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**Characterization of functional yogurt enriched with liquid sugarcane molasses:
physicochemical, sugar profile, color properties, and liquid chromatography-mass spectrometry
profiling of molasses phenolic acids**

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Abstract

Sugarcane molasses, a by-product of the sugar industry, is rich in carbohydrates, minerals, and nutritional fiber; it can be used as a useful component in dairy products. The purpose of the present work is to evaluate the effects of incorporating sugarcane molasses at 0%, 10%, and 20% on the chemical composition, sugar content, color properties, and antioxidant activity of yogurt samples. Increasing the concentration of sugarcane molasses resulted in higher content of crude ash, titratable acidity, yellowness index, and antioxidant activity, while significantly decreasing moisture, total fat, and crude protein contents. Depending on the level of incorporation, molasses, a source of sugars and bioactive compounds, had a substantial impact on the manufactured yogurts' proximate composition. Yogurt fortified with 20% molasses exhibited a higher yellow index and greater antioxidant activity than the control and other experimental groups. The incorporation of low-cost sugarcane molasses at appropriate concentrations can therefore improve the nutritional quality, enhance sensory properties, and consequently contribute to the preservation of yogurt.

Introduction

Yogurt's high nutritional content and well-recognized health benefits make it one of the most popular fermented dairy products worldwide. However, one of the main technological challenges is its texture, which can strongly influence consumer acceptance (Leal *et al.*, 2024). The symbiotic fermentation process responsible for yogurt production also contributes to its distinctive flavor. Yogurt consumption has increased markedly in recent years. Statista Market Insights (2024) estimates that the global yogurt market generated around US\$181.30 billion in revenue in 2024 and is expected to expand at a compound annual growth rate of 6.95%. Yogurt has numerous nutritional benefits. It can help alleviate gastrointestinal discomfort, eliminate lactose intolerance symptoms, support the immune system, protect against *Helicobacter pylori* infection and colon cancer, and increase metabolic rates, making it a vital component of a healthy diet (Gomaa *et al.*, 2024). In recent years, consumer dietary habits have evolved considerably, with increasing awareness of the harmful health effects of excessive sugar consumption. This concern drove the demand for low-sugar foods, which are perceived as more compatible with a balanced lifestyle. In this context, dairy products, particularly yogurts, have become a focus of innovation aimed at replacing refined white sugar with natural alternatives (Yang *et al.*, 2020). Among these alternatives, molasses stands out for its nutritional qualities and moderate sweetness, offering an attractive option.

Traditionally, molasses has been employed as a dietary supplement in place of refined sugar and as a functional ingredient in various food products, owing to its elevated content of nitrogenous compounds, mineral content, carbohydrates, and sweet flavor (Kassa *et al.*, 2024, Berrighi *et al.*, 2026). Historically, its high concentration of nitrogenous chemicals, carbohydrates, and sweet flavor, molasses has favored its use as a sugar substitute and as a common ingredient in many food preparations (Leal *et al.*, 2024). The incorporation of molasses in dairy products may therefore offer practical health benefits for consumers, particularly with regard to conditions such as diabetes, colon cancer, hyperlipidemia, and irritable bowel syndrome (Noureldin *et al.*, 2020). In addition, molasses enhances the color of yogurt, imparting a distinctive, desirable, and more natural appearance than that of artificially colored alternatives (Rashwan *et al.*, 2023). Moreover, the valorization of sugar industry by-products has emerged as a promising strategy to support the sustainable development of dairy products in the context of rapid expansion of global sugar industry production (Tarchi *et al.*, 2024). Consequently, greater attention should be given to the rational utilization of such resources for the development of value-added products. Zhu *et al.* (2023) have shown that employing natural resources to fortify yogurt could enhance

its functional and textural properties with minimal adverse effects. Yogurt is commonly enriched with a variety of natural and synthetic additives to improve its physicochemical, textural, sensory, and rheological characteristics. According to Bankole *et al.* (2023), these additives serve a number of technological uses, including as thickeners, colorants, flavoring agents, stabilizers, and gelling agents. In recent years, increasing interest has focused on the use of naturally occurring ingredients added to food and nutritious ingredients in yogurt flavors. To create innovative formulations for both adults and infants, flavored syrups or fruit concentrates are added to cultured milk either before or after incubation (Salehi, 2021). Molasses, also known as syrup, is a fruit juice concentrate made from a variety of fruits high in sugar and stands out as one of the most cost-effective sources of carbohydrates. Furthermore, it is a good source of phenolic compounds with health-promoting properties such as antimicrobial, antioxidant, and anticancer activities (Shalabi, 2022). Yogurt is regarded as a functional fermented dairy product because lactic acid bacteria increase lactose digestion, alter intestinal microbiota, and produce bioactive metabolites during fermentation (Marette and Picard-Deland, 2014). Significant levels of sucrose, glucose, fructose, minerals, and phenolic compounds with antioxidant and potentially health-promoting properties can be found in sugarcane molasses (Asikin *et al.*, 2016). Therefore, adding molasses to yogurt may offer complementary benefits by combining the probiotic and nutritional qualities of fermented milk with the antioxidant and micronutrient contribution of molasses. Yogurt formulations frequently include technological additives. These include stabilizers (pectin, gelatine, starch, and gums), which increase viscosity and reduce syneresis; sweeteners (including sucrose, honey, and fruit syrups), which enhance taste; and natural functional ingredients such as aromatic plants, herbs, spices, fruit extracts, and plant-derived syrups that offer antioxidant, antimicrobial, flavoring, and nutritional benefits are frequently found in yogurt formulations (Tamime and Robinson, 2007). According to Al-Farsi *et al.* (2007), date syrup can provide natural sweetness, minerals, and phenolic compounds with antioxidant action. Furthermore, phenolic chemicals and essential oils found in aromatic herbs like mint, rosemary, and thyme may have antibacterial and antioxidant properties (Nieto *et al.*, 2018). Although sugarcane molasses may function as a natural ingredient by offering sweetness, minerals, and antioxidant phenolics, its primary functional role is related to nutritional enrichment and antioxidant capacity rather than texture stabilization or potent antimicrobial preservation (Asikin *et al.*, 2016). The aim of this study was to replace white sugar and chemical additives with sugarcane molasses in the development of a bioenergetic, healthy yogurt with higher consumer acceptability, and to evaluate the impact of this replacement on color, physicochemical, and antioxidant activity of the yogurt.

Materials and Methods

Molasses

During sugar production from sugarcane, cane sugar molasses is generated as a by-product. To extract the juice, sugarcane is first harvested, cleaned, and crushed (Preston, 1986). The juice is then clarified using heat and lime to eliminate contaminants before the sugar solution is concentrated by evaporation. In the concentrated syrup, centrifugation and crystallization are used to separate the sugar crystals from the remaining liquid (Santos *et al.*, 2020). Cane molasses is the dark, viscous syrup that remains after several cycles of crystallization. It is used in the food, fermentation, and animal feed sectors and contains sugars, minerals, and organic compounds (Honig, 1974). After the last stage of sugar extraction, the concentrated black syrup that remains is collected as final molasses and utilized to make yogurt. Sugarcane molasses (1.5 L) was obtained from the GROS-BERRAHAL Group Sugar Refinery "RAM Sucre" located in the Ouest of Algeria in Mostaganem (Latitude: 35.9125° N, Longitude: 0.0768° E). The by-product was collected from the storage tank after recirculation in opaque plastic drums and then transported to the laboratory, where it was kept in the dark at room temperature until use for yogurt

manufacturing and analysis. Molasses chemical analysis was carried out in the Department of Chemistry at Tokat Gaziosmanpasa University, Turkey.

Yogurt manufacturing process

A total of 3 L of milk was purchased as pasteurized bottled milk, "Soummam, Algeria", with 1 L assigned to each treatment. The whole milk containing 2.8% total fat was transported to the laboratory and used for yogurt preparation. It was heated at 85°C for 30 min and then cooled to 42°C. After heat treatment, 1 g/L of starter culture was added, and the milk was incubated for 6 hours at 42°C until coagulation occurred. The yogurt was then cooled to 10°C. To produce the fortified flavored samples, molasses was incorporated into the cooled yogurt at concentrations of 0%, 10%, and 20%. After gentle stirring for 3 min, the flavored yogurt was dispensed and stored under refrigeration at 4°C (Figure 1a). The obtained yogurt from each treatment was directly analyzed in triplicate after 6 days of refrigerated storage. This storage period was selected to evaluate early changes in yogurt quality during refrigerated storage after molasses fortification.

Yogurt and sugarcane molasses physicochemical analyses

The chemical composition of all samples and moisture and ash contents were assessed according to the AOAC (2006) method. Total fat and crude protein levels were measured using the Folch and Kjeldahl techniques. Dornic acidity was measured by adding a drop of an alcoholic solution containing 1% phenolphthalein in 1 mL of milk as a color indicator. Subsequently, the sample was titrated with 0.01 mL of sodium hydroxide N/9 (NaOH) until a steady color shift from white to pale pink was attained. One Dornic acidity (°D) is required for every 0.05 mL of NaOH to cause this shift. Yogurt is divided into three groups based on its levels of Dornic acidity: top-grade yogurt (<70°D); intermediate-quality yogurt, which has Dornic acidity between 70°D and 90°D; and yogurt unfit for human consumption (>90°D) (Tchakouteu *et al.*, 2024). A calibrated Mettler Toledo SevenEasy Digital pH Meter w/PSU 8603 Monitor (USA) was used to measure the pH of 10 mL of milk samples that had been thoroughly homogenized with 20 mL of distilled water. Density was measured using a hydrometer in accordance with the NF V 04–204 standard (AFNOR, 1980). The method consists of submerging a densimeter in a 100 mL test tube containing the milk sample. The value was recorded directly once the reading stabilized.

Determination of antioxidant activity

Spectrophotometry, which employs the decolorization of 2,2-diphenyl-1-picrylhydryl (DPPH) purple solution as a reagent, was employed to assess the samples' capacity to scavenge free radicals. A 0.5 g yogurt sample was included in 10 mL of 80% methanol solution at 25°C (Xu *et al.*, 2019). The extraction solvent selected was aqueous methanol, which is commonly utilized to extract antioxidant compounds from food matrices. Since the aim was to assess the overall antioxidant capacity of yogurt samples, no protein precipitation step was included. As a result, various soluble antioxidant compounds, such as phenolic compounds, organic acids, peptides, and other soluble components that may contribute to DPPH radical-scavenging activity, are likely present in the extracts. To reduce interference arising from the dark color and high concentration of soluble solids in sugarcane molasses samples, an appropriate dilution was carried out prior to extraction. The same 80% methanol extraction method was then applied to the diluted molasses sample, and the supernatant was used for the determination of antioxidant activity. The mixture was then left in the dark for 24 hours. A methanolic solution of DPPH was prepared by dissolving 3.94 mg of DPPH in 100 mL of methanol. Subsequently, ~0.2 mL of the resulting extract was combined with 1.5 mL of 0.1 mM methanolic DPPH solution. The mixture was vortexed and incubated in the dark at room temperature (23±1°C) for 60 min to facilitate complete interaction between

antioxidants and DPPH radicals. A Hitachi U-2900 spectrophotometer was used to measure the absorbance at 517 nm. The negative control was prepared by mixing 1.5 mL of DPPH solution with 0.2 mL of methanol. Eq. 1 was used to calculate the percentage inhibition of DPPH free radicals to assess the antioxidant activity (Xu *et al.*, 2019).

$$\text{Antioxydant activity (\%)} = \frac{\text{Abs DPPH} - \text{Abs sam}}{\text{Abs DPPH}} \times 100 \quad [\text{Eq. 1}]$$

where: Abs_{DPPH} is the absorbance of DPPH solution and Abs_{sam} is the absorbance of the sample.

Phenolic acids determination by LC-MS

Approximately 20 mg of the samples were weighed into 2 mL Eppendorf tubes. Each tube received one mL of methanol to obtain a homogenous solution. Lipophilic molecules were removed by liquid-liquid extraction with 1 mL of hexane. The mixture was then centrifuged at 9000 rpm for 15 minutes to achieve phase separation. After centrifugation, 10 µL of the methanolic phase was collected and diluted to a final volume of 1000 µL by adding 495 µL of ultrapure water and 495 µL of methanol to obtain a concentration appropriate for analysis. The resulting solution was passed through a 0.25 µm membrane filter to reduce particle-derived interference in the produced solution. Individual phenolic component in the sugarcane molasses extracts was analyzed using an ultra-high-performance liquid chromatography system coupled to tandem mass spectrometry (UHPLC–MS/MS). An Inertsil ODS-4 C18 analytical column (150 × 2.0 mm, 3 µm), kept at 35 °C, was employed for chromatographic separation. The mobile phase consisted of (A) 10 mM ammonium formate in water and (B) methanol, delivered as a binary gradient. The gradient program started at 5% B, increased linearly to 95% B over 8.0 minutes, and was held at this composition for 12.0 minutes. The system was then returned to its initial conditions (5% B) for column re-equilibration, resulting in a run time of 15.0-minute. Ten microliters were injected, and the flow rate was maintained at 0.40 mL per minute. Between injections, the needle was rinsed with 50% methanol (Stalmach *et al.*, 2009).

Sugar profile determination by quantitative NMR analysis

NMR experiments were performed at 298.1 ± 0.1 K on a Bruker Avance 400 spectrometer equipped with a 5 mm inverse broadband (BBI) probe, an autosampler, and an automated system for tuning and shimming. Data acquisition was carried out using TOPSPIN 3.0 software (Bruker BioSpin GmbH, Rheinstetten, Germany). The pulse sequence adopted to record the 1D spectra was a 1H NOESY with presaturation of water and the parameters are listed below: pulse program = noesygppr1d; size of fid (TD) = 128 K data points; spectral width (SW) = 20 ppm; transmitter offset = 4.70 ppm; dummy scans (ds) = 4; number of scans (ns) = 64; acquisition time = 8.12 s; mixing time (d8) = 0.01 s; recycle delay (d1) = 30 s (Navarro *et al.*, 2020).

Yogurt color measurements

The color characteristics were assessed using a colorimeter (Minolta CR 300 Series, Minolta Camera Co., Ltd., Osaka, Japan). Parameters for chromaticity and lightness (L^*) After the yogurt samples were put on a white standard plate, a^* (red–green) and b^* (yellow–blue) were measured ($L^* = 93.49$, $a^* = -0.25$, and $b^* = 0.1$). The following equation [Eq. 2] were used to determine the total color difference (ΔE) (Chouchouli *et al.*, 2013):

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad [\text{Eq. 2}]$$

Statistical analysis

To determine statistical significance, one-way analysis of variance was used. Statistica software (version 10) was employed for *post hoc* mean comparisons using Fisher's test. Every measurement was done three times. The mean $\pm$ standard deviation is used to express the values. Statistically significant differences were indicated by $p < 0.05$.

Results and Discussion

Proximate composition and phenolic profile of sugarcane molasses

The main compositional characteristics of sugarcane molasses are presented in Table 1. The moisture content, which can vary with evaporation intensity, storage conditions, and processing parameters, fell within the range typically reported for commercial sugarcane molasses (Shalabi, 2022). The comparatively high ash percentage suggests the presence of significant concentrations of mineral components during sugar production, as inorganic components become concentrated in the final molasses fraction following sucrose crystallization. Ash content is frequently associated with potassium, calcium, magnesium, and iron retained from sugarcane juice during processing; similar mineral-rich compositions have been reported for sugarcane molasses (Eggleston, 2018). Although variances may arise due to cultivar variability, soil composition, and industrial processing conditions, similar ash values have been observed for sugarcane molasses (Payet *et al.*, 2005). The lower crude protein and total fat contents can be attributed to the removal of most proteins and lipids during the clarification and crystallization stages of sugar production. However, residual nitrogen-containing compounds may contribute to the formation of antioxidant chemicals during heating reactions (Eggleston, 2018). Sugarcane molasses extract exhibited significant antioxidant capacity, with a DPPH radical scavenging activity of 68.23%. This activity is primarily attributed to phenolic compounds that can donate electrons or hydrogen atoms to neutralize free radicals. Owing to its high phenolic acid and flavonoid contents, sugarcane molasses has been shown in previous studies to possess strong antioxidant activity; however, variations among studies may occur due to differences in sugarcane variety, geographical origin, processing conditions, extraction method, and analytical techniques (Li *et al.*, 2013). LM-LS analysis revealed that sugarcane molasses contains a range of bioactive phenolic compounds. Apigenin-7-glucoside, trans-ferulic acid, quinic acid, and quercetin were the next most abundant compounds, whereas trans-sinapic acid, sinapic acid, diosmetin, and chlorogenic acid were present in lower amounts. Given that quercetin is known to be a strong antioxidant flavonoid owing to its hydroxyl groups and capacity to donate electrons, its high concentration of quercetin may have had a major role in the DPPH scavenging activity observed in this investigation (D'Archivio *et al.*, 2007). According to Hewawansa *et al.* (2024), ferulic acid, chlorogenic acid, and sinapic acid have also been identified as significant phenolic antioxidants in sugarcane-derived products that contribute to radical scavenging activity through their phenolic structures. The identification of flavonoids such as diosmetin and flavonoid glycosides such as apigenin-7-glucoside further enhances the functional potential of sugarcane molasses. These compounds have been associated with antioxidant, anti-inflammatory, and protective biological effects. Variations in sugarcane cultivar, molasses type (final versus intermediate molasses), processing temperature, and extraction conditions may all account for the differences in phenolic compound concentrations observed relative to previous research (Payet *et al.*, 2005). Overall, the findings show that sugarcane molasses is a valuable source of phenolic compounds with strong antioxidant activity. Its potential use as a functional ingredient in food formulations, such as yogurt, is supported by its strong DPPH radical scavenging capacity and the presence of a variety of phenolic acids and flavonoids. The functional properties of yogurt can be enhanced by the incorporation of liquid sugarcane molasses, owing to its content of antioxidants and phenolic compounds. Molasses-derived phenolic compounds may augment the antioxidant capacity of

the final product, a key attribute of functional foods. Furthermore, the naturally occurring sugars and minerals in molasses may improve the nutritional profile of yogurt and serve as an energy source.

Physicochemical characteristics of yogurt

Cane molasses is generally considered slightly acidic. The results in Table 2 show a decrease in pH values, which indicate that the incorporation of molasses at higher concentrations (Y_{20%}) tends to increase titratable acidity ($p < 0.001$), density and decrease pH after storage compared to a non-fortified yogurt (Y_{0%}). This can be attributed to the high mineral content of molasses and its buffering capacity, which partially neutralizes the bacteria in the yogurt while the fermentable sugars in molasses stimulates lactic acid bacteria. However, high ash content may result in a lower— or more stable pH— than in the control samples, depending on the fermentation conditions, although it generally contribute to increased titratable acidity. These findings are in agreement with those obtained by Kazemi *et al.* (2022), who suggested that molasses provides extra fermentable sugars (such as sucrose, glucose, and fructose) that stimulate lactic acid bacteria, thereby enhancing lactic production during storage. Increasing the rate of molasses incorporation in yogurt reduces its moisture content compared with yogurt made without sugarcane molasse (Y_{0%}) (Table 2), although highly significant differences were observed ($p < 0.001$). This may be due to the richness of molasses in total solids (TS), which are transferred into yogurt and consequently lower the moisture percentage. These results are consistent with those reported by Priyashantha *et al.* (2025). Total fat and crude protein contents of yogurt were found to decrease with increasing molasses concentration in most treatments (Table 2). These may be attributed to the nonfat, nonprotein contents in molasses. Similar results were reported by Nouredin *et al.* (2020), who observed a comparable trend in yogurt with molasses at a rate of 2%. The fat content of the yogurts gradually decreased in this study, which investigated the enrichment of yogurt-type dairy products with low-fat plant-based ingredients such as molasses. Specifically, fat content declined from 2.98% in the control group (Y_{0%}) to 2.78% and 2.57% in the yogurts enriched with 10% and 20% cane molasses, respectively. This reduction can be attributed to a dilution effect, given that the molasses used contained only 0.87% fat. Increasing the level of molasses incorporation partially replaces milk, which is inherently richer in fat, with an ingredient predominantly composed of carbohydrates and minerals, and containing very little fat. Consequently, the fat content in the final product decreases as the concentration of molasses increases. Yogurt's non-fat components are increased by molasses, which is low in lipids and high in sugars, phenolic compounds, minerals, and water. As a result, during lactic fermentation, the fat is diluted without lipid formation (Karaca *et al.*, 2012). Differences in the nutritional quality of fortified products depend on the chemical composition of the molasses incorporated, which consists of 75% carbohydrates, 22% water, 0% protein and very low-fat content (0.1%) (Kassa *et al.*, 2024, Berrighi *et al.*, 2026). The antioxidant activity of the yogurt samples is given in Table 2. The incorporation of 20% liquid sugarcane molasses into yogurt resulted in a statistically significant increase in antioxidant activity, by approximately threefold ($p < 0.001$). The highest antioxidant activity was observed in Y_{20%}, whereas the lowest was recorded in the control yogurt (Y_{0%}). The results in Table 1 show that molasses contains substantial levels of several phenolic acids, which greatly enhance the antioxidant activity of yogurt. Antioxidant activity increases proportionally with the level of molasses incorporation. On the other hand, yogurt formulated with white sugar exhibits lower antioxidant activity, as it lacks the advantageous phenolic compounds. These findings are in agreement with those found by Lee *et al.* (2018). The efficient transfer of bioactive compounds from molasses into the dairy matrix is associated with the enhanced antioxidant activity seen in yogurt enriched with molasses. This enhancement implies that, in comparison to conventional yogurt, the new yogurt may offer extra protection against oxidative stress. Its promise as a functional dairy product is supported by the changes in chemical composition, which show that adding

molasses not only affects technological properties but also enhances nutritional value (Berrighi *et al.*, 2026).

Sugar profile

Molasses, as a source of sugar and bioactive compounds with health-promoting properties, including antimicrobial, antioxidant, and anticancer activities (Shalabi, 2022) exerts a significant influence on the proximate composition of the formulated yogurt, depending on the rate of incorporation. The Nuclear Magnetic Resonance (NMR) spectroscopy conditions enabled the profiling of sugars (Figure 2) in both molasses and yogurt samples. Most sugars were eluted within 7 mins to 13 mins. The major peaks corresponded to sucrose, followed by glucose and fructose (Figure 2a). The values obtained in the present study are higher than those reported by Palmonari *et al.* (2020), who found that cane molasses exhibited wide variability, with minimum values of 2.3% and 1.3% of dry matter for fructose and glucose, respectively, and maximum values of 14.3 and 12.1% of dry matter. This could be related to the extraction process applied as well as to the origin of the molasses. Molasses, is rich in sucrose relative to other sugars, acting as a primary concentrated source of this disaccharide as well as minerals, and offering various benefits often overlooked it is regarded merely as a sweetener (Rashwan *et al.*, 2023). The results for sugar content show highly significant differences ($p < 0.001$) among all yogurt fortified with different levels of molasses, with $Y_{20\%}$ showing the highest values (Figure 2b). The sucrose content in yogurt fortified with molasses was higher than that of control yogurt ($Y_{0\%}$), particularly for $Y_{20\%}$ (Figure 2b), due to the high sugar concentration in molasses-based yogurt. Because sugarcane molasses naturally contains significant levels of sucrose, glucose, and fructose, increasing its level from 0% to 20% in the current investigation resulted in the anticipated increase in the concentrations of these sugars. According to earlier studies (Gahruiea *et al.*, 2015; Ranadheera *et al.*, 2017), carbohydrate-rich fortifying ingredients increase the sugar content of yogurt in proportion to their level of inclusion. In contrast, yogurt not fortified with molasses contains only lactose, the natural milk sugar and the basic product for lactic fermentation (Morais *et al.*, 2022). The research on yogurt fortification with natural carbohydrate-rich substances is in line with the reported variations in the sugar profile of yogurts fortified with sugarcane molasses. Due to their natural contents of sucrose, glucose, fructose, and other soluble carbohydrates, previous research has demonstrated that addition of fruit purees, fruit syrups, fruit extracts, plant-derived sweeteners, dietary fibers, and other plant-based ingredients can significantly alter the carbohydrate composition of yogurt (Ranadheera *et al.*, 2017). Depending on the type and quantity of fortifying ingredient, these sugars may also be metabolized by yogurt starter cultures during fermentation and subsequent storage, leading to variations in individual sugar concentrations (Shah, 2017). Yogurt possesses a strong ability to incorporate additional ingredients that enhance its nutritional profile; it can be supplemented with molasses or date syrup, which serve as primary sources of carbs, minerals, and other nutrients (Karaca, 2013). yogurts enhanced with date syrup, honey, grape products, berry preparations, banana, pumpkin, apple, and other fruit concentrates have shown similar patterns, with the natural sugar content of the added ingredients being the main cause of the increases in total sugars (Gahruiea *et al.*, 2015). Conversely, dietary fibers with low digestible carbohydrate content, including inulin and oat fiber, typically improve texture and water-holding capacity while having little influence on sugar composition (Dabija *et al.*, 2018). As a result, the effect of fortification on the sugar profile depends not only on the concentration of the added ingredient but also on its chemical composition and interaction with yogurt starter microorganisms (Shah, 2017). The sugar profile analysis confirmed that molasses contributed naturally occurring carbs to the yogurt system. Because glucose, fructose, and sucrose serve as readily available sources of metabolic energy, their presence may enhance the product's ability to provide energy. Thus, adding molasses may support the development of a bioenergetic yogurt with both functional and nutritional benefits.

Color measurement of yogurts

Consumer preference and appreciation are directly impacted by food color features. Table 3 displays the color values (L^* , a^* , and b^*) of the molasses enhanced yogurt. The result show that increasing molasses incorporation levels in yogurt gives higher yellowness compared with the control treatments ($p < 0.001$). These results show that addition of molasses altered the yogurt's color from white to reddish-brown and yellow. Similar color changes have been reported in dairy products fortified with plant-derived ingredients rich in natural pigments and colored phytochemicals have been shown to exhibit these changes, which is consistent with the inherent color of molasses. As the fortification level increased, redness and yellowness increased proportionately, whereas lightness decreased (Gahruiea *et al.*, 2015). According to El-Sayed *et al.* (2025), the color of yogurt may change significantly from white to yellow, or brown, depending on the type and concentration of molasses added. The lightness (L^*) of the yogurt decreased significantly with increasing concentrations of sugarcane molasses ($p < 0.001$), whereas their yellowness (b^*) and ΔE^* values increased ($p < 0.001$). The control yogurt made with white sugar exhibited the highest brightness, consistent with the typically bright white color of plain yogurt. In contrast, yogurts with 10% and 20% molasses, showed significantly lower L^* values, suggesting progressively darker goods. This reduction in lightness is expected, as sugarcane molasses naturally contains dark brown pigments, such as caramelized chemicals, melanoidins produced during sugar production, and phenolic molecules that absorb visible light (Asikin *et al.*, 2016, Berrighi *et al.*, 2026). Consequently, brown-colored yogurts were obtained (Figure 1a) which can be attributed to the elevated levels of bioactive substances in molasses, particularly melanoidins and phenolic compounds formed during sugar processing (Cabral *et al.*, 2022). The natural, dark brown pigments found in the molasses itself act as a natural colorant, reducing the yogurt's whiteness (lightness), and explaining the shift in color parameters compared with the control group. As molasses treatments increased, the total color difference (ΔE) increased as well, indicating that color alterations became more noticeable with larger molasses assimilation. ΔE values below 1 are regarded as imperceptible, values between 1 and 2 are only detectable by trained observers, values between 2 and 3.5 represent noticeable differences, and values above 5 indicate clearly visible color changes that consumers can easily perceive, according to widely accepted CIELAB color difference interpretation criteria (Mokrzycki and Tatol, 2011). As a result, the study's ΔE values show that, in comparison to the control sample, both molasses-containing yogurts had quite noticeable color changes. The gradual rise in ΔE shows that color intensity is dependent on concentration and represents the cumulative influence of the colored components found naturally in molasses (Pathare *et al.*, 2013). Overall, these findings show that sugarcane molasses enriches yogurt with bioactive compounds and functions as a natural coloring ingredient. However, the darker appearance at higher inclusion levels may affect consumer acceptance and should be taken into account when optimizing product formulation (Gahruiea *et al.*, 2015). The higher ΔE value of 20% molasses yogurt indicates that molasses has a concentration-dependent impact on color development, mainly due to the contribution pigments of molasses and brown colored compounds (Table 3). A higher concentration of molasses led to a larger decrease in brightness and an increase in chromatic intensity, resulting in a yogurt that was darker and browner. Similar effects have been documented in food and dairy products with dark-colored plant-derived ingredients, where pigment molecules have a significant impact on how consumers perceive appearance (Pathare *et al.*, 2013). The noticeable color variations seen at greater molasses levels may have a major impact on product acceptance and perceived distinctiveness because color is one of the first quality criteria customers evaluate (Francis and Clydesdale, 1975; Du *et al.*, 2021). Moreover, the addition of molasses either during or after pasteurization and fermentation can enhance the technological and organoleptic properties of yogurt, including color, microstructure, texture, and other sensory attributes, due to its richness in bioactive compounds (Ning *et al.*, 2021). Overall, the findings show that adding molasses at 10% and 20% levels significantly affect yogurt appearance, producing

color changes that are not only statically measurable but also visually relevant from consumer perception. The natural pigments and Maillard reaction products found in sugarcane molasses are responsible for the color change of yogurt once molasses is added. These substances may also be linked to antioxidant activity in addition to aiding in visual separation, which would reinforce the evolved yogurt's functional qualities. All things considered, the findings show that sugarcane molasses can be effectively utilized as a natural functional ingredient for the production of yogurt. Molasses added naturally occurring bioactive chemicals and carbs to yogurt, enhancing its antioxidant capacity and potentially improving its nutritional and energy-related qualities.

Conclusions

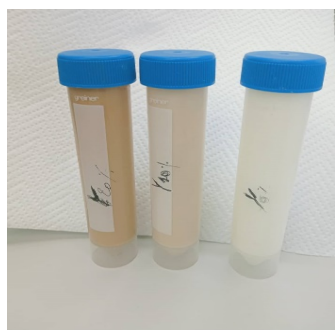
Molasses, a by-product of sugarcane, is rich in nutrients and antioxidants, making it ideal for enriching foods with energy and bioactive compounds. Its intense color, and sweetness make it an attractive ingredient in the production of dairy products, particularly yogurt, offering a highly palatable alternative to refined sugar. Moreover, it represents a promising innovation for the valorization of local products and by-products while enhancing nutritional quality. The incorporation of sugarcane molasses affects the physicochemical properties, improves the antioxidant activity, imparts a characteristic color, and may provide minerals, and may contribute to the nutritional value of the product. It thus constitutes a functional and original alternative to conventional sweeteners. Further studies on yogurt fortified with molasses should focus on optimizing consumer acceptance and monitoring microbiological growth, viscosity, sensory attributes, and polyphenol content after 30 days of refrigerated storage.

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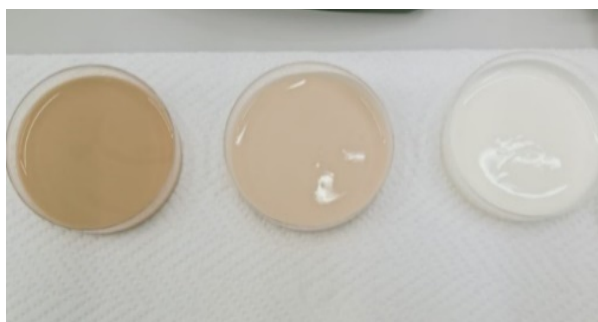
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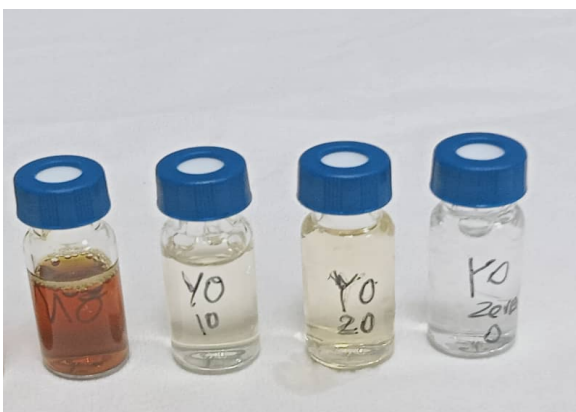
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(a)



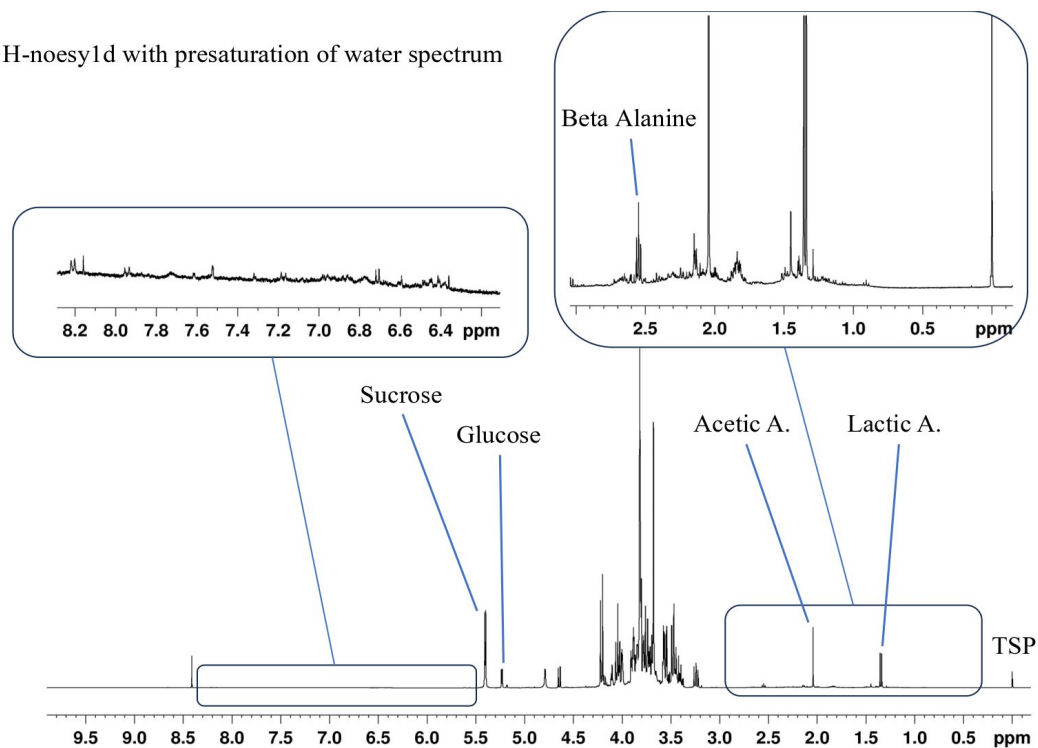
(b)



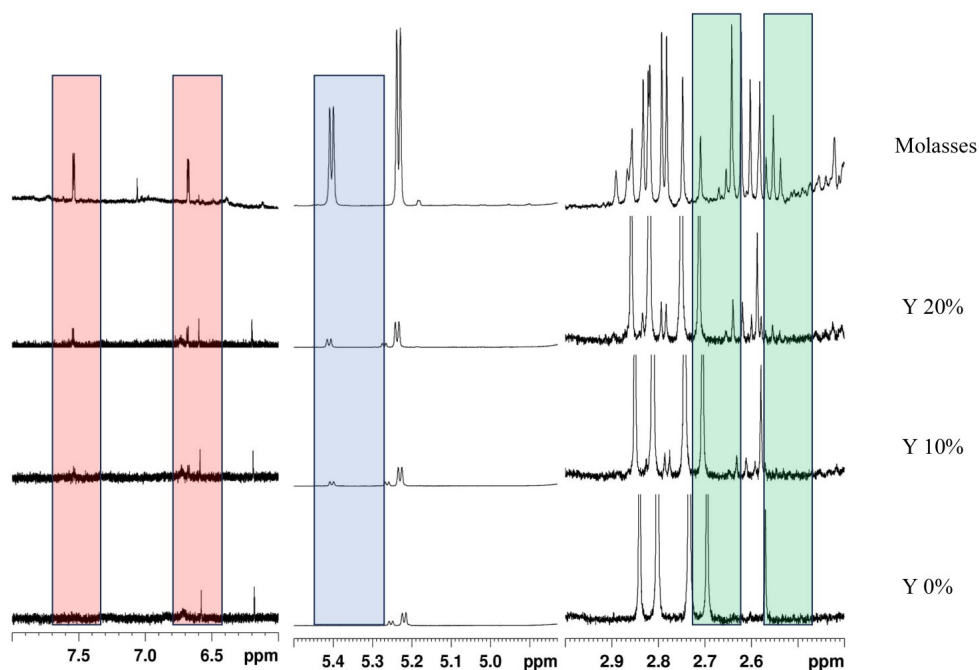
(c)

Figure 1. Yogurt samples containing different levels of sugarcane molasses (0%, 10%, and 20%) (a) prepared for color (b) and sugar profile analyses (c).

Molasses ^1H -noesy1d with presaturation of water spectrum



(a)



Malic acid, sucrose and polyphenols, are markers of molasses.
The comparison of ^1H NMR spectra of liquid extracts confirmed the addition of molasses was successfully achieved.
The figure shows the comparison of three portions of ^1H spectra relative to aromatic signals of polyphenols (RED), the anomeric proton of sucrose (BLUE), and methylene protons of malic acid (GREEN).

(b)

Figure 2. ^1H -qNMR spectra of sugars in molasses (a) and yogurt samples (b). $n=3$; $Y_0\%$: yoghurt nonfortified sugarcane molasses; $Y_{10}\%$: yoghurt fortified with sugarcane molasses at rate of 10%; $Y_{20}\%$: yoghurt fortified with sugarcane molasses at rate of 20%

Table 1. Physicochemical characteristics and phenolic compounds of cane sugar molasses.

	MO
pH	05.20±0.26
Acidity (°D)	29.00±1
Density	01.35±0.05
Moisture (%)	25±11
Crude ash (%)	09.94±0.08
Crude protein (%)	01.37±0.14
Total fat (%)	0.18±0.07
Antioxidant activity (%)	68.23±0.18
Phenolic acid compounds (µg/g extract)	
Quinic acid	92.94
Chlorogenic acid	23.74
Trans sinapic	04.48
Sinapic acid	04.31
Apigenin-7-Glucoside	87.29
Trans ferulic acid	55.91
Quercetin	288.935
Diosmetin	23.18

Values are presented as mean ± standard deviation of triplicate determinations, n=1; MO, sugarcane molasses.

Table 2. Effect of liquid sugarcane incorporation level on the physicochemical composition of yogurt.

	Y _{0%}	Y _{10%}	Y _{20%}	p-value
pH	04.89 ± 0.11	04.78 ± 0.07	04.63 ± 0.15	NS
Acidity (°D)	75.11 ± 0.18 ^c	83.24 ± 0.29 ^b	90.01 ± 0.02 ^a	***
Density	01.03 ± 0.04	01.38 ± 0.06	01.48 ± 0.10	NS
Moisture (%)	84.33 ± 0.29 ^a	82.56 ± 0.14 ^b	70.23 ± 0.12 ^c	***
Crude ash (%)	0.77 ± 0.06 ^b	0.89 ± 0.06 ^b	0.95 ± 0.02 ^a	**
Crude protein (%)	03.55 ± 0.09 ^a	03.50 ± 0.03 ^a	03.44 ± 0.03 ^b	**
Total fat (%)	02.98 ± 0.08	02.78 ± 0.07	02.57 ± 0.08	NS
Antioxidant activity (%)	19.05 ± 0.09 ^c	24.34 ± 0.08 ^b	28.45 ± 0.07 ^a	***

Values are presented as mean ± standard deviation of triplicate determinations, n=3; Y_{0%}: yoghurt nonfortified sugarcane molasses; Y_{10%}: yoghurt fortified with sugarcane molasses at rate of 10%; Y_{20%}: yoghurt fortified with sugarcane molasses at rate of 20%; NS: no significant. **,***p<0.01, 0.001. ^{a-d}Means with the same superscripts in a row are not significantly different (p >0.05)

Table 3. Effect of sugar molasses rate incorporation on the color attributes of the yogurts.

	Y _{0%}	Y _{10%}	Y _{20%}	p-value
L*	97.36 ± 0.18 ^a	81.90 ± 0.76 ^b	68.07 ± 2.48 ^c	***
a*	01.35 ± 1.17 ^c	02.62 ± 1.05 ^b	06.61 ± 3.77 ^a	***
b*	05.14 ± 4.45 ^b	18.87 ± 1.38 ^a	20.53 ± 1.49 ^a	***
ΔE*	00 ^c	22.24± 1.52 ^b	42.53 ± 3.17 ^a	***

Values are mean ± standard deviation of triplicate determinations. n=3; Y_{0%}: yoghurt nonfortified sugarcane molasses; Y_{10%}: yoghurt fortified with sugarcane molasses at rate of 10%; Y_{20%}: yoghurt fortified with sugarcane molasses at rate of 20%.

^{a-d}Means with the same superscripts in a row are not significantly different (p>0.05); L*, luminosity; a*, redness indices; b*, yellowness indices; ΔE* – total color change.