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Functional and antiglycation properties of cow milk set yogurt enriched with *Nyctanthes arbor-tristis* L. flower extract

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ABSTRACT

The fortification of yogurt with natural herbs to improve its nutritional and health benefit are an emerging trend. Hot infusions of *Nyctanthes abo-tristis* flowers are a traditionally used herb against diabetes. This study was designed to develop a novel yogurt fortified (FY) with 3, 3.5, and 6% *N.arbor-tristis* flower extract (NFE) to determine its suitability as a fortifier *en* route to innovative functional products. Upon fermentation, the highest sensory scores were obtained for the 3.5% NFE-FY with 11-, 6-, 3-fold higher antiglycation (NFE- IC_{50}: 28.04 ± 1.13 μg/mL and 3.5% NFE-FY IC_{50}: 46.80 ± 0.92 μg/mL) activity, free radical scavenging potentials, and total phenolic content, respectively. An improvement of texture profile values was observed in 3.5% NFE-FY compared to the control with 3.00 ± 0.1% fat, 3.88 ± 0.23% crude protein, 77.94 ± 0.09 moisture, 14.97 ± 0.27 total soluble solids, and 0.7637 ± 0.03 ash. The 3.5%NEF-FY also exhibited a longer shelf life and less microbial growth than the control. The GC-MS analysis of the NFE indicated the presence of phytochemicals essential for the observed bioactivity. The results confirmed that the *N.arbor-tristis* flower extract increased the overall quality of the yogurt and tested bioactivity. NFE-FY could be used in yogurt production to optimize the health benefits with improved functional characteristics to prevent diet-driven glycation activity.

1. Introduction

Glycation is the key molecular basis of diabetic complications such as diabetic retinopathy, nephropathy, and atherosclerosis associated with diabetes mellitus. Glycation is the non-enzymatic formation of adducts between amino groups and the carbonyl groups of reducing sugars. (Grzegorczyk-Karolak, Gołąb, Gburek, Wysokińska, & Matkowski, 2016). In addition, AGEs can also be formed from lipid-protein interactions under oxidative conditions (Sajithlal & Chandrakasan, 1999). Lipids and proteins are the primary targets of oxidative reactions. Reactive oxygen species may attack the side chains of amino acids and the peptide backbone during protein oxidation, resulting in the formation of carbonyl compounds, which leads to Maillard reaction followed by the formation of AGEs (Silva, Estévez, Ferreira, Silva, Lemos, Shimokomaki, & Madruga, 2018). Therefore, the advanced glycation end products (AGEs) generated in the human body can alter the structure and function of proteins. AGEs are slowly formed during the aging process, and the speed of the formation is increased in particular conditions, including diabetes and tissue oxidative stress. However, a chronic increase in intracellular oxidative stress and hyperglycaemic condition accelerates the formation of AGEs and leads to deposition in different parts of the body. Oxidative stress is further increased by generating reactive oxygen species and the formation of AGEs via the non-enzymatic, covalent attachment of glucose molecules to circulating proteins. The formation of AGEs is an irreversible reaction that affects the different biochemical reactions of the body (Jung, Park, Jung, Kim, & Kim, 2019). AGEs increase with chronic hyperglycemia associated with diabetes mellitus and an increase in oxidative stress. AGEs are fluorescent and non-fluorescent complex adducts that accumulate predominantly on long-lived proteins, compromising their physiological functions. An increase in AGE levels in the body is a significant cause of micro-and macrovascular complications. Inhibition of AGE formation is a therapeutic approach to prevent diabetic complications (Wakeman

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Abbreviations

AGEs	Advanced glycation end products
CY	Control yogurt
DPPH ●●	2,2-diphenyl-1-picrylhydrazyl radical
NFE	*Nyctanthes abor-tristis* flower extract
NFE-FY	*Nyctanthes* flower extract fortified yogurt
TTA	Titratable acidity analysis

and Archer, 2020).

Recent investigations have reported that plants with antiglycation properties with minimal side effects can be used as effective means to manage glycation-induced molecular damage. Plants with antidiabetic effects are used in traditional medicine without side effects (Gunathilaka, Samarakoon, Ranasinghe, & Peiris, 2019). Therefore, scientific research has extended the search for dietary plants, fruits, and herbal medicines that effectively inhibit AGE formation (Gunathilaka et al., 2019). Due to therapeutic safety, efficacy, minimal adverse effects, and low cost, the reputation of herbal remedies has increased globally.

Preventive medicine has made significant advances in the last decade, particularly in developed countries. Nutrition, according to research, plays a critical role in the prevention of chronic diseases, as the majority of them are diet-related. Functional food is defined as food necessary for survival and as a source of mental and physical well-being. It contributes to preventing and reducing risk factors for various diseases or enhancing certain physiological functions (Lobo, Patil, Phatak, & Chandra, 2010).

Yogurt is a popular functional food due to its exceptional taste and high nutritional properties. Recent evidence suggests that consuming yogurt poses numerous health benefits, including strengthening the immune system, reducing constipation, colon cancer, inflammatory bowel disease, improving lactose digestion, and managing blood glucose (Moore, Horti, & Fielding, 2018). Although the health benefits of yogurt are well documented, high protein and nucleic acid contents in milk could induce non-enzymatic reactions of reducing sugars with free amino groups, leading to the formation of AGEs (Grzegorczyk-Karolak et al., 2016). In today's nutrition food system, dairy products are a different carrier that has been successfully used to deliver phytochemicals and other nutrients. Furthermore, the addition of herbs or their extracts to various dairy products transforms these products into carriers for nutraceuticals. As a result, fortifying dairy foods with herbal extracts may aid in the development of functional dairy products with nutritional and medicinal value (El-sayed & Youssef, 2019). Recently, increasing consumer attention has focused on the role of plant-based food in health benefits.

In the South Asian traditional medicinal system, hot infusion of *Nyctanthes arbor-tristis* L (family Nycantheaseae) has been used to manage hyperglycemic conditions. The hot infusion of *N. arbor-tristis* is safe for oral consumption and elicits promising hypoglycemic activity (Rangika, Dayananda, & Peiris, 2015) and anti-obesity effects and potent anti-apoptotic activity of aqueous flower extract against human primary leukemia cell lines (Heendeniya et al., 2020). The authors further showed that the flower extract is rich in many bioactive compounds. Probiotic yogurt enriched with phenolic/polyphenolic extracts of rice berries has improved antioxidant capacity in healthy volunteers (Anuyahong, Chusak, Thilavech, & Adisakwattana, 2020). However, in Sri Lanka, research on obtaining yogurt produced with enhanced nutrients and bioactivity is scarce.

Pharmaceutical technology advancements are intended to discover new drugs and innovative approaches to administering them. There is a global trend toward using functional foods, which provide physiological or metabolic health benefits and their standard nutritional value (Alongi & Anese, 2021). Thus, the objective of the present study ~~aimed~~ is to manufacture yogurt incorporated with aqueous flower extract of *N. arbor-tristis* (NFE) to improve antiglycation activity. The acceptability and quality of yogurt fortified with different concentrations of *Nyctanthes* flower extract can be evaluated by sensory analysis and texture analysis. The fortified yogurt passing the acceptance test was used to determine the effect of extract additives on physicochemical properties, antiglycation and antioxidant activities, microbiological characteristics, and shelf life of fortified yogurt.

2. Materials and methods

2.1. Sample collection

Fresh *N. arbor-tristis* flowers were collected early in the morning (6.00–7.00 a.m.) from the Ayurvedic herbal gardens of Navinna (6.8533° N, 79.9151° E), Sri Lanka, between June and November 2019 and were authenticated by the Department of Botany. The flowers were thoroughly washed with tap water and shade-dried overnight. The dried flowers were ground to a fine powder using a mechanical grinder. Two grams of powder was heated with 100 mL of deionized water at 80 °C for 20 min. The resulting aqueous flower extract of *N. arbor-tristis* was filtered using a muslin cloth, freeze-dried at −70 °C, and kept at 4 °C until further analysis.

2.2. Preparation of yogurt

Yogurt samples were prepared without and with three different concentrations of flower extracts (0% (control), 3%, 3.5%, and 6% *N. abor-tristis* flower extract incorporated yogurt) according to the method described by Zhang et al. (2019). Briefly, fresh cow milk having 3% milk fat percentage, with fat and milk percentages were adjusted to 3%, and 15% using 2% skim and prewarmed (42 °C). Standardized milk supplemented with 0.4% gelatin and 5.02% sugar was homogenized for 5 min followed by pasteurization at 95 °C for 5 min. Subsequently, samples were cooled down to 42 °C in a water bath, and the mixture was inoculated with 3% (v/v) bacterial cultures (lactic acid bacteria; *S. thermophiles*, *L. bulgaricus*), along with 0.1% coloring, 0.1% (v/v) vanilla extract and different concentrations of *N. arbor-tristis* aqueous flower extracts (3%, 3.5%, and 6% v/v). Samples were incubated at 42 °C for 2 h until ~~the~~ pH reached 4.5, and they were chilled for 2 h (fresh yogurt) and stored up to 28 days at 4 °C.

2.3. Sensory evaluation

The sensory evaluation concerning the acceptance test of the yogurts (0, 3, 3.5~~,~~ and 6% of *N. abor-tristis* flower extract incorporated) was carried out by employing a 20 member-trained sensory panel and using 5 points hedonic scale, according to ISO guidelines. The purpose of this trial was to determine the taint potential of yogurt samples, and before evaluation, panelists were given 3 sessions to be familiarized with the parameters. The assessment included the sensory attributes of color, texture, aroma, taste, aftertaste, and overall acceptability. A 5-point hedonic scale (liked very much: 5, liked: 4, neither liked nor disliked: 3, disliked: 2, and extremely disliked: 1) was adopted for sensory evaluation (Dimitrellou, Kandylis, & Kourkoutas, 2019). Each panelist was randomly given three coded samples with a scorecard and a cup of distilled water at room temperature. Panelists were asked to provide scores according to their perceptual responses. Respondents were instructed to wash their mouth with distilled water after testing each product and rest for 2–3 min to overcome the sensory fatigue. The sessions were replicated, and the mean score was calculated.

2.4. Proximate composition

The proximate analysis of the control yogurt (CY) and sensorial accepted *Nyctanthes* flower extract fortified yogurt (3.5% NFE-FY) was

determined according to the methods described in AOAC (AOAC, 2006). Moisture and ash contents were determined by the gravimetric (AOAC 925.09) and dry incineration (AOAC 923.03) methods. Protein content (in terms of nitrogen content) was determined by the Micro-Kjeldahl method (AOAC 939.02). The obtained results expressed the total nitrogen content multiplied by 6.25 to obtain the total protein content. The crude fat content was determined using the Gerber method, and the percent crude fat was determined by AOAC 2000.18 (AOAC, 2006). Total soluble solids were analyzed using a Brix meter by spreading the yogurt sample on the surface of the prism and taking the reading (AOAC 913 2.0).

2.5. Texture analysis

Texture analysis of the yogurt samples (CY and 3.5% NFE-FY) was performed using a CT3 analyzer (Brookfield Engineering Laboratories Inc.) and a cylindrical probe (TA4/1000) equipped with the software. Before analysis, the samples were compressed twice with the deformation target at a pretest speed of 1 mm/s, and the recovery time between compressions was zero seconds (Mudgil, Barak, & Khatkar, 2017). All the samples were assayed in triplicate.

2.6. Syneresis

A sample of 25 g was centrifuged at 350 rpm for 30 min at room temperature. The weight of the supernatant was taken, and the syneresis of the yogurt sample (CY and 3.5% NFE-FY) was calculated using the following equation (Jovanović et al., 2020).

$$\text{Syneresis } \% = \text{Weight of released whey/Weight of yogurt sample X } 100$$

2.7. Shelf-life evaluation

The shelf-life evaluation of the yogurt samples (CY and 3.5% NFE-FY) was tested from time to time over three weeks. The pH and titratable acidity analysis (TTA) was carried out for shelf life from time to time (Al-Kadamany, Khattar, Haddad, & Toufeili, 2003). The pH of the yogurt samples was measured at three-day intervals over 21 days using a pH meter (pH 900, Precisa Co, Dietikon, Switzerland).

The titratable acidity (TTA) was determined via neutralization titration using 0.1 N NaOH in distilled water until the pH reached 8.3 (9 g of stored yogurt sample in 1 mL of water). The samples (CY and 3.5% NFE-FY) were measured at three-day intervals over four weeks. The amount of NaOH needed was calculated by the amount of lactic acid (%) formed (Mohamed Ahmed et al., 2021).

2.8. Coliform, yeast, and mold analysis

Total coliform, yeast, and mold analyses were conducted according to standard methods to examine dairy products using violet red bile agar and acidified potato dextrose agar (PDA). According to the pour plate technique, 1 mL of each sample (CY and 3.5% NFE-FY) was transferred into sterile plates followed by 12 mL of the media and mixed well. Upon incubation for 24 h at 37 °C, pink colonies formed were counted to determine the total coliform count. The test was repeated on days 1, 7, 14, and 21 in triplicate (Al-Kadamany et al., 2003).

Approximately 1 mL of sample (yogurt samples with and without *N. arbor-tristis*) was taken and diluted to 10 mL by adding peptone water. Subsequently, 1 mL of the diluted sample was inoculated on PDA plates and incubated at 25 °C for five days. Upon incubation, the number of yeast and mold colonies formed was counted separately. The procedure was tested for both samples on days 1, 7, 14, and 21 in triplicate.

2.9. Antioxidant activity

The antioxidant activity of the accepted yogurt sample (3.5% NFE-FY), control yogurt (CY), and the *Nyctanthes arbor-tristis* L. flower extract were determined using a (DPPH ●●) (2,2-diphenyl-1-picrylhydrazyl radical) scavenging assay (Mohamed Ahmed et al., 2021). A concentration series of the yogurt samples were prepared in methanol, and absorbance before adding the DPPH ● solution was taken at 517 nm. Subsequently, DPPH ● solution was added and incubated in a dark area for 15 min at 25 °C. Upon incubation, absorbance was taken at 517 nm. Gallic acid (GA) was used as the standard, and the DPPH ● radical scavenging activity was expressed as mg of GA equivalent per 1 g. Samples were analyzed in triplicate.

2.10. Antiglycation potential

The antiglycation potential of the 3.5% NFE-FY, control yogurt (CY), and the flower extract of *N. arbor-tristis* was measured using the method described by Matsuura et al. (2002) with modification. The concentration series (15.625, 31.25, 62.5, 125, 250, 500, and 1000 μg/mL) of 3.5% NFE-FY, control yogurt (CY), and the flower extract of *N. arbor-tristis* were prepared by dissolving with phosphate buffer saline (pH 7.4). Bovine serum albumin (80 μL), sample (80 μL), 400 mmol/L glucose (360 μL), and 50 mmol/L phosphate buffer saline (PBS-pH 7.4) were incubated at 60 °C for 72 h. After incubation, the solution was cooled, and 200 μl of 50% Trichloroacetic acid was added and centrifuged at 15,000 rpm at 4 °C for 4 min. The resulting precipitate was dissolved in PBS (pH 10), and the fluorescence intensity was measured at an excitation wavelength of 370 nm and an emission wavelength of 440 nm (Spectra Max Gemini EM). A sample control was conducted by replacing freeze-dried samples with 50 mmol/L PBS. Negative control was carried out simultaneously with BSA, PBS, and freeze-dried sample (3.5% NFE-FY, CY, and the extract of *N. arbor-tristis*) incubated under the same conditions. Rutin was used as the reference standard, and antiglycation potential (% inhibition) was calculated.

2.11. Total phenolic content and gas chromatography-mass spectroscopy (GC-MS) analysis

The phenolic content of 3.5% NFE-FY, control yogurt (CY), and the flower extract of *N. arbor-tristis* was performed using the Folin–Ciocalteu method (n = 3) according to the method described by Chang, Yang, Ucen, and Chern (2002). 3.5% NFE-FY, control yogurt (CY), and the flower extract of *N. arbor-tristis* were diluted with distilled water to prepare the concentration of 1 mg/mL of the above samples. The sample (20 μL), diluted Folin–Ciocalteu reagent (110 μl), and 10% sodium carbonate solution (70 μL) were mixed and incubated at room temperature for 30 min. The absorbance was measured at 760 nm in a UV visible spectrophotometer against a blank solution. Gallic acid was used as the standard, and the total phenolic content was expressed in milligram equivalents of gallic acid. (GAE) per gram of extract.

The GC-MS analysis was performed for the 90% methanol extract of the *N. arbor-tristis* flower extract using a 5975C gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and an HP-5MS capillary column (30 m × 25 μm with a film thickness of 0.25 μm). The sample (1 L) was injected into the HP-5MS capillary column and exposed to a temperature of 70 °C for 2 min. Then, the temperature was increased from 70 °C to 200 °C at a rate of 3 °C/min^{-1} and held at 200 °C for 15 min. Helium was used as the carrier gas with a flow rate of 1 mL/min. After obtaining the chromatogram, the mass spectrum of the unknown component was identified using the NIST (NIST 17) library.

2.12. Statistical analysis

The sensory evaluation was analyzed using the Friedman test. All other experiments were analyzed using a one-way analysis of variance

(ANOVA) and Tukey's multiple range tests to determine significance among the mean values. The SPSS Statistics 23.0 software package was used for data analysis (SPSS Inc., Chicago, IL, USA). All data from three replicates are presented as the mean ± standard deviation (SD). However, for sensory evaluation, 20 replicates were used. The proximate analysis parameters and the texture parameters were evaluated by principal component analysis (PCA) by Minitab software version 202. The level of statistical significance was set at $P < 0.05$. IC_{50} values were calculated using AAT Bioquest.

3. Results and discussion

3.1. Sensory attributes

Sensory analysis is very important for assessing the consumer acceptability of yogurt fortified with *Nyctanthes arbor-tristis* flower extract. The sensory study also confirms that additives have no adverse impacts on the organoleptic parameters of the final yogurt product. The sensory properties, such as appearance, texture, aroma, taste, after taste, and overall acceptability of yogurt samples made using flower extracts ranging between 3%, 3.5%, and 6% v/v, were analyzed by employing 20 trained sensory panelists. The sensory analysis data indicating consumer preference for each sensory attribute are illustrated in Table 1 and Fig. 1.

According to the results, the yogurt sample fortified with 3.5% *Nyctanthes arbor-tristis* flower extract (v/v) had the highest mean rank in all the sensory attributes except for texture. The appearance ranking of the FY increased in the order of 6% < 3% < 3.5% with a significant p-value of 0.001. A higher amount of *N. arbor-tristis* extract in 6% NFE-FY gave an unappealing color to the yogurt, thus ranking the lowest. The distributions of mean ranking of all the yogurt samples were more or less indicating that the addition of NFE had no significant effect on odor attribute.

Mean ranks for taste and after taste, and overall acceptability decreased in the order of 3.5% > 0% > 3% > 6%. The CY ranked lowest among the yogurt samples for overall acceptability, while the 3.5% NFE-FY ranked the highest. Thus, the addition of NFE had a positive impact on cumulative response compared to the control.

At a 6% extract concentration, the flower extract caused a bitter taste and an undesirable color in the yogurt. This evaluation was carried out to determine the optimum concentration of *N.s arbor-tristis* flower extract added to yogurt. When preparing *Nyctanthes arbor-tristis* flower-fortified yogurt on the laboratory scale, syneresis occurs due to the high moisture content from the high amount of *N. arbor-tristis* flower extract. It significantly affects the taste, color, and texture of the 6% NFE-FY. For the first time, this study reports the acceptability and quality of yogurt fortified with *Nyctanthes* flower extract, thus supporting the regular consumption of NFE-FY. According to the results, the sensory qualities improved with the addition of 3.5% NFY, and the results are in agreement with the results obtained by (Mohamed Ahmed et al., 2021) with Argel leaf extract and with pomegranate juice (Pan, Liu, Luo, & Luo, 2019) fortified yogurts.

According to Fig. 1 the overall acceptance of the samples showed that 3.5% NFY. had a more expressive mean rank for acceptance, being placed in the category between "liked = 4.0" and "neither liked nor disliked = 3.0", and differing from samples CY, 3% NYY, and 6% NYF, which received mean ranks of 1.70, 2.68 and 1.88, respectively. 3.5% NYY had the highest mean rank in all the sensory attributes except for texture.

The sample CY received the worse average of overall acceptability, a mean rank of 1.70, thus being classified in the category between "disliked = 2.0" and "extremely disliked = 1.0". Therefore, the addition of NFE had a positive impact on cumulative response compared to the control. The 6% NYY, which was awarded the lowest mean rank values for appearance, texture, taste, and after taste (Fig. 1 and Table 1), indicated the bitter and most undesired yogurt, showing the least appealing color and the sample with the lowest taste.

The sample 3.5% NYF showed the most distinct sensory profile, notably compared to other samples. The 3.5% NYF was mainly described using appealing appearance, color, pleasant aromas, better taste, and after taste with the highest overall acceptability. Thus it can be inferred that the appearance, aroma, and flavor influenced the acceptance of the 3% NYF. To obtain a better product from the sensory point of view, one should use yogurt samples fortified with 3.5% *Nyctanthes arbor-tristis* flower extract (v/v).

3.2. Texture profile and proximate analysis

It is known that the texture properties of fermented dairy products are determined by their structural arrangement and protein microstructure network. (Mudgil et al., 2017). Thus, the texture profile can prove whether the yogurts can meet better stiffness and firmness of (emulsion) gel network with sufficient dispersion of functional compounds. The texture profiles (hardness, chewiness, gumminess, and springiness) of NFE-FY and the control are given below in Table 2. The texture properties of yogurt are significant indicators of yogurt quality (Lunardello, Yamashita, De ToledoBenassi, DeRensis, & Vasconellos, 2012). The texture results can be explained by different yogurt types, properties, and different concentrations of added ingredients.

Hardness is the most significant characteristic in determining yogurt firmness, and it is the force required to attain a given distortion (Mudgil et al., 2017). According to the results, the hardness values of the control and NFE-FY samples were 193.33 ± 1.52 and 176.33 ± 1.52, respectively. The softness of the NFE-FY sample increased by 8% with the addition of the flower extract. Similar results were obtained by Azari-Anpar, Payeinmahali, DaraeiGarmakhany, and SadeghiMahounak (2017) with Aloe Vera gel-incorporated yogurt. They observed that salicylic acid and antimicrobial agents in Aloe Vera gel fortified yogurt reduced initial culture bacterial growth. Also, the reduction in firmness may be due to the weakness of the casein protein network in the NFE-FY sample due to interaction between the phenolic compounds and casein protein (Mudgil et al., 2017).

The gumminess increased slightly when *N. arbor-tristis* flower extract was added to the yogurt. Gumminess is the force essential for breaking semi-hard food into smaller fragments (Dar & Light, 2014). It is typical for semisolid foods to exhibit a low degree of hardness and a high degree of cohesiveness. It is also defined as the energy required to break down semisolid food. According to the results, the gumminess increased slightly by 1% in yogurt fortified with the flower extract, which was not significant. A similar slight increase in gumminess was observed by Domagała, Sady, Grega, and Bonczar (2006) with Amaranthus

Table 1
Sensory evaluation of yogurts fortified with 3%, 3.5%, and 6% *Nyctanthes arbor-tristis* flower extract.

Extract concentration	Appearance	Texture	Aroma	Taste	After taste	Overall acceptability
0% (control)	2.10	2.82	2.35	2.28	2.62	1.70
3%	2.80	3.30	2.02	2.45	2.02	2.68
3.5%	3.58	2.42	2.92	3.75	3.60	3.75
6%	1.52	1.45	2.70	1.52	1.75	1.88
p value	.0001	.0001	.074	.0001	.0001	.0001

The results are expressed as the mean rank according to the Friedman test. The significance level was set to $p < 0.05$.

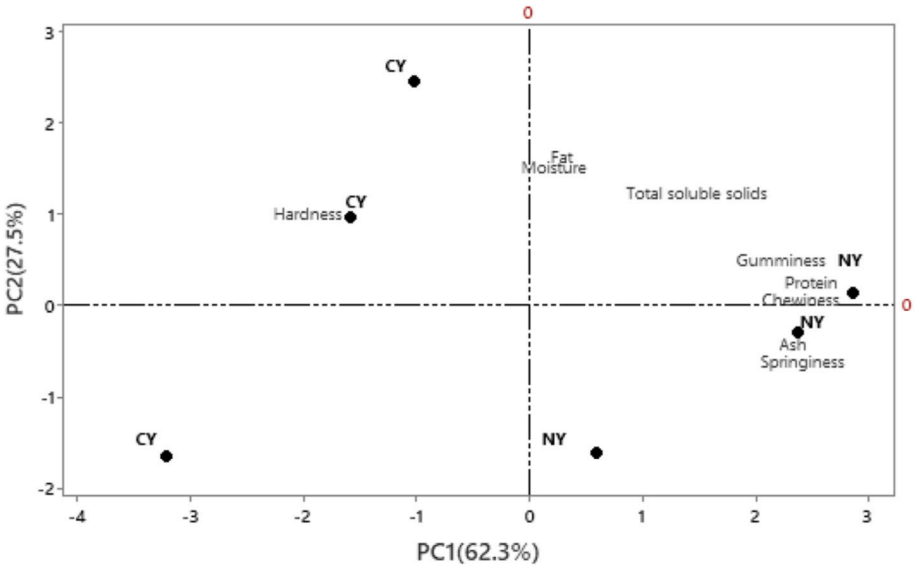

Fig. 1. The principal component analysis of textural profile parameters and the proximate analysis parameters obtained from control yogurt samples and yogurt samples fortified with 3.5% *Nyctanthes arbor-tristis* flower extract (v/v). **Captions: CY** = control yogurt samples. **NY** = 3.5% NFE-FY (yogurt samples fortified with 3.5% *Nyctanthes arbor-tristis* flower extract (v/v)).

Table 2
Texture profile and proximate analysis of yogurt-fortified with *Nyctanthes arbor-tristis* flower extract (3.5% NFE-FY) and the control.

Parameter	Control	3.5% NFE-FY
Texture Profile		
Hardness (g)	193.33 ± 1.52	176.33 ± 1.52
Chewiness (Mj)	87.66 ± 0.91	90.17 ± 1.11
Gumminess (g)	155.74 ± 0.86	157.20 ± 0.67
Springiness (mm)	37.86 ± 1.02*	55.1 ± 1.67*
Proximate Analysis (%)		
Fat	3.0 ± 0.20	3.0 ± 0.10
Protein	3.5 ± 0.12	3.88 ± 0.231
Ash	0.63 ± 0.015	0.764 ± 0.027
Moisture	78.46 ± 2.0	77.94 ± 0.078
Total soluble solids	14.87 ± 0.23	14.97 ± 0.27

The results are expressed as the mean ± SD; n = 3. *: $p < 0.05$ high springiness exerted by *Nyctanthes arbor-tristis* flower extract (3.5% NFE-FY) compared to the control.

seed-fortified yogurt. The authors also found a correlation between the fermentation rate and gumminess.

Chewiness can be defined as the time required to masticate a food sample to enhance consumption. Chewiness is also related to firmness, cohesiveness, and elasticity (Mudgil et al., 2017). The chewiness of NFE-FY was slightly increased by 2% compared to the control yogurt. The chewiness is directly related to gumminess (Azari-Anpar et al., 2017), slightly increased in the present study. The slight increase in chewiness was obtained by Mousavi, Heshmati, DaraeiGarmakhany, Vahidinia, and Taheri (2019) with flaxseed fortified yogurt. The increase in chewiness could be due to the gooeyness effect of *Nyctanthes* flowers, which further enhanced the structure of the yogurt sample (Mousavi et al., 2019).

Springiness is the degree to which a distorted material returns to its original condition once the force is removed. Springiness was increased significantly ($p < 0.05$) by 45.5% when *N. arbor-tristis* flower extract was added to the yogurt. Similar results were obtained with the addition of flaxseed (Mousavi et al., 2019). Springiness depends on heat, the protein interface, flexibility, and the degree of the unfolding of the protein (Mudgil et al., 2017). The increased springiness could be attributed to the increased texture integrity observed with the NFE-FY samples.

Pearson's correlation coefficients (Table S1) were used to understand the use of *N. arbor-tristis* flower extract in yogurt between proximate analysis and texture parameters. A negative correlation was observed between the chewiness and hardness parameters ($r = -0.751$); the higher the yogurt hardness value, the lower the time required to masticate the yogurt sample to enhance consumption. A positive correlation was observed between chewiness and gumminess ($r = 0.886$). The chewiness is directly related to gumminess (Azari-Anpar et al., 2017). The higher the yogurt chewiness value (the time required to masticate a food sample to enhance consumption), the higher the force essential for breaking semi-hard food into smaller fragments.

Proximate analysis was conducted for the best accepted *Nyctanthes* fortified (NF) and control yogurts. The results are shown in Table 2. According to the standards set by Sri Lanka Standard Institute SLS 824 part 2 (SLSI, 2016), yogurts' acceptable milk fat levels should be equal to or less than 3.0. And the yogurts prepared in the present study fell within the acceptable levels. The fat, protein, ash, moisture, and total soluble solid contents were more or less equal in both *Nyctanthes*-fortified and control yogurt samples.

The proximate analysis and texture parameters obtained were evaluated by principal component analysis (PCA) to eliminate multicollinearity between features. The PCA was carried out on 2 principal components that were chosen by the common rules. Fig. 2 represents the plot of PC1 versus PC2. This analysis explained 89.8% of the data variance. Protein (0.408), Ash (0.391), Chewiness (0.412), Gumminess (0.398) and Springiness (0.396) were positively correlated with PC1, while Fat (0.554), Moisture (0.544), Total soluble solids (0.432), Hardness (0.335) were positively correlated with PC2. According to Fig. 2, the addition of 3.5% *N. arbor-tristis* flower extract to the yogurt contributed to higher protein content, ash content, chewiness, gumminess, and springiness than the CY, which is elucidated on the right side of the chart. The hardness of 3.5% is illustrated on the right side of the chart, indicating that the 3.5% NFY sample exhibited lower hardness than CY.

The nutrient composition of yogurt is based on the nutrient composition of the milk from which it is derived. The other variables that play a crucial role during milk processing are the temperature, duration of heat exposure, exposure to light, and storage conditions. In addition, the changes in milk constituents that occur during lactic acid fermentation influence the nutritional and physiological value of the finished yogurt product. The final nutritional composition of yogurt is also affected by the species and strains of bacteria used in the fermentation, the source

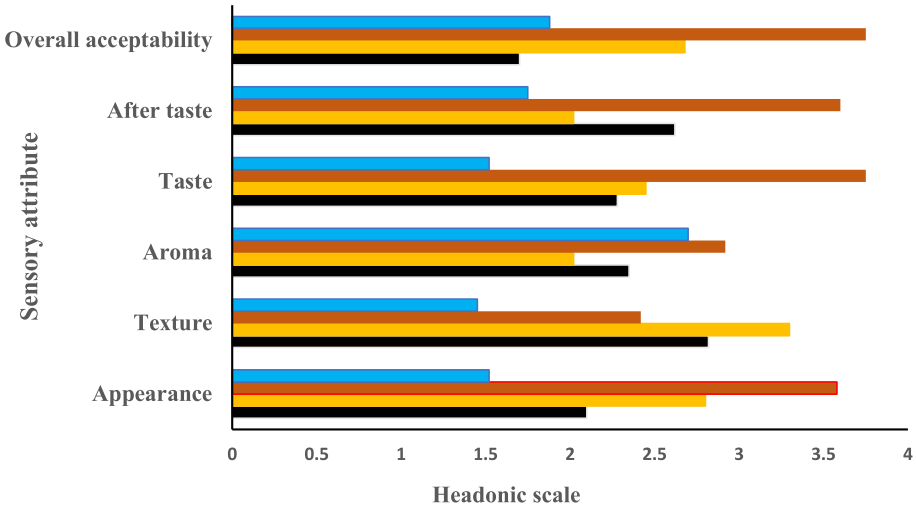

Fig. 2. Results of the Sensory evaluation of control yogurt and yogurts fortified with 3%, 3.5%, and 6% *Nyctanthes arbor-tristis*. A 5-point hedonic scale (liked very much: 5, liked: 4, neither liked nor disliked: 3, disliked: 2, and extremely disliked: 1) was adopted for sensory evaluation. **Captions:** ■ **CY:** control yogurt samples; ■**NY1**: yogurt samples fortified with 3% *N. arbortristis* flower extract (v/v); ■**NY2**: yogurt samples fortified with 3.5% *N. arbor-tristis* flower extract (v/v); ■**NY3**: yogurt samples fortified with 6% *N. arbor-tristis* flower extract (v/v).

and type of milk solids that may be added before fermentation, and the temperature and duration of the fermentation process (Aguirre-Ezkauriatza et al., 2008).

3.3. Physicochemical properties

The pH value changed significantly during storage for 21 days at 4 °C ± 1, and the pH values are given in Table 3. The pH range during the storage period was within the acceptable range. As expected, a post-acidification effect was observed in yogurts, resulting in low pH values during storage. The pH variation in both samples showed a similar pattern, indicating that the *Nyctanthes* flower extract did not affect yogurt pH during storage. The observed slight reduction in pH with time may be due to the rapid fermentation rate and action of lactic acid bacteria on lactose, producing lactic acid using lactose (Bakirci & Kavaz, 2008). Changes in pH during shelf life depend on culture type and storage temperature. When the temperature is higher than 4 °C, fermenting microbes increase and result in a low pH value. Nevertheless, if yogurts are stored at a low temperature just after incubation, this leads to a high pH due to the lower action of fermenting microbes (Staffolo, Bertola, Martino, & Bevilacqua, 2004).

Although the addition of the *Nyctanthes* flower extract did not affect the acidity of the yogurt compared to the control, storage for 21 days at 4 °C ± 1 significantly decreased the acidity in both samples. The acidity of the control yogurt and NFE-FY varied between 0.65-0.95 and 0.64–0.95, respectively. Enhancement of yogurt acidity is mainly due to the conversion of lactose to lactic acid by bacterial culture (Bakirci & Kavaz, 2008). The results are consistent with the study conducted by García-Pérez et al. (2005) with yogurt fortified with apple and bamboo. According to the Sri Lankan Standard specifications, the acidity of yogurt should be in the range of 0.8%–1.25% (SLSI, 2016), and the results obtained in this study fall within the standard range. Further increase in acidity and decreased pH in NFE-FY could be due to enhancement of metabolism due to the presence of phenolic, flavonoids compounds, and organic acids (Zhang et al., 2019).

Table 3
Effect of storage on pH values and acidity of yogurts with (3.5% NFE-FY) and without (CY) *Nyctanthes arbor-tristis* flower extract.

Days	pH		Acidity (% Lactic acid)	
	CY	NFE-FY	CY	NFE-FY
1	4.92 ± 1.0	4.95 ± 1.4	0.65 ± 0.4	0.64 ± 0.2
3	4.88 ± 1.3	4.89 ± 1.0	0.71 ± 0.2	0.7 ± 0.6
6	4.71 ± 1.4	4.72 ± 1.3	0.76 ± 0.3	0.75 ± 0.3
9	4.66 ± 1.3	4.66 ± 1.0	0.82 ± 0.6	0.83 ± 06
12	4.43 ± 1.6	4.42 ± 1.3	0.90 ± 0.6	0.92 ± 0.7
15	4.39 ± 1.3	4.4 ± 1.5	0.95 ± 0.4	0.95 ± 0.5
18	3.99 ± 1.0	3.98 ± 1.1	1.00 ± 0.4	0.99 ± 0.8
21	3.58 ± 1.0	3.56 ± 1.0	1.24 ± 0.9	1.25 ± 0.8

The results are expressed as the mean ± SD; n = 3.

The coliform count was determined to monitor the microbiological standards of the NFE-FY and the control. The findings are essential to evaluate the quality of yogurts and their suitability for human consumption, and they depend on hygiene, processing, and postprocessing contamination (Mohamed Ahmed et al., 2021). Based on the standard stipulated by the Matinal Agency of Food and Drug Administration Control, coliform in any 100 mL yogurt sample should be negative (Mbaeyi-Nwaoha & Egbuche, 2012). According to the results, the coliform counts in both samples were negative up to 28 days of storage, attributed to hygienic preparations, handling, and preparation.

Yeast and mold contamination is a leading cause of spoilage in yogurt and other cultured dairy products. Mold and yeast contamination is responsible for deterioration and changes in biochemical characteristics and flavors, leading to product downgrades by producing an adverse appearance. The results obtained for yeast contamination are displayed in Fig. 3.

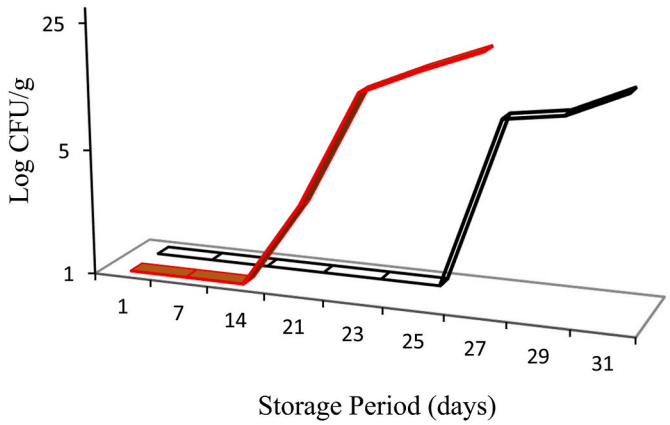

Fig. 3. The yeast and mold load of 3.5% *Nyctanthes abor-tristis* flower extract fortified (NFE-FY) control (CY) yogurts. . CY NEF-FY: Abbreviations of the yogurt samples. Results are expressed as mean + SD.

According to the results, the yeast and mold counts of control yogurt increased after the 14th day of storage and increased exponentially between days 20 and 38 of shelf life. In contrast, the mold count in the NFE-FY sample became evident after day 25 of shelf life. Spoilage is evident when yeast populations reach 5 to 6 (log CFU/ml), which can be seen as a swelling of the yogurt package due to gas production by yeast fermentation. Spoilage is accompanied by a fermented odor, flavor, and a gassy appearance, which eventually ruptures due to the accumulation of yeast colonies (Warde-Farley et al., 2010). In the control sample, spoilage was evident by 22 days, whereas in the NFE-FY sample, yeast fermentation started to appear only after 28 days. Yeast and mold grow well in an acidic environment, and they convert acid into nonacidic products. There was no yeast or mold due to hygienic preparation and a low acid environment. However, the acidity increased during storage, which favors the multiplication of yeasts and molds. Various microbes succeed one another as the chemical environment of the yogurt changes. The stages of microbial growth are *Streptococcus*, followed by *Lactobacillus,* yeasts, molds, and finally *Bacillus*. *Streptococci* convert lactose into lactic acid. The acidity of milk increases to the point where further *Streptococci* growth is inhibited by the acidic condition in the environment itself. After that, *Lactobacillus* begins to grow and convert the remaining lactose into lactic acid. This lactic acid gives a sour taste to yogurt (Bintsis, 2018). According to published data, *N. arbor-tristis* flowers possess high antioxidants (Heendeniya et al., 2020) and antimicrobial (Haque, Sultana, Abedin, Hossain, & Kabir, 2020) activities, which may be responsible for the longer shelf life observed. The GC-MS analysis also indicated the presence of compounds with antimicrobial and antioxidant compounds (Table 5).

Syneresis or whey separation is the major visible defect that occurs during set yogurt storage. This defect can affect the final product acceptance due to unfavorable expression and limit shelf life (Dimitrellou et al., 2019). Syneresis occurs due to the loss of yogurt gel capacity to entrap the serum phase by weakening the gel network, resulting in the whey separation of the yogurt. Total solids and protein content and milk type could affect the syneresis of yogurt. According to Table 4, the control and 3.5% flower extract-fortified yogurt's whey separation was not significantly different. Recorded studies showed a positive correlation between increased syneresis of yogurt and the addition of fruit juices accompanied by reduced viscosity (Bierzuńska, Cais-Sokolińska, & Yiğit, 2019).

In contrast, in the present study, the syneresis of NFE-FY was approximately 1% lower than that of control yogurt. Therefore, it is clear that the aqueous flower extracts dispersed in the whole system without imposing a negative impact on the emulsion stability. The difference in syneresis may be due to the addition of *N. arbor-tristis* hot infusion, which increased water content and viscosity.

3.4. Total phenols, GC- MS analysis, antioxidant, antiglycation

The total phenolic content obtained increased in the order of CY < 3.5% NFE-FY < NFE, with respective contents of 46 ± 0,03, 138 ± 0.09, and 151 ± 0.09, respectively (Table 4).

The antioxidant activity of the *Nyctanthes* water extract, CY, and NFE-FY was measured by DPPH ● radical scavenging activity, and the results are shown in Fig. 4. According to the results for NFE, the best accepted *Nyctanthes*-fortified (3.5% NFE-FY) and control yogurts (CY) exhibited IC_{50} values of 2.33 ± 0.09, 0.54 ± 0.02, and 13.58 ± 1.68 mg/mL, respectively. The free radical scavenging ability of NFE-FY was approximately 8% and 24% higher than that of the crude NFE and the control yogurt. Results of person correlation shows (Table 5) that there is a strong correlation between 3.5% NFE-FY and total polyphenolic content compared to CY and NFE. Hence, the high total polyphenolic content in the *N. arbor-tristis* can be attributed to the crude extract's observed higher scavenging potential. According to the results, the scavenging activity was increased in a dose-dependent manner. The ability to quench free radicals is also high in fermented dairy products are due to the presence of amino acids with sulfur, vitamins A and E, carotenoids, selenium, superoxide dismutase, glutathione peroxidases, milk oligosaccharides, and peptides (Khan et al., 2019). The recorded literature showed that the scavenging activity of the *N. arbor-tristis* flower extracts was in the order of stalk > flower > petals, thus indicating that the main antioxidant activity is in the orange-colored stem of the flower (Thakur & Jaiswal, 2017). The GC-MS analysis demonstrated the presence of several compounds with the ability to quench free radicals (Table 5) through the donation of electrons and hydrogen atoms, chelate transition metals, or inhibit lipid peroxidation. Hence, the observed high antioxidant capacity in NFE-FY can be due to the cumulative effect of the free radical scavenging ability of both *N. arbor-tristis* flowers and compounds present in the fermented milk.

The significant peaks obtained in the gas chromatogram (Fig. S1) indicated 17 main compounds in the extract (Table 6). Of these compounds, all had reported biological activity. Ten combinations had antioxidant activity, while four compounds had recorded antiglycation and anti-diabetes activities.

Table 4
Total phenolic content and whey separation of yogurt fortified with 3.5% *Nyctanthes* flower extract (NFE-FY) and the control sample.

Extract/Yogurt type	Total phenolic content mg GAE/g	Whey separation (mL/100 g)
Nyctanthes flower extract	151 ± 0.09**	NA
3.5% NFE-FY	138 ± 0.16*	9.82 ± 1.05
Control yogurt	46 ± 0.03	9.92 ± 1.02

The results are expressed as the mean of three ± standard deviations. NA: Not applicable. *: $p < 0.05$; **$p < 0.01$ compared to the control yogurt.

Table 5
Pearson correlation of *Nyctanthes abor-tristis*, 3.5% *Nyctanthes* fortified yogurt (3.5%- NFY) and control yogurt (CY) between antioxidant, antiglycation with total phenolic compounds (TPC).

Sample	Pearson Correlation		P value
	TPC & antioxidant activity	TPC & antiglycation activity	
N. abor-tristis flower extract	0.2	0.5	p < 0.01
3.5% NFE-FY	−0.90	0.95	p < 0.01
CY	−0.38	−0.48	p < 0.01

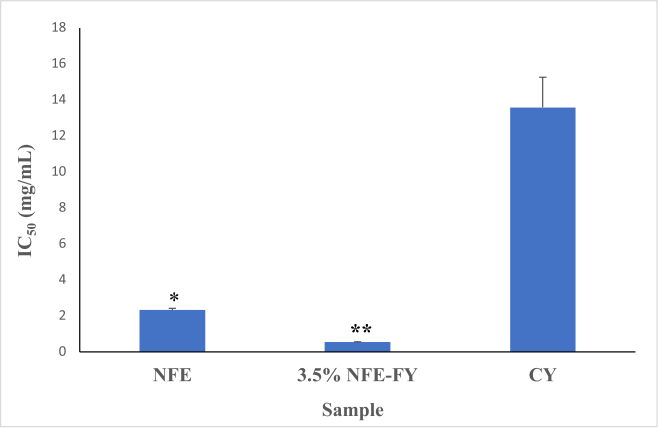

Fig. 4. IC50 values of antioxidant activities exhibited by NFE (*Nyctanthes abor-tristis* flower extract), 3.5% NFE-FY (3.5% *Nyctanthes* flower extract fortified yogurt), and CY (control yogurt). Data are expressed as mean + standard error. *<0.01; ***P < 0.001 as compared with the control.

Table 6
Active compounds identified in *Nyctanthes arbor-tristis* flower extract by chromatography/mass spectrometry (GC/MS) analysis.

Retention Time	Compound	Molecular Formula	Class	Reported activity
8.467	adipic acid-ethyl propargyl ester	$C_{11}H_{16}O$	Fatty acid esters	Anti-glycation, Antidiabetes
10.480	2-tetradecene	$C_{14}H_{28}$	Hydrocarbon derivative	Antioxidant, Antidiabetes, Anti-obesity
11.36	2,5-cyclohexdine-1,4, dione, 2-6-bis (1,1-dimethylethyl)	$C_{14}H_{20}O_2$	Hydrocarbon derivative	Antioxidant
12.034	phenol,2,5-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	Phenolic compound	Anti-glycation, Antidiabetes Antioxidant, Anticancer
12.953	7-hexadecene	$C_{16}H_{32}$	Hydrocarbon derivatives	Antioxidant
13.166	diethyl Pthalate	$C_{12}H_{14}O_4$	Diester of phthalic acid	Anticancer Antimicrobial
13.345	2-chloropropionic acid, octadecyl ester	$C_{21}H_{41}ClO_2$	Esters	Antioxidant
15.172	1-Octadecene	$C_{18}H_{36}$	Hydrocarbon derivative	Antioxidant, Anticancer
15.017	3,5-di-tert-Butyl-4-hydroxybenza-ldehyde	$C_{15}H_{22}O_2$	Hydroxybenzaldehyde derivative	Antioxidant, Anticancer
16.760	3,5-bis(1,1-dimethylethyl)-4-4 hydroxy-methyl ester	$C_{18}H_{28}O_3$	Benzene-propanic acid	Antioxidant
17.182	1-eicosene	$C_{20}H_{40}$	Alkene	Antimicrobial
18.636	hentriacontane	C31H64	Alkane	Anticancer
19.075	1-heptacosanol	C27H56O	Fatty alcohol	Antimicrobial, Antioxidant, Anticancer
20.603	1-dococene	$C_{22}H_{44}$	Alkane	Antibacterial
20.721	hexanedioic acid, bis (2-ethylhexyl) ester	$C_{22}H_{42}O_4$	Methyl esters	Antioxidant, Anti-obesity, Antiglycation
20.754	pentafluoropropionic acid, hexadecyl ester	$C_{19}H_{33}F_5O_2$	Fatty acid esters	Antioxidant, Anti-glycation
20.760	hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	Fatty acid & their esters	Antioxidant

The antiglycation potential of *Nyctanthes*-fortified yogurt (NFE-FY) and control yogurt (CY) samples was measured by bovine serum albumin. The results are depicted in Fig. 5. According to the results, among the tested yogurt samples and the *Nyctanthes* hot fusion extract, the most potent antiglycation activity was observed in the hot infusion (IC_{50}: 28.04 ± 1.13 μg/mL), followed closely by the 3.5% NFE-FY (IC_{50}: 46.80 ± 0.92 μg/mL). The CY sample exhibited the weakest antiglycation potential (IC_{50}: 568.51 ± 2.47 μg/mL). The antiglycation inhibition displayed by NFE-FY was 11 times higher than that displayed by CY.

When the *Nyctanthes arbor-tristis* flower extract was added to the system with the same constituents (BSA and glucose), it significantly suppressed emission, inhibiting glycated and amadori product formation. The reduction in emission of the glycated product revealed the potential of the *Nyctanthes arbor-tristis* flower extract sample to inhibit the formation of advanced glycated end products (AGEs). It is suggested that the ability to inhibit the formation of AGEs is closely related to the antioxidant properties of the plant extracts to scavenge radicals formed during the Maillard reaction, which includes glycation. The present study is the first to report the inhibitory activity of NFE-FY on the formation of AGE products. The antiglycation activity of the hot infusion and NFE-FY may be due to phenolic and flavonoid compounds, which significantly correlate with anti-glycation activity (Grzegorczyk-Karolak et al., 2016). Glycation and AGE formation are associated with increased free radical production. Glycation is a significant source of reactive carbonyl and oxygen species generated by oxidative and nonoxidative pathways. Inhibitors of AGE products may act as inhibitors of dicarbonyl intermediates and as antioxidants or metal ion chelators. Therefore, compounds with antioxidant activity could also inhibit AGE formation (Gunathilaka et al., 2019). According to Table 5, a significant correlation was observed between the total polyphenolic compounds and the antiglycation activity of 3.5% NFY. Polyphenolic compounds are known to possess potent radical scavenging ability thus confirming that yogurt fortified with 3.5% NFE could inhibit AGE formation. Rangika et al. (2015) also found that NFE exerts its antidiabetic activity by inhibiting α-amylase, inhibiting glucose diffusion by adsorbing and trapping glucose into the fiber matrix, and increasing glucose transport across the cell membranes. According to the GC-MS analysis of the *Nyctanthes arbor-tristis* flower extract, compounds such as adipic acid-ethyl propargyl ester; 2-tetradecene; phenol,2,5-bis(1,1-dimethylethyl); hexanedioic acid, bis (2-ethylhexyl) ester; and pentafluoropropionic acid, hexadecyl ester showed potent antiglycation, antidiabetic, and anti-obesity activity (Odjakova, Popova, Al, & Mironov, 2012), which led to observed antiglycation activity in the NFE-FY. As the popularity of yogurt continues to grow, scientists continuously investigate value-adding ingredients such as different kinds of plant extracts to produce functional yogurt with extra beneficial properties.

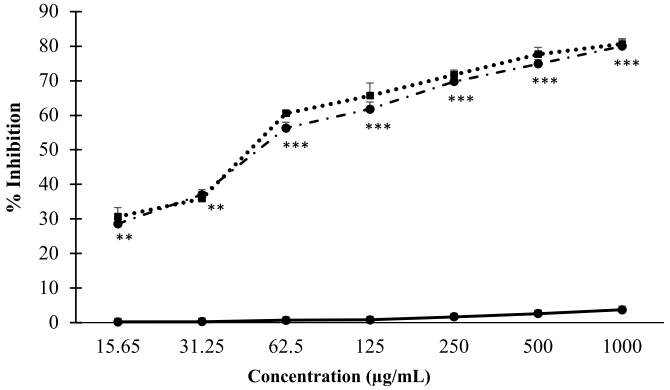

Fig. 5. Dose response relationship of *N. abor-tristis* flower extract, 3.5% *Nyctanthes* fortified yogurt (NFY), and control yogurt (CY) for antiglycation activity determined by glucose-induced protein glycation and formation of protein bound fluorescent advanced glycation end products. Data presented as mean of three samples + standard deviation. **<0.01; ***P < 0.001 as compared with the control. (Flower extract NFY CY).

4. Conclusion

Based on these results, the sensorial accepted sample of 3.5% NFE-FY possesses 11-, 6-, and 3-fold higher antiglycation activity, free radical scavenging ability, and total phenolic content, respectively, than the control yogurt. Furthermore, the addition of the extract (3.5%NFE-FY) had no significant effect on yogurt physicochemical characteristics, proximate composition, and texture profile with respect to control yogurt. Therefore, *Nyctanthes* extract-fortified yogurt can be considered a promising candidate for developing novel dairy products to increase functional characteristics and prevent diet-driven glycation activity. However, *in vivo* studies are warranted to confirm the potential health benefits of NFE-FYs.

CRediT authorship contribution statement

D.B.T. Amadarshanie: Methodology, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft. **T.L. Gunathilaka:** Investigation, Investigation, Data curation, Formal analysis, Writing – original draft. **Rajitha M. Silva:** Formal analysis, Writing –

review & editing. **S.B. Navaratne:** Resources, Conceptualization, Methodology, Supervision, Writing – review & editing. **L. Dinithi C. Peiris:** Conceptualization, Project administration, Supervision, Resources, Writing – review & editing.

Declaration of competing of interest

The authors declare that they have no known competing financial or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.112910.

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