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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 ± 0.04 $Pa.s^n$) and antioxidant activity (60.08± 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* *absinthium* displayed antifungal action too which makes this plant an essential natural product in pharmaceuticals, cosmetics and food industries (Batiha et al., 2020).

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

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

MATERIALS AND METHODS

Yoghurt manufacturing

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

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

Fortified yoghurt with 2 % of *Artemisia* powder (YD_1); Fortified yoghurt with 4 % of *Artemisia* powder (YD_2); Fortified yoghurt with 6 % of *Artemisia* powder (YD_3). First, the sensory analysis was performed on the control and the fortified yoghurts in order to choose the optimal dose. The parameters of fermentation were, then, evaluated during 6 hours of fermentation on control and selected fortified yoghurts. Also, physico-chemical, rheological and microbiological properties of all analyzed yoghurts were evaluated, during 28 days of storage at + 4 °C. The sampling was performed on days 1, 7, 14, 21 and 28 of storage.

Sensorial analysis

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

Physico-chemical analyses

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

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

Antioxidant activity determination

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to evaluate the free radical scavenging ability of yoghurt extracts. The DPPH assay was performed as described by Elfahri et al. (2016) with some modifications. A total of 800 µl of the DPPH solution (0.1 mM DPPH in 95% methanol) was added to 200 µl of each yoghurt extract. Then, the mixture was centrifuged at 9200 rpm / 2 min and kept in the dark during 30 minutes. The absorbance was measured against a blank containing distilled water and DPPH solution, using a JENWAY 6305 spectrophotometer at a wavelength of 517 nm. The radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

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

Rheological analysis

The rheological properties were determined according to the method described by Ben Moussa et al. (2019). Briefly, yoghurt samples were analyzed with a rotary viscometer Rheometric RM180 (Rheomat, Caluire, France), using a coaxial cylinders' geometry. The bob and the cup used had 15.18 (R_1) and 21 mm (R_2) radius, respectively, giving a ratio R_1/R_2=0.72. Viscosity measurements were between 0.01 and 500 s^{-1}. The viscometer was controlled by RSI Orchestrator v6.5.8 software. Flow properties were maintained at 4°C.

Microbiological analyses

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

Shelf life prediction

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

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

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

$$K = K0\, e^{(-Ea/RT)} \quad (2)$$

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

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

Statistical analysis

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

RESULTS AND DISCUSSION

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

The results of the sensorial profile (Figure 1), showed significant differences ($p < 0.05$) between the fortified yoghurts with *Artemisia* leaf powder (YD_1, YD_2 and YD_3) and the control (YC). All analyzed yoghurts were different ($p<0.05$) in terms of odor intensity with attributed notes of about 0; 1.87; 4.62 and 7.75, respectively for YC, YD_1, YD_2 and YD_3. For the intensity of white color, the four yoghurt samples had shown different and variable colors with scores of 9.00; 6.87; 4.00 and 1.50, respectively. In fact, fortified yoghurt with the highest dose of *Artemisia* (YD_3) was the greenest and the most bitter (7.12). This was due to the presence of high content of chlorophyll pigments responsible for the green color of *Artemisia absinthium*. Regarding consistency, results showed that YC and YD_1 were the most appreciated. In addition, YD_3 was the less appreciated by panelists in terms of mouthfeel descriptor. Concerning the bitterness and acidic taste, these descriptors were more noticeable in YD_2 and YD_3 which could be attributed to the aromatic compounds of *Artemisia absinthium* (Boulares et al., 2023). For the syneresis phenomenon, no whey exudation was observed on the first day of storage for all analyzed yoghurts. Also, it can be concluded that the overall appreciation decreased with the increase of *Artemisia absinthium* powder dose. Thereby, yoghurt (YD_1) fortified with the lowest *Artemisia* powder dose was the most appreciated by the panel. In this regard, in the rest of the study, only YD_1 and YD_2 were retained for evaluation of their qualities during fermentation and refrigerated storage.

Effect of *Artemisia absinthium* powder incorporation on fermentation parameters

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

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

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

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

Effect on pH and post-acidification variations

Quality parameters of control and fortified yoghurts evaluated during 28 days of refrigerated storage are reported in Table 1. During storage, a decrease in pH and an increase in acidity were recorded for all tested yoghurts. In fact, pH values decreased significantly to reach the lowest value of 4.20±0.01 in untreated control at the final day of storage. Moreover, initial acidity value (77 °D ± 0.01) increased for control yoghurt to reach a value of 106 °D ± 0,01, at the 28th day of storage. It should be noted that fortified yoghurt (YD_2) with the highest dose of *Artemisia* powder presented a significant lower value (92 °D ± 0,29) at the end of storage when compared to other analyzed yoghurts. These results were in agreement with those of Zhang et *al.* (2019) suggesting a post-acidification of yoghurt during 3 weeks of storage at + 4 °C as a result of the proliferation of acid-forming bacteria producing lactic acid during storage. More, similar findings were found by Ben Abdesslem et al. (2020) reporting that titratable acidity of control yoghurt was higher than that of fortified yoghurt with fennel essential oil due to the presence of natural compounds having antimicrobial activity and preventing acid production which confirm the protective role of *Artemisia absinthium* powder due to its richness in bioactive compounds.

Effect on syneresis variation

As shown in table 1, no whey separation was observed in all analyzed yoghurts at the beginning of storage. However, syneresis levels increased significantly ($p < 0.05$) during storage to reach the lowest whey separation rates of about 6.50 ± 0.10 % and 8 ± 0.00 %, respectively for the fortified yoghurts YD_1 and YD_2 when compared to control (14 ± 0.80 %). These results were in perfect agreement with those of Zhang et al. (2019) reporting that the syneresis decreased in yoghurt fortified with 0.2 % of moringa compared to the control yoghurt. It was interesting to note that incorporation of wormwood powder improved the protein matrix of the yoghurt and reduced the proteolysis which contribute to the reduction of serum release and as consequence the consistency improvement and the gel stability. This finding could be attributed to interactions between *Artemisia* components and yoghurt proteins as well as the lower acidity leading to caseins micelles stabilization and shelf life improvement (Srisuvor et al., 2013).

Effect on color parameters variations

The evolution of yoghurt color parameters during storage are shown in Table 1. At the beginning of refrigerated storage, fortified yoghurt with the highest *Artemisia* dose presented the initial lowest lightness L^* (40.86 ± 0.14). It was noticed that lightness L* of yoghurt decreased with the increase of *A. absinthium* powder with the highest values registered for the control during all storage period. In addition, during storage, luminosity L*, red color a* and yellow color b* decreased for all analyzed samples. In fact, negative a* values confirmed the dominance of the green color in fortified yoghurts. These findings were attributed to the initial green color of wormwood and its richness in chlorophyll pigments.

Effect on viscosity variation

The results of consistency coefficients (K) of control and fortified yogurts are illustrated in Figure 3. These values were obtained based on the flow curves showing the shear stress as function of shear rate and showing that all studied yoghurts presented non-Newtonian pseudoplastic flow behavior (Data not shown). In this study, a significant ($p < 0.05$) decrease of the consistency was observed in all yoghurt samples, during the 28 days of storage which can be related to the proteolysis phenomenon during refrigerated storage. These results were in agreement with those of Tokusoglu (2013) reporting a decrease in the viscosity of a fermented milk product during its storage due to the proteolysis of milk caseins. More, fortified yoghurts with wormwood powder presented better viscosity ($p < 0.05$) when compared to the control, during all storage period. In fact, at the end of storage, the highest consistency value (9.50 ± 0.02 $Pa.s^n$) was observed for the YD_2 yoghurt followed by the fortified yoghurt YD_1 (8.98 ± 0.04 $Pa.s^n$) and the control (7.72 ± 0.02 $Pa.s^n$). This finding can be attributed to the water retention capacity of *Artemisia* fibers and proteins which contribute to a formation of a strong firm gel and an increase of the resistance of yoghurt to flow (Zannini et al., 2018). This result was in agreement with that of Cordova-Ramos et al. (2018) reporting that jumbo powder improved the viscosity of fortified yoghurts due to the development of a strong network between milk and proteins which improve the rates of aggregation in the casein gels and the structural arrangement.

Effect on antioxidant activity variation

Data on antioxidant activity evolution in control and fortified yoghurts during storage, are illustrated in Figure 4. At the first day of storage, a significant difference ($p < 0.05$) was observed between fortified yoghurts YD_1 and YD_2 with respective inhibition percentage of about 60.08± 3.61 % and 69.79 ± 0.52 %. Also, a significant ($p < 0.05$) initial lower percentage was noted for control yoghurt (13.84 ±1.95 %). Besides, it was noted that the antioxidant activity decreased significantly ($p<0.05$) during refrigerated storage for all analyzed yoghurts. However, inhibition percentage remained higher in fortified yoghurts when compared to control confirming the intense biological activity of *Artemisia absinthium* due to its richness in natural antioxidants such as phenolic compounds and flavonoids (Ahamad et al., 2019).

Effect on microbiological quality variations

In this study, the counts of all enumerated bacterial flora are shown in Table 2. It was noted that fecal coliforms, total coliforms and yeast and molds remained absent in all analyzed yoghurts during refrigerated storage period which indicate the good hygienic practice during the manufacturing of yoghurt and the satisfactory quality of produced yoghurts. This finding can be explained by the presence of lactic acid bacteria inhibiting coliform growth as has been described in the study of Ben Moussa et al. (2019). More, initial mesophilic aerobic plate counts increased in YC, YD_1 and YD_2 yoghurts to reach, respective counts of 4.2 ± 0.02; 3.56 ± 0.02 and 3.44 ± 0.01 log CFU/g, at the end of the storage period. In fact, the control yoghurt had the highest microbial load during the whole storage period. This result could be probably attributed to the strong antimicrobial activity of the natural bioactive compounds of wormwood.

Concerning the evolution of lactic acid bacteria counts (Table 2), an increase in the numbers of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was observed until the 14th day of storage, in the control and fortified yoghurts with *Artemisia* powder which was explained by the presence of essential nutrients for their growth. Then, lactic starter counts decreased slightly toward the end of the storage period to reach lowest counts about 8.71 ± 0.02 log CFU/g and 8.46 ± 0.06 log CFU/g in fortified yoghurt YD_2, respectively for *St. thermophilus* and *Lb. bulgaricus.* This result could be due to the post-acidification of yoghurt which causes a retro-inhibition of lactic acid bacteria (Ben Moussa et al., 2019). Moreover, the counts of *Lb. bulgaricus* and *S. thermophilus* were maintained more than 8 log CFU/g during the four weeks of storage revealing a good quality of the prepared final products as recommended by the Codex Alimentarius (CODEX STAN 243-2003) that established a number of lactic acid bacteria which should be higher than 10^7 CFU / g.

Shelf life assessment

The estimation of the shelf life of control and fortified yoghurts with *Artemisia absinthium* powder was carried out using Arrhenius model in order to study the effect of this incorporation on the improvement of physicochemical (pH and acidity) and microbiological (yeasts and molds and coliforms) properties of final products during 28 days of storage at different temperatures (4 °C, 14 °C and 24 °C) (Data not shown). In the current study, data showed an increase in microbial counts and acidity values and a decrease in pH values for all analyzed yoghurts. As expected, for all analyzed yoghurts, the shelf life decreased with the increase of the storage temperature. In fact, the best quality and the highest shelf life were registered when yoghurts were stored at 4 °C when compared to the stored yoghurts at 14°C and 24 °C. Based on different tested parameters, the predicted shelf lives of fortified yoghurts were higher than that of control yoghurt (YC) and this for the four studied parameters. Indeed, the shelf life of fortified yoghurt with the lowest dose of *Artemisia* (YD_1) ranged from 32 (pH) to 45 days (coliforms). However, the shelf life of control yoghurt was about 28 days for all tested parameters. These findings demonstrated the antimicrobial effect of bioactive wormwood compounds leading to an increase of the shelf life from 4 to 7 days. To conclude, the incorporation of *Artemisia absinthium* powder in yoghurt represents a promising way to improve the conservation of dairy products by extending their shelf life.

CONCLUSIONS

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

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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	D_1	4.57 ± 0.01 ^{aA}	4.58 ± 0.01 ^{aA}	4.58 ± 0.00 ^{aA}
	D_7	4.48 ± 0.01 ^{aB}	4.51 ± 0.01 ^{bB}	4.53 ± 0.00 ^{bB}
	D_{14}	4.32 ± 0.02 ^{aC}	4.41 ± 0.01 ^{bC}	4.43 ± 0.01 ^{bC}
	D_{21}	4.18 ± 0.01 ^{aD}	4.26 ± 0.00 ^{bD}	4.33 ± 0.00 ^{cD}
	D_{28}	4.20 ± 0.01 ^{aD}	4.27 ± 0.00 ^{bD}	4.34 ± 0.01 ^{cD}
Acidity (D°)	D_1	77.00 ± 0.60 ^{aA}	77.00 ± 0.00 ^{aA}	76.00 ± 0.50 ^{aA}
	D_7	88.66 ± 0.88 ^{bB}	83.66 ± 0.33 ^{aB}	83.00 ± 0.00 ^{aB}
	D_{14}	96.00 ± 0.58 ^{cC}	92.00 ± 0.58 ^{bC}	89.00 ± 0.60 ^{aC}
	D_{21}	110.00 ± 0.00 ^{cD}	100.00 ± 0.00 ^{bD}	94.00 ± 0.29 ^{aD}
	D_{28}	106.00 ± 0.80 ^{cE}	97.66 ± 0.33 ^{bE}	92.00 ± 0.30 ^{aE}
Synersis (%)	D_1	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}	0.00 ± 0.00 ^{aA}
	D_7	5.00 ± 0.60 ^{cB}	2.00 ± 0.00 ^{bB}	1.60 ± 0.10 ^{aB}
	D_{14}	8.00 ± 0.00 ^{cC}	4.00 ± 0.10 ^{bC}	3.60 ± 0.00 ^{aC}
	D_{21}	12.0 ± 1.20 ^{cD}	6.50 ± 0.20 ^{aD}	5.00 ± 0.30 ^{aD}
	D_{28}	14.00 ± 0.80 ^{cE}	8.00 ± 0.00 ^{bE}	6.50 ± 0.10 ^{aE}
Color L*	D_1	57.12 ± 0.00 ^{cA}	50.07 ± 0.03 ^{bA}	40.86 ± 0.14 ^{aA}
	D_7	55.00 ± 0.06 ^{cB}	49.03 ± 0.00 ^{bB}	39.65 ± 0.00 ^{aB}
	D_{14}	52.03 ± 0.02 ^{cC}	46.97 ± 0.00 ^{bC}	37.00 ± 0.01 ^{aC}
	D_{21}	51.77 ± 1.66 ^{cC}	43.99 ± 0.00 ^{bD}	34.00 ± 0.00 ^{aD}
	D_{28}	47.94 ± 0.02 ^{cD}	41.88 ± 0.00 ^{bE}	30.04 ± 0.00 ^{aE}
a*	D_1	-0.61 ± 0.00 ^{cA}	-0.803 ± 0.00 ^{bA}	-0.846 ± 0.00 ^{aA}
	D_7	-0.79 ± 0.02 ^{bB}	-0.870 ± 0.00 ^{aB}	-0.890 ± 0.01 ^{aB}
	D_{14}	-0.87 ± 0.01 ^{bC}	-0.940 ± 0.02 ^{aC}	-0.953 ± 0.01 ^{aC}
	D_{21}	-0.96 ± 0.00 ^{bD}	-1.006 ± 0.02 ^{aD}	-1.020 ± 0.01 ^{aD}
	D_{28}	-1.06 ± 0.00 ^{aE}	-1.086 ± 0.00 ^{aE}	-1.096 ± 0.03 ^{aE}
b*	D_1	13.15 ± 0.01 ^{aA}	14.55 ± 0.00 ^{bA}	15.9 ± 0.00 ^{cA}
	D_7	12.75 ± 0.01 ^{aB}	14.51 ± 0.01 ^{bB}	15.9 ± 0.01 ^{cA}
	D_{14}	12.60 ± 0.00 ^{aC}	14.44 ± 0.01 ^{bC}	15.89 ± 0.00 ^{cA}
	D_{21}	12.40 ± 0.02 ^{aD}	14.32 ± 0.00 ^{bD}	15.7 ± 0.01 ^{cB}
	D_{28}	12.00 ± 0.00 ^{aE}	14.24 ± 0.00 ^{bE}	15.73 ± 0.01 ^{cC}

YC: Control yoghurt, YD_1: Fortified yoghurt with 2% of *Artemisia absintium* powder; YD_2: Fortified yoghurt with 4% of *Artemisia absinthium* powder.
Data are presented as the mean ± standard deviation of three experiments. Means with different superscripts are significantly different ($p<0.05$). Mean values with different lowercase letters (a, b, c, d) indicate a significant difference between the different analyzed samples. Mean values with different uppercase letters (A, B, C, D) indicate a significant difference between the same sample during storage period.

Table 2: Evolution of microbial flora (log CFU/g) of control and fortified yoghurts during 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}
Lactobacillus bulgaricus	**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}
Streptococcus thermophilus	**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}

YC: Control yoghurt, YD_1: Fortified yoghurt with 2% of *Artemisia absintium* powder; YD_2: Fortified yoghurt with 4% of *Artemisia absinthium* powder.
Data are presented as the mean ± standard deviation of three experiments. Means with different superscripts are significantly different ($p<0.05$). Mean values with different lowercase letters (a, b, c, d) indicate a significant difference between the different analyzed samples. Mean values with different uppercase letters (A, B, C, D) indicate a significant difference between the same sample during storage period.

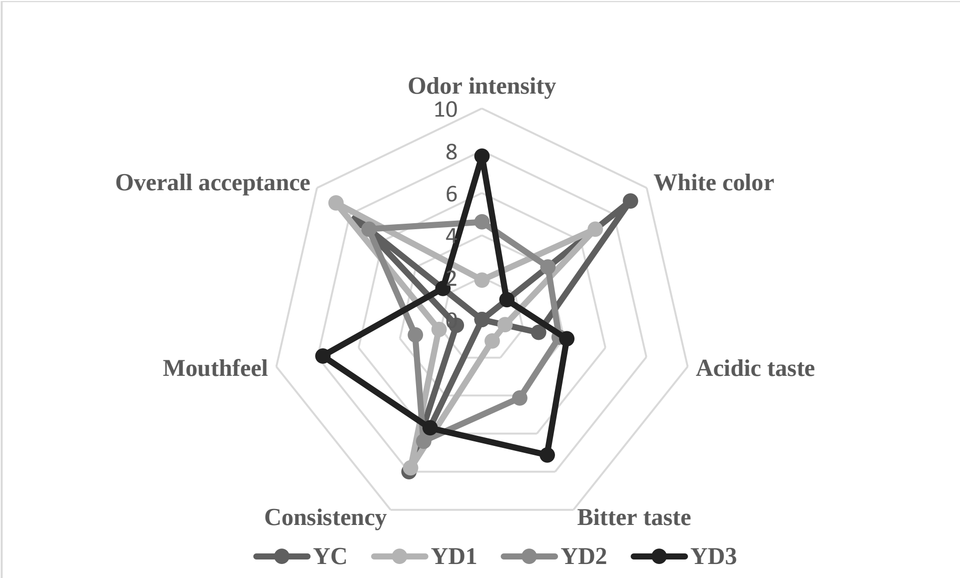
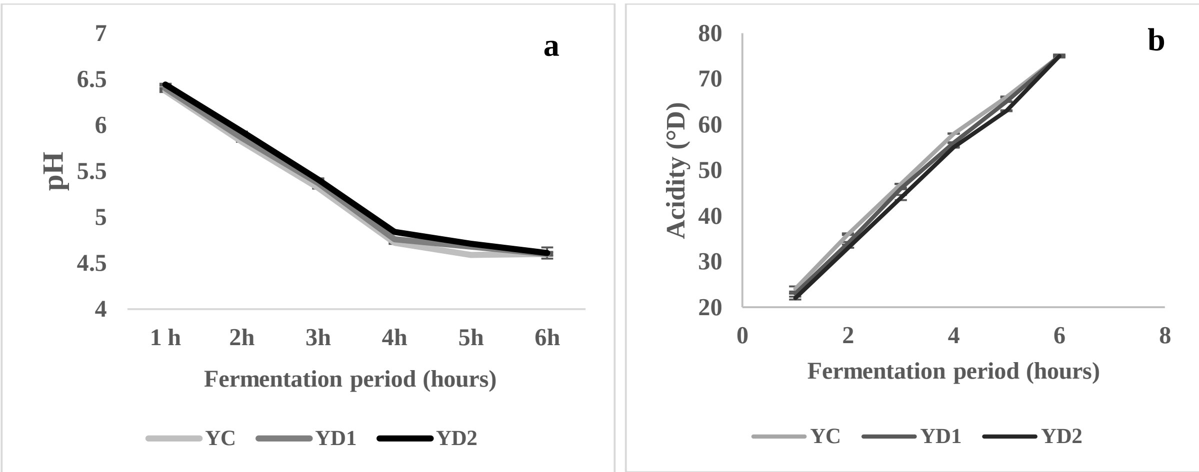


Figure 1: Sensorial properties of control and fortified yoghurts at the first day of storage. YC: Control yoghurt, YD_1: Fortified yoghurt with 2% of *Artemisia absintium* powder; YD_2: Fortified yoghurt with 4% of *Artemisia absinthium* powder; YD_3: Fortified yoghurt with 6% of *Artemisia absinthium* powder.



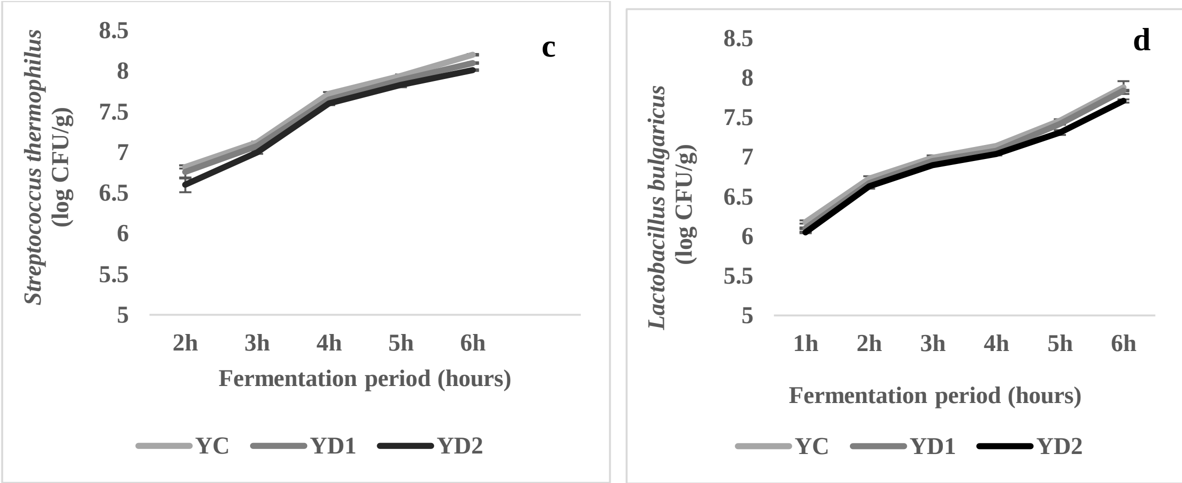


Figure 2: Evolution of fermentation parameters of control and fortified yoghurts. (a) pH, (b) acidity, (c) *Streptococcus thermophilus* and (d) *Lactobacillus bulgaricus*. YC: Control yoghurt, YD_1: Fortified yoghurt with 2% of *Artemisia absintium* powder; YD_2: Fortified yoghurt with 4% of *Artemisia absinthium* powder.

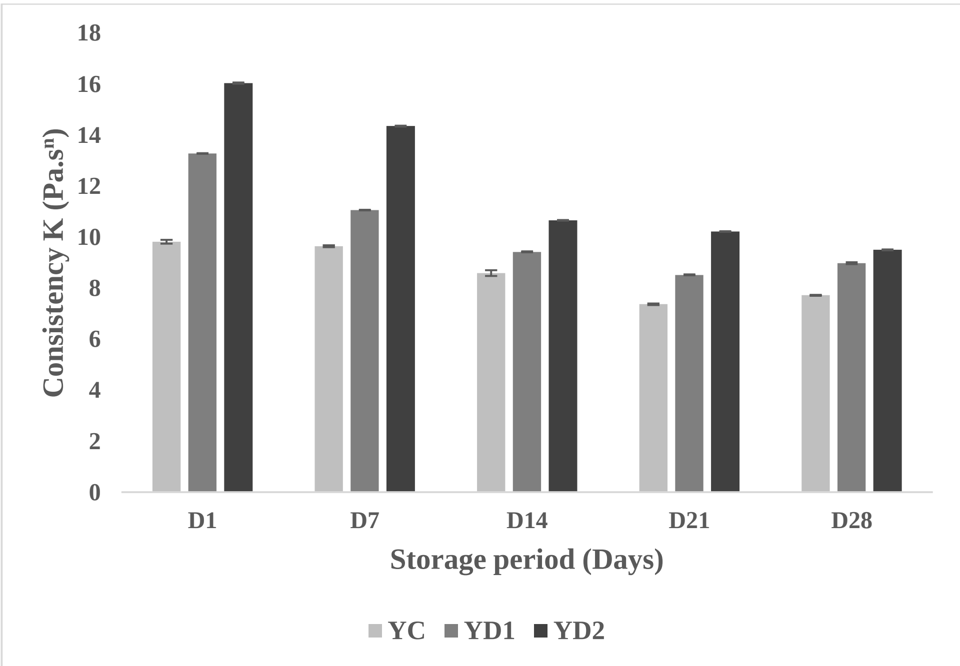


Figure 3: Evolution of consistency coefficient (Pa.s^n) of control and fortified yoghurts during refrigerated storage. YC: Control yoghurt, YD_1: Fortified yoghurt with 2% of *Artemisia absintium* powder; YD_2: Fortified yoghurt with 4% of *Artemisia absinthium* powder.

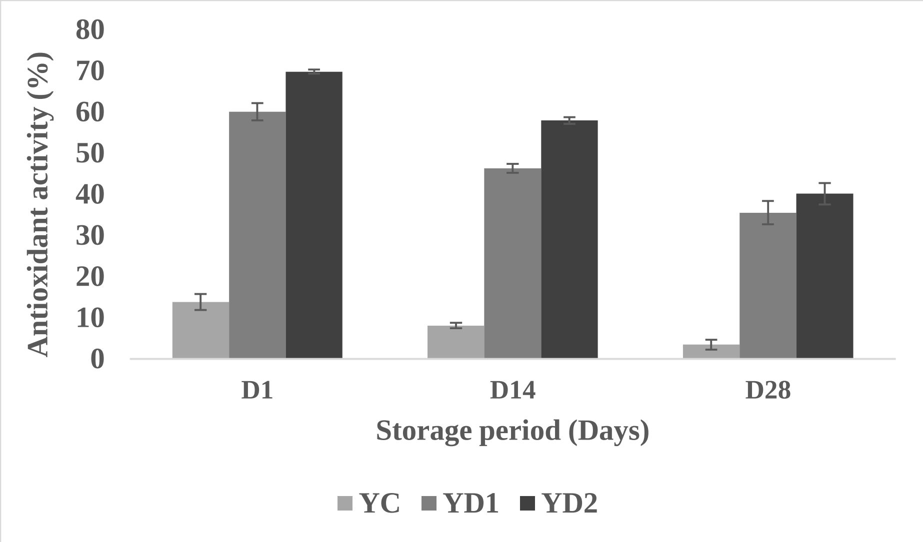


Figure 4: Evolution of the antioxidant activity (%) of control and fortified yoghurts during refrigerated storage. YC: Control yoghurt, YD_1: Fortified yoghurt with 2% of *Artemisia absintium* powder; YD_2: Fortified yoghurt with 4% of *Artemisia absinthium* powder.