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The Effect of Using Demineralized Whey Powder and Buttermilk Powder Instead of Skimmed Milk Powder on Physicochemical, Microbiological, and Biochemical Properties of Yogurt

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ABSTRACT

This study aimed to increase the total solids amount of raw milk by adding demineralized whey powder (d-WP), buttermilk powder (BMP), and skimmed milk powder (SMP) thereby producing yogurt with improved physicochemical, microbiological, and biochemical properties. d-WP and BMP were mixed with SMP at seven different ratios to produce yogurt. The physicochemical, microbiological, and biochemical properties of the yogurt samples (A, B, C, D, E, F, G) were analyzed during the storage period (28 days). Sample B (3.0% SMP, 0.5% d-WP, 0.5% BMP) has the highest results for viscosity and water-holding capacity, while sample C (2.0% SMP, 1.0% d-WP, 1.0% BMP) has the lowest acidity and highest pH. The concentrations of orotic, hippuric, and propionic acids in yogurts were determined to be statistically significant ($p< 0.01$). All 20 amino acids were detected at different ratios in all yogurt samples. It was determined that G (2.0% SMP, 2.0% d-WP) had the highest total amino acid content and F (2.0% SMP, 2.0% BMP) had the lowest total amino acid content during the storage period. It was determined that acetaldehyde (21.53±2.27 mmol kg^{-1}), which creates the typical aroma of yogurt, was produced more in sample D containing 1.0% SMP, 1.5% d-WP, 1.5% BMP. Using d-WP and BMP in appropriate combinations (0.5–1.5%) with SMP can produce a more functional yogurt with higher nutritional value, without causing any negative effects on the physicochemical, microbiological, and biochemical properties of yogurt.

1 | Introduction

Yogurt is a fermented dairy product with high functional properties and nutritional value, which is formed by the conversion of lactose to lactic acid by yogurt starter bacteria (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) added to milk that has undergone necessary pretreatments (clarification, separation, homogenization, heat treatment) (Garavand et al. 2023). Recently, the production of low- or reduced-fat dairy products has increased significantly worldwide. The production of yogurt from skimmed milk resulted in a bland taste and texture. It also caused problems in rheological and functional properties. In order to eliminate these problems, increasing milk's total solids has been a very important issue. Dairy byproducts (such as buttermilk powder [BMP], whey powder [WP]) used to increase the total solids reduce the industrial cost and enable the production of low-fat yogurt with low serum separation and high viscosity that consumers like (Lesme et al. 2020).

In yogurt production, dairy byproducts such as BMP and WP, in addition to skimmed milk powder (SMP), are used to improve physical and sensory quality. These products make a positive contribution to the characteristics of yogurt such as consistency, stability, and nutrient content (Silva and O'Mahony 2017). The use of various whey derivatives (such as WP, whey protein isolate, and whey protein concentrate) in yogurt production increases the water-holding capacity (WHC), viscosity, and emulsion properties of yogurt and results in a microstructurally better product (Laiho et al. 2017). Demineralized whey powder (d-WP), which is obtained by removing the minerals of WP with high mineral content, prevents salty taste in yogurt and improves sensory properties (Ramos et al. 2015). BMP contains all water-soluble milk components, including milk protein, lactose, and minerals. The phospholipid and phosphorus content of buttermilk is known to be higher than whole milk. Milk fat globule membrane (MFGM) components, which are incorporated into the buttermilk during churning and have important human health benefits, make it a unique product (Krebs et al. 2021). Having important functional properties such as water retention and emulsifying capacity, BMP is used in yogurt production in certain quantities as a fat substitute (Garczewska-Murzyn et al. 2022). The addition of BMP to yogurt prevents syneresis low-fat yogurt and hence improving consistency and taste. It also makes an important contribution to improving taste and consistency (Zhao et al. 2020).

The quality of yogurt is determined by its physicochemical, microbiological, and sensory quality and acceptance. Properties such as pH, viscosity, total solids, activity of starter cultures, taste, odor, and consistency of yogurt have a significant impact on the quality and likability of the product (Pourahmad and Assadi 2005). Nonvolatile organic acids (lactic, pyruvic, succinic, and oxalic acid) and carbonyl compounds (acetaldehyde, diacetyl, and acetone) produced by starter bacteria have a significant effect on the taste and aroma of yogurt. In addition, the various amino acids formed, those that are essential, make an important contribution to the flavor of yogurt (Garavand et al. 2023). The methods applied to increase the total solids of yogurt milk cause the formation of different types and proportions of taste-aroma compounds (Cheng 2010).

In this study, it was aimed to produce a product with higher nutritional value, textural properties, and flavor profile by increasing the total solids of yogurt. SMP, d-WP, and BMP powder, which have functional properties and have an important place in our nutrition due to their phospholipids, serum proteins, and other nutrients, were mixed in seven different ratios and used in yogurt production. Some physicochemical and microbiological properties of the yogurt samples were examined on the first, seventh, 14th, 21st, and 28th days of storage and the biochemical properties were examined on the first, 14th, and 28th days of storage.

2 | Materials and Methods

2.1 | Research Material

Raw cow's milk used in yogurt production was obtained from Atatürk University Food and Livestock Application and Research Center (Erzurum, Türkiye). The yogurt starter culture YC-350 (Chr. Hansen A/S, Hoersholm, Denmark) was composed of *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. SMP, d-WP (70%), and BMP were supplied from Slava Süt (Karaman, Türkiye).

TABLE 1 | Codes of yogurt samples and ratios of SMP, d-WP, and BMP in yogurt milks.

Code	SMP (%)	d-WP (%)	BMP (%)
A	4.0	0	0
B	3.0	0.5	0.5
C	2.0	1.0	1.0
D	1.0	1.5	1.5
E	0.0	2.0	2.0
F	2.0	0.0	2.0
G	2.0	2.0	0.0

2.2 | Yogurt Production

The raw cow's milk (total solid 11.43±0.03%, protein 3.11±0.01%, milk fat 2.80±0.00%, ash 0.66±0.06%, pH 6.65±0.07, titratable acidity [lactic acid %] 0.18±0.00%, and density 1.030±0.00 g/mL) used for yogurt production was subjected to various pretreatments (clarification, separation, and homogenization). Afterward, heat treatment was applied for 10 min at 90°C and the yogurt milk was cooled to 44±1°C. The milk was divided into seven parts. SMP (total solids 95.82±0.03%, fat 1.10±0.01%, protein 36.46±0.65%, ash 6.91±0.02%, lactose 51.36±0.66%, pH 6.60±0.01, titratable acidity 0.14±0.01%), d-WP (total solids 97.10±0.14%, fat 0.66±0.00%, protein 12.10±0.00%, ash 5.05±0.07%, lactose 79.29±0.07%, pH 6.25±0.00, titratable acidity 0.16±0.00%), and BMP (total solids 97.22±0.02%, fat 7.35±0.06%, protein 36.41±0.01%, ash 6.96±0.01%, lactose 46.51±0.06%, pH 6.54±0.00, titratable acidity 0.12±0.00%) were added to the cooled milk in the proportions given in Table 1 and mixed. Yogurt culture (*L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) was inoculated at a rate of 0.01% (w/v) and filled into sterile glass jars (200, 100, and 50 mL). Then, the inoculated milk was incubated at 44±1°C until the pH reached 4.6±0.1. Experimental yogurts, whose incubation was completed, were stored at 4±1°C for analysis. Yogurt samples were prepared with milk taken at two different times and in two replications at different times.

2.3 | Physicochemical Analyses

Total solids and ash contents were analyzed by the gravimetric method, fat content by the Gerber method, and protein by the Kjeldahl method (AOAC 2005). Lactose content was calculated by subtracting fat, protein, and ash from total solids. The apparent viscosity of the yogurts was measured by a viscometer (Brookfield DV-II+Pro, Stoughton, MA, USA) using spindle no 6, at 50 rpm (3±1°C) (Hashim et al. 2021). The apparent viscosity values are given in Pa.s. The WHC of yogurt samples was determined by modifying the method used by Krebs et al. (2021). Twenty grams of yogurt sample was centrifuged (Beckman Coulter, Allegra X-30R, Germany) at 5000 rpm for 20 min (4°C). The supernatant was removed and yogurt samples were weighed. The percentage of WHC was defined according to the following equation: WHC (%) = [weight of the remaining sample after centrifugation (g) / weight of the sample (g)] × 100.

The titratable acidity (lactic acid %) of the yogurts was measured using 0.1 N sodium hydroxide and phenolphthalein indicator (Garavand et al. 2023). The pH value was measured using a Mettler Toledo pH-meter (Mettler-Toledo, Seven CompactTM S210, Schwerzenbach, Switzerland).

2.4 | Microbiological Analyses

The counts of *L. delbruekii* subsp. *bulgaricus*, *S. thermophilus*, yeast, and molds were determined in yogurt samples. The count of *L. delbruekii* subsp. *bulgaricus* was determined after 72 h of incubation (anaerobic conditions) at 37±1°C on MRS agar (Chemsolute, Renningen) and the count of *S. thermophilus* was determined after 48 h of incubation (aerobic conditions) at 37±1°C on M17 agar (Biokar, France). PDA agar (Condalab, Spain) acidified with 10% tartaric acid (Carlo Erba, Val de Reuil Cedex) was used to determine the count of yeast and molds, and colonies that developed after 5 days at 20–25°C were counted (Yıldız and Bakırcı 2019).

2.5 | Biochemical Analyses

2.5.1 | Determination of Organic Acid Profile

The organic acid content of yogurt samples was determined by modifying the method used by Çelik et al. (2022). The following acids: lactic acid, pyruvic acid, citric acid, orotic acid, uric acid, and hippuric acid concentrations of yogurts were determined using the Agilent 6460 Triple Quadrapole LC-MS/MS system, ZORBAX Eclipse XDB C-8 (5 µm, 4.6 ×150 mm) column, 0.1% formic acid mobile phase, 0.5 flow rate, 10 L min^{-1} gas flow rate. Acetic and propionic acids were determined using the Shimadzu Prominence LC-20A HPLC system, ODS-3 (5 µm, 4.6 ×150 mm) column, SPD-M20A detector, D2&W lamp, 10 mM NaH_2PO_4 mobile phase, 0.8 mL min^{-1} flow rate. For the extraction of organic acids, 5 g yogurt sample was weighed, 5 mL methanol was added and shaken for 1 min. After centrifugation (5000 rpm-5 min), 100 µL of the resulting supernatant was taken and 900 µL of 0.2% formic acid solution was added. Afterward, it was shaken and filtered (0.45 µm), transferred to vials, and given to the system. The organic acid concentration is given in mmol kg^{-1}.

2.5.2 | Determination of Amino Acid Profile

Agilent 6460 Triple Quadrapole LC-MS/MS system (Jasem AA column, Jasem AA kit mobile phase, flow rate 0.7 mL min^{-1}, gas temperature 150°C, gas flow 11 mL min^{-1}) was used to determine the free amino acid content in yogurt samples. For extraction, 20 g of yogurt was centrifuged at 8000 rpm for 6 min. Six hundred and fifty microliters amino acid solvent (mobile phase A: mobile phase B, v:v, 1:4) and 50 µL internal standard were added to 100 µL supernatant. After shaking for 1 min, it was centrifuged at 10,000 rpm for 8 min. The supernatant was passed through a 0.45 µm filter and transferred to vials (Atila et al. 2021). The results were expressed in nmol µL^{-1}.

2.5.3 | Determination of Aroma Compounds

The volatile compounds of yogurts were determined using the method given by Yüksel and Bakırcı (2015) with some modifications. Five grams of yogurt sample was taken into vials and given to the Headspace-GC/FID (Shimadzu, QP2010) system. DB-WAX Ultra Inert column (Agilent, 30 m, 0.25 mm, 0.25 um) and Helium gas (1.7 mL dak^{-1}) were used. The oven temperature was started at 40°C, held at this temperature for 2 min, and then increased to 200°C at a rate of 5°C min^{-1}. Acetaldehyde, diacetyl, and acetoin concentrations of yogurts are given as mmol kg^{-1}.

2.6 | Statistical Analysis

The study was carried out in a factorial arrangement (7×5), with seven different levels of SMP, d-WP, and BMP, and five different storage periods (1, 7, 14, 21, 28), with two replications, according to the Randomized Complete Block Design. The obtained data were analyzed by variance analysis using the SPSS package program (Version 20.0). Duncan's Multiple Range Test was used to determine statistically different groups.

3 | Results and Discussion

3.1 | Physicochemical Parameters

In Table 2, the physicochemical properties of yogurt samples are shown. The addition of SMP, d-WP, and BMP caused differences ($p< 0.01$) in the total solids, protein, ash, apparent viscosity, WHC, titratable acidity, and pH results of the yogurts. The difference between the fat values of the samples was found to be statistically insignificant. The effect of storage time on total solids, fat, apparent viscosity, WHC, pH, and titratable acidity values was found to be significant ($p< 0.05$; $p< 0.01$), while the effect on protein and ash was not statistically significant.

The total solids content of sample A with only SMP was lower than the other samples. This is thought to be due to the fact that the total solids content of d-WP and BMP is about 1% higher than the total solids content of SMP. It is thought that the decreases in total solids content during storage are due to the conversion of lactose in yogurt to lactic acid by *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. Indeed, similar results were suggested by Ahmed and Razig (2017).

Fat is one of the important components of yogurt total solids. It was found that the fat content of yogurts varied due to the difference in the combination of added SMP, d-WP, and BMP. It has been reported that the decrease in fat content with the progression of storage time is related to the breakdown of fat during the fermentation process. Also, the fat content slightly decreased due to fat hydrolysis and liberation of free acids that

TABLE 2 | Physicochemical analytical results of yogurt samples (mean±SD).

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Total solids (%)	1	14.26±0.06^{a,A}	14.33±0.38^{a,A}	14.42±0.03^{a,A}	14.41±0.07^{a,A}	14.59±0.16^{a,A}	14.45±0.18^{a,AB}	14.29±0.09^{a,A}
	7	14.11±0.08^{a,A}	14.23±0.08^{ab,A}	14.47±0.05 ^{cd,AB}	14.54±0.11^{d,AB}	14.61±0.13^{d,A}	14.31±0.00^{abc,A}	14.44±0.11^{bcd,A}
	14	14.15±0.11^{a,A}	14.18±0.02^{a,A}	14.59±0.13^{b,AB}	14.64±0.04^{b,B}	14.66±0.04^{b,A}	14.59±0.04^{b,B}	14.51±0.01^{b,A}
	21	14.05±0.13^{a,A}	14.19±0.03^{ab,A}	14.40±0.09 ^{cd,A}	14.40±0.04 ^{cd,A}	14.55±0.03^{d,A}	14.42±0.06 ^{cd,AB}	14.30±0.07^{bc,A}
	28	14.00±0.13^{a,A}	14.09±0.09^{a,A}	14.81±0.26^{c,B}	14.38±0.02^{b,A}	14.55±0.01^{b,A}	14.52±0.00^{bc,AB}	14.44±0.11^{b,A}
Sign	Samples				**			
	Storage				*			
Fat (%)	1	3.20±0.14^{a,A}	2.70±0.00^{a,A}	2.80±0.14^{a,AB}	2.90±0.28^{a,A}	2.80±0.14^{a,BC}	3.00±0.14^{a,A}	2.80±0.42^{a,A}
	7	2.90±0.00^{a,A}	2.90±0.28^{a,A}	3.00±0.14^{a,B}	2.90±0.28^{a,A}	2.90±0.00^{a,C}	3.00±0.14^{a,A}	3.00±0.14^{a,A}
	14	2.88±0.53^{a,A}	2.60±0.14^{a,A}	2.50±0.00^{a,A}	2.70±0.28^{a,A}	2.30±0.00^{a,A}	2.60±0.14^{a,A}	2.60±0.14^{a,A}
	21	2.70±0.28^{a,A}	2.70±0.00^{a,A}	2.60±0.14^{a,A}	2.80±0.14^{a,A}	2.60±0.14^{a,B}	2.70±0.28^{a,A}	2.70±0.00^{a,A}
	28	2,90±0.28^{a,A}	2.90±0.28^{a,A}	2.80±0.14^{a,AB}	2.90±0.28^{a,A}	2.60±0.14^{a,B}	3.00±0.14^{a,A}	2.80±0.14^{a,A}
Sign	Samples				not significant			
	Storage				**			
Protein (%)	1	4.76±0.15^{a,A}	4.68±0.23^{a,AB}	4.54±0.03^{a,A}	4.66±0.33^{a,A}	4.56±0.07^{a,A}	4.92±0.00^{a,BC}	4.79±0.01^{a,B}
	7	4.64±0.18^{a,A}	4.93±0.00^{b,B}	4.56±0.03^{a,A}	4.48±0.09^{a,A}	4.56±0.05^{a,A}	4.63±0.00^{a,AB}	4.45±0.05^{a,AB}
	14	4.66±0.05^{ab,A}	4.60±0.19^{ab,AB}	4.54±0.01^{a,A}	4.85±0.02^{ab,A}	4.63±0.30^{ab,A}	5.05±0.17^{b,C}	4.59±0.31^{ab,AB}
	21	4.81±0.16^{d,A}	4.72±0.04 ^{cd,AB}	4.47±0.05^{ab,A}	4.50±0.16^{abc,A}	4.67±0.06^{bcd,A}	4.53±0.01^{abc,A}	4.38±0.04^{a,A}
	28	4.80±0.04^{b,A}	4.50±0.03^{ab,A}	4.74±0.10^{ab,B}	4.62±0.26^{ab,A}	4.39±0.07^{a,A}	4.67±0.23^{ab,AB}	4.66±0.01^{ab,AB}
Sign	Samples				**			
	Storage				not significant			
Ash (%)	1	0.97±0.04^{ab,A}	0.95±0.03^{ab,A}	1.02±0.01^{ab,A}	0.96±0.02^{ab,A}	0.99±0.05^{ab,A}	1.03±0.01^{b,A}	0.95±0.02^{a,A}
	7	1.00±0.01^{a,A}	1.01±0.01^{a,BC}	0.99±0.05^{a,A}	0.97±0.00^{a,A}	0.97±0.01^{a,A}	1.01±0.03^{a,A}	0.98±0.00^{a,A}
	14	1.00±0.01b^{c,A}	1.01±0.01^{bc,BC}	1.01±0.01^{bc,A}	0.97±0.02^{a,A}	0.98±0.01^{abc,A}	1.01±0.01^{c,A}	0.97±0.01^{ab,A}
	21	1.02±0.02^{bc,A}	1.02±0.00^{c,C}	1.00±0.01^{bc,A}	0.99±0.01^{b,A}	0.99±0.01^{b,A}	1.02±0.00^{c,A}	0.95±0.00^{a,A}
	28	0.96±0.01^{a,A}	0.97±0.01^{a,AB}	1.02±0.01^{a,A}	0.98±0.01^{a,A}	0.99±0.02^{a,A}	1.00±0.03^{a,A}	1.00±0.06^{a,A}
Sign	Samples				**			
	Storage				not significant			

(Continues)

TABLE 2 | (Continued)

	Storage time (days)	Yogurt samples						
		A	B	C	D	E	F	G
Lactose (%)	1	5.34±0.28^{a,A}	6.00±0.63^{ab,A}	6.07±0.13^{ab,AB}	5.90±0.13^{ab,A}	6.24±0.18^{b,A}	5.50±0.03^{ab,A}	5.75±0.52^{ab,A}
	7	5.58±0.09^{ab,A}	5.39±0.37^{a,A}	5.92±0.17^{bcd,A}	6.19±0.09^{d,A}	6.19±0.09^{d,A}	5.67±0.11^{abc,A}	6.01±0.01 ^{cd,A}
	14	5.62±0.67^{a,A}	5.98±0.06^{ab,A}	6.55±0.13^{b,B}	6.13±0.25^{ab,A}	6.75±0.33^{b,B}	5.93±0.26^{ab,AB}	6.35±0.17^{ab,A}
	21	5.53±0.27^{a,A}	5.76±0.06^{ab,A}	6.34±0.11^{c,AB}	6.15±0.25^{bc,A}	6.29±0.04^{c,AB}	6.17±023^{bc,B}	6.27±0.03^{c,A}
	28	5.34±0.40^{a,A}	5.72±0.39^{ab,A}	6.26±0.30^{bc,AB}	5.88±0.55^{abc,A}	6.56±0.06^{c,AB}	5.85±0.11^{abc,AB}	5.98±0.01^{abc,A}
Sign	Samples				**			
	Storage				**			
Apparent viscosity (Pa.s)	1	8.38±2.03^{c,A}	9.38±1.94^{c,A}	8.03±1.91^{bc,A}	6.30±0.15^{abc,A}	4.56±0.55^{a,A}	6.32±0.18^{abc,A}	4.83±0.96^{ab,A}
	7	11.08±1.70^{b,A}	11.15±0.59^{b,A}	9.95±0.80^{b,A}	7.22±0.00^{a,A}	6.49±0.30^{a,A}	7.86±0.96^{a,AB}	6.19±0.42^{a,AB}
	14	10.49±0.64^{d,A}	9.35±0.72 ^{cd,A}	8.72±2.07^{bcd,A}	7.69±1.56^{abc,A}	5.92±0.09^{a,A}	7.73±0.64^{abc,AB}	6.01±0.41^{ab,AB}
	21	11.55±0.06 ^{cd,A}	12.97±2.04^{d,A}	9.93±2.33^{bcd,A}	8.07±0.55^{ab,A}	6.58±0.40^{a,A}	8.50±0.88^{abc,B}	7.60±0.46^{ab,BC}
	28	10.92±0.37^{b,A}	11.40±1.02^{b,A}	10.25±1.00^{ab,A}	8.90±1.53^{ab,A}	7.19±2.06^{a,A}	9.73±1.03^{ab,B}	8.88±0.94^{ab,C}
Sign	Samples				**			
	Storage				**			
Water-holding capacity (%)	1	47.40±0.26^{c,A}	47.77±0.96^{c,A}	47.66±1.20^{c,AB}	44.50±0.35^{bc,A}	41.53±0.11^{ab,A}	42.00±1.79^{ab,A}	40.65±2.99^{a,A}
	7	46.29±0.11^{b,A}	45.43±1.92^{ab,A}	42.58±2.59^{ab,A}	40.84±0.48^{a,A}	40.65±1.29^{a,A}	42.71±2.06^{ab,A}	43.56±3.19^{ab,A}
	14	44.17±2.83^{a,A}	46.45±3.22^{a,A}	43.45±0.15^{a,AB}	43.32±3.51^{a,A}	41.98±1.56^{a,A}	41.27±0.90^{a,A}	42.10±2.08^{a,A}
	21	45.25±1.47^{ab,A}	46.73±2.89^{b,A}	44.67±2.54^{ab,AB}	43.17±2.79^{ab,A}	41.92±1.87^{ab,A}	42.94±2.16^{ab,A}	39.95±0.50^{a,A}
	28	46.54±0.58^{abc,A}	49.11±2.52^{c,A}	49.00±3.30^{bc,B}	47.88±4.65^{abc,A}	42.39±1.24^{a,A}	42.68±1.27^{ab,A}	43.94±1.32^{abc,A}
Sign	Samples				**			
	Storage				**			
Titratable acidity (%)	1	1.29±0.00^{d,A}	1.20±0.01^{c,A}	1.02±0.01^{a,A}	1.05±0.01^{a,A}	1.13±0.04^{b,A}	1.12±0.01^{b,A}	1.11±0.01^{b,A}
	7	1.37±0.02^{d,B}	1.34±0.00^{d,B}	1.18±0.01^{a,B}	1.18±0.00^{a,B}	1.19±0.01^{a,A}	1.22±0.01^{b,B}	1.29±0.01^{c,B}
	14	1.50±0.01^{e,C}	1.46±0.01^{d,C}	1.25±0.01^{a,C}	1.27±0.00^{a,C}	1.31±0.01^{b,B}	1.32±0.01^{b,C}	1.39±0.01^{c,C}
	21	1.51±0.01^{e,C}	1.47±0.00^{d,C}	1.28±0.01^{a,C}	1.29±0.01^{a,C}	1.34±0.03^{b,B}	1.37±0.01^{bc,D}	1.39±0.03^{c,C}
	28	1.63±0.02^{d,D}	1.52±0.01^{c,D}	1.33±0.01^{a,D}	1.31±0.01^{a,D}	1.41±0.01^{b,C}	1.41±0.01^{b,E}	1.43±0.03^{b,C}
Sign	Samples				**			
	Storage				**			

(Continues)

TABLE 2 | (Continued)

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
pH	1	$4.53 \pm 0.01^{d,D}$	$4.47 \pm 0.00^{c,D}$	$4.54 \pm 0.00^{de,D}$	$4.56 \pm 0.02^{e,C}$	$4.38 \pm 0.01^{a,D}$	$4.54 \pm 0.01^{de,D}$	$4.45 \pm 0.01^{b,D}$
	7	$4.32 \pm 0.01^{b,C}$	$4.21 \pm 0.03^{a,C}$	$4.46 \pm 0.01^{d,C}$	$4.38 \pm 0.01^{c,B}$	$4.26 \pm 0.04^{a,C}$	$4.34 \pm 0.01^{bc,C}$	$4.25 \pm 0.01^{a,C}$
	14	$4.20 \pm 0.00^{b,B}$	$4.20 \pm 0.00^{a,B}$	$4.37 \pm 0.01^{d,B}$	$4.30 \pm 0.00^{c,A}$	$4.18 \pm 0.01^{a,B}$	$4.29 \pm 0.02^{c,B}$	$4.17 \pm 0.00^{A,B}$
	21	$4.19 \pm 0.00^{c,B}$	$4.15 \pm 0.00^{a,B}$	$4.36 \pm 0.01^{f,B}$	$4.30 \pm 0.01^{e,A}$	$4.16 \pm 0.01^{ab,B}$	$4.28 \pm 0.01^{d,B}$	$4.17 \pm 0.01^{b,B}$
	28	$4.11 \pm 0.01^{b,A}$	$4.08 \pm 0.01^{a,A}$	$4.31 \pm 0.01^{e,A}$	$4.28 \pm 0.01^{d,A}$	$4.08 \pm 0.01^{a,A}$	$4.24 \pm 0.00^{c,A}$	$4.11 \pm 0.00^{b,A}$
Sign	Samples				**			
	Storage				**			

Note: Means with different capital letters in the same column and different lowercase letters in the same row are statistically different.
$^{**}p < 0.01$; $^{*}p < 0.05$.

escape determination by Gerber method (Ahmed and Razig 2017). A decrease in fat content in yogurt during the storage period could be attributed to lipolysis in yogurt.

The protein content of the yogurt samples varied depending on the proportions of SMP, d-WP, and BMP added. It has been reported that the decrease in protein content during the storage period is related to the decrease in total solids content and the degradation of amino acids by yogurt bacteria (Salwa et al. 2004). The protein ratios obtained in our study are consistent with the results obtained in similar studies (Ünal and Akalin 2013).

The ash content of yogurt is affected by the source and composition of milk. Therefore, it is thought that the high ash ratios determined in our study are due to the dairy byproducts (SMP, d-WP, and BMP) added to yogurt milk. Similar results were obtained by Aziznia et al. (2008) and Bakırcı and Arslaner (2007). In addition, the use of d-WP prevented problems (such as salty taste) related to the amount of mineral substances in yogurt. As shown in Table 2, the ash content of sample F without d-WP was found to be higher.

The lactose content of the yogurt samples varied according to the combination of SMP, d-WP, and BMP added. In relation to the total solids content of the samples, the lactose content of sample A was lower and the lactose content of sample E was higher (Table 2).

It is thought that the combination of SMP, d-WP, and BMP added to sample B affected the apparent viscosity of yogurt stronger than the other combinations, while the apparent viscosity of sample E was low due to the absence of SMP. It was observed that the apparent viscosity values of the yogurt samples were higher than the apparent viscosity values (cow 3.254±0.476 Pa.s, goat 6.412±0.934 Pa.s) of the yogurts made from natural composition cow and goat milk (Erkaya and Şengül 2012). Therefore, it was determined that the increase in milk solids with SMP, d-WP, and BMP positively affected the apparent viscosity of the yogurt. In the studies conducted, the increase in viscosity at the end of storage is due to the increase in the water retention capacity of proteins and the tightening of the gel structure as a result of cold storage (Arab et al. 2023; Elkashef et al. 2022).

There was a decrease in the WHC of yogurts due to increasing d-WP powder addition (E and G). Indeed, similar results were reported by Lesme et al. (2020). SMP, d-WP, and BMP have different water retention capacities. Higher WHC values were determined in the samples where all three powders were added. The low protein content of d-WP caused a decrease in WHC. The WHC value determined on the 28th day in all samples (except A) was higher than the value determined on the first day. This is explained by the increase in the WHC of proteins and the tightening of the gel structure as a result of the increase in acidity as reported by Akın and Konar (2001). In addition, WHC increased, especially with the increased hydration capacity of MFGM proteins and phospholipids (Garczewska-Murzyn et al. 2022).

When the titratable acidity values of yogurts were examined, it was observed that sample A had a higher titratable acidity value than the other samples (Table 2). Öztürk (2013) reported that higher titratable acidity was observed in yogurt samples produced using milk powder and BMP. The increase in titratable acidity levels of the samples during 28 days of storage (Table 2) was found to be consistent with the literature data (Tarakçı and Demirkol 2016). Casarotti et al. (2014) reported that the decrease in acidity toward the end of storage is due to the slowing down of metabolic activities of lactic acid bacteria and less lactic acid production as storage continues.

It was observed that the pH of sample E (with 2% BMP addition) was lower than the other samples (Table 2). It was reported that the MFGM components in BMP increased the activity of yogurt cultures at the beginning of fermentation, so yogurt samples with BMP addition had high acidity and low pH values (Garczewska-Murzyn et al. 2022). In addition, the higher lactose content of E may be related to its lower pH. The change in pH values of the experimental yogurts was slow. This may be due to the decrease in nutrients utilized by lactic acid bacteria and the buffering capacity of proteins (Krebs et al. 2021). The increase in lactic acid content was significantly slower at the chilled storage temperature (approximately 4°C) (Table 2) compared to the typical ripening temperature for yogurt starter cultures, as reflected in the changes in pH values.

In yogurt samples, a decrease in pH and an increase in titratable acidity were observed due to the activity of lactic acid bacteria that continued to convert lactose into lactic acid during storage.

3.2 | Microbiological Counts

The counts of *L. delbrueckii* subsp. *bulgaricus*, *S. thermophilus*, yeast, and molds in experimental yogurt samples are shown in Table 3. It was determined that the ratio of SMP, d-WP, and BMP used and storage period were significant on the *L. delbrueckii* subsp. *bulgaricus* counts of the yogurts at $p < 0.05$ level (Table 3). The highest *L. delbrueckii* subsp. *bulgaricus* count was determined in G and the lowest *L. delbrueckii* subsp. *bulgaricus* count in F. This indicates that d-WP is more effective in the growth of *L. delbrueckii* subsp. *bulgaricus* than BMP. Several researchers have reported an increase in the counts of *L. delbrueckii* subsp. *bulgaricus* in yogurts to which milk powder and whey concentrate were added during storage (Ünal and Akalin 2013). It is suggested that this is mainly due to the whey concentrate or powder, especially the nonprotein nitrogenous components in the composition of these products (Gantumur et al. 2024).

The effect of added SMP, d-WP, BMP ratio, and storage time on *S. thermophilus* counts was found to be statistically significant ($p < 0.01$) (Table 3). The *S. thermophilus* count and lactose content of sample E were higher than other samples on the first day of storage. Therefore, the high lactose content supported the growth of *S. thermophilus*. It was also observed that *S. thermophilus* growth was supported by the addition of d-WP and BMP at the same rate (2%). The counts of *S. thermophilus* detected in our study are generally similar to the probiotic yogurts with the addition of inulin and d-WP produced by Yüksel and Bakırcı (2014). Toward the end of the storage period, *S. thermophilus* counts detected in yogurt samples were lower than *L. delbrueckii* subsp. *bulgaricus* counts. It was also determined that the pH values of the samples decreased toward the end of the storage period. Indeed, Ozer (2006) reported that the optimum growth pH (6.5) of *S. thermophilus* was higher than that of *L. delbrueckii* subsp. *bulgaricus* (optimum pH 5.8). Additionally, cold storage (< 10°C) greatly slows down the metabolic activities of yogurt bacteria.

Yeast and mold counts were < 2 log cfu/g in all samples during storage. Yeast and mold growth affects the shelf life and is undesirable in the production of quality yogurt.

3.3 | Organic Acid Profile

Organic acids in milk and dairy products are formed as a result of the growth of starter bacteria or through the natural biochemical mechanism of the dairy animal. The organic acids formed prevent the growth of microorganisms (spoilage) and support the formation of the characteristic taste and aroma of the products. Organic acid production in fermented dairy products such as yogurt varies depending on the amount and type of nutrients to be used by starter cultures (Bangar et al. 2022). The concentrations of organic acids in yogurt samples are shown in Table 4.

Lactic acid is the organic acid with the highest level in yogurt composition. It is corroborated that the dominant organic acid was lactic acid in yogurt samples (Table 4). The difference between the lactic acid concentrations of the samples was found to be statistically insignificant. The concentration of lactic acid increased during storage compared to the concentration detected on the first day ($p < 0.01$). It has been reported that lactic acid continues to be produced during storage by Ndhlala et al. (2022). It has been reported that *L. delbrueckii* subsp. *bulgaricus* is more resistant to acidic environments and can convert lactose into lactic acid during storage (Ongol et al. 2007).

Acetic acid is generated by the conversion of acetaldehyde formed as a result of the breakdown of pyruvic acid. The determination of the concentration of acetic acid above a certain value may have negative effects on the taste and aroma of dairy products (Papaioannou et al. 2021). The addition of SMP, d-WP, and BMP had an insignificant effect on the acetic acid formation of the samples, while the effect of storage on the acetic acid formation of the samples was significant at $p < 0.01$ level (Table 4). It was determined that the acetic acid concentration of the samples was higher on the 28th day of storage than on the first day, while the acetaldehyde concentration was lower. This supports the formation of acetic acid via the conversion of acetaldehyde.

Pyruvic acid is formed by the breakdown of lactose as a result of bacterial fermentation in milk and then converted into lactic acid and other metabolites by various enzymes (Bangar et al. 2022). The difference between the samples in terms of pyruvic acid was found statistically insignificant. While there was a similarity between the first and 14th days of the storage period, it was observed that the concentration of pyruvic acid decreased ($p < 0.05$) on the 28th day (Table 4). Indeed, Beshkova et al. (2003) reported that the decrease in the concentration of pyruvic acid during storage was due to the formation of products such as

TABLE 3 | Microbiological analysis results of yogurt samples (mean±SD).

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
***L. delbrueckii* subsp. *bulgaricus* Count (log cfu/g)**	1	8.23±0.05^{a,A}	8.00±0.04^{a,A}	8.17±0.12^{a,A}	7.99±0.07^{a,A}	7.87±0.41^{a,A}	7.88±0.33^{a,A}	8.22±0.08^{a,A}
	7	8.40±0.20^{a,AB}	8.55±0.02^{a,B}	8.31±0.17^{a,AB}	8.03±0.88^{a,A}	8.09±0.93^{a,A}	8.14±0.93^{a,A}	8.65±0.07^{a,B}
	14	8.58±0.12^{a,B}	8.48±0.20^{a,AB}	8.45±0.21^{a,AB}	8.12±0.99^{a,A}	8.21±0.82^{a,A}	8.14±0.52^{a,A}	8.74±0.06^{a,B}
	21	9.25±0.11^{c,C}	8.66±0.30^{abc,B}	8.80±0.38^{bc,B}	8.42±0.11^{ab,A}	8.12±0.59^{ab,A}	8.02±0.01^{a,A}	8.58±0.04^{abc,B}
	28	8.63±0.04^{bc,B}	8.44±0.20^{bc,AB}	8.58±0.05^{bc,AB}	8.75±0.04^{c,A}	8.41±0.32^{bc,A}	7.87±0.06^{a,A}	8.24±0.19^{ab,A}
Sign	Samples				*			
	Storage				*			
***S. thermophilus* Count (log cfu/g)**	1	8.50±0.35^{c,C}	6.74±0.14a^{,A}	7.73±0.01^{b,B}	8.15±0.00^{bc,B}	8.60±0.15^{c,C}	8.14±0.14^{bc,D}	7.51±0.55^{b,A}
	7	8.32±0.34^{c,C}	7.87±0.08^{abc,CD}	7.45±0.18^{a,AB}	8.00±0.09^{abc,B}	8.18±0.10^{bc,BC}	7.81±0.20^{abc,CD}	7.58±0.50^{ab,A}
	14	8.20±0.27^{b,BC}	8.17±0.24^{b,D}	7.30±0.03^{a,A}	8.16±0.20^{b,B}	8.13±0.08^{b,B}	7.39±0.11^{a,AB}	8.00±0.35^{b,A}
	21	7.37±0.02^{ab,A}	7.30±0.01^{ab,B}	7.51±0.07^{b,AB}	7.38±0.13^{ab,A}	7.20±0.22^{ab,A}	7.13±0.21^{a,A}	7.30±0.05^{ab,A}
	28	7.58±0.11^{a,AB}	7.57±0.06^{a,BC}	7.49±0.18^{a,AB}	7.43±0.23^{a,A}	7.51±0.23^{a,A}	7.66±0.07^{a,BC}	7.51±0.04^{a,A}
Sign	Samples				**			
	Storage				**			
Yeast and molds count (log cfu/g)	1	< 2	< 2	< 2	< 2	< 2	< 2	< 2
	7	< 2	< 2	< 2	< 2	< 2	< 2	< 2
	14	< 2	< 2	< 2	< 2	< 2	< 2	< 2
	21	< 2	< 2	< 2	< 2	< 2	< 2	< 2
	28	< 2	< 2	< 2	< 2	< 2	< 2	< 2

Note: Means with different capital letters in the same column and different lowercase letters in the same row are statistically different.
**$p < 0.01$; *$p < 0.05$.

TABLE 4 | Organic acid profiles of yogurt samples (mean±SD).

	Storage time (days)	Yogurt samples						
		A	B	C	D	E	F	G
Lactic acid (mmol kg^{-1})	1	1025.33±97.58^{a,A}	1007.92±40.19^{a,A}	1007.08±91.71^{a,A}	893.25±95.77^{a,A}	942.94±106.66^{a,A}	1018.05±12.74^{a,A}	1019.60±61.90^{a,A}
	14	1175.62±49.32^{a,A}	1152.89±70.82^{a,A}	1148.44±35.69^{a,A}	1093.41±91.47^{a,A}	1068.79±78.48^{a,A}	1040.50±12.68^{a,AB}	1091.60±69.40^{a,A}
	28	1029.69±23.07^{a,A}	1107.59±43.48^{a,A}	1136.27±15.48^{a,A}	1069.49±35.61^{a,A}	1034.02±124.17^{a,A}	1058.93±7.40^{a,B}	1110.27±60.49^{a,A}
Sign	Samples				not significant			
	Storage				**			
Acetic acid (mmol kg^{-1})	1	20.94±2.40^{a,A}	17.46±5.94^{a,A}	12.26±7.44^{a,A}	11.50±4.90^{a,A}	13.71±8.49^{a,A}	13.39±0.50^{a,A}	17.08±3.33^{a,A}
	14	43.22±0.41^{a,C}	41.40±10.92^{a,AB}	44.35±13.06^{a,AB}	33.13±18.19^{a,A}	34.42±17.69^{a,A}	25.49±1.69^{a,B}	36.39±7.45^{a,AB}
	28	33.42±3.38^{a,B}	52.24±9.46^{a,B}	55.96±10.58^{a,B}	44.84±21.56^{a,A}	45.03±35.96^{a,A}	38.79±6.09^{a,C}	54.16±9.32^{a,B}
Sign	Samples				not significant			
	Storage				**			
Pyruvic acid (mmol kg^{-1})	1	20.61±5.66^{a,A}	22.25±1.55^{ab,A}	25.59±8.11^{ab,A}	20.50±0.28^{a,A}	23.82±3.32^{ab,A}	31.96±5.14^{b,B}	20.55±1.40^{a,A}
	14	20.54±5.49^{a,A}	22.42±0.95^{a,A}	22.12±1.13^{a,A}	25.05±7.71^{a,A}	26.05±4.84^{a,A}	25.66±3.70^{a,AB}	20.87±4.97^{a,A}
	28	12.39±2.51^{a,A}	22.23±1.01^{ab,A}	19.62±7.49^{ab,A}	24.50±6.38^{b,A}	18.50±4.82^{ab,A}	18.58±2.40^{ab,A}	18.63±2.91^{ab,A}
Sign	Samples				not significant			
	Storage				*			
Orotic acid (mmol kg^{-1})	1	113.29±21.57^{ab,A}	122.41±3.44^{ab,A}	123.96±23.20^{ab,A}	99.91±6.34^{a,A}	114.28±1.47^{ab,A}	141.18±5.37^{b,A}	126.47±12.24^{ab,A}
	14	122.89±5.90^{a,A}	134.91±0.85^{ab,B}	139.45±0.25^{ab,A}	136.54±7.22^{ab,B}	135.89±10.56^{ab,A}	139.84±1.59^{b,A}	132.66±9.96^{ab,A}
	28	90.05±1.01^{a,A}	123.45±4.34^{b,A}	129.07±11.43^{b,A}	129.60±12.09^{b,B}	124.12±5.51^{b,A}	139.17±1.85^{b,A}	130.50±80.14^{b,A}
Sign	Samples				**			
	Storage				**			
Citric acid (mmol kg^{-1})	1	377.14±11.77^{a,B}	370.88±19.29^{a,A}	347.50±70.31^{a,A}	348.94±2.92^{a,A}	313.00±28.56^{a,A}	261.24±10.78^{a,A}	367.11±28.46^{a,A}
	14	402.92±0.08^{a,C}	397.27±12.97^{a,A}	396.86±3.57^{a,A}	383.53±26.10^{a,A}	179.20±23.47^{a,A}	384.91±22.22^{a,A}	385.99±19.06^{a,A}
	28	351.14±2.00^{a,A}	406.42±19.63^{c,A}	385.83±7.20^{abc,A}	374.40±14.94^{abc,A}	364.28±25.67^{ab,A}	364.10±6.35^{ab,A}	401.42±18.99^{bc,A}
Sign	Samples				not significant			
	Storage				**			

(Continues)

TABLE 4 | (Continued)

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Uric acid (mmol kg^{-1})	1	4.58±1.42^{a,A}	11.95±1.35^{ab,B}	11.67±1.15^{ab,B}	19.23±7.11^{b,A}	20.41±6.71^{b,A}	12.94±2.56^{ab,A}	12.82±4.22^{ab,A}
	14	6.03±0.60^{a,A}	7.58±0.73^{ab,A}	6.,25±1.45^{a,A}	12.13±4.36^{ab,A}	7.12±5.03^{ab,A}	14.07±2.56^{b,A}	10.01±1.75^{ab,A}
	28	12.58±0.62^{a,B}	7.50±0.81^{a,A}	6.11±1.34^{a,A}	11.35±2.28^{a,A}	12.24±8.95^{a,A}	11.39±1.77^{a,A}	5.59±2.83^{a,A}
Sign	Samples				*			
	Storage				**			
Hippuric acid (mmol kg^{-1})	1	0.37±0.00^{ab,A}	0.37±0.00^{ab,A}	0.38±0.00^{b,B}	0.38±0.00^{b,A}	0.37±0.00^{a,A}	0.39±0.00^{c,B}	0.37±0.00^{ab,A}
	14	0.37±0.00^{ab,A}	0.38±0.00^{ab,A}	0.37±0.00^{a,A}	0.38±0.00^{ab,A}	0.37±0.00^{ab,A}	0.38±0.00^{b,AB}	0.37±0.00^{ab,A}
	28	0.37±0.00^{a,A}	0.38±0.00^{a,A}	0.37±0.00^{a,A}	0.38±0.00^{a,A}	0.37±0.01^{a,A}	0.38±0.00^{a,A}	0.38±0.00^{a,A}
Sign	Samples				**			
	Storage				not significant			
Propionic acid (mmol kg^{-1})	1	0.00±0.00^{a,A}	0.00±0.00^{a,A}	0.00±0.00^{a,A}	0.00±0.00^{a,A}	0.00±0.00^{a,A}	0.00±0.00^{a,A}	0.00±0.00^{a,A}
	14	0.00±0.00^{a,A}	7.49±0.43^{b,B}	7.92±0.09^{bc,A}	0.00±0.00^{a,A}	9.68±0.07^{d,C}	0.00±0.00^{a,A}	8.40±0.40^{c,B}
	28	0.00±0.00^{a,A}	8.01±0.44^{a,B}	8.01±11.33^{a,A}	7.33±0.04^{a,B}	5.22±0.07^{a,B}	0.00±0.00^{a,A}	7.87±1.96^{a,B}
Sign	Samples				**			
	Storage				**			

Note: Means with different capital letters in the same column and different lowercase letters in the same row are statistically different.
**$p < 0.01$; *$p < 0.05$.

acetaldehyde and diacetyl with increased conversion of pyruvic acid to lactic acid.

Orotic acid is a product known as an intermediate in nucleic acid biosynthesis and is involved in the development of yogurt starter bacteria (Czauderna et al. 2021). It was determined that the effect of added SMP, d-WP, BMP, and storage time on orotic acid formation of the samples was significant at $p < 0.01$ level (Table 4). It is thought that the orotic acid detected in yogurts varies depending on the concentration of orotic acid contained in raw milk and bacterial activity. Indeed, Güzel-Seydim et al. (2000) reported that orotic acid increased slightly during storage. In similar studies on the subject, the concentration of orotic acid was determined as 106–116 mg kg^{-1} (Güler 2013) and 39–45 mg kg^{-1} (Serra et al. 2009).

Citric acid is used as a starting material by starter cultures in fermented dairy products and supports the formation of taste and aroma substances. It prevents the flocculation of milk proteins during the heating and freezing processes (Garavand et al. 2023). While the effect of added SMP, d-WP, and BMP on citric acid formation of the samples was found to be statistically insignificant, the effect of the storage period was found to be significant ($p < 0.01$) (Table 4). Citric acid formation varies depending on the type of starter culture and the ratio between cultures (Silva et al. 2023). It is reported that citric acid is naturally present in milk, some concentration is used during fermentation, and there is no major change in its concentration during storage (Gueimonde et al. 2003).

Uric acid is used by lactic acid bacteria as a nitrogen source to form carbon dioxide and ammonia. Therefore, its concentration decreases during the storage period (Navrátilova et al. 2022). It was observed that the SMP, d-WP, and BMP used were effective on the uric acid formation of the samples at $p < 0.05$ level, while the storage period was effective at $p < 0.01$ level (Table 4). In the study conducted by Özer (2020), it was reported that the uric acid content of yogurt samples was determined in the range of 4.38–17.09 mg kg^{-1} and decreased during storage.

The formation of hippuric acid in milk occurs as a result of fermentation by lactic acid bacteria, followed by conversion to benzoic acid. The natural hippuric acid content of milk varies according to the type of animal from which the milk is obtained (Bartáková et al. 2021). The addition of SMP, d-WP, and BMP affected on the hippuric acid concentration in the samples at $p < 0.01$ level (Table 4). It was determined that the change in hippuric acid during the storage period was insignificant. This is explained by the conversion of hippuric acid to benzoic acid. The concentration of hippuric acid was determined as 1–32 μg g^{-1} in yogurt samples produced using different starter cultures by Torre et al. (2003) and 0–2.35 μg g^{-1} in yogurt samples produced using d-WP and inulin by Kavaz and Bakırcı (2014).

Propionic acid is naturally present in low amounts in milk. In products such as yogurt and cheese, its amount increases as a result of bacterial fermentation (Navrátilova et al. 2022). The effect of the combination used and the storage period on the propionic acid formation of the samples was statistically significant ($p < 0.01$) (Table 4). Propionic acid was not detected in A and F during the storage period; therefore, it was determined that d-WP was more effective than SMP and BMP on the concentration of propionic acid in yogurt. It is thought that the lactose concentration in d-WP is higher than SMP and BMP, thus increasing the production of propionic acid. Propionic acid was not detected on the first day of the storage period. Afterward, it showed an increase. Adhikari et al. (2002) reported that propionic acid was not detected at the beginning of fermentation and 542 mg kg^{-1} propionic acid was formed on the 22nd day of storage in yogurts.

3.4 | Free Amino Acid Profile

Yogurt starter bacteria utilize nutrients such as peptides and proteins in milk for their growth. In addition, free amino acids resulting from proteolysis in yogurt are used as a starting product for the synthesis of flavors (Garavand et al. 2023). The concentration of free amino acids in yogurt varies with environmental factors such as lactation period, milk type, heat treatment intensity, bacterial strain, storage conditions, and production technology. *S. thermophilus* has a weaker proteolytic activity than *L. delbrueckii* subsp. *bulgaricus*. Therefore, *L. delbrueckii* subsp. *bulgaricus* is primarily responsible for the increase in the free amino acid content of yogurt. Milk proteins are first degraded into peptides by proteinases of *L. delbrueckii* subsp. *bulgaricus*, and then further hydrolyzed to amino acids by peptidases of *S. thermophilus* (Ulmer et al. 2022).

The concentrations of 20 free amino acids, including essential (lysine, valine, phenylalanine, leucine, isoleucine, histidine, tryptophan, threonine, methionine) and nonessential (serine, glutamic acid, proline, tyrosine, cysteine, glycine, alanine, aspartic acid, arginine, asparagine, glutamine) determined in yogurts are given in Table 5. It was observed that the combinations were effective on all 20 amino acids formed in yogurts at $p < 0.01$ level. It was observed that the storage time had no statistically significant effect on the formation of cysteine and glycine, the effect on arginine was significant at $p < 0.05$ level, and the effect on other amino acids was significant at $p < 0.01$ level. The concentration of valine determined in all samples was statistically different ($p < 0.01$). In terms of total phenylalanine formation during the storage, A, B, C, D, E, and F were similar to each other, while G was found to be different ($p < 0.01$) from these two sample groups. When the formation of lysine, valine, leucine, phenylalanine, methionine, threonine, serine, and aspartic acid were examined during the storage period, it was observed that the concentrations determined in all days of storage were different ($p < 0.01$). The concentration of glycine determined in all days of storage was statistically similar.

In all yogurt samples, essential amino acids, which have an important place in nutrition, were detected in different amounts. Among arginine and histidine, which are considered essential in childhood, arginine was present in high concentrations in all samples, whereas histidine showed high levels in sample E (no SMP), followed by sample A (only SMP). The highest concentration of arginine was determined on the 14th day of storage. Similarly, Isık et al. (2023) detected essential amino acids in all fermented milk samples produced using five different lactic

TABLE 5 | Free amino acid profiles of yogurt samples (mean±SD).

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Lysine (nmol μL^{-1})	1	$3.23\pm0.05^{d,A}$	$1.41\pm0.23^{c,A}$	$0.95\pm0.09^{ab,A}$	$0.79\pm0.09^{a,A}$	$1.02\pm0.07^{ab,A}$	$1.19\pm0.17^{bc,A}$	$1.90\pm0.02^{ab,A}$
	14	$3.75\pm0.23^{c,B}$	$1.91\pm0.11^{b,A}$	$2.03\pm0.03^{b,B}$	$1.20\pm0.16^{a,AB}$	$1.00\pm0.09^{a,A}$	$1.74\pm0.24^{b,B}$	$1.74\pm0.12^{b,C}$
	28	$3.03\pm0.07^{d,A}$	$2.92\pm0.25^{cd,B}$	$2.12\pm0.06^{b,B}$	$1.28\pm0.15^{a,B}$	$1.42\pm0.35^{a,A}$	$2.59\pm0.00^{c,C}$	$1.29\pm0.11^{a,B}$
Sign	Samples				**			
	Storage				**			
Valine (nmol μL^{-1})	1	$2.01\pm0.09^{b,A}$	$3.71\pm0.18^{c,B}$	$4.07\pm0.10^{d,A}$	$6.06\pm0.03^{f,B}$	$4.75\pm0.01^{e,A}$	$1.29\pm0.23^{a,A}$	$6.54\pm0.07^{g,A}$
	14	$2.48\pm0.02^{b,B}$	$2.99\pm0.01^{c,A}$	$4.51\pm0.18^{d,B}$	$6.40\pm0.11^{e,C}$	$7.48\pm0.01^{g,C}$	$1.68\pm0.03^{a,AB}$	$6.92\pm0.07^{f,B}$
	28	$2.04\pm0.09^{a,A}$	$3.85\pm0.04^{b,B}$	$5.73\pm0.03^{c,C}$	$5.68\pm0.04^{c,A}$	$6.40\pm0.14^{d,B}$	$1.96\pm0.24^{a,B}$	$8.10\pm0.16^{e,C}$
Sign	Samples				**			
	Storage				**			
Leucine (nmol μL^{-1})	1	$1.68\pm0.18^{c,A}$	$1.35\pm0.08^{bc,A}$	$2.63\pm0.25^{e,A}$	$2.14\pm0.16^{d,A}$	$0.85\pm0.04^{a,AB}$	$1.07\pm0.05^{ab,A}$	$2.64\pm0.01^{e,A}$
	14	$1.93\pm0.17^{b,A}$	$1.82\pm0.07^{b,A}$	$2.60\pm0.44^{c,A}$	$2.01\pm0.10^{b,A}$	$0.77\pm0.08^{a,A}$	$1.60\pm0.03^{b,B}$	$3.99\pm0.07^{d,B}$
	28	$2.54\pm0.14^{b,B}$	$2.45\pm0.32^{b,B}$	$3.39\pm0.63^{c,A}$	$3.56\pm0.35^{c,B}$	$1.11\pm0.13^{a,B}$	$2.05\pm0.15^{b,C}$	$4.34\pm0.23^{d,B}$
Sign	Samples				**			
	Storage				**			
Isoleucine (nmol μL^{-1})	1	$1.67\pm0.00^{a,A}$	$2.86\pm0.35^{c,A}$	$1.60\pm0.07^{a,A}$	$3.88\pm0.06^{d,B}$	$2.37\pm0.02^{b,A}$	$1.37\pm0.07^{a,A}$	$5.46\pm0.23^{e,A}$
	14	$1.51\pm0.02^{a,A}$	$2.73\pm0.03^{b,A}$	$3.03\pm0.13^{b,B}$	$3.85\pm0.26^{c,AB}$	$3.79\pm0.15^{c,C}$	$1.76\pm0.00^{a,B}$	$7.05\pm0.06^{d,B}$
	28	$1.55\pm0.18^{a,A}$	$3.07\pm0.21^{c,A}$	$3.67\pm0.01^{d,C}$	$3.36\pm0.04^{c,A}$	$3.19\pm0.10^{c,B}$	$2.05\pm0.13^{b,C}$	$6.98\pm0.05^{e,B}$
Sign	Samples				**			
	Storage				**			
Phenylalanine (nmol μL^{-1})	1	$0.57\pm0.18^{ab,A}$	$0.53\pm0.00^{ab,A}$	$0.48\pm0.03^{ab,A}$	$0.39\pm0.12^{a,A}$	$0.35\pm0.07^{a,A}$	$0.42\pm0.10^{a,A}$	$0.70\pm0.03^{b,A}$
	14	$0.86\pm0.03^{cd,AB}$	$1.00\pm0.12^{d,B}$	$0.84\pm0.08^{bcd,B}$	$0.67\pm0.12^{ab,A}$	$0.63\pm0.03^{a,B}$	$0.72\pm0.04^{abc,AB}$	$1.29\pm0.00^{e,B}$
	28	$1.19\pm0.05^{b,B}$	$0.91\pm0.07^{a,B}$	$1.22\pm0.02^{b,C}$	$1.07\pm0.02^{b,B}$	$0.88\pm0.00^{a,C}$	$0.89\pm0.14^{a,B}$	$1.55\pm0.05^{c,C}$
Sign	Samples				**			
	Storage				**			

(Continues)

TABLE 5 | (Continued)

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Tryptophan (nmol μL^{-1})	1	$0.12\pm0.01^{b,A}$	$0.11\pm0.00^{b,B}$	$0.12\pm0.00^{bc,A}$	$0.09\pm0.00^{a,A}$	$0.10\pm0.01^{a,A}$	$0.12\pm0.00^{b,A}$	$0.13\pm0.00^{c,A}$
	14	$0.14\pm0.00^{b,B}$	$0.13\pm0.00^{b,C}$	$0.12\pm0.02^{ab,A}$	$0.11\pm0.01^{a,A}$	$0.11\pm0.00^{a,AB}$	$0.13\pm0.00^{b,B}$	$0.15\pm0.00^{c,B}$
	28	$0.15\pm0.00^{e,B}$	$0.10\pm0.00^{a,A}$	$0.13\pm0.01^{bcd,A}$	$0.11\pm0.01^{ab,A}$	$0.12\pm0.01^{abc,B}$	$0.13\pm0.01^{cd,B}$	$0.15\pm0.00^{de,B}$
Sign	Samples				**			
	Storage				**			
Methionine (nmol μL^{-1})	1	$0.15\pm0.00^{c,A}$	$0.16\pm0.02^{c,A}$	$0.09\pm0.00^{b,A}$	$0.10\pm0.01^{b,A}$	$0.11\pm0.02^{b,A}$	$0.15\pm0.00^{c,A}$	$0.05\pm0.00^{a,A}$
	14	$0.24\pm0.03^{d,AB}$	$0.20\pm0.00^{c,A}$	$0.16\pm0.01^{b,B}$	$0.24\pm0.01^{d,B}$	$0.11\pm0.01^{a,A}$	$0.35\pm0.00^{e,B}$	$0.21\pm0.00^{c,B}$
	28	$0.37\pm0.09^{bc,B}$	$0.34\pm0.03^{bc,B}$	$0.28\pm0.01^{b,C}$	$0.40\pm0.03^{c,C}$	$0.17\pm0.02^{a,B}$	$0.40\pm0.01^{c,C}$	$0.36\pm0.05^{bc,C}$
Sign	Samples				**			
	Storage				**			
Threonine (nmol μL^{-1})	1	$1.41\pm0.29^{a,A}$	$2.41\pm0.00^{b,A}$	$2.65\pm0.29^{b,A}$	$3.60\pm0.46^{c,A}$	$2.69\pm0.02^{b,A}$	$1.30\pm0.21^{a,A}$	$3.63\pm0.07^{c,A}$
	14	$1.70\pm0.11^{a,A}$	$2.59\pm0.21^{b,A}$	$3.78\pm0.79^{c,A}$	$5.04\pm0.04^{d,B}$	$5.59\pm0.31^{d,B}$	$1.42\pm0.25^{a,A}$	$3.79\pm0.15^{c,A}$
	28	$2.01\pm0.15^{ab,A}$	$3.96\pm1.16^{c,A}$	$3.45\pm0.41^{abc,A}$	$3.81\pm0.18^{bc,A}$	$7.85\pm1.48^{d,B}$	$1.73\pm0.23^{a,A}$	$5.07\pm0.63^{c,B}$
Sign	Samples				**			
	Storage				**			
Histidine (nmol μL^{-1})	1	$5.68\pm0.14^{d,C}$	$0.03\pm0.01^{a,A}$	$0.05\pm0.01^{a,A}$	$0.04\pm0.00^{a,A}$	$7.05\pm0.86^{e,B}$	$1.46\pm0.18^{b,A}$	$2.31\pm0.23^{c,B}$
	14	$4.06\pm0.04^{c,B}$	$0.02\pm0.00^{a,A}$	$0.42\pm0.16^{a,B}$	$1.57\pm0.34^{b,B}$	$5.04\pm0.60^{d,AB}$	$1.96\pm0.22^{b,AB}$	$4.55\pm0.57^{cd,C}$
	28	$0.02\pm0.01^{a,A}$	$0.08\pm0.01^{a,B}$	$2.13\pm0.06^{c,C}$	$0.97\pm0.08^{b,B}$	$3.02\pm0.62^{d,A}$	$2.42\pm0.01^{c,B}$	$0.73\pm0.06^{b,A}$
Sign	Samples				**			
	Storage				**			
Serine (nmol μL^{-1})	1	$7.07\pm0.71^{b,A}$	$9.24\pm0.04^{c,B}$	$7.48\pm0.27^{b,A}$	$1.75\pm0.09^{a,A}$	$1.69\pm0.19^{a,A}$	$2.12\pm0.20^{a,A}$	$9.34\pm0.74^{c,A}$
	14	$7.51\pm0.36^{b,A}$	$6.83\pm0.68^{b,A}$	$7.40\pm0.33^{b,A}$	$3.46\pm0.19^{a,B}$	$2.94\pm0.17^{a,B}$	$2.92\pm0.35^{a,B}$	$14.51\pm1.79^{c,B}$
	28	$8.46\pm0.51^{c,A}$	$9.03\pm0.37^{c,B}$	$10.88\pm1.03^{d,B}$	$4.89\pm0.36^{b,C}$	$3.53\pm0.36^{a,B}$	$2.90\pm0.15^{a,AB}$	$9.52\pm0.35^{c,A}$
Sign	Samples				**			
	Storage				**			

(Continues)

TABLE 5 | (Continued)

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Glutamic acid (nmol µL^{-1})	1	7.14±1.50^{b,B}	6.77±0.21^{b,A}	7.90±0.50^{b,A}	1.72±0.34^{a,A}	1.96±0.10^{a,A}	2.89±0.08^{a,A}	10.35±0.11^{c,A}
	14	2.64±0.09^{a,A}	9.46±0.60^{bc,B}	8.15±0.69^{b,A}	10.52±0.12^{c,C}	2.93±0.21^{a,B}	3.54±0.55^{a,AB}	13.73±1.96^{d,A}
	28	2.19±0.01^{a,A}	8.02±0.55^{b,AB}	8.07±0.94^{b,A}	4.47±0.07^{a,B}	4.83±0.32^{a,C}	4.54±0.61^{a,B}	14.35±2.99^{c,A}
Sign	Samples				**			
	Storage				**			
Proline (nmol µL^{-1})	1	18.14±0.51^{bcd,A}	17.73±2.79^{bcd,A}	16.23±0.48^{bc,A}	19.83±0.50^{d,AB}	18.74±0.69^{bc,A}	9.81±0.13^{a,A}	15.63±0.46^{b,A}
	14	18.23±0.72^{bc,A}	16.05±2.45^{b,A}	19.67±4.02^{bc,A}	21.53±1.30^{c,B}	26.56±0.11^{d,C}	10.53±0.05^{a,AB}	20.24±0.82^{bc,B}
	28	17.23±2.49^{b,A}	17.07±0.98^{b,A}	18.75±2.97^{b,A}	17.82±0.25^{b,A}	23.68±0.05^{c,B}	11.10±0.55^{a,B}	17.39±1.38^{b,AB}
Sign	Samples				**			
	Storage				**			
Tyrosine (nmol µL^{-1})	1	0.55±0.04^{a,A}	1.73±0.03^{e,A}	1.63±0.02^{d,A}	0.82±0.03^{b,A}	0.83±0.06^{b,A}	1.22±0.04^{c,B}	0.48±0.02^{a,A}
	14	0.54±0.02^{a,A}	2.44±0.02^{d,A}	2.48±0.24^{d,B}	1.60±0.05^{c,B}	1.35±0.03^{bc,B}	1.32±0.05^{b,B}	0.59±0.14^{a,A}
	28	0.49±0.03^{a,A}	2.51±0.45^{d,A}	3.05±0.38^{d,B}	1.64±0.05^{c,B}	1.68±0.04^{c,C}	0.23±0.07^{a,A}	1.06±0.14^{b,B}
Sign	Samples				**			
	Storage				**			
Cysteine (nmol µL^{-1})	1	0.00±0.00^{a,A}	1.86±0.07 ^{cd,B}	0.61±0.03^{ab,C}	0.96±0.03^{ab,C}	0.43±0.09^{ab,A}	2.87±1.29^{d,A}	1.64±0.02^{bc,C}
	14	0.13±0.00^{a,A}	1.12±0.06^{abc,A}	0.05±0.05^{a,A}	0.29±0.08^{ab,B}	1.39±0.09^{bc,C}	2.19±1.18^{c,A}	0.64±0.01^{ab,A}
	28	2.48±0.31^{c,B}	1.05±0.04^{b,A}	0.20±0.00^{a,B}	0.08±0.03^{a,A}	1.02±0.10^{b,B}	1.14±0.47^{b,A}	1.49±0.00^{b,B}
Sign	Samples				**			
	Storage				not significant			
Glycine (nmol µL^{-1})	1	1.54±0.09^{a,A}	2.40±0.38^{ab,A}	2.44±0.69^{ab,A}	3.50±0.37^{b,AB}	2.59±1.46^{ab,A}	1.12±0.07^{a,A}	3.88±0.15^{b,A}
	14	1.26±0.17^{a,A}	2.34±0.38^{ab,A}	3.62±0.30 ^{cd,A}	4.58±0.20^{d,B}	3.03±1.18^{bc,A}	1.16±0.23^{a,A}	4.60±0.38^{d,A}
	28	1.59±0.43^{a,A}	2.08±0.23^{ab,A}	3.05±0.03^{b,A}	2.91±0.72^{b,A}	3.22±0.35^{b,A}	1.67±0.24^{a,A}	4.52±0.84^{c,A}
Sign	Samples				**			
	Storage				not significant			

(Continues)

TABLE 5 | (Continued)

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Alanine (nmol µL^{-1})	1	$7.61\pm0.28^{e,B}$	$1.51\pm0.20^{cd,A}$	$1.28\pm0.18^{bc,A}$	$2.02\pm0.50^{d,A}$	$0.51\pm0.03^{a,A}$	$0.80\pm0.08^{ab,A}$	$1.14\pm0.06^{bc,A}$
	14	$2.21\pm0.12^{b,A}$	$3.38\pm0.29^{c,B}$	$2.10\pm0.21^{b,AB}$	$2.01\pm0.83^{b,A}$	$0.74\pm0.10^{a,A}$	$1.18\pm0.02^{a,B}$	$2.48\pm0.01^{b,B}$
	28	$3.15\pm0.65^{c,A}$	$2.75\pm0.14^{abc,B}$	$3.67\pm0.91^{c,B}$	$1.88\pm0.39^{ab,A}$	$1.72\pm0.22^{a,B}$	$1.57\pm0.06^{a,C}$	$3.03\pm0.46^{bc,B}$
Sign	Samples				**			
	Storage				**			
Aspartic acid (nmol µL^{-1})	1	$6.86\pm0.74^{b,A}$	$2.74\pm0.20^{a,A}$	$0.08\pm1.29^{b,B}$	$2.39\pm0.14^{a,A}$	$1.58\pm0.60^{a,A}$	$2.28\pm0.89^{a,A}$	$9.13\pm2.37^{b,A}$
	14	$7.30\pm0.52^{bc,A}$	$7.78\pm0.19^{bc,C}$	$4.98\pm0.26^{ab,A}$	$3.23\pm0.17^{a,A}$	$4.18\pm0.32^{a,B}$	$2.79\pm0.88^{a,A}$	$8.87\pm2.85^{c,A}$
	28	$15.59\pm1.22^{d,B}$	$4.70\pm0.46^{ab,B}$	$8.99\pm0.56^{c,B}$	$3.97\pm0.84^{a,A}$	$4.04\pm0.69^{a,B}$	$2.68\pm0.92^{a,A}$	$7.41\pm2.51^{bc,A}$
Sign	Samples				**			
	Storage				**			
Arginine (nmol µL^{-1})	1	$1.76\pm0.59^{abc,A}$	$5.74\pm0.28^{d,A}$	$3.28\pm0.97^{c,AB}$	$1.01\pm0.02^{a,A}$	$1.88\pm1.06^{abc,A}$	$1.44\pm0.56^{ab,A}$	$2.89\pm0.14^{bc,A}$
	14	$5.18\pm1.56^{c,A}$	$4.12\pm1.17^{bc,A}$	$4.91\pm1.17^{bc,B}$	$1.06\pm0.05^{a,A}$	$3.10\pm0.94^{abc,A}$	$2.55\pm0.73^{ab,A}$	$3.01\pm0.28^{abc,A}$
	28	$5.48\pm1.72^{c,A}$	$4.42\pm0.12^{c,A}$	$1.98\pm0.24^{a,A}$	$1.21\pm0.01^{a,B}$	$2.57\pm0.69^{ab,A}$	$2.08\pm0.38^{a,A}$	$4.06\pm0.00^{bc,B}$
Sign	Samples				**			
	Storage				*			
Asparagine (nmol µL^{-1})	1	$1.53\pm0.28^{b,A}$	$1.68\pm0.08^{b,A}$	$1.65\pm0.40^{b,A}$	$0.48\pm0.03^{a,A}$	$1.43\pm0.28^{b,A}$	$9.46\pm0.26^{c,B}$	$1.38\pm0.12^{b,A}$
	14	$2.07\pm0.21^{b,A}$	$1.74\pm0.07^{b,A}$	$2.28\pm0.31^{b,A}$	$0.99\pm0.16^{a,A}$	$2.28\pm0.15^{b,B}$	$9.38\pm0.38^{c,B}$	$1.06\pm0.00^{a,A}$
	28	$1.55\pm0.02^{ab,A}$	$2.20\pm0.09^{bc,B}$	$2.53\pm0.27^{c,A}$	$1.46\pm0.65^{ab,A}$	$1.21\pm0.09^{a,A}$	$1.70\pm0.08^{ab,A}$	$2.60\pm0.31^{c,B}$
Sign	Samples				**			
	Storage				**			
Glutamine (nmol µL^{-1})	1	$4.31\pm0.55^{e,A}$	$3.53\pm0.18^{cd,A}$	$2.67\pm0.43^{b,A}$	$3.90\pm0.07^{de,B}$	$3.10\pm0.03^{bc,A}$	$0.84\pm0.12^{a,A}$	$2.95\pm0.19^{bc,A}$
	14	$4.51\pm0.06^{c,A}$	$3.52\pm0.51^{b,A}$	$3.49\pm0.60^{b,A}$	$4.16\pm0.06^{b,B}$	$4.58\pm0.01^{c,C}$	$1.17\pm0.13^{a,A}$	$3.74\pm0.15^{b,B}$
	28	$4.03\pm0.65^{b,A}$	$3.77\pm0.27^{b,A}$	$3.47\pm0.57^{b,A}$	$3.41\pm0.18^{b,A}$	$4.09\pm0.15^{b,B}$	$1.88\pm0.18^{a,B}$	$3.70\pm0.02^{b,B}$
Sign	Samples				**			
	Storage				**			

Note: Means with different capital letters in the same column and different lowercase letters in the same row are statistically different.
**$p < 0.01$; *$p < 0.05$.

acid bacteria strains and reported that histidine and arginine concentrations were high.

Lysine, valine, leucine, isoleucine, threonine, serine, glutamic acid, proline, glycine, alanine, aspartic acid, arginine, and glutamine were found to be high in all yogurt samples (Table 5). Proline was detected in higher concentrations in all yogurt samples compared to other amino acids. Similar results were also reported by Boycheva et al. (2012) and Keser (2018). Tamime and Robinson (1999) attributed the high levels of serine, glutamic acid, proline, and alanine in yogurt to the fact that *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus* do not use these amino acids during fermentation and storage. Terzioglu and Bakirci (2023) explained the high aspartic acid concentration in yogurt by the conversion of glutamic acid and asparagine into aspartic acid during the deamination process. Amino acids found in higher concentrations in yogurt samples are as reported in the literature (Table 5).

When the change in the amino acids detected in the experimental yogurts during the storage period was examined, it was determined that there was a general increase in the formation of amino acids and no significant change in some of them. The increase in the content of amino acids during the storage is explained by Terzioglu and Bakirci (2023) who stated that their rate of formation is higher than their rate of use in reactions. Gu et al. (2020) stated that the concentration of free amino acids in yogurt samples produced using different lactic acid combinations showed little change during storage.

It was observed that the concentration of isoleucine and valine occurred at higher concentrations in D, E, and G with a high d-WP ratio (1.5–2%) compared to other samples (Table 5). As reported in the literature, whey is rich in lysine, leucine, and isoleucine (Bilal and Altıner 2017). In addition, Yüksel et al. (2019) have stated that the concentration of methionine and cystine in whey is also high.

The total amino acid concentration determined in the experimental yogurt samples varied between 43.20 and 103.14 nmol μL^{-1}. In terms of total amino acid concentration, it is noteworthy that sample G contained higher concentrations of amino acids in all storage periods (Table 5). It is observed that the formation of amino acids during storage is lower in F, which contains 2% BMP. Novokshanova et al. (2019) reported that although the protein content of skimmed milk and buttermilk were close to each other, the essential amino acid content of buttermilk was lower, while the essential amino acid content of whey protein hydrolysate was higher. The findings obtained show that the high amino acid content of yogurts produced by adding SMP, d-WP, and BMP contributes to the amount of essential and nonessential amino acids taken into the body.

3.5 | Aroma Compounds

Acetaldehyde is the main aroma component of yogurt and has an important role in the formation of the characteristic yogurt flavor. In yogurt, acetaldehyde can be synthesized from carbohydrates, proteins, lipids, and nucleic acids through different metabolic pathways (Krastanov et al. 2023). For the characteristic taste and aroma to be formed in yogurt, the concentration of acetaldehyde must be more than 8 ppm (Papaioannou et al. 2021). It is known that the use of both yogurt starter bacteria together develop more taste and aroma (Chen et al. 2017).

It was observed that SMP, d-WP, BMP, and storage period had a significant ($p < 0.01$) effect on the acetaldehyde levels of the samples (Table 6). It was observed that the concentration of acetaldehyde produced was higher in D and E, where the ratio of d-WP was higher (1.5–2%), whereas the concentration of acetaldehyde produced was lower in sample F, which did not contain d-WP. Indeed, Gallardo-Escamilla et al. (2005) reported that the acetaldehyde content of yogurts fortified with whey was higher than those fortified with SMP and that this was due to the fact that whey is rich in threonine, which is used in acetaldehyde production. The generally high acetaldehyde formation in yogurts is thought to be related to the enrichment of yogurt milk in terms of total solids content. It was observed that the concentration of acetaldehyde formed on the first day of the storage period was higher and differed ($p < 0.01$) from the concentration formed on the 14th and 28th days. Dan et al. (2017) and Krastanov et al. (2023) suggested that acetaldehyde is reduced to ethanol during storage and the acetaldehyde ratio decreases.

Diacetyl is a product involved in the formation of taste and aroma together with acetaldehyde in yogurt (Tian et al. 2024). It is reported that diacetyl production at the level of 0.2–3 mg kg^{-1} is sufficient for the formation of the desired taste and aroma in yogurt. Both citrate and pyruvate produced by lactose metabolism play an important role in the formation of diacetyl. In the absence of citrate, diacetyl and acetoin formation does not occur (Papaioannou et al. 2021).

It was determined that the addition of SMP, d-WP, and BMP had an effect on the diacetyl concentrations of yogurts at $p < 0.01$ level (Table 6). Erkaya and Şengül (2011) reported the concentration of diacetyl in yogurt made from cow's milk as $0.11 \pm 0.20 - 2.32 \pm 2.25$ depending on the peak area; Saçak (2022) reported the average concentration of diacetyl in fresh yogurt as 0.099 mg kg^{-1}. In sample F, which has low acetaldehyde content, a higher concentration of diacetyl formation occurred compared to other samples. Gürsoy-Balcı (2008) stated that diacetyl contributes to the aroma in yogurt production and its presence gains more importance in cases where acetaldehyde level is low. The diacetyl concentration of the experimental yogurts was found to be higher on the first day of storage. The decrease in the concentration of diacetyl determined during storage was statistically significant ($p < 0.01$). It was observed that the decrease in diacetyl concentration during storage was in agreement with literature data (Baranowska 2006).

Acetoin is a carbonyl compound. It is produced by some *S. thermophilus* strains. It is also produced in very small concentrations by *L. delbrueckii* subsp. *bulgaricus*. The conversion of citrates and the breakdown of lactose under certain conditions leads to the formation of acetoin and diacetyl. Acetoin is formed by the enzyme (diacetyl reductase) mediated breakdown of diacetyl or by decarboxylation α-acetolactic acid (Zhao et al. 2023).

It was determined that the effect of added SMP, d-WP, BMP, and storage period on the acetoin concentrations of the samples

TABLE 6 | Aroma compounds of yogurt samples (mean±SD).

	Storage time (days)	Yogurt samples						
		A	**B**	**C**	**D**	**E**	**F**	**G**
Acetaldehyde (mmol kg^{-1})	1	$18.55 \pm 0.48^{b,B}$	$18.77 \pm 0.42^{b,A}$	$19.51 \pm 1.67^{b,A}$	$24.20 \pm 0.86^{c,B}$	$23.32 \pm 0.32^{c,B}$	$9.38 \pm 1.85^{a,A}$	$18.09 \pm 1.84^{b,A}$
	14	$15.81 \pm 1.40^{bc,AB}$	$14.54 \pm 2.74^{b,A}$	$16.25 \pm 2.56^{bc,A}$	$21.00 \pm 0.43^{d,A}$	$18.59 \pm 0.02^{cd,A}$	$9.12 \pm 0.52^{a,A}$	$17.41 \pm 0.93^{bcd,A}$
	28	$12.96 \pm 2.17^{b,A}$	$14.41 \pm 2.10^{bc,A}$	$16.65 \pm 0.25^{cd,A}$	$19.40 \pm 0.96^{d,A}$	$18.02 \pm 0.26^{d,A}$	$7.93 \pm 1.17^{a,A}$	$15.96 \pm 1.62^{bcd,A}$
Sign	Samples				**			
	Storage				**			
Diacetyl (mmol kg^{-1})	1	$0.96 \pm 0.05^{ab,B}$	$0.65 \pm 0.06^{a,A}$	$0.65 \pm 0.03^{a,B}$	$0.53 \pm 0.00^{a,B}$	$0.44 \pm 0.02^{a,B}$	$1.36 \pm 0.56^{b,A}$	$0.56 \pm 0.11^{a,A}$
	14	$0.54 \pm 0.05^{ab,A}$	$0.74 \pm 0.06^{b,A}$	$0.47 \pm 0.02^{ab,A}$	$0.29 \pm 0.03^{ab,A}$	$0.26 \pm 0.05^{a,A}$	$1.17 \pm 0.49^{c,A}$	$0.40 \pm 0.03^{ab,A}$
	28	$0.51 \pm 0.02^{bc,A}$	$0.58 \pm 0.04^{cd,A}$	$0.54 \pm 0.08^{bcd,AB}$	$0.59 \pm 0.04^{cd,B}$	$0.32 \pm 0.01^{a,A}$	$0.69 \pm 0.12^{d,A}$	$0.38 \pm 0.07^{ab,A}$
Sign	Samples				**			
	Storage				**			
Acetoin (mmol kg^{-1})	1	$43.00 \pm 2.90^{a,A}$	$55.62 \pm 3.14^{b,B}$	$59.97 \pm 7.75^{b,A}$	$74.85 \pm 1.08^{c,C}$	$58.47 \pm 0.90^{b,B}$	$62.22 \pm 0.38^{b,A}$	$46.25 \pm 0.31^{a,A}$
	14	$35.04 \pm 7.83^{a,A}$	$38.69 \pm 0.05^{a,A}$	$44.77 \pm 4.98^{ab,A}$	$40.63 \pm 0.17^{ab,A}$	$49.76 \pm 1.22^{bc,A}$	$59.91 \pm 2.66^{c,A}$	$38.13 \pm 6.25^{a,A}$
	28	$35.79 \pm 7.52^{a,A}$	$35.43 \pm 0.63^{a,A}$	$43.42 \pm 2.18^{a,A}$	$53.66 \pm 0.07^{b,B}$	$60.63 \pm 0.20^{b,B}$	$58.16 \pm 6.74^{b,A}$	$37.91 \pm 1.62^{a,A}$
Sign	Samples				**			
	Storage				**			

Note: Means with different capital letters in the same column and different lowercase letters in the same row are statistically different.

**$p < 0.01$.

was significant at $p < 0.01$ level (Table 6). While D, E, and F were similar in terms of acetoin formation, A, B, C, and G were statistically different ($p < 0.01$) from each other and from this group. It is thought that the addition of BMP (1.5–2%) in yogurt samples contributed to acetoin formation. It was detected that the concentration of acetoin was higher in yogurt samples than diacetyl. Similar results were reported by Güler and Gürsoy-Balcı (2011) and Özer (2020).

4 | Conclusion

The results showed that the addition of SMP, d-WP, and BMP improved the physicochemical and biochemical properties of the yogurt and maintained the viability of yogurt cultures. It was determined that the viscosity and WHC of sample B were higher than the other samples during the storage period. It was determined that the pH value of sample C was higher and its acidity was lower. It was observed that the orotic, hippuric, and propionic acid concentrations of the yogurt samples were significant during the storage period ($p < 0.01$). While the highest amino acid concentration was detected in sample G; acetaldehyde formation occurred more in sample D. In conclusion, it was determined that the use of SMP, d-WP, and BMP in appropriate ratios and combinations in yogurt production generally gave more favorable results on the parameters examined than when they were used alone. It was also concluded that a yogurt with better nutritional and functional properties could be produced. The findings of this study will be a valuable resource to improve product quality in the yogurt industry and to provide consumers with healthier and more balanced nutrition options. The results obtained can guide the choice of additives in industrial applications and help to optimize the yogurt production process.

Author Contributions

Neslihan Yıldız Küçük: Validation, formal analysis, investigation, methodology, writing – original draft. **İhsan Bakırcı**: Conceptualization, methodology, supervision, visualization, writing – review and editing.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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