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Research Article

Effects of Guarana Aqua Extracts on Fermentation Kinetics and Quality Properties of Set-Type Yogurts

Evangelia Kourouniotou,[1] Nikoleta Karagianni,[1] Anastasia Bari,[1] Aikaterini Diseri,[2] Marianthi Hatziioannou,[3] and Persephoni Giannouli[1]

[1]*Department of Biochemistry and Biotechnology, School of Health Sciences, University of Thessaly, Viopolis, 41500 Larissa, Greece*
[2]*Faculty of Medicine, School of Health Science, Laboratory of Biochemistry, University of Thessaly, Viopolis, 41500 Larissa, Greece*
[3]*Department of Ichthyology and Aquatic Environment, School of Agricultural Sciences, University of Thessaly, Fytoko Street, 38 446 Nea Ionia, Magnesia, Greece*

Correspondence should be addressed to Persephoni Giannouli; pergian@uth.gr

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The combination of bioactive phytochemicals with yogurt in the form of plant extracts or essential oils stimulates the scientific community's interest and consumers' preferences. Extracts from guarana are considered bioactive agents and have important health protective properties. In this study, concentrations of guarana aqua extracts 0%, 0.5%, 1%, 2%, 3%, and 4% w/w were added to milk and yogurt fermentation kinetics, acidity, color, water-holding capacity, hardness, and microstructure and were evaluated. The presence of guarana aqua extract reduced the time needed for the completion of yogurt fermentation compared to the control samples. A drop in L^* and an increase in a^* parameters were also observed with increased guarana aqua extract concentrations. The lowest concentration of guarana aqua extract decreased titratable acidity, hardness, and syneresis. While different casein networks were observed in microscopy, yogurt without and yogurt with 3% w/w guarana extract had the same acidity and hardness. Finally, this research indicates the suitability of guarana extracts in the production of functional set-type yogurt and increases the knowledge on the effects of plant extracts on milk fermentation and yogurt quality characteristics.

1. Introduction

Functional foods can positively affect one or more targeted functions of the human body and also meet nutritional needs by improving health and/or reducing the risk of disease [1]. Yogurt is considered one of the most nutritious dairy products, as it is rich in protein, calcium, riboflavin, vitamin B6, and vitamin B12 [2]. Studies have shown that the daily consumption of fermented milk products have a positive effect in health and reduce the risk of many diseases as well as diabetes 2 [3], cardiovascular disease, and colon cancer [4]. In addition, systematic consumption of yogurt for years (for more than 4 years) helps to reduce weight gain [5]. In addition, when nutritional compounds were incorporated into the yogurt matrix in the form of essential oil or vegetable extracts, yogurt is transformed to a popular functional food.

Guarana seeds are estimated to contain high levels of caffeine, methylxanthine, and tannins, as well as other compounds such as saponins, polysaccharides, proteins, and fatty acids [6]. Guarana seeds also contain trace elements such as manganese, rubidium, nickel, and strontium [7]. However, the caffeine concentration depends on the preparation of guarana seeds, as it provides an average of about 50 mg of caffeine per gram. Guarana acts as a bioactive agent with antiproliferative and antilipogenic properties[8]. Numerous studies indicate that roasted guarana seeds contain antioxidants [9], antimicrobials [10], hypocholesterolemia [11], anti-inflammatory [12], and health-protective properties [13]. The chemical composition of guarana extract and the yield of extract vary according to the method and conditions used. Some common industrial methods for extracting phytochemicals from guarana seeds are enzyme assist extraction, solvent extraction, supercritical fluid extraction, etc. The main bioactive molecules derived by the above methods are caffeine, tannins, catechins, epicatechin, and epicatechin gallate [8]. Today, there are only a few studies on the incorporation of guarana extracts on food products. Lamb burgers which were fortified with freeze-dried hydroethanolic extractions from ground guarana seeds and pitanga leaf extracts, showed an increased shelf life without physicochemical and sensory changes [14]. In addition, coencapsulation of guarana peel extract (GPE) with *Bifidobacterium animalis* subsp. *lactis* BLC-1 was applied in peanut butter and without affecting product characteristics. This research showed that bifidobacteria were protected and, also, the overall acceptability of peanut butter [15]. A recent research on fortified yogurt drink with microcapsules of guarana hydroalcoholic extracts of milled dried seed with *Lacticaseibacillus paracasei BGP-1* has shown to prevent postfermentation of guarana extracts [16].

Although, the combination of bioactive phytochemicals with yogurt stimulates the interest of the scientific community and consumers' preferences. Nowadays, there are no research work on set-type yogurt with guarana aqua extracts. In order to incorporate guarana powder into yogurt, in this study, we investigate the compatibility of the direct incorporation of guarana aqua extract (GAE) into milk aiming to design a new set-type yogurt. More specifically, this research is aimed at exploring the effects of adding aqua guarana extracts on the fermentation kinetics, color, titratable acidity, water retention, syneresis, hardness, and microstructure analysis of set-type yogurt.

2. Materials and Methods

2.1. Preparation of Guarana Aqua Extracts. Guarana aqua extracts (GAE) were prepared by commercial bio guarana powder, (country of origin Brazil). Guarana powder was boiled in distilled water at 100°C for 1 hour [17]. The mixture was then cooled to 40°C and centrifuged at 11,800 × g for 20 minutes at 24°C. The supernatant was filtered.

2.2. Preparation of Yogurt Samples. The commercial skim milk powder which was used for the preparation of yogurt samples contained fat 0.8% w/w, sugars 52% w/w, proteins 36% w/w, and salt 1.1% w/w. Yogurts were made according to the method of Pan et al. and Mohamed Ahmed et al. [18, 19]. Guarana aqua extracts were homogenized with commercial skimmed milk powder and distilled water, creating milk mixtures with guarana concentrations of 0% (control), 0.5%, 1%, 2%, 3%, and 4% w/w. Then, mixtures were pasteurized at 90°C for 15 minutes, cooled until the temperature dropped to 40°C, and then inoculated with 3% w/w plain commercial yogurt [20]. The inoculated milk was distributed in plastic cylindrical containers of 30 g each. Samples were incubated at 37°C until the pH of the mixtures reached 4.6. The fermentation process was then stopped, cooling the mixtures to 4°C for further analysis. A total of six independent experimental groups of milk gels (yogurt type) were prepared (Figure 1) and analyzed after one day of storage.

2.3. Determination of Fermentation Kinetics. During the yogurt incubation, the pH was measured by a digital pH meter LAB-860 at regular intervals in each treatment until the value reached 4.6. The maximum acidification rate (Vmax) was calculated as the time variation of pH (dpH/dt) and expressed in 10^{-3} units pH/min, from the fermentation data. In addition, the following kinetic parameters were calculated during the incubation period: (i) tv_{max} (min), the time needed for V to reach its top value; (ii) pHv_{max}, the pH value when yogurt is at its maximum acidification rate; (iii) tpH_5 (min), the time required to reach pH 5; (iv) $tpH_{4.6}$ (min), the time required to reach pH 4.6 (i.e., to complete fermentation) [21, 22]. The pH measurements of the kinetics of each experimental group of treatments were performed 4 times.

2.4. Determination of Titratable Acidity, Water-Holding Capacity, and Syneresis. In order to evaluate the acidity of the treatments, 25 g of yogurt was stirred with 25 mL of distilled water and titrated with 0.1 N NaOH solution. Phenolphthalein solution was used as an indicator.

The results were given according to the relation:

$$\text{Total acidity} = \left[\frac{(\text{V} \times \text{NaOH factor} \times A \times D)}{\text{volume of sample}}\right] \times 100, \quad (1)$$

where V is the volume of NaOH added (mL), A is the conversion factor (0.009 for lactic acid), and D is the dilution factor [23, 24].

Syneresis and water-holding capacity (WHC) were evaluated [25]. Specifically, 10 g of yogurt was centrifuged for 20 minutes at 5000 × g at 4°C by using the high-speed refrigerated centrifuge Hitachi model CR22N (Koki Co., Ltd. Tokyo, Japan). Then, the supernatants (whey) and pellets were weighed. The measurements were performed after 1 day of storage in all samples and repeated 3 times for each experimental group of milk gels. Syneresis and water-holding capacity were calculated from the following formulas [19, 24].

$$\text{Syneresis (\%)} = \left(\frac{\text{weight of the supernatant (g)}}{\text{weight of the yogurt (g)}}\right) * 100,$$
$$\text{WHC (\%)} = \left(\frac{\text{weight of precipitate (g)}}{\text{weight of yogurt (g)}}\right) * 100. \quad (2)$$

All measurements were conducted in triplicates.

2.5. Color Determination. The color measurement was performed on a flat surface of 60 g of each treatment according to the International de L'Eclairage (CIE) $L^*a^*b^*$ system, and the color parameters values were determined by using a colorimeter (HunterLab MiniScan XE Plus, USA). Specifically, L^* or brightness represents darkness in light on a scale of 0 to 100. The value a^* or redness corresponds to green to

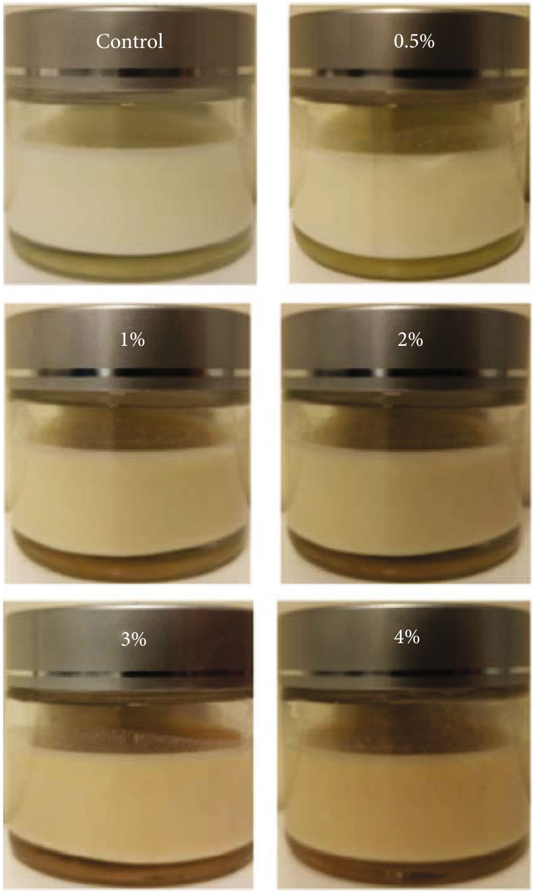

Figure 1: Pictures of set-type yogurts containing 0, 0.5, 1, 2, 3, and 4% w/w guarana extract (GAE).

red on a scale of -60 to +60. The value b* or yellowing represents blue to yellow on a scale of -60 to +60. In addition, the hue or hue angle was calculated by the equation arctangent (b*/a*), and Chroma or the color saturation index was measured by the equation $\sqrt{(a^*2\,b^*2)}$ by the method of AMSA [26, 27]. All measurements were repeated 3 times for each experimental treatment.

2.6. Hardness Determination. Hardness measurements were performed using a structure analyzer (Admet Texture Analyzer eXpert 5601, AdMEt, Inc., USA) with a 20 mm diameter cylindrical probe. The measurements were performed as a structure profile analysis (TPA) with 75% compression and at a rate of 100 mm/min. The diameter of the samples was 40 mm, and their height was 30 mm. The texture attribute "hardness" is defined as the maximum force of the 1st compression (Fmax) [28–31]. Average Fmax values were evaluated. The measurements were made at 4°C and repeated 3 times for each experimental group of yogurts.

2.7. Microstructure Analysis. Prefermented guarana extract—milk (0.5 mL)—was transferred to a microscopic slide, incubated at 40°C until the pH reached 4.6., and then sealed and stored at 4°C. One day after the preparation of the yogurts, the microstructure was determined [16, 32]. The observation of the microstructure was performed using a ZEISS Primovert Inverted microscope equipped with an Axiocam 105 color (Zeiss, Germany). Images were captured at a magnification of 10x. The sample microstructure was analyzed by the ImageJ software (ImageJ 1.53f51, Wayne Rasband and contributors, National Institutes of Health, USA). The light goes through the slide in the areas where loose or no gel is formed whereas the darker zones in the pictures represent dense compact yogurt gel. Particles were studied by circularity index (CI) which is defined in ImageJ as

$$\text{Circularity index} = \frac{4\pi \times \text{particle area}}{\text{particle perimeter}^2}. \quad (3)$$

CI value varies from 0 (noncircular shape) to 1 (perfect circular shape) [32, 33]. The total area % of the white areas was also calculated. All measurements were repeated 3 times for each experimental yogurt sample.

2.8. Statistical Analysis. The mean values of the measurements were compared by applying one-way analysis of variance (ANOVA) at a confidence level of 95% ($p < 0.05$) and LSD (least significant difference) test using IBM SPSS Statistics 26 software. The mean value of the measurements was determined for variability by calculating the standard error or standard deviation of the values. All the experiments were repeated three times, each with three replicates. The data were expressed as means ± standard errors of triplicate determinations.

3. Results and Discussion

3.1. Kinetic Measurements. The rate of acidification is an important factor in the preparation of yogurts because a balance must be struck between the strength of the casein network and the commercially viable fermentation time. When milk is acidified by lactic acid bacteria, aggregation of casein particles leads to gel formation. In the present study, the kinetics of milk fermentation was studied as affected by the addition of different concentrations of guarana extracts. Due to the fact that kinetics refers to the change in the pH of yogurts over time, it should be mentioned that the initial pH value of the yogurts was not affected by the addition of different concentrations of guarana extracts.

The values of the maximum acidification rate Vmax between the control (0% w/w) and the different concentrations of guarana (0.5%, 1%, 2%, 3%, and 4% w/w) in the prepared yogurts are shown in Table 1. No significant differences were noticed for yogurt samples containing 0% w/w and 0.5% w/w GAE ($p > 0.05$). The maximum Vmax value was observed for yogurt containing 4% w/w GAE ($11.60 \pm 0.00^* 10 - 3$ upH/min) which was significantly different ($p < 0.05$) compared to the rest of the samples. The effect of GE on milk fermentation also showed a pick of the acidification rate at 1% w/w GAE ($9.90 \pm 0.00^* 10 - 3$ upH/min) followed by drop for the following concentrations 2% and 3% w/w GAE. Probably, for guarana extract, the contained fiber is not the only factor responsible for the acceleration rate of acidification of milk. This is in contrast with other research

Table 1: Measurement of the maximum acidification rate, the time of the maximum acidification rate, the pH at the maximum acidification rate, the pH value when it is equal to 5, the pH value when it is equal to 4.6, in yogurts enriched with guarana extracts GAE (0%, 0.5%, 1%, 2%, 3%, 4% w/w).

Treatment GAE % w/w	Vmax (10^{-3} upH/min)	pH Vmax	TpH 5 (min)	TpH 4.6 (min)
0.0%	6.00 ± 0.00^{e}	5.30 ± 0.01^{f}	198.00 ± 1.00^{a}	331.00 ± 1.00^{a}
0.5%	6.40 ± 0.00^{e}	5.13 ± 0.02^{e}	137.67 ± 2.08^{d}	237.67 ± 2.08^{c}
1.0%	9.90 ± 0.00^{d}	5.23 ± 0.01^{d}	163.00 ± 2.00^{b}	263.00 ± 2.00^{b}
2.0%	7.80 ± 0.00^{c}	5.38 ± 0.02^{c}	133.33 ± 0.57^{e}	213.33 ± 0.57^{e}
3.0%	8.10 ± 0.00^{bc}	5.43 ± 0.01^{b}	143.33 ± 1.52^{c}	223.33 ± 1.52^{d}
4.0%	11.60 ± 0.00^{a}	5.58 ± 0.01^{a}	119.33 ± 1.52^{f}	186.33 ± 1.52^{f}

^{a,b,c,d,e}Values with different superscript letters indicate significant differences ($p < 0.05$) among treatments.

where the addition of moringa extract on yogurt fermentation [17] increase fermentation Vmax according to moringa extract concentration.

The time required for yogurts with GAE in concentrations of 0%, 0.5%, 1%, 2%, 3%, and 4% w/w, to reach pH 5, was also described (Table 1). For the control sample, the necessary time was the highest one (198 ± 1.00 min), while yogurt with guarana concentration 4% w/w needed the least time (119.33 ± 1.52^{b} min) ($p < 0.05$). In general, the highest time needed for samples with guarana extracts to reach pH 5 was the yogurt with 3% w/w. In addition, yogurts with guarana concentrations 0% (control), 0.5%, 1%, 2%, 3%, and 4% w/w reached pH 4.6 in different times. The yogurt with the 0% w/w concentration of guarana (control) approaches the desired pH value more slowly (331 ± 1.00 min), compared to the rest of the treatments ($p < 0.05$). Yogurt with 4% w/w guarana concentration arrived faster (186.33 ± 1.52^{b} min), which shows statistically significant differences ($p < 0.05$) with the control. Generally, the presence of guarana extracts in yogurt samples needs less time to reach pH 4.6 compared to control samples. Probably, active ingredients and indigestible carbohydrates of the guarana extract promoted the growth of lactic acid bacteria. Similar observations were recorded in studies with yogurts fortified with moringa extract [17], with *Pleurotus ostreatus* extract [22], and with green tea [34].

Milk acidification is a process that occurs when the action of casein micelles changes as the pH of the milk decreases. Lowering the pH separates the micelles from the colloidal calcium phosphate. Thus, the negative charge of the casein micelles is neutralized due to the hydrogen ions H+ that are released during acidification, enhancing the attraction and accumulation of proteins. When room temperature conditions are present and the pH is equal to 5.2, casein micelles form strong covalent bonds with the denatured whey proteins. Then, as the isoelectric point of the casein micelles approaches, that is, as the pH approaches 4.6, complete agglomeration of the micelles is induced [35]. Guarana extract has a rich phenolic composition, a high concentration of starch [8], sugars, etc. and provides many factors to influence starter culture. Therefore, guarana extract provides the available carbon source for increasing bacterial growth and reducing fermentation time such as glucose [36]. These results are similar with other researches where green tea powder [34] or red ginseng extract was incorporated in yogurt and decreased fermentation time as viable counts increased [37].

3.2. Titrated Acidity, Water-Holding Capacity, and Syneresis Measurements. The activity of lactic acid bacteria during low-temperature storage causes the production of lactic acid, and thus, the percentage of lactic acid as well as titratable acidity increases. In this research, the results of titratable acidity (TA) expressed in % lactic acid in yogurts with 0% (control), 0.5%, 1%, 2%, 3%, and 4% w/w guarana concentrations are presented in Figure 2. The highest TA values are shown in yogurt with 0% w/w guarana concentration (control) ($0.62 \pm 0.05\%$), while the lowest value was found in yogurt with 0.5% w/w guarana concentration ($0.53 \pm 0.15\%$). Yogurt with 0.5% w/w guarana concentration shows statistically significant differences ($p < 0.05$) compared to sample concentrations 0%, 2%, and 3% w/w GAE which showed similar titrated acidity ($p > 0.05$). The same was found in recent research on the enrichment of yogurt with flaxseed powder [38] and during the addition of edible rose extract to set-type yogurts [24]. Similar results were also reported in research on the enrichment of yogurt with flaxseed and fiber-rich pineapple peel powder [27, 38].

Syneresis and water-holding capacity are also two very important quality characteristics of yogurt. Syneresis is the process of extracting fluid from the yogurt network, leading to its separation from the whey. It happens mainly when the weakened yogurt gel network does not have the ability to trap the liquid phase of the serum, resulting in the separation of the yogurt whey [39]. Water-holding capacity refers to the amount of water that the yogurt sample has after the application of external force. Syneresis and water-holding capacity are two important quality characteristics that characterize yogurt, because the separation of whey on the surface of yogurt can cause negative impressions on consumers [39]. In this research, the effects of GAE concentrations on yogurt syneresis and water-holding capacity are presented in Figure 3. More specifically, the highest value of % syneresis appears in yogurt with 3% w/w guarana concentration (70.33 ± 0.58^{b} %), while the lowest in 0% w/w (62.00 ± 0.00^{a} %) ($p < 0.05$). Yogurts with 2% and 4% w/w guarana concentration showed similar syneresis values and do not differ statistically ($p > 0.05$). The yogurt with 1%

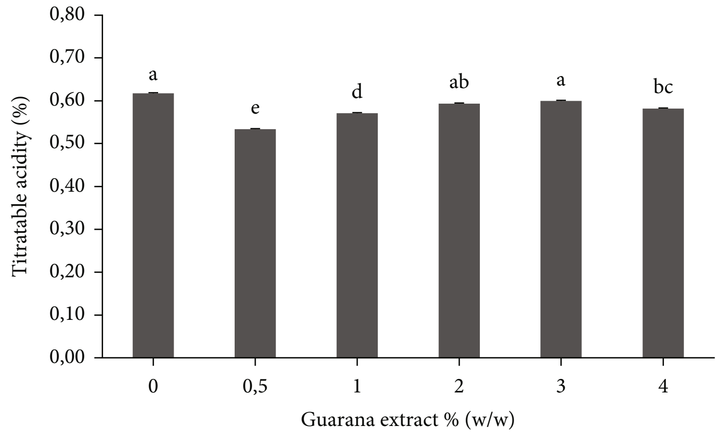

Figure 2: Titratable acidity (lactic acid %) of set-type yogurts containing 0, 0.5, 1, 2, 3, and 4% w/w guarana extract (GAE). Different letters (a–e) within the columns indicate significant differences ($p < 0.05$) among treatments. All measurements were conducted in triplicates.

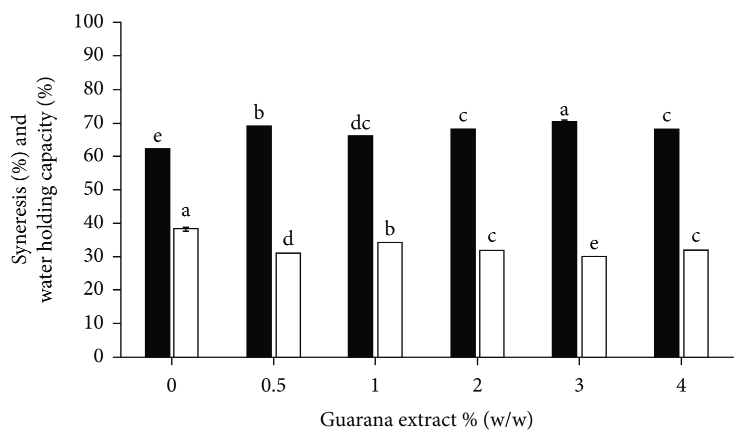

Figure 3: Syneresis (%) and water-holding capacity (%) of set-type yogurts with 0, 0.5, 1, 2, 3, and 4% w/w guarana extract (GAE). Black columns indicate syneresis values (%); white columns indicate water-holding capacity values (%). Different letters (a–e) within the columns indicate significant differences ($p < 0.05$) among treatments. All measurements were conducted in triplicates.

GAE showed the highest % of water-holding capacity compared to the rest of the samples with GAE. The sample with a concentration of 0% w/w GAE has the lowest value of water-holding capacity (38.33 ± 0.57^{a} %), while the sample with 3% w/w has the lowest ($p < 0.05$). Finally, the same values were found for % water-holding capacity for the 2% yogurts and 4% w/w guarana concentration ($p > 0.05$) (Figure 3). The results probably occurred due to the thermodynamic incompatibility between guarana polysaccharide [40] with milk proteins. In addition, due to the unbalanced osmotic potential, after the depletion of casein micelles in the presence of nonabsorbable polymers (dietary fibers), a contraction of the casein network protein occurred [41]. This agrees with many recent studies that showed that the addition of edible rose extract [24], date palm [41], moringa [17], and pineapple peel powder [27] to yogurt reduced syneresis and increased water-holding capacity.

3.3. Color Measurements. Color is one of the main quality characteristics of dairy foods because it could influence consumer taste. Storage and self-life of yogurt are factors which effect yogurt color [41]. In Figure 3, photos of samples with the different guarana extracts were presented, and color parameters L^*, a^*, b^*, Chroma and hue are shown in (Table 2). More clearly, the highest lightness value appeared in yogurt with 0% w/w guarana concentration (control) (83.66 ± 0.95), while lowest L^* was found in yogurt with 4% w/w guarana concentration (73.90 ± 0.03) ($p < 0.05$). As the concentration of GE to yogurt was increasing, the values of L^*were decreasing with statistically significant differences ($p < 0.05$) between them.

Parameter a^* in samples was also increased as the concentration of guarana extracts increased. The lowest values were observed for the yogurt 0% w/w (control) (-2.36 ± 0.04), while the highest in yogurt 4% w/w guarana concentration (3.85 ± 0.02), ($p < 0.05$). On the other hand, color parameter b^* showed the highest value when 3% w/w guarana extract was added to yogurt and the lowest in the control sample (0% w/w GE).

Chroma* and hue* angle values were also affected by the different concentrations of guarana extract. The highest Chroma* values were observed for the yogurt samples

Table 2: Color parameters L*, a*, b*, Chroma, and hue* angle of yogurt samples enriched with guarana extracts GAE (0%, 0.5%, 1%, 2%, 3%, and 4% w/w).

Treatment GAE % w/w	L*	a*	b*	Chroma*	Hue*
0.0%	83.66 ± 0.95^a	-2.36 ± 0.04^f	10.78 ± 0.34^f	11.03 ± 0.04^f	178.65 ± 0.01^a
0.5%	79.30 ± 0.05^b	1.25 ± 0.01^e	14.59 ± 0.02^e	14.64 ± 0.04^e	1.49 ± 0.01^b
1.0%	76.50 ± 0.09^c	2.91 ± 0.01^d	17.34 ± 0.03^d	17.58 ± 0.03^d	1.41 ± 0.01^d
2.0%	76.45 ± 0.09^d	3.40 ± 0.03^c	19.14 ± 0.15^c	19.44 ± 0.02^c	1.40 ± 0.01^e
3.0%	75.66 ± 0.08^e	3.41 ± 0.01^b	22.09 ± 0.01^a	22.35 ± 0.01^a	1.42 ± 0.01^c
4.0%	73.90 ± 0.03^f	3.85 ± 0.02^a	20.15 ± 0.07^b	20.52 ± 0.07^b	1.38 ± 0.01^f

[a,b,c,d,e]Values with different superscript letters indicate significant differences ($p < 0.05$) among treatments.

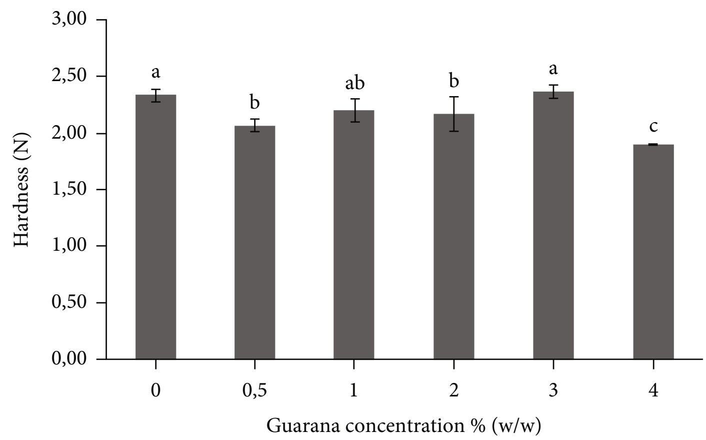

Figure 4: Hardness of set-type yogurts containing 0, 0.5, 1, 2, 3, and 4% w/w guarana extract (GAE). Different letters (a–c) within the columns indicate significant differences ($p < 0.05$) among treatments. All measurements were conducted in triplicates.

containing 4% w/w guarana extract (22.35 ± 0.01). Chroma* values were increasing with the increase of the guarana extract content. The opposite was observed for the hue* angle values where the greatest values were observed for the control sample (178.65 ± 0.01) and the lowest to yogurt with 4% GE (1.38 ± 0.01). Significant differences were observed among all treatments ($p < 0.05$).

In general addition of guarana extracts to yogurts, reduced the brightness (L*) and increased +a* and b*. Similar results were found in studies with type yogurts fortified with argel leaf extract [19], with chia seed extract [42] and with aqueous extract of *Pleurotus ostreatus* [43]. The results found in this research could be attributed to the color of the guarana extract (brown). In particular, the increase in the value of b* and the decrease in L* in yogurts may be due to the formation of brown polymers, which are resulted from the degradation of phenolic substances. Anthocyanins also were contained in guarana, and the fermentation process could influence the creation of a wide range of pH-related colors. Therefore, the variable a* increased due to the decrease in pH, after the acidification of the yogurts by the added cultivation.

3.4. Hardness Measurements. Textural properties of fermented dairy products depend on the arrangement and network of the protein microstructure, and texture analysis can determine whether yogurts can better respond to the stiffness and stability of the emulsion network with sufficient dispersion of functional compounds [28]. In general, there are many factors that affect yogurt hardness such as the type and composition of culture, milk origin, presence of fat, and structure of the emulsion. Consequently, all these parameters influence yogurt mouth feel, appearance, and overall consumer acceptability [44].

In this research, skim milk powder was used in the production of samples, and GE was incorporated in different concentrations. More specifically, in Figure 4, the hardness of yogurts with 0% (control), 0.5%, 1%, 2%, 3%, and 4% w/w guarana extracts was described. It was observed that the addition of 0.5% w/w guarana concentration reduces the hardness of yogurt compared to control (0% w/w) ($p < 0.05$). This may be due to the development of weak interactions between the phenolic compounds of the extract and the casein, leading to a decrease in the hardness values of yogurt [28, 29]. The highest hardness values appear in yogurts with concentrations of 0% and 3% w/w guarana (2.33 ± 0.06^a, 2.37 ± 0.06^{ab}). These two samples did not show statistically significant differences ($p > 0.05$). This is contrary with another study [45] where yogurts fortified with passion fruit peel powder had an average higher hardness compared to controls. In this case, the textural characteristics were affected by a combination of parameters such as culture composition, composition of passion fruit peel

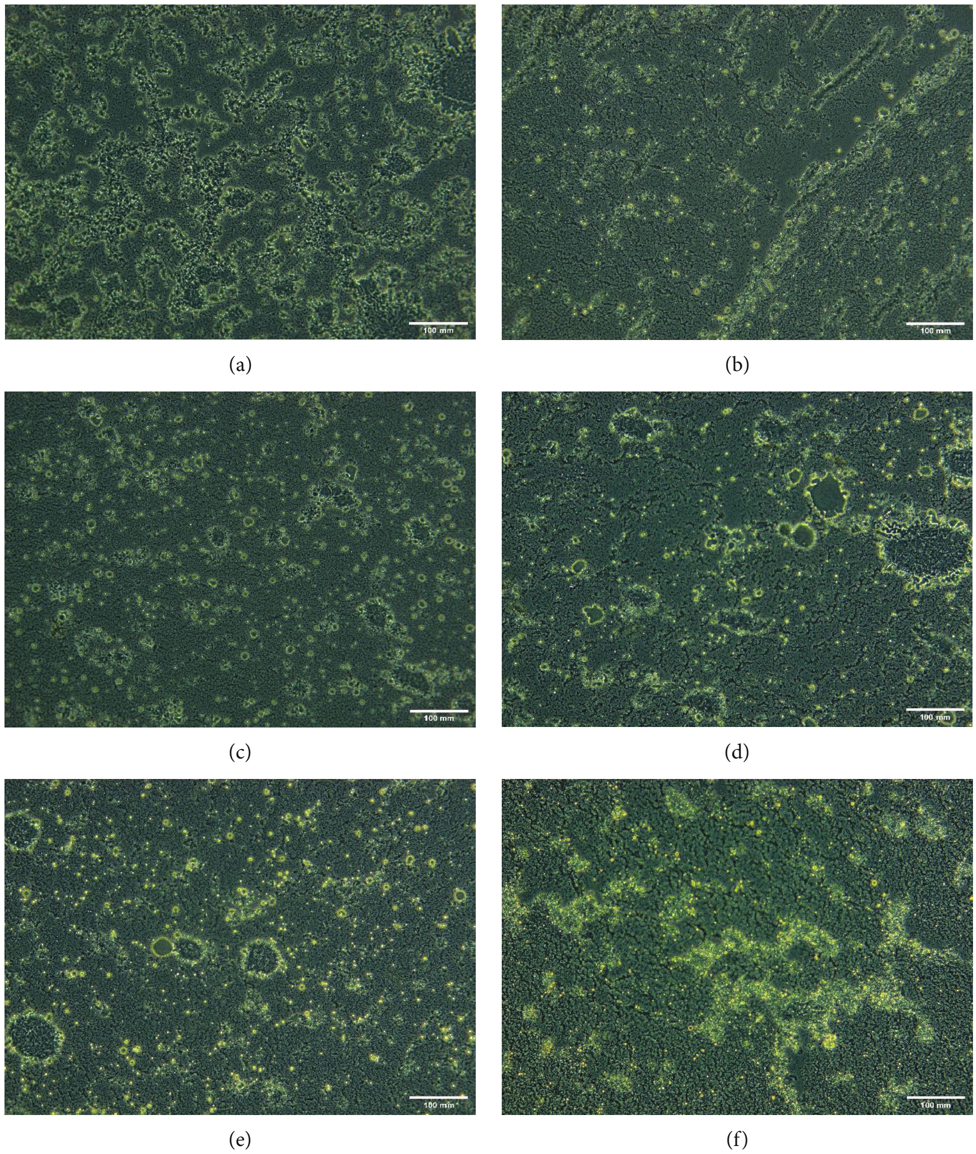

Figure 5: Microscopic images of set-type yogurts after a day of storage containing 0, 0.5, 1, 2, 3, and 4% w/w guarana extract (GAE). The white scale bar represents 100 μm. (a) Yogurt sample with 0% w/w GAE; (b) yogurt sample with 0.5% w/w GAE; (c) yogurt sample with 1% w/w GAE; (d) yogurt sample with 2% w/w GAE; (e) yogurt sample with 3% w/w GAE; (f) yogurt sample with 4% w/w GAE.

powder, and milk type [30, 44, 45]. Consequently, if guarana powder was added in set-type yogurts, the results probably could be similar to passion fruit peel powder [45] as the addition of powders in the yogurt formulation tends to increase the hardness of the gel. In this research with aqua guarana extracts, none of the fortified yogurts showed higher hardness than the control sample. Also, the lowest value in hardness was found in yogurts with guarana extract 4% w/w ($p < 0.05$). This sample had the shortest fermentation time which could be the reason for the low values in hardness [30] as the quick time to reach pH 4.6 was not sufficient to develop a casein network.

3.5. Microstructure Analysis. The microstructures of yogurts enriched with concentrations of 0% (control), 0.5%, 1%, 2%, 3%, and 4% w/w guarana extracts are presented in Figure 5, and differences were observed. These differences in the structures of yogurt gels were shown, in term of compact shape of the casein micelle network and the size of the pores. The dark zone is considered intact aggregated clusters from the set yogurts. The surface of these gel fragments was determined by ImageJ software [46]. Measurements of total area (%) and circularity index of yogurt particles containing (0.5, 1, 2, 3, and 4% w/w) guarana extract are presented in Figures 6(a) and 6(b), respectively. In particular, yogurt with 0% w/w guarana concentration presented a branched protein network with empty irregularly shaped areas, which led to the rearrangement and contraction of the protein. By adding 0.5% w/w guarana extract, the branched network disappeared, and some linear structures were created. This agrees with the drop, in hardness that was found for a sample with 0.5% w/w GAE. Both yogurt

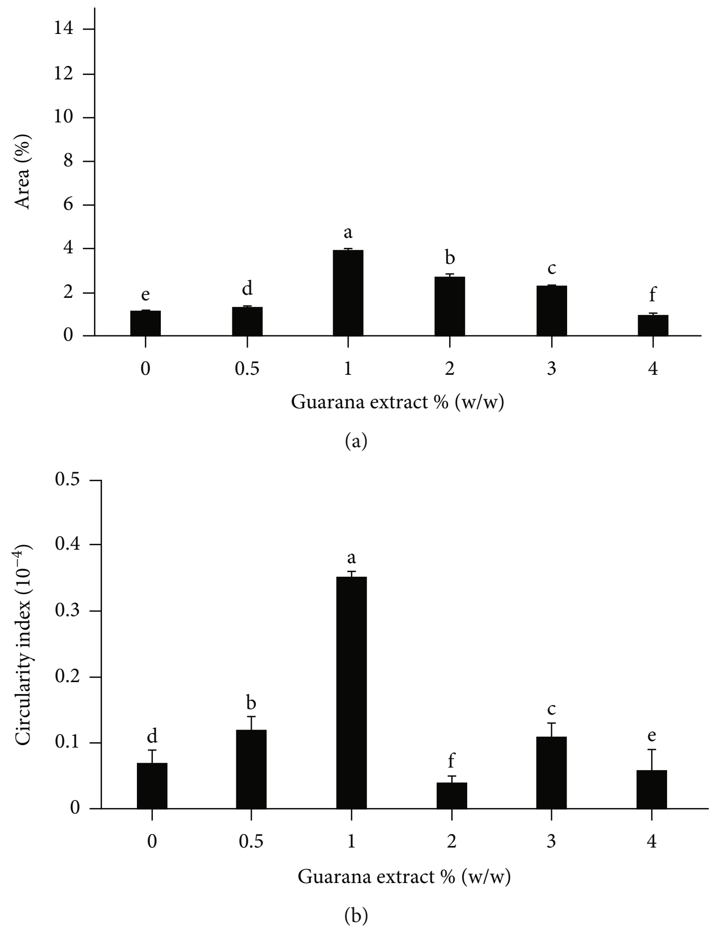

FIGURE 6: (a) Total area (%) and 6. (b) Circularity index of yogurt samples containing 0.5, 1, 2, 3, and 4% w/w guarana extract (GAE). Different letters (B–F) within the columns indicate significant differences ($p < 0.05$) among treatments. All measurements were conducted in three independent experiments.

control and 3% w/w guarana extract showed a more compact network of casein micelles, but the network has been arranged in a different way. More specifically, total area % of empty spaces was the highest for yogurt with 1% w/w GAE ($p < 0.05$) and the lowest for the sample with 4% GAE ($p < 0.05$). Also, sample with a guarana concentration of 4% w/w showed in microscopy the lowest value for total area % of empty spaces, and a different network of casein micelles was formed compared to the rest samples. The microstructure appears to be directly related with the yogurt hardness as the sample with 4% w/w GAE has the smallest hardness probably due to the small fermentation time and the presence of guarana. For both measurements, total area % and circularity index, the greatest values were observed for the yogurt samples containing 1% w/w GAE, and their values were significantly different ($p < 0.05$) from the other treatments. Yogurt with 1% w/w GAE had also the highest syneresis and the largest fermentation time compared to the other samples containing guarana extract ($p < 0.05$). Many studies are in accordance with our findings as the highest hardness showed a more homogeneous and compact structure [46] and in other studies of fortified yogurts such as yogurts with edible rose extract, pineapple peel powder, and moringa seed extract [24, 27, 47].

4. Conclusions

In this study, guarana aqua extract (GAE) at different concentrations was successfully incorporated in set-type yogurt. Small amount of GAE extract (0.5% w/w) alter casein network, and it formed a yogurt with a different microstructure, titratable acidity, and hardness compared to one without GAE. The addition of a high concentration in GAE extract (4% w/w) decreases fermentation time and forms a compact microstructure with small hardness. As it is important to incorporate plant extracts and phytochemicals in yogurt in order to create functional products without altering the quality characteristics that the consumers expect, our study demonstrates that the addition of 3% w/w GAE in yogurt is the most ideal concentration as it showed the most similar characteristics to the yogurt without GAE and smaller fermentation time. Consequently, these new data in yogurts fortified with GAE could be interesting for research and development in the dairy industry as the production of functional guarana yogurts could also stimulate consumers' preferences.

Data Availability

Data are available upon request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

E.K. performed the experiments and data analysis, N.K. assisted in experiments, A.B. did the color and texture experiments and data analysis, K. D. conducted the microscopy experiments and ImageJ analysis, M.H. supervised texture experiments and revised the manuscript draft, and P.G. designed, supervised experiments and data analysis, and wrote the paper.

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