

**Antioxidant, Syneresis and Sensory Characteristics of Probiotic Yogurt
Incorporated with *Agave tequilana* Aqueous Extract**

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Running title: Antioxidant effect of agave extract in yogurt

20 **Abstract**

BACKGROUND: The growing consumer interest in healthy food has encouraged the industry to search for food products that exhibit additional functional effects over nutritional value. Yogurt is the best carrier for beneficial nutrients such as probiotics and antioxidants. *Agave tequilana* is a plant that contains phytochemicals comprising flavonoids that show antioxidant activity.

25 **OBJECTIVES:** In this work, the effects of incorporation of *Agave tequilana* Aqueous Extract (ATAE) on antioxidant, syneresis and sensorial properties of probiotic yogurt were investigated.

METHODS: Radical scavenging ability was evaluated against the DPPH (2,2-diphenyl-1-picrylhydrazyl). Amounts of total phenolic compounds (TPC) were determined using the Folin Ciocalteu method. Syneresis was determined using the centrifugal technique. The sensory
30 evaluation was carried out using a five-point hedonic scale.

RESULTS: The addition of ATAЕ in yogurts exhibited a dose-dependent relationship and had significantly ($P \leq 0.05$) higher TPC and DPPH scavenging ability than control yogurts. The value of TPC and DPPH scavenging ability of 0.5, 1, and 1.5% ATAЕ-fortified yogurts were 389.9, 629.2 and 905.6 mg GAE/kg yogurt and 283, 480, and 617 mgBHT eq./kg yogurt, respectively.

35 The addition of ATAE increased syneresis of yogurt samples ($P \leq 0.05$). Although the sensory properties of symbiotic yogurt samples were lower than plain and probiotic treatments, their scores were still above the acceptable level.

CONCLUSIONS: As the results of this study indicated improvement of antioxidant properties of the yogurt incorporated with AEAT, its potential application as a functional food formulation
40 is recommended.

Keywords: Synbiotic yogurt; *Agave tequilana* extract; Antioxidant activity; Total phenol; syneresis.

Introduction

45 *Agave tequilana* is a plant of an angiosperm species that belongs to the family *Asparagaceae*. It is commonly used for the production of Tequila, an alcoholic beverage (López and Urías-Silvas, 2007). Several biological properties, including antibacterial, antioxidant and anti-inflammatory properties of this plant are reported (Sahnoun *et al.*, 2017). Some agave species demonstrated antioxidant activity (Romero-Lopez *et al.*, 2015). Agave syrup contains phytochemicals
50 comprising flavonoids, polycosanols and sapogenins, which show anticarcinogen and antioxidant activity (Narvaez-Zapata and Sanchez-Teyer, 2009). *Agave* juice is used for the production of syrup and fructooligosaccharides (FOS) powder due to its high content of fructans (Salazar-

Leyva *et al.*, 2016). Fructans are the principal photosynthetic composition of this plant (Lopez *et al.*, 2003). Fructans are used as food ingredients including sweeteners, texture modifiers, and fat-replacer in food products (Salazar-Leyva *et al.*, 2016). The prebiotic effect of agave fructans have been well known by several researches (López and Urias-Silvas, 2007; Marquez-Aguirre *et al.*, 2013; Ramnani *et al.*, 2015; Urias-Silvas *et al.*, 2008; Zamora-Gasga *et al.*, 2015).

In recent years, consumers are concerned about the beneficial value of food and are seeking healthier options (Kowaleski *et al.*, 2020). This growing consumer interest in healthy food has encouraged the industry to search and develop food products that show additional functional/health effects over common nutritional values. Antioxidants such as polyphenols and carotenoids are popular functional ingredients that represent different health benefits including anti-cancer, eye-protective, heart-protective and anti-diabetic properties. Furthermore, probiotic food products are among the most popular functional foods marketed worldwide. The growing consumer interest in probiotic products is because probiotic microorganisms exhibit different health benefits to human by improving lactose digestion, preventing intestinal infections, preventing cancers, modulating the immune system and lowering cholesterol (Aryana and McGrewa, 2007).

Yogurt is the most popular fermented dairy product market in the world due to its therapeutic, nutritional, and sensory properties. From a nutritional point of view, yogurt is a nutrient-dense food, as it contains protein, riboflavin, vitamins B6 and B12, and calcium. Yogurt and fermented

milk products are among the best carrier of beneficial nutrients such as probiotics and antioxidants (O'Sullivan *et al.*, 2016).

In this research, the effect of ATAE in levels 0.5, 1 and 1.5% was investigated on selected physicochemical and sensory properties of probiotic yogurt during the storage

Material and methods

Preparation of *Agave tequilana* aqueous extract

The *A. tequilana* was prepared from a plant garden in Tehran province of Iran. All the leaves were cut into small pieces and dried for two weeks in the shade at environmental temperature. It was then comminuted using a mechanical grinder (Moulinex, Paris, France). For extract preparation, 50 g of the grinded plant was soaked in 450 mL water and let shake for 48 h at 250 rpm, followed by filtration through filter paper of Whatman No. 1, then vaporized at 50 °C using a rotary evaporator (Buchi Rotavapor R-114, Switzerland) and further dried at 40 °C. The extract powder was refrigerated at 4 °C till running the experiments (Dahikar *et al.*, 2010).

Preparation of probiotic bacteria

The probiotic bacteria including *Lactobacillus acidophilus* (La5) and *Bifidobacterium bifidum* (Bb-12) prepared from CHR Hansen (Horsholm, Denmark) were used in this study. Freeze-dried bacteria were added to the sterile MRS-Broth medium and incubated for 48 h at 37 °C in aerobic

90 conditions and anaerobic jar for *L. acidophilus* and *B. bifidum*, respectively. Bacterial cultures were harvested by centrifugation at 4000 ×g at 4 °C for 10 min and washed twice with sterile saline and collected by centrifugation. A bacterial suspension with optical density (OD) of 0.1 at 600 nm was prepared and the cell numbers were determined using the surface plate count technique by preparing serial dilutions and plating on MRS agar. The plates were then incubated
95 at 37 °C for 3 days in aerobic and anaerobic conditions for *L. acidophilus* and *B. bifidum*, respectively, as mentioned above. The bacterial number was calculated by colony counter.

Yogurt preparation

Yogurts (set style) were made from cow's milk contains 1.5% (w/w) fat and 12 % (w/v) total solids. Raw milk was treated at 85 °C for 15 min, and then cooled to 42°C (for fermentation). All
100 cultures were used according to the manufacturer's instructions. Milk was inoculated with yogurt starters, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, (Delvo, USA) and probiotic cultures (10^8 cfu/ml) and mixed thoroughly; immediately ATAE (0, 0.5, 1 and 1.5%) was added to the milk and incubated at 42 °C. A plain yogurt without probiotic bacteria and AEAT was prepared as control. Fermentation continued up to the pH of 4.5 ± 0.02 . After fermentation,
105 yoghurt samples were cooled to 4 °C and stored at this temperature for 21 days.

Physicochemical and sensory analysis

Syneresis was determined using a centrifuge by Najgebauer-Lejko *et al.* (2014) and Sahan *et al.* (2008) methods. Syneresis extent was calculated as the weight percentage of whey released after
110 centrifugation.

Total phenolics content (TPC) was estimated using Folin-Ciocalteu reagent and Gallic acid as standard as described by Shori and Baba, (2013).

Determination of radical scavenging activity was done by DPPH method (Brand-Williams *et al.*, 1995) were evaluated. TPC was measured as described by Shori and Baba, (2013). BHT was
115 used as a standard antioxidant and the antioxidant activity of the yogurt samples was expressed as mg BHT equivalent/kg yogurt.

A panel composed of 5 experienced members was used to evaluate the yogurts characteristics for appearance, flavor, texture, and overall acceptability with a point scale from 0 to 5 (0 means s unacceptable).

120 All analyses were performed on the first, 7th and 21st days of storage.

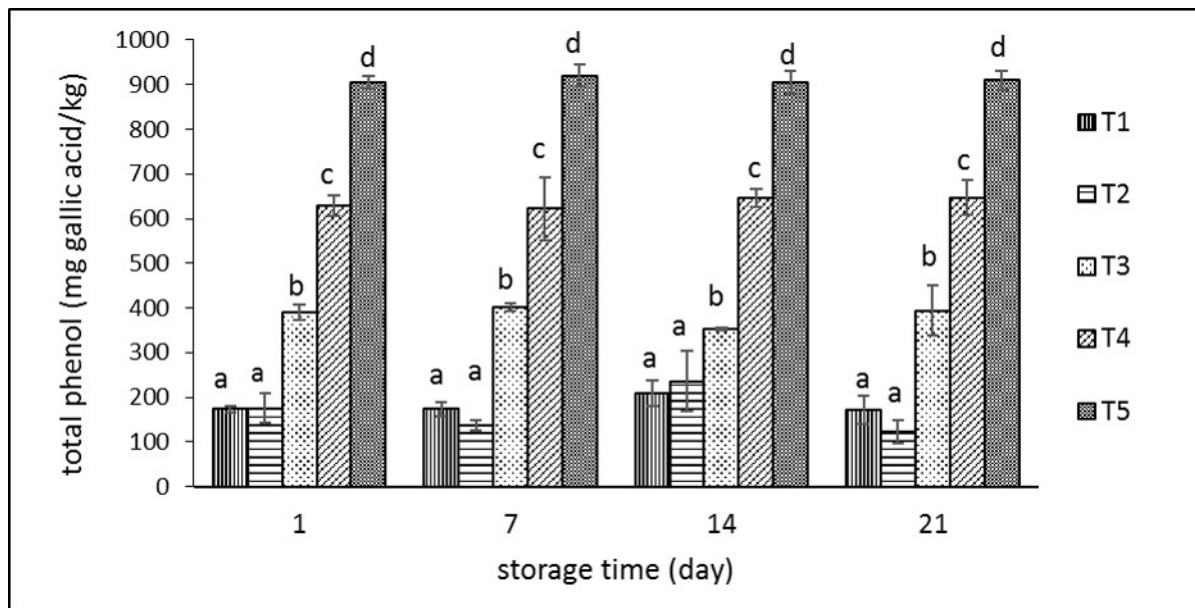
Statistical analysis

Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) procedure using SPSS 20 (Chicago, IL, USA). Duncan test was used to compare the difference among mean values at the significant level of 0.05 ($p < 0.05$). All experiments were replicated three times.

Results

According to Figure 1, the presence of ATAE caused a significant increase of TPC in the yogurts ($P \leq 0.05$); at day 1, the lowest and highest TPC were observed in T3 (389.9 mg gallic acid/ kg) and T5 (905.6 mg gallic acid/ kg), respectively. The lowest TPCs were observed in control probiotic yogurt (175.2 mg gallic acid/ kg) and plain yogurt sample (173.2 mg gallic acid/ kg), respectively, without any significant difference with each other ($P > 0.05$). Storage did not cause the significant increase of TPC in the yogurts ($P > 0.05$).

Figure 1 TPC of yogurt containing ATAE during storage.



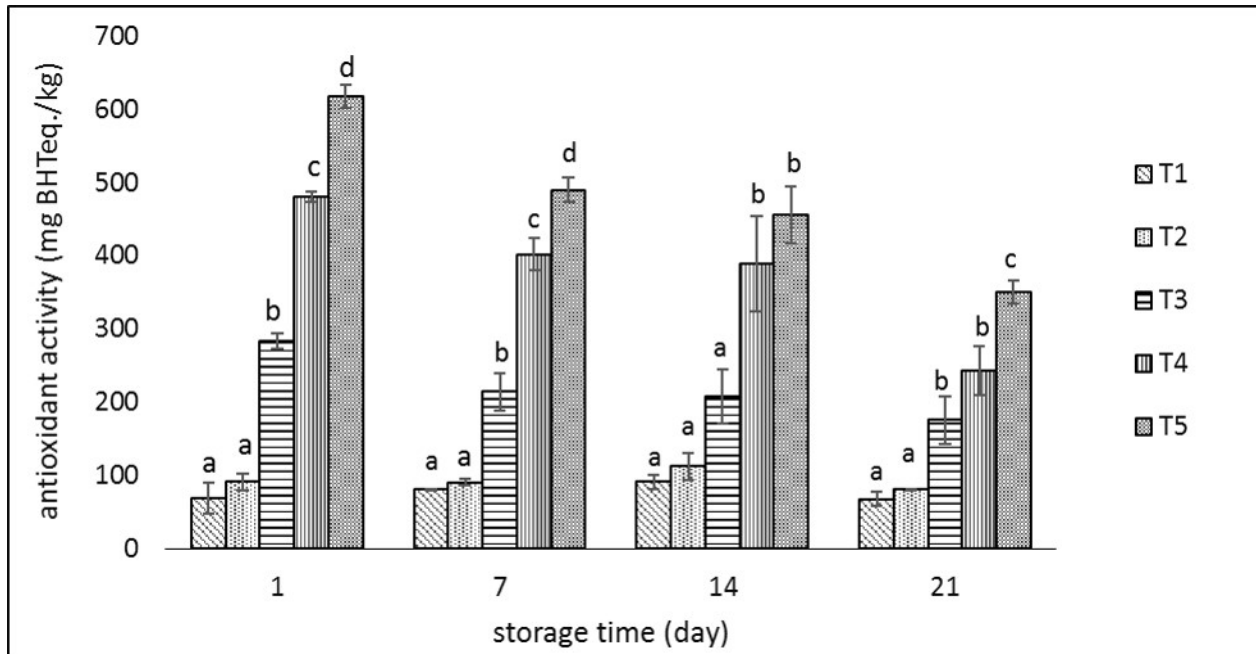
T1: plain yogurt, T2: probiotic yogurt, T3: synbiotic yogurt containing 0.5% AEAT, T4: synbiotic yogurt containing 1% AEAT, T5: synbiotic yogurt containing 1.5% AEAT. Different letters show statistically significant between different treatments at each day ($p < 0.05$).

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As shown in Figure 2, the antioxidant activity (AA) increases with increasing ATAE concentration ($P \leq 0.05$). The AA of plain yogurt was measured 69 mg BHT eq./kg on day 1 which remained constant during the storage. During 21 days of storage, no statistically significant difference was observed in AA of the control probiotic yogurt compared to plain yogurt ($P > 0.05$). The AA was decreased during the time ($P \leq 0.05$).

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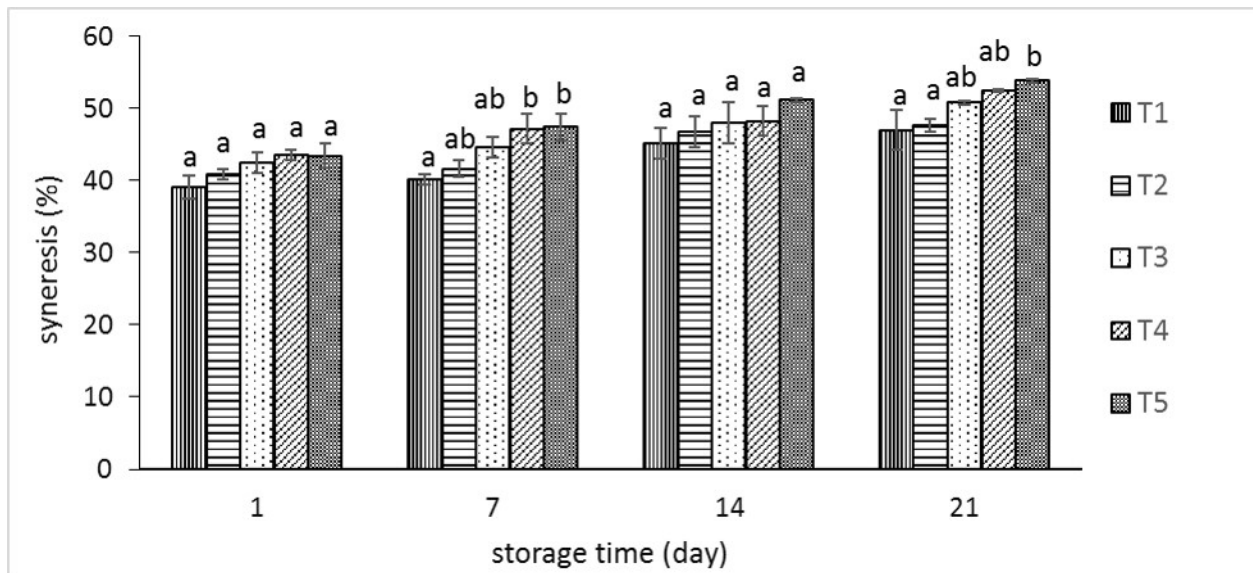
Figure 2 AA (mg BHT eq./kg) of synbiotic yogurt containing ATAE during storage.



T1: plain yogurt, T2: probiotic yogurt, T3: synbiotic yogurt containing 0.5% AEAT, T4: 150 synbiotic yogurt containing 1% AEAT, T5: synbiotic yogurt containing 1.5% AEAT. Different letters show statistically significant between different treatments at each day ($p < 0.05$).

The results of syneresis of the yogurt samples during the storage are indicated in Figure 3. The plain yogurt showed 39.1% syneresis on the first day, which increased to 47% on day 21. No 155 statistically significant difference was found in syneresis of probiotic yogurt compared to plain yogurt ($P > 0.05$), however the syneresis was enhanced during the time.

Figure 3 Syneresis (%) of synbiotic yogurt containing ATAE during storage.



160 T1: plain yogurt, T2: probiotic yogurt, T3: synbiotic yogurt containing 0.5% AEAT, T4:
 synbiotic yogurt containing 1% AEAT, T5: synbiotic yogurt containing 1.5% AEAT. Different
 165 letters show statistically significant between different treatments at each day ($p < 0.05$).

The results of the organoleptic assessment of the yogurt samples are shown in Table 1. The
 165 sensory properties of the probiotic yogurt including appearance, flavor, texture, and overall
 acceptability were comparable to the plain yogurt. Addition of AEAT induced significant
 darkening of the yogurt compared to the control yogurt ($P \leq 0.05$), and this effect was
 concentration dependent. Furthermore, an extract flavor was found by panelists in synbiotic
 yogurt containing different concentrations of AEAT. The synbiotic yogurt showed less softness
 170 and consistency compared to control yogurt ($P \leq 0.05$).

Table 1 Organoleptic characteristics of synbiotic yogurt containing ATAE during storage.

treatment	appearance	flavor	texture	Overall acceptance
T1	4.9 ± 0.32 ^a	5 ± 0.00 ^a	5 ± 0.00 ^a	4.8 ± 0.42 ^a
T2	4.8 ± 0.42 ^a	4.9 ± 0.32 ^a	4.9 ± 0.67 ^a	4.8 ± 0.42 ^a
T3	4.1 ± 0.57 ^b	4.3 ± 0.82 ^b	4 ± 0.67 ^b	3.9 ± 0.57 ^b
T4	3.4 ± 0.52 ^c	3.2 ± 0.63 ^c	3.3 ± 0.32 ^c	3.2 ± 0.63 ^c
T5	3 ± 0.47 ^c	3 ± 0.32 ^c	3.1 ± 0.32 ^c	3 ± 0.53 ^c

T1: plain yogurt, T2: probiotic yogurt, T3: synbiotic yogurt containing 0.5% AEAT, T4:
175 synbiotic yogurt containing 1% AEAT, T5: synbiotic yogurt containing 1.5% AEAT. Different
superscript letters at each column show statistically significant between different treatments ($P <$
0.05).

Discussion

180 Several studies have reported increase of phenolic content in yogurt due to fortification with
different plant extracts (Farvin *et al.*, 2010; Mosiyani, 2017; Shori, 2013; Shori and Baba, 2013).
In the present study, the polyphenol content of the yogurt samples did not change during the
storage. It seems that the effect of storage time depends on the several factors including bacterial
ability to metabolized polyphenols (Shori and Baba, 2013) and the extent of interaction between

185 phenolic compounds and proteins; the more connection between phenolic and proteins the more decrease in phenolic recovery during the time as reported by Vital *et al.* (2015).

It is reported that the AA of the yogurt is associated to the release of a large number of peptides and amino acids by lactic acid bacteria during fermentation of the milk (Farvin *et al.*, 2010; Kudoh *et al.*, 2001; Madhu *et al.*, 2012; Pena-Ramos *et al.*, 2004). The results revealed a direct relationship between TPC and AA in 0.5, 1, and 1.5% ATAE-fortified yogurts. Increases in AA of the plant preparations is related to the TPC content of their composition (Muniandy *et al.*, 2016; Vasco *et al.*, 2008). An increase in the concentration of ATAE induced an increase in AA; the value of DPPH scavenging ability of 0.5, 1, and 1.5% ATAE-fortified yogurts was 283, 480, and 617 mgBHT eq./kg yogurt, respectively. This is in accordance with other studies, which have shown that incorporation of different sections of the plant and fruit detect a significant difference between AA of yogurts fortified with them and that of the control (El-Said *et al.*, 2014; Yadav *et al.*, 2018; Hashemi *et al.*, 2016; stevia; de Carvalho *et al.*, 2019; Liu, 2018; Gaglio *et al.*, 2019; Shori and Baba, 2013; Shori, 2013; Mosiyani *et al.*, 2017). However, the antioxidant effects of the synbiotic yogurt samples showed a significant decrease up to 50% after 21 days of storage. Decrease in antioxidant activity of yogurts during storage were reported by many studies (Jozve-zargharabadi *et al.*, 2020; Cho *et al.*, 2020; Bchir *et al.*, 2019; Kim *et al.*, 2019; Mosyani *et al.*, 2017; Oh *et al.*, 2016). This may be resulted from the formation of a

complex between polyphenols and milk proteins (Kim *et al.*, 2019; Bchir *et al.*, 2019; Oksuz *et al.*, 2019; Sánchez-Bravo *et al.*, 2018; Helal *et al.*, 2018; Lamothe *et al.*, 2014).

Addition of AEAT to the probiotic yogurt significantly elevated syneresis extent compared to the plain yogurt and probiotic control yogurt ($P \leq 0.05$). This result is in agreement with results obtained by Michael *et al.* (2010), Faraki *et al.* (2020), Sengul and Yildiz (2012), and Ramirez-Santiago *et al.* (2010), who found that olive, garlic, onion and citrus extracts, *Auricularia auricula* aqueous extract, sour cherry pulp, and *Pachyrhizus erosus* L fibers, respectively, in yogurts led to an increase in syneresis. The increase of whey expulsion from the yogurt may be explained by weakening casein network due to interaction with active groups of the extract, thermodynamic incompatibility between polysaccharide of the extract and milk proteins, and unbalanced osmotic potential due to depletion flocculation of the casein micelles in the presence of non-adsorbing polymers such as dietary fiber (Michael *et al.*, 2010). The polyphenol-protein interaction which played an important role in serum separation depends on various factors including proteins and polyphenol nature, temperature, and presence of other bioactive compounds (Vital *et al.*, 2015). However, this observation is not in line with results of Huang *et al.* (2020), Guo *et al.* (2018), Amirdivani *et al.* (2013) and Narayana and Gupta, (2013), who demonstrated positive effect of the plant preparations on the decrease of syneresis. Increased syneresis in the yogurt samples over storage time can be explained by protein re-arrangement, which weaken the casein network (Everett and Mcleod, 2005). The increase of syneresis during

the storage time was reported earlier (Alighazi *et al.*, 2021; Amirdivani *et al.*, 2013; Zare *et al.*,
225 2011).

The reduction of organoleptic acceptability of the yogurt incorporated by red ginseng extract
(Jung *et al.*, 2016), *Hibiscus sabdariffa* (Iwalokun and Shittu, 2007), aqueous extract of basil and
savory (Mosyani *et al.*, 2017), *Chlorella vulgaris* and *Arthrospira platensis* (Beheshtipour and
230 Motazavian, 2012) and green tea extract (Shokery *et al.*, 2017) was reported. However, some
experiments showed improvement of sensorial parameters of yogurt which was fortified by
soybean extract (Park and Oh, 2007), agave fructans (Crispín-Isidro *et al.*, 2014), grape and
callus extracts (Karaaslan *et al.*, 2011), and cinnamon ethanol extract (Jin *et al.*, 2016).
Although, the overall acceptability of synbiotic yogurt containing AEAT was lower than that of
235 plain and probiotic control yogurt, they were still acceptable.

Conclusion

In this study, we presented a novel functional yogurt by incorporating ATAE to probiotic
yogurt. The results of this study showed a significant increase in TPC and AA of yogurt by
240 addition of AEAT. Generally, our results proposed use of this yogurt formulation as a functional
product for improving consumer health. However, as addition of the extract increased syneresis

and impaired sensorial acceptance of the yogurt, investigations on the application of encapsulated *A. tequilana* extract in symbiotic yogurt is recommended.

245 **Acknowledgment**

The authors wish to thank the Mabna Tashkhis Laboratory staffs for their hospitality and technical assistance in this program.

Conflict of Interest

250 The authors declared no conflict of interest.

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مطالعه اثر عصاره آبی آگاو تکیلانا بر خواص آنتی اکسیدانی، آب اندازی و خصوصیات حسی ماست

پروبیوتیک

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چکیده

505 زمینه مطالعه: علاقه رو به رشد مصرف کنندگان به غذای سالم، صنعت را تشویق کرده است تا محصولات غذایی را جستجو کند که اثر عملگرایی فراتر از ارزش غذایی دارند. ماست یکی از بهترین حامل ها برای مواد غذایی کاربردی از جمله پروبیوتیک ها و آنتی اکسیدان ها است. آگاو تکیلانا یک گیاه حاوی فیتوکمیکال های حاوی فلاونوئیدها است که فعالیت آنتی اکسیدانی نشان می دهد.

هدف: در این مطالعه، اثرات اضافه کردن عصاره آبی آگاو تکیلانا (AEAT) بر خواص آنتی اکسیدانی، آب اندازی و خصوصیات حسی ماست پروبیوتیک مورد بررسی قرار گرفت.

510 روش کار: محتوای فنلی کل نمونه های ماست به روش فولین سیوکالتیو اندازه گیری شد. فعالیت آنتی اکسیدانی نمونه های ماست با استفاده از روش DPPH تعیین شد. آب اندازی نمونه های ماست با استفاده از روش سانتریفیوژ اندازه گیری شد. آنالیز حسی با استفاده از آزمون هدونیک پنج نقطه ای انجام شد.

نتایج: محتوای فنلی به ترتیب 389/9، 629/2 و 905/6 میلی گرم اسید گالیک در کیلوگرم در غلظت‌های 0/5، 1 و 1/5 درصد عصاره بود. فعالیت آنتی اکسیدانی ماست با افزایش غلظت عصاره افزایش یافت، به طوری که نمونه های ماست سین بیوتیک حاوی غلظت های 0/5، 1 و 1/5 درصد AEAT به ترتیب 283، 480 و 617 mgBHT eq./kg فعالیت آنتی اکسیدانی را نشان دادند. نمونه های ماست سین بیوتیک در مقایسه با نمونه های ساده و پروبیوتیک آب اندازی بالاتری نشان دادند. اگرچه ویژگی‌های حسی نمونه‌های ماست سین بیوتیک کمتر از تیمارهای ساده و پروبیوتیک بود، اما امتیاز آن‌ها همچنان بالاتر از حد قابل قبول بود.

نتیجه گیری نهایی: از آنجایی که نتایج این مطالعه نشان دهنده بهبود خواص آنتی اکسیدانی ماست غنی شده با عصاره آبی آگاو است، کاربرد بالقوه آن به عنوان یک فرمولاسیون غذایی عملگرا توصیه می شود.

واژه های کلیدی: ماست سین بیوتیک، عصاره آگاو تکیلانا، فعالیت آنتی اکسیدانی، فنل کل، آب اندازی