Electronic Journal of Biotechnology 57 (2022) 24–30

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage:

Research Article

Storage quality and antioxidant properties of yogurt fortified with polyphenol extract from nutmeg, black pepper, and white pepper

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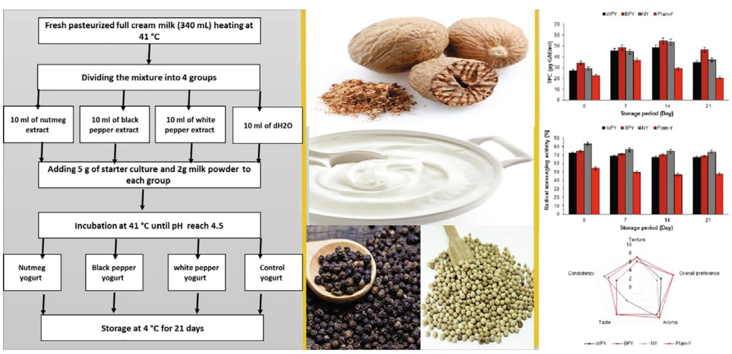
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 November 2021
Accepted 18 March 2022
Available online 23 March 2022

Keywords:
Antioxidant
Antioxidant-rich foods
Black pepper
Herbal yogurt
Nutmeg
Polyphenol compounds
Polyphenol extracts
Storage quality
White pepper
Yogurt

ABSTRACT

Background: The consumption of antioxidant-rich foods can lower the risk of chronic diseases. In the present work, plain yogurt (control) and three kinds of herbal yogurts fortified with a polyphenol extract from nutmeg, black pepper, and white pepper were prepared. Post-acidification, proteolysis, total phenolic content (TPC), and antioxidant activity of yogurt samples were studied over 21 days. Additionally, all yogurt samples were subjected to sensory evaluations on the first day of storage.
Results: Higher production ($p < 0.05$) of lactic acids was observed in all herbal yogurts than in plain yogurt over 21 days of storage. Black pepper and nutmeg enhanced ($p < 0.05$) the proteolytic activity of yogurt as compared to control, with the maximum values at 21 days. All three herbal yogurts showed the highest TPC (48–55 µg GAE/ml) at day 14 of storage. The radical scavenging activity of yogurt was positively affected ($p < 0.05$) by the presence of the three kinds of herbal extracts (72–83%; day 0), with the highest values observed in nutmeg yogurt. Ferric reducing antioxidant potential (FRAP) value of nutmeg yogurt was the highest ($p < 0.05$) among the other samples at day 7 of storage. The ferrous ion chelating (FIC) ability of all the three herbal yogurts ranged from 80% to 83% during 2 weeks. The highest overall preference scores among the three kinds of herbal yogurts were recorded for black pepper yogurt.
Conclusions: Polyphenol compounds derived from nutmeg, black pepper, and white pepper extracts could enrich yogurt with antioxidant properties, with nutmeg yogurt showing the best effect.
How to cite: Shori AB. Storage quality and antioxidant properties of yogurt fortified with polyphenol extract from nutmeg, black pepper, and white pepper. Electron J Biotechnol 2022;57. https://doi.org/10.1016/j.ejbt.2022.03.003

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso
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https://doi.org/10.1016/j.ejbt.2022.03.003
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1. Introduction

Secondary antioxidants, also known as non-enzymatic defense compounds, include carotenoids and chlorophyll derivatives, nitrogen compounds, and phenolic compounds, such as vitamin E, vitamin C, flavonoids, and phenolic acids [1]. Secondary antioxidants act as oxygen scavengers or chelating agents that can retard the rate of initiation and propagation of free radicals [2]. In addition, primary antioxidants are regenerated by secondary antioxidants through electron donation or hydrogens and are commonly added to foods to prevent lipid peroxidation [1].

Polyphenols are secondary metabolites found in the plant's cell walls. A polyphenol is an aromatic compound with different amounts of hydroxyl groups, which is linked by a glycoside or free glycoside [3]. The chemical structure of polyphenol can be divided into flavonoids (flavonol, isoflavones, and anthocyanin), phenolic acid, tannin, and lignin [4]. Polyphenol contains biologically active substances that have antioxidant properties, which have been regarded as the "seventh type of nutrient" for health [5]. Recently, polyphenols have been reported to exert positive effects on health and may reduce the use of synthetic drugs to alleviate metabolic complications [6,7]. Consequently, researchers have focused on identifying natural products that possess high antioxidant potential [8,9]. Black and white peppers are natural products that have both culinary and health applications. They cover about one-third of all global spice production, making them the most widely used spice crop [10,11,12,13]. Unlike black pepper produced from unripe and fully developed berries, white pepper is made from berries that are fully ripe [14]. Both black and white peppers contain lignans, alkaloids, flavonoids, aromatic compounds, and amides, and both peppers are known for their preservative [9], antioxidant [10,11,12,13], and antimicrobial [12,13] activities.

Nutmeg seed is known for its medicinal value and is used in culinary to enhance the flavor and aroma of food [15]. It contains α-pinene, β-pinene, p-cymene, β-caryophyllene, and carvacrol, which exhibit strong antioxidant properties [9,15]. It is also used as an antifungal, antimicrobial, anti-inflammatory, and even to reduce chronic liver disease [15]. Several studies have shown that phenolic antioxidants present in foods are potentially beneficial for human health [16,17,18]. Furthermore, phenolic antioxidants from plants can be used to promote the quality of dairy products [17].

Health-conscious consumers have long been considered yogurt to be a health-promoting product and could improve the digestive system [19,20]. Presently, yogurt with different formulas has been produced to enhance the product's health properties. This includes natural antioxidants derived from plant sources and/or bioactive peptides derived from milk proteins during yogurt fermentation [21,22]. Since the high demand for multicultural consumers for functional foods, dairy foods have driven the development of commercial new products in the market. Herbal supplements that contain significant amounts of biologically active compounds with a high antioxidant activity can be formulated into yogurt as a food supplement to provide health properties. Therefore, the purpose of this study is to investigate the development of post-acidification, proteolysis, total phenolic content (TPC), and antioxidant activity of yogurt by the addition of nutmeg, black pepper, and white pepper extracts as a source of polyphenols over 21 days of storage (4°C). Additionally, all yogurt samples were subjected to sensory evaluations on day 1 of storage.

2. Materials and methods

2.1. Herbal water extracts preparation

Dried seeds of nutmeg (*Myristica fragrans* Houtt.), black pepper, and white pepper (*Piper nigrum* L.) were purchased from a local herb store and ground into powder. The ground seeds (10 g) were mixed thoroughly with distilled water (dH_2O) at a ratio of 1:10. A hot water bath (70°C) was used to incubate the mixture for 12 h [23]. The centrifuge machine (Eppendorf®5804 R; 5000 *g*) was used for 15 min at 4°C to harvest the clear supernatant, and the extracts were used within a week.

2.2. Preparation of starter culture

Preparation of the starter culture followed the procedure described by Shori et al. [23]. One liter of fresh full-cream milk containing 4% fat was combined with a mixture of *Lactobacillus acidophilus* LA-5, *Bifidobacterium bifidum* Bb 12, *Lactobacillus casei* LC-01, *Streptococcus thermophilus* Th-4, and *Lactobacillus bulgaricus* in the ratio of 4:4:1:1:1 and incubated at 41°C for 12 h.

2.3. Preparation of probiotic yogurt

Probiotic plain yogurt and three types of herbal yogurt (nutmeg, black pepper, and white pepper yogurts) were prepared by mixing fresh pasteurized full cream cow milk (340 mL) and 20 g of starter culture [23]. The content of solids was adjusted by adding 8 g full cream milk powder (4% fat). The mixture was homogenized and divided into four portions, with each portion receiving 10 ml of nutmeg and 0.1 g/ml of black or white pepper water extract. The fourth portion was used as a control and 10 ml of dH_2O was added in place of herbal extract. All probiotic yogurt samples were incubated under the anaerobic condition at 41°C. The pH of the yogurt was determined every 30 min, and the incubation was terminated at pH 4.5 by placing the yogurt in a cold water bath for 1 h. The obtained yogurt samples were packed in plastic cups (100 mL) and refrigerated at 4°C for up to 21 days.

2.4. Determination of pH and titratable acidity

The pH and the titratable acidity (TA) of yogurt samples were determined according to Muniandy et al. [24].

2.5. Preparation of yogurt water extract

Plain or herbal yogurt (10 g) was homogenized with 2.5 ml dH_2O by using a homogenizer (Polytron® PT 1200 E), at the highest setting for 10 s [25]. The pH of the samples was acidified to pH 4.0 by adding HCl (0.1 M) and incubated in a water bath at 45°C for 10 min. The samples were centrifuged (5000 *g*, 4°C) for 10 min, and the supernatant was then adjusted to pH 7.0 using NaOH (0.1 M). This was followed by second centrifugation (5000 *g*, 4°C) for 10 min, and the clear supernatant was used for further analysis.

2.6. O-phthaldialdehyde assay

One milliliter of O-phthaldialdehyde (OPA) reagent (25 ml of 1000 mM sodium tetraborate, 2.5 ml of 20% (w/w) sodium dodecyl sulfate, 40 mg OPA dissolved in 1 ml of methanol, and 100 μl of β-mercaptoethanol, and the volume was made up to 50 ml by adding dH_2O to the yogurt extract (30 μl) in a 1.5 ml quartz cuvette. The absorbance of the solution was determined at 340 nm after 2 min of sitting at room temperature. The peptide concentration was evaluated against the tryptone standard curve (0.25–1.50 μg/ml; [25]).

2.7. Determination of total phenolic content

The TPC was achieved as described by Muniandy et al. [21].

2.8. Antioxidant properties

2.8.1. DPPH radical scavenging assay

The DPPH radical scavenging assay was determined according to Muniandy et al. [21].

2.8.2. Ferric reducing antioxidant potential assay

The ferric reducing antioxidant potential (FRAP) assay was conducted as described by Muniandy et al. [21].

2.8.3. Ferrous ion chelating ability assay

The ferrous ion chelating (FIC) ability assay was performed as described by Muniandy et al. [21].

2.9. Sensory evaluation

The sensory assessment of yogurt was conducted on the first day of storage at 4°C. A 12-member untrained panel conducted the assessment [26,27]. The assessment consists of five elements: texture, consistency, taste, and aroma. Each part was assessed based on a 10-point system (9–10 = very good, 7–8 = good, 5–6 = satisfactory, 3–4 = fairly satisfactory, 1–2 = unsatisfactory, and 0 = defective).

2.10. Statistical analysis

All experiments were completed in three separate batches. Every batch experiment was performed in duplicate. Data were expressed as mean ± standard error (SEM). One-way analysis of variance (ANOVA) and the significance of differences between means were determined at a level of $p < 0.05$. Statistical analysis was performed using IBM SPSS Statistics version 20.0 software.

3. Results and discussions

3.1. Post-acidification activity of herbal yogurt

The addition of nutmeg and white pepper in yogurt (0 day) significantly affected ($p < 0.05$) the pH reduction (4.47 ± 2.7 and 4.48 ± 2.2, respectively) as compared to control (4.56 ± 1.8; as shown Fig. 1). However, black pepper had no effect ($p > 0.05$) on the pH of yogurt at day 0 of storage. The pH values of herbal yogurt and control did not significantly differ after 7 days of storage (Fig. 1). However, it was found that white pepper yogurt displayed a significant reduction ($p < 0.05$) in pH value (4.42 ± 2.8) when compared with control (4.47 ± 3.1) after 14 days of storage. The pH reduction of plain and herbal yogurts was unaffected by further storage for 21 days.

According to the present study, nutmeg, black pepper, and white pepper extracts altered the acidity levels of yogurt significantly ($p < 0.05$) when compared with the control (Fig. 2). Similar results of total acidity were observed for all three kinds of herbal yogurts at 0 and 7 days of storage (0.72 and 0.81% LAE, respectively). The total acidity increased ($p < 0.05$) by 1.08%, 0.99%, and 1.08% LAE for nutmeg, black pepper, and white pepper yogurt, respectively, while control showed total acidity of 0.81% LAE after storage for 21 days (Fig. 2).

Fermentation of milk occurs because of the growth of microorganisms that utilize lactose to yield glucose and galactose by the enzyme lactase secreted by microorganisms to catalyze the reaction [24]. This glucose was catabolized for energy production; meanwhile, organic acids such as lactic acid were produced as by-products [28]. The increase in total acidity can be used as an indicator of microbial growth during fermentation which continued to increase during refrigeration [29]. In the present study, black pepper did not enhance pH reduction compared to plain yogurt during storage. Black pepper is composed of 5–9% of alkaloids, including piperidine, piperettine, and piperanine. These alkaloids could readily neutralize the lactic acids produced by lactic acid bacteria (LAB) in yogurt [30]. However, higher production ($p < 0.05$) of lactic acids was observed in all herbal yogurts than plain yogurt during storage periods (Fig. 2). It could be due to the metabolic activity of the starter culture which produced lactic acids and other organic acids [31]. It means that nutmeg, black pepper, and white pepper extracts enhanced the viability of starter culture in yogurt, resulting in higher production of lactic acids. This observation was consistent with previous studies that showed that plant extracts in yogurt enhanced the viability of LAB [23,31,32,33,34]. Further study is needed to investigate the viability of starter culture in herbal yogurt during fermentation and storage.

3.2. Peptide concentration of herbal yogurt

The presence of black pepper and nutmeg increased ($p < 0.05$) peptide concentration of fresh yogurt (0.44 ± 0.7 and 0.39 ± 0.5 m g/ml, respectively) compared to control (0.31 ± 0.4 mg/ml; as shown in Fig. 3). However, fresh white pepper yogurt showed no effect on peptide concentration ($p > 0.05$) in comparison with control. A significant increase in peptide concentration was observed ($p < 0.05$) for all three herbal yogurts ranging from 0.54 to

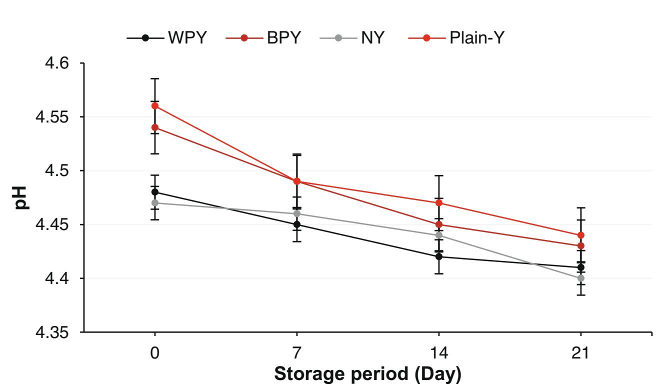

Fig. 1. The pH values of nutmeg (N), black pepper (B), and white (W) pepper (P) yogurts (Y) compared to that of plain yogurt (control) during 21 days of refrigerated storage at 4°C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period.

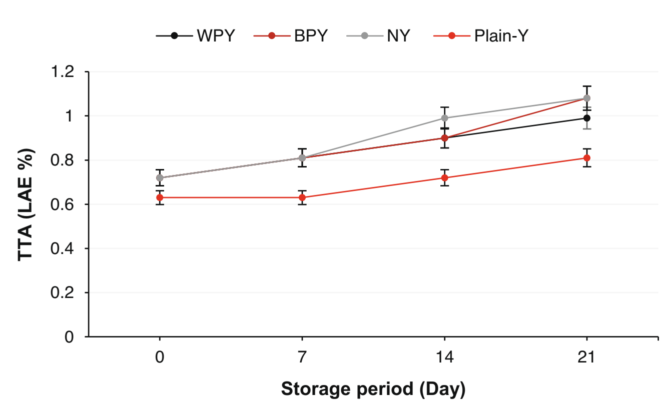

Fig. 2. Titratable acidity (TA; lactic acid equivalent %) of nutmeg (N), black pepper (B), and white (W) pepper (P) yogurts (Y) compared to those of plain yogurt (control) during 21 days of refrigerated storage at 4°C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period.

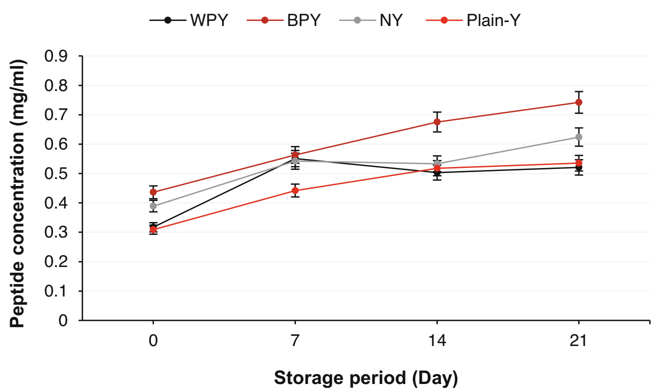

Fig. 3. Peptide concentration (mg/g) of nutmeg (N), black pepper (B), and white (W) pepper (P) yogurts (Y) compared to that of plain yogurt (control) during 21 days of refrigerated storage at 4°C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period.

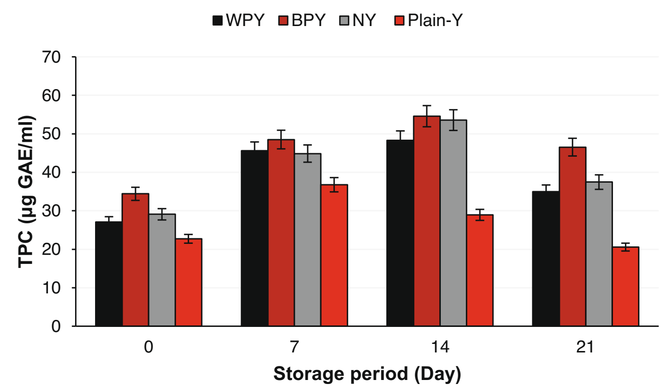

Fig. 4. Total phenolic content (TPC; µg GAE/ml) of nutmeg (N), black pepper (B), and white (W) pepper (P) yogurts (Y) compared to plain yogurt (control) during 21 days of refrigerated storage at 4°C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period.

0.56 mg/ml compared to control (0.44 ± 1.0 mg/ml) at 7 days of storage. On day 14 of storage, black pepper yogurt registered the highest peptide concentration (0.68 ± 1.2 mg/ml; $p < 0.05$) among samples that showed almost similar values (Fig. 3). The peptide concentration increased ($p < 0.05$) only for black pepper and nutmeg yogurts after 21 days of storage (Fig. 3).

The proteolysis of milk proteins by LAB can deliver a wide range of bioactive peptides [35]. There are multiple therapeutic benefits conferred by these peptides, such as antioxidant activity [36]. A previous study reported that bioactive peptides with antioxidant properties found in yogurt prevented free radical formation or scavenged free radicals and oxygen species that cause oxidative damage to biomolecules and cause cancer, heart disease, stroke, and arteriosclerosis [37]. According to this study, a higher peptide content in herbal yogurts was an indicator of increased metabolic activities of LAB. This speculation was supported by TA measurement (Fig. 2) which recorded an increase in the acidity of all herbal yogurts during the storage periods. In addition, the highest peptide content was shown in black pepper yogurt during the entire storage period (Fig. 3). Black pepper water extract was found to contain a higher amount of peptide (3.74 ± 2.4 mg/ml; data not shown) than other herbal extracts (0.83 ± 1.7 and 0.98 ± 2.1 mg/ml for white pepper and nutmeg, respectively). No significant differences in peptide content were found between white pepper yogurt and control during the storage periods. It may be due to the low level of peptides in white pepper extract (0.83 ± 1.7 mg/ml; data not shown). Milk proteins are known as a key source for a variety of bioactive peptides encrypted within native protein sequence and can thus be degraded during proteolytic activity [38]. Therefore, nutmeg, black pepper, and white pepper extracts may induce LAB in yogurt to produce more peptides that may contribute to health benefits.

3.3. Total phenolic content of herbal yogurt

Fresh nutmeg, black pepper, and white pepper yogurts had significantly higher TPC (29.09 ± 1.0, 34.41 ± 1.9, and 27.08 ± 1.4 µg GAE/ml, respectively) than control (22.69 ± 0.8 µg GAE/ml; as shown in Fig. 4). A significant increase in TPC was observed for all three herbal yogurts, ranging between 45–48 µg GAE/ml as compared to that in the control (36.75 ± 1.1 µg GAE/ml) on day 7 of storage. TPC of all herbal yogurts improved ($p < 0.05$) compared to control at 14 days of storage (Fig. 4). A significant reduction in TPC was observed for all yogurt samples at the end of storage. However, black pepper yogurt had the highest TPC (46.53 µg GAE/ml; $p < 0.05$) followed by nutmeg (37.45 µg GAE/ml; $p < 0.05$) and white pepper (34.96 µg GAE/ml; $p < 0.05$) yogurts, respectively, at 21 days of storage, while control yogurt had TPC of 20.587 µg GAE/ml.

Phenolic compounds have antioxidant activity and possess anti-inflammatory, anticancer, antimicrobial, antitumor, and antiulcer properties [17]. Therefore, phenolic compounds in plants may act as antioxidants. In the present study, a positive effect on TPC exerted by the yogurt fortified with nutmeg, black pepper, and white pepper water extracts was noted compared to control (Fig. 4). These findings may be attributed to the high content of polyphenols found in nutmeg, black pepper, and white pepper extracts (133.44, 155.34, and 34.522 µg GAE/ml, respectively; as shown in Table 1). According to Agbor et al. [14], black pepper extracts contain significantly ($p < 0.001$) more polyphenols than white pepper extracts. A total of 186 phenolic and polyhydroxy compounds were found in black pepper extract, including anthocyanins, proanthocyanidins, catechin derivatives, flavanones, flavones, flavonols, isoflavones, 3-O-p-coumaroyl quinic acid, O-hexoside, quinic acid (polyhydroxy compounds), etc. [39]. The water extract of black pepper had the highest TPC among other herbal extracts (Table 1); however, there was no difference in the TPC between black pepper yogurt and the other two herbal yogurts at days 7 and 14 of storage. A possible explanation for this could be the interaction between milk proteins (caseins) and phenol compounds [21]. A previous study found an increase in the size of casein aggregates when green, tea, grape, or cranberry extracts were mixed with casein due to phenolic compounds and casein interactions [40]. In addition, the authors have concluded that phenolic compounds that interacted with the protein would remain in the protein matrix, increasing its peak size and resulting in a decrease in the phenolic peak intensity.

A rise in TPC was observed in the three herbal yogurts after 7 and 14 days of refrigeration. The degradation of milk proteins by yogurt bacteria releases some phenolic compounds [17,21]. The amount of TPC present in plain yogurt is associated with non-phenolic compounds such as sugars, proteins, amino acids, and small peptides that may interfere with the measurement of the TPC [41].

3.4. Antioxidant activity of herbal yogurt

The presence of all three kinds of herbal extracts positively affected ($p < 0.05$) the scavenging ability of yogurt during the storage periods (Fig. 5). Plain yogurt showed radical scavenging activity of 54.52% (0 day). Nutmeg, black pepper, and white pepper

Table 1
Total phenolic content (TPC), DPPH radical scavenging activity, ferric reducing antioxidant potential (FRAP), and ferrous ion chelating (FIC) of black pepper (B), white (W) pepper (P), and nutmeg (N) water extracts (WE). Data are presented as mean ± SEM.

Samples	TPC (µg GAE/ml)	DPPH radical scavenging activity (%)	FRAP (µM Fe2 + E/ml)	FIC (%)
NWE	133.44 ± 0.7	93.04 ± 1.5	2594.29 ± 1.9	97.32 ± 1.1
BPWE	155.34 ± 0.4	81.74 ± 1.2	4051.43 ± 2.1	84.56 ± 0.7
WPWE	34.52 ± 0.35	76.37 ± 1.9	2794.29 ± 2.7	95.54 ± 0.9

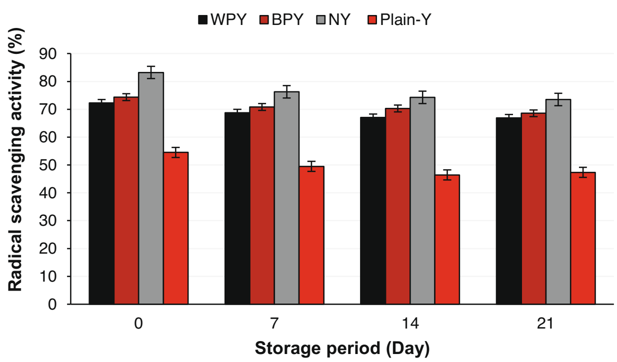

Fig. 5. Radical scavenging activity (%) of nutmeg (N), black pepper (B), and white (W) pepper (P) yogurts (Y) compared to that of plain yogurt (control) during 21 days of refrigerated storage at 4°C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period.

improved significantly the radical scavenging ability of yogurt to 83.2%, 74.4%, and 72.3%, respectively, on 0 day of storage. All yogurt's radical scavenging activity declined slightly ($p > 0.05$) during the 3 weeks of refrigeration (Fig. 5).

Herbal yogurts had no significant effect ($p > 0.05$) on FRAP when compared with control during storage periods (Fig. 6). However, nutmeg yogurt showed higher FRAP value (7401.43 ± 1.8 µM Fe^{2+} E/ml; $p < 0.05$) than control (5337.14 ± 1.2 µM Fe^{2+} E/ml) after 7 days of storage.

Plain yogurt showed an FIC ability of 52.06 ± 0.8% (0 day; as shown in Fig. 7). The presence of nutmeg, black pepper, and white pepper extracts in yogurt improved ($p < 0.05$) FIC ability to 79.76%, 75.64%, and 78.81%, respectively, on 0 day of storage. A sustained increase in FIC ability for all the three herbal yogurts ranged from 80% to 83% was seen during two weeks, whereas the FIC ability of control was decreased ($p > 0.05$) from 47.96% to 42.94% (Fig. 7). FIC

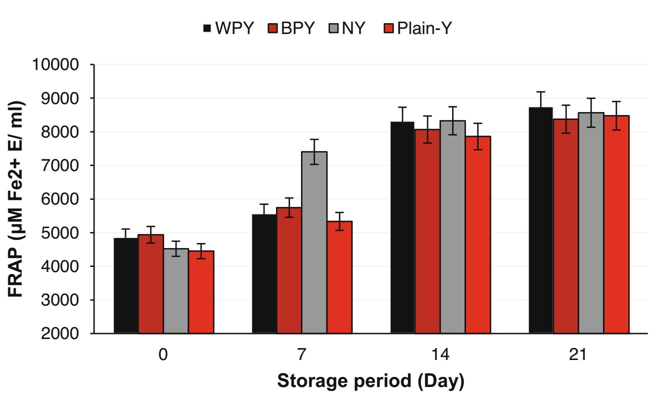

Fig. 6. Ferric reducing antioxidant potential (FRAP; µM Fe2 + E/ ml) of nutmeg (N), black pepper (B), and white (W) pepper (P) yogurts (Y) compared to that of plain yogurt (control) during 21 days of refrigerated storage at 4°C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period.

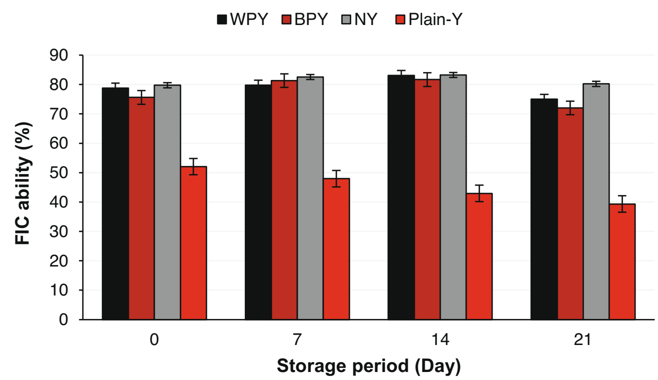

Fig. 7. Ferrous ion chelating (FIC; %) of nutmeg (N), black pepper (B), and white (W) pepper (P) yogurts (Y) compared to that of plain yogurt (control) during 21 days of refrigerated storage at 4°C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period.

ability in nutmeg, black pepper, and white pepper yogurts was reduced ($p < 0.05$) to 80%, 72%, and 75%, respectively, at day 21 of storage.

Based on the current study, the addition of nutmeg, black pepper, and white pepper extracts to yogurt significantly enhanced DPPH radical scavenging capability and FIC ability throughout storage periods (Fig. 5 and Fig. 7). The DPPH radical scavenging properties of nutmeg, black pepper, and white pepper extracts were 93.04%, 81.74%, and 76.37%, respectively (Table 1). In addition, the FIC ability was 97.32%, 84.56%, and 95.54% for nutmeg, black pepper, and white pepper extracts, respectively (Table 1). Moreover, the FRAP of nutmeg, black pepper, and white pepper extracts was found to be 2594.29% ± 1.9, 4051.43% ± 2.1, and 2794.29% ± 2. 7, respectively (Table 1). The FRAP results of all herbal yogurts did not differ significantly from plain yogurt except for 7-day-old nutmeg yogurt, which showed a significant ($p < 0.05$) improvement (Fig. 6). The elevation in the latter was associated with an increase in peptide content (Fig. 3). LAB has been shown to release bioactive peptides with antioxidant properties in yogurt [42]. Kennas and Amellal-Chibane [43] reported that yogurt containing 0.5% of pomegranate peel powder and 5% of honey had 18% DPPH radical scavenging capability on day 1. However, the present results showed higher radical scavenging activity in nutmeg, black pepper, and white pepper yogurts ranging between 72% and 83% on day 0 of storage. On the other hand, the addition of rosemary, dill, and oregano water extracts increased ($p < 0.05$) the radical scavenging activity of fresh yogurt to almost 90% [44]. Higher ($p < 0.05$) FIC ability was observed in coriander and cumin seeds yogurts reached 90% [38], which is almost comparable to the current results.

The results obtained for antioxidant activity can serve as the basis for the assessment of the preventative role of the three kinds of herbal yogurts against free radical-mediated lipid peroxidation, which is linked to many diseases, and thus is able to enhance the therapeutic effect of the yogurt.

The oxidation of lipids in foods causes the formation of undesirable compounds with off-flavors which may be harmful to health. Antioxidants are used during food processing to retard the oxida-

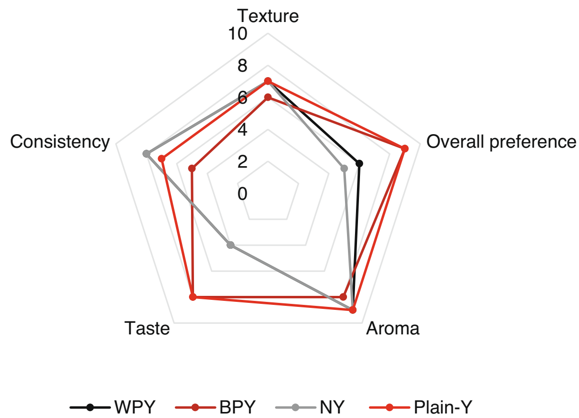

Fig. 8. Sensory evaluation of nutmeg (N), black pepper (B), and white (W) pepper (P) yogurts (Y) and plain yogurt (control) on the first day of refrigerated storage at 4°C. Data are presented as mean ± SEM. The level of significance was preset at $p < 0.05$ compared to control at the same storage period.

tion process [44]. Therefore, nutmeg, black pepper, and white pepper extracts can extend the shelf life of yogurt, as they possess high antioxidant activity and can remain active during storage.

3.5. Sensory evaluation of herbal yogurt

White pepper and nutmeg yogurts showed similar texture and consistency scores (7–8, good) compared to control, whereas black pepper yogurt showed less score (5–6, satisfactory; as shown in Fig. 8). In addition, white pepper and nutmeg yogurts scored lower (3–4, fairly satisfactory) for taste than black pepper yogurt, which was scored the same as the control (8, good). The score of aroma was not affected by the addition of white pepper and nutmeg to yogurt as compared to that of control (very good), except for black pepper yogurt which showed a good score (Fig. 8). The highest overall preference scores among the three kinds of herbal yogurts were recorded for black pepper yogurt (very good).

The "taste" was considered as food is always chosen for its good taste. In the present study, the lower scores for taste with the addition of nutmeg and white pepper to yogurt suggested that consumers did not prefer these yogurts. However, Illupapalayam et al. [45] reported a slightly higher taste score for nutmeg yogurt containing *L. acidophilus* or *Bifidobacterium animalis* ssp. *lactis* (5.30 ± 0.3 and 5.96 ± 0.4, respectively) based on 9 points hedonic scale. The low scores for texture and consistency of black pepper yogurt suggested that black pepper was less effective at improving texture and consistency than nutmeg and white pepper. Based on the present findings, black pepper yogurt had a higher acceptance rate by consumers than the other two herbal yogurts.

4. Conclusions

The presence of nutmeg, black pepper, and white pepper extracts enhanced pH reduction in yogurt except for black pepper yogurt over 21 days of storage. Moreover, the highest proteolytic activity was observed in 21-day-old black pepper and nutmeg yogurts. The highest TPC was found in all three herbal yogurts on day 14. The radical scavenging activity of yogurt was positively affected by the presence of the three kinds of herbal extracts. In addition, the FRAP value of 7-day-old nutmeg yogurt was the highest among all samples. The FIC ability for all three herbal yogurts was significantly improved compared to plain yogurt. Black pepper yogurt was well accepted by the panel as compared to the other yogurts. Polyphenol compounds from nutmeg, black pepper, and white pepper affected the functional and nutritional values of yogurt and contributed to antioxidant properties. Further analysis is needed to determine properties such as syneresis, water-holding capacity, color, and texture in herbal yogurt.

Financial support

This project was funded by Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No. (D-324-247-1443). The authors, therefore, gratefully acknowledgment the DSR technical and financial support.

Conflict of interest

There is no conflict of interest.

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