ACCEPTED ARTICLE

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

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Abstract

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

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

Introduction

The *Quercus* genus belonging to *Fagaceae* family includes several species (Korus et al., 2015). This variability leads to significant differences in the biochemical composition of acorns from different species (Zarroug et al., 2020). The specie *Quercus suber* L. is growing abundantly in the Tunisian coastal-forestry regions (Masmoudi et al., 2020). In general, cork oak acorn (*Quercus suber* L.) fruit is used both for feeding animals and in human diet but it remained under exploited and needs more valorization (Vinha et al., 2016; Masmoudi et al., 2020). This fruit is considered as functional food having high nutritional value due to its richness in valuable compounds such proteins, fibers, vitamins, minerals and starch as well as natural bioactive compounds known for their good antioxidant activity (Masmoudi et al., 2020; Zarroug et al., 2020). Thus, cork oak acorns containing about 63% of starch (Masmoudi 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 food industries responsible for interesting functional, nutritional and textural properties of food products (Perez-Pacheco et al., 2014). It's a biodegradable carbohydrate polymer which 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 large 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), exopolysaccharides (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 obtained starch sediment was suspended in the distilled water and then, centrifuged at 4000 rpm for 15 min. This procedure was repeated many times until white layer disappeared totally. The supernatant containing starch was dried at 45°C during 12h and crushed into a fine powder before being stored at room temperature until use.

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 min and cooled down to 43°C. Three batches were prepared. One batch was served as control (CY) and the two others were incorporated with acorn starch (YA) and industrial modified starch (YM), respectively, at a raison of 1%. All batches were, then, 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). 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 physico-chemical and technological characteristics of 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°C and 37°C (Mahmoudi et al., 2021). In this study, sampling was performed every 30 min during 210 min 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 min at 12075 g at 4°C, according to Ben Moussa et al. (2020). Syneresis (%) was calculated as weight of separated serum after centrifugation, relating 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 to Mahmoudi et al. (2021). The enumeration of yeasts and molds, total and fecal coliforms 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 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 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 behaves 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 and 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 studied characteristics was determined by means of the Arrhenius equation: $\mathbf{k = k_0 \exp(-Ea/RT)}$ allowing indicating the end of the shelf life.

Were 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. More, 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 was 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 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 control and treated products which reached the same acidity value of 75°D after 210 min. These data showed that the incorporation of starch did not affect the acid production.

In this study, the initial counts of *Streptococcus thermophilus* (5.4 log UFC/g) and *Lactobacillus bulgaricus* (5.08 log UFC/g) increased significantly ($p < 0.05$) during fermentation for all tested dairy products (Data not shown). After 210 min of fermentation, lactic starter cultures reached 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 analyzed samples. During storage at 4°C, pH decreased significantly to reach the highest value of 4.30±0.00 in 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 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 were 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 control product. At the end of storage, significant differences ($p < 0.05$) were observed between 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 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 leaded 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, 2018) 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 produced stirred yogurts are shown in Table 1. In this study, consistency coefficients were obtained from the flow curves of shear stress (P) as function of shear rate (s^{-1}). All samples showed shear thinning or non-Newtonian pseudoplastic flow behavior due to the breakdown of the caseins-gel structure and thus, the reduction of viscosity with shear rate increase (Data not shown). These findings were in a great agreement with those reported in the literature on yoghurt samples (Ilyasoglu and Yilmaz, 2018; Ben Moussa et al., 2019 a, b). The highest initial consistency value (9.65 ± 0.03 $Pa.s^n$), consisting of gel 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 which contributed to form a strong firm gel and to increase consistency of the prepared product. The incorporation of acorn starch increased the pseudoplasticity 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 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. It was noted that, at the end of storage period, the highest consistency (7.54 ± 0.08 $Pa.s^n$) was registered after incorporation of acorn starch when compared to 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 in this study, 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 two other 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 14^{th} day of storage and 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 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 (2018) and recommended by the Codex Alimentarius (more than 10^7 CFU/g).

The control product exhibited the higher total flora counts from the first day of storage (3.24±0.02 log CFU/g) until the 28^{th} day (3.94 ± 0.02 log CFU/g). 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 yogurt incorporated with acorn starch (Data not shown). Also, results demonstrated the satisfactory quality of control and added product with modified starch with an absence of these bacterial flora and particularly fecal coliforms during all storage period (Data not shown). These findings could be attributed, first, 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.

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 other samples (4.78± 0.40 and 4.00± 0.00, respectively, for 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 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 in microbial 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°C and 24 °C during the whole storage time. Moreover, the better quality was assigned to samples added with starches showing close shelf lives, when compared to 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 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.

ACKNOWLEDGEMENTS

This study was sponsored by the "Tunisian Institution of Agricultural Research and Higher Education" and «Tunisian Ministry of Higher Education and Scientific Research» from University of Carthage.

REFERENCES

American Public Health Association (APHA) 2001. Compendium of Methods for the Microbiological Examination of Foods, 4th Edition, APHA, Washington DC, 2001, p. 676.

Ben Abdessalem, S., Ben Moussa, O., Boulares, M., Elbaz, M., Chouaibi, M., Ayachi, S. and Hassouna, M. 2019. Evaluation of the effect of fennel (*Foeniculum vulgare* Mill) essential oil addition on the quality parameters and shelf-life prediction of yoghurt. International Journal of Dairy Technology 70: p 1-8, DOI: 10.1111/1471-0307.12667.

Ben Moussa, O., Boulares, M., Chouaibi, M., Mzoughi, M. and Hassouna, M. 2019a. Effects of lactulose levels on yogurt properties. Italian Journal of Food Science 31: 782-799.

Ben Moussa, O., Mzoughi, M., Chouaibi, M., Boulares, M. and Hassouna, M. 2019b. The combined effect of phytosterols and lactulose supplementation on yoghurt quality. Journal of Food and Nutrition Research 7 (4): 261-269.

Ben Moussa, O., Rouissi, E., Boulares, M. and Hassouna, M. 2020. Effects of chia seed levels on quality and bio-functional profile of stirred yoghurt. Acta Alimentaria 49 (4): 398–405.

Chen, C., Zhao, S., Hao, G., Yu, H., Tian, H. and Zhao, G. 2017. Role of lactic acid bacteria on the yogurt flavour: A review. International Journal of Food Properties 20 (1): 316-330.

Codex Alimentarius (2010): Codex standard 243-2003. Codex Standard for fermented milks. p 11 in Milk and milk products, 2nd ed, Codex Alimentarius commission, Rome, Italy.

Correia, P. R., Nunes, M. C., and Beirão-da-Costa, M. L. 2013. The effect of starch isolation method on physical and functional properties of Portuguese nut starches. II. *Q. rotundifolia Lam*. and *Q. suber Lam*. acorns starches. Food Hydrocolloids 30: 448-455.

Ilyasoglu, H., and Yilmaz, F. 2019. Characterization of yoghurt enriched with polyunsaturated fatty acids by using walnut slurry. International Journal of Dairy technology 72 (1): 110-119.

ISO 13299 .2016. Sensory analysis-Methodology-General guidance for establishing a sensory profile.

Korus, J., Witczak, M., Ziobro, R. and Juszczak, L. 2015. The influence of acorn flour on rheological properties of gluten free dough and physical characteristics of the bread. European Food Research and Technology 6: 35- 43.

Mahmoudi, I., Telmoudi, A., Ben Moussa, O., Chouaibi, M. and Hassouna, M. 2021. Quality characteristics of goat yogurt containing Lactobacillus probiotic bacteria. Journal of Agricultural Science and Technology 23(1): 83-96.

Masmoudi, M., Besbes, S., Bouaziz, M. A., Khlifi, M., Yahyaoui, D. and Attia, H. 2020. Optimization of acorn (*Quercus suber* L.) muffin formulations: Effect of using hydrocolloids by a mixture design approach. Food Chemistry 328: DOI: 10.1016/j.foodchem.2020.127082.

Perez-Pacheco, E., Moo-Huchin, V. M., Estrada-Leon, R.J., Ortiz-Fernandez, A., May-Hernandez, L. H., Rios-Soberanis, C. R. and Betancur-Ancona. D. 2014. Isolation and characterization of starch obtained from *Brosimum alicastrum* Swarts Seeds. Carbohydrate Polymers 101: 920–927.

Vinha, A. F., Barreira, J. C. M., Costa, A. S. G., Beatriz, M. and Oliveira, P. P. 2016. A New Age for Quercus spp. Fruits: Review on Nutritional and Phytochemical Composition and

Related Biological Activities of Acorns. Comprehensive reviews in food science and food safety 15 (6): 947-981.

Zarroug, Y., Boulares, M., Mejri, J., Slimi, B., Hamdaoui, G., Djebi, S., Saidi, F., Nasri, H., Terras Sfayhi, D. and Kharrat, M. 2020. Extraction and Characterization of Tunisian Quercus ilex Starch and Its Effect on Fermented Dairy Product Quality. Hindawi International Journal of Analytical Chemistry Volume 2020, Article ID 8868673, 9 pages DOI.org/10.1155/2020/8868673.

Zarroug, Y., Boulares, M., Terras Sfayhi, D. and slimi, B. 2022. Comparative study of the physiochemical composition and techno-functional properties of two extracted Tunisian acorn starches. In: Starch - Evolution and Recent Advances", ISBN 978-1-83969-891-0. DOI: 10.5772/intechopen.101562.

Zhang, L., Folkenberg, D. M., Amigo, J. M. and Ipsen, R. 2015. Effect of exopolysaccharide-producing starter cultures and post-fermentation mechanical treatment on textural properties and microstructure of low fat yoghurt. International Dairy Journal 53: 10-19.

Zhang, P., Wang, L., Qian, Y., Wang, X., Zhang, S. Chang, J., Ruan, Y. and Ma, B. 2019. Influences of extraction methods on physicochemical and functional characteristics of three new bulbil starches from Dioscorea opposita Thunb, cv Tiegun. Molecules, 24 (2019), pp. 1-16, 10.3390/molecules24122232P.

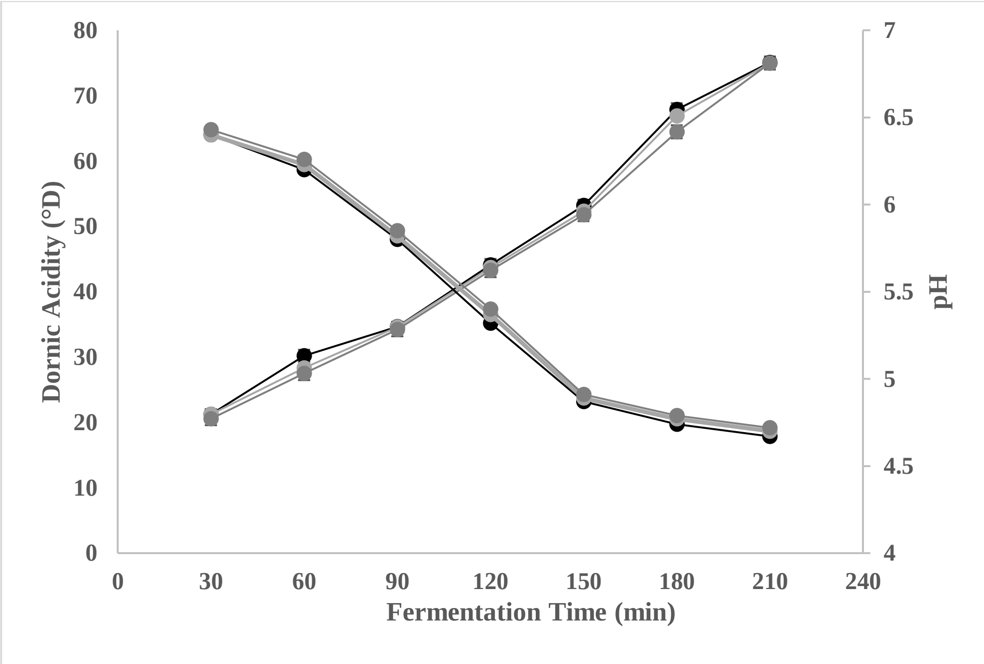
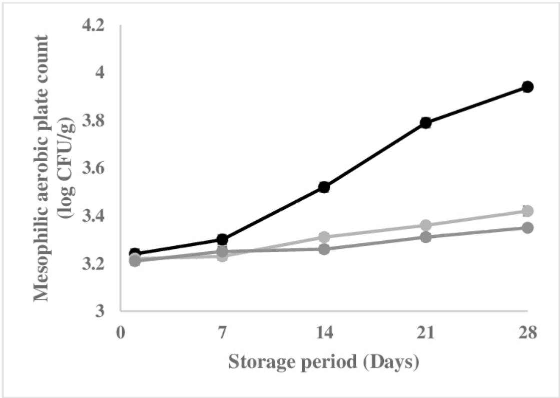
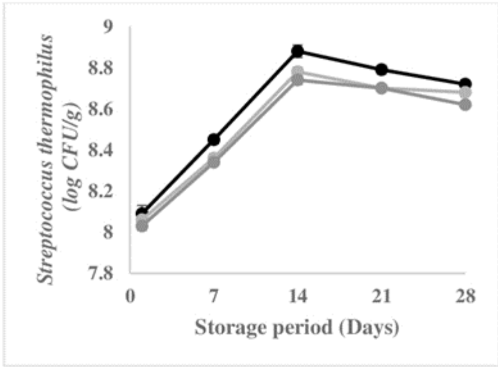
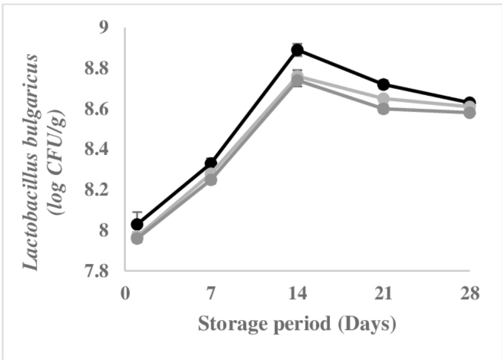


Figure 1 - Dornic acidity and pH changes during fermentation of stirred yogurts.
● Control stirred yogurt; ● Stirred yogurt incorporated with modified starch; ● Stirred yogurt incorporated with acorn starch.





(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.

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

		Stirred yogurts		
Parameter	**Storage period (Days)**	**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 ± 0.01^{bAB}	50.43 ± 0.00 ^{cA}
	14	52.00 ± 0.01 ^{aC}	50.01 ± 0.01^{bBC}	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 ± 0.01^{bC}	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 .01^{aC}	13.02 ± 0.01^{bC}	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}

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

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

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

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

	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}

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

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