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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, the treated stirred yogurt exhibited a lower syneresis value and a higher consistency when compared to the 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±0.5), acorn-starch-added yogurt product gained the highest overall acceptability scores (4.11±0.6) by the panel.

Keywords: Cork oak acorn, Stirred yogurt, Yogurt shelf life.

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 remains 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 *et al*., 2020) can constitute a new resource of highly-valued food ingredients, particularly starch, which represents an undervalued carbohydrate. In this context, particular and further interest must be given to acorn starch extraction and valorization. In fact, search for new promising ingredient for food industry with high potential for commercial use is increasing to develop new industrial applications and competitive market products (Vinha *et al*., 2016). In general, starch is the principal component in many

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food industries responsible for interesting functional, nutritional and textural properties of food products (Perez-Pacheco *et al*., 2014). It's a biodegradable carbohydrate polymer that has been widely studied due to its availability, price, properties and extensive industrial use. However, chemical composition, techno-functional properties, structure and crystallinity of starch granules are depending mainly on their botanical origin and growth conditions. These characteristics are essentially related to the amylose content, starch molecule's ability to hold water, hydrogen bonding and the degree of crystallinity (Correia *et al*., 2013; Zarroug *et al*., 2022). Thus, owing to its interesting properties such as high resistance and paste consistency, acorn starch can be used as thickening and stabilizing agents in food formulations (Vinha *et al*., 2016; Zarroug *et al*., 2020).

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

To the best of our knowledge, many researchers studied the use of acorn flour and several hydrocolloids in bread. However, there is no study about the effect of the incorporation of *Quercus suber* L. starch on technological properties of foods and yogurts during storage. In this regard, in the present study, the effect of acorn starch incorporation in milk during manufacturing of stirred yogurt was evaluated on its rheological properties as well as its shelf life.

MATERIALS AND METHODS

Acorn Starch Extraction

First, acorn starch used in this study was extracted from cork oak acorn (*Quercus suber* L.) using water soaking method as described by Zhang *et al*. (2019). The slurry was filtered and the obtained starch sediment was suspended in the distilled water, then, centrifuged at 4,000 rpm for 15 minutes. This procedure was repeated many times until the white layer disappeared completely. The supernatant containing starch was dried at 45°C during 12 hours and crushed into a fine powder before being stored at room temperature until use.

The extracted acorn starch was characterized for biochemical analysis and color parameters (Zarroug *et al*., 2020).

Stirred Yogurt Production

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

Skim milk powder was added to skimmed milk for standardization. Milk was homogenized, pasteurized at 95°C for 3 minutes and cooled down to 43°C. Three batches were prepared. One batch was served as Control (CY) and the two

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others were incorporated with Acorn starch (YA) and industrial Modified starch (YM), respectively, at a raison of 1%. Then, all batches were inoculated with a freeze-dried mixed starter culture at 2% (*S. thermophilus* and *Lb. bulgaricus*) and incubated at 43 °C until the gel structure was formed (pH reached 4.6 and acidity reached 75 °D). The obtained gels were broken by stirring in the yogurt vats for 5 min at a speed of 60 rpm, distributed in flasks, and stored at a refrigerator (4°C) overnight, before analyses.

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

Analysis of Stirred Yogurt Characteristics during Fermentation

The pH of the prepared samples was measured with a pH-meter (WTW portable pH meter, 315i/SET. Wissenschaftlich). The Dornic acidity (expressed as g lactic acid per 100 mL) was determined by the alkaline titration (Mahmoudi *et al*., 2021). For starter culture enumeration, *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbruekii* subsp. *bulgaricus* were enumerated on M17 agar and MRS agar (Biokar Diagnostics, France) during 48 hours, respectively, at 44 and 37°C (Mahmoudi *et al*., 2021). In this study, sampling was performed every 30 minutes during 210 minutes of fermentation.

Analysis of Stirred Yogurt Characteristics during Refrigerated Storage

Post-Acidification and Total Solids Measurements

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

Syneresis Measurements

The stirred gel was centrifuged for 20 minutes at 12,075×g at 4°C, according to Ben Moussa *et al*. (2020). Syneresis (%) was calculated as weight of the separated serum after centrifugation, relative to the total mass of gel that was centrifuged.

Rheological Analyses

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

Color measurements

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

Microbiological Analysis

The total number of mesophilic organisms was enumerated using Plate Count Agar (Oxoid, Ltd, Basingstoke, England) at 30 °C for 48 h. (Ben Abdessalem *et al*., 2019). Enumeration of lactic strains was performed as described before in this study according

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to Mahmoudi *et al*. (2021). The enumeration of yeasts, molds, and coliforms (total and fecal) were enumerated according to APHA (2001).

Descriptive Sensorial Analysis

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

Shelf Life Prediction

To predict the shelf life of the control and prepared stirred yogurts, the data on Arrhenius model, which is based on the accelerated shelf-life simulation test, were used to evaluate how the deterioration process behaved as a function of storage time (Ben Abdessalem *et al*., 2019). In this study, all products were stored at various temperatures (4, 14 and 24°C) in high-precision (±0.2°C) incubators, during 28 days. Thereafter, they were subjected to microbiological (coliforms, yeast, and molds) and physicochemical (pH and acidity) (dependent variables) analyses and they were sampled in appropriate time intervals to allow an effective kinetic analysis (0, 3, 6, 9, 12, 15, 18, 21 and 28 days). The effect of temperature on the rate of the studied characteristics was determined by means of the Arrhenius equation:

$$k = k_0 \exp(-Ea/RT).$$

Where, k is the reaction rate constant, R is the universal gas constant (8.31 J K^{-1} mol^{-1}), T is the absolute Temperature (°K), Ea is the activation Energy (J mol^{-1}) of the studied action, and k_0 is the pre-exponential factor of the frequency factor.

Statistical Analysis

All tests were possessed in three replications and the results were presented as mean and Standard Deviation (SD). An analysis of variance (ANOVA) in SPSS software version 26.0 (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

Characterization of Extracted Acorn Starch

In this study, starch content in cork oak acorn (*Quercus suber* L.) fruit was about 48.32±2.05%. Extracted acorn starch presented a beige to yellow color with $L*$ of 60.36±0.28 and $b*$ of 15.70±0.06. It was characterized by low moisture (12.36±0.10%), proteins (0.29±0.01%), lipids (0.25±0.01%) and ash (0.29±0.02%) contents, allowing its safe storage and showing its high purity. Moreover, acorn starch showed a high stability during heating and a strong gel formation capacity (Data not shown).

Influence of Acorn Starch Incorporation on Fermentation Parameters of Stirred Yogurt Properties

The evolution of pH and Dornic acidity values during fermentation of control and new stirred yogurt is given in Figure 1. Initially, no significant differences ($P > 0.05$) were noted between all samples. Initial pH value of about 6.40±0.05 pH units dropped significantly during fermentation to reach

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the highest value (4.72±0.01 pH units) in the product incorporated with acorn starch. Moreover, the variations in Dornic acidity confirmed the evolution of pH. In fact, acidity increased during fermentation with no significant differences registered between the control and treated products, which reached the same acidity value of 75 °D after 210 minutes. These data showed that the incorporation of starch did not affect the acid production.

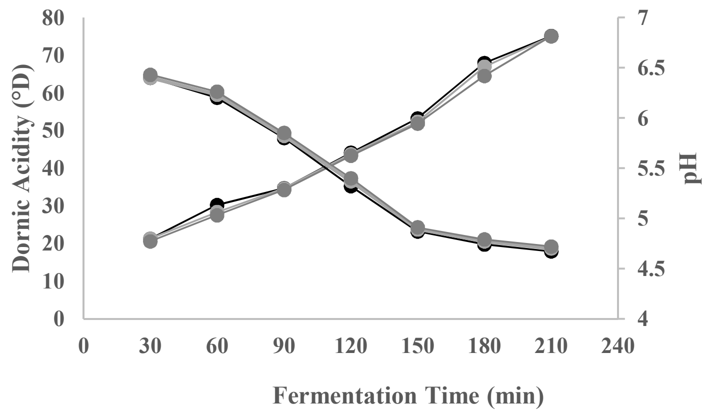

Figure 1. Dornic acidity and pH changes during fermentation of stirred yogurts.
● Control stirred yogurt; ● Stirred yogurt incorporated with modified starch; ● Stirred yogurt incorporated with acorn starch.

In this study, the initial counts of *Streptococcus thermophilus* (5.4 log UFC g^{-1}) and *Lactobacillus bulgaricus* (5.08 log UFC g^{-1}) increased significantly ($P< 0.05$) during fermentation for all tested dairy products (Data not shown). After 210 minutes of fermentation, lactic starter cultures reached the nearest counts in all products, suggesting that the incorporation of starches did not affect their proliferation. This finding was in accordance with that of Zarroug *et al*. (2020) reporting that acorn starch had no significant effect on lactic starter viability and on acid production.

Influence of Acorn Starch Incorporation on Stirred Yogurt Quality during Refrigerated Storage

pH and Post-Acidification Variations

In the present study, pH and Dornic acidity values of control and stirred yogurts added with starches are shown in Table 1. At the beginning of storage, no significant differences were observed between initial pH and Dornic acidity values of all the analyzed samples. During storage at 4°C, pH decreased significantly to reach the highest value of 4.30±0.00 in the prepared product with acorn starch. These values were actually desired in modern dairy industry in order to produce soft yoghurts recommended by consumers. Moreover, Dornic acidity increased significantly in all products. However, the highest values were always recorded in the control yoghurt. The variations in pH and Dornic acidity values were attributed to the proliferation of acid-forming bacteria producing lactic acid during storage. At the 28th day of storage, the highest (108.6°D±0.01) and lowest (94°D±0.00) post-acidification values were registered, respectively, in the control and the sample added with acorn starch. These findings were partially in line with those reported by Ben Moussa *et al*. (2019b) who showed that no significant differences in

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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.[a]

Parameter	Storage period (Days)	Stirred yogurts CP	MSP	ASP
pH	1	4.58 ± 0.34 aA	4.58 ± 0.01 aA	4.59 ± 0.01 aA
	7	4.45 ± 0.01 aAB	4.48 ± 0.00 aAB	4.49 ± 0.00 aAB
	14	4.30 ± 0.01 aBC	4.34 ± 0.01 aB	4.37 ± 0.01 aB
	21	4.25 ± 0.00 aC	4.29 ± 0.01 aB	4.32 ± 0.01 aB
	28	4.21 ± 0.01 aC	4.27 ± 0.00 aB	4.30 ± 0.00 aB
Dornic acidity (°D)	1	77.00 ± 0.00 aA	77.06 ± 0.05 aA	75.50 ± 0.00 aA
	7	85.00 ± 0.00 aB	83.49 ± 0.00 aB	83.00 ± 0.00 aB
	14	97.49 ± 0.00 aC	95.00 ± 0.00 aC	91.10 ± 0.10 bC
	21	99.50 ± 0.00 aD	96.66 ± 0.01 bCD	94.00 ± 0.10 cCD
	28	108.60 ± 0.01 aE	102.50 ± 0.01 bD	96.00 ± 0.00 bD
Total solids (%)	1	22.50 ± 0.10 aA	22.70 ± 0.00 aA	22.87 ± 0.01 aA
	7	21.61 ± 0.02 aB	22.43 ± 0.01 abAB	22.68 ± 0.02 bA
	14	21.15 ± 0.02 aB	22.30 ± 0.01 abAB	22.52 ± 0.01 bA
	21	20.85 ± 0.01 aBC	21.97 ± 0.01 abB	22.07 ± 0.06 bAB
	28	20.51 ± 0.01 aC	21.71 ± 0.01 bB	21.81 ± 0.01 bB
Syneresis (%)	1	1.00 ± 0.00 aA	0.00 ± 0.00 aA	0.00 ± 0.00 aA
	7	4.47 ± 0.00 aB	2.53 ± 0.00 bB	1.17 ± 0.01 cB
	14	7.1 ± 0.01 aC	3.00 ± 0.00 bB	2.07 ± 0.00 cC
	21	11.47 ± 0.01 aD	5.00 ± 0.01 bC	3.57 ± 0.01 cD
	28	13.4 ± 0.01 aE	6.93 ± 0.00 bD	4.83 ± 0.01 cE
Consistency coefficient K ($Pa.s^n$)	1	7.63 ± 0.02 aA	8.37 ± 0.45 bA	9.65 ± 0.03 cA
	14	6.12 ± 0.02 aB	7.64 ± 0.02 bB	9.24 ± 0.04 cB
	28	4.78 ± 0.04 aC	5.86 ± 0.36 bC	7.54 ± 0.08 cC
L*	1	57.11 ± 0.02 aA	52.18 ± 0.00 bA	51.01 ± 0.01 cA
	7	55.01 ± 0.01 aB	51.13 ± 0.01bAB	50.43 ± 0.00 cA
	14	52.00 ± 0.01 aC	50.01 ± 0.01bBC	49.39 ± 0.00 bB
	21	50.59 ± 0.00 aD	49.00 ± 0.00 bC	49.01 ± 0.01 bB
	28	48.50 ± 0.00 aE	48.01 ± 0.01bC	47.82 ± 0.01 bC
b*	1	13.11 ± 0.01 aA	13.29 ± 0.00 bA	13.40 ± 0.00 cA
	7	12.51 ± 0.01 aB	13.25 ± 0.00 bA	13.38 ± 0.01 bA
	14	12.40 ± 0.00 aC	13.21 ± 0.01 bB	13.35 ± 0.00 bAB
	21	12.39 ± 0.01 aD	13.12 ± 0.01 bC	13.32 ± 0.01 bB
	28	12.23 ± 0 .01aC	13.02 ± 0.01bC	13.29 ± 0.01 cbB
a*	1	-0.61 ± 0.00 aA	-0.783 ± 0.00 bA	-0.866 ± 0.01 cA
	7	-0.79 ± 0.01 aB	-0.840 ± 0.01 bB	-0.980 ± 0.01 cB
	14	-0.83 ± 0.01 aC	-0.903 ± 0.01 bC	-0.946 ± 0.00 bC
	21	-0.96 ± 0.01 aD	-0.970 ± 0.01 aD	-1.020 ± 0.02 bD
	28	-1.04 ± 0.00 aE	-1.064 ± 0.00 bE	-1.081 ± 0.00 cE

[a] Data are mean±standard deviation, n= 3. Means with different superscripts are significantly different ($P< 0.05$). Lowercase letters (a, b, and c) represent the statistical difference between samples; Upper case letters (A, B, and C) represent the statistical difference between the same samples during storage period. CP: Control stirred yogurt; MSP: Stirred yogurt incorporated with Modified Starch, ASP: Stirred yogurt incorporated with Acorn Starch.

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terms of post acidification were noted between the control yoghurt and that added with lactulose. Furthermore, data on post acidification confirmed the results obtained during fermentation, showing that acorn starch did not affect the viability of lactic starter cultures (Vinha *et al*., 2016).

Total Solids Variations

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

Syneresis Variations

In this study, the whey separation levels of all prepared dairy products are shown in Table 1. The results showed that, initially, no syneresis was observed in samples added with starches with a very low percentage (1%) of whey separation in the control. This finding was in disagreement with the literature reporting initial syneresis levels in the control and treated yoghurts varying from 32.15% to 63.34% (Ben Abdessalem *et al*., 2019; Ben Moussa *et al*., 2020). During storage, syneresis levels increased in all samples. This finding was in perfect agreement with those of the cited studies. Furthermore, it can be observed that products incorporated with starches, particularly acorn starch, had significantly lower ($p<0.05$) syneresis values throughout refrigerated storage as well as at the end of storage period (4.83 ± 0.01%). This result could be related to the increase of total solids in starches added products (Zarroug *et al*., 2020). Thus, addition of acorn starch led to syneresis reduction and gel stability improvement due to the fact that hydroxyl groups of amylose and amylopectin bind water into molecules (Zarroug *et al*., 2020). This decrease in serum release could be, also, attributed to the higher pH values and lower acidity after incorporation of acorn starch, reducing caseins micelles destabilization and leading to a good preservation and an improvement of shelf life (Mahmoudi *et al*., 2020).

Viscosity Variations

The viscosity of stirred yogurts similar to yoghurt is considered as the most important criteria affecting the texture (Ilyasoglu and Yilmaz, 2019) and the sensorial characteristics of the product as well as the consumer's pleasure of eating and preferences. The results of consistency coefficients (K) of the produced stirred yogurts are shown in Table 1. In this study, consistency coefficients were obtained from the flow curves of shear stress (P) as a function of shear rate (s^{-1}). All samples showed shear thinning or non-Newtonian pseudo-plastic flow behavior due to the breakdown of the caseins-gel structure, thus, the reduction of viscosity with shear rate increase (Data not shown). These findings were in agreement with those reported in the literature on yoghurt samples (Ilyasoglu and Yilmaz, 2019; Ben Moussa *et al*., 2019 a, b). The highest initial consistency value (9.65±0.03 $Pa.s^n$), consisting of gel

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formation of milk proteins, was observed in the product added with acorn starch followed by the product incorporated with modified starch (8.37±0.45 Pa.s^n) and then the control (7.63±0.02 Pa.s^n) (Table 1). These values were higher than that (1.73 Pa.s^n) registered by Zarroug *et al*. (2020) when incorporating 1% of acorn starch extracted from *Quercus ilex* variety. Thus, this concentration of added starch improved the rates of aggregation in the casein gels and the structural arrangement that contributed to form a strong firm gel and to increase consistency of the prepared product. The incorporation of acorn starch increased the pseudo-plasticity of the final product and its viscosity, which could be attributed to the increase of total solid content as reported by Ben Moussa *et al*. (2019a), showing similar observation on yoghurt added with lactulose. During all storage period, the consistency values of the products added with starches were improved when compared to the control, which exhibited the lowest values. The consistency decreased significantly ($P< 0.05$) in all analyzed samples, which can be assigned to the proteolysis phenomenon during refrigerated storage. At the end of storage period, it was noted that the highest consistency (7.54±0.08 Pa.s^n) was registered after incorporation of acorn starch when compared to the control (4.78±0.04 Pa.s^n) and product added with modified starch (5.86±0.36 Pa.s^n).

Color parameters variations

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

Microbiological Quality Variations

The counts of total mesophilic aerobic flora and lactic starter cultures are presented in Figure 2. During storage, the counts of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* increased significantly ($P< 0.05$) until the 14th day of storage, then, decreased slightly toward the end of the storage period due to the retro-inhibition of lactic acid bacteria caused by post-acidification. This trend was similar ($P> 0.05$) in all analyzed products, during the entire storage period, indicating that lactic starter cultures could survive in the presence of starches (Zarroug *et al*., 2020). This result was partially in line with that of Vinha *et al*. (2016), showing that this polysaccharide can be used as a prebiotic growth promoter. 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 reported before by Ilyasoglu and Yilmaz (2019) and recommended by the Codex Alimentarius (more than 10^7 CFU g^{-1}).

The control product exhibited the higher total flora counts from the first day of storage (3.24±0.02 log CFU g^{-1}) until the 28th day (3.94±0.02 log CFU g^{-1}). A significant increase of the counts of this flora was observed in all samples throughout the refrigerated storage. However, incorporating acorn starch in milk maintained the lowest microbial charges in the final product. In this study, yeasts, molds, total and fecal coliforms were absent during all the storage period in the stirred

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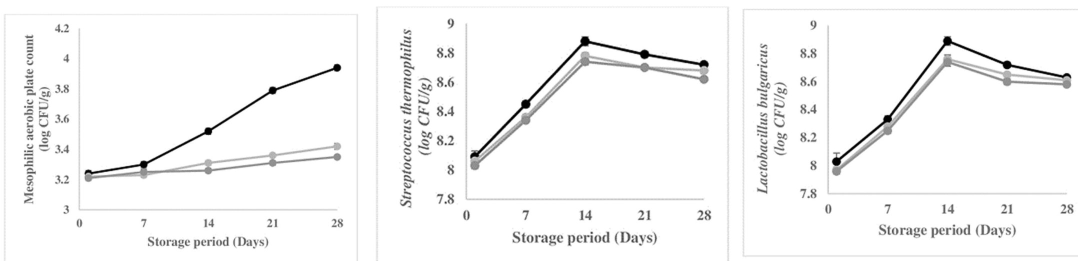
yogurt incorporated with acorn starch (data not shown). Also, results demonstrated the satisfactory quality of control and added product with modified starch with the absence of these bacterial flora and, particularly, fecal coliforms during all storage period (data not shown). These findings could be attributed to the presence of lactic acid bacteria and the high produced acidity in this stirred yogurt inhibiting undesirable bacterial growth and, also, the water holding capacity of acorn starch reducing the water content in the product and thus retarding microbial growth.



(a) (b) (c)

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.

Sensorial Properties

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

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

Shelf Life Assessment

In the current study, the Arrhenius model was used to determine the shelf life of the control and stirred yogurts added with acorn and modified starches. Based on the regression coefficient, the zero-order dynamic model was used for the variable acidity. However, the first-order reaction model was applied for all other tested variables (pH and microbiological counts). During 28 days of storage, data showed an increase in Dornic acidity and microbial

Table 2. Sensorial properties of stirred yogurts at the first day of storage at +4°C.[a]

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

[a] Lowercase letters (a, b, c) represent the statistical difference between samples. CP: Control stirred yogurt; MSP: Stirred yogurt incorporated with Modified Starch, ASP: Stirred yogurt incorporated with Acorn Starch.

charges and a decrease in pH values for all tested samples. However, the lowest Dornic acid values and microbial counts were registered when samples were stored at 4°C as compared to the samples stored at 14 and 24 °C during the whole storage time. Moreover, the better quality was assigned to the samples added with starches showing close shelf lives, when compared to the control. Depending on different tested parameters, it was noted that the predicted shelf-life of the control ranged from 25 days (pH and acidity) to 27 days (coliforms). However, the stirred yogurt added with acorn starch presented respective shelf lives of about 31 days (pH), 32 days (yeast and molds), and 33 days (acidity and coliforms) (data not shown). Thus, starch incorporation extended the shelf life of the stirred yogurt by about 6 days.

CONCLUSIONS

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

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ارزش گذاری نشاسته بلوط تونسی "*Quercus suber* L." در ماست هم‌زده

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چکیده

هدف پژوهش حاضر، ابتدا ارزش‌گذاری نشاسته بلوط چوب پنبه‌ای تونس (Tunisian cork oak) است که به روش خیساندن در آب در صنایع لبنی استخراج می‌شود. همچنین تأثیر ترکیب آن با شیر بر تکامل خواص رئولوژیکی، حسی و میکروبیولوژیکی ماست هم‌زده (stirred yogurt) در طول نگهداری در یخچال ارزیابی شد. هیچ اثر قابل توجهی بر پارامترهای تخمیر محصولی که نشاسته بلوط به آن اضافه شده بود مشاهده نشد. در طول ۲۸ روز نگهداری در یخچال، اختلاط نشاسته بلوط بر محصول پس از اسیدی شدن (post-acidification) و زنده ماندن کشت های آغازگر لاکتیکی (lactic starter cultures) تاثیری نداشت. افزون بر این، ماست هم زده تیمار شده در مقایسه با شاهد تیمار نشده وتیمار ترکیب شده با نشاسته اصلاح شده صنعتی، ارزش سینرزیس کمتر و قوام بالاتری را نشان داد. یافته ها نشان داد که ترکیب نشاسته بلوط عمر مفید محصول نهایی را حدود ۶ روز افزایش می دهد. علیرغم رنگ کم‌ترخوشایند آن (۳.۶۷±۰.۵)، محصول ماست اضافه شده با نشاسته بلوط بالاترین امتیاز پذیرش کلی (۴.۱۱±۰.۶) را به دست آورد.

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