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Quality characteristics and antioxidant activity of goat milk yoghurt fortified with *Lycium ruthenicum* Murr. fruit

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Abstract: This study aimed to analyse the influence of adding *Lycium ruthenicum* Murr. fruit (LRF) on physicochemical, sensory characteristics and antioxidant capacity in goat milk yoghurt (GMY) during 21 days of refrigerated storage. Three different yoghurt formulations were processed using varying amounts of added LRF (1.0, 1.5, and 2.0%). LRF supplementation improved some of the physicochemical characteristics and sensory characteristics of GMY. With the increase of LRF addition, the pH of GMY showed a downward trend, and the titrated acidity showed an upward trend. The corresponding number of colonies in the storage period was higher than that of yoghurt without LRF. The addition of a concentration of 1.0–2.0% LRF played a role in the proliferation of lactic acid bacteria, and viscosity and hardness increased with increasing LRF addition. Although the susceptibility to syneresis of GMY showed a downward trend with increasing LRF addition, the scavenging rate of DPPH (2,2-diphenyl-1-picrylhydrazyl), •OH (hydroxyl free radicals), and the reducing power of Fe^{3+} of the corresponding concentration of GMY increased. In general, GMY with LRF addition can combine the two nutrients and have higher nutritional value, which is a good supplement to the same types of products in the market.

Keywords: fruit; dairy foods; physicochemical properties; sensory attributes; anthocyanin activity

Lycium ruthenicum Murr. (LR) is a functional food that belongs to the genus *Lycium* of the family *Solanaceae*. Its fruit is locally named Hei guo gou qi (Heigouqi) or black goji berry (Islam et al. 2017; Vidana et al. 2023). It has been used as a remedy since ancient times in China for its emmenagogue, diuretic, antipyretic, tonic, aphrodisiac, hypnotic, and hepatoprotective effects (Altintas et al. 2006; Tao et al. 2022). During the past few decades, researchers have found several biologically active molecules in the fruit of LR, such as polysaccharides, pigments, essential oils, carotenoids, vitamin C, and phenolic compounds (Wang et al. 2018). Bioactive properties of *Lycium ruthenicum* Murr. fruit (LRF), including immuno-enhancement effects, anti-inflammatory activity, anti-oxidation, anti-ageing, anti-fatigue, and hypoglycemic activity has been widely investigated (Peng et al. 2023; Vidana et al. 2023). Furthermore, it is used as a unique

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nutritional food and can be eaten as a fruit or used as a raw material for beverages due to the characteristics of sweetness and juiciness (Lv et al. 2013; Wang et al. 2018).

The demand for new functional dairy foods has increased recently (Vasquez 2018; Abdel-Hamid et al. 2020). Various types of dairy products exist throughout the world, mainly from bovine milk. However, there is an increase in the use of goat milk. Goat milk is increasing, goat milk is used as an alternative to bovine milk in human nutrition, particularly in infant nutrition (Rutherfurd et al. 2006). Goat milk is an attractive source for infant feeding due to its high nutritional values, easy digestibility, and lower allergenic properties compared to bovine milk (Le Parc et al. 2014). However, goat milk has an undesirable 'goaty' flavour and some consumers express a dislike of goat milk products (Foda et al. 2009). Goat milk yoghurt (GMY) shows a lower overall acceptance compared with bovine milk yoghurt (Costa et al. 2014; Eissa et al. 2010). A further problem is softer curd during the fermentation process, and it is difficult to produce GMY with good consistency compared to bovine milk yoghurts (Clark et al. 2017), mainly due to the impact of naturally low α_{sl}-casein content and seasonally changing composition on the coagulation properties of goat milk (Farnsworth et al. 2006). Thus, it is not suitable for processing techniques used in the case of bovine milk to produce GMY without considering the difference in species.

In this context, various attempts have been made to improve the nutritional, texture, sensory features, and biological activities of GMY, including the incorporation of fruit and/or its derivatives, vegetables, or herbs. For example, adding jujube pulp improved the quality characteristics and antioxidant activity of GMY (Feng et al. 2019). Recently, we have assessed the effect of passion fruit pulp supplementation on antioxidant activity, and physico-chemical and sensory properties of GMY during storage (Yang et al. 2023). Furthermore, other ingredients were also added during goat milk fermentation, such as gums, honey, cyclodextrins, and transglutaminase (Young et al. 2012; Domagała et al. 2013; Machado et al. 2017). Park et al. (2019) have reported that locust beans and xanthan gums could be used in the production of GMY with enhanced textural properties. Furthermore, adding goji to the yoghurt caused a stimulation of the bacterial population from yoghurt (Rotar et al. 2014), and it has also been reported that encapsulated extracts of Goji berries could be used as prebiotic additives in food or nutraceuticals (Skenderidis et al. 2019). However, studies on the use of *L. ruthenicum* fruit (LRF) as an ingredient in dairy product formulation are still scarce and to our knowledge, there is no report on the incorporation of LRF in GMY. Considering the potential health benefits of biologically active molecules derived from LRF, the present work was carried out to produce a new dairy product with nutraceutical properties by GMY production with LRF addition. The physicochemical characteristics (pH, syneresis, hardness, viscosity), lactic acid bacterial counts, sensory features, and antioxidant activities [2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl free radicals (•OH), and Fe^{3+} reducing power] of GMY were evaluated after production during storage. The effect of LRF on the physicochemical properties, sensory attributes, and antioxidant activity of GMY was also investigated.

MATERIAL AND METHODS

Reagents and material. DPPH was purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals were analytical grade and were obtained from Aolong Chemical Co. (Luoyang, China). Dried fruits of LR were purchased from Dazhang Group (Luoyang, China).

Preparation of yoghurts. The physicochemical characteristics of LRF and goat milk were determined according to Pedro et al. (2019) and the National Food Safety Standard [(No. GB 19301 (2010)], respectively. The mean values of the physicochemical parameters of LRF and goat milk are shown in Table 1.

Table 1. Mean values of physicochemical parameters of *Lycium ruthenicum* Murr. fruit (LRF) and goat milk (GM) used for goat milk yoghurt

Parameters	Mean value
LRF	
Total solids (%)	81.54
Protein (%)	13.08
Fat (%)	0.68
pH	6.94
Anthocyanin ($mg \cdot g^{-1}$)	14.44
GM	
Non-fat solids (%)	8.80
Protein (%)	3.30
Fat (%)	3.57
pH	6.88
Lactose (%)	5.27

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The LRF was ground before use. Fresh goat milk was obtained in clean plastic containers from the local farm (Donggang, Luoyang, China). It was filtered through gauze to remove some impurities, and 7% cane sugar was added and stirred continuously. The mixture was then pasteurised at 95 °C for 5 min. The samples were packed separately in sterile glass bottles and cooled to 45 °C. LRF was added to the experimental milk samples at 0, 1, 1.5, and 2% (*w*/*v*) levels. The yoghurts were inoculated with 3% reactivated YO-MIX187 starter (*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; Danisco, Denmark) and incubated at 42 °C until coagulation, and then stored at 4 °C for 21 days. Each group had 3 parallel replicates.

Physicochemical analyses. Physicochemical analyses were done at 0, 3, 7, 14, and 21 days of refrigerated storage in triplicates for each sample. The pH values of the GMY samples were measured using a glass electrode pH meter (PHS-3C; Shanghai Leici Instruments Co., Ltd., China) and the titratable acidity value (expressed as Thorner degrees, °T) of each GMY sample was determined using a titrator system according to Li et al. (2022) method. Briefly, 10 g (accurate to 0.001 g) of GMY sample was placed into a 150 mL erlenmeyer flask and added with 20 mL of newly boiled water cooled to room temperature. The mixture was then titrated with standard sodium hydroxide solution using phenolphthalein as an indicator.

The susceptibility to syneresis (STS) of all yoghurt samples was determined using a drainage method according to a modification of Hassan et al. (1996). The test was carried out at 4 °C. Each sample was transferred into a funnel fitted with a 120-mesh stainless steel screen. The volume of whey collected over 2 h was measured in a 100 mL graduated cylinder.

The hardness analysis of all yoghurt samples was determined using a texture analyser (TA-XT plus; Stable Micro Systems Ltd., England) following a method described by Varghese and Mishra (Varghese and Mishra 2008) with a little modification, equipped with a 1 kg head. The probe (P/0.5) penetrated the samples to a depth of 15 mm (50% compression) at a test speed of 1.0 mm·s^{-1}. The force exerted on the probe was automatically recorded.

The apparent viscosity of all yoghurt samples was measured with a digital viscometer (NDJ-8S; Shanghai Fangrui Instrument Co., Ltd., China) with rotor number 3, and the shear velocities were 12 round·min^{-1}.

Microbiological analyses. Lactic acid bacteria (LAB) were counted according to the procedures of Ye et al. (2013). Viable counts of LAB were performed on 0, 7, 14, and 21 days in triplicate. Serial dilutions (10^{-1} to 10^{-7}) in MRS (de Man, Rogosa, Sharpe) agar (AOBOX Biotechnology Co., Ltd., China) were made for each GMY sample. Plates containing 20–200 colonies were selected, and the colonies were enumerated. The results were obtained as logarithms of the number of colony forming units (CFU) per mL (log CFU·mL^{-1}) of yoghurt.

Sensory evaluation. For sensory evaluation, 10 trained panellists (5 males and 5 females, aged between 18 and 25) from College of Food and Bioengineering, He'nan University of Science and Technology (Luoyang, China) were selected according to the scheme described by El-Sayed et al. (2002) with some modifications. Panellists were trained about basic tastes, aromas, texture, and flavours (García-Gómez et al. 2019). Briefly, a sensory analysis was carried out in a sensory evaluation room, yoghurt samples were delivered to the room in the glass bottles and kept refrigerated at 4 °C until needed. Sensory evaluation was begun after the sample was taken out and left at room temperature for 20 min, and the panellists individually tasted each yoghurt. Samples were evaluated after 0, 3, 7, 14, and 21 days of refrigerated storage through acceptability tests, focusing on attributes of appearance (20 points), texture (20 points), flavour (15 points), aroma (30 points), and taste (15 points).

Antioxidant capacity assay. The effects of the antioxidant capacity of LRF on yoghurt samples were determined with the DPPH method according to Zhao et al. (2020). The Fe3^{+} reducing power of all yoghurt samples was determined according to the method of Nguyen and Hwang (2016). •OH scavenging activity of all yoghurt samples was assayed as described by Sah et al. (2016).

Statistical analyses. All the data was entered to Microsoft Office Excel (software version 2013) and statistical analyses were carried out with SPSS (software version 25.0), and data were presented as mean ± standard deviation (SD). Data were compared using a one-way analysis of variance with Duncan's multiple range test, P < 0.05 was considered statistically significant for all analyses.

RESULTS AND DISCUSSION

Physicochemical analyses of LRF-GMY during storage. Figure 1 demonstrated the effects of added LRF on the pH values and titratable acidity of GMY during the storage period. The pH values of all yoghurt treatments ranged from 4.07 to 4.12 (Figure 1A). The GMY sample with 1% LRF addition did not show

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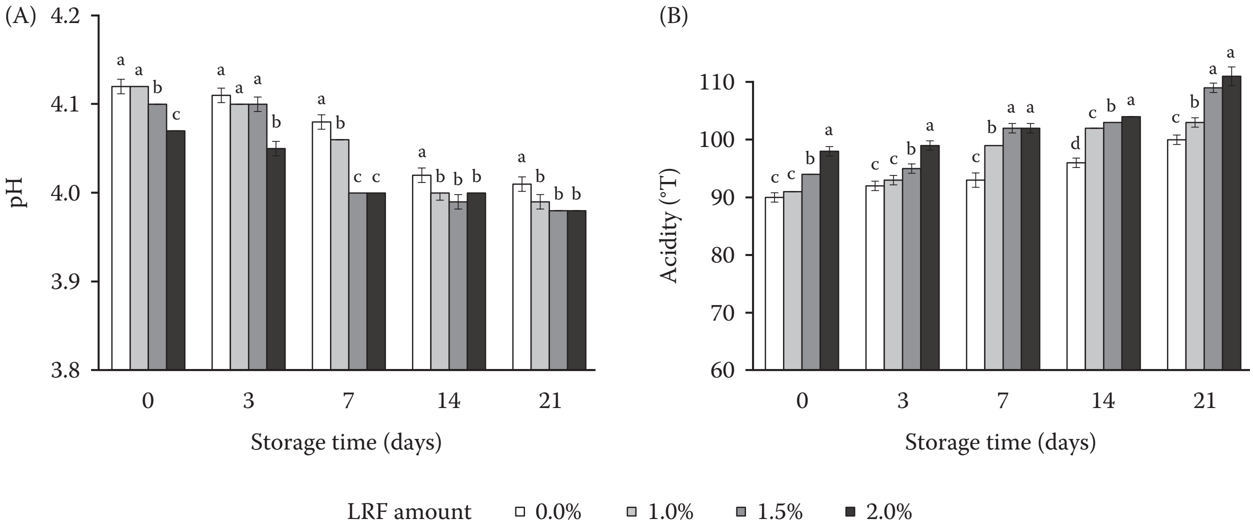

Figure 1. Effects of added *Lycium ruthenicum* Murr. fruit (LRF) on (A) pH and (B) titratable acidities values of goat milk yoghurt (GMY) during storage

a–d – significant differences in addition of LRF during each storage period ($P < 0.05$)

significant differences in pH compared to the control treatment ($P > 0.05$), meanwhile, increasing the amount of LRF (1.5% and 2%) significantly reduced the pH ($P < 0.05$) at 0 days. However, with the increase of LRF addition amount, the control treatment and the treatment group changed obviously. With the extension of storage time, the pH became lower, and there was no significant difference between different LRF addition amounts. This effect may be attributed to the stimulation of the bacterial growth resulting from LRF supplementation. Similar results were reported for probiotic yoghurt supplemented with *Siraitia grosvenorii* fruit, passion fruit peel powder, goji or pineapple waste powder (do Espírito Santo et al. 2012; Rotar et al. 2014; Sah et al. 2016; Abdel-Hamid et al. 2020). Additionally, the titratable acidity of GMY with the addition of LRF gradually increased with the extended storage time (Figure 1B), and the pH values showed a corresponding decreasing trend (Figure 1A). Similarly, a phenomenon was reported by Machado et al. (2017), who studied the trend of pH in honey GMY and found that honey had a positive effect on the acidification rate of GMY, while the pH values of the GMY formulations assessed continuously decreased during the entire storage period. However, Feng et al. (2019) found no significant differences between the formulations and the control during the storage period observed in both acidities and pH values of jujube GMY.

The hardness is an important parameter to evaluate the texture of yoghurt. The effect of LRF on the surface of yoghurt was shown in Figure 2. As shown in Figure 2, the hardness of GMY containing LRF first increased and then decreased with the extension of the storage period and increased overall with increasing concentration of LRF. The firmness of fermented milk was reported to be highly related to the composition of culture and protein content of the product, which confirmed our results. On the 21st day of storage, the hardness of all GMY formulations ranged from 26.7 to 35.3 g, which was lower than the values (48.4 g) of GMY obtained from Miocinovic et al. (2016). However, these results were significantly lower than that of cow milk yoghurt (329.46 g), which showed that GMY had a softer gel compared to cow milk yoghurt (Feng et al. 2019). Previous studies have also suggested that GMY has prolonged fermentation and soft curds, and the addition of bioactive components was found to be useful in making up for the two defects of GMY (Zhao et al. 2022). In the case of the same amount of LRF, with the extension of the storage period, the viscosity of GMY with the addition of LRF showed a trend of first increasing (0–14 days) and then falling (14–21 days) (Figure 3). During the same storage time, overall, the viscosity of GMY with LRF addition showed an upward trend as the amount of LRF increased (Figure 3). It may be due to the fiber contained in LRF that acted as a cutter disrupting the protein-protein interaction, with extension of storage time, the structure of yoghurt was destroyed, which damaged the protein-protein interaction in the test process, resulting in a decrease in viscosity (Espírito-Santo et al. 2013).

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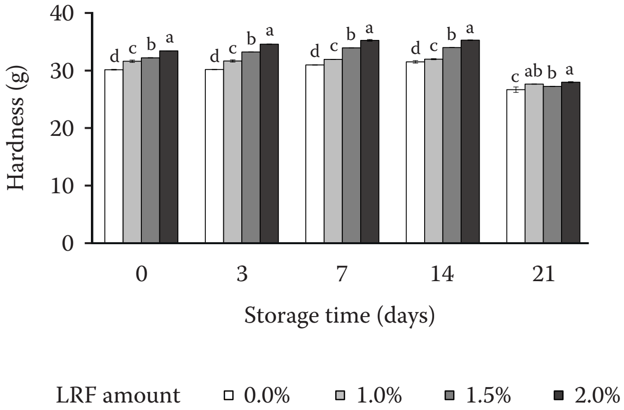

Figure 2. Effects of added *Lycium ruthenicum* Murr. fruit (LRF) on hardness of goat milk yoghurt (GMY) during storage

a–d – significant differences in addition of LRF during each storage period ($P < 0.05$)

Syneresis is an important defect in set yoghurt, as it can lead to poor consumer acceptance (Madhubasani et al. 2019). The syneresis of control and GMY with LRF addition is shown in Figure 4. Within the measurement range, there was no obvious difference between the samples with or without LRF addition except for 21 days, the STS of the samples with LRF addition was significantly higher than that of the control treatment at 21 days. The syneresis of GMY increased from 38.42% to 64.93% in 21 days of storage while the syneresis of GMY with 1% LRF addition increased from 41.79% to 72.47%. The addition of LRF affected the gel network structure of GMY and resulted in increased GMY STS. This phenomenon may be due to post acidification of GMY during storage first (Espírito-Santo et al. 2013). Evenmore with the LRF addition, the interaction may occur between protein and polysaccharides from LRF or produced by LAB in the yoghurt system. The increase in STS may be because of the increasing crosslinking levels between the protein aggregates and the polysaccharides in pores separated from the protein network structure (Arab et al. 2022). A similar result was already pointed out by Hassan et al. (2003), who reported the incompatibility of the exopolysaccharides with the protein aggregates in milk.

Microbiological analyses. The results of the viable counts of the starter bacteria inoculated in GMY containing or not containing added LRF during refrigerated storage are presented in Table 2. For all GMY, the maximum viable counts of the bacterial cultures were observed at 3 d. For GMY with LRF addition, the LAB count decreased from 8.00 to 7.45 log CFU·mL^{-1}, while for control yoghurt, the LAB count decreased from

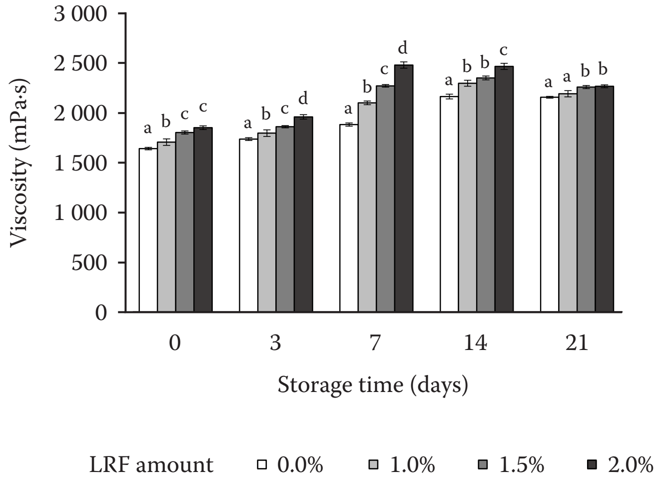

Figure 3. Effects of added *Lycium ruthenicum* Murr. fruit (LRF) on viscosity of goat milk yoghurt during storage

a–d – significant differences in addition of LRF during each storage period ($P < 0.05$)

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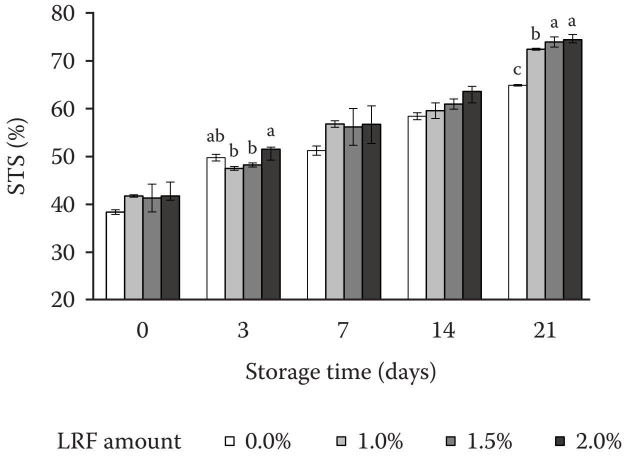

Figure 4. Effects of added *Lycium ruthenicum* Murr. fruit (LRF) on STS of goat milk yoghurt (GMY) during storage

a–c – significant differences in addition of LRF during each storage period ($P < 0.05$); STS – susceptibility to syneresis

7.90 to 7.08 log CFU·mL^{-1}. The decrease in LAB count for both yoghurts follows a similar trend. This shows that the addition of 1–2% LRF may have a certain probiotic effect on LAB, and the addition of LRF affects the viability of LAB through the protective effect of polysaccharides produced by it. This is consistent with the continuous decrease in the pH of GMY during storage. Recently study has also shown that *Lycium barbarum* polysaccharide can promote the growth of LAB in a certain concentration (Xu et al. 2022).

Sensory evaluation. The sensory properties of the yoghurt supplemented with LRF during storage are shown in Figure 5. The yoghurt with 1.5% LRF exhibited the highest appearance, texture, flavour, and aroma among all experimental yoghurts after 7 days of storage (Figure 5C). With increasing storage days, the colour of yoghurt with different additional amounts of LRF was getting deeper and deeper, which could be caused by the precipitation of pigments such as anthocyanin in LRF. The degree of sourness and sweetness changes from mild to moderate, delicate, and delicious, and finally to excessive acidity, which may be related to the fermentation and growth of lactic acid bacteria in yoghurt. With the increase of days, lactic acid bacteria consume sugar and other substances to produce lactic acid and then produce excessive acid. Meanwhile, the tissue state of yoghurt changes from the initial coagulation type to good coagulation, without whey precipitation. Due to the excessive acid production of yoghurt, the tissue state developed poor coagulation and precipitation of whey during 14–21 days. The smell of yoghurt gradually thickens and then fades with increasing storage time. This may be due to the reduction of aromatic substances in LRF. The flavour of yoghurt is the same as the taste, which is related to the growth of lactic acid bacteria. The aroma gradually thickens at first, then gradually fades at the later stage (14–21 days), it may be related to the post-acidification of yoghurt (Espírito-Santo et al. 2013).

Antioxidant activity of yoghurts. It can be seen in Figure 6, with the extension of the storage period, the DPPH free radical scavenging rate with or without LRF addition showed an upward trend caused by protein hydrolysis and increased organic acid content

Table 2. Changes in lactic acid bacteria (LAB) number (log CFU·mL^{-1}) of yoghurt during refrigerated storage

LRF concentrations (%)	Storage period (days)				
	0	3	7	14	21
control	7.90 ± 0.02$^{c, A}$	7.93 ± 0.02$^{c, A}$	7.83 ± 0.03$^{b, B}$	7.73 ± 0.03$^{b, C}$	7.08 ± 0.05$^{b, D}$
1.0	8.00 ± 0.03$^{b, AB}$	8.08 ± 0.02$^{b, A}$	7.96 ± 0.02$^{a, AB}$	7.92 ± 0.01$^{a, B}$	7.45 ± 0.11$^{a, C}$
1.5	8.11 ± 0.04$^{ab, AB}$	8.20 ± 0.02$^{ab, A}$	7.97 ± 0.04$^{a, AB}$	7.99 ± 0.05$^{a, B}$	7.48 ± 0.12$^{a, C}$
2.0	8.07 ± 0.02$^{a, AB}$	8.15 ± 0.04$^{a, A}$	8.00 ± 0.02$^{a, BC}$	7.95 ± 0.05$^{a, C}$	7.59 ± 0.09$^{a, D}$

$^{a–c}$ Mean values within a variable LRF concentration with the same lowercase superscript letters are not significantly different from each other ($P > 0.05$); $^{A–D}$ mean values within a row with the same uppercase superscript letters are not significantly different from each other ($P > 0.05$); CFU – colony forming unit; LRF – *Lycium ruthenicum* Murr. fruit

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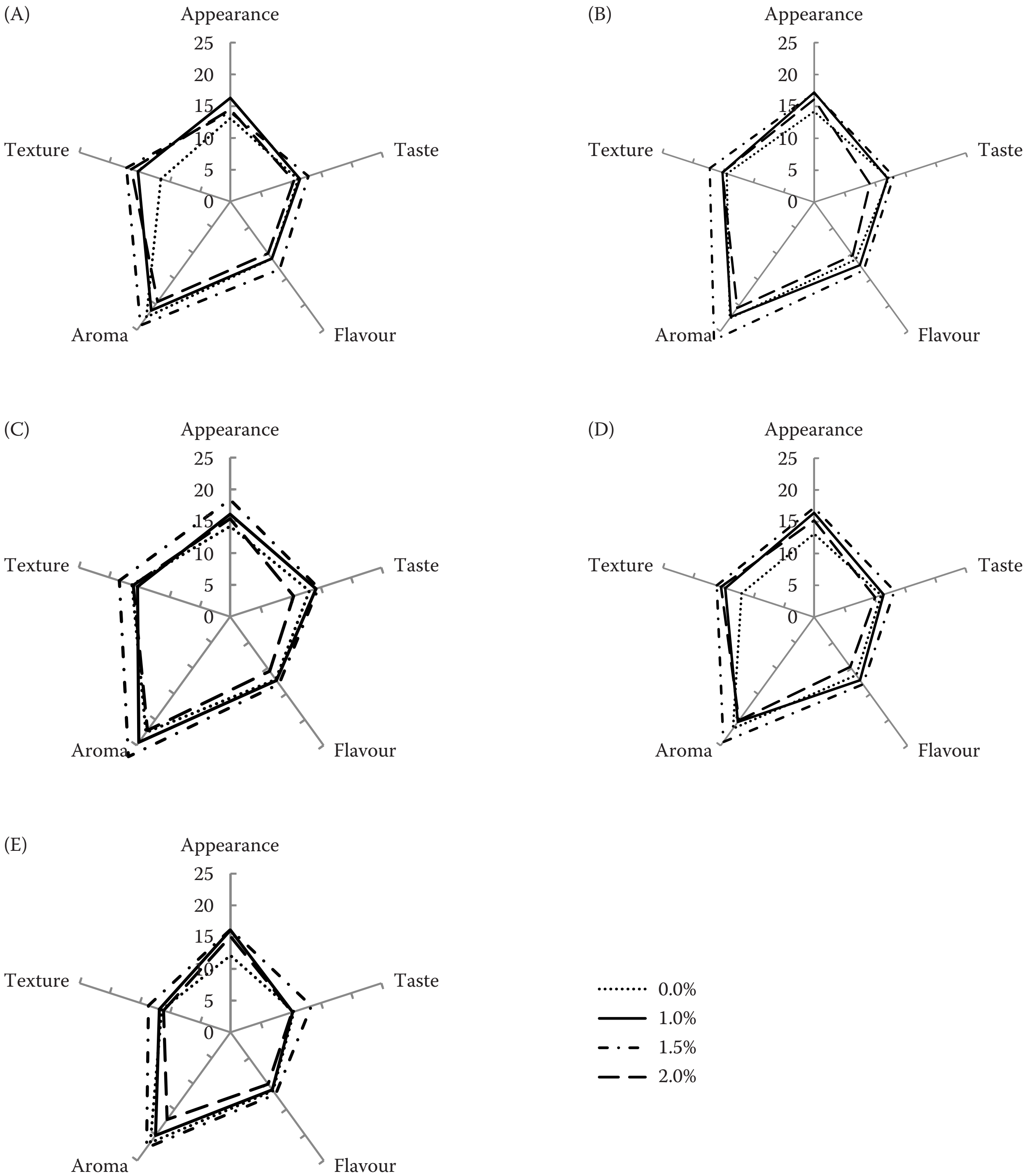

Figure 5. Sensory evaluation of goat milk yoghurt (GMY) supplemented with *Lycium ruthenicum* Murr. fruit (LRF) and control during storage at (A) 0 days, (B) 3 days, (C) 7 days, (D) 14 days, and (E) 21 days

caused by post acidification during storage, and the addition of LRF increased the DPPH radical scavenging ability of GMY; Meanwhile, with the increased content of LRF, the DPPH free radical scavenging rate of GMY gradually increased (Figure 6A). It was consistent with the changing trend of the DPPH free radical scavenging rate of LRF, which indicated that yoghurt could contain antioxidant substances.

With an extension of the storage period, the Fe^{3+} reducing power of GMY with added LRF was increased. With increasing LRF addition, the Fe^{3+} reducing power increased gradually, which was consistent with the

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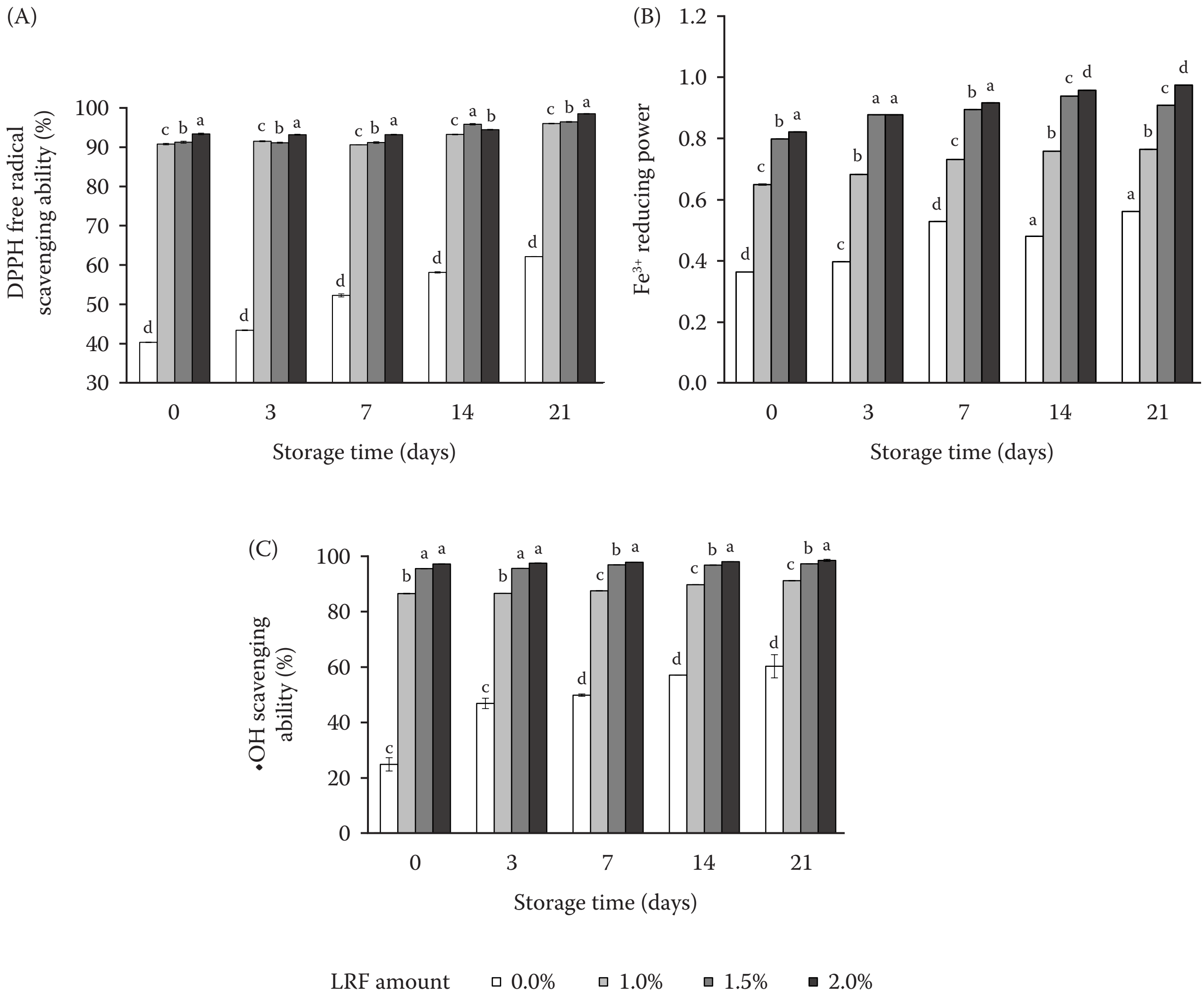

Figure 6. Effects of added *Lycium ruthenicum* Murr. fruit (LRF) on antioxidant activities of goat milk yoghurt (GMY) during storage: (A) DPPH free radical scavenging ability, (B) Fe^{3+} reducing power, and (C) •OH scavenging ability

a–d – significant differences in addition of LRF during each storage period ($P < 0.05$); DPPH – 2,2-diphenyl-1-picrylhydrazyl; •OH – hydroxyl free radicals

changing trend of the Fe^{3+} reducing power of LRF (Figure 6B). The reduction of Fe^{3+} to Fe^{2+} requires electron absorption, which indicates that LRF may contain this kind of electron-supplying substance.

With the prolongation of the storage period, the scavenging rate of the •OH of GMY with LRF addition increased gradually, and the scavenging rate of the •OH of GMY without LRF increased gradually; with LRF increase, it can be seen that the scavenging rate of the •OH of GMY increased (Figure 6C). The scavenging ability of free radicals is stronger, which is consistent with the changing trend of the scavenging rate of the •OH in LRF. These substances can be anthocyanins in LRF, which can enhance the scavenging ability of GMY to the •OH.

Previous studies have reported the antioxidant activity of LRF and GMY, respectively (Zheng et al. 2011; Liu et al. 2020; Papadaki et al. 2022). LRF has anthocyanin compounds and other bioactive compounds, and they have potential antioxidant activities. A similar result has been reported by Jaster et al. (2018), and the researchers found that yoghurt with a high anthocyanin content and higher antioxidant activity was produced. In summary, the antioxidant capacity was higher when GMY was fortified with LRF. With an increase in the content of LRF, the scavenging rate of DPPH and •OH and Fe^{3+} reducing the power of GMY increased and was significantly higher than the control yoghurt. It also indicated that the antioxidant activity (including the scaveng-

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ing rate of DPPH and •OH and Fe^{3+} reducing power) of GMY increased with the extension of storage time and LRF addition.

CONCLUSION

In conclusion, LRF is potentially useful in manufacturing GMY to improve its quality characteristics, and the nutritional and healthcare function of this product is in line with expectations. The results suggest that the incorporation of LRF into the GMY tested in this study has the potential to be a new, potentially functional goat dairy product.

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