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Salma Ben-Harb, Anne Saint-Eve, Maud Panouillé, Isabelle Souchon, Pascal Bonnarme, Eric Dugat-Bony, Françoise Irlinger*

[a]UMR GMPA, AgroParisTech, INRA, Université Paris-Saclay, 78850, Thiverval-Grignon, France

*Corresponding author. Email: francoise.irlinger@inra.fr

Abstract

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

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

1. Introduction

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

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

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

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

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

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

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

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

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

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

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

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

2. Materials and Methods

2.1. Ingredients/raw materials

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

2.2. Preparation of the two pea protein emulsions

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

2.3. Strains and inoculum preparation

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

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

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

2.4. Experimental design of pea and mixed emulsion fermentations

Pea and mixed emulsions were mixed with physiological saline containing microbial solutions in order to 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 incubation at 28 °C for 72 h, the fermented emulsions were compared with each other and with the control emulsion (i.e., without microbial inoculum) obtained under the same conditions. All samples were stored at -20°C before amplicon sequencing analysis, whereas sensory and microbiological analyses were carried out immediately.

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

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

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

2.5. Microbial analyses, cell counting and pH measurements

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

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

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

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

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

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

2.7. Sensory analysis

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

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

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

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

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

3. Results

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

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

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

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

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

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

3.2. Fermentation of emulsions by allochthonous microbial consortia

3.2.1. pH values and growth of the microbial consortia

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

Growth on the PE (Figure 2): After 72 hours of fermentation on PE at 28 °C, the counts of bacteria from the 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 $\log_{10}$ CFU/g (Figure 2B). For some samples (V6, V8 and V3), these counts were slightly lower than those generally found in cheese or fermented vegetables. In the control sample (VT), contamination by *Bacillus* (8 $\log_{10}$ CFU/g) was detected.

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

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

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

Growth on the ME (Figure 3): After 72 hours of fermentation at 28 °C, counts of bacteria from consortia 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 (Figure 3B). These concentrations were similar to those generally found in cheese or fermented vegetables during a ripening cycle. After incubation of the control sample (MT), various bacterial contaminants were detected at a count of around 8.7 $\log_{10}$ CFU/g.

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

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

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

3.2.2. Sensory properties of fermented emulsions

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

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

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

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

3.2.3. Volatile organic compounds identified in fermented PE and ME

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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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.

* indicates a species that was not deliberately inoculated and was therefore probably an endogenous contaminant of pea protein isolates.

B Relative abundance of fungal community species, in CFUs. The number of CFUs for each species was determined by plating serial dilutions of homogenized fermented emulsions on YGCA incubated at 28°C.

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

Figure 3 Distribution and abundance of species on the ME fermented by ten allochthonous microbial consortia (M1 to M10).

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

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

* indicates a species that was not deliberately inoculated and was therefore probably an endogenous contaminant of pea protein isolates.

B Relative abundance of fungal community species, in CFUs. The number of CFUs for each species was determined by plating serial dilutions of homogenized fermented emulsions on yeast glucose chloramphenicol agar incubated at 28°C.

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

Figure 4 The first two dimensions of a correspondence analysis of aroma attribute data (CATA method) for PE emulsions **(A)** fermented by ten microbial consortia, referred to as Vegan, V1 to V10 and the control non-fermented PE emulsion, as well as for ME emulsions **(B)** fermented by ten microbial consortia, referred to as Mixed, M1 to M10 and the control non-fermented ME emulsion. Blue: samples (V1 to V10 and M1 to M10). Red: aroma attributes, Black: control (non-fermented) emulsion samples.

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

assigned volatile compounds.

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

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

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

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

Supplementary material

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

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

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

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

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**

722 **Table S2**

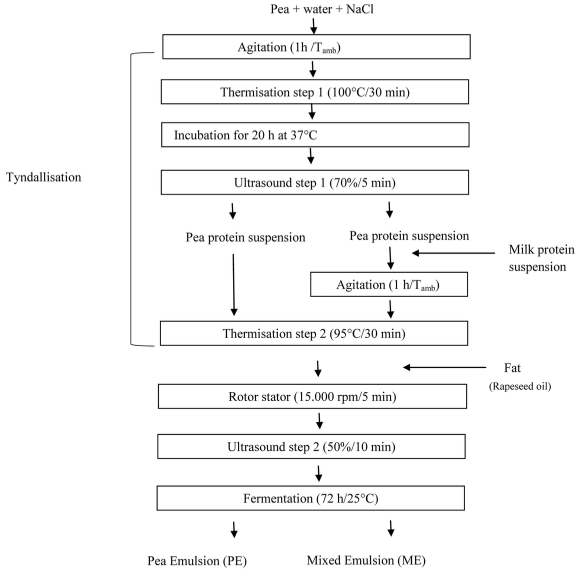
		G1									G2								G3														
Emulsion type	name of microbial consortia	263	ATCC 334	CNRZ 211T	CNRZ212	S3	NCW1	Com1	Ca2	3385	3M05	CNRZ918	CIP102111	ATCC 9172	2M01	Re117	GB01	91	ExfComLD	DH304	47(8)	ATCC 204307	38(10)	CLIB196	3550	UBOCC-A-109206	UBOCC-A-109052	UBOCC-A-109198	UBOCC-A-101359	177	CLIB183	26(8)	Main aromatic sensory attributes describing the fermented PE and ME emulsions
		Bacillus altitudinis	*Lactobacillus casei*	*Lactobacillus plantarum*	*Lactobacillus rhamnosus*	*Lactococcus lactis*	*Leuconostoc lactis*	*Staphylococcus xylosus*	*Staphylococcus lentus*	*Weissella cibaria*	*Acinetobacter johnsonii*	*Brevibacterium antiquum*	*Brevibacterium casei*	*Brevibacterium linens*	*Corynebacterium casei*	*Glutacimibacter arilaitens*	*Hafnia alvei*	*Psychrobacter celer*	*Candida catenulata*	*Debaryomyces hansenii*	*Debaryomyces hansenii*	*Geotrichum candidum*	*Kluyveromyces marxianus*	*Kluyveromyces lactis*	*Kluyveromyces lactis*	*Mucor brunneogriseus*	*Mucor brunneogriseus*	*Mucor circinelloides*	*Mucor hiemalis*	*Pichia fermentans*	*Yarrowia lipolytica*	*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																																	

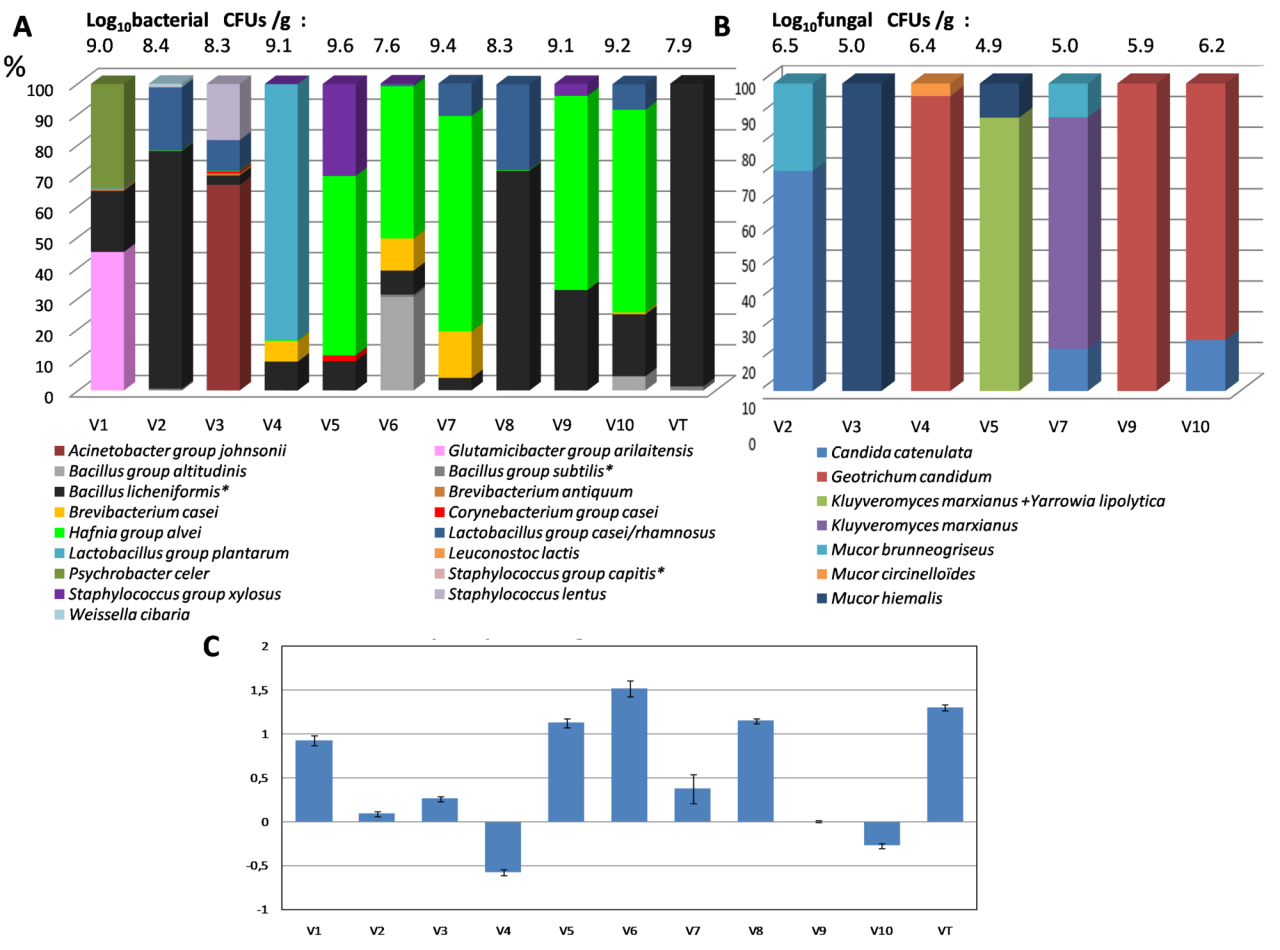
723

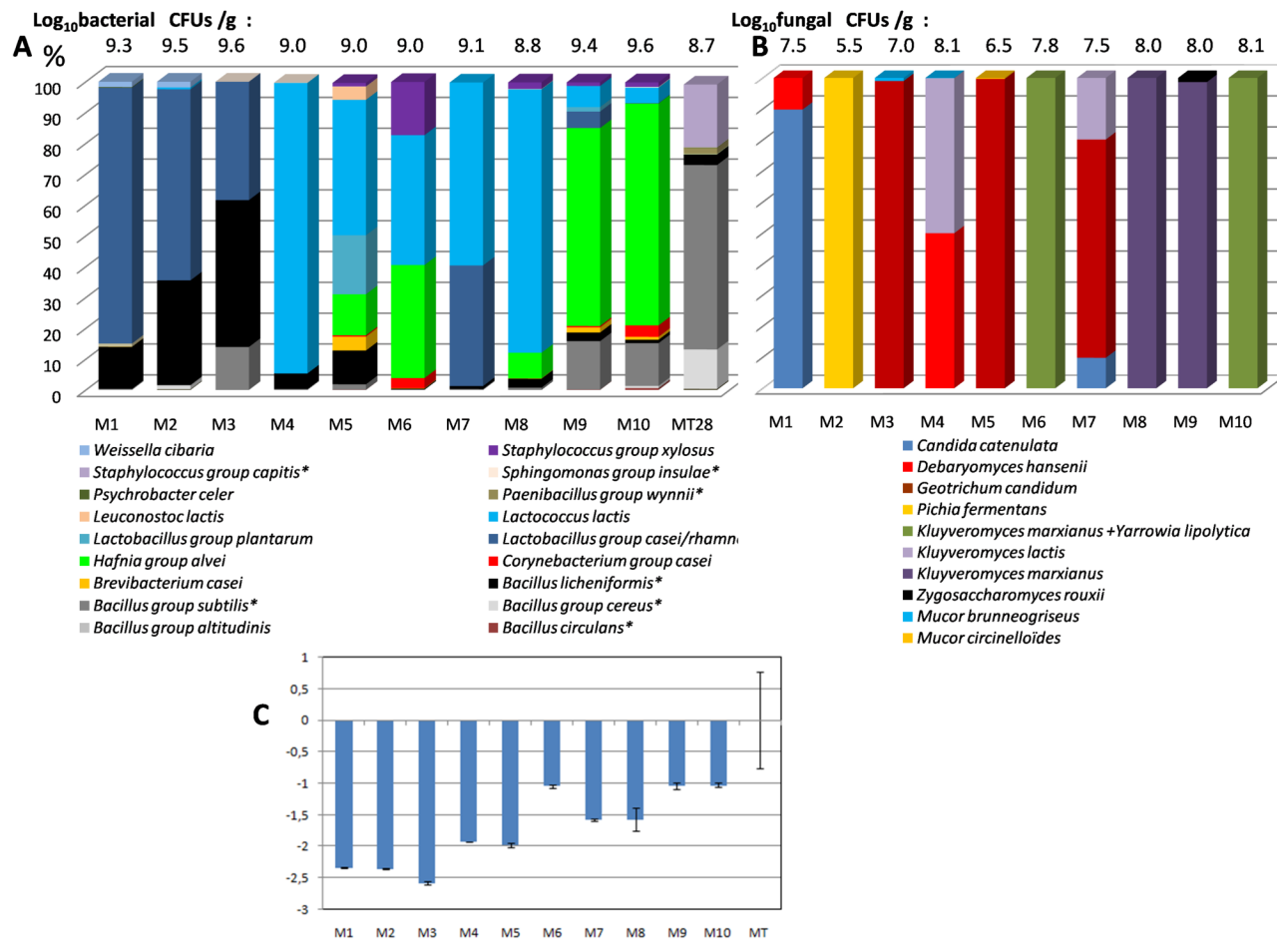
724

Table S3

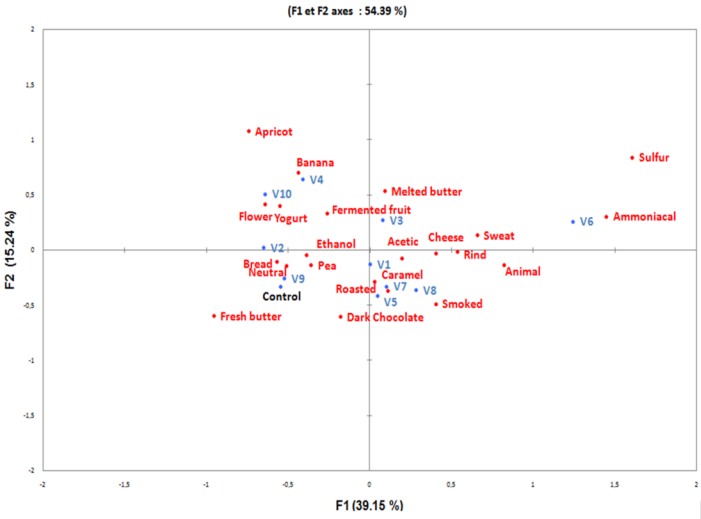
Attribute classes	**Vegetal**	**Roasted/Grilled**	**Floral**	**Lactic**	**Fruity**	**Animal**	**Other**
Descriptors	Pea	Dried fruit	Flower	Fresh milk	Citrus	Animal	Ammoniacal
	Garlic/Onion	Smoked	Honey	Cooked milk	Red fruit	Meat broth	Sulfur
	Potato	Dark chocolate		Fresh cream	Banana	Sweat	Acetic
	Herb	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					


Pea + water + NaCl
Agitation (1h /T$_{amb}$)
Thermisation step 1 (100°C/30 min)
Incubation for 20 h at 37°C
Tyndallisation
Ultrasound step 1 (70%/5 min)
Pea protein suspension
Pea protein suspension
Milk protein suspension
Agitation (1 h/T$_{amb}$)
Thermisation step 2 (95°C/30 min)
Fat
(Rapeseed oil)
Rotor stator (15.000 rpm/5 min)
Ultrasound step 2 (50%/10 min)
Fermentation (72 h/25°C)
Pea Emulsion (PE)
Mixed Emulsion (ME)

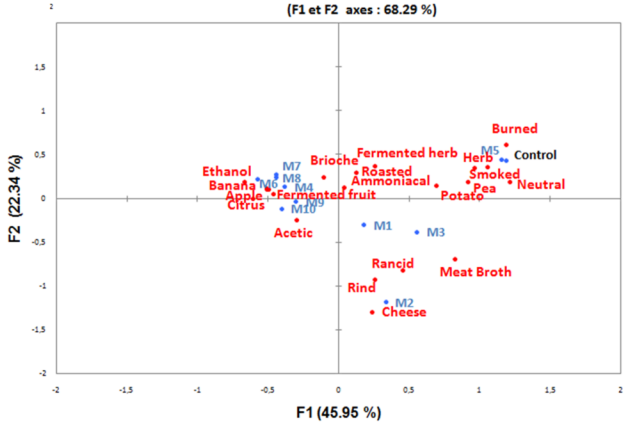

A
Log10bacterial CFUs /g :
9.0 8.4 8.3 9.1 9.6 7.6 9.4 8.3 9.1 9.2 7.9
%
100
90
80
70
60
50
40
30
20
10
0
V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 VT
Acinetobacter group johnsonii
Bacillus group altitudinis
Bacillus licheniformis*
Brevibacterium casei
Hafnia group alvei
Lactobacillus group plantarum
Psychrobacter 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
Log10fungal 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
2
1,5
1
0,5
0
-0,5
-1
V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 VT

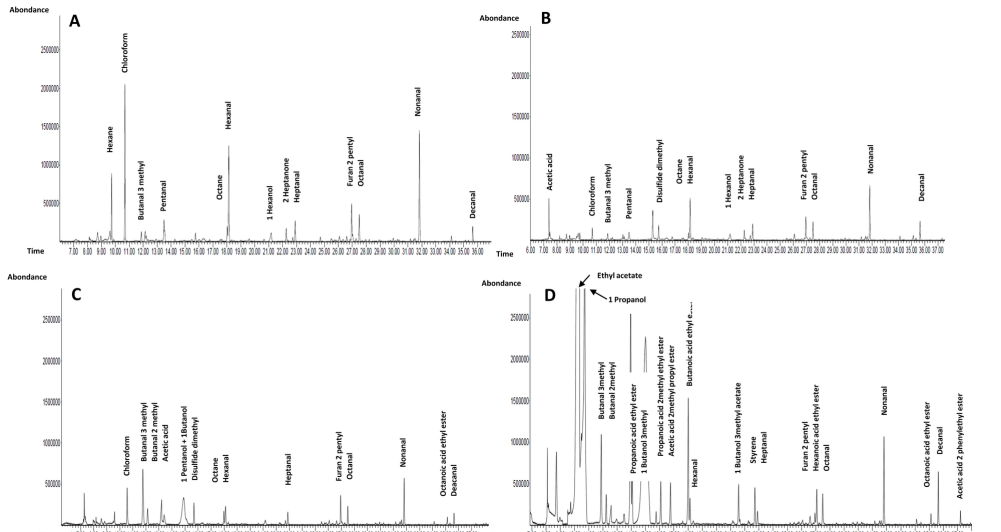

A
Log10bacterial CFUs /g :
9.3 9.5 9.6 9.0 9.0 9.0 9.1 8.8 9.4 9.6 8.7
%
100 90 80 70 60 50 40 30 20 10 0
M1 M2 M3 M4 M5 M6 M7 M8 M9 M10 MT28
Weissella cibaria
Staphylococcus group xylosus
Staphylococcus group capitis*
Sphingomonas group insulae*
Psychrobacter celer
Paenibacillus group wynnii*
Leuconostoc lactis
Lactococcus lactis
Lactobacillus group plantarum
Lactobacillus group casei/rhamn
Hafnia group alvei
Corynebacterium group casei
Brevibacterium casei
Bacillus licheniformis*
Bacillus group subtilis*
Bacillus group cereus*
Bacillus group altitudinis
Bacillus circulans*
B
Log10fungal CFUs /g :
7.5 5.5 7.0 8.1 6.5 7.8 7.5 8.0 8.0 8.1
M1 M2 M3 M4 M5 M6 M7 M8 M9 M10
Candida catenulata
Debaryomyces hansenii
Geotrichum candidum
Pichia fermentans
Kluyveromyces marxianus +Yarrowia lipolytica
Kluyveromyces lactis
Kluyveromyces marxianus
Zygosaccharomyces rouxii
Mucor brunneogriseus
Mucor circinelloïdes
C
1 0,5 0 -0,5 -1 -1,5 -2 -2,5 -3
M1 M2 M3 M4 M5 M6 M7 M8 M9 M10 MT

A


(F1 et F2 axes : 54.39 %)
F2 (15.24 %)
F1 (39.15 %)
Apricot
Banana
V4
Sulfur
Melted butter
V10
Flower Yogurt
Fermented fruit
V3
V6
Ammoniacal
Ethanol
Acetic
Cheese
Sweat
V2
Rind
Bread
Pea
V1
Animal
Neutral
V9
Caramel
Control
Roasted
V7
V8
Smoked
V5
Fresh butter
Dark Chocolate

B


(F1 et F2 axes : 68.29 %)
F2 (22.34 %)
F1 (45.95 %)
Burned
M5
Control
Fermented herb
Brioche
Herb
M7
Roasted
Smoked
Ethanol
M8
Ammoniacal
Banana
M6
M4
Pea
Neutral
Apple
Fermented fruit
Potato
Citrus
M9
M10
M1
M3
Acetic
Rancid
Meat Broth
Rind
M2
Cheese


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 2methyl
Acetic acid
1 Pentanal + 1Butanol
Disulfide dimethyl
Octane
Hexanal
Heptanal
Furan 2 pentyl
Octanal
Nonanal
Octanoic acid ethyl ester
Desacanal
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
Butanoic acid ethyl e...
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