Fermentation of texturized pea protein in combination with proteases for aroma development in meat analogues

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ABSTRACT

The potential use of texturized pea protein in meat analogues was investigated by comparing the effects of fermentation on pea and myofibrillar pork proteins in a model system including additives, microbial starters and proteases. Model fermentation was controlled for 15 days by pH decrease, microbial counts and free amino acids increase. Besides, volatile production and sensory properties were evaluated at the end of fermentation. Protein type affected free amino acid generation and volatile profile. Models supplemented with proteases showed an increase in amino acid derived compounds (branched aldehydes and alcohols) and fruity odor notes. During fermentation, protease addition significantly reduced the production of linear aldehydes (pentanal, hexanal and octanal) in vegetal models, while pyrazine compounds were not affected. This changes in the volatile profile reduced the legume-beany odor, although increased the perception of toasted cereal-like notes generated by the texturization process.

KEYWORDS: meat analogue, plant based, pea protein isolate, fermentation, off-flavor

1. INTRODUCTION

Flavor is an essential issue in the development of meat- and processed-meat analogues[1]. Changes in the ingredients or processing affect greatly the flavor of these products and, consequently, consumer preference, which is highly influenced by cultural habits and experience[2]. The main components in the formulation of meat analogues are plant protein-rich ingredients, such as plant protein isolates and soy or wheat concentrates, as well as legumes like pea and lupine, rice or potato[3]. Peas belong to the *Fabaceae* family and are popular for their low cost, and high protein content[4]. The protein ingredients are the most important component for differentiation of meat analogues, because of their ability to provide meat-like structure and nutritional health[5].

Numerous studies have demonstrated that the inclusion of plant protein in food products formulation is the origin of undesirable volatile flavors[6]. The removal or covering of the plant protein off-flavors, as well as those originated from flavor interactions with the plant proteins, are key in the study of meat analogues flavor[7,8]. Indeed, many studies have focused on the flavor of cooked meat analogues, and the effect of the addition of flavorings and aroma precursors during their manufacture[9]. In case of fermented and dry-cured products, aroma seems to be the biggest challenge for the formulation of meat analogues.

The fermentation of plant proteins has the potential to produce pleasant aroma compounds of interest in the design of fermented dry sausage analogues. The fermentation of plant-based foods to generate different flavor profiles is widely known in Asia since ancient times. Several of these fermented foods have been described as having a taste profile with umami characteristics. Moreover, many of these foods have been characterized in terms of their aroma profile and taste, as in case of Chinese fermented soybean curd or white sufu and the Japanese fermented soybean paste miso[10].

The protein sources most widely used in meat analogues are soy and pea isolates [11]. However the application of a fermentative process of these protein sources for production of fermented meat analogues has been scarcely investigated. Recent studies have proposed the fermentation of pea protein with a combination of lactic acid bacteria (LAB) and yeast starter cultures to reduce the off-flavors produced by the presence of hexanal and other oxidation products like 2-pentylfuran, (E, E)-2,4-decadienal, hexanal, nonanal, (E, E)-2,4-nonadienal, octanal, (E)-2-nonenal and (E)-2-octenal[12,13]. Furthermore, the application of microbial consortia (LAB and molds) in the fermentative process in combination with enzymatic hydrolysis has been proposed as a way to improve the taste of soy protein isolates [14]. This improvement was observed in both the taste and functionality (emulsifying and foaming properties) of the protein isolate and, in addition, the fermented protein isolates showed a reduced beany flavor.

In traditional fermented dry-cured products, flavor generation depends on precursors produced during the fermentative process and the activity of microbial starters selected to ferment animal proteins. The ability of these starters to generate precursors and aromas has not been tested on plant proteins. Moreover, their activity may be hindered by their ability to hydrolyze vegetal proteins, which could be improved applying exogenous proteolytic enzymes. In summary, the aim of this study was to determine the functionality of microbial starters, combined with proteases, in the fermentation of texturized pea proteins. The fermentation process and its outcome were compared with that of an identical model system formulated with extracted pork meat proteins undergoing the same treatment. The results of this study could provide information about the potential use of texturized pea proteins in dry-cured meat analogues manufacturing.

2. MATERIAL AND METHODS

2.1 Isolation of myofibrilar proteins from pork meat. The isolation of myofibrillar proteins was performed following the method of Molina & Toldrá[15] using the muscle *Longissimus thoracis et lumborum*. The process consisted on the homogenization of the meat with 0.03 N phosphate buffer pH 7.4 using a stomacher (IUL masticator, Barcelona, Spain) followed by a centrifugation process at 10.000 g during 20 min. The pellet was collected and the process was repeated three times for the removal of sarcoplasmic proteins. The final pellet was resuspended in a solution containing 0.1 N buffer phosphate, 0.7 M potassium iodide and 0.02 % sodium azide at pH 7.4, and then filtered through glass wool and diluted again in a solution containing 0.1 N buffer phosphate and 0.02% sodium azide at pH 7.4. Finally, the suspension was removed by centrifugation and the pellet containing myofibrillar proteins was collected and used in the formulation of the models.

2.2. Preparation of vegetal and animal fermentation models. The fermentation model systems included animal or vegetal proteins together with common additives used in the fermentation of meat products (salt, glucose and nitrifying agents), previously dissolved in distilled water and filter (0.22 μm) sterilized (Grynia, Labbox, Barcelona, Spain), and microbial starters. A commercial protease (Flavourzyme >500 U/g, Sigma, Merck, Germany) was applied as flavouring enzyme in some of the models as described in Table S1. Two models, animal (A) and vegetal (V), were prepared. The animal model (A) was formulated with the extracted myofibrillar proteins (8 % w/v), while the vegetal model (V) was prepared with texturized pea protein (Manufacturas Ceylan, Valencia, Spain) (8 % w/v) previously homogenized in a blender. Two additional models containing the flavouring enzyme (0.02 %) were prepared from the animal (AE) and vegetal (VE) models. The ingredients (3 % NaCl, 2 % Glucose, 0.015 % $NaNO_2$, 0.015 % KNO_3) of the four models were homogenized in a blender and inoculated with the commercial starter TRADI-302 (0.0125 %), containing, *Lactobacillus sakei, Staphylococcus carnosus,* and *Staphylococcus xylosus*, (Chr. Hansen,

Hoersholm, Denmark) and yeast *D. hansenii* (L5, 10^6 cells/ml)[16] as indicated in Table S1. The fermentation experiments of the four models (A, AE, V and VE) were prepared in triplicate. The models were incubated at 25 °C in a heater (Incuterm Digit, Raypa, Barcelona, Spain) and samples were taken at days 0, 3, 8 and 15. The evolution of the fermentation was followed by the decrease in pH, microbial counts, and free amino acids production. The sample for microbial analysis was homogenized with saline solution in a sampling bag with a side filter (Scharlab, Barcelona, Spain) using a Pulsifier II (Microgen Bioproducts, Camberley, UK) (3 pulses of 30 s). The sample for physic-chemical analysis was centrifuged at 10.000 g for 20 min and the supernatant filtered through a 0.2 µm filter (Minisart NML, Sartorius, Göttingen, Germany) and used for pH measurement with a portable pH-meter (HI 99163, Hanna Intruments Inc., Hoonsocket, USA). The supernatant was further used for free amino acids and volatile compound analysis. The samples for volatiles analysis were acidified using 200 µL of tricloroacetic acid to inactivate protease activity, then neutralized with 1 N NaOH and kept at -20 °C until further analysis. Additionally, at the end of the fermentation (15 days) the remaining fermented model was kept for sensory analysis.

2.3. Microbiological analysis. The analysis was performed as described by Belloch et al.[17], . In summary, the homogenized samples were used to prepare decimal dilutions which were subsequently spread in triplicates on the appropriate media plates for microbial counts as follows: total mesophilic bacteria (TMB) on Plate Count Agar (PCA) (Condalab, Madrid, Spain) at 30 °C for 2 days, LAB on MRS Agar (Scharlau, Barcelona, Spain) at 30 °C for 2 days, Gram positive cocci (GC+) on Mannitol Salt Agar (MSA) (Scharlau, Barcelona, Spain) at 30 °C for 2 days, enterobacteria (E) on Violet Red Bile Glucose Agar (VRBGA) at 37 °C for 24 h, and yeasts and moulds (YM) on Rose Bengal Agar Chloramphenicol (Scharlau, Barcelona, Spain) at 30 °C for 3 days. Results from the microbial counts were expressed as log CFU/g.

2.4. Volatile compounds analysis. Volatile compounds present in the headspace of the liquid sample were analyzed as described in Perea-Sanz et al.,[16] by extracting the compounds with a solid phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA). Samples consisting of 4 mL of supernatant previously defrosted, were placed in a headspace vial (20 ml, Gerstel, Germany) containing 1.88 g NaCl and equilibrated at 37°C during 30 min. Then, the volatile compounds were extracted for 1 h at 37°C under shaking at 250 rpm using the SPME fibre (85 μm, CAR/PDMS StableFlex fibre, 1cm). The extracted volatile compounds were analyzed in an Agilent HP 7890 series II GC with an HP 5975C mass selective detector (Hewlett-Packard Palo Alto, CA, USA) and a Gerstel MPS2 multipurpose sampler (Gerstel, Germany). The fiber was desorbed in the GC injection port at 240°C for 5 min in splitless mode. Volatile compounds were separated using a DB-624 capillary column (30 m x 0,25 mm, 1,40 μm Agilent Technologies, USA) and analyzed using the MS detector in SCAN mode. Volatile compounds were identified by comparison with mass spectra from the library database (Nist'17), by linear retention indices calculated using the series of n-alkanes C8-C22 (Aldrich, Merck, Germany)[18], and by comparison with authentic standards (Table S5). Quantification was performed in SCAN mode using either total or extracted ion area (TIC or EIC) on an arbitrary scale. Each model supernatant was analyzed in triplicate and the results were expressed as abundance units (AU) 10^{-5} per g of protein in the media, and the differences in volatiles produced depending on the protein source, animal or vegetal, were determined.

2.5. Free amino acid analysis. The abundance of free amino acids released from the proteolytic activity in the liquid sample was measured following the methodology described by Aristoy and Toldrá[19], which includes the deproteinization and derivatization of the sample. Norleucine (10 mM in 0.01 M HCl) was used as internal standard. The separation of free amino acids was done by reversed-phase HPLC chromatography in an Agilent Series 1100 equipment (Agilent, CA, USA) equipped with a Waters Nova Pack® C18 column (3.9 × 300

159 mm; Waters Corporation, MA, USA) at 52°C using a photodiode array detector[20]. The
160 separated amino acids were detected at 254 nm. Each medium supernatant was analyzed in
161 triplicate. Identification of amino acids was achieved by comparison against a solution of
162 mixed standards (Sigma, Merck, Germany), and quantification was based on the calculated
163 response factors. They were calculated using five amino acid standard levels in the presence
164 of the added internal standard (norleucine). The final results were expressed as mg of free
165 amino acid per g of protein in the model, and the differences in released free amino acids
166 depending on the protein source, animal or vegetal, were determined.

167 **2.6. Sensory analysis.** The sensory analysis was done from model samples at the end of the
168 fermentation process (15 days) using the detection frequency method[21] to reveal the aroma
169 impact of volatile compounds in the models. Odors were evaluated by six trained panellists,
170 4 females and 2 males with an average of 40 years old, who evaluated the odors by smelling
171 the model samples as reported in Belloch et al.[22] The aroma descriptors were recorded and the
172 results were expressed as the number of times a descriptor was detected by the panellists [21,23].

173 **2.7. Statistical analysis.** Data were analyzed using the Generalised Linear Model (GML)
174 procedure in the statistical software XLSTAT 2018 (Addinsoft, Barcelona, Spain). Data
175 analysis, using the linear mixed model, included two factors: protein source (vegetal or
176 animal) and enzyme as fixed effects, and replicates as random effects. Differences between
177 sample means were analysed according to Tukey's test, when a significant effect of the
178 treatment group was detected ($P < 0.05$). Principal component analysis (PCA) was performed
179 to evaluate the relationships between variables (pH, microbial counts, free amino acids and
180 volatiles) and models at the four sampling times. Heatmaps plotted using XLSTAT 2018 were
181 based on the relative abundance of identified volatile compounds in the models at the four
182 sampling times.

183

184 ## 3. RESULTS

185 The evolution of the fermentation, free amino acids content, changes in the volatile profile
186 and sensory analysis of the fermentative process in the vegetal and animal models,
187 supplemented or not with a protease, was monitored. The analyses were done at the beginning
188 of the fermentation (day 0), at the middle (day 3 and 8), and at the end of the process (day 15).

189 **3.1. Evolution of the fermentation: pH and microbial counts**. The evolution of pH and
190 microbial counts in the fermented models is shown in Figure 1 and Tables S2 and S3. Values
191 of pH decreased significantly during the fermentation of the animal (Fig 1A and 1B) and
192 vegetal models (Fig 1C and 1D). Moreover, the addition of the proteolytic enzyme accelerated
193 significantly the pH decrease (Table S2). Fermentation time increased significantly microbial
194 counts, usually at days 3 or 8 of fermentation. Microbial counts were lower in the animal (Fig
195 1A) than in the vegetal model (Fig 1C). In contrast, the addition of the protease decreased
196 bacterial counts in both models (Fig 1B and 1D; Table S3). This decrease was significant in
197 case of GC+ counts in the animal model (AE), while in the vegetal model (VE) the effect was
198 observed in both GC+ and in LAB counts. In contrast, the differences in PCA and YM counts
199 between models with or without enzyme were not significant. No enterobacteria were detected
200 along the fermentation process.

201 **3.2. Determination of free amino acids in the models along the fermentation.** The total
202 content of free amino acids along the fermentation is reported in Figure 2, while the values
203 for individual amino acids are in Tables S4 and S5. In general, free amino acid content
204 significantly increased in all models along the fermentation time (Fig 2), except for few amino
205 acids (glu, his, thr, met, phe, and trp) in the animal models (Table S4). The addition of enzyme
206 also increased significantly the amino acid content in both, vegetal and animal, models (Tables
207 S4 and S5). This increase was about 100 times higher in the vegetal than in the animal models
208 (Fig 2). In the animal model the addition of enzyme significantly increased the production of

amino acids ala, pro, val, ile, leu, orn and lys, but the amount produced was only 2 to 3 times higher than at the initial time. In contrast, the amount of free amino acids produced by enzyme addition in the vegetal model was higher, around 8 fold in case of try, ala, thr and glu and 12 fold in case of phe, ile, leu and val.

3.3. Differences in the volatiles profile between the models along the fermentation. The volatile organic compounds (VOCs) profile was very different in the vegetal and animal models (Fig 3 and 4; Tables S6-S8). Sixty-two VOCs were identified in the model's headspace, but the chemical structure was confirmed in only 54 of them (Table S6). Eight VOCs, including 3 pyrazines, were tentatively identified by mass spectrometry. The main difference between the vegetal and animal models was the presence of pyrazines in the vegetal models, which were absent in the animal models. Also, four additional compounds, 3-methyl-3-buten-1-ol, 3-methyl-1-butanol acetate, ethylbenzene and 3-pentanone were only detected in the vegetal models (Table S6).

The evolution of the VOCs profile classified by chemical group (Fig 3) along the fermentation of all models indicates that alcohols constituted the most abundant group, followed by aldehydes and ketones. The evolution of the fermentation can be recognized by the significant increase of alcohols with time in all models (Fig. 3), being this increase higher in the vegetal (Fig 3C) than in the animal model (Fig 3A). Besides, the addition of enzyme impacted differently the vegetal and animal models. In the vegetal models (Table S8), alcohols such as ethanol and 2-ethyl-1-hexanol were the most abundant compounds found in the V model, while in the VE model increased several methyl branched alcohols (2- and 3-methyl-1-butanol) and phenylethyl alcohol. Similarly, branched aldehydes 2-methyl and 3-methyl butanal, were in higher abundance in the VE than in the V model. The abundance of ketone compounds generally increased in the VE model respect to the V model (Figure 3D). Few changes were observed in pyrazines abundance along the fermentation and the addition of

234 enzyme did not produce a clear trend. In contrast, the addition of enzyme in the animal model
235 did not cause many significant differences in the volatile profile (Table S7). The main
236 differences were the increase in branched aldehydes (2-methyl and 3-methyl butanal) in the
237 AE model, as happened in the vegetal model VE (Figure 3A and 3B, Table S7).

238 A more comprehensive comparison of the compounds constituting the volatile profile of the
239 models was plotted in a heatmap with hierarchical clustering (Fig 4). The dendrogram at the
240 top shows that the models are divided in two groups by the type of protein employed, animal
241 (right) vs. vegetal (left). Moreover, differences within each group can also be observed. In the
242 vegetal model, the effect of the enzyme had larger impact than the fermentation time, as
243 samples VE3, VE8 and VE15 appear separated from the rest of the samples. In the animal
244 model the main impact was caused by the fermentation time as samples A15, AE8, AE15 were
245 separated (left) from the rest. The dendrogram on the left shows which compounds support
246 the differences between and within the models. The presence of pyrazines and few ketones
247 constitute the core of cluster E, which separates between the vegetal and animal models. The
248 remaining clusters of compounds account for the main differences within the models. Cluster
249 D composed by several alcohols and ketones separates samples A8, A15 and AE15 from the
250 other samples in the animal model, as well as the VE from the V samples in the vegetal model.
251 The separation of A8, A15 and AE15 samples is also supported by compounds in cluster C,
252 composed by several ketones, branched aldehydes and alcohols. Finally, cluster B constituted
253 mainly by linear aldehydes separates initial samples 0 and 3 from later samples 8 and 15 in
254 the V model.

255 A further analysis of the data was applied to study the effect of time and enzyme addition on
256 the fermentation of pea protein vs. pork myofibrillar protein, and the results were plotted in a
257 principal components analysis (Fig 5). The PCA explained 62.8 % of the variability. The first
258 factor (42.5%) separated the animal samples from the vegetal samples, whereas the second

factor (20.3 %) separated the samples containing enzyme by fermentation time. Microbial counts and free amino acids were clearly related to the V model samples. Moreover, it is worth to note that all free amino acids are closely related to the VE model samples. Regarding the volatile compounds, pyrazines seem to be the main variable separating V from A models, while alcohols and aldehydes separate VE from the V model.

3.4. Sensory properties of the fermented models. The odor profile of the models was evaluated at the end of the fermentation time (15 days) (Figure 6), and significant ($P < 0.05$) differences were found among all models. The animal models were defined by descriptors fruity, sour, and cooked vegetal, while the vegetal models were described by toasted cereal, legume, cocoa and cheesy odor notes in addition to fruity and sour. The addition of enzyme had a significant impact on the odor profile of the vegetal model. The legume and cocoa notes in the V model were replaced by toasted cereal, cheesy and fruity notes in the VE model. Furthermore, the addition of enzyme significantly decreased the sour odors. In case of the animal models, the addition of enzyme (AE model) only increased the fruity and cooked vegetal odors already present in the A model.

4. DISCUSSION

In order to develop attractive plant-based fermented meat analogs, we have evaluated the potential of pea protein isolates fermentation in combination with enzymatic proteolysis, to improve flavor. Moreover, we have compared these findings with those obtained applying a similar fermentative process using extracted meat proteins. The results from our study show (Fig 1) that the fermentation process progressed in a similar way using texturized pea protein or myofibrillar pork proteins, although the presence of the enzyme (protease) accelerated the process. This may be due to the increase in free amino acids produced by the proteolytic

activity (Fig 2), which would increase the metabolic activity of LAB and, consequently, the decrease in pH. Enzyme addition (VE and AE models) caused a slight negative effect on LAB counts in both models; however, this decrease did not seem to have a large impact neither in the pH decrease nor in the fermentation progress. On the contrary, yeast growth was not affected by the presence of enzyme in the models, which could have important consequences for aroma generation[24]. The most important difference between the models, animal (A) and vegetal (V), was the higher microbial counts in the vegetal model, which might indicate that the texturized pea protein is more accessible to the microorganisms, thus facilitating proteolysis activity. This agrees with the slight increase in free amino acid abundance in the V model respect to the A model (Figure 2, Tables S4 and S5). Previous studies have demonstrated that hydrolysis of myofibrillar proteins using *Staphylococcus carnosus* exoproteases highly increases the concentration of free amino acids Glu and Gly, and moderately in case of His, Thr, Val, Leu, Phe and Lys [25,26]. In contrast, the addition of the starter culture (A model), which also includes *S. carnosus*, did not produce a significant increase of protein hydrolysis, and only the addition of the commercial protease (AE model) produced a significant increase of the proteolytic activity against myofibrillar pork proteins. In agreement with previous studies, some of the most abundant free amino acids produced in the AE model (animal model with enzyme) (Table S4) were the same as those produced by hydrolysis of myofibrillar proteins using *S. carnosus* exoproteases[26]. The texturized pea protein (VE model) underwent a similar proteolysis and fermentative process than the animal model (AE) but, in comparison, the free amino acid yield in the former was significantly higher than in the latter. This result would indicate that the pea protein is more accessible to enzymatic activity than the myofibrillar pork proteins. Moreover, the large proteolysis yield of the vegetal model (VE) would suggest that not only exopeptidase activities are present. Furthermore, the amino acid composition of plant proteins can be very different from the one found in meat proteins[26] and, in case of pea proteins,

308 the most abundant amino acids are Glu, Arg, Leu, Lys, whereas the less abundant are Met and
309 Cys, in agreement with previous studies[27].

310 The generation of free amino acids is closely related to the formation of volatile compounds
311 affecting aroma. For example, in fermented meat products the generation of sulfur amino acids
312 promotes formation of sulfur compounds which contribute to savory properties of the meat
313 product[28]. An important result from our study was that the fermented models, animal and
314 vegetal, generated different volatile profiles which were derived from the different amino acid
315 composition of the proteins present in the models. The volatile profile of hydrolyzed
316 myofibrillar proteins using *S. carnosus* exoproteases[26] has been reported to include VOCs such
317 as linear aldehydes, alcohols and ester compounds after only 2 h of hydrolysis. Among these
318 compounds, two were found derived from phenylalanine, benzenacetaldehyde and phenylethyl
319 alcohol. The generation of these two compounds was also observed in the animal models (A
320 and AE) (Table S7). However, benzeneacetaldehyde was absent or scarcely produced in the
321 vegetal models (V and VE), whereas phenylethyl alcohol was found abundantly in the VE
322 model (Table S8). The main differences between a purely enzymatic hydrolysis[26] and our study
323 are the addition of microbial starters, and the longer incubation times (up to 15 days). These
324 differences were responsible in the VE model for the generation of compounds derived from
325 phenylalanine (benzenacetaldehyde and phenylethyl alcohol), as well as those derived from
326 isoleucine and leucine, like branched aldehydes (2-methyl- and 3-methyl butanal) and their
327 respective alcohols (Figure 4).

328 The effect of the long fermentation time, applied in our models, on the volatile profile is not
329 easy to analyze since from the beginning of fermentation (day 0) both models, animal and
330 vegetal, had a very dissimilar volatile profile. The largest difference was the presence in the
331 pea protein models of odor-active carbonyl compounds (linear aldehydes and 2-pentyl furan,

Figure 4) responsible for the beany flavor[29], and pyrazine compounds derived from the degradation of fatty acids and amino acids, respectively[30]. The presence of different aldehydes, ketones and pyrazines responsible for the beany flavor in the vegetal models largely depends on the initial pea protein composition[31] and texturization process[29], but also on the volatile extraction technique employed during analysis, which affects the VOCs profile qualitative and quantitatively[32]. The large influence of these factors on VOCs profile limits comparisons of results between studies using the same extraction conditions. Nevertheless, odor compounds responsible for the pea protein isolate flavor such as hexanal, benzaldehyde, heptanal and 1-octen-3-ol, derived from lipid oxidation processes [33,34], were also present in the vegetal models (Figure 4). Regarding the pyrazines, the ones present in vegetal models may be derived from Maillard reactions during the texturization process as 2,5-dimethyl-pyrazine[30]. Other pyrazines are inherent constituents of the pea protein as methoxypyrazines[35], while 2-isobutyl-3-hydroxypyrazine vary during the isolation process of pea proteins and affect the aroma profile.[34]

The contribution of microbial starters to food aroma has been widely explored[36]. Moreover, their application in fermented meat products for their ability to transform free amino acids, generated by the endogenous proteolytic system, into volatile compounds has been amply proven[37]. In case of vegetal proteins, most efforts have focused on the removal of beany off flavors, especially on the transformation of aldehydes and ketones into alcohols or carboxylic acids by the activity of alcohol dehydrogenases (ADH) and aldehyde dehydrogenases (ALDH) present in microorganisms[31]. Among the most studied microorganisms for this application are LAB (*Lactobacillus acidophilus*, *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum* and *Streptococcus thermophilus*) and *Saccharomyces cerevisiae*. In case of the animal and vegetal models used in our study, both the formulation of the models and the

356 microbial starter were selected to imitate a fermented meat product, therefore bacterial
357 (TRADI-302, Chr. Hansen, Denmark) and fungal starters[16] used for that purpose were applied.

358 Since the beginning of the fermentation, ketones and aldehydes were detected in high
359 abundance in the vegetal models (V and VE) (Figure 4), as already observed in previous
360 studies[13]. Fermentation reduced aldehydes such as pentanal and nonanal in the V model, and
361 hexanal in VE model. Similar reductions of hexanal and nonanal have been attributed to *S.*
362 *cerevisiae* and *L. plantarum* fermentations of pea protein for 6 and 8 h, respectively[13]. However,
363 fermentation was not able to reduce ketones and pyrazines, specially 2,5-dimethylpyrazine,
364 which contributes to the nutty and cereal-like odor in fermented pea[30]. The alcohols increase
365 observed during fermentation of the vegetal models is in accordance with previous studies[13].
366 The presence in the VE model of methyl branched alcohols (2-methyl and 3-methyl butanol)
367 (Figure 4, Table S8) could be a direct consequence of the large amounts of free amino acids,
368 which made possible the generation of methyl branched alcohols by microbial activity.

369 The impact of the VOCs on the aroma of the fermented models can not be solely determined
370 by the calculation of the odor activity values (OAV). Besides, the extraction method employed
371 (SPME) only allows the comparison of the volatile profile among models and fermentation
372 time, and it requires the application of accurate quantitation methodologies[38]. These limitations
373 were overcome applying a sensory analysis of the models. This analysis revealed that fruitiness
374 and cooked vegetal odors detected in the AE model could be explained by the presence of D-
375 limonene, benzene, acetaldehyde, branched aldehydes and terpinen-4-ol, respectively (Figure
376 5). In the vegetal model (VE), the reduction of the legume and cocoa odor notes, as well as the
377 increase of toasted cereal notes was related to the reduction of aldehydes[29,31]. On the contrary,
378 pyrazines abundance was not affected by fermentation time or enzyme addition, and probably
379 increased the perception of the nutty and cereal-like odor in the vegetal models[30]. In this regard,

recent studies have revealed the potential of plant hydrolysates to simulate the meaty aroma by producing volatile compounds through Maillard reactions[39, 40]. The combination of Maillard reactions and protein hydrolysis, using the same enzyme applied in our study, on wheat and rice[40] and soy[39] revealed similar nutty and toasted aroma notes. These odors were attributed to alkyl pyrazines resulting from the Maillard reaction and derived from the free amino acids generated thorough hydrolysis. Similarly, in our study the presence of alkyl pyrazines was detected at the beginning of the fermentation, therefore their origin can be attributed mainly to the texturization process of pea proteins which employed high pressure and temperatures[29].

In conclusion, the potential of the fermented vegetal models to simulate the meaty aroma should be focused on the elimination not only of the beany compounds but also of the pyrazines producing toasted-cereal like odors. Moreover, the generation of volatiles which could reduce or mask these off-aromas in the vegetal model is largely affected by the level of proteolysis and generation of free amino acids, which are used as volatile precursors by the microbial starters. Finally, the whole food matrix composition and not only the proteins is a source of flavor compounds, therefore the interaction mechanisms between proteins, fat, and volatile compounds will affect flavor perception in plant-based foods[35]. In summary, these models are far from a real food system and the elucidation of the aroma impact of the compounds generated through fermentation of plant proteins should be done through proper quantitation on future developed plant-based foods.

ABBREVIATIONS AND NOMENCLATURE

Solid phase microextraction (SPME), total mesophilic bacteria (TMB), lactic acid bacteria (LAB), Gram positive cocci (GC+), enterobacteria (E), yeasts and molds (YM), volatile organic compounds (VOCs), odor activity values (OAV).

DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interest.

SUPPORTING INFORMATION.

Supplementary tables are included with the composition of animal and vegetal models, identification of volatile compounds, and the data of microbial counts, free amino acid content, and volatile compounds content in the models.

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REFERENCES

1. Flores, M. and Piornos, J.A. Fermented meat sausages and the challenge of their plant-based alternatives: A comparative review on aroma-related aspects. *Meat Sci.*, **2021**, 182, 108636. https://doi.org/10.1016/j.meatsci.2021.108636
2. Hartmann, C & Siegrist, M., Consumer perception and behaviour regarding sustainable protein consumption: A systematic review. *Trends Food Sci. Tech.,* **2017**, 61, 11-25. http://dx.doi.org/10.1016/j.tifs.2016.12.006
3. Fu, Q., Zhao, J., Rong, S., Han, Y., Liu, F., Chu, Q., Wang, S., and Chen, S. Research Advances in Plant Protein-Based Products: Protein Sources, Processing Technology, and Food Applications. *J Agric. Food Chem.* **2023**, 71, 15429−15444.
4. Boukid F. Plant-based meat analogues: from niche to mainstream. *Eur. Food Res. Tech.* **2021**, 247:297–308. https://doi.org/10.1007/s00217-020-03630-9

5.Bohrer, B.M. An investigation of the formulation and nutritional composition of modern meat analogue products. *Food Sci. Human Wellness* **2019**, 8, 320–329. https://doi.org/10.1016/j.fshw.2019.11.006

6. Singh, M., Trivedi, N., Enamala, M. K., Kuppam, C., Parikh, P., Nikolova, M. P., & Chavali, M. Plant-based meat analogue (PBMA) as a sustainable food: A concise review. *Eur. Food Res. Techn.*, **2021**, 247, 2499-2526. https://doi.org/10.1016/j.crfs.2022.06.001

7. Guo, J., He, Z., Wu, S., Zeng, M and Chen J. Effects of concentration of flavor compounds on interaction between soy protein isolate and flavor compounds. *Food Hydrocolloids*, **2020**, 100, 105388. https://doi.org/10.1016/j.foodhyd.2019.105388

8. Zhang, C., Hua, Y., Li, X., Kong, X., Chen, Y.et al.. Key volatile off-flavor compounds in peas (Pisum sativum L.) and their relations with the endogenous precursors and enzymes using soybean (Glycine max) as a reference. *Food Chem.*, **2020**, 333, 127469. https://doi.org/10.1016/j.foodchem.2020.127469.

9. He, J., Liu, H., Balamurugan, S., Shao, S. Fatty acids and volatile flavor compounds in commercial plant-based burgers. *J. Food Sci.,* **2021**, 86, 293-305. https://doi.org/10.1111/1750-3841.15594

10. Inoue, Y., Kato, S., Saikusa, M., Suzuki, C., otsubo, Y., Tanaka, Y., Watanabe, H., Hayase, F. Analysis of the cooked aroma and odorants that contribute to umami aftertaste of soy miso (Japanese soybean paste). *Food Chem.,* **2016**, 213, 521-528. https://doi.org/10.1016/j.foodchem.2016.06.106

11.Boukid F. and Castellari, M. Veggie burgers in the EU market: a nutritional challenge? Eur. Food Res. Tech. **2021**, 247:2445–2453 https://doi.org/10.1007/s00217-021-03808-9

12.El Youssef, C., Bonnarme, P., Fraud, S., Péron, A-C., Henlink, S., and Landaud, S. Sensory Improvement of a Pea Protein-Based Product Using Microbial Co-Cultures of Lactic Acid Bacteria and Yeasts. *Foods*, **2020**, 9, 349. https://doi.org/10.3390/foods9030349

13.Xiang, L., Zhu W., Jiang B., Chen J., Zhou L,. Zhong F. Volatile compounds analysis and biodegradation strategy of beany flavor in pea protein. *Food Chem.*, **2023**, 402, 134275. https://doi.org/10.1016/j.foodchem.2022.134275

14.Meinlschmidt, P., Schweiggert-Weisz, U., Eisner, P. Soy protein hydrolysates fermentation: Effect of debittering and degradation of major soy allergens. *LWT - Food Sci. Tech.*, **2016**, 71, 202–212. https://doi.org/10.1016/j.lwt.2016.03.026

15.Molina, I., & Toldrá, F. Detection of proteolytic activity in microorganisms isolated from dry-cured ham. *J. Food Sci.*, **1992**, 57(6), 1308-1310. https://doi.org/10.1111/j.1365-2621.1992.tb06843.x

16. Perea-Sanz, L., Peris, D., Belloch, C., Flores, M. *Debaryomyces hansenii* metabolism of sulfur amino acids as precursors of volatile sulfur compounds of interest in meat products. *J. Agric. Food Chem.*, **2019**, 67, 9335-9343. https://doi.org/10.1021/acs.jafc.9b03361

17. Belloch, C, Neef, A., Salafia, C., López-Díez, JJ., and Flores, M. Microbiota and volatilome of dry-cured pork loins manufactured with paprika and reduced concentration of nitrite and nitrate. *Food Res, Int*., **2021**, 149, 110691.

18. Van den Dool, H., & Dec Kratz, P. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *J. Chrom. A*, **1963**, 11, 463–471. https://doi.org/10.1016/S0021-9673(01)80947-X.

19.Aristoy, M. C., & Toldrá, F. Deproteinization techniques for HPLC amino acid analysis in fresh pork muscle and dry-cured ham. *J Agric. Food Chem.* **1991**, 39, 1792–1795. https://doi.org/10.1021/jf00010a020

20.Flores, M., Aristoy, M. C., Spanier, A. M., & Toldrá, F. Non-volatile components effects on quality of "Serrano" dry-cured ham as related to processing time. *J. Food Sci.*, **1997**, 62(6), 1235–1239. https://doi.org/10.1111/j.1365-2621.1997.tb12252.x

21.Pollien, P., Ott, A., Montigon, F., Baumgartner, M., Muñoz-Box, R. & Chaintreau, A. Hyphenated headspace-gas chromatography-sniffing technique: screening of impact odorants and quantitative aromagram comparisons. *J Agric. Food Chem.* **1997**, 45, 2630–2637. https://doi.org/10.1021/jf960885r

22. Belloch, C, Perea-Sanz, L, Gamero, A, Flores, M. Selection of Debaryomyces hansenii isolates as starters in meat products based on phenotypic virulence factors, tolerance to abiotic stress conditions and aroma generation. *J. Appl. Microbiol*. **2022**, 00:1–12. https://doi.org/10.1111/jam.15454

23.Campo, E., Do, B.V., Ferreira, V. & Valentin, D. Aroma properties of young Spanish monovarietal white wines: a study using sorting task, list of terms and frequency of citation. *Aust. J. Grape Wine Res.*, **2008**, 14, 104–115. https://doi.org/10.1111/j.1755-0238.2008.00010.x

24.Flores, M., Corral, S., Cano-García, L., Salvador, A., and Belloch, C. Yeast strains as potential aroma enhancers in dry fermented sausages. *Int. J. Food Microbiol.* **2015**, 212, 16–24. https://doi.org/10.1016/j.ijfoodmicro.2015.02.028

25.Wang, H., Li, Y., Xia, X., Liu, Q., Sun, F., Kong, B. Flavour formation from hydrolysis of pork meat protein extract by the protease from Staphylococcus carnosus isolated from Harbin dry sausage. *LWT - Food Sci. Tech.,* **2022a** 163, 113525. https://doi.org/10.1016/j.lwt.2022.113525

26.Wang, H., Li, Y., Xia, X., Liu, Q., Sun, F., Kong, B. Interaction between protease from Staphylococcus epidermidis and pork myofibrillar protein: Flavor and molecular simulation. *Food Chem.*, **2022b**, 386, 132830. https://doi.org/10.1016/j.foodchem.2022.132830.

27.Gorissen, SHM., Crombag, JJR., Senden, JMG., Waterval, WAH., Bierau, J., Verdijk, LB., van Loon, LJK. Protein content and amino acid composition of commercially available

496 plant-based protein isolates. *Amino Acids*, **2018**, 50:1685–1695.
497 https://doi.org/10.1007/s00726-018-2640-5
498 28. Li, L., Belloch C., and Flores, M. A comparative study of savory and toasted aromas in dry
499 cured loins versus dry fermented sausages. LWT-Food Sci. Tech., **2023**, 173, 114305.
500 https://doi.org/10.1016/j.lwt.2022.114305
501 29. Ebert, S., Michel, W., Nedele, A-K., Baune, M-C., Terjung, N., Zhang, Y., Gibis, M., Weiss,
502 J. Influence of protein extraction and texturization on odor-active compounds of pea
503 proteins. *J. Sci. Food Agric.,* **2022**, 102: 1021–1029. https://doi.org/10.1002/jsfa.11437
504 30.Schindler, S., Zelena, K., Krings, U., Bez, J., Eisner, P., and Berger, RF. Improvement of
505 the Aroma of Pea (Pisum sativum) Protein Extracts by Lactic Acid Fermentation. *Food*
506 *Biotech.,* **2012**, 26, 58-74. https://doi.org/10.1080/08905436.2011.645939
507 31.Fischer, E., Cayot, N., & Cachon, R. Potential of microorganisms to decrease the "beany"
508 off-flavor: A review. *J Agric. Food Chem.*, **2022**, 70, 4493–4508.
509 https://doi.org/10.1021/acs.jafc.1c07505
510 32.Murat, Ch., Bard, MH., Dhalleine, C., Cayot, N. Characterisation of odour active
511 compounds along extraction process from pea flour to pea protein extract. *Food Res. Int.,*
512 **2012**, 53, 31–41. https://doi.org/10.1016/j.foodres.2013.03.049
513 33.Utz, F.; Spaccasassi, A.; Kreissl, J.; Stark, T.D.; Tanger, C.; Kulozik, U.; Hofmann, T.;
514 Dawid, C. Sensomics-Assisted Aroma Decoding of Pea Protein Isolates (Pisum sativum L.).
515 *Foods*, **2022**, 11, 412. https://doi.org/10.3390/foods11030412
516 34.Benavides-Paz, YL., Ismail, BP., and Reineccius, GA. Monitoring the aroma profile during
517 the production of a pea protein isolate by salt solubilization coupled with membrane
518 filtration. *ACS Food Sci. & Tech.*, **2022**, 2, 280−289
519 https://doi.org/10.1021/acsfoodscitech.1c00384

520 35.Roland, W. S. U., Pouvreau, L., Curran, J., van de Velde, F., & de Kok, P. M. T. Flavor
521 aspects of pulse ingredients. *Cereal Chem. J.*, **2017**, 94, 58–65.
522 https://doi.org/10.1094/CCHEM-06-16-0161-FI
523 36.Kimura, Y., Aoki, R., Takayama, Y., Suzuki, C and Suzuki, Y. Quantification of Functional
524 Aromatic Amino Acid Metabolites in Fermented Foods and Their Production by Food
525 Microorganisms. *Food Sci. Tech. Res.*, **2020**, 26, 79-92. https://doi.org/10.3136/fstr.26.79
526 37.Flores M & Olivares A. Flavor (Chap. 25). In: *Handbook of Fermented Meat and Poultry*,
527 Second Edition. Edited by Fidel Toldrá. John Wiley & Sons, Ltd., **2015**, pp 217-225.
528 38.Jelén, HH. & Wieczorek, MN. Commentary: "Quantitative" vs quantitative Headspace
529 Solid-Phase Microextraction (HS-SPME) in food volatile and flavor compounds analysis. *J.*
530 *Food Comp. Anal.* **2023**, 115, 104955. https://doi.org/10.1016/j.jfca.2022.104955
531 39.Kim, Y-H., Kim, M-J., Oh, W-Y., Lee, J-H. Antioxidant effects and reaction volatiles from
532 heated mixture of soy protein hydrolysates and coconut oil. *Food Sci. Biotech.*, **2023**, 32,
533 309–317. https://doi.org/10.1007/s10068-022-01189-7
534 40.Chiang, J-H., Yun Yeo, M-T., Min Ong, D-S., Henry, C-J. Comparison of the molecular
535 properties and volatile compounds of Maillard reaction products derived from animal- and
536 cereal-based protein hydrolysates. *Food Chem.*, **2022**, 383, 132609.
537 https://doi.org/10.1016/j.foodchem.2022.132609
538

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FIGURE CAPTIONS

Figure 1. Effect of fermentation time and addition of enzyme on pH and microbial counts (log cfu/g) of the vegetal and animal models. The results from the animal models are in Figures A (without enzyme, A model) and B (with enzyme, AE model). The results from the vegetal models are in Figures C (without enzyme, V) and D (with enzyme, VE model). Symbols represent pH (□), TMB (△), LAB (●), GC+ (○) and Y&M (▼). Details about the individual variables and ANOVA results of the fermentation time and enzyme effects on the models are reported in Tables S2 and S3.

Figure 2. Evolution of total free amino acids content (mg/g protein) in animal and vegetal models. Figures: A, animal model without enzyme (A, ●) and animal model with enzyme (AE, ○), and B, vegetal model without enzyme (V, ●) and vegetal model with enzyme (VE, ○).

Figure 3. Abundance (AU x 10^5/g protein) of volatile compounds summarized by chemical group detected in the headspace of the animal and vegetal models along the fermentation. Figures: A (animal model without enzyme, A), B (animal model with enzyme, AE), C (vegetal model without enzyme, V) and D (vegetal model with enzyme, VE). Compounds: aldehydes (●), alcohols (○), esters (▼), alkanes (△), ketones (■), pyrazines (□), other (◊).

Figure 4. Heatmap representing the volatile profile of the animal and vegetal models during the fermentation. Samples: animal models without (A) and with enzyme (AE) and vegetal models without (V) and with enzyme (V). Numbers in the samples represent fermentation time in days. Colors in the heatmap indicate relative abundance of each volatile compound: blue, relatively high abundance; red, relatively low abundance.

Figure 5. Principal component analysis showing the relationship among variables, microbial counts, pH, free amino acids and volatile compounds, and the animal and vegetal models along the fermentation. Animal models are represented by samples A (without enzyme) and AE (with

567 enzyme, whereas vegetal models are represented by samples V (without enzyme) and V (with
568 enzyme). The numbers in the models represent the fermentation time in days of the samples.
569 **Figure 6.** Odor profile of the animal and vegetal models after 15 d of incubation. Animal
570 models are represented by A (blue) and AE (orange) lines, whereas vegetal models are
571 represented by V (gray) and VE (yellow) lines.
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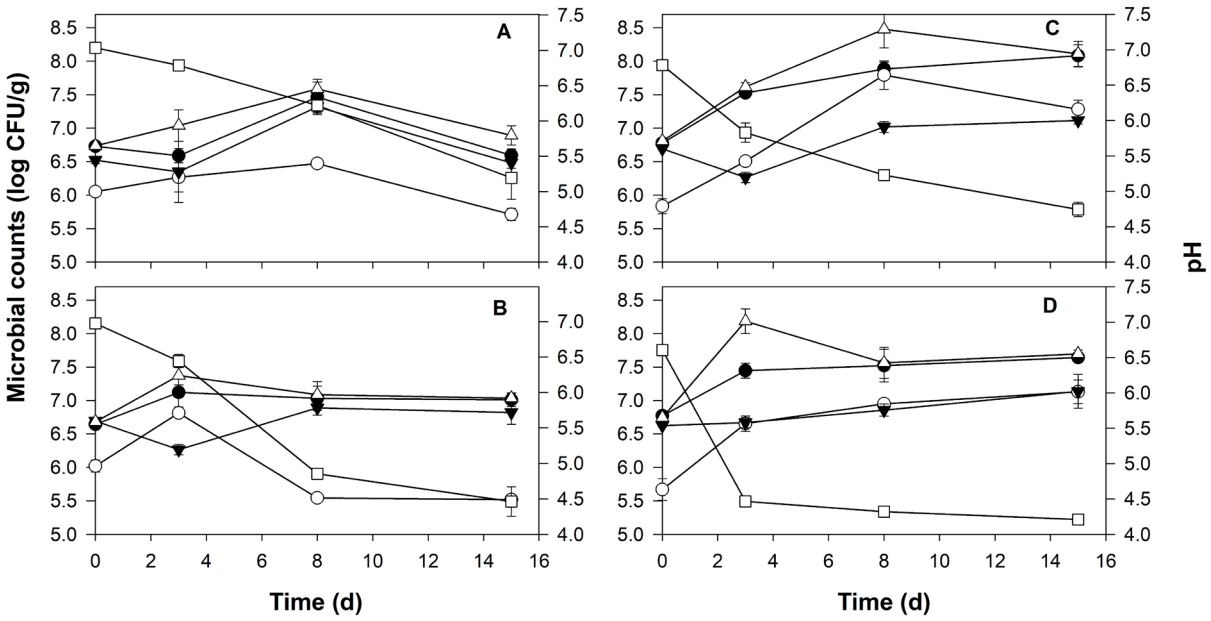
Figure 1.



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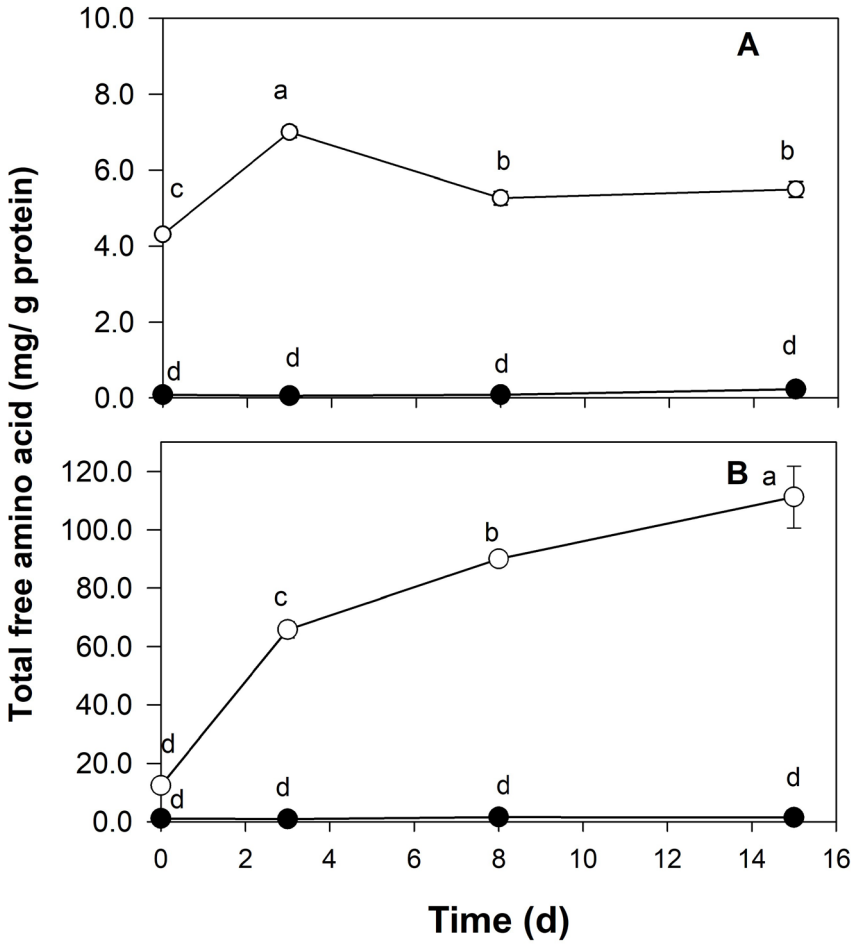

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591 **Figure 3.**

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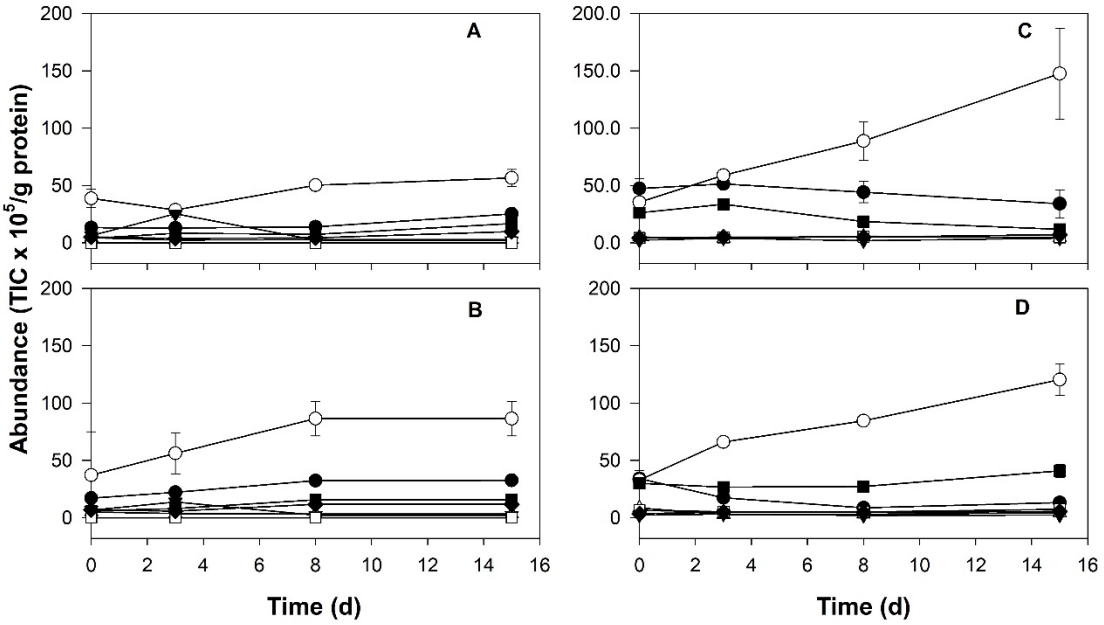

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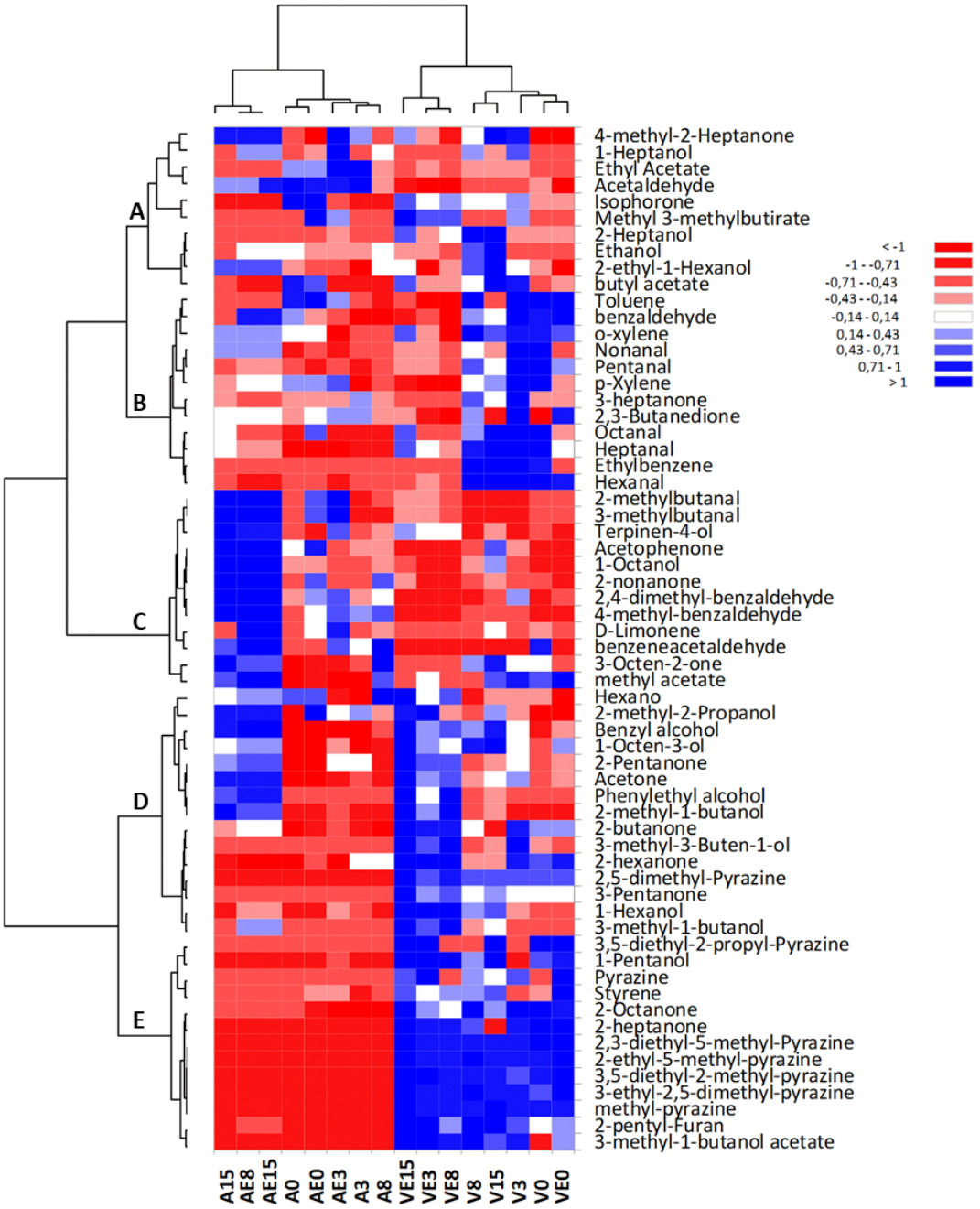

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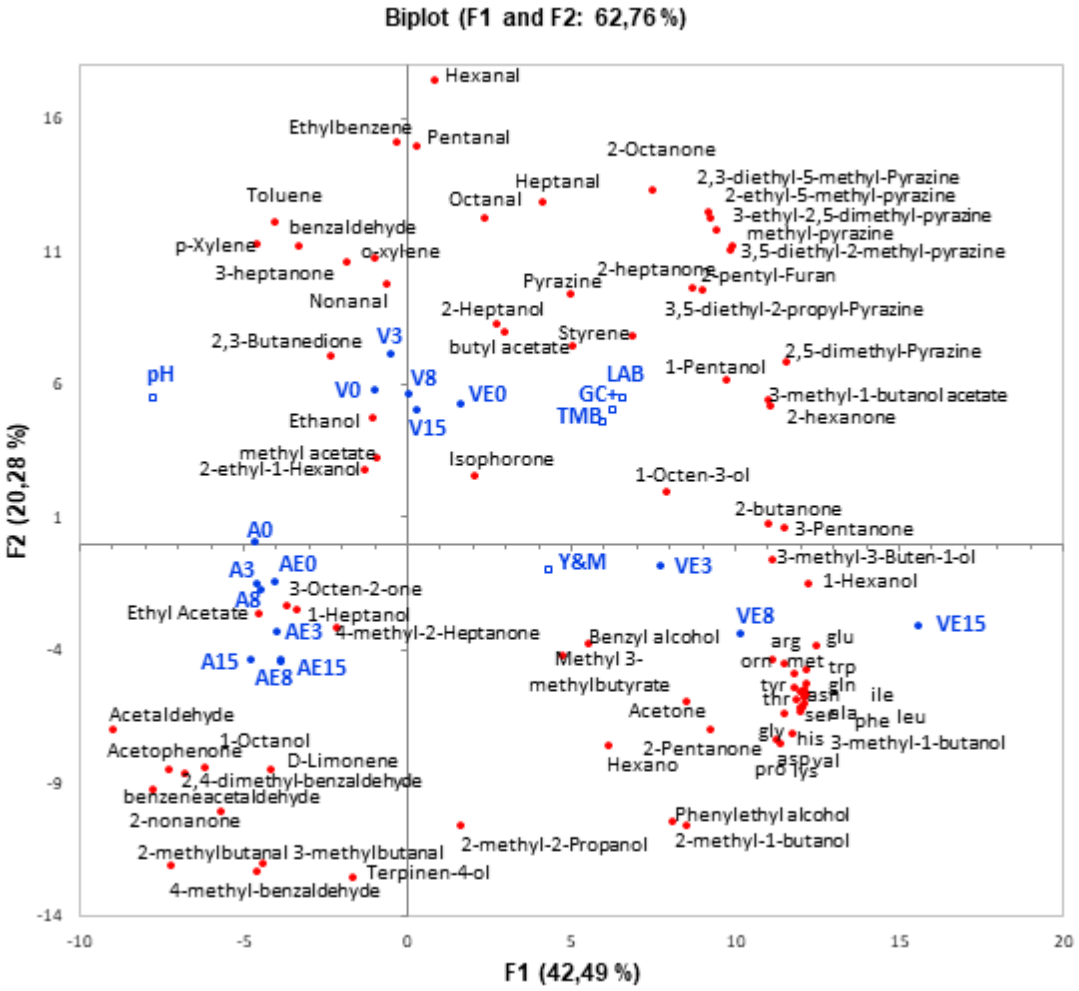

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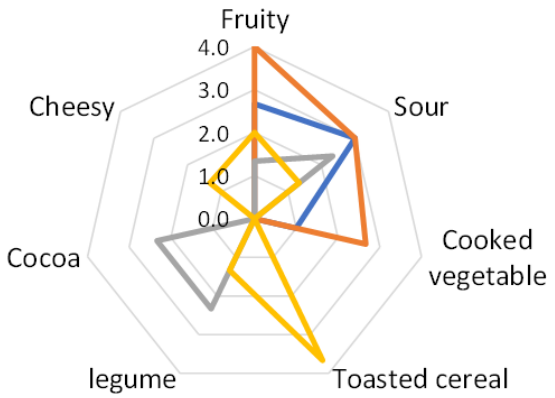
606 **Figure 6.**



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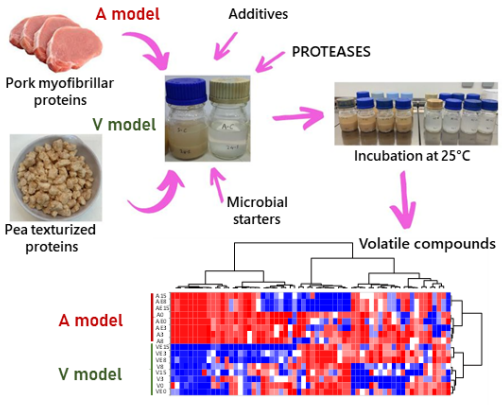
610 **Graphic for table of contents (TOC)**



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Supplementary Tables

Manuscript title: Fermentation of texturized pea protein in combination with proteases for aroma development in meat analogues

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Table S1. Composition of animal (myofibrillar pork protein) and vegetal (texturized pea protein) models.

	Models			
Composition (g/100ml)	**Animal A**	**Animal + Enzyme AE**	**Vegetal V**	**Vegetal + Enzyme VE**
Myofibrillar pork protein	8	8	-	-
Texturized pea protein			8	8
NaCl	3	3	3	3
Glucose	2	2	2	2
$NaNO_2$	0.015	0.015	0.015	0.015
KNO_3	0.015	0.015	0.015	0.015
Starter TRADI-302	0.0125	0.0125	0.0125	0.0125
D. hansenii (L5) (cells/ml)	10^6	10^6	10^6	10^6
Protease (Flavourzyme) (g/g protein)		0.02		0.02

Table S2. Microbial counts (log cfu/g) and pH from the fermentative process of the animal models without and with proteolytic enzyme. Samples were taken at 0, 3, 8 and 15 days of incubation.

	Animal model without enzyme								Animal model with enzyme											
	A0[1]		**A3**		**A8**		**A15**		**AE0**		**AE3**		**AE8**		**AE15**		**RMSE[2]**	***P*$_t$[3]**	***P*$_E$**	***P*$_{txE}$**
PCA	6.74	bc	7.04	abc	7.59	a	6.89	abc	6.68	c	7.4	ab	7.09	abc	7.04	abc	0.24	**	ns	ns
LAB	6.73	b	6.59	b	7.46	a	6.60	b	6.64	b	7.12	ab	7.03	ab	7.01	ab	0.24	**	ns	**
GC+	6.05	bcd	6.27	bc	6.47	ab	5.71	de	6.02	cd	6.81	a	5.54	e	5.52	e	0.15	***	*	***
YM	6.52	ab	6.35	c	7.32	a	6.48	ab	6.69	ab	6.27	b	6.89	ab	6.82	ab	0.31	**	ns	*
pH	7.04	a	6.79	ab	6.22	b	5.19	c	6.97	a	6.44	ab	4.85	cd	4.46	d	0.23	***	***	***

[1]Animal models containing myofibrillar proteins without (A) and with (AE) enzyme at 0, 3, 8 and 15 d of incubation. [2]RMSE: root mean square of the errors. [3]*Pf*: *P* value of the time effect, P_E*: P* value of enzyme effect, P_{txE}*: P* value of interaction between time and enzyme effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.5$; ns: $P > 0.05$. [4]Different letters in the same row indicate significant differences among models and sampling times.

Table S3. Microbial counts (log cfu/g) and pH from the fermentative process of the vegetal models without and with proteolytic enzyme. Samples were taken at 0, 3, 8 and 15 days of incubation.

	Vegetal model without enzyme								Vegetal model with enzyme											
	V0[1]		**V3**		**V8**		**V15**		**VE0**		**VE3**		**VE8**		**VE15**		**RMSE[2]**	***P*$_t$[3]**	***P*$_E$**	***P*$_{txE}$**
PCA	6.81	cd	7.62	bc	8.48	a	8.11	ab	6.8	d	8.2	ab	7.6	bcd	7.7	ab	0.29	***	ns	**
LAB	6.78	c	7.53	ab	7.88	ab	8.08	a	6.77	c	7.45	b	7.52	ab	7.64	ab	0.21	***	*	ns
GC+	5.84	de	6.51	cd	7.79	a	7.28	ab	5.67	e	6.65	bc	6.95	bc	7.13	abc	0.24	***	*	*
YM	6.69	ab	6.27	b	7.02	a	7.12	a	6.63	ab	6.67	ab	6.86	a	7.14	a	0.19	***	ns	ns
pH	6.78	a	5.83	b	5.23	c	4.74	d	6.61	a	4.47	de	4.32	e	4.21	e	0.12	***	***	***

[1]Vegetal models containing texturized pea protein without (V) and with (VE) enzyme at 0, 3, 8 and 15 d of incubation. [2]RMSE: root mean square of the errors. [3]*Pf*: *P* value of the time effect, P_E*: P* value of enzyme effect, P_{txE}*: P* value of interaction between time and enzyme effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.5$; ns: $P > 0.05$. [4]Different letters in the same row indicate significant differences among models and sampling times.

Table S4. Free amino acids content (mg/g protein) in the animal model without and with proteolytic enzyme. Samples were taken at 0, 3, 8 and 15 days of incubation.

	Animal model without enzyme				Animal model with enzyme							
	A0[1]	A3	A8	A15	AE0	AE3	AE8	AE15	RMSE	*Pt*	*PE*	*P txE*
Asp	0.01 d	0.01 d	0.00 d	0.01 d	0.12 c	0.22 a	0.17 b	0.11 c	0.02	***	***	***
Glu	0.00	0.00	0.00	0.02	0.08	0.16	0.08	0.08	0.067	ns	**	ns
Ser	0.00 e	0.00 e	0.00 e	0.00 e	0.16 b	0.24 a	0.11 c	0.04 d	0.013	***	***	***
Asn	0.00 e	0.00 e	0.00 e	0.00 e	0.18 b	0.28 a	0.13 c	0.05 d	0.013	***	***	***
Gly	0.00 d	0.00 d	0.00 d	0.00 d	0.07 c	0.15 a	0.10 b	0.11 b	0.012	***	***	***
Gln	0.00 d	0.00 d	0.00 d	0.00 d	0.24 b	0.36 a	0.13 c	0.08 c	0.02	***	***	***
His	0.00	0.00	0.00	0.00	0.05	0.11	0.08	0.11	0.040	ns	***	ns
Thr	0.07 b	0.05 b	0.07 b	0.16 ab	0.24 ab	0.33 a	0.23 ab	0.15 ab	0.068	ns	***	**
Ala	0.00 c	0.00 c	0.00 c	0.02 c	0.22 b	0.58 a	0.41 a	0.48 a	0.063	***	***	**
Arg	0.00 b	0.00 b	0.00 b	0.00 b	0.36 a	0.34 a	0.00 b	0.15 ab	0.112	*	***	*
Pro	0.00 c	0.00 c	0.00 c	0.00 c	0.05 c	0.17 b	0.34 a	0.36 a	0.032	***	***	***
Tyr	0.00 c	0.00 c	0.00 c	0.00 c	0.26 b	0.38 a	0.00 c	0.00 c	0.022	***	***	***
Val	0.00 d	0.00 d	0.00 d	0.00 d	0.33 c	0.57 b	0.60 b	0.66 a	0.014	***	***	***
Met	0.00 c	0.00 c	0.00 c	0.03 bc	0.16 a	0.16 a	0.08 abc	0.09 ab	0.031	ns	***	*
Ile	0.00 d	0.00 c	0.00 d	0.00 d	0.37 c	0.65 b	0.71 a	0.72 a	0.018	***	***	***
Leu	0.00 d	0.00 c	0.00 d	0.00 d	0.69 c	1.13 a	1.04 b	1.07 b	0.019	***	***	***
Phe	0.00 b	0.00 b	0.00 b	0.00 b	0.49 a	0.61 a	0.51 a	0.41 a	0.071	ns	***	ns
Trp	0.00 b	0.00 b	0.00 b	0.00 b	0.03 ab	0.01 ab	0.00 b	0.27 a	0.090	ns	*	ns
Orn	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b	0.06 ab	0.11 a	0.13 a	0.031	*	***	*
Lys	0.00 c	0.00 c	0.00 c	0.00 c	0.22 b	0.51 a	0.45 a	0.44 a	0.027	***	***	***
Total aac	0.08 d	0.06 d	0.08 d	0.23 d	4.31 c	7.00 a	5.27 b	5.50 b	0.189	***	***	***

[1]Animal models containing myofibrillar proteins without (A) and with (AE) enzyme at 0, 3, 8 and 15 d of incubation. [2]RMSE: root mean square of the errors. [3]*Pf*: *P* value of the time effect, P_E: *P* value of enzyme effect, P_{txE}: *P* value of interaction between time and enzyme effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.5$; ns: $P > 0.05$. [4]Different letters in the same row indicate significant differences among models and times.

Table S5. Free amino acids content (mg/g protein) in the vegetal model without and with proteolytic enzyme. Samples were taken at 0, 3, 8 and 15 days of incubation.

	Vegetal model without enzyme				Vegetal model with enzyme							
	V0	**V3**	**V8**	**V15**	**VE0**	**VE3**	**VE8**	**VE15**	**RMSE**	**Pt**	**PE**	**PtxE**
Asp	0.03 b	0.06 b	0.06 b	0.04 b	0.06 b	0.36 a	0.53 a	0.60 a	0.086	***	***	***
Glu	0.45 c	0.36 c	0.47 c	0.45 c	0.60 c	3.17 b	4.28 a	5.07 a	0.320	***	***	***
Ser	0.00 d	0.00 d	0.01 d	0.01 d	0.36 d	1.79 c	2.38 b	2.84 a	0.147	***	***	***
Asn	0.18 e	0.03 e	0.03 e	0.01 e	0.59 d	1.25 c	1.61 b	2.00 a	0.086	***	***	***
Gly	0.01 d	0.03 d	0.05 d	0.03 d	0.10 d	0.68 c	1.08 b	1.59 a	0.058	***	***	***
Gln	0.00 c	0.02 c	0.04 c	0.01 c	0.80 b	1.85 a	2.03 a	2.05 a	0.081	***	***	***
His	0.00 b	0.01 b	0.02 b	0.07 b	0.14 b	0.51 a	0.55 a	0.59 a	0.077	***	***	**
Thr	0.14 d	0.13 d	0.28 d	0.14 d	0.36 d	1.38 c	2.29 b	3.11 a	0.140	***	***	***
Ala	0.03 d	0.03 d	0.08 d	0.08 d	0.56 d	3.09 c	3.96 b	4.73 a	0.254	***	***	***
Arg	0.00 d	0.00 d	0.00 d	0.00 d	0.92 c	2.19 a	2.05 ab	1.66 b	0.151	***	***	***
Pro	0.02 b	0.01 b	0.10 b	0.07 b	0.18 b	0.66 a	0.89 a	1.08 a	0.155	***	***	**
Tyr	0.01 b	0.00 b	0.02 b	0.00 b	0.62 b	4.55 a	5.52 a	5.04 a	0.356	***	***	***
Val	0.02 c	0.00 c	0.01 c	0.04 c	0.78 c	5.63 b	7.31 ab	9.21 a	0.857	***	***	***
Met	0.13 d	0.10 d	0.02 d	0.03 d	0.12 d	0.56 c	0.92 b	1.22 a	0.088	***	***	***
Ile	0.00 c	0.00 c	0.06 c	0.05 c	1.09 c	7.08 b	9.63 ab	12.51 a	1.024	***	***	***
Leu	0.02 d	0.01 d	0.02 d	0.06 d	2.72 d	17.00 c	24.06 b	30.61 a	1.773	***	***	***
Phe	0.03 d	0.01 d	0.09 d	0.04 d	1.84 d	11.17 c	16.91 b	22.11 a	1.493	***	***	***
Trp	0.03 c	0.02 c	0.06 c	0.08 c	0.22 c	0.71 b	1.05 b	1.53 a	0.119	***	***	***
Orn	0.00 c	0.07 bc	0.16 bc	0.18 bc	0.00 c	0.18 bc	0.49 ab	0.79 a	0.153	***	***	*
Lys	0.01 e	0.00 e	0.01 e	0.04 de	0.29 d	1.97 c	2.45 b	2.92 a	0.097	***	***	***
Total aac	1.11 d	0.88 d	1.57 d	1.45 d	12.35 d	65.78 c	89.98 b	111.25 a	6.553	***	***	***

[1] Vegetal models containing texturized pea protein without (V) and with (VE) enzyme at 0, 3, 8 and 15 d of incubation. [2]RMSE: root mean square of the errors. [3]*Pf*: *P* value of the time effect, P_E: *P* value of enzyme effect, P_{txE}: *P* value of interaction between time and enzyme effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.5$; ns: $P > 0.05$. [4]Different letters in the same row indicate significant differences among models and times.

Table S6. Volatile compounds identified in the headspace of the animal and vegetal models. Animal models contain myofibrillar proteins without (A) and with (AE) enzyme. Vegetal models contain texturized pea protein without (V) and with (VE) enzyme.

	Compound	tr[1] (min)	LRI DB 624[2]	LRI std DB624[2]	RI[3]	Models A - AE	Models V - VE
	Aldehydes						
1	Acetaldehyde	2.13	469	466	a	s[4]	s
2	3-methylbutanal	11.02	690	687	a	s	s
3	2-methylbutanal	11.72	701	698	a	s	s
4	Pentanal	15.35	738	736	a	s	s
5	Hexanal	24.53	841	839	a	s	s
6	Heptanal	31.92	942	939	a	s	s
7	Octanal	39.41	1047	1044	a	s	s
8	Nonanal	46.27	1150	1148	a	s	s
9	Benzaldehyde	37.17	1017	1013	a	s	s
10	benzeneacetaldehyde	43.99	1109	1104	a	s	s
11	4-methyl-benzaldehyde	46.12	1148	-	b	s	s
12	2,4-dimethyl-benzaldehyde	52.79	1292	-	b	s	s
	Alcohols						
13	Ethanol	3.11	508	507	a	s	s
14	2-methyl-2-Propanol	4.62	569	-	b	s	s
15	3-methyl-3-Buten-1-ol	20.40	790	787	a	n	s
16	3-methyl-1-butanol	20.89	795	793	a	s	s
17	2-methyl-1-butanol	21.11	798	795	a	s	s
18	1-Pentanol	23.47	828	823	a	s	s
19	1-Hexanol	30.77	925	921	a	s	s
20	1-Heptanol	37.63	1024	1021	a	s	s
21	1-Octen-3-ol	38.12	1030	1028	a	s	s
22	2-Heptanol	32.40	949	947	a	s	s
23	Benzyl alcohol	44.70	1122	1120	a	s	s
24	1-Octanol	44.89	1126	1123	a	s	s
25	Phenylethyl alcohol	48.71	1195	1191	a	s	s
26	2-ethyl-1-Hexanol	42.21	1083	1083	a	s	s
	Ester compounds						
27	Methyl acetate	4.18	551	549	a	s	s
28	Ethyl Acetate	7.58	635	635	a	s	s
29	Methyl 3-methylbutirate	21.79	806	804	a	s	s
30	Butyl acetate	25.05	848	846	a	s	s
31	3-methyl-1-butanol acetate	29.56	907	905	a	n	s
	Alkanes						
32	Hexano	5.40	600	600	a	s	s
33	Toluene	20.18	788	790	a	s	s
34	Ethylbenzene	27.78	883	881	a	n	s
35	p-Xylene	28.42	891	893	a	s	s
36	o-xylene	30.24	917	915	a	s	s
37	Styrene	30.39	919	921	a	s	s

	Ketones						
38	Acetone	3.65	530	527	a	s	s
39	2,3-Butanedione	7.08	627	624	a	s	s
40	2-butanone	7.35	631	629	a	s	s
41	2-Pentanone	14.89	733	731	a	s	s
42	3-Pentanone	15.63	741	740	a	n	s
43	2-hexanone	24.12	836	835	a	s	s
44	3-heptanone	30.98	928	-	b	s	s
45	2-heptanone	31.49	936	933	a	s	s
46	4-methyl-2-Heptanone	34.56	981	-	b	s	s
47	2-Octanone	38.77	1038	1034	a	s	s
48	2-nonanone	45.79	1142	1139	a	s	s
49	3-Octen-2-one	43.13	1095	1094	a	s	s
50	2-pentyl-Furan	36.52	1009	1009	a	s	s
	Other compounds						
51	D-Limonene	39.15	1043	1046	a	s	s
52	Acetophenone	45.30	1133	1134	a	s	s
53	Isophorone	49.13	1203	1207	a	s	s
54	Terpinen-4-ol	50.18	1229	1228	a	s	s
	Pyrazines						
55	Pyrazine	18.63	772	772	a	n	s
56	Methyl-pyrazine (94)[5]	26.00	860	860	a	n	s
57	2,5-dimethyl-Pyrazine (108)	32.06	944	943	a	n	s
58	2-ethyl-5-methyl-pyrazine (121)	38.46	1034	1033	a	n	s
59	3-ethyl-2,5-dimethyl-pyrazine (135)	44.00	1109	1109	a	n	s
60	2,3-diethyl-5-methyl-Pyrazine (150)	48.08	1183	-	b	n	s
61	3,5-diethyl-2-methyl-pyrazine (150)	48.35	1188	-	b	n	s
62	3,5-diethyl-2-propyl-Pyrazine (122)	48.56	1192	-	b	n	s

[1]Tr: retention time, [2]LRI: Linear retention indices of the compounds (LRI DB624) or standards (LRI-std) eluted from GC-MS using a DB-624 capillary column. [3]Reliability of identification: a, identification by mass spectrum and by coincidence with the LRI of an authentic standard; b, tentatively identification by mass spectrum. [4](s) present in model, (n) absent in model. [5]Target ion (m/z in parenthesis) used to quantify the compound when the peak was not completely resolved.

Table S7. Volatile compounds content (AU 10^{-5}/g protein) in the headspace of the animal models (containing myofibrillar proteins) without and with proteolytic enzyme. Samples were taken at 0, 3, 8 and 15 days of incubation.

	Animal model without enzyme								Animal model with enzyme											
	A0[1]		A3		A8		A15		AE0		AE3		AE8		AE15		RMSE[2]	P_t[3]	P_E	P_{txE}
Aldehydes																				
Acetaldehyde	1.39		1.47		0.72		0.71		0.85		1.01		0.77		0.97		0.33	*ns*	*ns*	*ns*
3-methylbutanal	0.06	bc[4]	0.00	c	0.00	c	3.96	a	2.04	abc	3.79	a	3.05	abc	3.22	ab	0.93	*	***	*
2-methylbutanal	0.00	bc	0.00	b	0.43	ab	3.46	a	1.54	ab	3.25	a	2.79	a	3.00	a	0.70	*	***	*ns*
Pentanal	0.05	ab	0.00	b	0.23	ab	0.22	ab	0.00	b	0.24	ab	0.30	a	0.26	ab	0.06	*	*ns*	*ns*
Hexanal	1.30		2.49		2.25		1.70		1.29		1.03		1.10		1.10		0.65	*ns*	*	*ns*
Heptanal	0.23	b	0.29	ab	0.26	ab	0.59	a	0.16	b	0.22	b	0.47	ab	0.50	ab	0.10	**	*ns*	*ns*
Octanal	0.14		0.21		0.29		0.74		0.75		0.29		0.38		0.36		0.16	*ns*	*ns*	*ns*
Nonanal	0.58	b	0.82	b	1.34	b	2.64	a	1.02	b	0.45	b	2.66	a	2.66	a	0.76	**	*ns*	*ns*
Benzaldehyde	7.03	ab	3.61	b	3.68	b	5.16	ab	6.00	ab	5.19	ab	8.33	a	8.33	a	1.31	*ns*	*	*
Benzeneacetaldehyde	0.91		2.46		3.83		2.05		0.00		2.09		4.29		4.51		1.44	*ns*	*ns*	*ns*
4-methyl-benzaldehyde	0.90	c	2.53	abc	3.05	abc	5.10	ab	2.08	bc	2.98	abc	5.97	a	5.97	a	1.15	***	*	*ns*
2,4-dimethyl-benzaldehyde	1.20	b	1.03	b	1.32	ab	4.10	a	1.80	ab	2.17	ab	3.19	a	3.19	ab	0.85	**	*ns*	*ns*
Alcohols																				
Ethanol	6.78		5.12		7.61		4.28		6.54		5.78		10.46		9.56		6,15	ns	ns	ns
2-methyl-2-Propanol	0.00	e	0.23	c	0.17	d	0.29	b	0.31	a	0.19	d	0.30	ab	0.30	ab	0.00	***	***	***
3-methyl-3-Buten-1-ol																				
3-methyl-1-butanol	0.00	b	0.29	b	0.50	b	0.23	b	0.00	b	0.91	b	5.50	a	5.76	a	0.77	***	***	***
2-methyl-1-butanol	0.00	b	0.00	b	0.00	b	1.57	a	0.00	b	0.72	ab	1.56	a	1.63	a	0.26	***	***	**
1-Pentanol	0.00	b	0.00	b	0.00	b	0.01	b	0.00	b	0.32	a	0.00	b	0.00	b	0.02	***	***	***
1-Hexanol	1.50		0.64		0.50		0.78		0.39		3.93		3.61		3.52		1,36	*ns*	*	*ns*
1-Heptanol	0.00		0.00		6.99		3.55		5.27		29.25		12.18		9.85		10,48	*ns*	*	*ns*
1-Octen-3-ol	1.19	de	1.38	de	1.79	cd	3.07	ab	0.76	e	2.49	bc	3.38	a	3.38	a	0.26	***	***	***
2-Heptanol	0.44	ab	0.24	c	0.21	c	0.25	bc	0.27	bc	0.48	a	0.33	abc	0.33	abc	0.04	*ns*	*	**
Benzyl alcohol	0.00	b	0.48	b	0.81	b	1.96	a	0.00	b	0.00	b	2.28	a	2.40	a	0.22	***	**	**
1-Octanol	3.46	b	2.53	b	3.49	b	8.83	a	3.43	b	2.68	b	8.30	a	8.33	a	1.05	***	*ns*	**
Phenylethyl alcohol	0.00	b	0.00	b	0.21	b	4.59	a	0.00	b	0.00	b	6.48	a	6.57	a	0.53	***	***	***
2-ethyl-1-Hexanol	26.55		18.32		28.81		33.35		22.70		24.42		33.61		33.61		6.45	*	*ns*	*ns*
Esthers comp																				

Methyl acetate	0.33	b	0.49	ab	1.11	ab	1.10	ab	0.56	ab	0.35	b	1.42	a	1.42	a	0.30	***	*ns*	*ns*
Ethyl Acetate	5.55	c	24.84	a	1.10	c	0.65	c	5.18	c	13.16	b	0.57	c	0.57	c	1.71	***	***	***
Methyl 3-methylbutyrate	0.00	b	0.00	b	0.00	b	0.04	b	0.72	a	0.12	b	0.00	b	0.00	b	0.07	***	***	***
Butyl acetate	0.44	a	0.20	ab	0.17	b	0.24	ab	0.40	ab	0.18	ab	0.17	b	0.17	b	0.07	**	*ns*	*ns*
3-methyl-1-butanol acetate																				
Alkanes																				
Hexane	0.37		0.41		0.51		0.32		0.27		0.18		0.33		0.37		0.24	*ns*	*ns*	*ns*
Toluene	3.65	a	2.22	b	2.03	b	2.32	ab	4.09	a	3.11	ab	2.38	b	2.38	b	0.67	**	*ns*	*ns*
Ethylbenzene																				
p-Xylene	0.28		0.19		0.23		0.24		0.27		0.30		0.26		0.26		0.07	*ns*	*ns*	*ns*
o-xylene	0.15	a	0.09	b	0.13	a	0.17	a	0.14	a	0.00	b	0.18	a	0.17	a	0.02	***	*ns*	**
Styrene	0.25	b	0.00	d	0.11	c	0.30	b	0.57	a	0.49	a	0.21	bc	0.21	bc	0.01	***	***	***
Ketones																				
Acetone	0.23	b	1.65	b	0.76	b	4.85	a	0.25	b	1.24	b	4.45	a	4.45	a	0.49	***	**	***
2,3-Butanedione	0.63		0.80		0.56		0.67		0.64		0.79		0.72		0.72		0.23	*ns*	*ns*	*ns*
2-butanone	0.70	d	1.21	cd	0.80	d	2.03	ab	1.07	cd	1.62	bc	2.20	a	2.20	a	0.17	***	***	***
2-Pentanone	0.00	c	0.84	ab	0.42	bc	1.16	a	0.00	c	0.90	ab	1.22	a	1.23	a	0.15	***	**	**
3-Pentanone																				
2-hexanone	0.27		1.17		1.03		0.39		0.62		0.22		0.30		0.29		0.40	*ns*	*ns*	*ns*
3-heptanone	0.89		1.03		0.51		0.00		0.37		0.75		0.12		0.17		0.33	*ns*	*ns*	*ns*
2-heptanone	0.46	ab	0.41	b	0.40	b	0.75	a	0.51	ab	0.40	b	0.55	ab	0.55	ab	0.10	*	*ns*	*ns*
4-methyl-2-Heptanone	0.43	b	0.69	ab	0.45	b	0.83	a	0.40	b	0.97	a	0.87	ab	0.93	a	0.19	*	*ns*	*ns*
2-Octanone	0.42	ab	0.27	b	0.27	b	0.46	a	0.35	ab	0.28	b	0.45	a	0.46	a	0.05	**	*ns*	*
2-nonanone	0.81	b	0.83	b	2.43	ab	4.73	a	2.51	ab	0.83	b	3.68	a	3.68	a	1.40	*	*ns*	*ns*
3-Octen-2-one	0.00	b	0.75	ab	1.42	ab	1.81	a	0.70	ab	0.16	b	1.28	ab	1.39	ab	0.47	*	*ns*	*ns*
Other compounds																				
2-pentyl-Furan	0.00	b	0.00	b	0.00	b	0.00	b	0.00	b	0.00	b	0.16	a	0.14	a	0.03	**	***	**
D-Limonene	0.26	b	0.61	b	1.07	b	0.00	b	0.76	b	2.11	ab	4.47	a	4.63	a	0.87	*	***	*ns*
Acetophenone	4.16	ab	3.47	b	3.82	b	6.70	a	5.54	ab	3.26	b	6.53	a	6.53	a	1.42	*	*ns*	*ns*
Isophorone	0.84	a	0.00	c	0.00	c	0.00	c	0.85	a	0.28	b	0.00	c	0.00	c	0.07	***	*ns*	*ns*
Terpinen-4-ol	0.00		0.52		0.76		3.68		0.00		0.95		1.47		1.32		1.00	*ns*	*ns*	*ns*

[1]Animal models containing myofibrillar proteins without (A) and with (AE) enzyme at 0, 3, 8 and 15 d of incubation. [2]RMSE: root mean square of the errors. [3]*Pf*: *P* value of the time effect, P_E: *P* value of enzyme effect, P_{txE}: *P* value of interaction between time and enzyme effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.5$; ns: $P > 0.05$. [4]Different letters in the same row indicate significant differences among models and times.

Table S8. Evolution of the content of volatile compounds (AU 10^{-5}/g protein) in the headspace of vegetal models (containing pea protein) without and with proteolytic enzyme. Samples were taken at 0, 3, 8 and 15 days of incubation.

	Vegetal model without enzyme				Vegetal model with enzyme							
	V0[1]	V3	V8	V15	VE0	VE3	VE8	VE15	RMSE[2]	Pt[3]	PE	P txE
Aldehydes												
Acetaldehyde	0.47	0.45	0.44	0.37	0.26	0.24	0.21	0.27	0.13	ns	**	ns
3-methylbutanal	0.22 c[4]	0.00 d	0.00 d	0.00 d	0.37 c	0.86 a	0.40 c	0.59 b	0.06	***	***	***
2-methylbutanal	0.29 c	0.00 d	0.00 d	0.00 d	0.40 c	0.69 a	0.37 c	0.53 b	0.04	***	***	***
Pentanal	3.18 a	3.37 a	1.57 b	0.73 bc	1.10 bc	0.29 c	0.21 c	0.58 bc	0.38	***	***	***
Hexanal	24.53 a	25.01 a	29.98 a	29.20 a	19.43 ab	6.91 bc	1.06 c	1.72 c	5.32	ns	***	*
Heptanal	0.93 abc	1.16 a	0.88 abc	1.11 ab	0.55 bc	0.56 abc	0.44 c	0.77 abc	0.21	ns	***	ns
Octanal	1.19 abc	1.38 ab	0.86 abc	1.57 a	0.52 bc	0.41 c	0.43 bc	0.96 abc	0.33	*	***	ns
Nonanal	7.69 a	7.30 a	2.32 b	1.83 b	1.02 b	1.39 b	1.21 b	1.76 b	1.53	*	***	**
Benzaldehyde	8.25 abc	10.78 a	7.22 bcd	6.74 bcde	9.69 ab	4.97 cde	3.43 e	4.35 ed	1.19	***	***	***
Benzeneacetaldehyde	1.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.44	ns	ns	ns
4-methyl-benzaldehyde	0.35 c	0.89 a	0.87 a	0.71 ab	0.30 c	0.43 bc	0.31 c	0.61 abc	0.11	**	***	**
2,4-dimethyl-benzaldehyde	0.37 b	1.85 a	0.79 b	0.80 b	0.64 b	0.30 b	0.31 b	0.32 b	0.33	*	**	**
Alcohols												
Ethanol	0.60	1.67	15.21	55.14	0.70	4.91	2.53	3.17	21.82	ns	ns	ns
2-methyl-2-Propanol	0.00 b	0.16 ab	0.16 ab	0.27 a	0.00 b	0.28 a	0.18 ab	0.26 a	0.06	***	ns	ns
3-methyl-3-Buten-1-ol	0.59 cd	2.23 b	0.00 d	0.27 d	0.00 d	1.93 bc	3.88 a	5.17 a	0.39	***	***	***
3-methyl-1-butanol	0.00 c	0.98 c	1.70 c	4.43 bc	0.36 c	8.70 bc	14.94 ab	24.06 a	4.56	**	***	*
2-methyl-1-butanol	0.00 c	0.00 c	0.17 c	0.46 bc	0.00 c	1.05 bc	2.40 ab	3.84 a	0.70	**	***	**
1-Pentanol	2.08	0.00	1.17	3.12	2.13	2.71	2.32	2.41	1.12	ns	*	ns
1-Hexanol	0.98 f	5.17 e	9.25 d	11.45 c	2.58 f	16.55 b	17.64 b	27.36 a	0.69	***	***	***
1-Heptanol	2.84 bc	14.70 a	12.86 a	4.69 b	3.27 bc	2.89 bc	1.58 c	2.03 bc	0.85	***	***	***
1-Octen-3-ol	2.04 d	2.84 cd	4.22 abc	4.76 ab	3.42 bcd	3.16 bcd	2.91 cd	5.74 a	0.58	***	ns	**
2-Heptanol	0.34 b	0.57 b	4.95 ab	6.77 a	0.36 b	0.68 b	1.08 b	2.21 ab	1.60	*	*	ns
Benzyl alcohol	0.62 e	1.12 cde	1.50 bcd	1.81 ab	1.01 de	1.45 bcd	1.59 abc	2.13 a	0.19	***	**	ns
1-Octanol	2.14 bc	2.81 bc	3.21 ab	4.46 a	2.07 bc	2.19 bc	1.73 c	2.30 bc	0.46	**	***	**
Phenylethyl alcohol	0.00 f	0.00 f	0.02 ef	0.86 d	0.33 e	2.46 c	6.63 b	12.93 a	0.13	***	***	***
2-ethyl-1-Hexanol	25.69 bc	28.77 bc	34.78 b	54.60 a	17.75 c	18.65 c	25.73 bc	28.42 bc	4.06	***	***	**
Esthers comp	**2.31**	**4.41**	**2.71**	**4.33**	**2.61**	**3.30**	**2.11**	**2.42**				

Methyl acetate	1.13	ab	1.27	a	0.69	b	1.07	ab	1.32	a	0.89	ab	0.71	b	0.72	b	0.19	**	ns	*
Ethyl Acetate	0.99		2.12		1.25		2.26		0.81		1.31		0.42		0.00		0.76	ns	*	ns
Methyl 3-methylbutyrate	0.00	c	0.27	b	0.00	c	0.00	c	0.00	c	0.35	b	0.37	b	0.66	a	0.06	***	***	***
Butyl acetate	0.20	d	0.41	b	0.30	bcd	0.73	a	0.25	d	0.26	cd	0.27	cd	0.39	bc	0.05	***	***	***
3-methyl-1-butanol acetate	0.00	e	0.33	bcd	0.48	abc	0.27	cd	0.24	d	0.50	ab	0.35	bcd	0.65	a	0.07	***	***	***
Alkanes																				
Hexane	0.19		0.33		0.10		0.19		0.00		0.27		0.41		0.49		0.19	ns	ns	*
Toluene	3.94		3.52		4.08		2.44		4.14		1.41		1.41		2.39		0.85	*	**	*
Ethylbenzene	0.10	ab	0.22	a	0.17	ab	0.14	ab	0.00	b	0.00	b	0.00	b	0.00	b	0.06	ns	***	ns
p-Xylene	0.39	a	0.41	a	0.28	ab	0.29	ab	0.24	ab	0.19	b	0.24	ab	0.22	b	0.05	ns	***	ns
o-xylene	0.20	a	0.21	a	0.23	a	0.18	a	0.18	a	0.14	a	0.00	b	0.17	a	0.04	*	***	***
Styrene	0.89	b	0.51	b	1.06	b	1.27	b	4.12	a	0.86	b	1.03	b	1.31	b	0.64	**	**	**
Ketones																				
Acetone	1.82	b	3.22	b	2.15	b	2.88	b	2.00	b	3.32	b	4.32	b	8.58	a	1.17	***	***	***
2,3-Butanedione	0.46		2.14		0.95		0.27		1.10		0.35		0.36		0.70		0.70	ns	ns	*
2-butanone	2.95	ab	3.79	ab	2.33	ab	1.15	b	2.69	ab	3.55	ab	3.63	ab	6.18	a	1.46	ns	*	*
2-Pentanone	0.41	b	0.91	b	0.51	b	0.71	b	0.59	b	1.21	b	1.37	b	2.93	a	0.50	***	***	**
3-Pentanone	0.24	b	0.24	b	0.26	b	0.32	b	0.28	b	0.36	b	0.45	b	1.61	a	0.26	***	***	***
2-hexanone	1.72	bc	1.91	abc	1.03	c	0.93	c	1.89	abc	2.14	ab	2.39	ab	2.87	a	0.42	ns	***	**
3-heptanone	0.34	b	5.58	a	1.76	ab	1.17	b	0.46	b	0.00	b	0.00	b	0.00	b	1.45	*	**	*
2-heptanone	15.95	a	13.37	ab	10.64	ab	4.93	b	18.92	a	13.33	ab	12.73	ab	15.27	ab	3.37	*	*	ns
4-methyl-2-Heptanone	0.29	e	0.89	a	0.60	bc	1.05	a	0.29	e	0.50	cd	0.36	de	0.70	b	0.08	***	***	***
2-Octanone	1.09		1.14		0.94		0.79		0.94		0.75		0.67		1.05		0.19	ns	ns	*
2-nonanone	0.96	b	0.90	b	0.96	b	1.14	a	0.70	b	0.70	b	0.67	b	1.10	a	0.17	*	*	ns
3-Octen-2-one	0.76	b	0.77	b	0.96	b	1.28	a	0.52	c	0.52	c	0.41	c	0.47	c	0.10	***	***	***
Other compounds																				
2-pentyl-Furan	0.40	c	0.64	bc	1.08	a	0.84	ab	0.46	bc	1.05	a	0.50	bc	0.78	abc	0.13	***	ns	***
D-Limonene	0.52	ab	0.28	b	0.31	b	0.91	a	0.23	b	0.37	b	0.29	b	0.44	ab	0.17	*	*	ns
Acetophenone	2.63	b	3.49	b	3.03	b	4.79	a	2.62	b	2.71	b	2.63	b	2.73	b	0.41	***	***	***
Isophorone	0.26	ab	0.38	ab	0.31	ab	0.34	ab	0.17	b	0.32	ab	0.42	ab	0.54	a	0.12	*	ns	ns
Terpinen-4-ol	0.27	d	0.24	d	0.19	d	0.48	c	0.30	d	0.74	b	0.88	ab	0.99	a	0.11	***	***	***
Pyrazines																				
Pyrazine	0.00	b	0.31	ab	0.23	ab	0.11	b	0.91	a	0.40	ab	0.00	b	0.26	ab	0.23	ns	*	*
methyl-pyrazine (94)	0.29		0.29		0.30		0.33		0.30		0.32		0.32		0.36		0.04	ns	ns	ns

2,5-dimethyl-Pyrazine (108)	1.28		1.41		1.51		1.30		1.38		1.37		1.61		3.31		0.78	ns	ns	ns
2-ethyl-5-methyl-pyrazine (121)	0.58		0.60		0.67		0.56		0.67		0.58		0.55		0.64		0.08	ns	ns	ns
3-ethyl-2,5-dimethyl-pyrazine (135)	1.68		2.12		2.58		2.22		2.76		2.28		2.14		2.45		0.40	ns	ns	*
2,3-diethyl-5-methyl-Pyrazine (150)	0.12	ab	0.10	b	0.11	ab	0.11	b	0.14	a	0.11	ab	0.10	b	0.12	ab	0.01	*	ns	ns
3,5-diethyl-2-methyl-pyrazine (150)	0.21	abc	0.17	c	0.21	abc	0.20	bc	0.27	a	0.23	abc	0.22	abc	0.25	ab	0.02	ns	***	ns
3,5-diethyl-2-propyl-Pyrazine (122)	0.10	ab	0.00	c	0.08	b	0.09	b	0.12	a	0.08	b	0.00	c	0.10	ab	0.02	***	*	***

[1] Vegetal models containing pea protein without (V) and with (VE) enzyme at 0, 3, 8 and 15 d of incubation. [2]RMSE: root mean square of the errors. [3]*Pf*: *P* value of the time effect, P_E: *P* value of enzyme effect, P_{txE}: *P* value of interaction between time and enzyme effects. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.5$; ns: $P > 0.05$. [4]Different letters in the same row indicate significant differences among models and times.