

## The efficiency of natural plant extracts in improving storage stability, antioxidant activity, sensory evaluation, and physicochemical properties of date juice-based energy drink

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### Abstract

Energy drinks (EDs) are a type of sweetened and non-alcoholic beverage with high content of caffeine. EDs were prepared from date juice enriched with different concentrations of ginger rhizomes extract, moringa leaf extract, and caffeine. Physicochemical properties (pH, TSS, and color parameters), total phenolic content (TPC), antioxidant activity, microbiological quality, and sensory characteristics were evaluated for freshly prepared EDs and during storage. The results showed that freshly prepared EDs from date juice and natural extracts revealed lower pH, higher TSS, and lower color parameters than the control. The inclusion of date juice and natural extracts improved the TPC, antioxidant activity, and microbiological quality of freshly prepared EDs. Storage studies exhibited that EDs containing date juice and natural extracts were more stable during storage in terms of all quality parameters estimated. However, the ED made from date juice, ginger extract, moringa extract, and caffeine was the best in terms of all parameters evaluated with higher stability during 3 months of storage at room temperature. In general, by date juice and natural plant extracts addition, a desirable ED can be prepared.

**Keywords:** antioxidant activity; date juice; energy drink; natural extract; storage stability

### Introduction

Energy drinks (EDs) are a type of sweetened, non-alcoholic, carbonated, or non-carbonated beverages, with high content of caffeine and may or may not contain other stimulants such as taurine, guarana, or glucuronolactone (Selahvarzi *et al.*, 2021; Ariffin *et al.*, 2022; Oberhoffer *et al.*, 2022b). In the last few years, the consumption of EDs has increased particularly among children and teenagers. According to the European Food Safety Authority, about 66% of adolescents consume EDs (Oberhoffer *et al.*, 2022a). The global energy

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drinks market reached \$58.35 billion in 2022 and is expected to grow to \$76.60 billion in 2026 (Reportlinker, 2022). EDs are very popular products that are believed to boost energy, stamina, sports performance, and physical and mental fitness (Selahvarzi *et al.*, 2021; Ariffin *et al.*, 2022). On contrary, several studies revealed that the excessive and chronic consumption of EDs had some adverse impacts on human health i.e. cardiac arrhythmia, arterial hypertension or coronary artery dissection, cardiotoxicity, and the onset of cardiomyopathy (Oberhoffer *et al.*, 2022a).

Nowadays, the demand for natural ingredients with beneficial effects on human health in the food and beverages industry is increasing. Moreover, various reports stated that the regular consumption of fruits and vegetables can play an important role in reducing the risk of several chronic diseases such as cancer, cardiovascular disease, atherosclerosis, coronary heart disease, aging, neurodegenerative diseases, and inflammation (Al-Farsi *et al.*, 2005; Al-Farsi *et al.*, 2007). The protective effect of fruits and vegetables is mainly related to their high content of bioactive compounds, such as phenolics and antioxidants, besides dietary fiber, minerals, and other nutrients (Al-Farsi *et al.*, 2005). Date palm fruits (*Phoenix dactylifera* L.) are a rich source of carbohydrates (70-80%), phenolics, antioxidants, and other nutrients. It is considered a unique source of rapid energy because fructose and glucose, which are easily absorbed by the human body, represent the majority of the carbohydrate content in dates (Al-Farsi *et al.*, 2007). Ginger (*Zingiber officinale*), a popular rhizomatous herb, is extensively used worldwide as a spice in culinary, beverages, and herbal medicinal practices. It is effective against several diseases e.g., rheumatic disorders, cold and flu symptoms, fevers, gastrointestinal complications, bronchitis, diabetes, and cancer. Such benefits of ginger are related to its high content of bioactive compounds including 6-gingerol, 6-shogaol, zingerone, beside other phenolics and flavonoids (Ali *et al.*, 2018). Moreover, moringa (*Moringa oleifera*), a tree from the sub-Himalayan regions, has been used in indigenous medicine for various diseases e.g., inflammation, cardiovascular, gastrointestinal, hematological, and hepatorenal disorders. Additionally, fresh and dried moringa leaves represent a part of the diet in some African countries such as Nigeria, Ghana, and Ethiopia (González-Romero *et al.*, 2020). The nutritional and health benefits of moringa leaves are attributed to their high content of phenolic compounds, particularly flavonoids, besides other antioxidants, such as ascorbic acid and carotenoids.

Generally, several studies discussed the negative effects of the excessive and chronic consumption of EDs on human health (Oberhoffer *et al.*, 2022b, 2022a; Tomanic *et al.*, 2022). However, very few studies have been carried out on the development of EDs fortified with natural extracts (Selahvarzi *et al.*, 2021). In addition, there are no studies were conducted on the use of date juice in EDs production. Accordingly, the present research aimed to evaluate the physicochemical properties, antioxidant activity, sensory evaluation, and storage stability of EDs produced from date juice and enriched with ginger, and moringa extracts.

## Materials and Methods

### Materials

Fresh date fruits (*Phoenix dactylifera* L.) of Khalas type (in Khalal stage), fresh ginger rhizomes (*Zingiber officinale*), and fresh moringa leaves (*Moringa oleifera*) were obtained from a private farm, Al-Hofuf, Al-Ahsa, Saudi Arabia. Carbon dioxide gas (food grade) was procured from SIGAZ Company, Dammam, Saudi Arabia. Chemicals, microbial media, and other additives were obtained from Sigma-Aldrich Co., Steinheim, Germany. Commercial sucrose and plastic cans were purchased from the local market.

### Preparation date juice

Date fruits were washed, pitted, sliced, and mixed in a blender with distilled water (1:2; w/v) to obtain the date juice. Then, the juice was filtered in a vacuum filtration device using a Zeiss filter to obtain superior purification. Consequently, the filtered juice was concentrated to about 9.5° Brix using a rotary evaporator

(BUCHI, Switzerland, Rotavapor R215). Then, the juice was kept in brown glass bottles at  $5 \pm 1$  °C for further use (Bilek and Bayram, 2015).

#### *Preparation of ginger and moringa extracts*

Ginger rhizomes were washed, peeled, minced, and dried in the oven at 45 °C for 48 h. Then, they ground and sieved using a 0.25-mm sieve (60 mesh). About 100 g of ginger powder was mixed with 500 ml of distilled water and homogenized for 2 h at 50 °C using a magnetic stirrer. The extract was filtered using a Zeiss filter, concentrated in a rotary evaporator, and kept in a brown bottle at  $5 \pm 1$  °C for further use. The same method was followed to prepare moringa leaf extract (El-Gammal *et al.*, 2017; Sugimoto *et al.*, 2018).

#### *Experimental design*

The energy drinks (EDs) were prepared according to the standards of the Gulf Standardization Organization (GSO 05/FDS/1926:2021)(GSO, 2019). The detailed experimental plan of date fruit- natural extracts-based energy drinks is shown in Table 1. All treatments were subjected to sensory evaluation to select the optimum concentration of each group. Then, the selected treatments were kept at room temperature for further analysis. Physico-chemical properties, microbial analysis, and sensory properties were evaluated after processing and during the storage period of three months.

**Table 1.** Detailed experimental plan of date fruit- natural extracts-based energy drinks

Ingredients	Treatments*												
	Control	G <sub>1</sub>	G <sub>2</sub>	G <sub>3</sub>	G <sub>4</sub>	M <sub>1</sub>	M <sub>2</sub>	M <sub>3</sub>	M <sub>4</sub>	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>
Sucrose (g)	95	0	0	0	0	0	0	0	0	0	0	0	0
Ginger extract (mL)	0	2.5	5	7.5	10	0	0	0	0	5	5	5	5
Moringa extract (mL)	0	0	0	0	0	2.5	5.0	7.5	10	7.5	7.5	7.5	7.5
Caffeine (mg)	360	360	360	360	360	360	360	360	360	360	280	200	120
Vitamin B <sub>1</sub> (mg)	80	80	80	80	80	80	80	80	80	80	80	80	80
Vitamin B <sub>2</sub> (mg)	40	40	40	40	40	40	40	40	40	40	40	40	40
Vitamin B <sub>3</sub> (mg)	80	80	80	80	80	80	80	80	80	80	80	80	80
Vitamin B <sub>6</sub> (mg)	20	20	20	20	20	20	20	20	20	20	20	20	20
Vitamin B <sub>12</sub> (mcg)	20	20	20	20	20	20	20	20	20	20	20	20	20
Taurine (g)	4	4	4	4	4	4	4	4	4	4	4	4	4
Citric acid (mg)	600	600	600	600	600	600	600	600	600	600	600	600	600
Ascorbic acid (mg)	700	700	700	700	700	700	700	700	700	700	700	700	700
Sterilized distilled water (mL)	899.1	0	0	0	0	0	0	0	0	0	0	0	0
Date fruit juice (9.5° Brix) (mL)	0	991.6	989.1	986.6	984.1	991.6	989.1	986.6	984.1	981.6	981.7	981.8	981.9
Total volume (mL)**	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

\*G<sub>1</sub>, G<sub>2</sub>, G<sub>3</sub>, G<sub>4</sub>: ginger treatments; M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub>: moringa treatments; C<sub>1</sub>, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub>: caffeine treatments.

\*\*Trail batch of the preparations (1 liter of the final product).

#### *Physico-chemical properties*

The pH of EDs samples was determined using a digital pH meter (Denver Instruments Co., USA) while total soluble solids (TSS) were measured using an Abbe refractometer (AR4, Kruss, Germany) according to the

method described earlier (Raj *et al.*, 2019). The calorie content of EDs samples was calculated by following the method described by Saleh and Ali (2020).

#### *Total phenolic content*

The total phenolic content (TPC) was determined by following the method outlined by Bilek and Bayram (2015) using Folin-Ciocalteu's reagent. The absorbance readings were taken at 765 nm by a JENWAY spectrophotometer (Model 6405 UV/VIS, JENWAY®, Staffordshire, UK). The TPC values were expressed as milligrams of gallic acid equivalent (GAE) per mL sample.

#### *Antioxidant activity*

The antioxidant activity of EDs samples was assessed using DPPH radical scavenging assay according to Selahvarzi *et al.* (2021). The absorbance was determined with a spectrophotometer at 515 nm and the results were presented as the percentage of DPPH scavenging activity.

#### *Color parameters*

The color properties of EDs were determined using a colorimeter (CR-10, Konica Minolta, Japan) according to the method described earlier (Alqahtani *et al.*, 2021). The colour parameters were expressed as L\* (lightness; 0 = black, 100 = white), a\* (+60 = redness, -60 = greenness), and b\* (+60 = yellowness, -60 = blueness). In addition, the total color difference ( $\Delta E$ ) was calculated as follows:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

where  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  are the differences between the determined colour parameters of EDs and the standard (used as samples' background).

#### *Microbiological analysis*

The total bacterial count (TBC), yeasts and molds count, and coliform count of the EDs were determined by the plate counting method (Tomadoni *et al.*, 2017; Ismail, 2021).

#### *Sensory evaluation*

The sensory properties of EDs samples were evaluated based on the method described earlier (Jha & Gupta, 2016). Ten panelists from the staff members of Date Palm Research Center of Excellence, King Faisal University, Saudi Arabia, had been selected to conduct the test. The panelists were asked to evaluate the colour, taste, odour, and overall acceptability of EDs samples using the nine-point hedonic scale. Coded samples were presented to the panelists at room temperature ( $25 \pm 1$  °C) with normal light conditions.

#### *Statistical analysis*

All measurements of the present study were carried out in triplicates, presented as the mean  $\pm$  standard deviation, and subjected to analysis of variance (ANOVA). A significant difference ( $p < 0.05$ ) between means was determined via Duncan's multiple range test using SPSS software (SPSS Inc., version 16.0, Chicago, USA).

## **Results and Discussion**

### *Quality parameters of freshly prepared energy drinks*

Physicochemical properties, sensory evaluation, and microbiological analysis of freshly prepared EDs enriched with natural extracts are presented in Table 2. The samples that contained date juice showed lower pH values than the control sample. The lower pH of EDs prepared from date juice could be due to the acidic nature of fresh date juice as reported by Nadir *et al.* (2017). However, no significant differences in the pH

values were observed between the EDs samples made from date juice. As shown in Table 2, the TSS values of EDs samples ranged from 10.02 to 11.42. The C3 sample showed the highest TSS value, whereas the control showed the lowest. However, the TSS of all EDs samples was consistent with the values recommended by the standards of the Gulf Standardization Organization (GSO 05/FDS/1926:2021) (GSO, 2019). The TSS values of the EDs in the current study were close to those reported for EDs produced from agricultural wastes of melon seed powder and tea stalk caffeine by Selahvarzi *et al.* (2021). The calorie content of EDs ranged between 38.00 and 42.35 Kcal. The control sample showed the lowest calorie content while C3 showed the highest. However, slight differences in the calorie content were found between samples. Similar values of calorie content have been reported by Nowak and Gośliński (2020) for fruit-based energy drinks.

**Table 2.** Physiochemical properties, color parameters, antioxidant activity, and microbial analyses of fresh date fruit- natural extracts-based energy drinks

Parameter	Treatments			
	Control	G2	M3	C3
pH	5.61 ± 0.26 <sup>a</sup>	3.98 ± 0.15 <sup>b</sup>	3.92 ± 0.19 <sup>b</sup>	3.95 ± 0.22 <sup>b</sup>
TSS (%)	10.02 ± 0.24 <sup>b</sup>	10.53 ± 0.61 <sup>ab</sup>	10.67 ± 0.57 <sup>ab</sup>	11.42 ± 0.78 <sup>a</sup>
Calories (Kcal/100mL)	38.00 ± 0.95 <sup>c</sup>	40.13 ± 1.03 <sup>b</sup>	40.28 ± 1.15 <sup>b</sup>	42.35 ± 1.21 <sup>a</sup>
TPC mg GAE/mL	-	2.64 ± 0.28 <sup>c</sup>	3.35 ± 0.36 <sup>b</sup>	5.62 ± 0.44 <sup>a</sup>
DPPH (%)	14.23 ± 0.28 <sup>d</sup>	32.17 ± 0.92 <sup>c</sup>	39.45 ± 1.05 <sup>b</sup>	47.18 ± 1.13 <sup>a</sup>
L*	92.06 ± 1.19 <sup>a</sup>	31.15 ± 0.92 <sup>b</sup>	29.24 ± 0.77 <sup>c</sup>	29.38 ± 0.81 <sup>c</sup>
a*	7.23 ± 0.08 <sup>a</sup>	4.76 ± 0.25 <sup>b</sup>	3.29 ± 0.32 <sup>d</sup>	4.05 ± 0.62 <sup>c</sup>
b*	25.18 ± 0.64 <sup>a</sup>	12.65 ± 0.82 <sup>b</sup>	11.30 ± 0.90 <sup>b</sup>	11.93 ± 0.50 <sup>b</sup>
ΔE	24.18 ± 0.56 <sup>c</sup>	65.32 ± 0.75 <sup>b</sup>	66.92 ± 0.68 <sup>a</sup>	66.91 ± 0.79 <sup>a</sup>
Overall acceptability	8.20 ± 0.46 <sup>a</sup>	8.03 ± 0.38 <sup>a</sup>	8.23 ± 0.35 <sup>a</sup>	8.21 ± 0.43 <sup>a</sup>
Total bacterial count (log cfu/ml)	0.78 ± 0.03 <sup>a</sup>	0.26 ± 0.02 <sup>b</sup>	0.22 ± 0.01 <sup>c</sup>	0.13 ± 0.02 <sup>d</sup>
Yeasts and molds (log cfu/mL)	-	-	-	-
<i>Enterobacteriaceae</i> (log cfu/mL)	-	-	-	-

Results are expressed as the means ± standard deviation; n = 3. Values with different lowercase letters in the same row indicate significant differences ( $p < 0.05$ ) between treatments.

The results showed that the control sample was free of phenolic compounds, accordingly, it revealed the lowest antioxidant activity among all samples. On contrary, the EDs made from date juice and natural extracts (G2, M3, C3) showed higher total phenolic content (TPC) compared to the control. Since date fruits contain significant amounts of phenolic compounds (Al-Farsi *et al.*, 2007), this could be responsible for the higher TPC values of date juice-based EDs. Likewise, EDs samples prepared from date juice and natural extracts showed higher antioxidant activity than the control sample, which could be attributed to the higher TPC in date juice (Nadir *et al.*, 2017; Abdul-Hamid *et al.*, 2020). However, the C3 sample showed the highest TPC and antioxidant activity among all samples, which could be related to the presence of date juice, ginger, and moringa extracts, which in turn increased the TPC and consequently improved the antioxidant capacity. Our findings of TPC and antioxidant activity are consistent with those reported by Selahvarzi *et al.* (2021) who developed EDs with higher TPC and antioxidant activity from melon seed powder and tea stalks.

The colour parameters of freshly prepared EDs are displayed in Table 2. The EDs samples containing date juice exhibited lower L\*, a\*, and b\* values with higher ΔE values than the control, which could be attributed to the presence of natural pigments in date juice e.g., carotenoids and anthocyanins. The values of colour parameters obtained in the current study were close to those reported for date juice concentrate by Kulkarni *et al.* (2010). It is worth noting that the a\* value of the M3 sample was slightly lower than G2 and C3, which could be due to the presence of green pigments in moringa extract. Likewise, the b\* value of the G2 sample was slightly

higher than that obtained for M2 and C3, which may be due to the yellow pigments present in the ginger extract. Generally, the EDs containing date juice exhibited brownish color, which could be due to oxidation, caramelization, and Maillard reactions (Alqahtani *et al.*, 2021). However, the differences in colour values between EDs samples that contained date juice were insignificant.

Sensory evaluation results of freshly prepared EDs showed that the overall acceptability of all samples varied between 8.03 and 8.23 (Table 2). However, no significant differences in the overall acceptability scores were observed between samples. The microbiological quality of freshly prepared EDs was evaluated and the results are shown in Table 2. The total bacterial count (TBC) of EDs samples ranged from 0.13 to 0.78 log cfu/mL. In addition, all freshly prepared EDs samples including the control were free of yeasts, molds, and coliform growth. The samples containing date juice and natural extracts revealed lower TBC than the control, which could be due to their higher content of phenolic compounds compared to the control. Polyphenolic compounds have been well-recognized for antimicrobial activity (Selahvarzi *et al.*, 2021). Generally, C3 revealed the highest antimicrobial activity against bacteria, which could be due to the combined effect of ginger, moringa, and caffeine (Aziz, 2015; Quiñones-González *et al.*, 2019; Patil *et al.*, 2022). The antimicrobial effects of natural extracts were also observed by Selahvarzi *et al.* (2021) for melon seed powder and tea stalk-based Eds.

#### *Shelf-life studies*

##### Physiochemical properties

Changes in the physiochemical properties of EDs samples during storage are presented in Table 3. With storage, the pH values decreased significantly ( $p < 0.05$ ) for the control sample and insignificantly ( $p > 0.05$ ) for date fruit-natural extracts-based samples. It could be suggested that the decrease in pH values could be due to the Maillard reaction in which acidic groups are formed (Mtaoua *et al.*, 2017). Al-Sahlany *et al.* (2022) allotted the decrease in pH during storage to the fact that the sugars in date juice act as a substrate for lactic acid bacteria resulting in reduced pH. Similar findings were observed for the date, pineapple, pawpaw, and watermelon juices (Ibrahim, 2016; Al-Sahlany *et al.*, 2022). These authors found that the pH values of the developed juices decreased during storage. Generally, the decrease in the pH of date juice based-EDs samples was lower than that of the control. The C3 exhibited better stability of pH value among all samples during storage, which could be attributed to the effect of functional compounds in date, ginger, moringa, and caffeine.

The changes in the TSS of EDs samples were monitored throughout the storage period (Table 3). Generally, the TSS of all samples insignificantly changed ( $p > 0.05$ ) with the extended storage. Our findings in the current study agreed with Tomadoni *et al.* (2017) who reported that the TSS content of strawberry juice remained unchanged during storage.

The TPC and antioxidant activity of EDs samples were determined during the storage period (Table 3). At zero time, the control sample was free of phenolic compounds, while the TPC of date juice- natural extracts-based EDs samples varied between 2.64 and 5.62 mg GAE/mL. Likewise, the antioxidant activity was 14.23% for the control sample and varied from 32.17 to 47.18% for date juice- natural extracts-based EDs samples at zero time. Generally, the TPC and antioxidant activity of EDs samples decreased during storage. The decrease in TPC of the EDs could be due to the chemical reactions that occur as a consequence of processing and storage and the partial degradation of phenolic compounds by some enzymes such as polyphenol oxidase (Guiné and Barroca, 2014). The trends obtained in the present research were similar to those reported by Piljac-Žegarac *et al.* (2009); Mtaoua *et al.* (2017), who observed that the TPC and antioxidant activity of date juice and dark fruit juices decreased gradually with extended storage. The decrease in the antioxidant activity of EDs samples during storage is attributed to the decrease in the TPC. However, among all samples, C3 showed the best storage stability in terms of TPC and antioxidant activity, which could be attributed to its higher content of natural antioxidants.

**Table 3.** Changes in physicochemical properties, phenolic content, and antioxidant activity of date fruit-natural extracts-based energy drinks during storage at room temperature

Treatments	Storage period (month)			
	0	1	2	3
<b>pH</b>				
<b>Control</b>	5.61 ± 0.26 <sup>Aa</sup>	4.15 ± 0.30 <sup>Ab</sup>	3.51 ± 0.23 <sup>Ac</sup>	3.34 ± 0.17 <sup>Ac</sup>
<b>G2</b>	3.98 ± 0.15 <sup>Ba</sup>	3.83 ± 0.19 <sup>Ab</sup>	3.64 ± 0.24 <sup>Ab</sup>	3.47 ± 0.19 <sup>Ab</sup>
<b>M3</b>	3.92 ± 0.19 <sup>Ba</sup>	3.84 ± 0.22 <sup>Aa</sup>	3.72 ± 0.19 <sup>Aa</sup>	3.54 ± 0.32 <sup>Aa</sup>
<b>C3</b>	3.95 ± 0.22 <sup>Ba</sup>	3.89 ± 0.18 <sup>Aa</sup>	3.79 ± 0.31 <sup>Aa</sup>	3.66 ± 0.20 <sup>Aa</sup>
<b>TSS</b>				
<b>Control</b>	10.02 ± 0.24 <sup>Ba</sup>	10.00 ± 0.53 <sup>Ba</sup>	9.97 ± 0.60 <sup>Ba</sup>	9.95 ± 0.48 <sup>Ba</sup>
<b>G2</b>	10.53 ± 0.61 <sup>ABa</sup>	10.51 ± 0.62 <sup>ABa</sup>	10.49 ± 0.71 <sup>ABa</sup>	10.47 ± 0.62 <sup>ABa</sup>
<b>M3</b>	10.67 ± 0.57 <sup>ABa</sup>	10.64 ± 0.48 <sup>ABa</sup>	10.61 ± 0.53 <sup>ABa</sup>	10.60 ± 0.73 <sup>ABa</sup>
<b>C3</b>	11.42 ± 0.78 <sup>Aa</sup>	11.41 ± 0.54 <sup>Aa</sup>	11.38 ± 0.47 <sup>Aa</sup>	11.37 ± 0.55 <sup>Aa</sup>
<b>TPC</b>				
<b>Control</b>	-	-	-	-
<b>G2</b>	2.64 ± 0.28 <sup>Ca</sup>	2.43 ± 0.32 <sup>Cab</sup>	2.30 ± 0.19 <sup>Cab</sup>	2.12 ± 0.22 <sup>Cb</sup>
<b>M3</b>	3.35 ± 0.36 <sup>Ba</sup>	3.21 ± 0.25 <sup>Ba</sup>	3.04 ± 0.31 <sup>Ba</sup>	2.90 ± 0.26 <sup>Ba</sup>
<b>C3</b>	5.62 ± 0.44 <sup>Aa</sup>	5.51 ± 0.27 <sup>Aa</sup>	5.38 ± 0.18 <sup>Aa</sup>	5.27 ± 0.34 <sup>Aa</sup>
<b>DPPH</b>				
<b>Control</b>	14.23 ± 0.28 <sup>Da</sup>	11.37 ± 0.25 <sup>Db</sup>	7.15 ± 0.22 <sup>Dc</sup>	6.43 ± 0.28 <sup>Dd</sup>
<b>G2</b>	32.17 ± 0.92 <sup>Ca</sup>	27.23 ± 0.91 <sup>Cb</sup>	21.40 ± 0.84 <sup>Cc</sup>	14.62 ± 0.73 <sup>Cd</sup>
<b>M3</b>	39.45 ± 1.05 <sup>Ba</sup>	35.64 ± 0.77 <sup>Bb</sup>	30.73 ± 0.69 <sup>Bc</sup>	23.48 ± 0.64 <sup>Bd</sup>
<b>C3</b>	47.18 ± 1.13 <sup>Aa</sup>	43.57 ± 0.82 <sup>Ab</sup>	38.35 ± 0.75 <sup>Ac</sup>	32.17 ± 0.58 <sup>Ad</sup>

Results are expressed as the means ± standard deviation; n = 3. Values with different lowercase letters in the same row indicate significant differences ( $p < 0.05$ ) between treatments. Values with different capital letters in the same column indicate significant differences ( $p < 0.05$ ) concerning storage days

#### Color parameters

Color is one of the most important quality parameters of foods and beverages that, mainly, affect consumers' acceptability and preference for the product. Results regarding the changes in color parameters of date fruit-natural extracts-based EDs are shown in Table 4. It could be noticed that  $L^*$  values (lightness) of all EDs samples decreased insignificantly ( $p > 0.05$ ) during storage. Similar findings were reported by Mtaoua *et al.* (2017); Tomadoni *et al.* (2017); Illera *et al.* (2019) for the date, strawberry, and apple juices, respectively. These authors observed a decrease in the  $L^*$  values of the developed juices during storage. The decrease in  $L^*$  value during storage could be due to the dark compounds formed by the Maillard reaction, which may be enhanced during storage. Moreover, the decrease in  $L^*$  values can also be related to the partial precipitation of unstable suspended components in date juice (Mtaoua *et al.*, 2017). Similarly, the results of color measurements showed that the  $a^*$  and  $b^*$  values of EDs samples decreased slightly with extended storage. The trends obtained for the changes in the color parameters of the EDs samples in the current research were consistent with other reports (Mtaoua *et al.*, 2017). These authors observed a slight decrease in the  $a^*$  and  $b^*$  values of the date juice during storage. Generally, these changes were more obvious in the control sample. Such a decrease in  $a^*$  and  $b^*$  values could be related to the enzymatic degradation of natural pigments during storage. However, the changes in  $L^*$ ,  $a^*$ , and  $b^*$  values of the C3 sample were lower compared to other treatments which could be due to its richness in bioactive compounds.

**Table 4.** Changes in color parameters of date fruit- natural extracts-based energy drinks during storage at room temperature

Treatments	Storage period (month)			
	0	1	2	3
	<b>L*</b>			
<b>Control</b>	92.06 ± 1.19 <sup>Aa</sup>	91.43 ± 1.35 <sup>Aa</sup>	91.32 ± 1.23 <sup>Aa</sup>	86.15 ± 1.56 <sup>Ab</sup>
<b>G2</b>	31.15 ± 0.92 <sup>Ba</sup>	30.52 ± 0.83 <sup>Bab</sup>	30.17 ± 0.75 <sup>Bab</sup>	29.03 ± 0.87 <sup>Bb</sup>
<b>M3</b>	29.24 ± 0.77 <sup>Ca</sup>	28.64 ± 0.67 <sup>Cab</sup>	28.04 ± 0.78 <sup>Cab</sup>	27.52 ± 0.76 <sup>Bb</sup>
<b>C3</b>	29.38 ± 0.81 <sup>Ca</sup>	28.75 ± 0.55 <sup>Ca</sup>	28.46 ± 0.92 <sup>BCa</sup>	28.17 ± 0.59 <sup>Ba</sup>
	<b>a*</b>			
<b>Control</b>	7.23 ± 0.18 <sup>Aa</sup>	7.02 ± 0.26 <sup>Aa</sup>	6.75 ± 0.34 <sup>Aab</sup>	6.33 ± 0.50 <sup>Ab</sup>
<b>G2</b>	4.76 ± 0.25 <sup>Ba</sup>	4.63 ± 0.35 <sup>Bab</sup>	4.48 ± 0.18 <sup>Bab</sup>	4.12 ± 0.38 <sup>Bb</sup>
<b>M3</b>	3.29 ± 0.34 <sup>Da</sup>	3.20 ± 0.32 <sup>Da</sup>	3.04 ± 0.27 <sup>Da</sup>	2.90 ± 0.29 <sup>Ca</sup>
<b>C3</b>	4.05 ± 0.22 <sup>Ca</sup>	3.96 ± 0.14 <sup>Ca</sup>	3.85 ± 0.29 <sup>Ca</sup>	3.74 ± 0.32 <sup>Ba</sup>
	<b>b*</b>			
<b>Control</b>	25.18 ± 0.64 <sup>Aa</sup>	22.30 ± 0.72 <sup>Ab</sup>	20.62 ± 0.90 <sup>Ac</sup>	16.83 ± 0.65 <sup>Ad</sup>
<b>G2</b>	12.65 ± 0.82 <sup>Ba</sup>	10.28 ± 0.84 <sup>Bb</sup>	9.50 ± 0.65 <sup>BCbc</sup>	8.66 ± 0.73 <sup>Bc</sup>
<b>M3</b>	11.30 ± 0.90 <sup>Ba</sup>	10.55 ± 0.53 <sup>Bab</sup>	9.19 ± 0.82 <sup>Cbc</sup>	8.75 ± 0.58 <sup>Bc</sup>
<b>C3</b>	11.93 ± 0.50 <sup>Ba</sup>	11.43 ± 0.71 <sup>Ba</sup>	10.74 ± 0.59 <sup>Bab</sup>	9.57 ± 0.91 <sup>Bb</sup>
	<b>ΔE</b>			
<b>Control</b>	24.18 ± 0.56 <sup>Ca</sup>	21.52 ± 0.82 <sup>Cb</sup>	19.90 ± 0.70 <sup>Cc</sup>	18.34 ± 0.92 <sup>Cd</sup>
<b>G2</b>	65.32 ± 0.75 <sup>Bb</sup>	65.60 ± 0.90 <sup>Bab</sup>	65.85 ± 0.83 <sup>Bab</sup>	66.87 ± 0.77 <sup>Ba</sup>
<b>M3</b>	66.92 ± 0.68 <sup>Ab</sup>	67.42 ± 0.65 <sup>Aab</sup>	67.83 ± 0.85 <sup>Aab</sup>	68.32 ± 0.83 <sup>Aa</sup>
<b>C3</b>	66.91 ± 0.79 <sup>Aa</sup>	67.46 ± 0.73 <sup>Aa</sup>	67.65 ± 0.92 <sup>Aa</sup>	67.80 ± 0.78 <sup>ABa</sup>

Results are expressed as the means ± standard deviation; n = 3. Values with different lowercase letters in the same row indicate significant differences ( $p < 0.05$ ) between treatments. Values with different capital letters in the same column indicate significant differences ( $p < 0.05$ ) concerning storage days

#### Microbiological analysis

The principal factor responsible for limiting the shelf life of foods and beverages is microbial spoilage. Accordingly, the microbiological analysis [total bacterial count (TBC), yeasts and molds count, and coliform count] of prepared EDs samples was performed during storage (Table 5). The microbial quality of EDs samples at zero days was discussed previously (section 3.1). Generally, the TBC of all samples increased significantly ( $p < 0.05$ ) during storage, however, the values were within the acceptable range ( $< 6.0 \log \text{CFU/g}$ ) up to the end of storage (Seddiek *et al.*, 2022). The increase in TBC in the EDs samples that contained date juice and natural extracts was lower than the control. Moreover, the C3 exhibited the lowest TBC during storage, while the control sample revealed the highest. Singh *et al.* (Singh *et al.*, 2022) reported that the addition of date pulp improved the microbial quality and the shelf-life of date-based desserts due to their higher antioxidant and antimicrobial effects. In addition, a study conducted by Aziz *et al.* (Aziz, 2015) stated that ginger rhizomes extract revealed good antimicrobial activities due to its higher content of bioactive ingredients such as alkaloids, flavonoids, and saponins. Patil *et al.* (Patil *et al.*, 2022) reported that moringa leaves extract has strong antibacterial and antifungal effects against a wide range of microorganisms due to its higher content of bioactive compounds with antimicrobial effects such as pterygospermin, moringine, and benzyl isothiocyanate. Likewise, it is worth noting that caffeine has antimicrobial action against microorganisms as confirmed by the study conducted by Quiñones-González *et al.* (2019). Therefore, C3 showed the best storage stability in terms of microbiological quality, which could be due to the synergistic effect of date juice and the natural extracts of moringa, ginger, and caffeine. The growth of yeasts and molds was detected on control, G2 and M3, and C3 after 1, 2, and 3 months of storage, respectively. Regarding the coliform count, an indicator organism used to identify the hygienic conditions (Singh *et al.*, 2022), the EDs contained date juice and natural extracts were



free of coliform at zero time and through the storage period, while the growth of coliform was detected on the control sample after two months of storage. The trends obtained for the effects of storage time and natural extracts in the present study were consistent with the results reported by Selahvarzi *et al.* (Selahvarzi *et al.*, 2021), Singh *et al.* (2022), and Tomadoni *et al.* (2017) for melon seed powder- and tea stalk-based EDs, date-based dessert, and strawberry juice, respectively

**Table 5.** Changes in the microbial count of date fruit- natural extracts-based energy drinks during storage at room temperature

Treatments	Storage period (months)			
	0	1	2	3
	<b>Total bacterial count (log cfu/mL)</b>			
Control	0.78 ± 0.03 <sup>Ad</sup>	1.27 ± 0.04 <sup>Ac</sup>	2.00 ± 0.05 <sup>Ab</sup>	2.45 ± 0.06 <sup>Aa</sup>
G2	0.26 ± 0.02 <sup>Bd</sup>	0.65 ± 0.03 <sup>Bc</sup>	1.27 ± 0.03 <sup>Bb</sup>	1.69 ± 0.05 <sup>Ba</sup>
M3	0.22 ± 0.01 <sup>Cd</sup>	0.58 ± 0.01 <sup>Cc</sup>	1.14 ± 0.04 <sup>Cb</sup>	1.52 ± 0.04 <sup>Ca</sup>
C3	0.13 ± 0.02 <sup>Dd</sup>	0.34 ± 0.01 <sup>Dc</sup>	0.68 ± 0.02 <sup>Db</sup>	0.93 ± 0.02 <sup>Da</sup>
	<b>Yeasts and molds (log cfu/mL)</b>			
Control	-	0.31 ± 0.02 <sup>Ac</sup>	0.48 ± 0.03 <sup>Ab</sup>	0.66 ± 0.04 <sup>Aa</sup>
G2	-	-	0.19 ± 0.01 <sup>Bb</sup>	0.32 ± 0.03 <sup>Ba</sup>
M3	-	-	0.12 ± 0.02 <sup>Cb</sup>	0.27 ± 0.03 <sup>Ba</sup>
C3	-	-	-	0.14 ± 0.02 <sup>Ca</sup>
	<b>Coliform (log cfu/mL)</b>			
Control	-	-	0.57 ± 0.04 <sup>Ab</sup>	0.84 ± 0.05 <sup>Aa</sup>
G2	-	-	-	-
M3	-	-	-	-
C3	-	-	-	-

Results are expressed as the means ± standard deviation; n = 3. Values with different lowercase letters in the same row indicate significant differences ( $p < 0.05$ ) between treatments. Values with different capital letters in the same column indicate significant differences ( $p < 0.05$ ) concerning storage days

#### Sensory evaluation

The sensory quality attributes of foods and beverages are critical factors affecting consumers' acceptance (Seddiek *et al.*, 2022). Changes in sensory scores of EDs samples during storage at room temperature are presented in Table 6.

**Table 6.** Changes in sensory properties of date fruit- natural extracts-based energy drinks during storage at room temperature

Treatments	Storage period (month)			
	0	1	2	3
	<b>Color</b>			
Control	8.65 ± 0.52 <sup>Aa</sup>	8.12 ± 0.28 <sup>Ab</sup>	7.41 ± 0.19 <sup>Ab</sup>	6.57 ± 0.63 <sup>Ac</sup>
G2	8.31 ± 0.43 <sup>Aa</sup>	8.05 ± 0.42 <sup>Ab</sup>	7.65 ± 0.32 <sup>Ab</sup>	7.11 ± 0.70 <sup>Ab</sup>
M3	8.08 ± 0.37 <sup>Aa</sup>	7.93 ± 0.51 <sup>Ab</sup>	7.57 ± 0.40 <sup>Ab</sup>	7.08 ± 0.48 <sup>Ab</sup>
C3	8.25 ± 0.61 <sup>Aa</sup>	8.14 ± 0.39 <sup>Aa</sup>	7.74 ± 0.51 <sup>Aa</sup>	7.36 ± 0.29 <sup>Aa</sup>
	<b>Taste</b>			
Control	8.05 ± 0.51 <sup>Aa</sup>	7.65 ± 0.39 <sup>Ab</sup>	7.13 ± 0.29 <sup>Bbc</sup>	6.45 ± 0.52 <sup>Bc</sup>
G2	8.11 ± 0.45 <sup>Aa</sup>	7.82 ± 0.52 <sup>Ab</sup>	7.46 ± 0.18 <sup>ABab</sup>	7.10 ± 0.49 <sup>ABb</sup>
M3	8.47 ± 0.30 <sup>Aa</sup>	8.13 ± 0.80 <sup>Aa</sup>	7.81 ± 0.32 <sup>Aa</sup>	7.38 ± 0.61 <sup>ABa</sup>
C3	8.63 ± 0.37 <sup>Aa</sup>	8.32 ± 0.61 <sup>Aa</sup>	8.05 ± 0.41 <sup>Aa</sup>	7.73 ± 0.80 <sup>Aa</sup>
	<b>Odor</b>			
Control	8.11 ± 0.42 <sup>Aa</sup>	7.59 ± 0.70 <sup>Aab</sup>	7.04 ± 0.45 <sup>Abc</sup>	6.32 ± 0.24 <sup>Bc</sup>

<b>G2</b>	8.15 ± 0.37 <sup>Aa</sup>	7.73 ± 0.82 <sup>Aa</sup>	7.22 ± 0.71 <sup>Aa</sup>	7.00 ± 0.51 <sup>ABa</sup>
<b>M3</b>	8.62 ± 0.48 <sup>Aa</sup>	8.27 ± 0.56 <sup>Ab</sup>	7.86 ± 0.54 <sup>Ab</sup>	7.25 ± 0.75 <sup>ABb</sup>
<b>C3</b>	8.38 ± 0.25 <sup>Aa</sup>	8.11 ± 0.47 <sup>Ab</sup>	7.90 ± 0.36 <sup>Ab</sup>	7.46 ± 0.48 <sup>Ab</sup>
<b>Overall acceptability</b>				
<b>Control</b>	8.27 ± 0.46 <sup>Aa</sup>	7.79 ± 0.45 <sup>Ab</sup>	7.19 ± 0.28 <sup>Abc</sup>	6.45 ± 0.43 <sup>Ac</sup>
<b>G2</b>	8.19 ± 0.38 <sup>Aa</sup>	7.87 ± 0.77 <sup>Ab</sup>	7.44 ± 0.37 <sup>Ab</sup>	7.07 ± 0.55 <sup>Ab</sup>
<b>M3</b>	8.39 ± 0.35 <sup>Aa</sup>	8.11 ± 0.60 <sup>Ab</sup>	7.75 ± 0.40 <sup>Ab</sup>	7.24 ± 0.59 <sup>Ab</sup>
<b>C3</b>	8.42 ± 0.43 <sup>Aa</sup>	8.19 ± 0.54 <sup>Aa</sup>	7.90 ± 0.39 <sup>Aa</sup>	7.52 ± 0.46 <sup>Aa</sup>

Results are expressed as the means ± standard deviation; n = 3. Values with different lowercase letters in the same row indicate significant differences ( $p < 0.05$ ) between treatments. Values with different capital letters in the same column indicate significant differences ( $p < 0.05$ ) concerning storage days

The scores of sensory parameters [color, odor, taste, and overall acceptability] of all samples decreased during storage. The decrease in color scores could be related to the formation of dark compounds by the Maillard reaction, which may be motivated during storage. Mtaoua *et al.* (2017) interpreted the decrease in color scores of date juice during storage by the precipitation of unstable suspended components and the enzymatic degradation of natural pigments. Regarding taste, the results revealed a slight decrease in taste scores in all samples during storage. However, this decrease in taste scores could be related to the formation of a slight sour taste as a result of pH reduction with the extended storage. It could be noted that the findings of color and taste obtained by the sensory panel evaluation were following the instrumental results of color and pH. Likewise, the odor scores of EDs samples insignificantly ( $p > 0.05$ ) decreased with storage. The changes in odor scores may be due to the enzymatic and microbial degradation of volatile compounds and organic acids, which are mainly responsible for the odor of the product. Generally, the decrease in the sensory scores of date juice-based-EDs samples was lower than that of the control. In addition, the C3 revealed better storage stability in terms of sensory scores among all samples during storage, which may be due to the combined effect of functional compounds in date, ginger, moringa, and caffeine.

## Conclusions

In the present study, date juice- natural extracts-based energy drinks (EDs) were successfully prepared and evaluated. The freshly prepared EDs containing date juice and natural extracts revealed lower pH, higher TSS, and lower color parameters than the control. The total phenolic content (TPC), antioxidant activity, and microbiological quality of freshly prepared EDs were improved. During storage, the EDs samples contained date juice and natural extracts maintained their physicochemical properties (pH, TSS, and color parameters), TPC, antioxidant activity, microbiological quality, and sensory characteristics. Generally, the C3 revealed better storage stability among all samples in terms of the quality parameters evaluated. However, further studies about the purification of the juice, nutrients profile, and invitro digestibility, are needed.

## Authors' Contributions

Conceptualization: NKA and TMA; Data curation: HAM and SA; Formal analysis: FNMA and HAM; Funding acquisition: NKA; Investigation: FNMA and HAM; Methodology: FNMA and HAM; Project administration: NKA and TMA; Resources: FNMA and NKA; Software: SA; Supervision: NKA and HAM; Validation: SA; Visualization: HAM and SA; Writing - original draft: HAM and SA; Writing - review and editing: TMA. All authors read and approved the final manuscript.

### **Ethical approval** (for researches involving animals or humans)

Not applicable.

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### **Conflict of Interests**

The authors declare that there are no conflicts of interest related to this article.

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