

Effect of fortification with *Artemisia absinthium* leaf powder on yoghurt quality during storage

Mouna Boulares^{a*}, Asma Bezzezi^a, Imen Mahmoudi^a, Nour El Houda Aloui^a, Olfa Ben Moussa^a, Mnasser Hassouna^a

^a Research Unit 'Bio-Preservation and Valorization of Agricultural Products UR13-AGR 02', Higher Institute of Food Industries of Tunisia, Carthage University, Tunisia.

*Corresponding author; e-mail: boulares_mouna2006@yahoo.fr

Abstract

This study aimed to evaluate the sensorial, physico-chemical, rheological and microbiological properties of fortified yoghurt with *Artemisia absinthium* leaf powder during refrigerated storage. The valorization of this plant in food industry was realized by the incorporation of its dried leaf powder at various concentrations in order to produce a new functional yoghurt. It is interesting to note that the fortified yoghurt with the lowest *Artemisia* dose (2%) was the most preferred by the panel. More, this fortification did not affect the fermentation parameters nor the viability of lactic starter. During storage, the incorporation of *Artemisia* powder reduced syneresis and improved the microbiological properties of fortified yoghurts. Besides, at the end of storage, it was noted that fortified yoghurt with 2% of *Artemisia* powder presented the highest consistency ($8.98 \pm 0.04 \text{ Pa.s}^n$) and antioxidant activity ($60.08 \pm 3.61 \%$) when compared to control yoghurt. Finally, the accelerated shelf-life test showed the efficiency of *Artemisia absinthium* powder incorporation by increasing the shelf life of yoghurt by about 4 days.

Key words: *Artemisia absinthium*, leaf, functional yoghurt, quality, shelf life.

INTRODUCTION

Artemisia absinthium L. commonly known as wormwood, is an important perennial shrubby medicinal plant native to North Africa, Middle East, Europe, and Asia. *Artemisia* is one of the most predominant and widely distributed genus in Asteraceae family. Its leaves and flowers are very bitter and have a distinctive aroma (Batiha et al., 2020). *Artemisia absinthium* contains many phytochemical compounds such as terpenoids, organic acids, lactones, tannins, resins, and phenols. It also contains flavonoids and phenolic acids (coumaric, syringic, salicylic, chlorogenic, and vanillic acids) which contribute to free radical scavenging mechanism. The medicinal efficacy of this plant is often based on its bioactive ingredients. Actually, *Artemisia*

38 *absinthium* displayed antifungal action too which makes this plant an essential natural product
39 in pharmaceuticals, cosmetics and food industries (Batiha et al., 2020).

40 Yoghurt is one of the most popular fermented dairy products widely consumed all over the
41 world due to its nutritional and sensory characteristics and health benefits (Ben Moussa et al.,
42 2019). Yoghurt is produced by lactic fermentation of two specific strains: *Lactobacillus*
43 *delbrueckii* *ssp. bulgaricus* (*Lactobacillus bulgaricus*) and *Streptococcus salivarius* *ssp.*
44 *thermophilus* (*Streptococcus thermophilus*) (Obudi et al., 2019). So often, food hydrocolloids
45 or bioactive compounds are added to yoghurts to modify the texture, increase the stability or to
46 enhance their functionality, quality and therapeutic properties (Pirsa et al., 2018).

47 In this connection, the present study aimed to valorize *Artemisia absinthium* leaf powder by its
48 incorporation at different concentrations in order to formulate new functional yoghurt. **First,**
49 **the effect of *Artemisia absinthium* powder incorporation on fermentation parameters was**
50 **evaluated. Then, the influence of this fortification was studied on yoghurt quality during 28**
51 **days of refrigerated storage as well as on the shelf life produced functional product.**

52

53 MATERIALS AND METHODS

54 Yoghurt manufacturing

55 *Artemisia absinthium* L. was collected during January 2020 from Bizerte in north Tunisia and
56 identified by a specialist in botany. The leaves were dried at room temperature for three weeks.
57 *Artemisia absinthium* leaf powder was characterized by respective moisture and ash contents
58 of about 4.18 % and 3.34 %. Their protein, fat and carbohydrate contents were 11.97%, 8.8%
59 and 6.93 %, respectively.

60 The manufacturing of yoghurt was realized at an industrial scale in CLN from Delice group of
61 north Tunisia. The Fresh cow's milk with 15,15±0,01°D acidity (pH 6,67±0,01), 31,50±0,005
62 g/L fat and 47,38±0,005 g/L lactose was received. Milk was standardized, homogenized and
63 pasteurized at 90 °C for 5 min, and then cooled to 45 °C. It was then inoculated with 2% of
64 lyophilized starter culture (*S. thermophilus* and *L. bulgaricus*) (Chr. Hansen, Denmark) and
65 incorporated with *Artemisia absinthium* powder at the appropriate concentrations. After a
66 second homogenization process, inoculated milk was distributed into propylene containers and
67 incubated at a fermentation temperature of 45 °C for 6 h. Fermentation was stopped by rapid
68 cooling when the acidity reached 75°D, and the product was stored at +4 °C. The first yoghurt
69 sample was prepared without adding *Artemisia absinthium* powder, and it served as a control
70 (YC). Three other batches were incorporated with *Artemisia absinthium* powder to obtain:

71 Fortified yoghurt with 2 % of *Artemisia* powder (YD₁); Fortified yoghurt with 4 % of *Artemisia*
72 powder (YD₂); Fortified yoghurt with 6 % of *Artemisia* powder (YD₃). First, the sensory
73 analysis was performed on the control and the fortified yoghurts in order to choose the optimal
74 dose. The parameters of fermentation were, then, evaluated during 6 hours of fermentation on
75 control and selected fortified yoghurts. Also, physico-chemical, rheological and
76 microbiological properties of all analyzed yoghurts were evaluated, during 28 days of storage
77 at + 4 °C. The sampling was performed on days 1, 7, 14, 21 and 28 of storage.

78 Sensorial analysis

79 After the first day of storage at 4°C, the sensorial properties of control and fortified yoghurts
80 were evaluated by 8 expert panelists from CLN Dairy Industry, Delice group. The samples were
81 subjected to a descriptive sensory evaluation performed inside a uniformly illuminated room,
82 at approximately 25 °C. The obtained yoghurts were coded and, then, served to panelists in
83 randomized order to give a score for each descriptor ranging from zero to nine. The main
84 descriptors were odor intensity, white color, acidic taste, bitter taste, whey exudation,
85 mouthfeel, consistency and overall acceptance (ISO 22935-1: 2023).

86

87 Physico-chemical analyses

88 The physico-chemical characterization of studied yoghurts was evaluated. In fact, the pH
89 value was measured with a Microprocessor pH-meter BT-500 (Boeco, Hamburg, Germany).
90 The titratable acidity was expressed as Dornic degree (Mahmoudi et al., 2021). The syneresis
91 was calculated according to Ben Moussa et al. (2020). Briefly, the yoghurt was centrifuged
92 for 20 min at 12075 g at 4°C and syneresis (%) was calculated as weight of separated serum
93 after centrifugation related to the total mass of centrifuged gel.

94 The color parameters L* (lightness/darkness), a* (redness/greenness), and
95 b* (yellowness/blueness) were determined according to the CIELAB color space using a
96 colorimeter (Minolta Chroma Meter, CR-300, Tokyo, Japan) (Mahmoudi et al., 2021).

97

98 Antioxidant activity determination

99 The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to evaluate the free radical
100 scavenging ability of yoghurt extracts. The DPPH assay was performed as described by Elfahri
101 et al. (2016) with some modifications. A total of 800 µl of the DPPH solution (0.1 mM DPPH
102 in 95% methanol) was added to 200 µl of each yoghurt extract. Then, the mixture was
103 centrifuged at 9200 rpm / 2 min and kept in the dark during 30 minutes. The absorbance was

104 measured against a blank containing distilled water and DPPH solution, using a JENWAY 6305
105 spectrophotometer at a wavelength of 517 nm. The radical scavenging activity was expressed
106 as the inhibition percentage and was calculated using the following formula:

107 Antioxidant activity (%) = [(Absorbance of control – Absorbance of sample) / Absorbance of
108 blank] × 100.

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110

111 **Rheological analysis**

112 The rheological properties were determined according to the method described by Ben Moussa
113 et al. (2019). Briefly, yoghurt samples were analyzed with a rotary viscometer Rheometric
114 RM180 (Rheomat, Caluire, France), using a coaxial cylinders' geometry. The bob and the cup
115 used had 15.18 (R₁) and 21 mm (R₂) radius, respectively, giving a ratio R₁/R₂=0.72. Viscosity
116 measurements were between 0.01 and 500 s⁻¹. The viscometer was controlled by RSI
117 Orchestrator v6.5.8 software. Flow properties were maintained at 4°C.

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119 **Microbiological analyses**

120 Counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were enumerated,
121 respectively, on M17 agar and MRS agar (Biokard, diagnostics, Beauvais, France) during 48
122 hours, respectively at 44°C and 37°C (Mahmoudi et al., 2021). The mesophilic aerobic plate
123 count was enumerated using Plate Count Agar (Oxoid, Ltd, Basingstoke, England) at 30 °C for
124 48 h (Ben Abdessalem et al., 2020). Yeasts, molds and coliforms were enumerated according
125 to APHA (2001).

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127

127 **Shelf life prediction**

128 In this study, the shelf life estimation of analyzed yoghurts was studied using an Accelerated
129 shelf life test to evaluate how the deterioration process behaves during 28 days of storage at
130 various temperatures (4, 14 and 24 °C). Yoghurt samples were subjected to physicochemical
131 (pH and acidity) and microbiological (coliforms and yeast and molds) analyses. Sampling was
132 performed in appropriate time intervals to allow an effective kinetic analysis.

133 The equation (1) expressed the kinetic equation, and Equation (2) is the Arrhenius equation.

$$134 \quad A = A_0 e^{Kt} \quad (1)$$

$$135 \quad K = K_0 e^{(-Ea/RT)} \quad (2)$$

136 where K is the reaction rate constant, t is the time, A_0 is the product characteristic at initial
137 condition and A is the quality factor. K_0 is the pre-exponential factor of the frequency factor,
138 E_a is the energy of activation (J mol^{-1}), R is the universal gas constant ($8.31 \text{ J K}^{-1} \text{ mol}^{-1}$) and T
139 is the absolute temperature ($^{\circ}\text{Kelvin}$) (Boulares et al., 2022).

140 The shelf life of yoghurts can be finally predicted when determining the order kinetics equation
141 (zero-order or first-order reaction model) allowing to define the parameters indicating the end
142 of the shelf life based on the risk level.

143

144 **Statistical analysis**

145 The results related to all analyses were presented as mean and standard deviation. All tests
146 were possessed in three replications. An analysis of variance (ANOVA) in SPSS software
147 (SPSS IBM 2020) was performed with Duncan's test used at a significance level of 5% to
148 highlight significant differences among the produced samples and during storage time.

149

150 **RESULTS AND DISCUSSION**

151 **Effect of *Artemisia absinthium* powder incorporation on sensory quality of yoghurt**

152 The results of the sensorial profile (Figure 1), showed significant differences ($p < 0.05$) between
153 the fortified yoghurts with *Artemisia* leaf powder (YD₁, YD₂ and YD₃) and the control (YC).

154 All analyzed yoghurts were different ($p < 0.05$) in terms of odor intensity with attributed notes
155 of about 0; 1.87; 4.62 and 7.75, respectively for YC, YD₁, YD₂ and YD₃. For the intensity of
156 white color, the four yoghurt samples had shown different and variable colors with scores of
157 9.00; 6.87; 4.00 and 1.50, respectively. In fact, fortified yoghurt with the highest dose of
158 *Artemisia* (YD₃) was the greenest and the most bitter (7.12). This was due to the presence of
159 high content of chlorophyll pigments responsible for the green color of *Artemisia absinthium*.

160 Regarding consistency, results showed that YC and YD₁ were the most appreciated. In addition,
161 YD₃ was the less appreciated by panelists in terms of mouthfeel descriptor. Concerning the
162 bitterness and acidic taste, these descriptors were more noticeable in YD₂ and YD₃ which could
163 be attributed to the aromatic compounds of *Artemisia absinthium* (Boulares et al., 2023). For
164 the syneresis phenomenon, no whey exudation was observed on the first day of storage for all
165 analyzed yoghurts. Also, it can be concluded that the overall appreciation decreased with the
166 increase of *Artemisia absinthium* powder dose. Thereby, yoghurt (YD₁) fortified with the
167 lowest *Artemisia* powder dose was the most appreciated by the panel. In this regard, in the rest

168 of the study, only YD₁ and YD₂ were retained for evaluation of their qualities during
169 fermentation and refrigerated storage.

170 **Effect of *Artemisia absinthium* powder incorporation on fermentation parameters**

171 Initially, no significant difference ($p > 0.05$) was observed between the pH values of the control
172 yoghurt and those added with the two doses of *Artemisia absinthium* powder. The initial pH
173 value, which was around 6.37 ± 0.01 (Figure 2.a), decreased significantly ($p < 0.05$) during the
174 fermentation time for all analyzed yoghurts to reach a value of about 4.61 ± 0.01 pH units for
175 the control. This result was explained by the action of the lactic starter which degrade lactose
176 into lactic acid and cause a lowering of pH (Pernoud et al., 2005). This observation was in
177 agreement with that of Tokusoglu (2013) who noted a decrease in the pH of yoghurts during
178 fermentation with values ranging between 4.7 and 6.5. Furthermore, pH values of the control
179 remained lower than those of fortified yoghurts until the end of the fermentation. These results
180 were in agreement with those of Dhuol et al. (2013) reporting that the pH of a control fermented
181 milk product was lower than that of enriched product with cassava powder.

182 In addition, obtained results showed that initial acidity value (24 °D) increased significantly
183 ($p < 0.05$), during the fermentation (Figure 2.b). Even if acidity values remained higher in the
184 control when compared to the two fortified yoghurts until the 5th hour of fermentation, no
185 significant differences ($p > 0.05$) were observed. In fact, after 6 hours of fermentation, all tested
186 yoghurts, reached the same optimal acidity value (75 °D) confirming that the addition of
187 *Artemisia absinthium* powder did not affect significantly the acid production.

188 Concerning the evolution of the lactic starter, no significant differences ($p > 0.05$) were noted
189 between the control and fortified yoghurts with *Artemisia absinthium* powder, at the beginning
190 of the fermentation. More, a significant increase ($p < 0.05$) in the counts of *Streptococcus*
191 *thermophilus* (Figure 2.c) and *Lactobacillus bulgaricus* (Figure 2.d) was observed in all studied
192 yoghurts during fermentation. These findings were in accordance with those found by Joung et
193 al. (2016) reporting an increase of lactic starter loads during fermentation after addition of
194 persimmon leaf powder and white mulberry leaves extracts to yoghurt. Besides, nearest lactic
195 starter counts were noted in all studied yoghurts at the end of fermentation, confirming that the
196 addition of wormwood powder did not affect the viability of lactic starters.

198 **Effect of *Artemisia absinthium* powder incorporation on yoghurt quality during storage**

199 ***Effect on pH and post-acidification variations***

200 Quality parameters of control and fortified yoghurts evaluated during 28 days of refrigerated
201 storage are reported in Table 1. During storage, a decrease in pH and an increase in acidity were
202 recorded for all tested yoghurts. In fact, pH values decreased significantly to reach the lowest
203 value of 4.20 ± 0.01 in untreated control at the final day of storage. Moreover, initial acidity
204 value ($77 \text{ }^\circ\text{D} \pm 0.01$) increased for control yoghurt to reach a value of $106 \text{ }^\circ\text{D} \pm 0.01$, at the 28th
205 day of storage. It should be noted that fortified yoghurt (YD₂) with the highest dose of *Artemisia*
206 powder presented a significant lower value ($92 \text{ }^\circ\text{D} \pm 0.29$) at the end of storage when compared
207 to other analyzed yoghurts. These results were in agreement with those of Zhang et al. (2019)
208 suggesting a post-acidification of yoghurt during 3 weeks of storage at + 4 °C as a result of the
209 proliferation of acid-forming bacteria producing lactic acid during storage. More, similar
210 findings were found by Ben Abdesslem et al. (2020) reporting that titratable acidity of control
211 yoghurt was higher than that of fortified yoghurt with fennel essential oil due to the presence
212 of natural compounds having antimicrobial activity and preventing acid production which
213 confirm the protective role of *Artemisia absinthium* powder due to its richness in bioactive
214 compounds.

215

216 *Effect on syneresis variation*

217 As shown in table 1, no whey separation was observed in all analyzed yoghurts at the
218 beginning of storage. However, syneresis levels increased significantly ($p < 0.05$) during
219 storage to reach the lowest whey separation rates of about $6.50 \pm 0.10 \%$ and $8 \pm 0.00 \%$,
220 respectively for the fortified yoghurts YD₁ and YD₂ when compared to control ($14 \pm 0.80 \%$).
221 These results were in perfect agreement with those of Zhang et al. (2019) reporting that the
222 syneresis decreased in yoghurt fortified with 0.2 % of moringa compared to the control
223 yoghurt. It was interesting to note that incorporation of wormwood powder improved the
224 protein matrix of the yoghurt and reduced the proteolysis which contribute to the reduction of
225 serum release and as consequence the consistency improvement and the gel stability. This
226 finding could be attributed to interactions between *Artemisia* components and yoghurt proteins
227 as well as the lower acidity leading to caseins micelles stabilization and shelf life improvement
228 (Srisuvor et al., 2013).

229

230 *Effect on color parameters variations*

231 The evolution of yoghurt color parameters during storage are shown in Table 1. At the
232 beginning of refrigerated storage, fortified yoghurt with the highest *Artemisia* dose presented

233 the initial lowest lightness L^* (40.86 ± 0.14). It was noticed that lightness L^* of yoghurt
234 decreased with the increase of *A. absinthium* powder with the highest values registered for the
235 control during all storage period. In addition, during storage, luminosity L^* , red color a^* and
236 yellow color b^* decreased for all analyzed samples. In fact, negative a^* values confirmed the
237 dominance of the green color in fortified yoghurts. These findings were attributed to the initial
238 green color of wormwood and its richness in chlorophyll pigments.

239

240 *Effect on viscosity variation*

241 The results of consistency coefficients (K) of control and fortified yogurts are illustrated
242 in Figure 3. These values were obtained based on the flow curves showing the shear stress as
243 function of shear rate and showing that all studied yoghurts presented non-Newtonian
244 pseudoplastic flow behavior (Data not shown). In this study, a significant ($p < 0.05$) decrease of
245 the consistency was observed in all yoghurt samples, during the 28 days of storage which can
246 be related to the proteolysis phenomenon during refrigerated storage. These results were in
247 agreement with those of Tokusoglu (2013) reporting a decrease in the viscosity of a fermented
248 milk product during its storage due to the proteolysis of milk caseins. More, fortified yoghurts
249 with wormwood powder presented better viscosity ($p < 0.05$) when compared to the control,
250 during all storage period. In fact, at the end of storage, the highest consistency value ($9.50 \pm$
251 0.02 Pa.s^n) was observed for the YD₂ yoghurt followed by the fortified yoghurt YD₁ ($8.98 \pm$
252 0.04 Pa.s^n) and the control ($7.72 \pm 0.02 \text{ Pa.s}^n$). This finding can be attributed to the water
253 retention capacity of *Artemisia* fibers and proteins which contribute to a formation of a strong
254 firm gel and an increase of the resistance of yoghurt to flow (Zannini et al., 2018). This result
255 was in agreement with that of Cordova-Ramos et al. (2018) reporting that jumbo powder
256 improved the viscosity of fortified yoghurts due to the development of a strong network
257 between milk and proteins which improve the rates of aggregation in the casein gels and the
258 structural arrangement.

259

260 *Effect on antioxidant activity variation*

261 Data on antioxidant activity evolution in control and fortified yoghurts during storage, are
262 illustrated in Figure 4. At the first day of storage, a significant difference ($p < 0.05$) was observed
263 between fortified yoghurts YD₁ and YD₂ with respective inhibition percentage of about $60.08 \pm$
264 3.61% and $69.79 \pm 0.52 \%$. Also, a significant ($p < 0.05$) initial lower percentage was noted for
265 control yoghurt ($13.84 \pm 1.95 \%$). Besides, it was noted that the antioxidant activity decreased

266 significantly ($p < 0.05$) during refrigerated storage for all analyzed yoghurts. However, inhibition
267 percentage remained higher in fortified yoghurts when compared to control confirming the
268 intense biological activity of *Artemisia absinthium* due to its richness in natural antioxidants
269 such as phenolic compounds and flavonoids (Ahamad et al., 2019).

270

271 **Effect on microbiological quality variations**

272 In this study, the counts of all enumerated bacterial flora are shown in Table 2. It was noted that
273 fecal coliforms, total coliforms and yeast and molds remained absent in all analyzed yoghurts
274 during refrigerated storage period which indicate the good hygienic practice during the
275 manufacturing of yoghurt and the satisfactory quality of produced yoghurts. This finding can
276 be explained by the presence of lactic acid bacteria inhibiting coliform growth as has been
277 described in the study of Ben Moussa et al. (2019). More, initial mesophilic aerobic plate counts
278 increased in YC, YD₁ and YD₂ yoghurts to reach, respective counts of 4.2 ± 0.02 ; 3.56 ± 0.02
279 and 3.44 ± 0.01 log CFU/g, at the end of the storage period. In fact, the control yoghurt had the
280 highest microbial load during the whole storage period. This result could be probably attributed
281 to the strong antimicrobial activity of the natural bioactive compounds of wormwood.

282 Concerning the evolution of lactic acid bacteria counts (Table 2), an increase in the numbers of
283 *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was observed until the 14th day of
284 storage, in the control and fortified yoghurts with *Artemisia* powder which was explained by
285 the presence of essential nutrients for their growth. Then, lactic starter counts decreased slightly
286 toward the end of the storage period to reach lowest counts about 8.71 ± 0.02 log CFU/g and
287 8.46 ± 0.06 log CFU/g in fortified yoghurt YD₂, respectively for *St. thermophilus* and *Lb.*
288 *bulgaricus*. This result could be due to the post-acidification of yoghurt which causes a retro-
289 inhibition of lactic acid bacteria (Ben Moussa et al., 2019). Moreover, the counts of *Lb.*
290 *bulgaricus* and *S. thermophilus* were maintained more than 8 log CFU/g during the four weeks
291 of storage revealing a good quality of the prepared final products as recommended by the Codex
292 Alimentarius (CODEX STAN 243-2003) that established a number of lactic acid bacteria which
293 should be higher than 10^7 CFU / g.

294

295 **Shelf life assessment**

296 The estimation of the shelf life of control and fortified yoghurts with *Artemisia absinthium*
297 powder was carried out using Arrhenius model in order to study the effect of this incorporation
298 on the improvement of physicochemical (pH and acidity) and microbiological (yeasts and

299 molds and coliforms) properties of final products during 28 days of storage at different
300 temperatures (4 °C, 14 °C and 24 °C) (Data not shown). In the current study, data showed an
301 increase in microbial counts and acidity values and a decrease in pH values for all analyzed
302 yoghurts. As expected, for all analyzed yoghurts, the shelf life decreased with the increase of
303 the storage temperature. In fact, the best quality and the highest shelf life were registered when
304 yoghurts were stored at 4 °C when compared to the stored yoghurts at 14°C and 24 °C. Based
305 on different tested parameters, the predicted shelf lives of fortified yoghurts were higher than
306 that of control yoghurt (YC) and this for the four studied parameters. Indeed, the shelf life of
307 fortified yoghurt with the lowest dose of *Artemisia* (YD₁) ranged from 32 (pH) to 45 days
308 (coliforms). However, the shelf life of control yoghurt was about 28 days for all tested
309 parameters. These findings demonstrated the antimicrobial effect of bioactive wormwood
310 compounds leading to an increase of the shelf life from 4 to 7 days. To conclude, the
311 incorporation of *Artemisia absinthium* powder in yoghurt represents a promising way to
312 improve the conservation of dairy products by extending their shelf life.

313

314 CONCLUSIONS

315 The current study aimed to improve the quality of yoghurt by its fortification with
316 *Artemisia absinthium* leaf powder and to satisfy consumer demand for healthy products. Results
317 revealed the efficiency of use of *Artemisia* powder as natural additive in food industries. In fact,
318 the prepared yoghurt with appropriate *Artemisia* dose of about 2% was the most preferred by
319 panelists. Moreover, the incorporation of *Artemisia* powder did not affect significantly the
320 fermentation parameters such as lactic starter viability and acidity. During refrigerated storage,
321 whey exudation and microbial proliferation were reduced as a result of *Artemisia* incorporation.
322 Besides, the fortification of yoghurt with wormwood powder improved their antioxidant
323 activity and rheological properties during the whole storage period. Also, the shelf life of
324 fortified yoghurt was extended by about 4 days when compared to control. Finally, it was
325 concluded, in this study, that the fortification with *A. absinthium* leaf powder can be considered
326 as a promising method for the production of functional yoghurt with high quality and interesting
327 biological activities.

328

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334

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Table 1: Evolution of pH, titratable acidity (°D), syneresis (%) and color parameters of Control and fortified yoghurts during refrigerated storage

Analysis	Storage time (Days)	Yoghurt samples			
		YC	YD ₁	YD ₂	
pH	D₁	4.57 ± 0.01 ^{aA}	4.58 ± 0.01 ^{aA}	4.58 ± 0.00 ^{aA}	
	D₇	4.48 ± 0.01 ^{aB}	4.51 ± 0.01 ^{bB}	4.53 ± 0.00 ^{bB}	
	D₁₄	4.32 ± 0.02 ^{aC}	4.41 ± 0.01 ^{bC}	4.43 ± 0.01 ^{bC}	
	D₂₁	4.18 ± 0.01 ^{aD}	4.26 ± 0.00 ^{bD}	4.33 ± 0.00 ^{cD}	
	D₂₈	4.20 ± 0.01 ^{aD}	4.27 ± 0.00 ^{bD}	4.34 ± 0.01 ^{cD}	
Acidity (D°)	D₁	77.00 ± 0.60 ^{aA}	77.00 ± 0.00 ^{aA}	76.00 ± 0.50 ^{aA}	
	D₇	88.66 ± 0.88 ^{bB}	83.66 ± 0.33 ^{aB}	83.00 ± 0.00 ^{aB}	
	D₁₄	96.00 ± 0.58 ^{cC}	92.00 ± 0.58 ^{bC}	89.00 ± 0.60 ^{aC}	
	D₂₁	110.00 ± 0.00 ^{cD}	100.00 ± 0.00 ^{bD}	94.00 ± 0.29 ^{aD}	
	D₂₈	106.00 ± 0.80 ^{cE}	97.66 ± 0.33 ^{bE}	92.00 ± 0.30 ^{aE}	
Syneresis (%)	D₁	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	
	D₇	5.00 ± 0.60 ^{bB}	2.00 ± 0.00 ^{bB}	1.60 ± 0.10 ^{aB}	
	D₁₄	8.00 ± 0.00 ^{cC}	4.00 ± 0.10 ^{bC}	3.60 ± 0.00 ^{aC}	
	D₂₁	12.0 ± 1.20 ^{cD}	6.50 ± 0.20 ^{aD}	5.00 ± 0.30 ^{aD}	
	D₂₈	14.00 ± 0.80 ^{cE}	8.00 ± 0.00 ^{bE}	6.50 ± 0.10 ^{aE}	
Color	L*	D₁	57.12 ± 0.00 ^{cA}	50.07 ± 0.03 ^{bA}	40.86 ± 0.14 ^{aA}
		D₇	55.00 ± 0.06 ^{cB}	49.03 ± 0.00 ^{bB}	39.65 ± 0.00 ^{aB}
		D₁₄	52.03 ± 0.02 ^{cC}	46.97 ± 0.00 ^{bC}	37.00 ± 0.01 ^{aC}
		D₂₁	51.77 ± 1.66 ^{cC}	43.99 ± 0.00 ^{bD}	34.00 ± 0.00 ^{aD}
		D₂₈	47.94 ± 0.02 ^{cD}	41.88 ± 0.00 ^{bE}	30.04 ± 0.00 ^{aE}
	a*	D₁	-0.61 ± 0.00 ^{cA}	-0.803 ± 0.00 ^{bA}	-0.846 ± 0.00 ^{aA}
		D₇	-0.79 ± 0.02 ^{bB}	-0.870 ± 0.00 ^{aB}	-0.890 ± 0.01 ^{aB}
		D₁₄	-0.87 ± 0.01 ^{bC}	-0.940 ± 0.02 ^{aC}	-0.953 ± 0.01 ^{aC}
		D₂₁	-0.96 ± 0.00 ^{bD}	-1.006 ± 0.02 ^{aD}	-1.020 ± 0.01 ^{aD}
		D₂₈	-1.06 ± 0.00 ^{aE}	-1.086 ± 0.00 ^{aE}	-1.096 ± 0.03 ^{aE}
	b*	D₁	13.15 ± 0.01 ^{aA}	14.55 ± 0.00 ^{bA}	15.9 ± 0.00 ^{cA}
		D₇	12.75 ± 0.01 ^{aB}	14.51 ± 0.01 ^{bB}	15.9 ± 0.01 ^{cA}
		D₁₄	12.60 ± 0.00 ^{aC}	14.44 ± 0.01 ^{bC}	15.89 ± 0.00 ^{cA}
		D₂₁	12.40 ± 0.02 ^{aD}	14.32 ± 0.00 ^{bD}	15.7 ± 0.01 ^{cB}
		D₂₈	12.00 ± 0.00 ^{aE}	14.24 ± 0.00 ^{bE}	15.73 ± 0.01 ^{cC}

411 YC: Control yoghurt, YD₁: Fortified yoghurt with 2% of *Artemisia absinthium* powder; YD₂: Fortified yoghurt with
 412 4% of *Artemisia absinthium* powder.
 413 Data are presented as the mean ± standard deviation of three experiments. Means with different superscripts are
 414 significantly different (p<0.05). Mean values with different lowercase letters (a, b, c, d) indicate a significant
 415 difference between the different analyzed samples. Mean values with different uppercase letters (A, B, C, D)
 416 indicate a significant difference between the same sample during storage period.

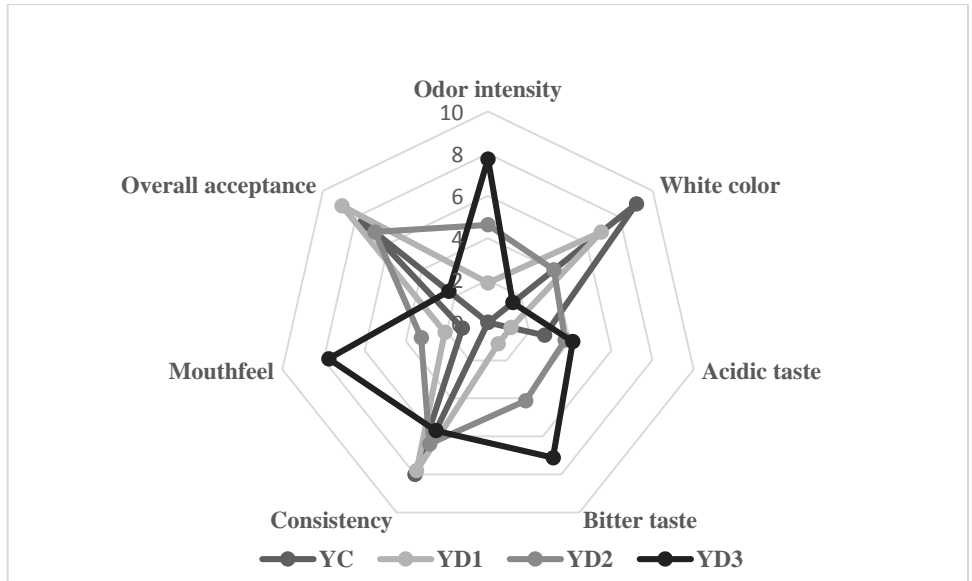
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425 **Table 2:** Evolution of microbial flora (log CFU/g) of control and fortified yoghurts during
 426 refrigerated storage.

Flora	Storage time (Days)	Yoghurt samples		
		YC	YD ₁	YD ₂
Mesophilic aerobic plate count	D ₁	3.35 ± 0.02 ^{bA}	3.23 ± 0.03 ^{aA}	3.24 ± 0.03 ^{aA}
	D ₇	3.41 ± 0.06 ^{bA}	3.27 ± 0.01 ^{aA}	3.24 ± 0.01 ^{aA}
	D ₁₄	3.69 ± 0.05 ^{bB}	3.35 ± 0.02 ^{aB}	3.29 ± 0.06 ^{aA}
	D ₂₁	3.87 ± 0.01 ^{bC}	3.43 ± 0.01 ^{aC}	3.40 ± 0.02 ^{aB}
	D ₂₈	4.21 ± 0.02 ^{cD}	3.56 ± 0.02 ^{bD}	3.44 ± 0.08 ^{aB}
<i>Lactobacillus bulgaricus</i>	D ₁	8.04 ± 0.03 ^{bA}	7.92 ± 0.02 ^{aA}	7.87 ± 0.06 ^{aA}
	D ₇	8.28 ± 0.01 ^{aB}	8.26 ± 0.01 ^{abB}	8.20 ± 0.03 ^{aB}
	D ₁₄	8.83 ± 0.17 ^{cC}	8.72 ± 0.01 ^{bC}	8.65 ± 0.01 ^{aC}
	D ₂₁	8.70 ± 0.01 ^{aCD}	8.66 ± 0.06 ^{aC}	8.55 ± 0.01 ^{aD}
	D ₂₈	8.60 ± 0.20 ^{aD}	8.51 ± 0.01 ^{aD}	8.46 ± 0.02 ^{aE}
<i>Streptococcus thermophilus</i>	D ₁	8.11 ± 0.01 ^{bA}	8.06 ± 0.02 ^{aA}	8.09 ± 0.01 ^{aA}
	D ₇	8.46 ± 0.06 ^{bB}	8.38 ± 0.00 ^{aB}	8.38 ± 0.00 ^{aB}
	D ₁₄	8.91 ± 0.08 ^{bC}	8.86 ± 0.01 ^{abC}	8.82 ± 0.06 ^{aC}
	D ₂₁	8.85 ± 0.03 ^{bC}	8.80 ± 0.08 ^{abCD}	8.79 ± 0.01 ^{aC}
	D ₂₈	8.77 ± 0.02 ^{bD}	8.73 ± 0.01 ^{abD}	8.71 ± 0.02 ^{aD}

427 YC: Control yoghurt, YD₁: Fortified yoghurt with 2% of *Artemisia absinthium* powder; YD₂: Fortified yoghurt with
 428 4% of *Artemisia absinthium* powder.
 429 Data are presented as the mean ± standard deviation of three experiments. Means with different superscripts are
 430 significantly different (p<0.05). Mean values with different lowercase letters (a, b, c, d) indicate a significant
 431 difference between the different analyzed samples. Mean values with different uppercase letters (A, B, C, D)
 432 indicate a significant difference between the same sample during storage period.

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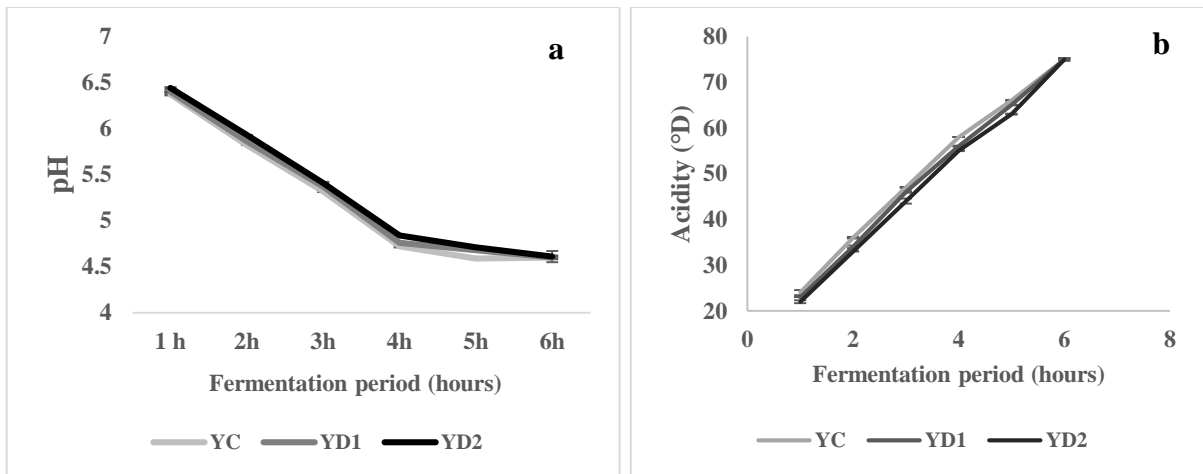
436 **Figure 1:** Sensorial properties of control and fortified yoghurts at the first day of storage. YC:
 437 Control yoghurt, YD₁: Fortified yoghurt with 2% of *Artemisia absinthium* powder; YD₂:
 438 Fortified yoghurt with 4% of *Artemisia absinthium* powder; YD₃: Fortified yoghurt with 6% of
 439 *Artemisia absinthium* powder.

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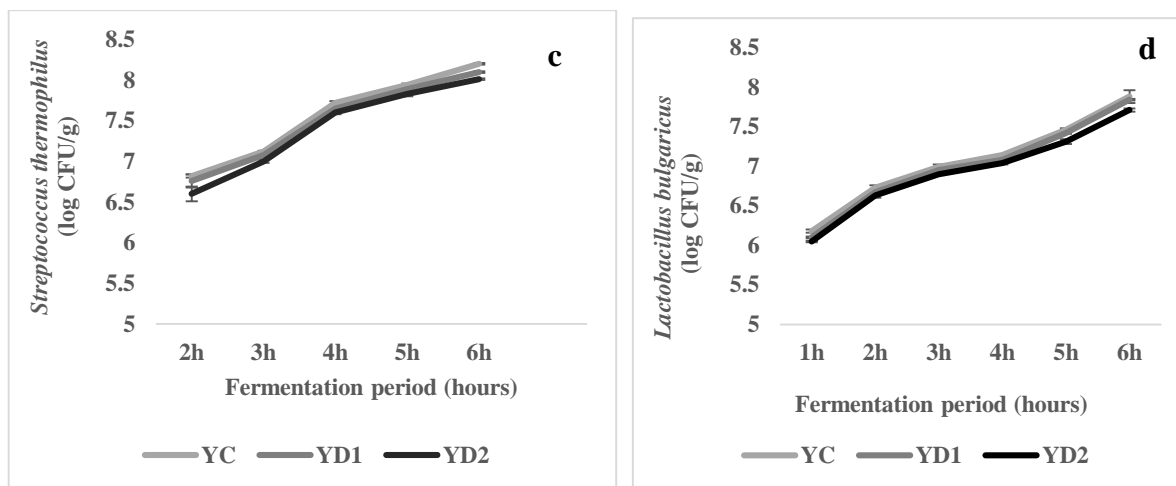
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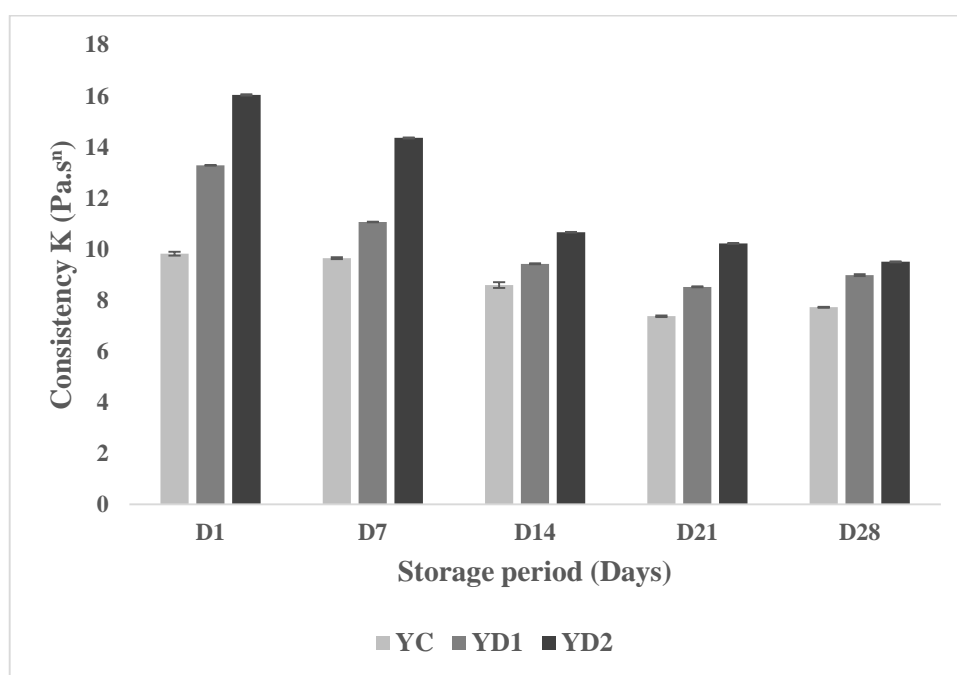


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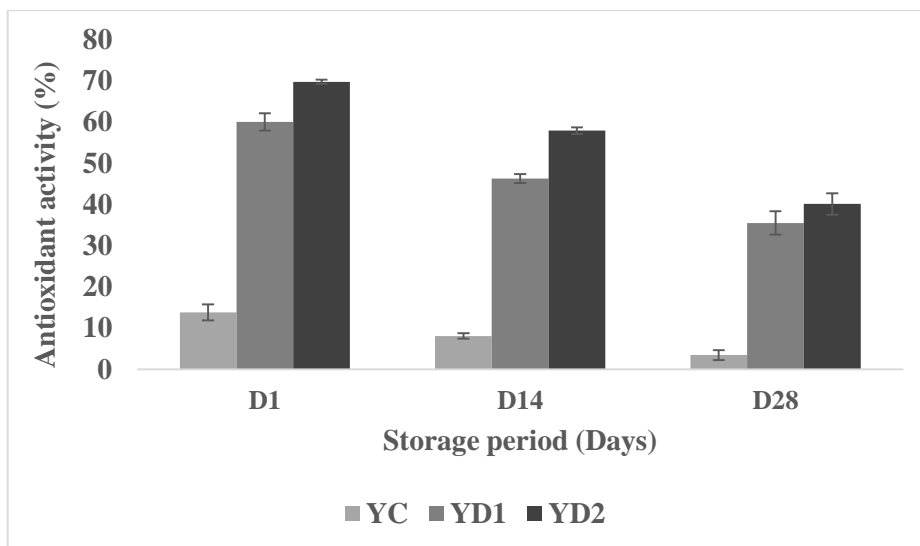
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Figure 2: Evolution of fermentation parameters of control and fortified yoghurts. (a) pH, (b) acidity, (c) *Streptococcus thermophilus* and (d) *Lactobacillus bulgaricus*. YC: Control yoghurt, YD₁: Fortified yoghurt with 2% of *Artemisia absinthium* powder; YD₂: Fortified yoghurt with 4% of *Artemisia absinthium* powder.



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Figure 3: Evolution of consistency coefficient (Pa.sⁿ) of control and fortified yoghurts during refrigerated storage. YC: Control yoghurt, YD₁: Fortified yoghurt with 2% of *Artemisia absinthium* powder; YD₂: Fortified yoghurt with 4% of *Artemisia absinthium* powder.



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458 **Figure 4:** Evolution of the antioxidant activity (%) of control and fortified yoghurts during
 459 refrigerated storage. YC: Control yoghurt, YD₁: Fortified yoghurt with 2% of *Artemisia*
 460 *absinthium* powder; YD₂: Fortified yoghurt with 4% of *Artemisia absinthium* powder.

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