

## Preliminary exploration of total antioxidant and oxidant status of novel high-protein milk products

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### Summary

This study aimed to determine the total antioxidant status (*TAS*) and total oxidant status (*TOS*) of the novel high-protein milk-products, which were designed to be protein-rich, lactose-free and reduced-fat milk-based. All different flavoured milk products with varying concentration of protein (52–82 g·l<sup>-1</sup>) of various brands were included. *TAS* and *TOS* were measured and oxidative stress indices (*OSI*) were calculated. Both *TAS* and *TOS* values of high-protein milk products with different aromas were found to be higher than conventional cows' milks ( $p < 0.05$ ). However, the *TOS* values of high-protein milk-products with vitamin addition appeared to be lower compared to milk with the same flavour but higher protein concentration ( $p < 0.05$ ). *OSI* value of peanut-banana-flavoured milk containing 52 g·l<sup>-1</sup> protein with B-complex vitamins and L-carnitine was  $6.05 \pm 1.05$ . However, the *OSI* value of coffee-flavoured milk product containing 76 g·l<sup>-1</sup> protein with vitamin B complex and vitamin D was the lowest with a value of  $1.36 \pm 0.05$ . In conclusion, this study determined that the antioxidant and oxidant capacities of high-protein milk products vary according to the brand, flavour, polyphenol concentration and, in some cases, depend on vitamin concentration. Vitamins for improvement of exercise performance and  $\beta$ -oxidation in exercise may also have a beneficial effect on oxidative stress, but the key-point seems to be their combination with components rich in polyphenols in milk-products.

### Keywords

high-protein milk; antioxidant; oxidant; oxidative stress

Modern nutritional science has begun to provide more information about the functions and possible mechanisms of specific food ingredients in health promotion and/or disease prevention [1]. Analysis of consumers' choices and expectations in the global market is of great significance in food innovations [2]. There is a worldwide tendency for food industries to transform nutritional information into consumer demand by improving food products that provide superior sensory appeal and nutritive as well as health benefits in response to increased health awareness. Recent innovations in food technologies include producing new health-promoting food ingredients together with reducing or removing undesirable food components, adding specific nutrients, modifying composition of food, masking undesirable flavours or stabilizing ingredients [1]. As an innovative model, milk and dairy products with increased protein concentration have recently gained increased consumer interest over the recent years [3].

Milk and dairy products are important for the nutrition of children, adults, elderly people, vegetarians and athletes, especially in terms of providing nutrients [4, 5]. Current data show that milk and dairy products contribute to meeting nutritional requirements and have protective effects against many chronic diseases [4]. Milk and dairy products are reported to protect against to some noncommunicable diseases such as primarily Type 2 diabetes, cardiovascular diseases, and some types of cancer (especially colorectal, bladder, gastric and breast cancer), and have positive impact on bone mineral density [4, 6]. Besides, it has recently become more prominent that milk and dairy products reduce childhood obesity and improve body composition in energy restriction applications, as well as help with loss of body weight in adults [7, 8]. Available data also indicate that consumption of milk proteins positively regulates human body composition. It has been stated that consumption of milk proteins in fat-restricted

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diets, particularly for body weight loss, reduces fat mass, increases lean body mass and indirectly contributes to metabolic health [9].

Milk has become a popular post-exercise recovery food nowadays [10]. Milk is a beverage that contains high-quality protein, carbohydrates, water, micronutrients and is almost isotonic. These features allow milk to be used in many cases for recovery purposes. Herein, its contributions to muscle protein synthesis or cycle, rehydration, management of exercise-related muscle aches and energy balance stand out [10]. Additionally, the issue that cows' milk has antioxidant properties that can reduce oxidative stress and carbohydrate-protein mixture consumption can decrease lipid peroxidation has recently gained popularity [11–13].

The relationship between exercise and oxidative stress is quite complicated and changes depending on the mode, intensity and duration of training [14]. It was reported that regular moderate exercises can be useful for health and oxidative stress which was defined as an imbalance between production of reactive oxygen species (ROS) and an adequate antioxidant defense. On the contrary, it is said that acute exercise is associated with an increase in oxidative stress [15]. Supporting the endogenous defense system with oral antioxidants can be essential in reducing or preventing oxidative stress through exercise. Compared to exogenous antioxidant supplements, natural foods can act as synergists to optimize the antioxidant effect [15]. In a supportive study, it was determined that the proteins and fat components, in particular casein, play a role in the antioxidant activities of whole milk, skim milk, acid whey and ultrafiltration permeate. Although the antioxidant capacities of beverages rich in polyphenols are four-fold greater than that of milk, antioxidant activity increases and they synergistically reduce toxic lipid peroxidation products in a combination of milk and polyphenols [16]. Still, there is almost no data on the antioxidant capacities of innovative milk and dairy products, which are often combined with a beverage with a high concentration of polyphenols and whose protein concentration is increased by ultrafiltration, and which are frequently recommended to be taken during and after exercise.

This study aimed to determine the total antioxidant status (TAS) and total oxidant status (TOS) of various types of novel high-protein milk products, which are designed to be protein-rich, lactose-free and reduced-fat milk-based available on the market for both athletes and non-athletes.

## MATERIALS AND METHODS

### Sample selection and acquisition

High-protein milk products and conventional ultra-high temperature-processed (UHT) cows' milk products (whole milk, reduced-fat milk, lactose-free milk) offered for sale in the markets in Turkey between January and July 2020 were included in the study (Tab. 1). In this context, ready-to-drink milk and milk produced with ultrafiltration and UHT technology available on the Turkish market countrywide was supplied from various supermarkets in Ankara, Turkey in special plastic bottles or Tetra Pak packaging (Tetra Laval, Pully, Switzerland). Twelve different high-protein milk products of seven different flavours and varying concentrations of proteins (10–30 g in product, resp. 52–82 g·l<sup>-1</sup>) from various brands were included in the study. This type of milk products includes lactose-free products and those fortified with Vitamin B<sub>1</sub>, Vitamin B<sub>6</sub>, Vitamin B<sub>12</sub>, Vitamin D and/or L-carnitine (Tab. 1). For comparison, conventional cows' milk (whole milk containing 3.0–3.3 % fat and reduced-fat milk containing 0.1 % fat) produced by the same companies were included in the study, if available. Since high-protein milk products are lactose-free, lactose-free milk products (containing 1.5 % fat) from the same brands were also included in the study for comparison. Each milk sample or brand were purchased in at least three different markets in Ankara, Turkey.

### Sample preparation

In order to determine TAS and TOS, all milk samples were centrifuged for 15 min at 15596 ×g and +4 °C and filtered through Chromafil Xtra PTFE filter (pore size 0.45 µm; Macherey Nagel, Düren, Germany). All analyses were carried out in duplicate.

### Total antioxidant status

TAS levels were measured using commercially available kits (Relassay, Gaziantep, Turkey) and Mindray BS300 Auto Biochemistry Analyzer (Mindray Bio-Medical Electronics, Shenzhen, China). The novel automated method is based on bleaching of the characteristic colour of a stable 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3 %. The change of absorbance at 660 nm is related to the total antioxidant level of the sample. The results were expressed as millimoles of Trolox equivalents per litre.

**Tab. 1.** General characteristics and nutritional facts of high-protein milk products.

| Product                             | 1         | 2         | 3          | 4             | 5         | 6       | 7         | 8         | 9         | 10          | 11        | 12       |
|-------------------------------------|-----------|-----------|------------|---------------|-----------|---------|-----------|-----------|-----------|-------------|-----------|----------|
| Protein in package [g]              | 10        | 30        | 26         | 26            | 27        | 12      | 25        | 26        | 27        | 30          | 25        | 30       |
| Flavour                             | Vanilla   | Vanilla + | Strawberry | Peanut-banana | Coconut   | Cocoa + | Cocoa     | Cocoa     | Cocoa     | Chocolate + | Coffee    | Coffee + |
| Company                             | A         | B         | A          | A             | C         | B       | D         | A         | C         | B           | D         | B        |
| Country of production               | Turkey    | Turkey    | Turkey     | Turkey        | Turkey    | Turkey  | Turkey    | Turkey    | Turkey    | Turkey      | Turkey    | Turkey   |
| Type of package                     | Tetra Pak | Bottle    | Tetra Pak  | Tetra Pak     | Tetra Pak | Bottle  | Tetra Pak | Tetra Pak | Tetra Pak | Bottle      | Tetra Pak | Bottle   |
| Package size [ml]                   | 200       | 500       | 500        | 500           | 330       | 200     | 330       | 500       | 330       | 500         | 330       | 500      |
| Declared production technology      | UHT, H    | UF, ST    | UHT, H     | UHT, H        | UHT       | UF, ST  | UHT       | UHT, H    | UHT       | UHT, SF     | UHT       | UHT, SF  |
| <b>Nutritional facts per 100 ml</b> |           |           |            |               |           |         |           |           |           |             |           |          |
| Energy [kJ]                         | 197       | 204       | 199        | 194           | 249       | 191     | 257       | 209       | 265       | 191         | 256       | 198      |
| Fat [g]                             | 0.3       | 0.1       | 0.3        | 0.3           | 0.3       | 0.1     | 1.0       | 0.3       | 0.5       | 0.1         | 1.0       | 0.1      |
| Saturated fat [g]                   | 0.2       | 0.1       | 0.2        | 0.2           | 0.2       | 0.1     | 0.7       | 0.2       | 0.3       | 0.1         | 0.7       | 0.1      |
| Carbohydrate [g]                    | 5.8       | 5.8       | 5.9        | 5.9           | 6.0       | 5.0     | 5.4       | 6.5       | 6.5       | 5.0         | 5.3       | 5.4      |
| Sugars [g]                          | 5.8 *     | 5.8*Suc   | 5.6 *      | 5.6 *         | 3.9       | 5.0*Suc | 5.4       | 5.6 *     | 4.2       | 5.0*Suc     | 5.3       | 5.4*Suc  |
| Proteins [g]                        | 5.2       | 6.0       | 5.2        | 5.2           | 8.2       | 6.0     | 7.6       | 5.2       | 8.2       | 6.0         | 7.6       | 6.0      |
| Vitamin B <sub>1</sub> [mg]         | 0.165     | ND        | 0.165      | 0.165         | ND        | ND      | 0.165     | 0.165     | ND        | ND          | 0.165     | ND       |
| Vitamin B <sub>3</sub> [mg]         | 2.4       | ND        | 2.4        | 2.4           | ND        | ND      | 2.4       | 2.4       | ND        | ND          | 2.4       | ND       |
| Vitamin B <sub>6</sub> [mg]         | 0.21      | ND        | 0.21       | 0.21          | ND        | ND      | 0.21      | 0.21      | ND        | ND          | 0.21      | ND       |
| Vitamin B <sub>12</sub> [µg]        | 0.375     | ND        | 0.375      | 0.375         | ND        | ND      | 0.375     | 0.375     | ND        | ND          | 0.375     | ND       |
| Vitamin D [µg]                      | ND        | ND        | ND         | ND            | ND        | ND      | 0.75      | ND        | ND        | ND          | 0.75      | ND       |
| L-carnitin [mg]                     | 30        | ND        | 30         | 30            | ND        | ND      | ND        | 30        | ND        | ND          | ND        | ND       |
| Calcium [mg]                        | 180       | 150       | 180        | 180           | 244       | 150     | 140       | 180       | 244       | 150         | 140       | 150      |

All products were lactose-free.

(+) – product contained additional ingredients (product 2 contained 0.1 % vanilla, product 6 contained 1 % cocoa, product 10 contained 0.5 % chocolate; product 12 contained 0.5 % soluble coffee).

A, B, C, D – encrypted producer names, \* – product with no added sugar, Suc – product contained sucralose, UHT – ultra-high temperature, H – homogenization, ST – sterilization, UF – ultrafiltration, ND – not declared.

### Total oxidant status

*TOS* levels were measured using commercially available kits (Relassay) and Mindray BS300 Auto Biochemistry Analyzer (Mindray Bio-Medical Electronics). In the method, oxidants present in the sample oxidized the ferrous ion in its complex with *o*-dianisidine to ferric ion. The oxidation reaction was enhanced by glycerol molecules present in the reaction medium. The ferric ion produced a coloured complex with xylenol orange in an acidic medium. The colour intensity, which could be measured spectrophotometrically in 530 nm, was related to the total level of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in micromoles of hydrogen peroxide equivalents per litre.

### Oxidative stress index

The ratio of *TOS* to *TAS* was accepted as the oxidative stress index (*OSI*). For calculation, the resulting unit of *TAS* was converted to micromoles per litre, and the *OSI* value (arbitrary unit) was calculated [17].

### Statistical analysis

Data were analysed using IBM SPSS Statistics for Windows, version 22 (SPSS, Armonk, New York, USA). Arithmetic mean  $\pm$  standard deviation values were given as descriptive statistics for variables. Mann-Whitney U-test or Kruskal-Wallis test were used to compare the *TAS*, *TOS* and *OSI* values of milk samples. Level of significance was set at  $p < 0.05$  for all of the analyses.

## RESULTS AND DISCUSSION

This study is the first to determine the total antioxidant and oxidant capacities of protein-enriched, lactose-free and fat-free milk products, mostly fortified with vitamins, which are sold in the category of 'high-protein milk products' and are sold for exercising individuals, athletes and individuals who want to lose weight.

In the present study, *TAS* values of conventional whole, reduced-fat and lactose-free cows' milks were  $0.54 \pm 0.08$  mmol·l<sup>-1</sup>,  $0.51 \pm 0.01$  mmol·l<sup>-1</sup>,  $0.53 \pm 0.07$  mmol·l<sup>-1</sup>, respectively (Tab. 2). These results are consistent with the knowledge that milk is a food that contains many essential nutrients as well as some components with antioxidant effects [18]. Protein fractions in milk, in particular casein, show antioxidant activity [19]. Antioxidant enzymes in milk, such as superoxide dismutase, catalase and glutathione peroxidase, together

with the conjugated linoleic acid, coenzyme Q10, vitamins C, E, A and D<sub>3</sub>, equol, uric acid, carotenoids and minerals contribute to the antioxidant activity of milk [18, 19]. In a previous study, total antioxidant capacities of milk obtained from three different animal species, i.e. goat, cow and sheep, were  $1.22 \pm 0.15$   $\mu$ mol·l<sup>-1</sup>,  $1.17 \pm 0.07$   $\mu$ mol·l<sup>-1</sup> and  $0.59 \pm 0.16$   $\mu$ mol·l<sup>-1</sup> (expressed as Trolox equivalent), respectively, and hence, it was concluded that antioxidant capacities values of milk might differ depending on the animal species and the amount of lactic acid, fat and dry matter in the milk [20]. In the present study, the antioxidant and oxidant capacity of milk products solely based on conventional cows' milk was evaluated, and therefore milk products of other animal species were not studied.

The *TAS* and *TOS* values of high-protein milk products are shown in Tab. 2. Comparing the *TAS* values of high-protein milk samples, the *TAS* value of 60 g·l<sup>-1</sup> protein vanilla milk (product 2) was higher than 52 g·l<sup>-1</sup> protein vanilla milk with vitamin B complex and L-carnitine (product 1),  $2.77 \pm 0.00$  mmol·l<sup>-1</sup> and  $1.62 \pm 0.02$  mmol·l<sup>-1</sup>, respectively ( $p < 0.05$ ). When comparing to cocoa milks, the *TAS* values of 60 g·l<sup>-1</sup> protein milk (product 6), 76 g·l<sup>-1</sup> protein milk with vitamin B complex and vitamin D (product 7), 52 g·l<sup>-1</sup> protein milk with B vitamin complex and L-carnitine (product 8) and 82 g·l<sup>-1</sup> protein milk (product 9) were not statistically different ( $p > 0.05$ ). Besides, the *TAS* value ( $2.88 \pm 0.00$  mmol·l<sup>-1</sup>) of 60 g·l<sup>-1</sup> protein chocolate milk (product 10) was not statistically different from kinds of cocoa milks ( $p > 0.05$ ). The *TAS* values of 76 g·l<sup>-1</sup> protein coffee milk with vitamin B complex and vitamin D (product 11) and 60 g·l<sup>-1</sup> protein coffee milk (product 12) were found the highest with the values of  $3.17 \pm 0.02$  mmol·l<sup>-1</sup> and  $2.88 \pm 0.00$  mmol·l<sup>-1</sup>, respectively ( $p > 0.05$ ).

In the present study, *TAS* values of all high-protein milk samples with various flavours and those enriched with dairy proteins were found to be higher than those of conventional cows' milk ( $p < 0.05$ ; Tab. 2). In the literature, it was reported previously that whey and casein fractions increase antioxidant activity in cell culture and animal studies [21, 22]. A study supporting this fact reported that milk proteins such as  $\alpha$ -caseins,  $\beta$ -caseins or  $\beta$ -lactoglobulin show in vitro antioxidant activity and reduce oxidative-induced aging damage [22]. The study concluded that whey protein intake for 8 weeks contributed to antioxidant defense in rats by reducing lipid and protein oxidation associated with weight lifting [21]. A study investigating the effect of whey and soya protein supplementation

**Tab. 2.** Comparison of total antioxidant and oxidant status of high-protein milk products and conventional milk samples.

| Product                           | Milk                          | Company    | Protein [g·l <sup>-1</sup> ] | <i>n</i> | Vitamins added | TAS [mmol·l <sup>-1</sup> ] | TOS [μmol·l <sup>-1</sup> ] | OSI                      |
|-----------------------------------|-------------------------------|------------|------------------------------|----------|----------------|-----------------------------|-----------------------------|--------------------------|
| <b>High-protein milk products</b> |                               |            |                              |          |                |                             |                             |                          |
| 1                                 | Vanilla                       | A          | 52                           | 3        | yes            | 1.62 ± 0.02 <sup>a</sup>    | 93.73 ± 0.70 <sup>a</sup>   | 5.78 ± 0.05 <sup>a</sup> |
| 2                                 | Vanilla (0.1 % vanilla)       | B          | 60                           | 3        | ND             | 2.77 ± 0.00 <sup>b</sup>    | 114.93 ± 11.80 <sup>b</sup> | 4.14 ± 0.43 <sup>b</sup> |
| 3                                 | Strawberry                    | A          | 52                           | 3        | yes            | 1.42 ± 0.02 <sup>a</sup>    | 56.98 ± 13.34 <sup>c</sup>  | 4.00 ± 1.02 <sup>b</sup> |
| 4                                 | Peanut-banana                 | A          | 52                           | 3        | yes            | 1.42 ± 0.00 <sup>a</sup>    | 86.32 ± 15.05 <sup>d</sup>  | 6.05 ± 1.05 <sup>c</sup> |
| 5                                 | Coconut                       | C          | 82                           | 3        | ND             | 2.12 ± 0.01 <sup>c</sup>    | 98.47 ± 3.48 <sup>e</sup>   | 4.64 ± 0.13 <sup>d</sup> |
| 6                                 | Cocoa (1 % cocoa)             | B          | 60                           | 3        | ND             | 2.87 ± 0.01 <sup>b</sup>    | 51.27 ± 26.92 <sup>f</sup>  | 1.78 ± 0.93 <sup>e</sup> |
| 7                                 | Cocoa                         | D          | 76                           | 3        | yes            | 2.87 ± 0.01 <sup>b</sup>    | 76.06 ± 4.20 <sup>g</sup>   | 2.65 ± 0.14 <sup>f</sup> |
| 8                                 | Cocoa                         | A          | 52                           | 3        | yes            | 2.86 ± 0.00 <sup>b</sup>    | 66.06 ± 0.95 <sup>h</sup>   | 2.30 ± 0.03 <sup>g</sup> |
| 9                                 | Cocoa                         | C          | 82                           | 3        | ND             | 2.88 ± 0.00 <sup>b</sup>    | 67.34 ± 13.06 <sup>i</sup>  | 2.33 ± 0.45 <sup>g</sup> |
| 10                                | Chocolate (0.5 % chocolate)   | B          | 60                           | 3        | ND             | 2.87 ± 0.02 <sup>b</sup>    | 90.62 ± 2.72 <sup>j</sup>   | 3.15 ± 0.09 <sup>h</sup> |
| 11                                | Coffee                        | D          | 76                           | 3        | yes            | 3.17 ± 0.02 <sup>b</sup>    | 43.27 ± 1.72 <sup>k</sup>   | 1.36 ± 0.05 <sup>i</sup> |
| 12                                | Coffee (0.5 % instant coffee) | B          | 60                           | 3        | ND             | 2.88 ± 0.00 <sup>b</sup>    | 72.73 ± 0.38 <sup>l</sup>   | 2.52 ± 0.01 <sup>j</sup> |
| <b>Conventional milks</b>         |                               |            |                              |          |                |                             |                             |                          |
| 13                                | Whole milk, plain             | A, B, C, D | 30                           | 4        | ND             | 0.54 ± 0.08 <sup>d</sup>    | 14.90 ± 3.52 <sup>m</sup>   | 2.78 ± 0.77 <sup>k</sup> |
| 14                                | Reduced-fat milk, plain       | A, B, C    | 29                           | 3        | ND             | 0.51 ± 0.01 <sup>d</sup>    | 15.01 ± 0.50 <sup>m</sup>   | 2.94 ± 0.18 <sup>l</sup> |
| 15                                | Lactose-free milk, plain      | A, B       | 28                           | 2        | ND             | 0.53 ± 0.07 <sup>d</sup>    | 14.50 ± 6.88 <sup>n</sup>   | 1.29 ± 0.28 <sup>m</sup> |

The mean ± standard deviation values of duplicates are presented. Different superscripts for the results of a specific parameter show a significant difference at the *p*-value of 0.05.

All high-protein milk products were lactose-free.

TAS – total antioxidant status (expressed in millimoles of Trolox equivalents per litre), TOS – total oxidant status (expressed in micromoles of hydrogen peroxide equivalents per litre), OSI – oxidative stress index.

A, B, C, D – encrypted producer names, ND – not declared.

on muscle redox parameters reported that whey proteins regulate tissue glutathione and free radical composition in a manner that prevents oxidative stress, and a synergistic redox effect was not observed in its combination with soya [23]. However, when high-protein milk products were evaluated in the present study, the stimulating effect of protein increase on TAS did not take place generally in all types of milk products (Tab. 2). Apparently, individual high-protein milk products differed in composition, which made any generalization difficult.

Comparing the TOS values of high-protein milk products, TOS value of 52 g·l<sup>-1</sup> protein vanilla milk with vitamin B complex and L-carnitine (product 1) was lower than 60 g·l<sup>-1</sup> protein vanilla milk (product 2), 93.73 ± 0.70 μmol·l<sup>-1</sup> and 114.93 ± 11.80 μmol·l<sup>-1</sup>, respectively (*p* < 0.05). In comparison, TOS value of 82 g·l<sup>-1</sup> protein coconut milk (product 5) was the highest among all milk products (98.47 ± 3.48 μmol·l<sup>-1</sup>, *p* < 0.05). The TOS value of 60 g·l<sup>-1</sup> protein milk with cocoa flavour (product 6) was found to be

51.27 ± 26.92 μmol·l<sup>-1</sup>. The TOS value of 76 g·l<sup>-1</sup> protein cocoa milk with vitamin B complex and vitamin D (product 7) was higher than 52 g·l<sup>-1</sup> protein cocoa milk with vitamin B complex and L-carnitine (product 8; 76.06 ± 4.20 μmol·l<sup>-1</sup> and 66.06 ± 0.95 μmol·l<sup>-1</sup>, respectively, *p* < 0.05). Moreover, the TOS value (90.62 ± 2.72 μmol·l<sup>-1</sup>) of 60 g·l<sup>-1</sup> protein chocolate milk (product 10) was higher than that of high-protein cocoa milks (*p* < 0.05). The TOS value of 76 g·l<sup>-1</sup> protein coffee milk with vitamin B complex and vitamin D (product 11) was lower compared to 60 g·l<sup>-1</sup> protein coffee milk (product 12) (43.27 ± 1.72 μmol·l<sup>-1</sup> vs 72.73 ± 0.38 μmol·l<sup>-1</sup>, *p* < 0.05). In addition, TOS values of all high-protein milk samples with increased protein concentration and a highly complex nutritional matrix were also found to be higher compared to conventional cows' milk samples (*p* < 0.05).

High-protein milk products are fortified with vitamins B<sub>6</sub>, vitamin D and L-carnitine (Tab. 1), which are frequently used in energy-providing systems and are needed during exercises and sports



[24–28]. Also considering the musculoskeletal and non-musculoskeletal (vitamin D receptor-mediated) positive effects, some milk products of certain brands were fortified with vitamin D [24]. The *TOS* values of high-protein milk products with L-carnitine, and vitamin addition (Tab. 1, Tab. 2) appeared to be lower compared to milk with the same flavour but higher protein concentration.

Regarding L-carnitine, in light- and medium-intensity exercises, the energy requirement is met by free fatty acids, and the highest fat-oxidation occurs in exercises performed at up to 50–60 % of the maximum oxygen consumption [25]. L-carnitine is an endogenous substance that is necessary for  $\beta$ -oxidation of fatty acids and has mainly been studied in the field of sports nutrition because of its glycogen-sparing effect [25, 26]. L-carnitine supplementation reportedly improved exercise performance [25]. In addition to physical performance, recent studies suggested that L-carnitine consumption induced the endogenous antioxidant defense system, prevented lipid peroxidation and inhibited oxidative stress [26, 27]. The present study supports the hypothesis that it acts not only on endogenous basis but on the exogenous one as well.

In the present study, the average *OSI* values of high-protein milk products were determined (Tab. 2). Accordingly, the *OSI* values of 52 g·l<sup>-1</sup> protein peanut-banana milk with vitamin B complex and L-carnitine (product 4) and 52 g·l<sup>-1</sup> protein vanilla milk with vitamin B complex and L-carnitine (product 1) were the highest among all kinds of milk products ( $6.05 \pm 1.05$  and  $5.78 \pm 0.05$ , respectively). Following to product 1 and 4, the *OSI* values of 82 g·l<sup>-1</sup> protein coconut milk (product 5), 60 g·l<sup>-1</sup> protein vanilla milk (product 2), 52 g·l<sup>-1</sup> protein strawberry milk with vitamin B complex and L-carnitine (product 3) were found as the other milk-products with the highest *OSI* value.

When the *OSI* values of cocoa milks were evaluated, the *OSI* value of 76 g·l<sup>-1</sup> protein milk with vitamin B complex and vitamin D (product 7) and 52 g·l<sup>-1</sup> protein milk with vitamin B complex and L-carnitine (product 8) were found to be  $2.65 \pm 0.14$  and  $2.30 \pm 0.03$ , respectively. While the *OSI* value of 82 g·l<sup>-1</sup> protein milk (product 9) was  $2.33 \pm 0.45$ , the *OSI* value of the 60 g·l<sup>-1</sup> protein milk (product 6) was  $1.78 \pm 0.93$ . However, 60 g·l<sup>-1</sup> protein chocolate milk (product 10) had an *OSI* value of  $3.15 \pm 0.09$  and was relatively higher compared to cocoa milks. Moreover, the *OSI* value of 76 g·l<sup>-1</sup> protein coffee milk with vitamin B complex and vitamin D (product 11) was the lowest with a value of  $1.36 \pm 0.05$ . The difference between the *OSI* values of high-protein

milk products with different flavours and various protein concentrations was statistically significant ( $p < 0.05$ ). Besides, most of the milk products with different flavours and various protein concentrations had higher *OSI* values than conventional whole cows' milk, reduced-fat cows' milk, and lactose-free cows' milk (Tab. 2).

To sum up, when the *OSI* values that allowed both *TAS* and *TOS* to be evaluated simultaneously are examined, the *OSI* values of some flavoured high-protein milk products, in particular those containing fat-based flavours, were significantly higher. However, the *OSI* values of those containing cocoa and coffee were generally found to be low as consistent with the previous knowledge in the literature [29–33].

In a study supporting the present study [29], total phenolic content (*TPC*) and total antioxidant activities of conventional cows' milk and flavoured UHT milks were determined by methods based on Trolox-equivalent antioxidant activity (*TEAC*) and oxygen radical absorbance capacity (*ORAC*). In terms of antioxidant activities and *TPC* ( $1587.52 \pm 229.84$  mg·l<sup>-1</sup> vs  $1030.10 \pm 19.31$  mg·l<sup>-1</sup>, expressed as gallic acid equivalents), the lowest values among the products were found in conventional cows' milk samples. Besides, total antioxidant activity values (*TEAC* of  $6.25 \pm 0.53$  mmol·l<sup>-1</sup> and  $4.31 \pm 0.51$  mmol·l<sup>-1</sup>; *ORAC* of  $4.31 \pm 0.57$   $\mu$ mol·ml<sup>-1</sup> and  $2.98 \pm 0.15$   $\mu$ mol·ml<sup>-1</sup>, respectively) were determined to be the highest in chocolate milk in the same study. Moreover, in a study conducted on natural cocoa powder [30], the *ORAC* value was determined as  $826 \pm 103$  mmol·kg<sup>-1</sup> (expressed as Trolox equivalent) and cocoa was determined to have a significant antioxidant activity. The results of the present study and accumulated literature can be attributed to the polyphenol concentration (12–18 % dry weight) of the cocoa bean, in particular the flavonoid concentration (catechins or flavan-3-ols ~37 %, anthocyanins ~4 % and proanthocyanidins ~58 %) with high antioxidant activities [31]. Similarly, due to the phytochemical concentration of coffee, it has high antioxidant activity capable of scavenging free radicals, donating hydrogen and electrons, providing reducing activity and also acting as a metal ion pro-oxidant chelator [32, 33].

## CONCLUSIONS

Results of this study demonstrated that the antioxidant and/or oxidant capacities of high-protein milk products, which are special products with increased protein concentration, vary accor-

ding to the brand, flavour and/or concentrations of polyphenols and, in some cases, depending on vitamin concentration. Individual milk products studied had unique characteristics, which made it difficult to evaluate the effect of protein concentrations on antioxidant and/or oxidant capacity. However, as the product range expands, the possibility of comparison may increase in the future with an increase in the number of high-protein milk products with a similar matrix and ingredients. Although this was not a randomized controlled clinical study, it provided information on the oxidative condition of a selection of innovative milk products for individuals and athletes who insist on consuming these types of milk and/or have an insufficient antioxidant intake, especially those whose diet is not adequately balanced. There are some limitations in this study. The first one is that characterization of nutrients and bioactive substances that may affect the antioxidant or oxidant capacity of high-protein milk products was not conducted in this study. Secondly, antioxidant or oxidant capacity values found in this study could not be compared since there was no similar study available in the literature. Thirdly, this study only reflected the milk products sold in Turkey in a specific period (between January and July 2020). It is recommended to consider these conditions in studies to be conducted in the future.

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