein
36 that require less energy and water use. One crucial challenge faced by food researchers is the need to increase the use
37 of sustainable plant proteinsthat have satisfactory nutritional and functional properties (Boland et al., 2013). Legumes
38 provide the dietary fiber as well as the high amounts of protein (18-32%), minerals and vitamins required for human
39 health. Furthermore, legumes possess functional properties such as water retention, fat binding, foaming and gelling –
40 all of which may be of value in the development of a broad variety of food products (Boye et al., 2010). Soy is the
41 most widely used source of plant proteins substituted for animal proteins. However, other sources of plant proteins
42 exist. Thanks to excellent yields, good availability, and cost-effective production, the pea (*Pisum sativum* L.) may
43 have a future as a sustainable human food supply. Furthermore, the food industry can use pea proteins to formulate
44 new food products because of the latter's high nutritive value and non-allergenic nature (Sabate and Soret, 2014).
45 However, the application of pea protein in food is limited by the persistence of green, beany off-flavors that are
46 rejected by consumers. These defects are either intrinsic to the growing plant itself, generated during fractionation of
47 the raw materials, or produced during the food product's final processing (Murat et al., 2013).

48 Fermentation might constitute a means of decreasing pea off-flavors and thus improving levels of consumer
49 acceptance. In fact, fermentation is one of the world's oldest food preservation techniques. By transforming the
50 chemical constituents of raw materials, functional microorganisms thereby enhance the bio-availability of nutrients,
51 enrich the sensory quality of the food, convey biopreservative effects (possibly improving food safety), degrade toxic
52 components and anti-nutritive factors, produce antioxidant and antimicrobial compounds, stimulate probiotic
53 functions, and fortify the food product with health-promoting bioactive compounds (Limón et al., 2015; Steinkraus,
54 2002; Tamang, 2015; Tamang et al., 2009, 2016a,). Successful fermentation mainly rely on the microbial biodiversity
55 and the microbial starter cultures used to induce an appreciated end-product.

56 The use of selected starter cultures is a common means of accelerating and guiding the fermentation process,
57 thus improving the quality of the end-products. A large number of species from three major groups (lactic acid
58 bacteria (LAB), acid-sensitive bacteria and fungi, including both yeasts and molds) have been developed as starters
59 for the fermentation of various foods.

60 LAB (primarly *Streptococcus*, *Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus* and
61 *Weissella* species) play pivotal roles in a broad spectrum of food fermentation processes (Chumchuere et al., 2000;
62 Peyer et al., 2016; Tamang et al., 2016b). One of the most important applications of LAB is their use as starter
63 cultures in the production of fermented dairy products. By fermenting lactose and hydrolyzing protein, the bacteria
64 influence the organoleptic characteristics of the final product. In fermented soy-based food, Meinlschmidt et al.
65 (2016) confirmed that fermentation with the LAB *L. helveticus* can decrease levels of bitter and beany off-flavors.
66 Furthermore, lactic fermentation also acts as a low-cost method for food preservation, since LAB inhibit pathogenic
67 and/or undesirable spoilage microbiota like *Listeria, Clostridium, Staphylococcus* and *Bacillus*. These food
68 preservation activities mainly result from acidification of the matrix, competition for nutrients and the production of
69 antimicrobial compounds such as bacteriocins (Tamang et al., 2009).

70 Acid-sensitive bacteria (including *Actinobacteria* such as *Corynebacterium* and *Firmicutes* such as
71 *Staphylococcus*) have been detected in various fermented foods. These bacteria are able to secrete degradative
72 enzymes and thus produce many volatile organic compounds (VOCs) and other substances from proteolysis and
73 lipolysis (such as peptides, amino acids and free fatty acids) in various fermented foods but most particularly in
74 cheeses and in fermented legumes such as doenjang-meju, kecap and kedong-sufu (Alexandraki et al., 2013; Feng et
75 al., 2013; Irlinger et al., 2015; Jung et al., 2014; 2016; Monnet et al., 2015; Tamang et al., 2016a; 2016b).

76 High levels of fungi may also play a crucial role in the fermentation of plant- and dairy-based foods
77 (Alexandraki et al., 2013; Irlinger et al., 2015; Rani and Soni, 2007). The fermentation of popular soybean foods
78 (such as koji) is based on filamentous fungi - mostly *Aspergillus, Mucor* and *Rhizopus* (Zhu and Tramper, 2013).
79 Yeasts (particularly *Geotrichum* and *Debaryomyces*) have also been detected in soybean foods (such as doenjang,
80 kecap and black lentils (bhallae)) and in cheese. In the later food product, yeasts are known to use various energy

81 sources and to be involved in proteolysis and the production of VOCs (Alexandraki et al., 2013; Monnet et al., 2015;
82 Rani and Soni, 2007; Sridevi et al., 2010).

83 The *Bacillus* group is known to be a strong colonizer in African and Asian fermented plant-based foods and is
84 even essential for the alkaline fermentation of various soy products (Steinkraus, 1997; Tamang et al., 2016b). Thus,
85 *Bacillus subtilis* and *Bacillus licheniformis* are the dominant fermentative organisms in *doenjang-* a traditional,
86 fermented soybean food product (Nam et al., 2012). *Bacillus*-derived enzymes hydrolyze proteins and lipids into
87 easily digestible free amino acids - some of which are then converted into flavors. With a known history of safe use
88 in foods, *Bacillu*s may also be a good producer of antimicrobial agents that act against many pathogenic
89 microorganisms (Compaoré et al., 2013).

90 Alternatively, studies of agave and sausage fermentation have demonstrated that the use of microbial consortia
91 is a promising strategy for controlling the fermentation process and improving flavor characteristics (Garcia-Aguirre
92 et al., 2009; Pérez-Chabela et al., 2013).

93 Little is known about the impact of fermentation - particularly by microbial consortia - on the overall sensory
94 characteristics of pea-protein-based products. In a recent study, ten starter cultures (comprising seven species of
95 *Streptococcus* and *Lactobacillus* LAB) were tested for yogurt production by inoculation into milk containing five
96 ratios of pea proteins, ranging from 0 to 40% (Yousseef et al., 2016). The increase in the pea protein concentration
97 resulted in products with higher acidity, greater syneresis and lower firmness than the reference product (yogurt). In
98 another study, lactic fermentation by *Lactobacillus plantarum* and *Pediococcus pentosaceus* strains improved the
99 aroma of pea protein extracts by decreasing the n-hexanal content and reducing or masking "green-note" off-flavors
100 (Schindler et al., 2012).

101 The primary objective of the present study was to design *de novo* microbial consortia by selecting strains that
102 were representative of diverse phylogenetic groups and that had been isolated from fermented foods or plants. These
103 strains then had to (i) colonize pea-protein-based products, and (ii) produce VOCs that helped to reduce or mask the
104 pea proteins' off-flavors. Two types of pea-based product were studied: a pure (100%) pea protein emulsion and a
105 mixed emulsion containing a 1:1 ratio of pea proteins to milk proteins. Fifty-five microorganisms isolated from dairy

106 or vegetable products were used alone or in combination to inoculate these suspensions. Total counts, microbial
107 diversity and sensory analyses were carried out in order to evaluate the changes in sensory perception and the
108 microbial composition of the various fermented emulsions. Lastly, VOCs produced by fermentation with two selected
109 microbial consortia were analyzed using gas chromatography-mass spectrometry (GC-MS) in order to characterize
110 changes in the suspension's aromatic composition corresponding to a decrease in the green note perceived by
111 panelists.

112 **2. Materials and Methods**

113 **2.1. Ingredients/raw materials**

114 Pea protein isolates (NUTRALYS® S85F; 69.8% of pea protein) were provided by Roquette Frères (Lestrem,
115 France). Skim milk powder (35.1% of milk protein; 56% of lactose) was purchased from Lactalis (Bourgbarré,
116 France), and rapeseed oil (Fleur de Colza, Lesieur, France) was purchased from a local supermarket.

117 **2.2. Preparation of the two pea protein emulsions**

118 Two types of emulsions were prepared under the same conditions with 10% (w/w) proteins. The first
119 contained 100% pea proteins (the pea emulsion, PE) and the second contained a 50:50 mixture of pea and milk
120 proteins (the mixed emulsion, ME) (**Table 1**). For both emulsions, proteins were first dispersed in a 1% NaCl
121 solution and stirred at room temperature for 1 h. For the ME, the suspensions of pea and milk proteins were mixed
122 together and stirred for 1 h. Fat was then added to each suspension, and the emulsion was homogenized with a rotor-
123 stator homogenizer (IKA©T25 Digital Ultra-Turrax) and an ultrasonic homogenizer (Branson-250/450 Sonifier). The
124 PE and ME preparations were subjected to tyndallization (successive heat treatments) in order to inactivate spores
125 (**Figure 1**) followed by another ultrasound decontamination step (ultrasound step 1). This process was required to
126 decrease the number of environmental *Bacillus*-type spore-forming bacteria to below 10 CFU/g (data not shown).

127 **2.3. Strains and inoculum preparation**

128 Fifty-five microbial strains, sourced from international and private collections (GMPA, Grignon, France, and
129 the LUBEM Laboratory, Brest, France) and isolated from dairy and vegetable products, were used in this study

130 (**Tables 2 and 3**). They are assigned to different phylogenetic groups (*Actinobacteria*, *Firmicutes*, *Proteobacteria*,
131 molds and yeasts) and are representative of the following genera: *Glutamicibacter*, *Brachybacterium*,
132 *Brevibacterium*, *Citricoccus*, *Corynebacterium*, *Microbacterium*, *Acinetobacter*, *Advenella*, *Alcaligenes*, *Hafnia*,
133 *Halomonas*, *Psychrobacter*, *Bacillus*, *Staphylococcus*, *Carnobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*,
134 *Streptococcus*, *Weissella*, *Mucor*, *Penicillium*, *Rhizopus*, *Candida*, *Debaryomyces*, *Geotrichum*, *Kluyveromyces*,
135 *Pichia*, *Saccharomyces*, *Yarrowia* and *Zyggosaccharomyces*.

136 Strains were cultured separately at 28 °C for 48 to 72 h on the following broth media: potato dextrose broth
137 (PDB) for yeasts and filamentous fungi, Man Rogosa and Sharpe (MRS) for *Lactobacillus, Lactococcus* and M17 for
138 *Streptococcus* strains, and brain heart infusion (BHI) for other bacteria. When the stationary phase of growth was
139 reached, cells were harvested by centrifugation (5000 x g, 10 min, 4 °C), washed in sterile physiological saline (NaCl
140 9 g/l) and re-suspended into sterile physiological saline at a cell density of 8.0 $\log_{10}$CFU/ml.

141 **2.4. Experimental design of pea and mixed emulsion fermentations**

142 Pea and mixed emulsions were mixed with physiological saline containing microbial solutions in order to
143 achieve an initial cell density of 6.0 $\log_{10}$CFU/g for each bacterium and 4.0 $\log_{10}$CFU/g for each fungus. After
144 incubation at 28 °C for 72 h, the fermented emulsions were compared with each other and with the control emulsion
145 (i.e., without microbial inoculum) obtained under the same conditions. All samples were stored at -20°C before
146 amplicon sequencing analysis, whereas sensory and microbiological analyses were carried out immediately.

147 The study was performed in two parts, four months apart. In the first part of the study, 55 strains were
148 evaluated for their ability to grow in the pea emulsions containing 100% pea proteins (PE) and mixed emulsions
149 containing a 50:50 mixture of pea and milk proteins (ME), and were characterized in sensory analyses. All strains
150 were individually tested in PE and in ME. Furthermore, 70 combinations (composed of three, six or nine strains) were
151 assembled and inoculated in both PE and ME. To do this, the 55 strains were grouped into three groups, i.e. Eukarya
152 (G3), *Firmicutes* (G1), and an acid-sensitive (*Actinobacteria*/*Proteobacteria*) group (G2). Three strains per group
153 were then randomly selected to build consortia containing either three strains (G1 or G2 or G3), six strains (G1+G2

154 or G1+G3 or G2+G3) or nine strains (G1+G2+G3), respectively. For each consortium size, ten combinations
155 (reshuffled replicates) were tested. The experimental design is summarized in Supplementary Table S1. Twenty
156 monocultures were replicated at least twice to check the fermentations' reproducibility.

157 In the second part of the study, 20 microbial combinations (comprising 14 fungal strains assigned to 11
158 species, nine strains assigned to nine *Firmicutes* species and eight strains assigned to three *Proteobacteria* species
159 and five *Actinobacteria* species respectively) were selected from the first step on the basis of their ability to grow on
160 at least one emulsion and to produce noticeable aromatic notes (Supplementary **Table S2**). All fermentations in this
161 second step were performed in triplicate.

162 **2.5. Microbial analyses, cell counting and pH measurements**

163 The fermented PE and ME preparations were homogenized, and approximatively 1 g per sample was
164 transferred to a sterile container. The sample was diluted 1:10 with sterile saline solution (8.5 g/l NaCl) and the
165 mixture was homogenized with an Ultra Turrax®device (Labortechnik, Germany) at 8000 rpm for 1 min. Total
166 bacteria (except LAB) were counted by surface plating in duplicate on BHI agar supplemented with 50 mg/l
167 amphotericin B after five days of incubation at 25°C. The fungal population was determined by surface plating in
168 duplicate using yeast-glucose-chloramphenicol agar (YGCA) supplemented with 0.01 g/l tetrazolium chloride (TTC)
169 after three days of incubation at 25 °C. Lactic acid bacteria were counted by surface plating in duplicate on Man
170 Rogosa and Sharpe (MRS) agar after two days of incubation at 30 °C. Each fungal species had a distinct morphotype
171 on YGCA supplemented with TTC, which allowed them to be quantified directly.

172 pH values were the arithmetic means of three measurements using a BlueLine 27 surface electrode (Schott).

173 **2.6. Metabarcoding analysis of theV3-V4 regions of the bacterial 16S rRNA gene**

174 First, DNA was extracted from 0.250 g of each fermented solution using the previously specified bead-
175 beating-based protocol (Monnet et al., 2006). The DNA concentration was determined with a Qubit fluorometer
176 (Thermo Fisher Scientific, USA), using a Broad Range DNA assay kit.

177 The PCR amplification, library preparation and sequencing were performed at the GeTPlaGe facility
178 (Toulouse, France), as previously described (Dugat-Bony et al., 2016).

179 Paired-end reads were merged using Flash (Magoč and Salzberg, 2011) and analyzed using FROGS (Escudié
180 et al., 2017), according to the standard operating procedure. Briefly, operational taxonomic units (OTUs) were built
181 using Swarm with an aggregation distance of 3 (Mahé et al., 2014), and each OTU accounting for less than 0.005% of
182 the total set of sequences was discarded, as recommended by Bokulich et al. (2013). Lastly, the OTU's affiliation was
183 checked using the EzBiocloud database (Yoon et al., 2017).

184 **2.7. Sensory analysis**

185 The non-fermented and fermented emulsions were characterized in an orthonasal sensory analysis. A
186 descriptive analysis was performed by a panel of 20 semi-trained judges (age range: 22 to 45), according to the
187 “check-all-that-apply”(CATA) method. All panelists were familiar with sensory analysis in general but had not been
188 specifically trained to evaluate pea products. The attributes listed in the CATA questionnaire were selected from the
189 literature and in preliminary testing. A total of 54 descriptors were thus selected and organized into seven classes
190 (Supplementary **Table S3**). In the first step, 10 sessions were performed and 36 samples were analyzed by each judge
191 in each session, resulting in a total of 360 samples tested. In the second step, two sessions were performed and 11
192 samples (ten microbial combinations and one control sample) were analyzed by each judge in each session. For
193 CATA testing, the panelist ticked the corresponding box in the questionnaire when he/she recognized the attribute in
194 the smell of the test product. The samples were blind-labeled with a three-digit number and the order of sample
195 presentation was randomized between subjects and sessions. All sensory analyses were carried out in individual
196 booths in an air-conditioned room at 20°C, under white light. All samples were analyzed in triplicate.

197 Sensory sessions were analyzed using Fizz software (version 2.47A, Biosystemes, France). To analyze the
198 CATA data, a Cochran test was performed to highlight any significant differences ($p<0.05$) between products.
199 Furthermore, a correspondence analysis was used to represent samples and aromatic descriptors.

200 **2.8. Extraction and identification of volatile flavor components from fermented emulsions**

201 To identify VOCs, the V10, M7, VT and MT emulsion samples were subjected to GC/MS analysis. The
202 selection of the two fermented emulsions (V10 and M7) was based on the following criteria: (i) strong growth of
203 added strains during fermentation (> 10^9UFC/g);(ii) absence or low presence of endogenous Bacillus and (iii)
204 generation of aromatic fruity notes. First, two duplicates of the same sample were mixed, vigorously shaken, and
205 diluted (1/30) with cold (4 °C) Milli-Q water (Merck Milipore, Merck KGaA, Germany). After homogenization (20
206 sec) with a Polytron© PT 2100 (VWR, Radnor, USA), the VOCs were extracted from 5 ml of the mixture, carried out
207 using a water-jacketed purge and trap concentrator (Tekmar-Dohrman 3100, Tekmar, USA) at 40 °C (purge: 40
208 °C/15 min; desorb: 225 °C/2 min) coupled to a gas chromatograph (Agilent Technologies 3800, USA) and a mass
209 spectrometer detector (MSD 5975C, Agilent Technologies, USA). The apparatus was equipped with a DB-5 polar
210 capillary column (30 m x 0.25 mm; film thickness: 0.25 µm; Agilent 122-5532, USA). The oven temperature was
211 increased from 40 °C to 250 °C at a rate of 4 °C/min and then maintained at 250 °C for 10 min.

212 Individual peaks were identified by comparing their retention indices and their mass spectra with those within
213 the mass spectral library database (Wiley 275 K and NBS 75 k). The retention indices of peaks from fermented
214 emulsions (V10 and M7) were then compared with those of control samples (VT and MT). The data were reported as
215 log (peak area/g) for each compound detected.

216 **3. Results**

217 **3.1. Initial screening for strains able to grow on PE and/or ME**

218 The growth ability of 55 strains from 49 microbial species depended on their phylogenetic group (group G1:
219 *Firmicutes*; G2: *Proteobacteria* and *Actinobacteria*; G3: yeast and molds), the type of microbial consortium (single
220 vs. multiple inoculated strains), and the type of emulsion (pea emulsion containing 100% pea proteins (PE) and
221 mixed emulsion containing a 50:50 mixture of pea and milk proteins (ME)).

222 **Growth on the PE (Table 2):** A small proportion of the strains (8 out of 20) from G2 (*Actinobacteria* and
223 *Proteobacteria*), including the species *Glutamicibacter arilaitensis*, *Brevibacterium antiquum, Brevibacterium casei,*
224 *Corynebacterium casei, Acinetobacter johnsonii, Alcaligenes faecalis, Hafnia alvei* and *Psychobacter celer,* were
225 able to grow on PE as single strains or when combined with other microorganisms. In contrast, most of the strains (12

226 out of 16) from the *Firmicutes* group, including the species *Bacillus altitudinis, Staphylococcus equorum,*
227 *Staphylococcus xylosus* and *Staphylococcus lentus, Carnobacterium maltaromaticum, Lactobacillus casei, plantarum*
228 and *rhamnosus, Lactococcus lactis, Leuconostoc lactis* and *Weissella cibaria*, were able to grow on PE as single
229 strains or when combined with other microorganisms. Similarly, 16 out of 19 strains from the fungal group G3 (other
230 than *Penicillium camemberti* and *Saccharomyces cerevisiae* species) could grow on PE as single strains or when
231 combined with other microorganisms.

232 **Growth on the ME (Table 3):** A small number (6 out of 20) of strains from G2, including those from the
233 species *Brevibacterium casei, Brevibacterium linens, Corynebacterium casei, Acinetobacter johnsonii, Hafnia alvei*
234 and *Psychrobacter celer*, were able to grow on ME as single strains or when combined with other microorganisms. It
235 is noteworthy that in contrast to the PE, the ME substrate allowed the majority (14) of the 20 single strains of
236 *Actinobacteria* and all *Proteobacteria* to grow as single cultures. In contrast, their growth was strongly compromised
237 when they were combined with LAB from G1. In fact, only five strains of G2 were selected for their ability to grow
238 in combination with LAB. Almost all strains (15 out of 16) belonging to G1 were able to grow in ME as a single
239 culture, with the exception of one strain of *Streptococcus thermophilus.* Nine strains belonging to the genera *Bacillus,*
240 *Staphylococcus, Lactococcus, Lactobacillus, Leuconostoc, Streptococcus* and *Weissella* showed strong growth as
241 single strains or when combined with other microorganisms, and were therefore selected for the assembly of
242 consortia. Likewise, most strains (16 out of 19) from the fungal group G3 were able to grow in the ME as single
243 strains or when combined with other microorganisms, with the exception of those belonging to the species
244 *Penicillium camemberti*, *Pichia fermentans* and *Saccharomyces cerevisiae*. The *Mucor* molds and yeasts of the
245 genera *Candida, Debaryomyces, Kluyveromyces, Geotrichum, Pichia*, *Yarrowia* and *Zygosaccharomyces* were able
246 to grow when combined with other microorganisms and were therefore selected for the second part of the experiment.

247 At the end of this screening step, 31 strains were selected for the assembly of 20 microbial consortia, referred
248 to as Vegan, V1 to V10 on PE and Mixed, M1 to M10 on ME, each of which comprised three to nine strains (**Table**
249 **S2**). The selection was based on the following criteria: (i) rapid growth during propagation;(ii) a high cell density and
250 (iii) various aromatic sensory attributes after incubation for three days in PE or ME.

251 **3.2. Fermentation of emulsions by allochthonous microbial consortia**

252 **3.2.1. pH values and growth of the microbial consortia**

253 Fermentation of PE and ME was performed using 20 allochthonous microbial consortia (V1 to V10 for PE,
254 and M1 to M10 for ME) and evaluating their ability to enhance the food safety and olfactory features of pea-based
255 products (e.g. the suppression of growth by endogenous microorganisms, the aromatic profile and green note
256 reduction). The bacterial and fungal consortia were added during the exponential growth phase at initial
257 concentrations close to those of starter cultures and ripening cultures used in dairy processes. Non-inoculated
258 preparations of PE and ME were used as controls (VT and MT, respectively).

259 **Growth on the PE (Figure 2):** After 72 hours of fermentation on PE at 28 °C, the counts of bacteria from the
260 consortia V1 to V10 ranged from 7.6 to 9.5 log_{10} CFU/g (Figure 2A), and the counts of fungi ranged from 4.9 to 6.5
261 log_{10} CFU/g (Figure 2B). For some samples (V6, V8 and V3), these counts were slightly lower than those generally
262 found in cheese or fermented vegetables. In the control sample (VT), contamination by *Bacillus* (8 log_{10} CFU/g) was
263 detected.

264 An analysis of the bacterial and fungal species' phylogenetic distribution **(Figure 2A and 2B)** showed that all
265 samples (V1 to V10, plus VT) were composed of species assigned to the expected bacterial phyla (*Actinobacteria,*
266 *Firmicutes* and *Proteobacteria*) and fungal genera (*Geotrichum, Kluyveromyces, Mucor* and *Candida*). In samples
267 V2, V8 and VT, contamination assigned to *Bacillus licheniformis* was dominant, since it accounted for 77%, 71% and
268 98%, respectively, of the detected sequences. It is worth noting that the relative abundances of *Bacillus licheniformis*
269 and the *Lactobacillus* group *casei/rhamnosus* were very similar in samples V2 and V8. The other microbial
270 populations (V1, V3-V7 and V9-V10) were dominated by the inoculated species, namely *Acinetobacter* group
271 *johnsonii* (67%), *Lactobacillus* group *plantarum* (83%), *Hafnia alvei* (49% to 70%), *Candida catenulata* (70%),
272 *Mucor hiemalis* (99%) *Kluyveromyces marxianus* (80%) and *Geotrichum candidum* (85% to 99%). In PE, using only
273 pea proteins as an energy source, these inoculates produced the most promising results with respect to (i) their own
274 microbial growth, and (ii) the suppression of growth by endogenous microorganisms. Conversely, some species

275 inoculated into the PE were not detected after three days of fermentation (*Brevibacterium antiquum, Leuconostoc*
276 *lactis, Weissella cibaria, Debaryomyces hansenii, Pichia fermentans* and *Yarrowia lipolytica*).

277 In the inoculated PEs V1, V5, V6 and V8 and the non-inoculated control PE (VT), the pH rose by 1 to 1.6
278 units (**Figure 2C**). The resulting alkaline PEs were characterized by bacterial dominance and limited fungal growth.
279 Conversely, the pH of inoculated PEs V2, V3, V7 and V9 did not significantly change (by less than 0.5 units). In
280 these cases, the overall action of these consortia maintained the pH value at around 6, without substantial
281 acidification or alkalization. Surprisingly, slight acidification of PEs V4 and V10 (both inoculated with *Geotrichum*
282 *candidum*) was observed.

283 **Growth on the ME (Figure 3):** After 72 hours of fermentation at 28 °C, counts of bacteria from consortia
284 M1 to M10 ranged from 8.8 to 9.6 $\log_{10}$ CFU/g (Figure 3A) and counts of fungi ranged from 5.5 to 8.1 $\log_{10}$ CFU/g
285 (Figure 3B). These concentrations were similar to those generally found in cheese or fermented vegetables during a
286 ripening cycle. After incubation of the control sample (MT), various bacterial contaminants were detected at a count
287 of around 8.7 $\log_{10}$ CFU/g.

288 An analysis of the phylogenetic distribution of the bacterial and fungal species(**Figures 3A and 3B**) showed
289 that all the samples (M1 to M10 and MT) were composed of species assigned to the expected bacterial phyla
290 (*Actinobacteria, Firmicutes* and *Proteobacteria*) and fungal genera (*Debaryomyces, Geotrichum, Kluyveromyces,*
291 *Mucor*, *Pichia* and *Candida*). Whereas little or no contamination (<1%) was observed in some samples (M4, M6, M7
292 and M8), other samples (M2, M3 and MT) were substantially contaminated by several species of *Bacillus* (*B.*
293 *licheniformis*, *B. cereus* and *B. subtilis*, accounting for 35%, 61% and 76%, respectively, of the detected sequences).
294 The low number of strains in these consortia (four in M2 and three in M3) from G1 (*Firmicutes*) and G3 (fungi)
295 appeared to favor the growth of endogenous *Bacillus* species. The other microbial populations (in M1 and M4 to
296 M10) were dominated by the inoculated LAB species, *i.e., Lactococcus lactis* (59% to 94%), *Lactobacillus group*
297 *casei/rhamnosus* (37% to 81%), *Proteobacteria* such as *Hafnia alvei* (8% to 72%) and fungi such as *Candida*
298 *catenulata* (82%), *Kluyveromyces marxianus* (80%), *Kluyveromyces lactis* (48%), *Debaryomyces hansenii* (42%),
299 *Pichia fermentans* (99%) and *Geotrichum candidum* (99%). These consortia produced the best results with respect to

300 (i) their noticeable growth in ME by using pea proteins, casein and lactose as energy sources and (ii) their
301 suppression of the endogenous microbiota. Conversely, some bacterial species initially added to the ME did not grow
302 after three days of fermentation (*Leuconostoc lactis, Psychrobacter celer* and *Staphylococcus lentus*). It is worth
303 noting that *Debaryomyces hansenii* and *Pichia fermentans* were able to colonize the ME (M1 and M2) but not the
304 pure PE (V2, V3 and V9). Conversely, *Psychrobacter celer* (34%) and *Staphylococcus lentus* (18%) grew on the PE
305 (V1 and V3) but not on the ME (M1 and M2).

306 The transformation of lactose into lactic acid by LAB led to significant acidification of the ME emulsions,
307 with the change in pH ranging from 1 to 2.5 for all inoculated ME samples but essentially zero for the non-inoculated
308 control sample (MT: pH 6.3) - thus favoring the growth of autochthonous species, e.g. *Bacillus subtilis* and *Bacillus*
309 *cereus,* known to be acid-sensitive (**Figure 3C**).

310 **3.2.2. Sensory properties of fermented emulsions**

311 The PE and ME preparations fermented by the different allochthonous microbial consortia presented marked
312 differences in aromatic perception by the panel. The preparations could be significantly discriminated with regard to
313 21 (for MEs) and 22 (for PEs) of the 54 attributes. Correspondence analysis (CA) maps drawn up for the significant
314 sensory descriptors ($P<0.05$) illustrated the sensory properties of the pea and mixed emulsions.

315 First, the sensory map for the fermented PEs (**Figure 4A**) explained 53.4% of the variance. With the
316 exception of the V9 emulsion (which was characterized by the same pea, neutral and bread aroma notes as the non-
317 inoculated control sample, and loaded negatively on the F1 and F2 axes), the other fermented emulsions formed three
318 clusters with distinct aromatic profiles. The cluster encompassing V6 was characterized by cheesy notes (ammonia
319 and sulfur). The cluster encompassing V1, V3, V5, V7 and V8 had bland sensory characteristics. Lastly, the cluster
320 encompassing V4 and V10 was characterized by flowery and fruity notes.

321 Secondly, the sensory map for the fermented MEs (**Figure 4B**) explained 68.3% of the variance. These
322 emulsions also formed three clusters. The cluster encompassing M5 and MT was characterized by smoked, pea and
323 herb notes. The cluster encompassing M4, M6, M7, M8, M9 and M10 was characterized by fermented fruit and

324 ethanol notes. Lastly, the cluster group encompassing M1, M2 and M3 was perceived as having with strong cheese,
325 rind and rancid notes.

326 **3.2.3. Volatile organic compounds identified in fermented PE and ME**

327 The VOCs extracted from and identified in each type of emulsion before and after the fermentation with one
328 type of microbial consortium (V10 for the PE and M7 for the ME) are depicted in **Figure 5**. The differences between
329 the identified VOCs highlighted the impact of fermentation on the aroma profiles. In general, a significant increase in
330 the number and percentage of VOCs was obtained after fermentation. Different compounds, variously comprising
331 acids, alcohols, aldehydes, esters, and ketones, appeared or strongly arose in fermented V10 and M7 emulsions,
332 relative to the controls. This was especially true for 3-methylbutanal and 2-methylbutanal (**Figure 5C)** for V10, also
333 characterized by an increase in the level of dimethyl disulfide. The M7 fermented emulsion was characterized by the
334 accumulation of esters in general and ethyl acetate in particular (**Fig 5D**). It is worth noting that the levels of
335 aldehydes identified as hexanal and heptanal (known to be responsible for green off-flavors, and initially present in
336 the non-fermented samples) were much lower after fermentation. Some compounds appeared during the fermentation
337 process, depending on the type of emulsion (e.g., 3-methyl-1-butanol in the ME, and 2-methylpropanal and 2-
338 butanone in the PE), whereas others disappeared in the fermented ME (such as octane and 1-hexanol).

339 **4. Discussion**

340 Fermenting a product enriched with plant proteins in order to improve its sensory qualities is a real scientific
341 as well as technological challenge because even if knowledge about traditional fermented products exists, the design
342 of new fermented products and the strategy leading to the design of appropriate microbial combinations, remain
343 poorly documented The present study investigated the feasibility of fermenting plant products with allochthonous
344 microorganisms by combining them into promising new microbial consortia not previously found in food systems.

345 A preliminary subtractive screening based on the ability to colonize two pea-based products, namely ME
346 (containing salt, vegetable oil, lactose, and pea and milk proteins), and PE (containing salt, vegetable oil and pea
347 proteins), was applied to 55 microbial strains isolated from dairy products and vegetables. Strains belonging to

348 various phylogenetic groups (*Firmicutes* (G1), *Actinobacteria* and *Proteobacteria* (G2), and eukaryotes (G3)) were
349 tested on PE and ME emulsions, in pure culture or in combination with three to nine strains, for a total of 320
350 associations. The assembly strategy of these strains was based on the balanced distribution according to their
351 phylogenetic group. This screening led to the selection of 10 associations for each of the two emulsions, PE and ME,
352 in which 31 strains were distributed and had the best growth. These associations were further assessed and compared
353 on the basis of their growth and their ability to modulate the aromatic profile (odors) of these emulsions.

354 The presence of carbohydrates in the ME markedly influenced the fermentation performed by the tested
355 microorganisms, since lactic acid fermentation strongly reduced the pH of the medium. In contrast, the absence of
356 carbohydrates in the PE promoted alkaline fermentation. The alkalization of the PE observed here has previously
357 been reported in fermentation studies concerning various legumes with high protein contents. Many fermented foods
358 produced from soy, lupin and pea in Africa and Asia (such as doenjang, douchi, natto and meju) are traditionally
359 fermented by alkalizing bacteria - generally species from the *Bacillus* genus, such as *Bacillus amyloliquefaciens,*
360 *Bacillus circulans, Bacillus coagulans, Bacillus firmus, Bacillus licheniformis, Bacillus megaterium, Bacillus*
361 *pumilus, Bacillus subtilis,* and *Bacillus thuringiensis* (Kim et al., 2004; Tamang et al., 2016b). Although these species
362 have been isolated from naturally fermented legume products (Chettri and Tamang, 2015b; Wang et al., 2006), *B.*
363 *subtilis* is the dominant functional bacterium and may be used as commercial starter in Asian fermented soybean
364 foods (Tamang et al., 2016b). It is worth noting that the *Bacillus* genus's strong adaptation to this habitat was
365 confirmed in our study by the omnipresence and growth of endogenous communities predominantly composed of
366 *Bacillus licheniformis* and *Bacillus subtilis* -especially in the non-inoculated samples. In most PE samples, an
367 increase in pH was observed after fermentation. This alkalization probably resulted from the proteolysis of pea
368 proteins, the only energy source in the PE. Our results suggest that *Bacillus* species and certain selected microbial
369 consortia are involved in this catabolism, leading to the release of small peptides and free amino acids that can then
370 be transformed into alcohols, ammonia or aldehydes via decarboxylation and/or deamination. The latter compounds
371 could then be responsible for alkalization (Chen et al., 2012, Shrestha et al., 2013).

372 Most of the eukaryotes tested here (and particularly *Rhizopus, Mucor* and *Geotricum*) were able to grow in 373 PE. Our results are in line with the data in the literature (Jung et al. 2014; Nout and Kiers, 2005*;* Tamang et al., 374 2016b) showing that these genera are dominant in tempeh (an Indonesian cake made from soybeans) and in 375 traditional Korean fermented soybean. Moreover, in our study, most of the bacteria assigned to the *Actinobacteria* 376 and *Proteobacteria* (particularly *Hafnia alvei*, *Acinetobacter johnsonii* and *Glutamicibacter arilaitensis*) exhibited 377 strong growth on the PE when combined in consortia of three to nine strains, and also competed well against 378 endogenous Bacillus.

379 One novel feature of the current study was the absence of added carbohydrates in the fermented PEs. Some 380 LAB species (such as *Lactobacillus casei, Lactobacillus rhamnosus* and *Lactobacillus plantarum*) were able to grow 381 on pea-based products, which indicated that pea proteins are suitable substrates for some LAB. Similar results were 382 reported by Schindler et al. (2012), showing that *Lactobacillus plantarum* (frequently isolated from plants and leaves) 383 is able to colonize pea proteins and helps to improve the flavor by either reducing off-flavor formation or masking 384 undesirable green notes. Furthermore, some *L. plantarum* strains exhibit other important biological properties such as 385 probiotic activity (Stefanovic et al., 2017) and the ability to reduce (i) undesired oligosaccharides, (ii) undesired 386 contaminations (Demarigny, 2012), and (iii) the allergenicity of pea proteins (Barkholt et al., 1998).

387 In the literature, the controlled, non spontaneous fermentation of legume proteins usually takes place through 388 acidification following the addition of LAB (Drake et al., 2000; Fritsch et al., 2015; Meinlschmidt et al., 2016; 389 Yousseef et al., 2016) and the addition of carbohydrates to stimulate the LAB and improve the product's sanitary 390 properties (Ravyts et al., 2012). The pH decrease is due to an accumulation of organic acids (particularly lactic acid 391 and acetic acid) via the consumption of lactose by LAB such as *Leuconostoc mesenteroides, Lactobacillus brevis,* 392 *Lactobacillus plantarum, Pediococccus cerevisiae, Streptococcus thermophilus, Lactococcus lactis, Lactobacillus* 393 *bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium bifidus* (Steinkraus K.H, 2002). Similarly, our present 394 results demonstrate that the presence of lactose-containing powdered milk in the ME provides interesting properties 395 when fermented with LAB from G1. From among the nine initially tested LAB, five species (*Lactococcus lactis,* 396 *Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus plantarum* and *Leuconostoc lactis*) were selected for

397 their strong growth and acidification capacities in ME. Lactic acid fermentation inhibited the growth of the
398 endogenous Bacillus that naturally contaminate pea protein extracts (e.g. *Bacillus subtilis* and *Bacillus licheniformis*)
399 as well as the growth of inoculated acid-sensitive species - most of which belong to the *Actinobacteria* and
400 *Proteobacteria* (e.g., *Brevibacterium casei, Corynebacterium casei* and *Staphylococcus lentus*).

401 During fermentation, microorganisms produce enzymes to break down food nutrients (e.g., proteins,
402 carbohydrates, lipids, organic and amino acids), and thus generate precursors of non-volatile and volatile compounds
403 affecting aroma, texture and flavor. These compounds are critical components for food acceptance by consumers. The
404 extent, sequence and time-scale of substrate use depend on the food product and microorganism in question. The
405 enzymatic degradation of proteins is particularly important in the fermentation of food stuffs with a high protein
406 content. Various peptides, free amino acids and α-keto-acids are generated by the respective actions of proteases,
407 amino carboxypeptidases and decarboxylases, and thus contribute to the fermented product's organoleptic properties
408 (e.g., aroma and texture) (Curioni et al., 2002; Visessanguan et al., 2005). In the pea, albumin and globulin account
409 for 15–25% and 50–60%, respectively, of the total protein, with a vicilin :legumin ratio of between 0.6 and 3.7 for the
410 globulins in smooth cultivars (Gueguen and Barbot, 1988). Pea albumins contain higher proportions of the essential
411 amino acids, tryptophan, lysine, threonine, cysteine and methionine, than globulins, whereas the latter are rich in
412 arginine, phenylalanine, leucine and isoleucine (Swanson, 1990).

413 Our analysis of aromas and VOCs showed that the increase in the diversity and abundance of aroma
414 compounds during fermentation depended on the type of emulsion. The fruity and flowery notes, detected in samples
415 V4 and V10 might have been generated by the yeast *Geotrichum candidum*, present in both of the emulsions, as well
416 as by the bacteria *Lactobacillus plantarum* and *Hafnia alvei*, which are the main bacterial species in the fermented V4
417 and V10 pea emulsions, respectively. All three species are known to possess strong proteolytic and deamination
418 activities (Boutrou et al., 2005; Irlinger et al., 2012; Peralta et al., 2016). Whereas all PE emulsions fermented with
419 V5, V6, V7, V9 and V10 consortia are dominated by *Hafnia alvei*, each of them are characterized by a different
420 sensory profile. These sensory differences may be due to the fungal composition that varies for each consortium
421 (*Kluyveromyces marxianus* and *Yarrowia lipolytica* (V5); no yeast (V6); *Kluyveromyces marxianus* (V7);

Geotrichum candidum (V9); and *Geotrichum candidum* and *Candida catenulata* (V10)), probably affecting changes in the pH value and metabolic responses of dominant microbial strains during fermentation, consequently leading to the generation of different metabolites and therefore, a particular aromatic profile for each given product. The sulfur notes perceived in the V6 sample are clearly due to the growth of *Hafnia alvei* (the dominant bacterial species) but may also be due to the presence of the *Bacillus subtilis* contaminant. This finding agrees with the observation of Irlinger et al. (2012) of a dramatic increase in volatile sulfur compound production in a model cheese inoculated with *H. alvei*. Compounds like dimethyl disulfide are produced by the degradation of sulfur amino acids and strongly contribute to cheese's aroma profile (Landaud et al., 2008).

The fruity aromatic notes in some ME samples (M6, M7 and M8) were probably generated by the growth of *Kluyveromyces* and *Geotrichum* strains, which are able to produce alcohol and esters through the catabolism of lactose and amino acids, respectively (Satyanarayana and Kunze, 2009). The presence of roasted/grilled notes in the fermented PE and ME emulsions might be associated with the proteolysis of pea vicilin by various microorganisms (e.g., LAB and yeasts). In the roasting process, it has been shown that hydrophobic free amino acids and hydrophilic peptides are responsible for the formation of cocoa-specific aroma components (Crafack et al., 2013). These components are generated from cocoa vicilin by the cooperative action of an aspartic endoprotease and a carboxypeptidase of microbial origin that are naturally present in ungerminated cocoa seeds (Ho et al., 2014; Voigt et al., 1994). In the present study, the selected microbial consortia tended to be associated with positive attributes such as "fruity" and/or "cheesy" for the ME, and "fruity" and "roasted" for the PE. These attributes differed from the ones observed for the non-fermented control samples (mainly "pea", "bread" and "herb"). This result is in line with Yousseef et al. (2016), who reported that pea products (40 g pea protein per 100 g total protein, $66.5^{0}/_{00}$ lactose) inoculated with LAB starters tended to have higher intensities of positive attributes (such as "creamy", "dairy" and "sweet") and lower intensities of negative descriptors (such as "vegetal", "earth" and "vinegar").

We used GC-MS to compare the VOC profiles for the control vs. fermented emulsions after three days of fermentation. Two microbial consortia (V10 for PE, and M7 for ME) were selected according to their rapid growth, high cell density, and aromatic sensory attributes. The control emulsions were mainly characterized by the presence

447 of aldehydes like hexanal, heptanal and pentanal, which originate from enzymatic and/or auto-oxidation of the fatty
448 acids - mainly linoleic and linolenic acids - present in peas (Murat et al., 2013) and are responsible for undesirable
449 green-beany flavors (Azarnia et al., 2011; Curioni and Bosset, 2002). Levels of these aldehydes were strongly
450 reduced by fermentation in both the PE and the ME. Conversely, other aldehydes were found to be present at higher
451 levels in fermented ME and PE preparations compared to the control emulsions. This was notably the case for 3-
452 methylbutanal and 2-methylbutanal - branched-chain aldehydes derived from the metabolism of the branched amino
453 acids, leucine and isoleucine and which significantly contribute malty, nutty and caramel notes to the aroma profile
454 (Curioni and Bosset, 2002; Smit et al., 2005). In the fermented V10 emulsion, a high level of dimethyl disulfide was
455 detected. This was probably produced by *G. candidum*, which is known to generate volatile sulfur compounds
456 through methionine catabolism (Arfi et al., 2002). A variety of esters accumulated in the M7 emulsion, among which
457 ethyl acetate was the most concentrated. The latter was probably produced by the yeast *K. lactis*, which is known to
458 produce esters from lactose (Arfi et al., 2002; Liu et al., 2004). It is well known that esters contribute typical fruity
459 notes to fermented products (Liu et al., 2004).

460 In conclusion, our present results showed how the assembly of multiple microbial cultures can be successfully
461 applied to processes to develop innovative food products. Several consortia characterized by a high diversity
462 composed of six to nine strains, including yeasts (*Geotrichum candidum, Kluyveromyces marxianus* and *Candida*
463 *catenulata*), lactic acid bacteria (*Lactococcus lactis, Lactobacillus plantarum* and *Lactobacillus casei*) and other
464 bacteria (*Hafnia alvei*) were found to actively ferment pea proteins. It is likely that the inoculation level, substrate
465 type (pea/milk/lactose), pH and composition of the microbial consortia may affect metabolic responses of strains -
466 leading to the generation of different metabolites and, consequently, a particular aromatic profile for each given
467 product. Further research is needed to determine how interactions between these species affect the fermentation
468 process and the quality of the pea end-product. Our findings also emphasize the need for more research on the
469 biochemical reactions that occur during fermentation, especially those affecting pH (use of lactose) and the release of
470 important aroma precursors (such as amino acids) but also those favoring the degradation of toxic components and

471 anti-nutritive factors. Finally, to go further, consumer test could be envisaged to evaluate the perception and liking of
472 these new fermented products.

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652

653 **Table and Figure legends**

654 **Table 1** Composition of pea and mixed emulsions (g /100 g)

655 **Table 2** List of microbial strains investigated in this study and used on emulsions of 100% pea proteins. The
656 following are indicated: the origin of the strain, the genus and species affiliation, with a recognized Qualified
657 Presumptive Safe (QPS) status according to EFSA (2017) (a) and with a known food usage, according to Bourdichon
658 et al. (2012) (b). The growth of individual strains, alone or in combinations of one to nine strains in the pea
659 emulsions, containing 100% pea proteins (PE) is reported. Red squares: no growth, or strain no longer detected.
660 Green squares: growth.

661 **Table 3** List of microbial strains investigated in this study and used on mixed emulsions of pea and milk proteins.
662 The following are indicated: the origin of the strain, the genus and species affiliation, with a recognized Qualified
663 Presumptive Safe (QPS) status according to EFSA(2017) (a) and with a known food usage, according to Bourdichon
664 et al. (2012) (b). The growth of individual strains, alone or in combinations of one to nine strains in the mixed
665 emulsions, containing a 50:50 mixture of pea and milk proteins (ME) is reported. Red squares: no growth, or strain
666 no longer detected. Green squares: growth.

667 **Figure 1** Preparation of fermented pea emulsions containing 100% pea proteins (PE) and mixed emulsions,
668 containing a 50:50 mixture of pea and milk proteins (ME).

669 **Figure 2** Distribution and abundance of species on the PE fermented by ten allochthonous microbial consortia (V1 to
670 V10).

671 **A** Relative abundance of bacterial species determined by amplicon sequencing of bacterial 16S DNA (bottom); each
672 column represents the different fermented emulsions. Only taxa detected at >0.5% relative abundance are shown.
673 Total bacterial cell counts were determined using a culture-dependent method on BHI incubated at 28°C and MRS
674 incubated at 30°C (top).

675 VT A non-inoculated PE sample was used as a control and subjected to the same incubation conditions.

676 * indicates a species that was not deliberately inoculated and was therefore probably an endogenous contaminant of

677 pea protein isolates.

678 **B** Relative abundance of fungal community species, in CFUs. The number of CFUs for each species was determined

679 by plating serial dilutions of homogenized fermented emulsions on YGCA incubated at 28°C.

680 **C** Change in pH in the PE after three days of fermentation by different microbial consortia (V1 to V10, plus VT).

681 **Figure 3** Distribution and abundance of species on the ME fermented by ten allochthonous microbial consortia (M1

682 to M10).

683 **A** Relative abundance of bacterial species, determined by amplicon sequencing of bacterial 16S DNA (bottom); each

684 column represents the different fermented emulsions. Only taxa detected at >0.5% relative abundance are shown.

685 Total bacterial cell counts were determined using a culture-dependent method on BHI incubated at 28°C and MRS

686 incubated at 30°C (top).

687 MT A non-inoculated ME sample was used as a control and subjected to the same incubation conditions.

688 * indicates a species that was not deliberately inoculated and was therefore probably an endogenous contaminant of

689 pea protein isolates.

690 **B** Relative abundance of fungal community species, in CFUs. The number of CFUs for each species was determined

691 by plating serial dilutions of homogenized fermented emulsions on yeast glucose chloramphenicol agar incubated at

692 28°C.

693 **C** Change in pH in the ME after three days of fermentation by different microbial consortia (M1 to M10 and MT).

694 **Figure 4** The first two dimensions of a correspondence analysis of aroma attribute data (CATA method) for PE

695 emulsions **(A)** fermented by ten microbial consortia, referred to as Vegan, V1 to V10 and the control non-fermented

696 PE emulsion, as well as for ME emulsions **(B)** fermented by ten microbial consortia, referred to as Mixed, M1 to M10

697 and the control non-fermented ME emulsion. Blue: samples (V1 to V10 and M1 to M10). Red: aroma attributes,

698 Black: control (non-fermented) emulsion samples.

699 **Figure 5** GC-MS chromatograms (full scan) of four representative emulsions before and after fermentation with

700 assigned volatile compounds.

701 **A** GC-MS chromatogram of the PE emulsion before fermentation.

702 **B** GC-MS chromatogram of the ME emulsion before fermentation.

703 **C** GC-MS chromatogram of the fermented PE emulsion (V10)

704 **D** GC-MS chromatograms of fermented ME emulsion (M7).

705

706 **Supplementary material**

707 **Table S1** Experimental design. Strains are grouped by their phylum and/or phylogenetic affiliation. For each
708 combination, one or two representatives of each phylum were selected. The process was repeated once (single
709 cultures) or ten times (consortia of three to nine species). The exact composition of each combination is available
710 upon request.

711 **Table S2** Assembling and composition of microbial consortia and sensory characterization of fermented pea
712 emulsions containing 100% pea proteins (PE) and mixed emulsions containing a 50:50 mixture of pea and milk
713 proteins (ME).

714

715 **Table S3** List of sensory attributes (54 descriptors organized into seven classes) used for the CATA questionnaire

716

717 **Table 1**

Emulsion type	Components						
	Milk protein	Pea protein	Fat	Lactose	Ash	Sodium	Fiber
Pea Emulsion (PE)	-	10	10	-	0.44	0.44	0.14
Mixed Emulsion (ME)	5	5	10	8	1.41	0.4	0.07

718

719 **Table S1**

Number of strains / combination	Number of combinations						Total	Number of reshuffled replicates	Number of identical replicates per re-shuffled replicate	Total consortia
	G1 (Firmicutes)		G2 (acid-sensitive bacteria)		G3 (Fungi)					
	Lactic acid bacteria n=10	*Staphylococcus/ Bacillus n=6*	*Actinobacteria n=14*	*Proteobacteria n=6*	*Yeast n=12*	*Mold n=7*				
1	**10**	**6**	**14**	**6**	**12**	**7**	**55**	1	1 to 3	90
3	**1** (2 LAB +1 *Staphylococcus* or *Bacillus*)		**1** (2 *Actinobacteria* + 1 *Proteobacteria*)		**1** (2 yeasts + 1 Mold)		**3**	10	1	30
6	**3 (G1+G2 and G1+G3 and G2+G3)**						**3**	10	1	30
9	**1 (G1+G2+G3)**						**1**	10	1	10
TOTAL							**62**			**160**

720
721

722 **Table S2**

Emulsion type	name of microbial consortia	G1									G2								G3														Main aromatic sensory attributes describing the fermented PE and ME emulsions
		263 *Bacillus altitudinis*	ATCC 334 *Lactobacillus casei*	CNRZ 211T *Lactobacillus plantarum*	CNRZ212 *Lactobacillus rhamnosus*	S3 *Lactococcus lactis*	NCW1 *Leuconostoc lactis*	Com1 *Staphylococcus xylosus*	Ca2 *Staphylococcus lentus*	3385 *Weissella cibaria*	3M05 *Acinetobacter johnsonii*	CNRZ918 *Brevibacterium antiquum*	CIP102111 *Brevibacterium casei*	ATCC 9172 *Brevibacterium linens*	2M01 *Corynebacterium casei*	Re117 *Glutacimibacter arilaitensis*	GB01 *Hafnia alvei*	91 *Psychrobacter celer*	ExfComLD *Candida catenulata*	DH304 *Debaryomyces hansenii*	47(8) *Debaryomyces hansenii*	ATCC 204307 *Geotrichum candidum*	38(10) *Kluyveromyces marxianus*	CLIB196 *Kluyveromyces lactis*	3550 *Kluyveromyces lactis*	UBOCC-A-109206 *Mucor brunneogriseus*	UBOCC-A-109052 *Mucor brunneogriseus*	UBOCC-A-109198 *Mucor circinelloides*	UBOCC-A-101359 *Mucor hiemalis*	177 *Pichia fermentans*	CLIB183 *Yarrowia lipolytica*	26(8) *Zygosaccharomyces rouxii*	
Pea protein emulsion (PE)	V1											■				■		■															Coffee, milk, butter, yogurt
	V2	■	■							■		■							■		■						■						Coffee, milk, hazelnut
	V3				■				■	■	■	■			■														■	■		■	Grilled hazelnut, caramel
	V4			■			■						■									■						■					Brioche, fermented fruit, apricot
	V5							■							■		■						■						■		■		Hazelnut, grilled peanut
	V6	■			■		■						■				■																Chocolate, flower
	V7	■			■		■						■				■		■				■				■						Sweet almond, sweat and apple
	V8	■	■							■																							Melted butter, cheese
	V9							■									■			■		■											Pea, Bread
	V10	■			■		■					■	■				■		■			■									■		Yogurt, Flower
Main aromatic sensory attributes describing the fermented PE emulsions by the pure culture																																	
Pea and Milk protein emulsion (ME)	M1	■	■							■								■	■		■						■						Yogurt, nutmeg and ammonia
	M2				■				■	■																				■			Grass cut, sour
	M3				■																	■					■						Grapefruit, celery
	M4					■														■				■		■							Apricot, chicory
	M5			■		■	■						■									■						■					Fermented fruit, pineapple, apple, banana
	M6					■		■						■	■		■						■						■		■		Fermented fruit, pineapple, apple, banana
	M7				■	■													■			■			■								Fermented fruit, pineapple, apple, banana
	M8					■	■	■					■				■						■										Grapefruit, leather
	M9					■							■				■						■									■	Fresh milk, vanilla, whey acid and chestnut
	M10					■								■	■		■						■								■		Orange, cheese rind and acid whey
Main aromatic sensory attributes describing the fermented ME emulsions by the pure culture																																	

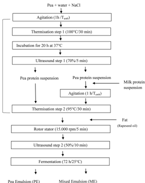
723

724

725 **Table S3**

Attribute classes	**Vegetal**	**Roasted/Grilled**	**Floral**	**Lactic**	**Fruity**	**Animal**	**Other**
Descriptors	Pea	Dried fruit	Flower	Fresh milk	Citrus	Animal	Ammoniacal
	Garlic/Onion	Smoked	Honey	Cooked milk	Red fruit	Meat broth	Sulfur
	Potato	Dark chocolate		Fresh cream	Banana	Sweat	Acetic
	Herb	Bread		Fresh butter	Pineapple		Rancid
	Fermented herb	Grilled		Melted butter	Apricot		Ethanol
	Woody	Roasted		Yoghurt	Apple		Propionic
		Coffee		Cheese	Fermented fruit		Moldy
		Spicy		Rind			Putrid
		Mint		Acid curd			Sour
		Caramel		Fresh curd			Neutral
		Burned		Whey			Butyric
		Chicory					
		Brioche					
		Vanilla					

726



Tyndallisation

A

Log$_{10}$bacterial CFUs /g :

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	VT
Log$_{10}$bacterial CFUs /g	9.0	8.4	8.3	9.1	9.6	7.6	9.4	8.3	9.1	9.2	7.9

%

100 90 80 70 60 50 40 30 20 10 0

V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 VT

- *Acinetobacter group johnsonii*
- *Bacillus group altitudinis*
- *Bacillus licheniformis**
- *Brevibacterium casei*
- *Hafnia group alvei*
- *Lactobacillus group plantarum*
- *Psychrobacter celer*
- *Staphylococcus group xylosus*
- *Weissella cibaria*
- *Glutamicibacter group arilaitensis*
- *Bacillus group subtilis**
- *Brevibacterium antiquum*
- *Corynebacterium group casei*
- *Lactobacillus group casei/rhamnosus*
- *Leuconostoc lactis*
- *Staphylococcus group capitis**
- *Staphylococcus lentus*

B

Log$_{10}$fungal CFUs /g :

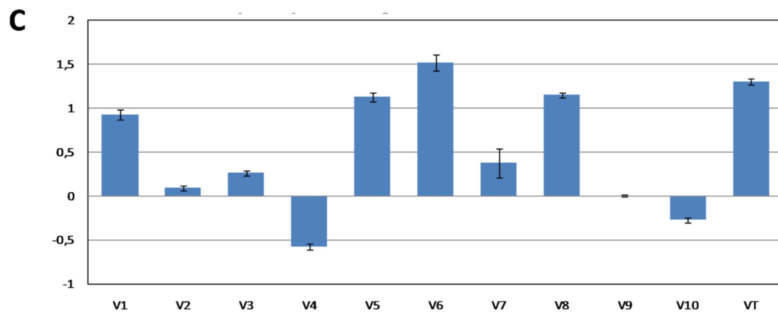
	V2	V3	V4	V5	V7	V9	V10
Log$_{10}$fungal CFUs /g	6.5	5.0	6.4	4.9	5.0	5.9	6.2

100 90 80 70 60 50 40 30 20 10 0

V2 V3 V4 V5 V7 V9 V10

- *Candida catenulata*
- *Geotrichum candidum*
- *Kluyveromyces marxianus +Yarrowia lipolytica*
- *Kluyveromyces marxianus*
- *Mucor brunneogriseus*
- *Mucor circinelloïdes*
- *Mucor hiemalis*

C



A

Log$_{10}$bacterial CFUs /g :

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	MT28
Log$_{10}$bacterial CFUs /g	9.3	9.5	9.6	9.0	9.0	9.0	9.1	8.8	9.4	9.6	8.7

%

0 10 20 30 40 50 60 70 80 90 100

- *Weissella cibaria*
- *Staphylococcus group capitis**
- *Psychrobacter celer*
- *Leuconostoc lactis*
- *Lactobacillus group plantarum*
- *Hafnia group alvei*
- *Brevibacterium casei*
- *Bacillus group subtilis**
- *Bacillus group altitudinis*
- *Staphylococcus group xylosus*
- *Sphingomonas group insulae**
- *Paenibacillus group wynnii**
- *Lactococcus lactis*
- *Lactobacillus group casei/rhamn*
- *Corynebacterium group casei*
- *Bacillus licheniformis**
- *Bacillus group cereus**
- *Bacillus circulans**

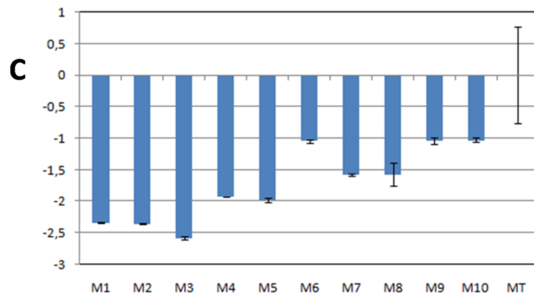
B

Log$_{10}$fungal CFUs /g :

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Log$_{10}$fungal CFUs /g	7.5	5.5	7.0	8.1	6.5	7.8	7.5	8.0	8.0	8.1

- *Candida catenulata*
- *Debaryomyces hansenii*
- *Geotrichum candidum*
- *Pichia fermentans*
- *Kluyveromyces marxianus +Yarrowia lipolytica*
- *Kluyveromyces lactis*
- *Kluyveromyces marxianus*
- *Zygosaccharomyces rouxii*
- *Mucor brunneogriseus*
- *Mucor circinelloïdes*

C



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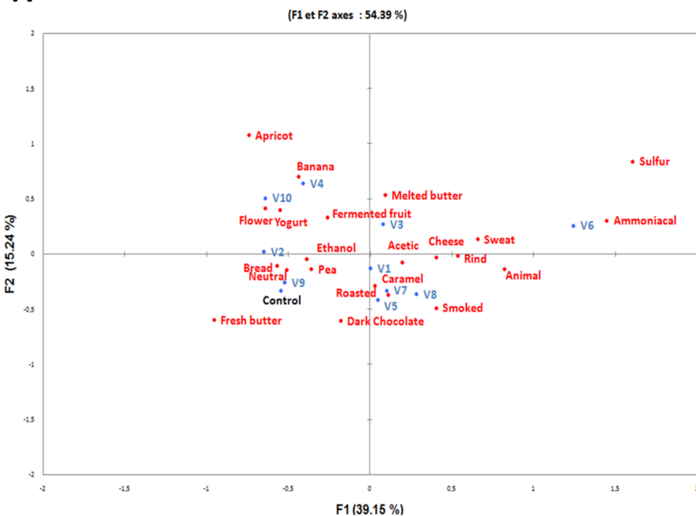
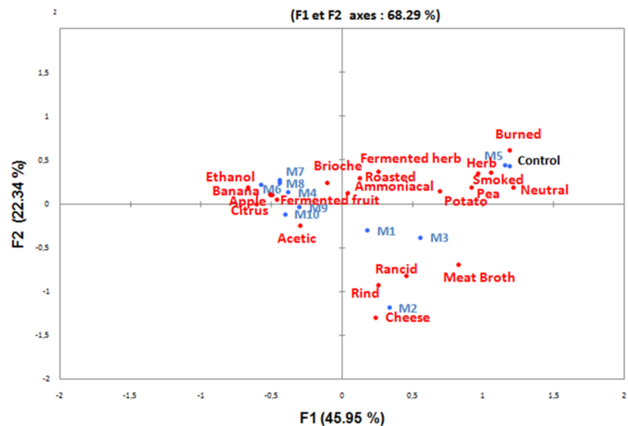
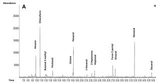
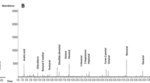
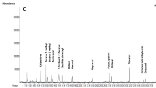


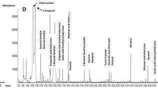
B




A
Abondance
Time
Hexane
Chloroform
Butanal 3 methyl
Pentanal
Octane
Hexanal
1 Hexanol
2 Heptanone
Heptanal
Furan 2 pentyl
Octanal
Nonanal
Decanal


B
Abondance
Time
Acetic acid
Chloroform
Butanal 3 methyl
Pentanal
Disulfide dimethyl
Octane
Hexanal
1 Hexanol
2 Heptanone
Heptanal
Furan 2 pentyl
Octanal
Nonanal
Decanal


C
Abondance
Time
Chloroform
Butanal 3 methyl
Butanal 2 methyl
Acetic acid
1 Pentanol + 1Butanol
Disulfide dimethyl
Octane
Hexanal
Heptanal
Furan 2 pentyl
Octanal
Nonanal
Octanoic acid ethyl ester
Decanal


D
Abondance
Time
Ethyl acetate
1 Propanol
Butanal 3methyl
Butanal 2methyl
Propanoic acid ethyl ester
1 Butanol 3methyl
Propanoic acid 2methyl ethyl ester
Acetic acid 2methyl propyl ester
Hexanal
1 Butanol 3methyl acetate
Styrene
Heptanal
Furan 2 pentyl
Hexanoic acid ethyl ester
Octanal
Nonanal
Octanoic acid ethyl ester
Decanal
Acetic acid 2 phenylethyl ester