Original Article

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

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ABSTRACT

Background: The growing consumer interest in healthy food has encouraged the industry to search for products that exhibit additional benefits 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.

Objectives: In this work, the effects of incorporating *A. tequilana* aqueous extract (ATAE) on the antioxidant properties, syneresis, and flavor of probiotic yogurt were investigated.

Methods: Radical scavenging ability was evaluated against the 2,2-diphenyl-1-picrylhydrazyl (DPPH). Amounts of total phenolic compounds (TPC) were determined using the Folin-Ciocalteu method. Syneresis was determined using the centrifugal technique. The sensory evaluation was carried out using a 5-point hedonic scale.

Results: The addition of ATAE in yogurts exhibited a dose-dependent relationship and had significantly ($P \leq 0.05$) higher TPC and DPPH scavenging ability than the control yogurts. The TPC and DPPH scavenging properties of 0.5%, 1%, and 1.5% ATAE-fortified yogurts were 389.9, 629.2 and 905.6 mg GAE/kg yogurt and 283, 480, and 617 mg BHT eq./kg yogurt, respectively. The addition of ATAE increased the syneresis of yogurt samples ($P \leq 0.05$). Although the sensory properties of synbiotic yogurt samples were lower than plain and probiotic treatments, their scores were still above the acceptable level.

Conclusion: As the results of this study indicated, it is recommended to improve the antioxidant properties of the yogurt by incorporating AEAT and its potential application as a functional food formulation.

Keywords: Synbiotic yogurt, *Agave tequilana* extract, Antioxidant activity, Total phenol, Syneresis

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1. Introduction

Agave tequilana is a plant of an angiosperm species that belongs to the family Asparagaceae. It is commonly used to produce Tequila, an alcoholic beverage (López & Urías-Silvas, 2007). Several biological properties of this plant, including antibacterial, antioxidant, and anti-inflammatory properties, have been reported (Sahnoun et al., 2017). Some Agave species demonstrated antioxidant activity (Romero-Lopez et al., 2015). Agave syrup contains phytochemicals comprising flavonoids, polycosanols, and sapogenins, which show anticarcinogen and antioxidant activities (Narvaez-Zapata & Sanchez-Teyer, 2009). Agave juice is used to produce syrup and fructooligosaccharides powder because of 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). Several research studies have well supported the prebiotic effect of Agave fructans (López & Urías-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 have been concerned about the beneficial value of food and are seeking healthier options (Kowaleski et al., 2020). This growing interest in healthy food has encouraged the industry to search for and develop products that show additional functional/health effects over common nutritional values. Antioxidants such as polyphenols and carotenoids are popular active ingredients representing 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 humans by improving lactose digestion, preventing intestinal infections, preventing cancers, modulating the immune system, and lowering cholesterol (Aryana & Mc Grewa, 2007).

Yogurt is the world's most popular fermented dairy product market 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 *A. tequilana* aqueous extract (ATAE) at 0.5%, 1%, and 1.5% concentrations was investigated on selected physicochemical and sensory properties of probiotic yogurt during the storage.

2. Materials and Methods

Preparation of *ATAE*

A. tequilana was prepared from a plant garden in the 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 mashed using a mechanical grinder (Moulinex, Paris, France). For extract preparation, 50 g of the grinded plant was soaked in 450 mL water and shaken 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) were prepared from CHR Hansen (Horsholm, Denmark). Freeze-dried bacteria were added to the sterile MRS Broth medium and incubated for 48 h at 37°C in aerobic conditions and an anaerobic jar for *L. acidophilus* and *B. bifidum*, respectively. Bacterial cultures were harvested by centrifugation at 4000×g at 4°C for 10 min, washed twice with sterile saline, and collected by centrifugation. A bacterial suspension with an optical density of 0.1 at 600 nm was prepared. Cell numbers were determined using the surface plate count technique by preparing serial dilutions and plating on MRS (from De Man, Rogosa, and Sharpe) agar. The plates were then incubated 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 and contained 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 cultures were used according to the manufacturer's instructions. Milk was inoculated with yogurt starters, *Streptococcus thermophilus, Lactobacillus bulgaricus* (Delvo, USA), and probiotic cultures (10^8 CFU/mL) and mixed thoroughly; im-

mediately ATAE (0%, 0.5%, 1%, and 1.5%) was added to the milk and incubated at 42°C. Plain yogurt without probiotic bacteria and AEAT was prepared as a control. Fermentation continued up to the pH of 4.5±0.02. After fermentation, yogurt 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 centrifugation.

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

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

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

All analyses were performed on storage's first, seventh, and twenty first days.

Statistical analysis

Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) procedure in SPSS software, version 20 (Chicago, IL, USA). Duncan's 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.

3. Results

According to Figure 1, the presence of ATAE significantly increased TPC in the yogurts ($P\leq0.05$); 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 samples (173.2 mg gallic acid/kg), respectively, without any significant difference from each other ($P>0.05$). Storage did not cause a significant increase in TPC in the yogurts ($P>0.05$).

As shown in Figure 2, the antioxidant activity (AA) increases with increasing ATAE concentration ($P\leq0.05$). The AA of plain yogurt was measured at 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 ($P\leq0.05$).

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

The results of the organoleptic assessment of the yogurt samples are presented in Table 1. The sensory properties of the probiotic yogurt, including appearance, flavor, texture, and overall acceptability, were comparable to the

Table 1. Organoleptic characteristics of synbiotic yogurt containing AEAT 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: Synbiotic yogurt containing 1% AEAT; T5: Synbiotic yogurt containing 1.5% AEAT.

Different superscript letters at each column show statistically significant between different treatments ($P<0.05$).

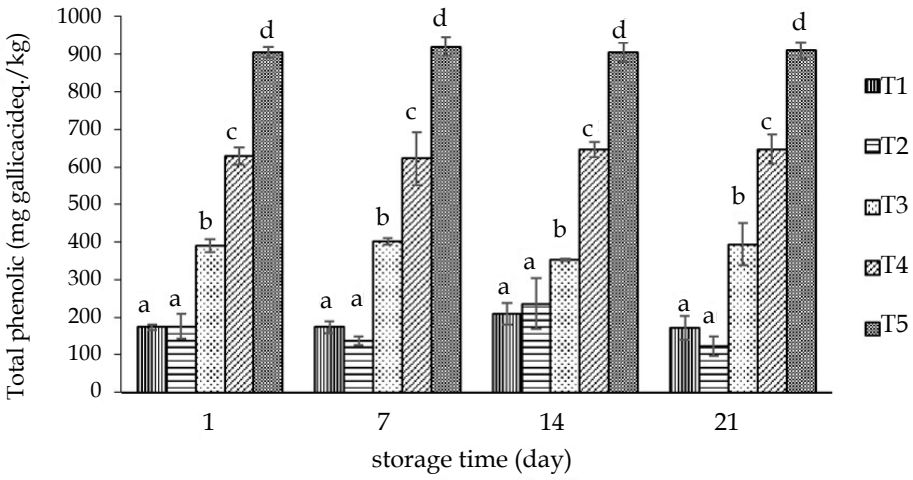

Figure 1. Total phenolic compounds of yogurt containing AEAT during storage

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 on each day (P<0.05).

plain yogurt. The addition of AEAT induced significant darkening of the yogurt compared to the control yogurt (P≤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 and consistency compared to the control yogurt (P≤0.05).

4. Discussion

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

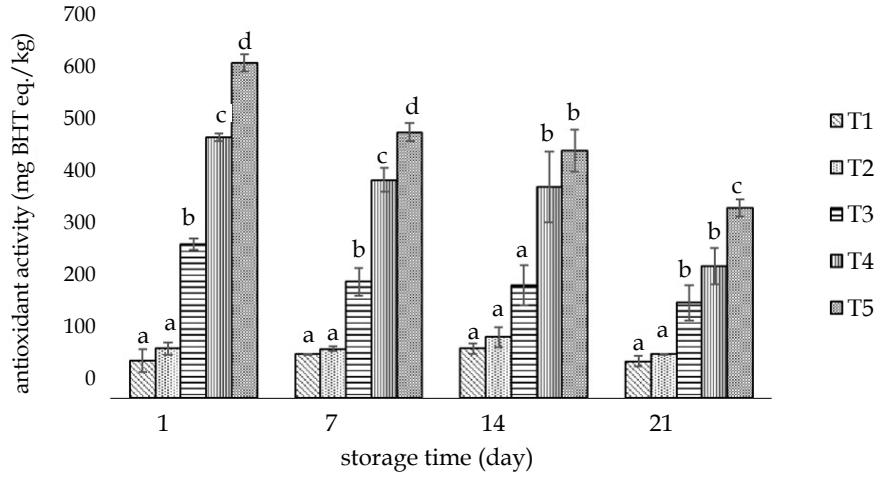

Figure 2. The antioxidant activity (AA) (mg BHT eq./kg) of synbiotic yogurt containing AEAT during storage

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 on each day (P<0.05).

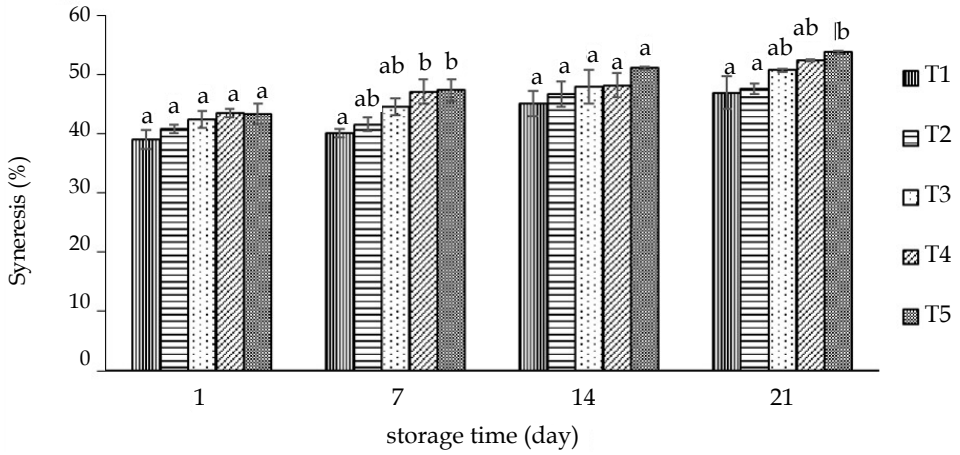

Figure 3. Syneresis (%) of synbiotic yogurt containing AEAT during storage

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.

[a, b, ab] Show statistically significant between different treatments on each day ($P<0.05$).

It is reported that the AA of yogurt is associated with the release of a large number of peptides and amino acids by lactic acid bacteria during the fermentation of the milk (Farvin et al., 2010; Kudoh et al., 2001; Madhu et al., 2012; Pena-Rasmos 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 are 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 mg BHT eq./kg yogurt, respectively. This finding is in accordance with other studies, which have shown that the incorporation of different sections of the plant and fruit creates a significant difference between the AA of yogurts fortified with them and that of the control (El-Said et al., 2014; Yadav et al., 2018; Hashemi et al., 2016; de Carvalho et al., 2019; Liu, 2018; Gaglio et al., 2019; Shori & Baba, 2013; Shori, 2013; Mosiyani et al., 2017). However, the antioxidant effects of the synbiotic yogurt samples showed a significant decrease of up to 50% after 21 days of storage. Many studies reported declining yogurts' antioxidant activity during storage (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 result from forming 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).

Adding AEAT to the probiotic yogurt significantly elevated syneresis extent compared to the plain yogurt and probiotic control yogurt ($P\leq0.05$). This result is in agreement with results obtained by Michael et al. (2010), Faraki et al. (2020), Sengul et al (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 rise of whey expulsion from the yogurt may be explained by a 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 plays an important role in serum separation, depends on various factors, including proteins and polyphenol nature, temperature, and other bioactive compounds (Vital et al., 2015). However, this observation is not in line with the results of Huang et al., 2020, Guo et al., 2018, Amirdivani et al., 2013, and Narayana and Gupta, 2013, who demonstrated the 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 weakens the casein network (Everett & Mcleod, 2005). The increase in syneresis during the storage time was reported earlier (Alighazi et al., 2021; Amirdivani et al., 2013; Zare et al., 2011).

The studies reported the reduction of organoleptic acceptability of the yogurt incorporated by red ginseng extract (Jung et al., 2016), *Hibiscus sabdariffa* (Iwalokun & Shittu, 2007), aqueous extract of basil and savory (Mosyani et al., 2017), *Chlorella vulgaris* and *Arthrospira platensis* (Beheshtipour & Motazavian, 2012) and green tea extract (Shokery et al., 2017). However, some experiments showed improvement in sensory parameters of yogurt which was fortified with soybean extract (Park & 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 plain and probiotic control yogurt, they were still acceptable.

5. Conclusion

In this study, we presented a novel, useful yogurt by incorporating ATAE into probiotic yogurt. This study showed a significant increase in TPC and AA of yogurt by adding AEAT. Generally, our results supported using this yogurt formulation as a useful product for improving consumer health. However, as adding the extract increased syneresis and impaired sensory acceptance of the yogurt, investigations on applying encapsulated *A. tequilana* extract in synbiotic yogurt are recommended.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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The paper was extracted from the MSc thesis of Melika Farzaneh at Department of Food Science and Technology, Shahr-e-Qods Branch, Islamic Azad University.

Authors' contributions

Conceptualization, supervision and writing-review & editing: Vajiheh Fadaei, and Hassan Gandomi; Visualization and writing-original draft: Melika Farzaneh; Resources: Vajiheh Fadaei; Formal analysis and methodology: Hassan Gandomi. Investigation: All authors.

Conflict of interest

The authors declare no conflict of interest.

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مقاله پژوهشی

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

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چکیده

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.

روش کار: محتوای فنلی کل نمونه‌های ماست به روش فولین سیوکالتیو اندازه‌گیری 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 ۳۸۹/۹، ۶۲۹/۲ و ۹۰۵/۶ میلی‌گرم اسید گالیک در کیلوگرم در غلظت‌های ۰/۵، ۱ و ۱/۵ درصد عصاره بود. فعالیت آنتی اکسیدانی ماست با افزایش غلظت عصاره افزایش یافت، به طوری که نمونه های ماست سین بیوتیک حاوی غلظت های ۰،۵، ۱ و ۱،۵ درصد AEAT به ترتیب ۲۸۳، ۴۸۰ و ۶۱۷ 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.

نتیجه‌گیری 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.

کلیدواژه‌ها: ماست سین بیوتیک، عصاره آگاو تکیلانا، فعالیت آنتی اکسیدانی، فنل کل، آب اندازی

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