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Effect of the incorporation of 'Marrubium Vulgare L.' in Yogurt: Physicochemical, nutritional and sensory properties

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Abstract. The research work on the incorporation of *Marrubium vulgare* extract in the yogurt formulations and its effects on physicochemical and sensory properties were conducted. The obtained results revealed that the incorporation of *Marrubium vulgare* extract did not negatively influenced physicochemical parameters, with pH values varied from 4.37 ± 0.01 in natural Yogurt to 4.31 ± 0.01 in the 4% *Marrubium* yogurt, and titratable acidity varied significantly from 99.3 ± 0.5°D to 105.3 ± 1.2°D ($p < 0.05$). The water holding capacity (WHC) improved slightly, but significantly from 20.40 ± 0.03% to 20.67 ± 0.07% ($p < 0.05$). The results suggest a slight increase in titratable acidity, WHC, and viscosity (especially, at higher extract concentrations); however, further research is needed to refine this trend. Nutritionally, total solids, protein and fat content remained largely unchanged ($p > 0.05$), but led to a significant increase in ash content, from 0.63 ± 0.03% to 0.93 ± 0.03% ($p < 0.05$). Additionally, the total phenolic content was significantly increased from 5.25 ± 0.31 mg per 100 g to 7.30 ± 0.27 mg per 100 g ($p < 0.05$) and the antioxidant activity from 2.71 ± 0.19 mg per 100 g to 4.42 ± 0.24 mg per 100g ($p < 0.05$). The sensory evaluation revealed that *Marrubium*-enriched Yogurt received significantly higher ratings in flavor and overall acceptability ($p < 0.05$) compared to plain Yogurt. Texture ratings remained similar ($p > 0.05$). This study highlights the potential of *Marrubium vulgare* as a functional ingredient for Yogurt fortification, enhancing antioxidant properties, mineral content, and consumer acceptability.

Key words: *Marrubium Vulgare*, nutritional properties, phytochemicals, sensory evaluation, Yogurt.

INTRODUCTION

Yogurt is both low in energy and rich in nutrients; as such, it is an ideal dietary component for prevention of obesity and other cardiovascular risks. This is primarily due to its high protein content, which promotes satiety, and its moderate fat and carbohydrate levels, particularly in low-fat and unsweetened varieties (Ibrahim et al., 2020; Sahingil & Hayaloglu, 2022). Yogurts are semi-solid fermented foods produced by fermentation of milk by dairy cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. There has been growing information that the consumption of yogurts as part of a healthy diet is associated with healthier metabolic profiles for children and adults (Zagorska et al., 2020; Vieira et al., 2022).

In addition to traditional dairy based yogurts, plant based alternatives are gaining popularity due to increasing consumer demand for lactose free and vegan options. These yogurts are usually produced with almond, soy, oat, cashew, or coconut milk, and their nutritional profile depends on their basis and fortification (Pachekrepapol et al., 2021; Dhakal et al., 2023). Recently, the application of plants (powder, fresh, extracts, essential oil) in dairy products has been reported (El-Sayed & Youssef, 2019) and is gaining attention (Tang et al., 2019). Plants have been used as flavor, colour and aroma enhancing agents and for preservation of several foods, including dairy products (El-Sayed & Youssef, 2019; Jiménez-Redondo et al., 2022).

Marrubium vulgare L. is distributed widely in tropical regions of North Africa, Central Asia, and Southern Europe. Horehound (*Marrubium vulgare*) is a wild flora, found in all parts of commercially influential Europe. The leaves, flowers, and young stems are harvested during the months of June through September for therapeutic use. Many of the claims made as to the traditional medicinal uses of Horehound have regained confirmation within the last years of scientific research and clinical studies.

Prior phytochemical studies of marrubium validated the characterisation and isolation of several flavonoids, including luteolin and apigenin and their derivatives, vitexin (Nawwar et al., 1989) as well as several labdane diterpenoids (Laonigro et al., 1979) and a small amount of an essential oil (Saleh & Glombitza, 1989; Boulila et al., 2015).

White horehound has long since been utilized for preparing cough medicines as well as for both acute and benign bronchial infection treatment. As far as bronchitis and throat inflammation are concerned, this remedy brings palpable relief (Hseini & Kahouadji, 2007).

The leaves of this plant, when made into a poultice, are also applied to the forehead for migraine and fever, as well as temple application for ear infections (Tahrı et al., 2012). In Germany, FIDMD's commission E states in its research that Horehound is administered for gastric sickness and appetite loss (Hseini & Kahouadji, 2007).

Various studies have reported the Marrubium beneficial effects, especially its anti-inflammatory, antimicrobial, and antioxidant activities. According to Aćimović et al. (2020), this plant's abundance of flavonoids and phenolic compounds contributes to its antioxidant qualities by playing a crucial role in scavenging free radicals. Furthermore, according to Yousefi et al. (2014), *Marrubium vulgare* extracts demonstrate substantial anti-inflammatory effects by decreasing the generation of pro inflammatory mediators, which can explain its traditional use in treating respiratory and gastrointestinal diseases.

Additionally, this plant exhibits strong antibacterial action against a range of pathogenic strains. Lodhi et al. (2017) highlighted the effectiveness of *Marrubium* *vulgare* extracts against several bacteria and fungi, suggesting its potential as a natural antimicrobial agent. Furthermore, a study by Djahra et al. (2013) found that this plant's bioactive substances specifically, flavonoids and diterpenes are responsible of its antibacterial and anti-inflammatory properties. These results support the traditional usage of *Marrubium vulgare* and its incorporation into innovative pharmacological uses, as well as the growing interest in this plant as a possible source of novel natural medicinal compounds.

Although *Marrubium vulgare* has a well-established pharmacological potential, its use in food matrices is still not fully understood. Although its medical properties have been the subject of numerous investigations, its inclusion into yogurt compositions has not yet been the subject of any research. Given the increasing consumer demand for functional foods with added health benefits, integrating *Marrubium vulgare* into Yogurt could offer a novel approach to enhancing its nutritional and sensory properties. Incorporating *Marrubium vulgare* into yogurt aims to offer consumers a product that not only retains the well-known benefits of yogurt, such as being a rich source of probiotics, proteins, and essential nutrients (Ibrahim et al., 2020; Sahingil & Hayaloglu, 2022), but also provides an added value through the marrubium bioactive compounds (Djahra et al., 2013; Yousefi et al., 2014; Lodhi et al., 2017; Aćimović et al., 2020).These compounds confer antibacterial, anti-inflammatory and antioxidant properties, which could help combat oxidative stress, a factor implicated in various chronic diseases. This functional yogurt could therefore serve as a preventive dietary strategy, promoting overall health while maintaining the sensory and nutritional qualities appreciated by consumers.

Thus, the purpose of this study was to examine the effects of adding Marrubium vulgare extract to yogurt formulations and evaluate its impact on the nutritional composition (total solids, protein, fat, and ash), physicochemical properties (pH, titratable acidity, water-holding capacity, and viscosity), total phenolic content, antioxidant activity, and sensory qualities. This research aims to bridge the gap in knowledge regarding the functional application of *Marrubium vulgare* in dairy products, providing valuable insights for the development of innovative, health-promoting fermented foods.

MATERIALS AND METHODS

Milk and lactic ferments

In this study, commercial full-fat milk powder (LOYA brand, Blida, Algeria) and lyophilized cultures of *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* from Chrhansen (France) were added as ferment after the heat treatment.

Yogurt preparation

In the laboratory, both control (natural Yogurt, NY) and experimental formulations incorporating *Marrubium vulgare* extract (MY) were produced following standardized Yogurt manufacturing protocols.

The yogurt production process started with milk standardization using commercial full-fat milk powder (LOYA brand, Blida, Algeria), reconstituted to 20% (w/v) in distilled water. Pasteurization was performed using a Julabo TW20 water bath (Germany) at 90 °C for 10 minutes. This thermal treatment eradicated undesirable microorganisms and denatured whey proteins, enhancing gel formation and overall texture. The milk was then rapidly cooled to 42 °C to optimize conditions for microbial inoculation and fermentation.

Starter cultures (*Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*) were inoculated at a concentration of 0.03% (w/v), sourced from CHR Hansen (France). In the MY formulations, *Marrubium vulgare* extract was incorporated at concentrations of 2%, 3%, and 4% post-heat treatment to evaluate its influence on physicochemical and sensory attributes. The extract was prepared using analytical-grade distilled water and filtered through Whatman No.1 paper before incorporation.

Fermentation was conducted at 42 ± 1 °C for 5 hours in a laboratory ventilated oven (Memmert Model UFB 400, with forced air circulation, GmbH +Go. Germany).

Upon completion of fermentation, the yogurt was rapidly cooled to 4 °C to halt bacterial activity and preserve its structural integrity. The final product was stored under refrigerated conditions (4 ± 1 °C) to ensure microbiological stability and extend shelf life.

The incorporation of *Marrubium vulgare* was executed post-thermal treatment. The formulation adhered to the methodology proposed by Amellal-Chibane & Benamara (2011) (Table 1).

Table 1. Recipe of standard natural yogurt (NY) and Yogurt with marrubium extract (MY), for 1l of milk

Samples	Milk powder (%)	Marrubium extract (%)	Lactic ferment (%)
NY	20	0	0.03
MY1	20	2	0.03
MY2	20	3	0.03
MY3	20	4	0.03

NY: natural Yogurt, MY1, MY2 and MY3: Yogurt with marrubium extract.

Plant sample preparation and extraction

The extract was prepared according to Amessis-Ouchemoukh et al. (2014). The leaves of marrubium were harvested from remote areas in Bajaia, Algeria.

Fresh leaves were airdried in the shade at room temperature. Upon drying, the plant material was powdered with an electric grinder of the IKA, A11 Basic, from Staufen, Germany, and subsequently, 4 g of this powder were exhaustively extracted by maceration with 50 mL of distilled water for 24 h at room temperature with continuous agitation.

In all cases, the solutions were filtered through Whatman No. 1 filter paper to remove solid residues.and concentrated to dryness under reduced pressure in a rotary evaporator (Rotavapor R-210, Büchi Advanced, Switzerland) at 40 °C. The extract was subsequently stored at 4 ± 1 °C until further use in Yogurt preparation.

Yogurt Physico-chemical parameters

All the yogurt measurements were performed after keeping 24 h at 4 °C.

The pH of each samples was determined according to AOAC (1998), at room temperature (25 ± 2 °C) using electrodes of a pH meter (PHM 210, Hanna instruments, France) placed directly into each sample. The pH meter with accuracy of 0.1 was first standardized using buffer solution of pH 4 and 9. The determination was performed in triplicate to find the mean pH of the sample

The lactic acid (titratable acidity) content was evaluated by titration of 1 g of Yogurt mixed with 9 mL of distilled water and a few drops of phenolphthalein (0.10%) against 0.1 M NaOH until the appearance of pink color (Skripleva & Arseneva, 2015).

The Water holding capacity was assessed by centrifuging 5 g of Yogurt at 4,500 rpm for 30 min at 10 °C followed by the quantification of the separated whey (Ladjevardi et al., 2016).

The viscosity was performed using a SNB-1 digital viscometer (Princeton Instruments), at room temperature. The measurements were made in triplicate to find the mean viscosity values. Total solids content was determined by gravimetric methodology (AOAC, 1998). The procedure consists of placing 2 g of the sample in a laboratory ventilated oven (Memmert) 105 ± 2 °C at atmospheric pressure, until constant weight is obtained. Each determination was made in triplicate.

Ash content was determined according to AOAC (1998). The sample (2 g) was accurately weighed into a pre-weighed crucible using an analytical balance and dried in a ventilated oven at 105 ± 3 °C. Samples were transferred to a muffle furnace (Nabertherm) and ashed at 520 ± 5 °C for 4 hours then cooled in a desiccator for 1 hour and weighed.

The Kjeldahl method (FIL-IDF 20B: 1993) was used to measure the protein content of yogurt samples. 0.5 g of yogurt was digested with concentrated sulfuric acid (H_2SO_4) at 420 °C, in the presence of a catalyst (potassium sulfate and copper sulfate), until a clear solution appeared. The sample was neutralized using sodium hydroxide (NaOH) and then distilled to release ammonia, which was absorbed with boric acid. The nitrogen content was determined by titration using 0.1 N hydrochloric acid (HCl) and the protein content (g per 100 g) was obtained with a conversion factor of 6.38.

The fat content of Yogurt samples was determined using the Gerber method (ISO 2446:2001). A measured quantity of Yogurt (10 mL) was mixed with 10 mL of sulfuric acid (90–91%) in a Gerber butyrometer. One milliliter of isoamyl alcohol was added, and the mixture was centrifuged at 1,100 rpm for 5 minutes at 65°C. The fat content was then read directly from the scale of the butyrometer and expressed as a percentage of fat.

Total phenolic content (TPC)

The TPC level was assessed via the Folin-Ciocâlteu method (Georgé et al., 2005). Each sample of 20 g was mixed with a 7:3 v/v solution of acetone-water and left for 30 min. Filtration of the obtained solutions was performed using a syringe filter (0.45 µm). Of this solution, 2.5 mL of the diluted Folin-Ciocâlteu reagent (1/10) was mixed with 200 µL of each extract and 300 µL of deionized water. This mixture was incubated for 2 min at room temperature, and then 2 mL of a 75 g L^{-1} solution of sodium carbonate was added. The mixture was then incubated in darkness for 15 min at 50 °C and cooled by an ice bath to room temperature. Finally, the absorbance was read immediately at 760 nm by a UV visible spectrophotometer (Spectroscan 50, Biotech Engineering Management Company Limited, UK). TPC was expressed as mg of gallic acid equivalent (GAE) per 100 g juice f.w., according to the calibration curve constructed with standard solutions of pure gallic acid.

Antioxidant activity

The DPPH free radical method was used to assess the antioxidant activity (Piga et al., 2003). Each extract (in a solution of acetone-water, 7:3, v/v) was added to an equal volume of methanol solution containing 6×10−5 M DPPH; after, the mixture was incubated for 15 minutes at room temperature in the dark. Absorbance values for the extracts were measured with a spectrophotometer (Spectroscan 50, Biotech Engineering Management Company Limited, UK).

Absorbances were converted to inhibition percentages of DPPH concentrations, so that results are expressed as mg GAE per 100 g juice f.w., according to a calibration curve established with standard solutions of pure gallic acid.

Sensorial properties

A scoring test with 1 being the worst and 9 being the best was used for the sensory evaluation of the two Yoghurt samples (plain Yoghurt and Marrubium Yoghurt). This evaluation was based on a 9-point hedonic scale, where 1 represented 'dislike extremely' and 9 represented 'like extremely'. The Yoghurt samples were rated with regard to color, flavor, texture, and overall acceptability on the first day of storage.

The sensory evaluation was performed by a panel of 20 trained panelists (10 males and 10 females) aged between 22 and 45 years, all with prior experience in dairy product evaluation and no allergic reactions to milk. The evaluation took place in a sensory analysis laboratory under controlled conditions, with standardized lighting and a neutral background to minimize external influences. Panelists tasted glasses of Yoghurt pots chilled to about 5 °C. Each panelist was seated in an individual booth to avoid external bias. A glass of water was provided to cleanse the palate in between Yoghurt formulations.

Color was assessed based on uniformity, brightness, and natural appearance. Flavor was evaluated by the balance between acidity and sweetness, as well as the presence of any off-flavors. Texture was judged on smoothness, creaminess, firmness, and the absence of syneresis (whey separation). Overall acceptability was determined based on the integration of color, texture, and flavor (da Costa et al., 2020; Lucatto et al., 2020; Akan et al., 2022).

Statistical analysis

The experimental results of the physico-chemical analyses were expressed as mean values ± standard deviation, for triplicate measurements. Differences among samples were determined using the one-way ANOVA, followed by the Tukey's honestly significant difference (HSD) at $p < 0.05$ using JMP program (version 7.0, USA).

RESULTS AND DISCUSSION

Physico-chemical parameters

The pH values of the Yogurt samples showed minor variations. The control sample exhibited a pH of 4.37 ± 0.01, while the samples containing 2%, 3%, and 4% extract showed pH values of 4.34 ± 0.02, 4.32 ± 0.02, and 4.31 ± 0.01, respectively (Fig. 1, A). The difference was statistically significant ($p < 0.05$) only between the yogurt control (NY) and the MY samples, but no significant differences were noticed among MY1, MY2, and MY3.

Titratable acidity showed slight variations among samples, with significant differences only at higher extract concentration (Fig. 1, B). The control Yogurt displayed an acidity of 99.3 ± 0.5, whereas the samples with 2%, 3%, and 4% extract had values of 100 ± 1.0, 101.3 ± 0.5, and 105.3 ± 1.2, respectively. However, statistical analysis showed that a significant difference ($p < 0.05$) was observed only when 4% *Marrubium* *vulgare* was added. That indicated the fermentation process was significantly affected when 4 % of marrubium extract was added to the composition.

The noticed significant increases ($p < 0.05$) in titratable acidity could have possibly been due to the impact of Marrubium's bioactive compounds on microbial metabolism, which might have altered the fermentation dynamics by promoting lactic acid bacterial activity. Phenolic compounds present in Marrubium extract may have acted as metabolic enhancers, thereby increasing acid production. This observation aligns with previous studies where plant's bioactive compounds impacted the acidification process in fermented dairy products (Mocanu et al., 2010; Daramola et al., 2013).

Water holding capacity is among the most significant properties that indicate yogurt quality through storage. It is a physical property that plays an important role in yogurt shelf life and acceptability (Adepoju & Selezneva, 2020).

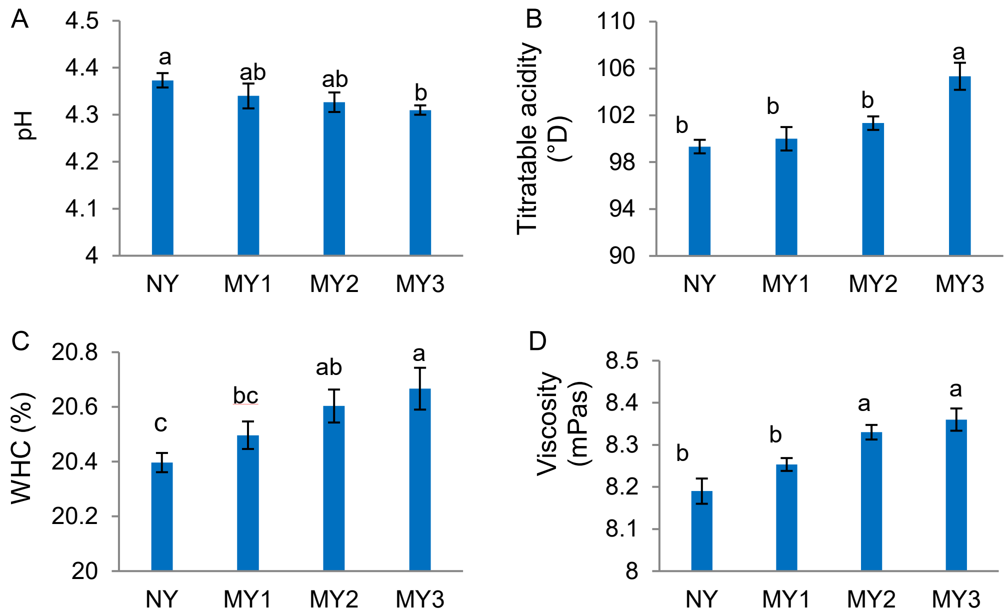

Figure 1. Physico-chemical parameters of yogurt simples: pH (A), Titrable acidity (B), WHC (C) and viscosity (D).

NY: natural Yogurt, MY1, MY2 and MY3: Yogurt with marrubium extracts (2, 3 and 4 % respectively).

In this study, as is seen in Fig. 1, C., Water-holding capacity (WHC) remained relatively stable across formulations. WHC values were 20.40 ± 0.03% in the control and 20.50 ± 0.04%, 20.60 ± 0.04%, and 20.67 ± 0.05% in the 2%, 3%, and 4% formulations, respectively. The differences were statistically significant ($p < 0.05$) only between NY and MY3, but the variation remained minimal.

Of the factors influencing the stability of Yogurt curd, the viscosity value came out as one. The control sample exhibited a viscosity of 8.19 ± 0.03 mPas, while samples with 2%, 3%, and 4% extract had values of 8.25 ± 0.02 mPas, 8.33 ± 0.02 mPas, and 8.36 ± 0.03 mPas, respectively (Fig. 1, D). A statistically significant difference ($p < 0.05$) was observed between the yogurt control and the *Marrubium vulgare* Yogurts. However, no significant difference ($p > 0.05$) was obtained between the MY2 (3%) and MY3 (4%) formulations.

The slight différence in WHC and viscosity between Yogurt control and 4% Marrubium yoghur (MY3), can be explaind by the presence of methylated phenolic compounds, which form hydrogen bonds with water molecules through their hydroxyl moieties (Daramola et al., 2013). *Marrubium vulgare* is known to contain various bioactive compounds, including phenolic acids and flavonoids (Aćimović et al., 2020). These methylated phenolics may enhance water retention by stabilizing the protein network in Yogurt. Further research is needed to refine the observed trends and to study the infuance of *Marrubium vulgare* extract on the probiotic bacteria viability (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*), as well as their interaction with WHC, viscosity and overall stability of the Yogurt.

Overall, the incorporation of *Marrubium vulgare* extract in Yogurt did not negatively affect the studied physicochemical parameters. These findings suggest that *Marrubium vulgare* can be used as a functional ingredient in fermented dairy products without compromising their physicochemical properties. However, further studies are needed to explore its impact on Yogurt stability during storage.

Nutritional parameters

Total solids content showed minor variations, with values of 22.23 ± 0.02% in the control, while MY1, MY2, and MY3 formulations exhibited values of 22.14 ± 0.02%, 22.11 ± 0.03%, and 22.01 ± 0.01%, respectively. A significant difference ($p < 0.05$) was observed only between the control and MY3 (Fig. 2, A).

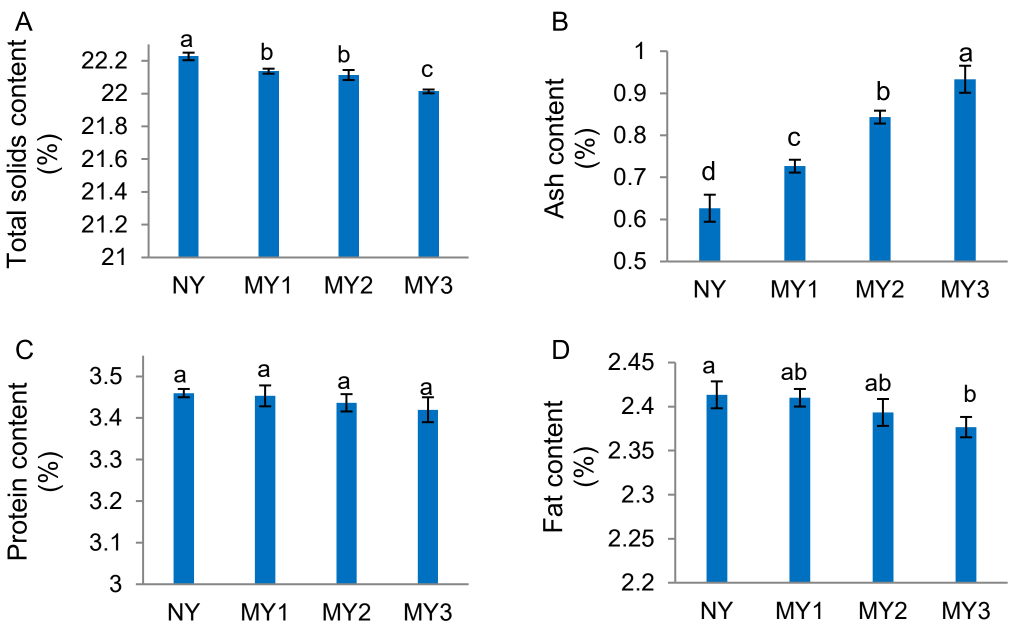

Figure 2. Nutritional properties of yogurt simples: Total soluble content (A), Ash content (B), Protein content (C) and Fat content (D).

NY: natural Yogurt, MY1, MY2 and MY3: Yogurt with marrubium extracts (2, 3 and 4 % respectively).

This implies a slight increase of water content in the product because of the addition of an aqueous extract. Similar results were also noted by Hasneen et al. (2020).

In addition, the ash quality of Yogurt compositions differed moderately (Fig. 2, B). Increasing the marrubium concentration in Yogurt yielded a significant difference in ash content ($p < 0.05$) in comparison with plain Yogurt, with values of 0.63 ± 0.03% in the control, compared to 0.76 ± 0.02%, 0.82 ± 0.03%, and 0.93 ± 0.03% in the MY1, MY2, and MY3 samples, respectively.

Besides, positive coefficients of correlation were ascertained between marrubium extract concentration and Yogurt ash content. This may be due to the intrinsic mineral composition of Marrubium. The literature abounds with references stating that marrubium contains several minerals with overexpression of potassium salts (Amri et al., 2017).

Further, adding plant extracts to Yogurt aided in mineral enrichment, claims several authors (Yadav & Shukla, 2014; Arslaner et al., 2021; Tami et al., 2022).

As observed in Fig. 2, C, the addition of marrubium extract did not have a significant consequence ($p < 0.05$) on the protein concentration of Yogurt samples showing 3.45 ± 0.01% in Yogurt control, while MY1, MY2, and MY3 samples had values of 3.48 ± 0.02%, 3.46 ± 0.02%, and 3.42 ± 0.03%, respectively.

However, against the fat concentration, a small reduction in the fat content was observed when comparisons were made between Yogurt with added marrubium extract and plain Yogurt, with the control at 2.41 ± 0.02%, and MY1, MY2, and MY3 at 2.41 ± 0.02%, 2.39 ± 0.02%, and 2.38 ± 0.02%, respectively (Fig. 2. D). A significant decrease ($p < 0.05$) was observed only in the MY3 sample (4% *Marrubium vulgare*). The little reduction could be explained by the fact that the marrubium extract itself has low fat. Comparable result was obtained in the study on the incorporation of plant extracts into Yoghurt (Hasneen et al., 2020). Overall, these findings suggest that the incorporation of *Marrubium vulgare* extract did not negatively impacted the nutritional components of Yogurt, as protein, total solids, and fat content remained largely stable across formulations. This highlights the potential of *Marrubium* as a functional ingredient that impouved certain physicochemical properties (such as ash content) without compromising the fundamental nutritional profile of Yogurt.

Further research is needed to refine the obtained trends and investigate a deeper understanding of the nutritional influance of *Marrubium vulgare* extract in Yogurt, in particular concerning its impact on macronutrient interactions and mineral bioavailability.

Phenolic compounds

As can be observed in Fig. 3, A, Yogurt formulations made with Marrubium indicated remarkably greater levels, with a significatif difference at $p < 0.05$ of phenolic compounds (7.30 ± 0.10 mg per 100 g) compared to the control Yogurt (5.25 ± 0.09 mg per 100 g). This increase in phenolic compounds appears to corroborate with research conducted previously that profiles phenolics out of *Marrubium vulgare* (Neamah et al., 2017). Yogurt with a Marrubium extract level of 4% provided the highest amount of phenolic compounds, revealing a correlation between the phenolic benefit and Marrubium concentration. Statistical analysis confirmed that these differences were significant ($p < 0.05$). This was consistent in many studies investigating plant-extract-enriched Yogurt (Daramola et al., 2013; Dabija et al., 2018; Bulut et al., 2021; Jiménez-Redondo et al., 2022).

Antioxidant activity

The antioxidant activity of the Yogurt formulations was studied using an assay based on DPPH radical scavenging activity (Fig. 3, B). Results showed that marrubium Yogurt (4.42 ± 0.16 mg per 100 g) had significantly (p <0.05) higher antioxidant activity than plain Yogurt (2.71 ± 0.13 mg per 100 g). Such a significant increase ($p < 0.05$) in the antioxidant activity is explicated in reference to the presence of the phenolic compounds in the extract of marrubium, presenting inherent antioxidant activity (Daramola et al., 2013; Dabija et al., 2018; Bulut et al., 2021; Jiménez-Redondo et al., 2022). Moreover, marrubium extract concentrations show strong correlation with antioxidant activity in Yogurt.

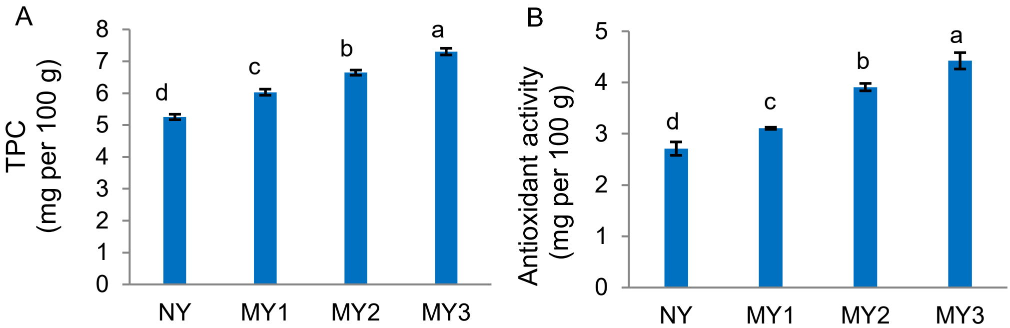

Figure 3. Total phenolic compounds (A) and antioxidant activity (B) of yogurt simples.
NY: natural Yogurt, MY1, MY2 and MY3: Yogurt with marrubium extracts (2, 3 and 4 % respectively).

These findings highlight the potential of *Marrubium vulgare* as a natural source of antioxidants in dairy formulations, contributing to an enhanced functional profile of yogurt. The incorporation of *Marrubium* could offer health benefits by improving the antioxidant capacity of yogurt, which may help in combating oxidative stress and promoting overall wellness. However, to fully validate these benefits, further research is needed to assess the stability of the antioxidant compounds during storage, their bioavailability upon digestion, and their potential interactions with dairy components.

The sensory analysis results

Sensory analysis results indicated significant differences in marrubium Yogurt relative to natural Yogurt (NY), particularly in regard to color, flavor, texture, and overall acceptability (Fig. 4).

Marrubium Yogurt exhibited a distinct hue, significantly different from natural Yogurt, mainly due to the addition of *Marrubium* extract. Panelists noted that the color was deeper and slightly greenish, which could be attributed to the natural pigments in *Marrubium*. The color was also evaluated for uniformity, and the *Marrubium* Yogurt showed a consistent distribution without any visible phase separation, ensuring a visually appealing appearance.

Marrubium Yogurt was rated significantly higher ($p < 0.05$) in flavor intensity compared to natural Yogurt, indicating a preference for the unique taste conferred by the *Marrubium* extract. The presence of herbaceous notes and a mild bitterness was noted by several panelists, contributing to a more complex and richer taste profile. This suggests that Marrubium extract enhances the overall sensory experience by introducing new flavor dimensions, making it more appealing to consumers looking for innovative Yogurt flavors.

Texture analysis showed no significant differences ($p > 0.05$) among the samples, indicating that Marrubium extract does not negatively impact Yogurt texture.

The Yogurt retained its smoothness, creaminess, and *Marrubium* Yogurt was rated significantly higher ($p < 0.05$) in flavor intensity compared to natural Yogurt, firmness, with no excessive syneresis (whey separation) observed. The panelists noted that the Marrubium Yogurt maintained a cohesive and stable structure, suggesting that the extract does not interfere with the gel network formation in Yogurt.

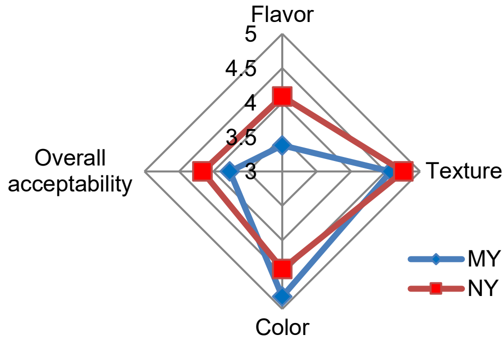

Figure 4. Sensory analysis results of Yogurt simples.
Color: uniformity, brightness, and natural appearance; texture: smoothness, creaminess, firmness, and the absence of syneresis; Flavor: Balance between acidity and sweetness, the presence of any off-flavors; Overall acceptability.
NY: natural Yogurt, MY: Yogurt with marrubium extract (4%).

The overall liking acceptability indicated that Marrubium Yogurt was significantly preferred ($p < 0.05$) over natural Yogurt. The harmonious integration of color, flavor, and texture contributed to its higher consumer appeal. Panelists particularly appreciated the unique sensory attributes introduced by Marrubium, suggesting that its incorporation enhances the acceptability and desirability of Yogurt as a functional dairy product.

These findings highlight the potential of Marrubium extract as a novel ingredient to improve the sensory appeal of dairy products. Its distinct color, enriched flavor profile, and maintained textural integrity make it a promising functional additive that can cater to evolving consumer preferences while adding potential health benefits.

CONCLUSIONS

This study demonstrated the potential of *Marrubium vulgare* extract as a functional ingredient in Yogurt formulations. However, it was revealed that its incorporation did not negatively affect key physicochemical properties, ensuring the structural integrity of the final product. These findings indicate that the *Marrubium vulgare* extract can be integrated into Yogurt formulations without compromising stability. However, further research is required to refine the formulation for improved functional and textural properties.

Moreover, the addition of *Marrubium vulgare* did not negatively impact the nutritional composition of the Yogurt, as total solids, protein, and fat content remained stable across formulations. However, a significant increase in ash content was observed, suggesting an enrichment in minerals.

Furthermore, the addition of *Marrubium vulgare* significantly increased total phenolic content and antioxidant capacity, reinforcing its role as a natural source of bioactive compounds with potential health benefits. Sensory evaluation confirmed that *Marrubium vulgare*-enriched Yogurt was well accepted by panelists, particularly in terms of flavor and overall acceptability.

This research study not only demonstrates that *Marrubium vulgare* improves yogurt sensory perception but also highlights its role in improving the nutritional value of the final product by increasing phenolic compound content and antioxidant activity. These findings emphasize the impact of adjusting the appropriate supplementation levels to increase both functional benefits and sensory attributes. They provide valuable insights for the dairy industry, paving the way for the innovation of fortified yogurts that successfully combine consumer preference with enhanced health-promoting properties.

Future research should focus on optimizing the concentration of *Marrubium vulgare* to maximize its functional benefits while maintaining optimal sensory properties. Additionally, further investigations are needed to evaluate its impact on mineral bioavailability, macronutrient interactions, and long-term Yogurt stability.

REFERENCES

Aćimović, M., Jeremić, K., Salaj, N., Gavarić, N., Kiprovski, B., Sikora, V. & Zeremski, T. 2020. Marrubium vulgare L.: A Phytochemical and Pharmacological Overview. *Molecules* **25**(12). 2898. doi: 10.3390/molecules25122898

Adepoju, F. & Selezneva, I. 2020. Comparative study of Yogurt enriched with Moringa powder in different concentrations. *AIP Conference Proceedings*. AIP Publishing. doi: 10.1063/5.0018035

Akan, E., Yerlikaya, O., Bayram, O.Y. & Kinik, O. 2022. Viability of probiotics, rheological and the sensorial properties of probiotic yogurts fortified with aqueous extracts of some plants. *Anais da Academia Brasileira de Ciencias* **94**(3), e20211274. doi: 10.1590/0001-3765202220211274

Amellal-chibane, H. & Benamara, S. 2011. Total contents of major minerals in the nature Yogurt and in the Yogurts with the date powder of three dry varieties. *Biodiversity* **1**(2), 74–78.

Amessis-Ouchemoukh, N., Abu-Reidah, I.M., Quirantes-Piné, R., Madani, K. & Segura-Carretero, A. 2014. Phytochemical profiling, in vitro evaluation of total phenolic contents and antioxidant properties of Marrubium vulgare (horehound) leaves of plants growing in Algeria. *Industrial Crops and Products* **61**, 120–129. doi: 1016/j.indcrop.2014.06.049

Amri, B., Martino, E., Vitulo, F., Corana, F., Kaab, L.B., Rui, M., Rossi, D., Mori, M., Rossi, S. & Collina, S. 2017. Marrubium vulgare L. Leave Extract: Phytochemical Composition, Antioxidant and Wound Healing Properties. *Molecules* **22**(11). doi: 10.3390/molecules22111851

AOAC. 1998. Official methods of analysis of AOAC International 16th ed AOAC Int. Gaithersburg

Arslaner, A., Salik, M.A. & Bakirci, I. 2021. The effects of adding Hibiscus sabdariffa L. flowers marmalade on some quality properties, mineral content and antioxidant activities of yogurt. *Journal of food science and technology* **58**, 223–233.

Boulila, A., Sanaa, A., Salem, I.B., Rokbeni, N., M'Rabet, Y., Hosni, K. & Fernandez, X. 2015. Antioxidant properties and phenolic variation in wild populations of Marrubium vulgare L. (Lamiaceae). *Industrial Crops and Products* **76**, 616–622. doi: 10.1016/j.indcrop.2015.07.069

Bulut, M., Tunçtürk, Y. & Alwazeer, D. 2021. Effect of fortification of set type Yogurt with different plant extracts on its physicochemical, rheological, textural and sensory properties during storage. *International Journal of Dairy Technology* **74**(4), 723–736. doi: 10.1111/1471-0307.12803 da Costa, G.M., de Paula, M.M., Costa, G.N., Esmerino, E.A., Silva, R., de Freitas, M.Q., Barao, C.E., Cruz, A.G. & Pimentel, T.C. 2020. Preferred attribute elicitation methodology compared to conventional descriptive analysis: A study using probiotic yogurt sweetened with xylitol and added with prebiotic components. *Journal of Sensory Studies* **35**(6), e12602. doi: 10.1111/joss.12602

Dabija, A., Codină, G.G., Ropciuc, S., Gâtlan, A.-M. & Rusu, L. 2018. Assessment of the Antioxidant Activity and Quality Attributes of Yogurt Enhanced with Wild Herbs Extracts. *Journal of Food Quality* **2018**, 1–12. doi: 10.1155/2018/5329386

Daramola, B., Oje, O.J. & Oduola, R.O. 2013. Phytochemical screening and application of extracts of selected plant foods in preparation of enhanced sensorial and healthier image Yogurt. *African Journal of Biotechnology* **12**, 96–102. doi: 10.5897/ajb12.750

Dhakal, D., Younas, T., Bhusal, R.P., Devkota, L., Henry, C.J. & Dhital, S. 2023. Design rules of plant-based yoghurt-mimic: Formulation, functionality, sensory profile and nutritional value. *Food Hydrocolloids* **142**, 108786. doi: 10.1016/j.foodhyd.2023.108786

Djahra, A., Bordjiba, O. & Benkherara, S. 2013. Extraction, separation and antibacterial activity of tannins of white horehound (Marrubium vulgare L.). *Phytothérapie* **11**, 348–352. doi: 10.1007/s10298-013-0819-1

El-Sayed, S.M. & Youssef, A.M. 2019. Potential application of herbs and spices and their effects in functional dairy products. *Heliyon* **5**(6), e01989.

Georgé, S., Brat, P., Alter, P. & Amiot, M.J. 2005. Rapid determination of polyphenols and vitamin C in plant-derived products. *Journal of Agricultural and Food Chemistry* **53**(5), 1370–1373. doi: 10.1021/jf048396b

Hasneen, D.F., Zaki, N.L., Abbas, M.S., Soliman, A.S., Ashoush, I.S. & Fayed, A.E. 2020. Comparative evaluation of some herbs and their suitability for skimmed milk Yogurt and cast Kariesh cheese fortification as functional foods. *Annals of Agricultural Sciences* **65**(1), 6–12. doi: 10.1016/j.aoas.2020.05.001

Hseini, S. & Kahouadji, A. 2007. Ethnobotanical study of medicinal flora in the Rabat region (Western Morocco). *Lazaroa* **28**, 79–93 (in French).

Ibrahim, M., Barakova, N. & Jõudu, I. 2020. Enrichment of the low-fat Yogurt with oat β-glucan and EPS-producing Bifidobacterium bifidum improves its quality. *Agronomy research* **18**(S3), 1689–1699. doi: 10.15159/AR.20.024

Jiménez-Redondo, N., Vargas, A.E., Teruel-Andreu, C., Lipan, L., Muelas, R., Hernández-García, F., Sendra, E. & Cano-Lamadrid, M. 2022. Evaluation of cinnammon (Cinnamomum cassia and Cinnamomum verum) enriched Yogurt during refrigerated storage. *LWT - Food Science and Technology* **159**, 113240. doi: 10.1016/j.lwt.2022.113240

Ladjevardi, Z.S., Yarmand, M., Emam-Djomeh, Z. & Niasari-Naslaji, A. 2016. Physicochemical properties and viability of probiotic bacteria of functional synbiotic camel yogurt affected by oat β-glucan during storage. *Journal of Agricultural Science and Technology* **18**(5), 1233–1246.

Laonigro, G., Lanzetta, R., Parrilli, M., Adinolfi, M. & Mangoni, L. 1979. The configuration of the diterpene spiroethers from Marrubium vulgare and from Leonotis leonurus. *Gazzetta Chimica Italiana* **109**, 145–150.

Lodhi, S., Vadnere, G.P., Sharma, V.K. & Usman, M.R. 2017. Marrubium vulgare L.: A review on phytochemical and pharmacological aspects. *J. Intercult. Ethnopharmacol* **6**(4), 429–452. https://doi.org/10.5455/jice.20170713060840

Lucatto, J.N., da Silva-Buzanello, R.A., de Mendonça, S.N.T.G., Lazarotto, T.C., Sanchez, J.L., Bona, E. & Drunkler, D.A. 2020. Performance of different microbial cultures in potentially probiotic and prebiotic Yogurts from cow and goat milks. *International Journal of Dairy Technology* **73**(1), 144–156. doi: 10.1111/1471-0307.12655

Mocanu, D., Rotaru, G., Botez, E., Andronoiu, D. & Nistor, O. 2010. Probiotic yogurt with medicinal plants extract: Physical–chemical, microbiological and rheological characteristics. *Journal of Agroalimentary Processes and Technologies* **16**(4), 469–476.

Nawwar, M.A., El-Mousallamy, A.M., Barakat, H.H., Buddrus, J. & Linscheid, M. 1989. Flavonoid lactates from leaves of Marrubium vulgare. *Phytochemistry* **28**, 3201–3206.

Neamah, S.I., Sarhan, I.A. & Al-Shaye'a, O.N. 2017. Bioactive compounds from Marrubium Vulgare L. based on in vitro antioxidant activity. *Journal of Global Pharma Technology* **10**(9), 183–188.

Pachekrepapol, U., Kokhuenkhan, Y. & Ongsawat, J. 2021. Formulation of yogurt-like product from coconut milk and evaluation of physicochemical, rheological, and sensory properties. International *Journal of Gastronomy and Food Science* **25**, 100393. doi: 10.1016/j.ijgfs.2021.100393

Piga, A., Caro, A.D., Pinna, I. & Agabbio, M. 2003. Changes in ascorbic acid, polyphenol content and antioxidant activity in minimally processed cactus pear fruits. *LWT - Food Science and Technology* **36**(2), 257–262. doi: 10.1016/S0023-6438(02)00227-X

Sahingil, D. & Hayaloglu, A.A. 2022. Enrichment of antioxidant activity, phenolic compounds, volatile composition and sensory properties of yogurt with rosehip (Rosa canina L.) fortification. *International Journal of Gastronomy and Food Science* **28**, 100514. doi: 10.1016/j.ijgfs.2022.100514

Saleh, M. & Glombitza, K. 1989. Volatile oil of Marrubium vulgare and its anti-schistosomal activity. *Planta Medica* **55**, 105–105.

Skripleva, E. & Arseneva, T. 2015. Optimization of the recipe of Yogurt with additives and control of some quality attributes of new Yogurt recipe. *Agronomy Research* **13**(4), 1086–1095.

Tahrı, N., El Bastı, A., Zıdane, L., Rochdı, A. & Douıra, A. 2012. Ethnobotanical study of medicinal plants in the province of Settat (Morocco). *Kastamonu University Journal of Forestry Faculty* **12**, 192–208 (in French).

Tami, S.H., Aly, E., Darwish, A.A. & Mohamed, E.S. 2022. Buffalo stirred Yogurt fortified with grape seed extract: new insights into its functional properties. *Food Bioscience* **47**, 101752. doi: 10.1016/j.fbio.2022.101752

Tang, P.-l., Hao, E.-w., Deng, J.-g., Hou, X.-t., Zhang, Z.-h. & Xie, J.-l. 2019. Boost anti-oxidant activity of yogurt with extract and hydrolysate of cinnamon residues. *Chinese Herbal Medicines* **11**(4), 417–422. doi: 10.1016/j.chmed.2019.05.007

Vieira, E.D.F., Styles, D., Sousa, S., Santos, C., Gil, A.M., Gomes, A.M. & Vasconcelos, M.W. 2022. Nutritional, rheological, sensory characteristics and environmental impact of a yogurt-like dairy drink for children enriched with lupin flour. *International Journal of Gastronomy and Food Science* **30**(10), 100617. doi: 10.1016/j.ijgfs.2022.100617

Yadav, K. & Shukla, S. 2014. Microbiological, physicochemical analysis and sensory evaluation of herbal yogurt. *The Pharma Innovation* **3**(10), 01–04.

Yousefi, K., Fathiazad, F., Soraya, H., Rameshrad, M., Maleki-Dizaji, N. & Garjani, A. 2014. Marrubium vulgare L. methanolic extract inhibits inflammatory response and prevents cardiomyocyte fibrosis in isoproterenol-induced acute myocardial infarction in rats. *BioImpacts: BI* **4**(1), 21–27. doi: 10.5681/bi.2014.001

Zagorska, J., Ciprovica, I., Straumite, E. & Majore, K. 2020. Acceptance of low-sugar Yogurt among Latvian teenagers. *Agronomy Research* **13**S3), 1086–1095. doi: 10.15159/AR.20.111