

## ACCEPTED ARTICLE

# Valorization of Tunisian acorn "*Quercus suber* L." starch in stirred yogurt

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## Abstract

The current research aimed first, to valorize Tunisian cork oak acorn starch extracted by water soaking method in dairy industry. The effect of its incorporation into milk on the evolution of rheological, sensorial and microbiological properties of stirred yogurt during refrigerated storage was, also, evaluated. No significant effect was observed on fermentation parameters of the acorn starch added product. During 28 days of refrigerated storage, the incorporation of acorn starch did not affect the post-acidification and the viability of the lactic starter cultures. Moreover, acorn starch added stirred yogurt exhibited a lower syneresis value and a higher consistency when compared to untreated control and that incorporated with industrial modified starch. The findings revealed that acorn starch incorporation extended the shelf life of the final product by about 6 days. Despite its less appreciated color ( $3.67 \pm 0.5$ ), acorn starch added product gained the highest overall acceptability scores ( $4.11 \pm 0.6$ ) by the panel.

**Keywords:** Cork oak acorn, quality, shelf life, starch, stirred yogurt.

## Introduction

The *Quercus* genus belonging to *Fagaceae* family includes several species (Korus et al., 2015). This variability leads to significant differences in the biochemical composition of acorns from different species (Zarroug et al., 2020). The specie *Quercus suber* L. is growing abundantly in the Tunisian coastal-forestry regions (Masmoudi et al., 2020). In general, cork oak acorn (*Quercus suber* L.) fruit is used both for feeding animals and in human diet but it remained under exploited and needs more valorization (Vinha et al., 2016; Masmoudi et al., 2020). This fruit is considered as functional food having high nutritional value due to its richness in valuable compounds such proteins, fibers, vitamins, minerals and starch as well as natural bioactive compounds known for their good antioxidant activity (Masmoudi et al., 2020; Zarroug et al., 2020). Thus, cork oak acorns containing about 63% of starch (Masmoudi

40 et al., 2020) can constitute a new resource of highly-valued food ingredients particularly starch  
41 which represents an undervalued carbohydrate. In this context, particular and further interest  
42 must be given to acorn starch extraction and valorization. In fact, search for new promising  
43 ingredient for food industry with high potential for commercial use is increasing to develop  
44 new industrial applications and competitive market products (Vinha et al., 2016). In general,  
45 starch is the principal component in many food industries responsible for interesting  
46 functional, nutritional and textural properties of food products (Perez-Pacheco et al., 2014).  
47 It's a biodegradable carbohydrate polymer which has been widely studied due to its  
48 availability, price, properties and extensive industrial use. However, chemical composition,  
49 techno-functional properties, structure and crystallinity of starch granules are depending  
50 mainly on their botanical origin and growth conditions. These characteristics are essentially  
51 related to the amylose content, starch molecule's ability to hold water, hydrogen bonding and  
52 the degree of crystallinity (Correia et al., 2013 ; Zarroug et al., 2022). Thus, owing to its  
53 interesting properties such as high resistance and paste consistency, acorn starch can be used  
54 as thickening and stabilizing agents in food formulations (Vinha et al., 2016; Zarroug et al.,  
55 2020).

56 Due to its sensory characteristics and nutritive value solicited by consumers, yoghurt is  
57 considered as one of the most popular fermented dairy products and is widely consumed  
58 around the world (Ben Moussa et al., 2019a). The name yoghurt is assigned only for fermented  
59 products by the mixed culture containing both *Streptococcus salivarius ssp. thermophilus* (*S.*  
60 *thermophilus*) and *Lactobacillus delbrueckii ssp. bulgaricus* (*L. bulgaricus*) (Chen et al., 2017;  
61 Ben Moussa et al., 2019a). It provides nutritional and health benefits for human diet such as  
62 improving digestibility and lactose utilization (Ben Moussa et al., 2019a, 2019b) and having  
63 strong hypocholesterolemic action (Ben Moussa et al., 2020). Nowadays, despite the large  
64 types of yoghurts produced by the dairy industries, new products are still demanded (Ben  
65 Abdessalem et al., 2019). Thus, a wide range of bioactive compounds are added to yoghurt to  
66 improve its therapeutic properties (Ben Moussa et al., 2020), to enhance its viscosity and  
67 sensorial properties and prevent syneresis (Zarroug et al., 2020). Among the incorporated  
68 substances, there are probiotics, phytosterols, seeds, prebiotics (Ben Moussa et al., 2019b,  
69 2020), fennel essential oil, (Ben Abdessalem et al., 2019), exopolysaccharides (Zhang et al.,  
70 2015) and *Quercus ilex* starch (Zarroug et al., 2020).

71 To the best of our knowledge, many researchers studied the use of acorn flour and several  
72 hydrocolloids in bread. However, there is no study about the effect of the incorporation of  
73 *Quercus suber* L. starch on technological properties of foods and yogurts during storage. In

74 this regard, in the present study, the effect of acorn starch incorporation in milk during  
75 manufacturing of stirred yogurt was evaluated on its rheological properties as well as its shelf  
76 life.

77

## 78 **Materials and methods**

### 79 ***Acorn starch extraction***

80 First, acorn starch used in this study was extracted from cork oak acorn (*Quercus suber* L.)  
81 using water soaking method as described by Zhang et al. (2019). The slurry was filtered and  
82 obtained starch sediment was suspended in the distilled water and then, centrifuged at 4000  
83 rpm for 15 min. This procedure was repeated many times until white layer disappeared totally.  
84 The supernatant containing starch was dried at 45°C during 12h and crushed into a fine powder  
85 before being stored at room temperature until use.

86 Extracted acorn starch was characterized for biochemical analysis and color parameters  
87 (Zarroug et al., 2020).

88

### 89 ***Stirred yogurt production***

90 The new dairy product was produced in Tunisian CLN-Delice industry according to the  
91 manufacturing process of stirred yoghurt.

92 Skim milk powder was added to skimmed milk for standardization. Milk was homogenized,  
93 pasteurized at 95°C for 3 min and cooled down to 43°C. Three batches were prepared. One  
94 batch was served as control (CY) and the two others were incorporated with acorn starch (YA)  
95 and industrial modified starch (YM), respectively, at a raison of 1%. All batches were, then,  
96 inoculated with a freeze-dried mixed starter culture at 2% (*S. thermophilus* and *Lb. bulgaricus*)  
97 and incubated at 43° C until the gel structure was formed (pH reached 4.6 and acidity reached  
98 75 °D). Obtained gels were broken by stirring in the yogurt vats for 5 min at a speed of 60 rpm,  
99 distributed in flasks and stored at a refrigerator (4°C) overnight before analyses.

100 Sampling was performed at the first day of storage and every 7 days during 28 days of  
101 refrigerated storage at 4 °C. All determinations of physico-chemical and technological  
102 characteristics of produced yoghurts were done in triplicate. Descriptive sensorial analysis was  
103 performed at the beginning of storage.

### 104 ***Analysis of stirred yogurt characteristics during fermentation***

105 The pH of the prepared samples was measured with a pH-meter (WTW portable pH meter,  
106 315i/SET. Wissenschaftlich). The Dornic acidity (expressed as g lactic acid per 100 mL) was  
107 determined by the alkaline titration (Mahmoudi et al., 2021). For starter culture enumeration,

108 *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus*  
109 were enumerated on M17 agar and MRS agar (Biokar Diagnostics, France) during 48 hours,  
110 respectively at 44°C and 37°C (Mahmoudi et al., 2021). In this study, sampling was performed  
111 every 30 min during 210 min of fermentation.

112

### 113 ***Analysis of stirred yogurt characteristics during refrigerated storage***

#### 114 ***Post-acidification and total solids measurements***

115 Post-acidification was evaluated by measurement of pH and Dornic acidity of all produced  
116 samples (expressed as degree Dornic) (Ben Moussa et al., 2020). Total solids was determined  
117 according to Mahmoudi et al. (2021).

118

#### 119 ***Syneresis measurements***

120 The stirred gel was centrifuged for 20 min at 12075 g at 4°C, according to Ben Moussa et al.  
121 (2020). Syneresis (%) was calculated as weight of separated serum after centrifugation,  
122 relating to the total mass of gel that was centrifuged.

123

#### 124 ***Rheological analyses***

125 The apparent viscosity was determined, each two weeks during 28 days of storage, with a  
126 rotary viscometer (Rheomat RM-180, Germany) using a cone-plate geometry (60 mm in  
127 diameter) as described by Ben Moussa et al. (2019a). Viscosity measurements were performed  
128 between 0.01 and 500 s<sup>-1</sup> with flow properties assessed at 4°C. Area of thixotropic hysteresis  
129 loop as difference between the area under the up-flow curve and the down-flow curve was  
130 computed by the use of RSI Orchestrator v 6.5.8 software.

#### 131 ***Color measurements***

132 The colorimetric parameters L\* (Darkness or lightness), a\* (greeness or redness) and b\*  
133 (blueness or yellowness) were performed on the surface of the different samples, using a  
134 colorimeter (Minolta Chroma Meter, CR-300, Tokyo, Japan) (Mahmoudi et al., 2021).

135

#### 136 ***Microbiological analysis***

137 The total number of mesophilic organisms was enumerated using Plate Count Agar (Oxoid,  
138 Ltd, Basingstoke, England) at 30 °C for 48 h. (Ben Abdessalem et al., 2019). Enumeration of  
139 lactic strains was performed as described before in this study according to Mahmoudi et al.  
140 (2021). The enumeration of yeasts and molds, total and fecal coliforms were enumerated  
141 according to APHA (2001).

142

### 143 *Descriptive Sensorial analysis*

144 The sensorial properties of the control and incorporated products with starches were analyzed  
145 at the first day of refrigerated storage. The samples were placed into coded cups with three  
146 random digit numbers and served to panelists in a randomized order. Nine experts evaluated  
147 all prepared products for the main descriptors used to evaluate appearance, taste and texture  
148 mainly odor intensity, white color, flavor, bitter taste, mouth feel, consistency, syneresis and  
149 overall acceptance. A six-point scale was used ranging from 0 (low intensity) to 5 (high  
150 intensity) (ISO 13299, 2016).

### 151 *Shelf life prediction*

152 To predict the shelf life of control and prepared stirred yogurts, the data on Arrhenius model  
153 which is based on the accelerated shelf-life simulation test were used to evaluate how the  
154 deterioration process behaves as a function of storage time (Ben Abdesslem et al., 2019). In  
155 this study, all products were stored at various temperatures (4, 14 and 24 °C) in high-precision  
156 ( $\pm 0.2$  °C) incubators, during 28 days. Thereafter, they were subjected to microbiological  
157 (coliforms and yeast and molds) and physicochemical (pH and acidity) (dependent variables)  
158 analyses and they were sampled in appropriate time intervals to allow an effective kinetic  
159 analysis (0, 3, 6, 9, 12, 15, 18, 21 and 28 days). The effect of temperature on the rate of studied  
160 characteristics was determined by means of the Arrhenius equation:  $k = k_0 \exp(-E_a/RT)$   
161 allowing indicating the end of the shelf life.

162 Where  $k$  is the reaction rate constant,  $R$  is the universal gas constant ( $8.31 \text{ J K}^{-1} \text{ mol}^{-1}$ ),  $T$  is the  
163 absolute temperature (°K),  $E_a$  is the activation energy ( $\text{J mol}^{-1}$ ) of the studied action and  $k_0$  is  
164 the pre-exponential factor of the frequency factor.

### 166 *Statistical analysis*

167 All tests were possessed in three replications and the results were presented as mean and  
168 standard deviation (SD). An analysis of variance (ANOVA) in SPSS software version 26.0  
169 (SPSS IBM 2020) was performed with Duncan's test used at a significance level of 5% to  
170 highlight significant differences among the produced samples and during storage time.

## 172 **Results and discussion**

### 173 *Characterization of extracted acorn starch*

174 In this study, starch content in cork oak acorn (*Quercus suber* L.) fruit was about  
175  $48.32 \pm 2.05\%$ . Extracted acorn starch presented a beige to yellow color with  $L^*$  of  $60.36 \pm 0.28$   
176 and  $b^*$  of  $15.70 \pm 0.06$ . It was characterized by low moisture ( $12.36 \pm 0.10\%$ ), proteins ( $0.29 \pm$   
177

178 0.01%), lipids ( $0.25 \pm 0.01\%$ ) and ash ( $0.29 \pm 0.02\%$ ) contents allowing its safe storage and  
179 showing its high purity. More, acorn starch showed a high stability during heating and a strong  
180 gel formation capacity (Data not shown).

181

182 ***Influence of acorn starch incorporation on fermentation parameters of stirred yogurt***  
183 ***properties***

184 The evolution of pH and Dornic acidity values during fermentation of control and new stirred  
185 yogurt was given in Figure 1. Initially, no significant differences ( $p > 0.05$ ) were noted  
186 between all samples. Initial pH value of about  $6.40 \pm 0.05$  pH units dropped significantly  
187 during fermentation to reach the highest value ( $4.72 \pm 0.01$  pH units) in the product  
188 incorporated with acorn starch. Moreover, the variations in Dornic acidity confirmed the  
189 evolution of pH. In fact, acidity increased during fermentation with no significant differences  
190 registered between control and treated products which reached the same acidity value of  $75^\circ\text{D}$   
191 after 210 min. These data showed that the incorporation of starch did not affect the acid  
192 production.

193 In this study, the initial counts of *Streptococcus thermophilus* ( $5.4 \log \text{ UFC/g}$ ) and  
194 *Lactobacillus bulgaricus* ( $5.08 \log \text{ UFC/g}$ ) increased significantly ( $p < 0.05$ ) during  
195 fermentation for all tested dairy products (Data not shown). After 210 min of fermentation,  
196 lactic starter cultures reached nearest counts in all products suggesting that the incorporation  
197 of starches did not affect their proliferation. This finding was in accordance with that of  
198 Zarroug et al. (2020) reporting that acorn starch had no significant effect on lactic starter  
199 viability and on acid production.

200

201 ***Influence of acorn starch incorporation on stirred yogurt quality during refrigerated***  
202 ***storage***

203 ***pH and post-acidification variations***

204 In the present study, pH and Dornic acidity values of control and stirred yogurts added with  
205 starches are shown in Table 1. At the beginning of storage, no significant differences were  
206 observed between initial pH and Dornic acidity values of all analyzed samples. During storage  
207 at  $4^\circ\text{C}$ , pH decreased significantly to reach the highest value of  $4.30 \pm 0.00$  in prepared product  
208 with acorn starch. These values were actually desired in modern dairy industry in order to  
209 produce soft yoghurts recommended by consumers. Moreover, Dornic acidity increased  
210 significantly in all products. However, the highest values were always recorded in the control  
211 yoghurt. The variations in pH and Dornic acidity values were attributed to the proliferation of

212 acid-forming bacteria producing lactic acid during storage. At the 28<sup>th</sup> day of storage, the  
213 highest ( $108.6^{\circ}\text{D}\pm 0.01$ ) and lowest ( $94^{\circ}\text{D}\pm 0.00$ ) post-acidification values were registered,  
214 respectively, in the control and the sample added with acorn starch. These findings were  
215 partially in line with those reported by Ben Moussa et al. (2019b) who showed that no  
216 significant differences in terms of post acidification were noted between the control yoghurt  
217 and that added with lactulose. Furthermore, data on post acidification confirmed the results  
218 obtained during fermentation showing that acorn starch did not affect the viability of lactic  
219 starter cultures (Vinha et al., 2016).

220

### 221 ***Total solids variations***

222 Results of total solids variation of all prepared dairy products during refrigerated storage were  
223 given in Table 1. Initial dry matter levels were  $22.50\pm 0.10\%$ ;  $22.7\pm 0.01\%$  and  $22.87\pm 0.01\%$ ,  
224 for control and products added with modified and acorn starches, respectively. These values  
225 were lower than those reported by Zarroug et al. (2020). During storage, total solids content  
226 dropped significantly for all analyzed samples. However, after starches incorporation, the  
227 values increased when compared to control product. At the end of storage, significant  
228 differences ( $p < 0.05$ ) were observed between control and treated samples in terms of total  
229 solids. The obtained contents were higher than those reported in the literature at the 28<sup>th</sup> day  
230 of refrigerated storage (Ben Abdessalem et al., 2019). Also, results were in accordance with  
231 those of Ben Moussa et al. (2019a) and Zarroug et al. (2020) who reported an increase in total  
232 solids levels when lactulose and acorn starch (1%) were added, respectively.

233

### 234 ***Syneresis variations***

235 In this study, the whey separation levels of all prepared dairy products are shown in Table 1.  
236 The results showed that, initially, no syneresis was observed in samples added with starches  
237 with a very low percentage (1%) of whey separation in the control. This finding was in  
238 disagreement with the literature reporting initial syneresis levels in control and treated yoghurts  
239 varying from 32.15% to 63.34% (Ben Abdessalem et al., 2019; Ben Moussa et al., 2020).  
240 During storage, syneresis levels increased in all samples. This finding was in perfect agreement  
241 with those of the cited studies. Furthermore, it can be observed that products incorporated with  
242 starches particularly acorn starch had significantly lower ( $p < 0.05$ ) syneresis values throughout  
243 refrigerated storage as well as at the end of storage period ( $4.83 \pm 0.01\%$ ). This result could be  
244 related to the increase of total solids in starches added products (Zarroug et al., 2020). Thus,  
245 addition of acorn starch led to syneresis reduction and gel stability improvement due to the

246 fact that hydroxyl groups of amylose and amylopectin bind water into molecules (Zarroug et  
247 al., 2020). This decrease in serum release could be, also, attributed to the higher pH values and  
248 lower acidity after incorporation of acorn starch, reducing caseins micelles destabilization and  
249 leading to a good preservation and an improvement of shelf life (Mahmoudi et al., 2020).

250

### 251 **Viscosity variations**

252 The viscosity of stirred yogurts similar to yoghurt is considered as the most important criteria  
253 affecting the texture (Ilyasoglu and Yilmaz, 2018) and the sensorial characteristics of the  
254 product as well as the consumer's pleasure of eating and preferences. The results of consistency  
255 coefficients (K) of produced stirred yogurts are shown in Table 1. In this study, consistency  
256 coefficients were obtained from the flow curves of shear stress (P) as function of shear rate ( $s^{-1}$ ).  
257 All samples showed shear thinning or non-Newtonian pseudoplastic flow behavior due to  
258 the breakdown of the caseins-gel structure and thus, the reduction of viscosity with shear rate  
259 increase (Data not shown). These findings were in a great agreement with those reported in the  
260 literature on yoghurt samples (Ilyasoglu and Yilmaz, 2018; Ben Moussa et al., 2019 a, b). The  
261 highest initial consistency value ( $9.65 \pm 0.03 \text{ Pa.s}^n$ ), consisting of gel formation of milk  
262 proteins, was observed in the product added with acorn starch followed by the product  
263 incorporated with modified starch ( $8.37 \pm 0.45 \text{ Pa.s}^n$ ) and then the control ( $7.63 \pm 0.02 \text{ Pa.s}^n$ )  
264 (Table 1). These values were higher than that ( $1.73 \text{ Pa.s}^n$ ) registered by Zarroug et al. (2020)  
265 when incorporating 1% of acorn starch extracted from *Quercus ilex* variety. Thus, this  
266 concentration of added starch improved the rates of aggregation in the casein gels and the  
267 structural arrangement which contributed to form a strong firm gel and to increase consistency  
268 of the prepared product. The incorporation of acorn starch increased the pseudoplasticity of the  
269 final product and its viscosity which could be attributed to the increase of total solid content as  
270 reported by Ben Moussa et al. (2019a) showing similar observation on yoghurt added with  
271 lactulose. During all storage period, the consistency values of the products added with starches  
272 were improved when compared to control which exhibited the lowest values. The consistency  
273 decreased significantly ( $p < 0.05$ ) in all analyzed samples which can be assigned to the  
274 proteolysis phenomenon during refrigerated storage. It was noted that, at the end of storage  
275 period, the highest consistency ( $7.54 \pm 0.08 \text{ Pa.s}^n$ ) was registered after incorporation of acorn  
276 starch when compared to control ( $4.78 \pm 0.04 \text{ Pa.s}^n$ ) and product added with modified starch  
277 ( $5.86 \pm 0.36 \text{ Pa.s}^n$ ).

278

279



### 280 ***Color parameters variations***

281 Data on color parameters ( $L^*$ ,  $a^*$  and  $b^*$ ) are displayed in Table 1. At the first day of storage,  
282 the product added with acorn starch showed the initial lowest lightness  $L^*$  ( $51.01 \pm 0.01$ ) and  
283 highest yellowness ( $13.40 \pm 0.00$ ). During refrigerated storage, luminosity  $L^*$ , red color  $a^*$   
284 and yellow color  $b^*$  decreased for all analyzed samples. As described before in this study,  
285 acorn starch was characterized by a yellow color. Thus, from the beginning and until the end  
286 of the storage period, yellowness of the stirred yogurt increased with incorporation of acorn  
287 starch into the dairy milk and the lightness and redness showed a decreasing trend when  
288 compared to the two other prepared products. This finding showed that the addition of acorn  
289 starch enhanced the yellowness of the final product and reduced its lightness and redness.

290

### 291 ***Microbiological quality variations***

292 The counts of total mesophilic aerobic flora and lactic starter cultures are presented in Figure  
293 2. During storage, the counts of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*  
294 increased significantly ( $p < 0.05$ ) until the 14<sup>th</sup> day of storage and then, decreased slightly  
295 toward the end of the storage period due to the retro-inhibition of lactic acid bacteria caused  
296 by post-acidification. This trend was similar ( $p > 0.05$ ) in all analyzed products, during the  
297 entire storage period, indicating that lactic starter cultures could survive in presence of starches  
298 (Zarroug et al., 2020). This result was partially in line with that of Vinha et al. (2016) showing  
299 that this polysaccharide can be used as a prebiotic growth promoter. Moreover, the counts of  
300 *Lb. bulgaricus* and *S. thermophilus* were maintained more than 8 log CFU/g during the four  
301 weeks of storage revealing a good quality of the prepared final products as reported before by  
302 Ilyasoglu and Yilmaz (2018) and recommended by the Codex Alimentarius (more than  $10^7$   
303 CFU/g).

304 The control product exhibited the higher total flora counts from the first day of storage  
305 ( $3.24 \pm 0.02$  log CFU/g) until the 28<sup>th</sup> day ( $3.94 \pm 0.02$  log CFU/g). A significant increase of  
306 the counts of this flora was observed in all samples throughout the refrigerated storage.  
307 However, incorporating acorn starch in milk maintained the lowest microbial charges in the  
308 final product. In this study, yeasts, molds, total and fecal coliforms were absent during all the  
309 storage period in the stirred yogurt incorporated with acorn starch (Data not shown). Also,  
310 results demonstrated the satisfactory quality of control and added product with modified starch  
311 with an absence of these bacterial flora and particularly fecal coliforms during all storage  
312 period (Data not shown). These findings could be attributed, first, to the presence of lactic acid  
313 bacteria and the high produced acidity in this stirred yogurt inhibiting undesirable bacterial

314 growth and also, the water holding capacity of acorn starch reducing the water content in the  
315 product and thus, retarding microbial growth.

316  
317 ***Sensorial properties***

318 The sensorial scores of all analyzed products are presented in Table 2. Panelists identified  
319 significant differences ( $p < 0.05$ ) between all samples in terms of white color, acidic taste and  
320 mouth feel, while all other attributes were not affected ( $p > 0.05$ ). With the incorporation of  
321 acorn starch, the color score decreased while the odor, flavor and acidic taste scores increased.  
322 As expected, the sample prepared with acorn starch, characterized by its yellow tone,  
323 presented the lowest color score ( $3.67 \pm 0.50$ ) when compared to other samples ( $4.78 \pm 0.40$   
324 and  $4.00 \pm 0.00$ , respectively, for the control and the product added with modified starch).

325 Since acorn starch did not affect the viability of lactic starter cultures, all stirred yogurts  
326 presented close scores for the majority of descriptors essentially the flavor. In fact, Chen et al.  
327 (2017) demonstrated that the lactic cultures produced the most key flavor components during  
328 fermentation of yogurt. Besides, the control presented less consistency acceptability and whey  
329 exudation acceptability with the lowest scores. Otherwise, the smooth oral texture score of the  
330 control was better ( $p < 0.05$ ) than the other samples which was attributed to the adjunction of  
331 starches. Based on these findings, the stirred yogurt added with acorn starch presented the  
332 highest overall acceptance ( $4.11 \pm 0.60$ ), in a maximum of 6 points scale.

333  
334 ***Shelf life assessment***

335 In the current study, the Arrhenius model was used to determine the shelf life of control and  
336 stirred yogurts added with acorn and modified starches. Based on the regression coefficient,  
337 the zero-order dynamic model was used for the variable acidity. However, the first-order  
338 reaction model was applied for all other tested variables (pH and microbiological counts).  
339 During 28 days of storage, data showed an increase in Dornic acidity and in microbial charges  
340 and a decrease in pH values for all tested samples. However, the lowest Dornic acid values  
341 and microbial counts were registered when samples were stored at 4 °C as compared to the  
342 samples stored at 14 °C and 24 °C during the whole storage time. Moreover, the better quality  
343 was assigned to samples added with starches showing close shelf lives, when compared to  
344 control. Depending on different tested parameters, it was noted that the predicted shelf-life of  
345 the control ranged from 25 days (pH and acidity) to 27 days (coliforms). However, the stirred  
346 yogurt added with acorn starch presented respective shelf lives of about 31 days (pH), 32 days

347 (yeast and molds) and 33 days (acidity and coliforms) (Data not shown). Thus, starch  
348 incorporation extended the shelf life of the stirred yogurt by about 6 days.

349

## 350 **CONCLUSIONS**

351 Results of the present study showed that cork oak acorn (*Quercus suber* L.) flour contained  
352 a high content of starch. This non valorized component was characterized by a yellow color.  
353 In order to improve its use in food industries, acorn starch was incorporated into milk during  
354 manufacturing of stirred yogurt and compared to a control and a product added with industrial  
355 modified starch. During fermentation, incorporation of acorn starch had no effect on the  
356 acidification rate and the fermentation time as well as the viability of lactic starter strains.  
357 During refrigerated storage, the treated products had similar post-acidification and close  
358 counts of lactic starter cultures to those of control. In general, the incorporation of acorn starch  
359 exhibited an increase of total solids leading to a consistency improvement and syneresis  
360 reduction in the final product regarding the other tested samples. The data showed that the  
361 incorporation of acorn starch enhanced the yellowness of the stirred yogurt which was less  
362 appreciated by the panel but affected positively its sensorial properties leading to the highest  
363 overall acceptability. Moreover, acorn starch improved the shelf life of the final product by  
364 about 6 days. In conclusion, acorn starch was shown to be an interesting ingredient in dairy  
365 industry and could be used in food industries in order to improve rheological and sensorial  
366 properties of final products.

367

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372

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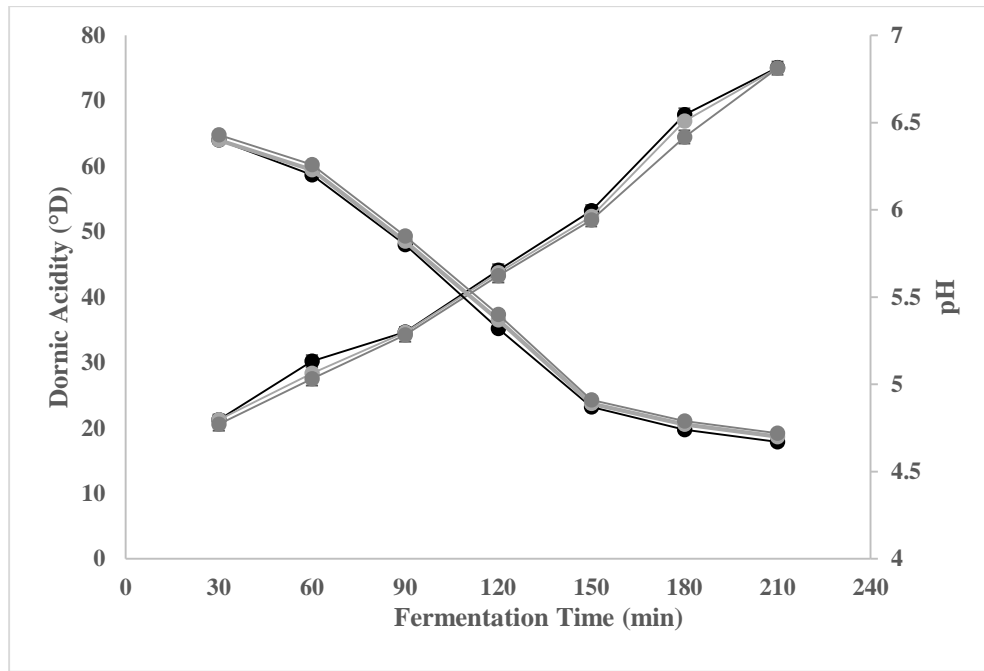
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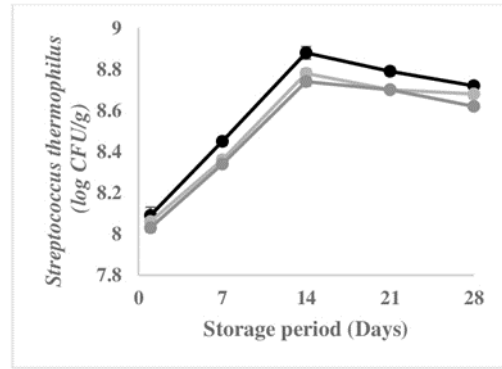
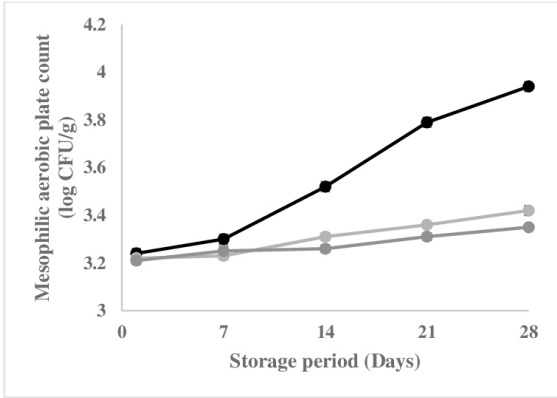
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**Figure 1 - Dornic acidity and pH changes during fermentation of stirred yogurts.**

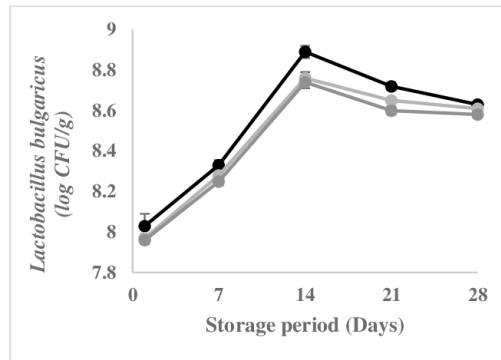
● Control stirred yogurt; ● Stirred yogurt incorporated with modified starch; ● Stirred yogurt incorporated with acorn starch.

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**Figure 2** - Evolution of mesophilic aerobic plate (a) *Streptococcus thermophilus* (b) and *Lactobacillus bulgaricus* (c) counts during refrigerated storage of stirred yogurts.

● Control stirred yogurt; ● Stirred yogurt incorporated with modified starch; ● Stirred yogurt incorporated with acorn starch.

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**Table 1.** Evolution of physicochemical properties, syneresis, consistency and color parameters of stirred yogurts during 28 days of storage at +4°C.

| Parameter   | Storage period (Days) | Stirred yogurts             |                              |                             |
|---|-----------------------|-----------------------------|------------------------------|-----------------------------|
|   |                       | CP                          | MSP                          | ASP                         |
| <b>pH</b>   | <b>1</b>              | 4.58 ± 0.34 <sup>aA</sup>   | 4.58 ± 0.01 <sup>aA</sup>    | 4.59 ± 0.01 <sup>aA</sup>   |
|   | <b>7</b>              | 4.45 ± 0.01 <sup>aAB</sup>  | 4.48 ± 0.00 <sup>aAB</sup>   | 4.49 ± 0.00 <sup>aAB</sup>  |
|   | <b>14</b>             | 4.30 ± 0.01 <sup>aBC</sup>  | 4.34 ± 0.01 <sup>aB</sup>    | 4.37 ± 0.01 <sup>aB</sup>   |
|   | <b>21</b>             | 4.25 ± 0.00 <sup>aC</sup>   | 4.29 ± 0.01 <sup>aB</sup>    | 4.32 ± 0.01 <sup>aB</sup>   |
|   | <b>28</b>             | 4.21 ± 0.01 <sup>aC</sup>   | 4.27 ± 0.00 <sup>aB</sup>    | 4.30 ± 0.00 <sup>aB</sup>   |
| <b>Dornic acidity (°D)</b>                          | <b>1</b>              | 77.00 ± 0.00 <sup>aA</sup>  | 77.06 ± 0.05 <sup>aA</sup>   | 75.50 ± 0.00 <sup>aA</sup>  |
|   | <b>7</b>              | 85.00 ± 0.00 <sup>aB</sup>  | 83.49 ± 0.00 <sup>aB</sup>   | 83.00 ± 0.00 <sup>aB</sup>  |
|   | <b>14</b>             | 97.49 ± 0.00 <sup>aC</sup>  | 95.00 ± 0.00 <sup>aC</sup>   | 91.10 ± 0.10 <sup>bC</sup>  |
|   | <b>21</b>             | 99.50 ± 0.00 <sup>aD</sup>  | 96.66 ± 0.01 <sup>bCD</sup>  | 94.00 ± 0.10 <sup>cCD</sup> |
|   | <b>28</b>             | 108.60 ± 0.01 <sup>aE</sup> | 102.50 ± 0.01 <sup>bD</sup>  | 96.00 ± 0.00 <sup>bD</sup>  |
| <b>Total solids (%)</b>                             | <b>1</b>              | 22.50 ± 0.10 <sup>aA</sup>  | 22.70 ± 0.00 <sup>aA</sup>   | 22.87 ± 0.01 <sup>aA</sup>  |
|   | <b>7</b>              | 21.61 ± 0.02 <sup>aB</sup>  | 22.43 ± 0.01 <sup>abAB</sup> | 22.68 ± 0.02 <sup>bA</sup>  |
|   | <b>14</b>             | 21.15 ± 0.02 <sup>aB</sup>  | 22.30 ± 0.01 <sup>abAB</sup> | 22.52 ± 0.01 <sup>bA</sup>  |
|   | <b>21</b>             | 20.85 ± 0.01 <sup>aBC</sup> | 21.97 ± 0.01 <sup>abB</sup>  | 22.07 ± 0.06 <sup>aBB</sup> |
|   | <b>28</b>             | 20.51 ± 0.01 <sup>aC</sup>  | 21.71 ± 0.01 <sup>bB</sup>   | 21.81 ± 0.01 <sup>bB</sup>  |
| <b>Syneresis (%)</b>                                | <b>1</b>              | 1.00 ± 0.00 <sup>aA</sup>   | 0.00 ± 0.00 <sup>aA</sup>    | 0.00 ± 0.00 <sup>aA</sup>   |
|   | <b>7</b>              | 4.47 ± 0.00 <sup>aB</sup>   | 2.53 ± 0.00 <sup>bB</sup>    | 1.17 ± 0.01 <sup>cB</sup>   |
|   | <b>14</b>             | 7.1 ± 0.01 <sup>aC</sup>    | 3.00 ± 0.00 <sup>bB</sup>    | 2.07 ± 0.00 <sup>cC</sup>   |
|   | <b>21</b>             | 11.47 ± 0.01 <sup>aD</sup>  | 5.00 ± 0.01 <sup>bC</sup>    | 3.57 ± 0.01 <sup>cD</sup>   |
|   | <b>28</b>             | 13.4 ± 0.01 <sup>aE</sup>   | 6.93 ± 0.00 <sup>bD</sup>    | 4.83 ± 0.01 <sup>cE</sup>   |
| <b>Consistency coefficient K (Pa.s<sup>n</sup>)</b> | <b>1</b>              | 7.63 ± 0.02 <sup>aA</sup>   | 8.37 ± 0.45 <sup>bA</sup>    | 9.65 ± 0.03 <sup>cA</sup>   |
|   | <b>14</b>             | 6.12 ± 0.02 <sup>aB</sup>   | 7.64 ± 0.02 <sup>bB</sup>    | 9.24 ± 0.04 <sup>cB</sup>   |
|   | <b>28</b>             | 4.78 ± 0.04 <sup>aC</sup>   | 5.86 ± 0.36 <sup>bC</sup>    | 7.54 ± 0.08 <sup>cC</sup>   |
| <b>L*</b>   | <b>1</b>              | 57.11 ± 0.02 <sup>aA</sup>  | 52.18 ± 0.00 <sup>bA</sup>   | 51.01 ± 0.01 <sup>cA</sup>  |
|   | <b>7</b>              | 55.01 ± 0.01 <sup>aB</sup>  | 51.13 ± 0.01 <sup>bAB</sup>  | 50.43 ± 0.00 <sup>cA</sup>  |
|   | <b>14</b>             | 52.00 ± 0.01 <sup>aC</sup>  | 50.01 ± 0.01 <sup>bBC</sup>  | 49.39 ± 0.00 <sup>bB</sup>  |
|   | <b>21</b>             | 50.59 ± 0.00 <sup>aD</sup>  | 49.00 ± 0.00 <sup>bC</sup>   | 49.01 ± 0.01 <sup>bB</sup>  |
|   | <b>28</b>             | 48.50 ± 0.00 <sup>aE</sup>  | 48.01 ± 0.01 <sup>bC</sup>   | 47.82 ± 0.01 <sup>bC</sup>  |
| <b>b*</b>   | <b>1</b>              | 13.11 ± 0.01 <sup>aA</sup>  | 13.29 ± 0.00 <sup>bA</sup>   | 13.40 ± 0.00 <sup>cA</sup>  |
|   | <b>7</b>              | 12.51 ± 0.01 <sup>aB</sup>  | 13.25 ± 0.00 <sup>bA</sup>   | 13.38 ± 0.01 <sup>bA</sup>  |
|   | <b>14</b>             | 12.40 ± 0.00 <sup>aC</sup>  | 13.21 ± 0.01 <sup>bB</sup>   | 13.35 ± 0.00 <sup>bAB</sup> |
|   | <b>21</b>             | 12.39 ± 0.01 <sup>aD</sup>  | 13.12 ± 0.01 <sup>bC</sup>   | 13.32 ± 0.01 <sup>bB</sup>  |
|   | <b>28</b>             | 12.23 ± 0.01 <sup>aC</sup>  | 13.02 ± 0.01 <sup>bC</sup>   | 13.29 ± 0.01 <sup>cbB</sup> |
| <b>a*</b>   | <b>1</b>              | -0.61 ± 0.00 <sup>aA</sup>  | -0.783 ± 0.00 <sup>bA</sup>  | -0.866 ± 0.01 <sup>cA</sup> |
|   | <b>7</b>              | -0.79 ± 0.01 <sup>aB</sup>  | -0.840 ± 0.01 <sup>bB</sup>  | -0.980 ± 0.01 <sup>cB</sup> |
|   | <b>14</b>             | -0.83 ± 0.01 <sup>aC</sup>  | -0.903 ± 0.01 <sup>bC</sup>  | -0.946 ± 0.00 <sup>bC</sup> |
|   | <b>21</b>             | -0.96 ± 0.01 <sup>aD</sup>  | -0.970 ± 0.01 <sup>aD</sup>  | -1.020 ± 0.02 <sup>bD</sup> |
|   | <b>28</b>             | -1.04 ± 0.00 <sup>aE</sup>  | -1.064 ± 0.00 <sup>bE</sup>  | -1.081 ± 0.00 <sup>cE</sup> |

**CP:** Control stirred yogurt ; **MSP:** Stirred yogurt incorporated with modified starch; **ASP:** Stirred yogurt incorporated with acorn starch.

Data are mean±standard deviation, n= 3. Means with different superscripts are significantly different (P< 0.05).

Lowercase letters (a, b, c) represent the statistical difference between samples; Uppercase letters (A, B, C) represent the statistical difference between the same sample during storage period.

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501 **Table 2:** Sensorial properties of stirred yogurts at the first day of storage at +4°C.

|            | <b>Odor intensity</b>  | <b>White Color</b>     | <b>Acidic taste</b>    | <b>Flavor</b>          | <b>Mouth feel</b>      | <b>Consistency</b>     | <b>Syneresis</b>       | <b>Overall acceptability</b> |
|------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------------|
| <b>CP</b>  | 3.00±0.00 <sup>a</sup> | 4.78±0.40 <sup>b</sup> | 3.11±0.33 <sup>a</sup> | 4.11±0.60 <sup>a</sup> | 4.33±0.50 <sup>b</sup> | 3.78±0.44 <sup>a</sup> | 4.67±0.50 <sup>a</sup> | 3.67±0.71 <sup>a</sup>       |
| <b>MSP</b> | 2.78±0.44 <sup>a</sup> | 4.00±0.00 <sup>a</sup> | 3.78±0.44 <sup>b</sup> | 4.00±0.70 <sup>a</sup> | 3.56±0.53 <sup>a</sup> | 3.89±0.33 <sup>a</sup> | 5.00±0.00 <sup>a</sup> | 3.89±0.78 <sup>a</sup>       |
| <b>ASP</b> | 3.22±0.44 <sup>a</sup> | 3.67±0.50 <sup>a</sup> | 3.89±0.33 <sup>b</sup> | 4.22±0.67 <sup>a</sup> | 3.78±0.67 <sup>a</sup> | 4.11±0.33 <sup>a</sup> | 5.00±0.00 <sup>a</sup> | 4.11±0.60 <sup>a</sup>       |

502 **CP:** Control stirred yogurt; **MSP:** Stirred yogurt incorporated with modified starch; **ASP:** Stirred yogurt  
 503 incorporated with acorn starch.

504 Lowercase letters (a, b, c) represent the statistical difference between samples.

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