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1 Title: Mixture design applied for formulation and characterization of vegetal-based fermented
2 products

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17 **Abstract**

18 The present study consists on the characterization of six different flours in order to evaluate
19 their ability to ferment alone and in mixtures. A discrepancy in the composition and physico-
20 chemical characteristics of the flours was recorded. Three of these flours (oats, chickpea and
21 coconut) were selected for the construction of the mixture design, which was used to
22 formulate a base for a fermented vegetable dessert. Oat and chickpea flours have an effect on
23 color accentuation ($\Delta E=17.37$) as well as on increasing water retention (75.31 g/100 g).
24 During storage, post-acidification of the media to pH 4.42 was recorded, with a water holding
25 capacity ranging from 56.64 to 66.12 g per 100 g, indicating a change in the texture of the
26 mixtures. The fermented plant product showed a high viability of lactic acid bacteria, more
27 than 10^7 CFU/mL for *L. delbrueckii subsp. Bulgaricus* and more than 10^8 CFU/mL for *S.*
28 *thermophiles* after 30 days of storage. The use of flours in the formulation of fermented
29 products was demonstrated as an alternative strategy for the development of new vegetable-
30 based desserts.

31 **Keywords:** flour, fermentation, texture, mixture design

32 **1. Introduction**

33 Today, people are aware of the health benefits of plant-based diets, the improvement and
34 sustainability of these foods and their impact on the environment (Jeske, Zannini, & Arendt,
35 2018) . There are different diets around the world, and there is an increasing trend towards
36 diets with less or no dairy products for health reasons such as lactose intolerance or allergy
37 problems or simply as a lifestyle choice.

38 According to de Gavelle (2019), the main protein intake in western diets is essentially of
39 animal origin and it should be noted that milk has long been considered as the only food
40 containing all the essential substances for human nutrition. Davoodi, Esmaeili, & Mortazavian
41 (2013) had grouped a number of studies that revealed the fact that certain constituents of milk
42 and common contaminants (such as pesticides, estrogens and insulin-like growth factor 1)
43 may be responsible for adverse health effects. Widely disseminated nutrition information
44 campaigns in the media and emerging studies raise awareness of the importance of nutrition
45 and reinforce the close link between food and health. The potential disadvantage of
46 commercially available plant substitutes is the variation in nutrient profiles, with low quality
47 or protein and mineral intakes. To promote these products as substitutes of cow's milk,
48 manufacturers must consider protein content and quality as well as enrichment. Attention
49 should be drawn to the possibilities to improve them by selecting raw materials based on their
50 nutritional quality (Mäkinen, Wanhalinna, Zannini, & Arendt, 2016).

51 Flour from different grains and seeds has been widely used in preparations because of its
52 health benefits. In addition to fibre, these flours provide vitamins, proteins, minerals and
53 carbohydrates (González-Montemayor, Flores-Gallegos, Contreras-Esquivel, Solanilla-
54 Duque, & Rodríguez-Herrera, 2019). Flours are the result from the grinding of cereal seeds,
55 leguminous plants, certain mealy plants, or other parts of plants dried and/or de-oiled (fruits,
56 leaves, nuts...). The Functional properties of flours reflect the complex interaction between

57 the composition, structure, molecular conformation, these physico-chemical characteristics
58 and the nature of the environment, in which they are associated and measured (Kumar,
59 Chandra, Chauhan, Singh, & Kumar, 2017).

60 Fermentation is a natural means of improvement, without the addition of additives or
61 preservatives in the food matrix. Thus, the industry uses defined starting cultures to better
62 control the quality of these products and ensure their reproducibility (Coda, Montemurro, &
63 Rizzello, 2017). There are various studies (Ferragut, Cruz, Trujillo, Guamis, & Capellas,
64 2009; İçier, Gündüz, Yılmaz, & Memeli, 2015; Mishra Pandey & Mishra, 2015; R. Wang &
65 Guo, 2016; da Silva Fernandes et al., 2017; Patrignani et al., 2018) on fermented plant
66 products, which are generally soybean-based. Despite this effervescence and a multitude of
67 studies highlighting the benefits of soybeans, the latter remains at the heart of some
68 controversies, notably because 82% of production is derived from genetically modified crops
69 (James, 2014). However, there are various alternatives in addition to soya, like cereals,
70 legumes, other plants or even vegetable proteins (Hickisch, Beer, Vogel, & Toelstede, 2016;
71 Akin & Ozcan, 2017; Gupta & Bajaj, 2017; Wang, Chelikani, & Serventi, 2018; Zannini,
72 Jeske, Lynch, & Arendt, 2018). As defined by Coda et al. (2017), the fermentation of cereal-
73 based beverages by LAB (Lactic Acid Bacteria) improves protein digestibility, nutritional
74 bioavailability of minerals, bio-accessibility of other nutrients (polyphenols, fiber), decreases
75 the glycemic index, increases shelf life and improves the organoleptic quality of products. To
76 achieve the nutritional and textural quality appreciated by the consumer, a formulation system
77 is needed that links the impact of each compound and their interaction. In contrast to the
78 classical approach with the study of one variable at a time, the use of a mixture design in this
79 type of formulation allows for statistically-based optimization to better understand the
80 dynamics of the process and to illustrate the effects and complex interactions between the

81 constituents (Gupta & Bajaj, 2017). Therefore, the answer depends on the proportions of the
82 different ingredients in the mixture.

83 The aim of this study is focused on two parts: the first one is the characterization of flours in
84 terms of composition, physico-chemical characteristics and fermentability. In the second one,
85 the creation and realization of a mixture design with three of these flours preselected for the
86 formulation of fermented desserts as an alternative to dairy products. Thus, the fermentability
87 of these mixtures, their effects on the physico-chemical parameters and on the texture of the
88 fermented products, the microbiological quality and the viability of the lactic acid bacteria
89 during the shelf life were developed.

90 2. Materials and methods

91 2.1. Raw material

92 Flours from oat (*Avena sativa* L.), rice (*Oryza Sativa* L.), quinoa (*Chenopodium quinoa*) of
93 the Celnat brand (Saint-Germain Laprade, France), coconut flour (*Cocos nucifera*) and lupin
94 (*Lupinus albus*) of the brand Ecoïdées (Soultz Sous Forets, France) and chickpea (*Cicer*
95 *arietinum*) of the brand Priméal (Peaugres, France), has been selected for this study. All were
96 from organic and fair farming.

97 Chemicals and reagents which were of analytical quality have been obtained from suppliers
98 such as Sigma Aldrich (Schnelldorf, Germany), Lybios (Pontcharra-Sur-Turdine, France),
99 Danisco France (Paris, France), and [Biokar \(Solabia group, France\)](#).

100 2.2. Chemical properties

101 The dry matter and ash content were determined by the standard method (AOAC, 1995). The
102 content of starch and sugar was determined by enzymatic kit Total starch HK and K-SUFRG
103 kit (Megazyme, Lybios, Pontcharra-Sur-Turdine, France). The protein content was analyzed
104 by the Kjeldahl method (using N×6.25 as a conversion factor) (AOAC, 1995). The fat was
105 extracted and measured by the Soxhlet method using a mixture of diethyl ether and petroleum
106 ether (1:1 v/v) as solvent.

107 2.3. Physical properties

108 2.3.1. Hydration properties

109 To characterize the hydration properties of the flours, the water absorption index (WAI), the
110 water solubility index (WSI) and the swelling power (SP) were determined using the method
111 described in Kraithong, Lee, & Rawdkuen (2018) and Lai (2001).

112 A 2.5 g sample of flour was dispersed in 30 mL of distilled water and heated at two
113 temperatures 30 °C and 90 °C independently, for 30 min in a water bath with stirring using a
114 vortex mixer every 10 min. The cooked dough was cooled to room temperature and then

115 centrifuged at 5732 g for 15 min (Centrifuge Heraeus Megafuge 8R, Fisher Scientific,
116 Göteborg - Sweden). The mass of dry solids in the supernatant was recovered by evaporation
117 overnight at 105 ± 1 °C and the pellet was weighed. The characteristic indexes were calculated
118 by following the Eq. (1), (2), (3).

$$119 \quad \text{WAI} = \frac{\text{Mass of the pellet (g)}}{\text{Mass of the sample (g)}} \quad (1)$$

$$120 \quad \text{WSI (g/100 g)} = \frac{\text{Mass of dissolved solids in the supernatant (g)} \times 100}{\text{Mass of the sample (g)}} \quad (2)$$

$$121 \quad \text{SP} = \text{WAI} \times \left(1 - \frac{\text{WSI}}{100}\right) \quad (3)$$

122 *2.3.2. Analysis of the particle size*

123 Mastersizer 3000 Laser Diffraction Scale provides particle size distributions by using the
124 Aero S dry dispersion unit (Malvern Instruments, United Kingdom).

125 The flour was put into the dry dispersion unit and the measurements were performed in
126 triplicate, with a refractive index of 1.52, 2.0×10^5 - 3.0×10^5 Pa of pressure, and opening of the
127 hopper of 3 mm. Then, the particle size distribution by decile (D_{10} , D_{50} , and D_{90}) was
128 recorded.

129 *2.3.3. Differential scanning calorimetry (DSC)*

130 The thermal characteristics of flours have been studied using differential scanning calorimetry
131 (DSC₂₅₀, TA Instruments, USA) calibrated using an empty pan.

132 For each flour 2 ± 0.5 mg were dispersed in distilled water to achieve the ratio of (30/70 w/w)
133 (flour: water), in a hermetically sealed aluminum capsule as it was described by Villanueva,
134 Harasym, Muñoz, & Ronda (2018). The samples were then held for 5 min at 30 °C and then
135 heated to 90 °C at a rate of 20 °C/min.

136 The TRIOS software was used to determine the starting temperature (T_0), the peak
137 temperature (T_p), and the ending temperature of the gelatinization (T_f), as well as the ΔH_{Gel}
138 (gelatinization enthalpy).

164 rapidly cooled to 45 °C, which is the inoculation temperature. A quantity of lactic acid
165 bacteria (0.3 DCU/kg) was regenerated in a sterile bottle with 10 mL of plant-based juice 30
166 min before inoculation. The flasks were mixed regularly and gently to rehydrate the LAB
167 strains and thus obtain a homogeneous solution without residue. The pH changes were
168 recorded by a D291 consort program (Consort bvba, Turnhout, Belgium), with a 30 min
169 interval between two measurements over a total duration of 6 h.

170 The pH and acidity measurements were performed once the fermented beverages were
171 processed and stored at 4 °C. The pH measurement was taken at room temperature with a pH-
172 meter (Model: Mettler Toledo F20-Kit FiveEasy™ Benchtop). Acidity is the measure of the
173 quantity of acid (mL NaOH 0.1 N/10 g, expressed in degree of acidity).

174 *2.5. Characteristics of fermented desserts during storage*

175 The stability of the fermented products was evaluated at 4 °C for 30 d. The samples were
176 analyzed at 24 h, 15 d, and 30 d after manufacture.

177 *2.5.1. Texture analysis*

178 The analysis of the texture of the fermented desserts was carried out using a Lloyd
179 traction/compression analyzer (AMETEK S.A.S, Elancourt, France) equipped with a 10 N
180 cell force, by carrying out the TPA texture profile analysis procedure, according to the
181 method of Wang & Guo (2016) with minor modifications.

182 Each sample was compressed twice to 40 % of the original height with a cylindrical probe (1
183 cm in diameter, 10 cm long) at a crosshead speed of 2 mm/s.

184 *2.5.2. Water Holding Capacity (WHC)*

185 Water holding capacity (WHC) is defined as the ability of the sample to contain all or a
186 portion of its water. The WHC of the samples was determined by a centrifugation method
187 described by Wang et al., (2017) with slight modifications. The fermented products (5 g) were

188 centrifuged at 6368 g at 4 °C for 20 min. WHC (g/100 g) is defined according to the
189 following Eq. (5).

$$190 \quad WHC(g/100 g) = \frac{Mass_{sediment}}{Mass_{gel}} * 100 \quad (5)$$

191 *2.5.3. Color analysis*

192 Color is measured by the standard method established by the "Commission Internationale de
193 l'Eclairage" (CIE), based on the determination of tristimulus values (XYZ), from a three-
194 dimensional space, called CIELAB. This space is a non-linear transformation of the
195 tristimulus values and each color is defined by the brightness value L* and the color channels
196 a* (red-green) and b* (blue-yellow). The mixtures color was analyzed using a colorimeter
197 (Konica Minolta CR-400 series, Roissy CDG Cedex) using a white ceramic plate as a
198 reference for calibration.

199 The Total Color Difference (ΔE_{76}) is a measure of the visual difference between the color of
200 each sample relative to the reference color in a CIELAB space. The values of delta (ΔE_{76}) are
201 obtained according to the following Eq. (6).

$$202 \quad \Delta E_{76} = \sqrt{(L_{ref}^* - L_s^*)^2 + (a_{ref}^* - a_s^*)^2 + (b_{ref}^* - b_s^*)^2} \quad (6)$$

203 Where the (ref) index indicates the sample values used as a reference and the (s) index for the
204 sample values.

205 *2.6. Microbiological analysis*

206 A single product was defined for the realization of these experiments. It was determined
207 according to the optimal paths of the mixture design, by selecting the optimal parameters of
208 the results obtained previously. The selected product corresponds to the E1 mixture of the
209 previously prepared mixture design, which is composed in proportion of dry matter of: 60 %
210 chickpeas, 20 % coconut and 20 % oat. A sample (1 mL) of the fermented mixture was mixed
211 with 9 mL of sterilized tryptone salt broth (NaCl 0.85 % (w/v)). A series of appropriate

212 dilutions of the sample was individually inoculated in triplicate on selective mediums. Man,
213 Rogosa and Sharp agar (MRS) were used for enumeration of *Lactobacillus delbrueckii subsp.*
214 *bulgaricus* and M-17 agar for enumeration of *Streptococcus thermophilus* and then
215 incubated at 37 °C for 48 and 72 h respectively according to the method of Li et al. (2017).
216 After incubation, petri dishes with 30 to 300 colonies were counted and expressed as log₁₀
217 colony forming units per milliliter (CFU/mL) of sample. The Total Aerobic Flora (Yeasts,
218 Molds and Enterobacteriaceae) and Thermoresistant, (Spores, Thermophilic and Mesophilic
219 Aerobic Flora) were determined on the media Petrifilm™3M™ and the Mesophilic Flora non-
220 lactic in an agar gelysate medium (SFA medium for the enumeration of contaminating germs
221 in dairy products) of the brand Grosseron (Grosseron, 44220 Couëron, France).

222 2.7. Statistical analysis

223 The statistical analyses were performed using NemrodW software (NemrodW® 27 Traverse
224 de la Malvina Bât. B, 13012 Marseille, France), which is a software package for designing
225 and analyzing experimental and mixture design. All experiments were run at least three times
226 and allowed for the determination of mean values and standard deviations. ANOVA tests
227 were performed to compare the means as well as the correlation coefficients between the
228 other parameters. The variances were considered to be significant at the threshold of 95 % (p
229 < 0.05).

230 3. Results and discussion

231 3.1. Composition of the flours

232 The composition of the six different flours have been grouped in Table 2, the results obtained
233 show that there was a difference in their composition. The dry matter content varies from
234 89.87 ± 0.13 g/100 g in the oat flour to 96.35 ± 0.25 g/100 g in the coconut flour. The variability
235 of the composition is linked to the variation in the origin of the seeds that make up the flours.
236 The highest protein contents were obtained in the *Fabaceae* (lupin flour) 39.17 ± 1.75 g/100 g.
237 The fat composition was variable from 2.50 ± 0.40 g/100 g in rice to 17.0 ± 0.40 g/100 g in
238 coconut, and between 6 and 11 g/100 g in the other flours. In terms of carbohydrate content,
239 the highest concentrations of starch were recorded in cereals (oats and rice) and pseudo-
240 cereals (quinoa), a value that reaches 72.49 g/100 g in rice flour, in contrast to the contents of
241 sugar, which are the lowest in rice and oat flour, the highest value 14.32 g/100 g was recorded
242 in coconut. The overall composition corresponds to a certain extent to literature results
243 (Trinidad et al., 2006; Nowak, Du, & Charrondière, 2016; S. Wang & Zhu, 2016; Sultan,
244 Wani, & Masoodi, 2018; Zhang, Li, Ma, & Hu, 2019; Olukomaiya et al., 2020) and producer
245 claims for each flour type.

246 3.2. Physicochemical characteristics

247 The grinding process used in the production of flour can be different from one seed to another
248 which can be the source of size differences in studied flours (Benković, Belščak-Cvitanović,
249 Bauman, Komes, & Srećec, 2017). Analyses have shown a significant effect of the type of
250 flour on the particle size (Table 2). The particle diameter varied from 11.27 ± 0.06 to
251 971.67 ± 58.24 μm , with a median diameter (D_{50}) between 36.63 ± 1.40 μm in chickpea and
252 313.33 ± 4.73 μm in oat. The distribution was monomodal in coconut and rice flours, bimodal
253 for oat and lupin, and multimodal for quinoa and chickpea flours. [González-Montemayor et al. \(2019\)](#) have reported that particle size affects the physico-chemical properties of flours,

255 increasing the surface area per unit volume, and the distribution of macronutrients and
256 functional components is expected to be different.

257 The DSC analysis provided the starch gelatinization parameters. According to Ndangui
258 (2015), the presence of a single endothermic peak during DSC analysis is typical of starch
259 gelatinization in the presence of excess water. This continuous endothermic transition
260 indicates a swelling of the granule and a progressive disruption of the starch crystal lattice in
261 the gelatinization temperature range. This phenomenon was not observed on flours without
262 starch (i.e. lupin and coconut), unlike oat, quinoa, chickpea and rice flours. Enthalpy, which
263 corresponds to the internal energy of the system resulting from the heat treatment, is
264 proportional to the starch content. The lowest value is recorded with chickpea (0.80 ± 0.09 J/g)
265 which has the lowest starch content, followed by oats (1.36 ± 0.15 J/g), quinoa (1.99 ± 0.10 J/g)
266 and the highest value with rice (2.04 ± 0.02 J/g) which has the highest starch content. The
267 results of characteristic temperatures (T_0 , T_p , and T_f) obtained correspond to what is reported
268 in the literature by Kaur & Singh (2005) for chickpea flour and Ye, Wang, Wang, Zhou, &
269 Liu (2016) for rice flour. Therefore, the ΔH_{Gel} values recorded are lower in all the flours
270 studied compared to these publications which are between 3 and 6 J/g. This difference may be
271 due to the flour/water ratio used. According to Zhu (2017), amylopectin content affects the
272 reduction of gelatinization parameters as well as certain grinding parameters such as time.

273 The water absorption index (WAI), the water solubility (WS) and the swelling power (SP)
274 characterize the interaction of the product with water and are often important indicators that
275 affect the subsequent treatment food. The results of WAI, WS, and SP at 30 and 90 °C are
276 shown in Fig.1. An increase in water absorption was observed in all flours with an increase in
277 hydration temperature, followed by an increase in swelling power and a decrease in solubility.
278 The results were partly in agreement with the literature (Kraithong et al., 2018; Lai, 2001;
279 Maqbool, Naik, Hussain, & Rather, 2017), which reported WAI values of 2.38-2.75 g/g, WSI

280 values of 9.84-20.10 g/100 g and SP values of 2.15-2.42 in rice and chickpea flours.
281 According to Kraithong et al. (2018) a high WAI can be explained by the presence of a large
282 number of hydrophilic groups in starch molecules, high sugar and protein content. In the case
283 of chickpea flour, a high absorption index, greater than 7 (g/g), was observed as the presence
284 of significant levels of starch and protein. The latter may have promoted a strong involvement
285 of hydrogen bonds with polar or charged side chains. Also, a large particle size contributes to
286 low absorption, just as a high lipid content would block hydration with hydrophobic parts as it
287 can be observed for lupin flour characterized by the lowest WAI at 90 °C, with a high lipid
288 content and a very low starch content.

289 *3.3. Fermentability of flours*

290 The evolution of acidification in the six flours studied was compared in Table 2. A decrease
291 in pH was observed after the addition of the **LAB strains**, resulting an acidification of the
292 medium with a final pH ranging from 4.81 ± 0.06 to 4.40 ± 0.07 . The decrease in pH could be
293 attributed to the increase in organic acids produced by microbial activities during the
294 degradation of carbohydrates (Olukomaiya et al., 2020). Although, the use of quinoa flour,
295 chickpea and coconut allows a reduction of the pH, in less than 16 h of incubation, oat, rice,
296 and lupin flours have a prolonged latency phase, with pH above 4.5, even after 21 h of
297 incubation. This delay could be attributed to the slow metabolism of the strains present in the
298 **LAB strains**, the nature of the substrates consumed, or the incubation temperature (Hickisch
299 et al., 2016). A variation which is also found on the average speeds which is at least of the
300 order of -0.05 ± 0.004 pH unit/hour in lupin flour, and which can expect at most -0.29 ± 0.01 pH
301 unit/hour in quinoa flour.

302 3.4. *Mixture design*

303 3.4.1. *Choice of ingredients*

304 The previous step specified above allowed the selection of three compounds for the
305 formulation of a fermented vegetable product, so the choice is to focus on oat, chickpea, and
306 coconut flour. The combination of these flours will give the products a firm texture without
307 the addition of gelling agents because of their starch composition and their water absorption
308 properties during gelling in the presence of water at high temperatures. The combination of
309 these flours will allow fermentation and bring nutritional value to the final products with
310 protein, fat, and fiber content. Oats and chickpeas are pregnant foods and among the most
311 consumed cereals and legumes in the world for their rich nutritional intake. They provide
312 carbohydrates, mainly in the form of starch, soluble dietary fiber, lipids, balanced proteins,
313 and several vitamins. Coconut flour is a real economic interest as a residue generated during
314 the extraction of coconut milk and coconut oil. This flour is not only abundant in dietary fiber
315 but also trans fatty acids and low in carbohydrates. The consumption of oat, chickpea or
316 coconut products has been associated with various health impacts such as the reduction of
317 cholesterol, the risk of cardiovascular disease, gastrointestinal disorders, as well as cancer
318 prevention (Trinidad et al., 2006; Sreerama, Sashikala, Pratapa, & Singh, 2012; Martínez-
319 Villaluenga & Peñas, 2017).

320 3.4.2. *Acidification kinetics*

321 Fermentation kinetics were carried out to determine the pH evolution during a 6 h incubation
322 at 45 °C. The results are shown in Fig. 2.

323 The pH varies from 6.48 ± 0.12 to 6.08 ± 0.03 after 30 min from the start of fermentation, and
324 from 4.76 ± 0.04 to 4.58 ± 0.06 after 6 h of fermentation depending on the formulation. In the
325 case of fermentation of flour mixtures (oats, chickpeas, coconut), acidification is more
326 accelerated, unlike fermentation of flours alone. Mixing would have reduced the time required

327 to reach a target pH close to 4.5. This may be related to the composition of the raw materials
328 (presence of different sugars), which are consumed by the LAB strains, resulting in a
329 reduction of the pH of the medium (da Silva Fernandes et al., 2017). The presence of legumes
330 that are a source of complex carbohydrates (fiber, starch and oligosaccharides), which also
331 contain growth factors and prebiotic components for the bacteria, also promotes a gentle
332 fermentation process (Akin & Ozcan, 2017). The pH decreased after 24 h of storage, ranging
333 from 4.42 ± 0.01 to 4.56 ± 0.00 and the acidity from 3.73 ± 0.06 to 4.80 ± 0.20 , to stabilize during
334 the month of storage at 4 °C. According to Gupta & Bajaj (2017), a very small variation in pH
335 of fermented oat flour beverage products stored under refrigerated conditions can be recorded,
336 attributed to the low metabolic activity of the LAB strains, and therefore to the low acid
337 production during the storage period. By reference to the type of compound and their
338 interactions, the pH increases and the acidity decreases with the addition of oats. An effect
339 that is also important during the interaction of the 3 ingredients (oat, chickpea and coconut).
340 However, the interactions among the flours in pairs had negative effects on the pH response
341 and positive effects on the acidity response.

342 *3.4.3. Influence of flour composition on color difference*

343 According to the results obtained, there is a significant effect ($p < 0.001$) of the flours used on
344 the color of the mixtures. The results (Table 3) indicate that product E1 had the highest ΔE
345 (17.37 ± 0.01), the furthest difference from the reference white, while E11 was the product
346 with the lowest ΔE (14.92 ± 0.01). The color differences obtained in the mixtures (oats,
347 chickpea, coconut) are lower than those reported by Luana et al., (2014), ($\Delta E > 18$) in the
348 fermented oat flake products. This shows the interaction between the three flours used and
349 their impact in the reduction of ΔE . Consequently, the final color of the product can be
350 predicted according to the composition using the response surfaces presented in Fig. 3.

351 The ΔE did not change during the month of storage, the largest variation is less than 1 unit,
352 which is not detectable with the naked eye. Analysis after 24 h showed that the increase in oat
353 content plays a role in the final color of the product. This influence is half as important for
354 coconuts and up to four times less for chickpeas. The oat-coconut interaction had a negative
355 effect which is most significant on the color difference.

356 *3.4.4. Influence of flour composition on water holding capacity*

357 After fermentation, the water retention rate varies between 58.03 ± 0.21 g/100 g and
358 75.31 ± 0.72 g/100 g in the products obtained. Statistical analysis shows that these values
359 increase significantly with the increase in oat and chickpea flours. After 30 d of storage at 4
360 °C, the WHC was between 56.64 ± 0.10 g/100 g and 66.12 ± 0.09 g/100 g, reflecting an increase
361 in syneresis. The result is a small water pill on the surface of the jars in products where water
362 retention is less than 65 g/100 g, a phenomenon also common in dairy yogurt. According to
363 Shi Wang et al. (2018), a fermented plant product containing starch may undergo
364 retrogradation during cooling, causing water to be expelled from the gel, resulting in
365 syneresis. The WHC in mixtures can decrease by up to 13 g/100 g during 30 d storage at 4 °C.
366 These results are close to those obtained by Coda et al. (2017), which indicate a decrease in
367 WHC of 10 g/100 g under the same conditions and for the same storage time.

368 *3.4.5. Influence of flour composition on texture*

369 There was a significant effect of composition on the hardness and cohesion of processed
370 products (Tables 2 and 3). The study by Hickisch et al. (2016), on alternatives to yogurt
371 showed that heat treatment and the choice of fermentation micro-organism had an impact on
372 the physical characteristics of the products. It was observed that at 24 h of storage, hardness
373 varies from 0.10 ± 0.00 N to 0.23 ± 0.01 N and cohesion from 0.31 ± 0.10 to 0.69 ± 0.03 . After 30
374 d of storage, the hardness increases slightly from 0.02 to 0.12 N, and the cohesion decreases
375 from 0.01 to 0.22.

376 The addition of oats resulted in a firmer gel due to the ability of this flour to retain water. On
377 the other hand, the cohesion of the gel increased considerably with the oat-coconut
378 interaction. The firmness of the yogurt is directly related to its total solids content, particularly
379 its protein content and type (Mishra Pandey & Mishra, 2015). A higher content of different
380 proteins, but also the effect of pH would lead to a higher degree of cross-linking of the gel
381 network, which would result in an increase in structural strength, potentially leading to an
382 increase in water holding capacity, firmness and viscosity of the gels (Mishra Pandey &
383 Mishra, 2015; Akin & Ozcan, 2017; Shi Wang et al., 2018).

384 *3.5. Microbiological quality and viability of lactic strains*

385 The heat treatment applied on the mixture before fermentation, confirmed the microbiological
386 quality during storage. Indeed, an acidification kinetics of the E1 mixture during fermentation
387 (shown in fig.4) compared to a control mixture without LAB strains, which didn't
388 significantly acidify. Through the first week of storing at 4 °C, there was no microbial
389 contamination. The load is as low, after 30 days, except for mesophilic non-lactic flora,
390 moulds and yeast, which was 41, 11 and 10 germs/mL respectively, a very low microbial
391 charge. Olukomaiya et al. (2020) had indicated that, the number and increase of bacteria, and
392 more particularly yeasts and moulds, can be attributed to variable reactions and changes in the
393 pH and other biochemical parameters in the fermented product.

394 According to Jeske et al. (2018) the enumeration of *Streptococcus thermophilus* and
395 *Lactobacillus bulgaricus* used in the production of yoghurt from cow's milk reaches a
396 population of 20×10^8 cells/mL, which corresponds to about $9.30 \log_{10}$ CFU/mL. The
397 enumeration of lactic acid bacteria strains was carried out during four weeks of storage (Table
398 5). The quantity of both strains of bacteria at 24 h after the end of fermentation was 8.04 ± 0.04
399 \log_{10} CFU/mL for *L. delbrueckii subsp. Bulgaricus* and $8.10 \pm 0.06 \log_{10}$ CFU/mL for *S.*
400 *thermophilus*. These values were stable during one month of storage at 4 °C, with a slight

401 decrease in *L. delbrueckii subsp. Bulgaricus* count to $7.92 \pm 0.05 \log_{10}$ CFU/mL. Considering
402 that a probiotic must be a fermented product containing a minimum number of $6 \log_{10}$
403 CFU/mL microorganisms throughout its shelf life (da Silva Fernandes et al., 2017).

404 **4. Conclusion**

405 The aim of the present study was to highlight the influence of a given concentration of three
406 ingredients individually as well as their interaction on the physicochemical and textural
407 properties of fermented vegetable desserts. It was shown that there is a significant variation
408 between the different flours studied, especially for composition such as starch content, which
409 has a direct impact on properties such as gelatinization temperatures and water absorption.

410 The individual fermentation tests of the flours with the [LAB strains](#) were positive with a
411 decrease in pH down to 4.40. The comparison of the composition, hydration parameters,
412 starch gelatinization and acidification kinetics allowed the choice of three ingredients: oat,
413 chickpea and coconut flours. An optimization in term of physico-chemical term was then
414 done by mixture design with constraints.

415 The results showed that the choice of the applied mixture plays a decisive role on the
416 acidification of the mixture, the color, the water retention and the texture of the final products.
417 Thus, acidification is faster in a mixture than individual flour fermentation. The response
418 surface showed a significant effect of oat-coco interaction on color, and more specifically the
419 effect of oat concentration on water retention which impacts firmness and gel stability during
420 storage.

421 The next step of this study is the selection and optimization of an ideal product, combining
422 the information obtained, in order to provide the best solutions for the formulation of new
423 vegetable alternatives to dairy products.

424 **Author contributions:**

425 A.C.A., L.M., designed the study. A.C.A., carried out the experiment, wrote the manuscript
426 with support from K.C.J.F., and L.M. K.C.J.F., provided critical feedback and helped shape
427 the research, analysis and manuscript. L.M., designed the model and the computational
428 framework, analysed the data and supervised the work. P.E., F.M., L.B., C.C., helped
429 supervise the project. All authors final approval of the version to be submitted.

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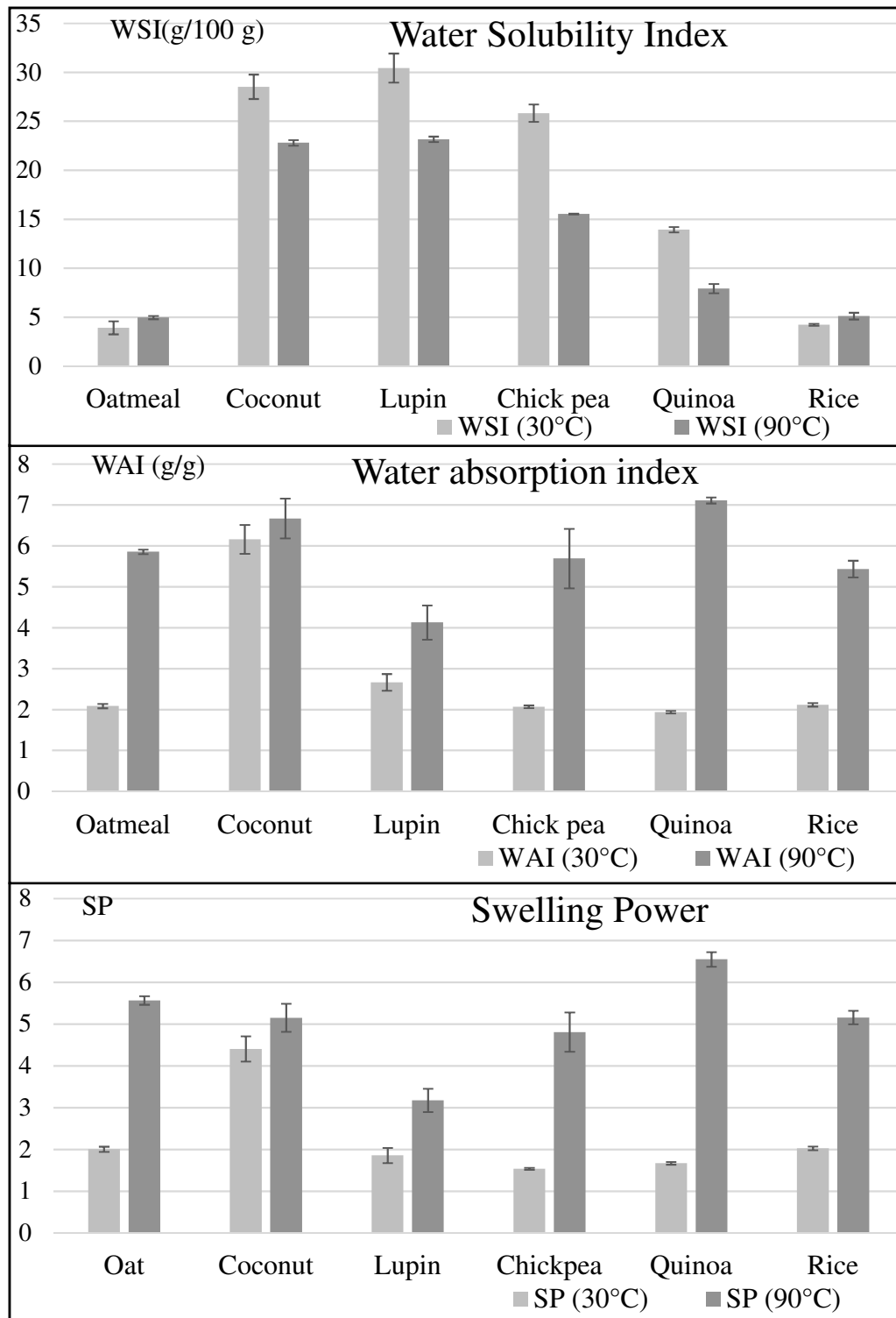


Fig. 1. Hydration properties, water absorption index (WAI), water solubility index (WSI) and swelling power (SP) of flours.

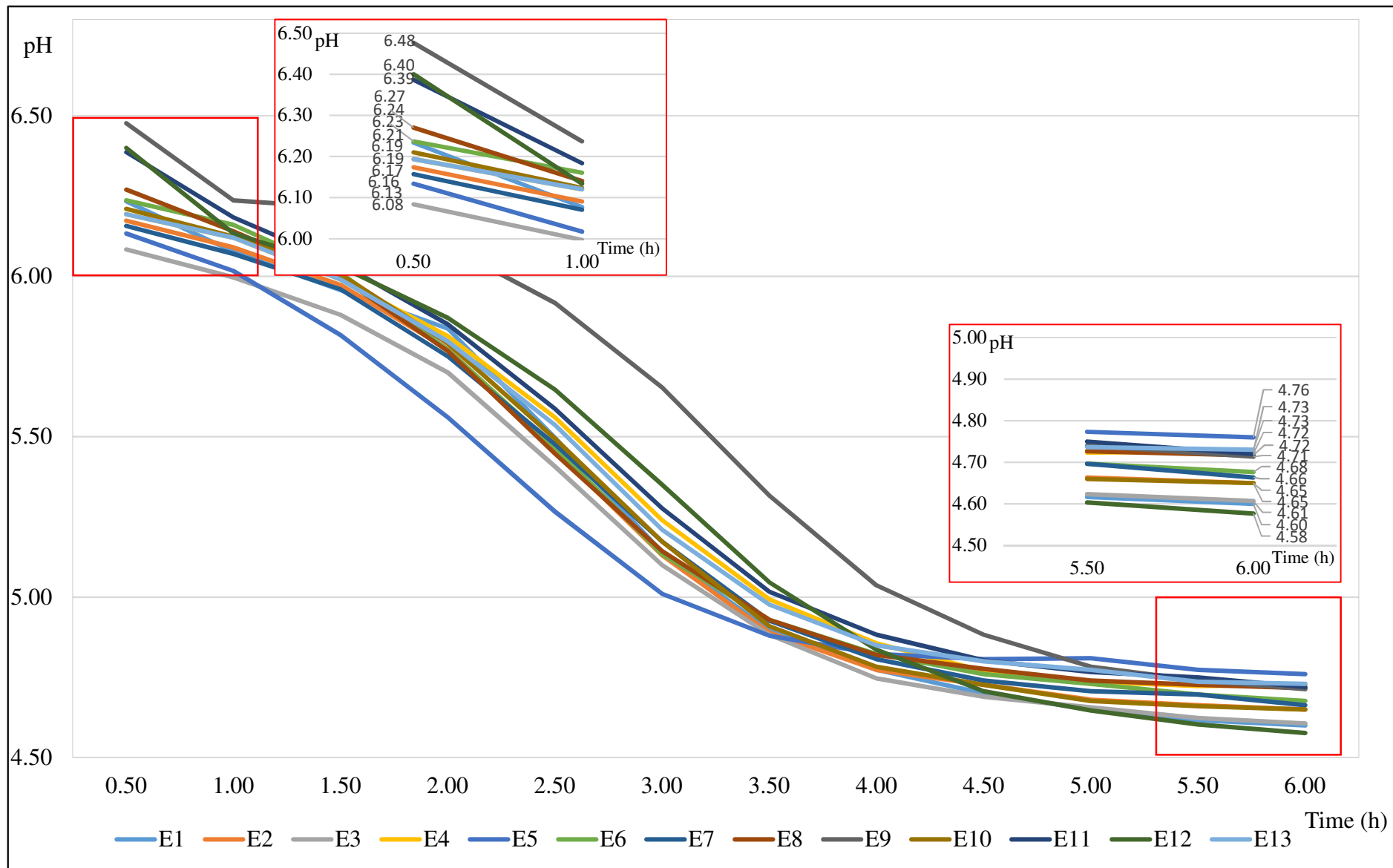


Fig. 2. Kinetics of acidification of the products in the mixture design plan during 6 hours of fermentation. (color)

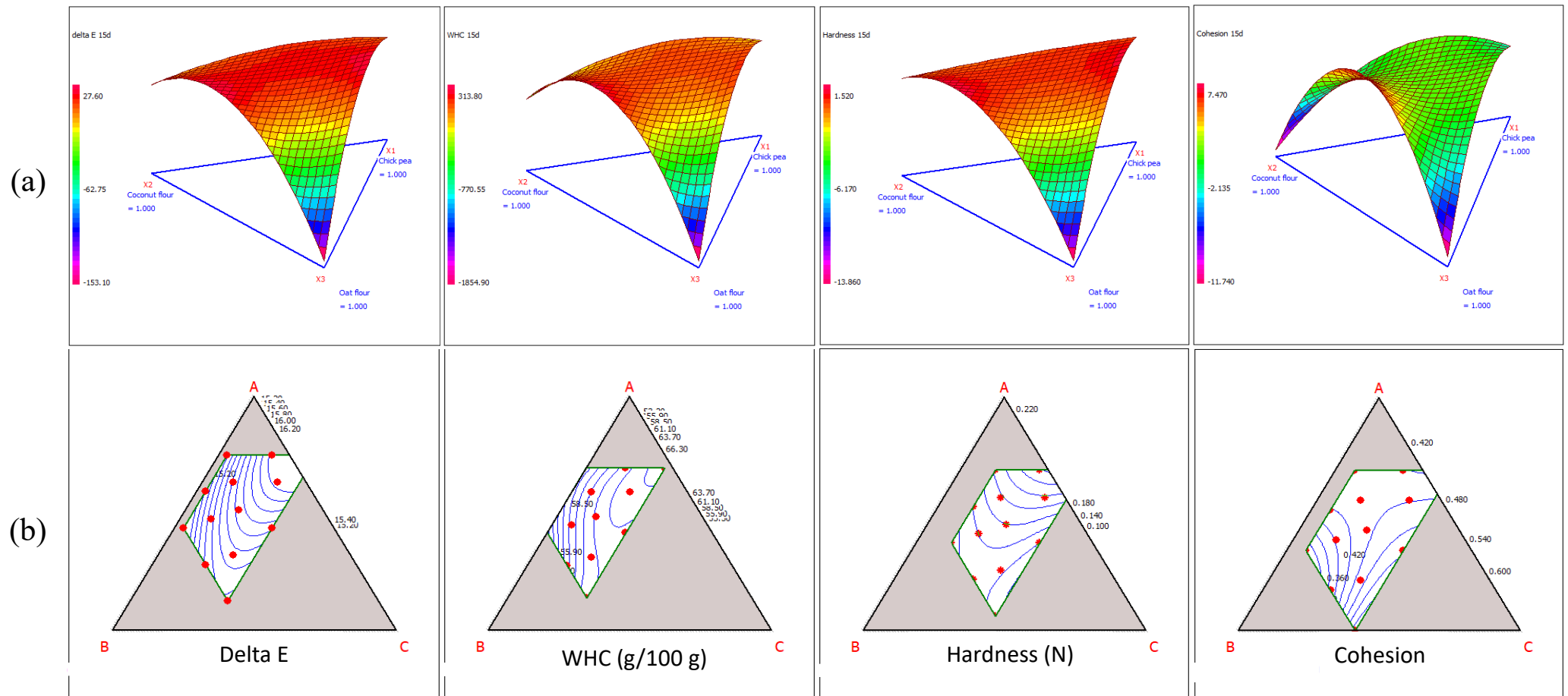


Fig. 3. (a) 3D and (b) 2D contour plot Variation of responses, color difference (ΔE), water holding capacity (WHC) and texture (hardness, cohesion) depending on the amount of flour at 15 d of storage. (Color).

A: Oat, B: Coconut, C: Chickpea

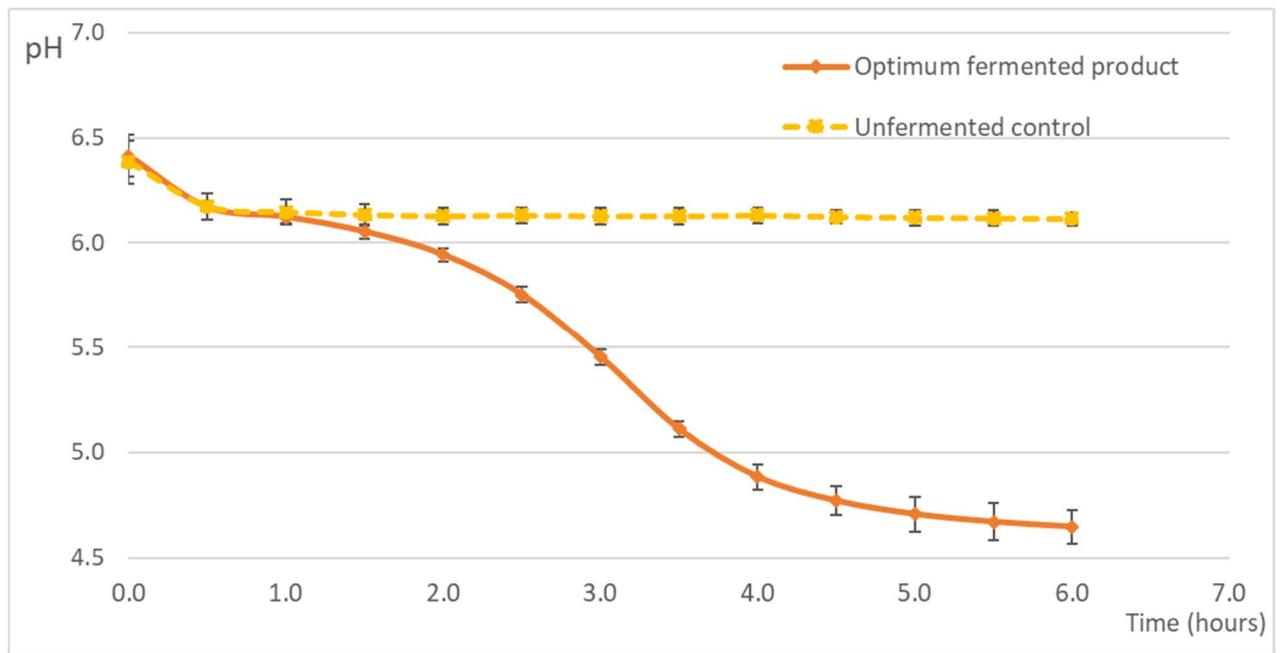


Fig. 4. Acidification kinetics during 6 hours of fermentation with and without lactic acid bacteria stains (control)

Table 1

Mixture design of 13 formulations based on 3 compounds with constraints.

No.exp.	Name	Chickpea	Coconut	Oat
1	E1	0.6000	0.2000	0.2000
2	E2	0.5000	0.4000	0.1000
3	E3	0.4000	0.4000	0.2000
4	E4	0.6000	0.3000	0.1000
5	E5	0.5000	0.3000	0.2000
6	E6	0.6000	0.2500	0.1500
7	E7	0.4500	0.4000	0.1500
8	E8	0.5500	0.3500	0.1000
9	E9	0.5250	0.3250	0.1500
10	E10	0.5625	0.2625	0.1750
11	E11	0.5125	0.3625	0.1250
12	E12	0.4625	0.3625	0.1750
13	E13	0.5625	0.3125	0.1250

The mixture results were then analyzed by NemrodW software with a cubic model.

Table 2

Chemical composition and physico-chemical characteristics of the different flours

	Oat	Coconut	Quinoa	Lupin	Chick pea	Rice
Composition (g/100g)						
Dry matter^a	89.87±0.13	96.35±0.25	90.59±0.29	93.70±0.35	90.60±0.56	90.15±0.44
Proteins^a	8.17±0.64	15.79±0.69	12.79±0.60	39.17±1.75	18.65±0.48	6.29±0.56
Carbohydrates^b :						
Starch	64.66	0	67.36	0.39	38.52	72.49
Sugar	1.23	14.32	3.38	2.85	2.36	1.06
Lipids and fats^a	7.06±0.22	17.4±0.40	6.14±0.21	11.40±0.87	7.80±0.17	2.50±0.42
Ash^a	1.08±0.12	4.42±0.11	2.31±0.06	3.98±0.13	2.90±0.23	1.77±0.06
Particle size (µm)^a						
D₁₀	18.57±0.25	67.30±1.41	11.27±0.06	12.00±0.10	12.03±0.06	15.53±0.15
D₅₀	313.33±4.73	244.67±6.51	68.93±0.83	56.33±0.70	35.63±1.40	63.13±0.81
D₉₀	949.67±32.02	566.67±18.56	692.33±4.51	367.67±8.50	971.67±58.24	203.00±8.66
Gelification properties^a						
T₀ (°C)	55.71±0.47	no gelatinization	55.55±0.22	no gelatinization	65.74±1.24	63.14±0.94
T_p (°C)	62.98±0.61		64.42±0.83		71.50±1.19	70.32±0.72
T_f (°C)	68.79±0.30		72.26±1.51		76.04±1.70	76.42±0.62
ΔH_{gel} (J/g)	1.36±0.15		1.99±0.10		0.80±0.09	2.04±0.02
Kinetics of acidification^a						
Initial pH	6.15 ±0.03	6.32±0.02	6.33±0.03	5.61 ±0.03	6.31±0.01	6.48±0.03
Final pH	4.60±0.07	4.45±0.10	4.40±0.07	4.81±0.06	4.44±0.04	4.73±0.05
Average speed (pH unit /h)	-0.27±0.01	-0.15±0.02	-0.29±0.01	-0.05±0.00	-0.15±0.01	-0.14±0.01

D₁₀: Diameter corresponding to 10% cumulative of the particle size distribution, D₅₀: Diameter corresponding to 50% cumulative of the particle size distribution, D₉₀: Diameter corresponding to 90% cumulative of the particle size distribution.

T₀: onset temperature; T_p: peak temperature; T_f: conclusion temperature ΔH_{gel}: enthalpy of gelatinization.

Initial pH: the pH after adding the ferments; Final pH: the pH after 21 hours of fermentation; Average speed: the average rate of acidification.

a: analyzes were performed in triplicate, b: analyzes were performed twice.

Table 3

Color difference (ΔE), water holding capacity (WHC) and texture (hardness, cohesion) of mixture design products at different storage times.

	Storage time	ΔE	WHC (g/100 g)	Hardness (N)	Cohesion
E1	24 h	17.37±0.01	70.62±0.43	0.23±0.01	0.51±0.06
	15 d	16.76±0.00	67.05±0.29	0.26±0.03	0.41±0.06
	30 d	17.37±0.00	65.92±0.32	0.30±0.01	0.34±0.11
E2	24 h	15.72±0.00	58.03±0.21	0.10±0.00	0.69±0.03
	15 d	15.26±0.01	54.57±1.25	0.12±0.00	0.37±0.08
	30 d	15.67±0.05	57.20±0.03	0.14±0.00	0.56±0.04
E3	24 h	16.24±0.01	75.31±0.72	0.14±0.01	0.41±0.03
	15 d	15.63±0.00	63.93±0.34	0.11±0.01	0.56±0.07
	30 d	16.11±0.01	63.02±0.87	0.14±0.01	0.35±0.10
E4	24 h	15.62±0.00	68.07±0.39	0.11±0.00	0.31±0.10
	15 d	15.35±0.02	58.46±1.19	0.19±0.01	0.42±0.08
	30 d	15.76±0.01	56.64±0.10	0.15±0.01	0.48±0.04
E5	24 h	16.29±0.01	74.28±0.22	0.14±0.01	0.53±0.06
	15 d	15.84±0.00	64.87±0.57	0.11±0.01	0.52±0.07
	30 d	16.52±0.01	60.97±0.27	0.12±0.01	0.58±0.02
E6	24 h	16.72±0.01	73.02±0.40	0.22±0.01	0.48±0.06
	15 d	16.53±0.07	65.33±0.31	0.26±0.01	0.44±0.03
	30 d	16.78±0.01	66.12±0.09	0.31±0.02	0.34±0.08
E7	24 h	15.57±0.01	66.66±0.63	0.10±0.00	0.59±0.18
	15 d	15.30±0.01	59.09±0.91	0.09±0.00	0.40±0.08
	30 d	15.47±0.01	58.02±0.72	0.16±0.03	0.38±0.04
E8	24 h	15.40±0.00	64.73±0.26	0.10±0.00	0.49±0.17
	15 d	15.29±0.01	58.11±0.50	0.15±0.01	0.42±0.12
	30 d	15.46±0.00	61.18±1.83	0.16±0.01	0.47±0.06
E9	24 h	15.95±0.00	69.40±0.30	0.14±0.01	0.48±0.04
	15 d	16.19±0.01	63.96±0.55	0.17±0.01	0.53±0.02
	30 d	16.49±0.01	62.72±0.22	0.22±0.01	0.46±0.03
E10	24 h	16.03±0.01	72.75±0.48	0.16±0.01	0.43±0.06
	15 d	16.31±0.01	65.24±0.45	0.20±0.02	0.48±0.02
	30 d	16.72±0.01	64.64±0.65	0.29±0.02	0.42±0.06
E11	24 h	14.92±0.01	66.15±0.36	0.11±0.01	0.50±0.05
	15 d	15.19±0.01	61.97±0.38	0.16±0.00	0.40±0.09
	30 d	15.54±0.00	61.93±0.95	0.17±0.01	0.49±0.04
E12	24 h	15.64±0.00	67.90±0.53	0.13±0.01	0.44±0.06
	15 d	15.89±0.00	62.29±0.59	0.17±0.01	0.45±0.07
	30 d	15.89±0.00	65.04±0.63	0.17±0.00	0.44±0.01
E13	24 h	15.52±0.00	65.17±0.28	0.13±0.01	0.54±0.03
	15 d	15.85±0.00	61.32±0.42	0.12±0.00	0.48±0.06
	30 d	15.92±0.01	60.47±0.42	0.23±0.12	0.40±0.11

All analyses were performed in triplicate.

Table 4.

Estimation of the coefficients of the mathematical model for the answers: Color difference (ΔE), water retention capacity (WHC) and texture (hardness, cohesion) at different storage times.

	Storage time	ΔE	WHC (g/100 g)	Hardness (N)	Cohesion
Variance analysis	24 h	<0.01***	<0.01***	<0.01***	3.32*
	15 d	<0.01***	<0.01***	<0.01***	0.49**
	30 d	<0.01***	0.04***	<0.01***	<0.01***
b₁	24 h	54.34**	174.71	0.10	-5.26
	15 d	0.37	-206.03**	-0.01	-2.50
	30 d	16.00	-349.05*	-4.04	8.68**
b₂	24 h	102.07*	29.94	-1.03	-17.39
	15 d	-20.28	-641.09***	-1.10	-11.75
	30 d	14.71	-866.42*	-9.52	20.74**
b₃	24 h	230.58	369.79	-12.62	-39.30
	15 d	-153.23	-1855.05***	-13.87	-11.29
	30 d	5.02	-2857.33**	-49.85*	91.74***
b₁₂	24 h	-240.66*	-282.78	1.13	44.62
	15 d	85.21	1756.12***	1.71	28.35
	30 d	-4.62	2500.65**	24.50	-52.39**
b₁₃	24 h	-416.47	-927.02	20.48	82.41
	15 d	315.73	3832.90***	22.88	27.68
	30 d	39.55	5908.72**	90.75*	-171.49***
b₂₃	24 h	-632.67	-144.77	29.61	147.03
	15 d	414.73	6001.88***	30.71	75.95
	30 d	23.96	8693.57**	123.11	-260.44***
b₁₂₃	24 h	935.01	1569.31	-36.40	-253.91
	15 d	-558.82	-9196.49***	-38.56	-133.60
	30 d	-51.90	-14004.66**	-172.13	410.18***
R²	24 h	0.87	0.91	0.86	0.42
	15 d	0.88	0.95	0.74	0.60
	30 d	0.91	0.70	0.76	0.87

b₁: Chickpea; b₂: Coconut; b₃: Oat; *, significant effect (p<0.05); **, significant effect (p<0.01), ***: significant effect (p<0.001); R²: the coefficient of determination.

Table 5: Control of the viability of the lactic acid bacteria strains in the optimum product during one month of storage

Storage time	Viable counts of strains [\log_{10} CFU/mL]	
	<i>L. delbrueckii subsp. Bulgaricus</i>	<i>S. thermophiles</i>
24 h	8.04±0.04	8.10±0.06
7 d	8.09±0.06	7.99±0.02
15 d	8.05±0.07	8.21±0.05
30 d	7.92±0.05	8.10±0.05

All analyses were performed in triplicate