



Evaluation of Physicochemical Properties of Yogurt Fortified with Four Varieties of Common Bean Whey (*Phaseolus vulgaris*)

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ABSTRACT

Background: In recent years, the role of novel foods in public health has rapidly grown due to increased life expectancy, rising medication costs and consumers' focus on obtaining health benefits.

Methods: This study evaluates the acid production, microbial growth, physicochemical and textural properties of common bean-fortified yogurt (CBWIFY) during 28 days of cold storage.

Result: All varieties of CBWIFY showed higher acidity and lower pH value, resulting in a higher microbial population after production and during 28-day storage. The color values "a" and "L" did not vary significantly in all samples and remained steady after 28 days, while the "b" value increased. The highest syneresis (14.86%) was found in white bean whey, while the lowest syneresis (1.81%) was observed in kidney bean whey. In terms of texture, the cohesiveness of CBWIFY and the control sample decreased, while the hardness, springiness and gumminess improved during cold storage. Remarkably, cranberry bean whey had higher values (cohesiveness, springiness and gumminess) despite the hardness found in white bean whey.

Key words: Common bean, *L. Bulgaricus*, Physicochemical properties, Yogurt.

INTRODUCTION

Interest in creating healthy foods is growing, whereas the global flavored milk market is projected to reach USD 68.8 billion by 2024 (Sawale *et al.*, 2020). Attention to plant-based dairy products has grown due to concerns over health, animal welfare and environmental awareness. These products incorporate a variety of legumes, seeds, grains, nuts, vegetables and fruits (Aydar *et al.*, 2021). Food security is a major concern, especially the need to address protein malnutrition immediately (Pradeepkiran, 2019). Food producers are increasingly turning to plant-based proteins and pulses are a sustainable and high-quality option (Bessada *et al.*, 2019). Pulses are a low-cost source of plant protein, with 18.5-32% protein content. They're considered "the meat of the poor" and are popular in developing countries. Due to their many benefits, pulses have a promising future in the food industry. Plant-based milk, derived from peas, peanuts, lentils, almonds, corn and soy, is becoming increasingly popular in yogurt production and food manufacturing (Emkani *et al.*, 2022).

Yogurt is made by fermenting milk with a culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*. Starter cultures facilitate milk acid production and synthesis of aromatic compounds. This enhances our knowledge of developing functional foods by utilizing the probiotic role of lactic acid bacteria (LAB) and their "generally recognized as safe" (GRAS) position (Yankey *et al.*, 2023). Due to its sensory properties, it is widely consumed as a nutritious and healthy food. Yogurt is categorized as plain or flavored based on its composition. Plain yogurt contains only dairy constituents, while flavored yogurt contains additives (Li *et al.*, 2019).

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Adding pulses to the daily diet can lower the risk of chronic illnesses (Peer Khan *et al.*, 2021). Several interests have been shown in developing novel pulse-supplemented products, such as a bean curd made from protein whey of various pulses (Kleintop *et al.*, 2013). To the best of our

knowledge, there is a lack of information regarding the effect of storage time on common bean whey-fortified yogurt (CBWFY). With its high protein and fiber content, storage may alter the physicochemical properties of the CBWFY and thus requires investigation. Therefore, in this study the acid production, growth of LAB counts, pH, syneresis, color and texture properties of CBWFY immediately after production as well as during 1 month of storage were studied.

MATERIALS AND METHODS

The experiment was carried out during the storage month of 2019-11 at the research laboratory of the College of Food Science and Technology, Nanjing Agricultural University. The preparation of CBWFY, enumeration of viable cell counts ((colony forming unit/gram) CFU/g), the pH and acidity analysis, syneresis and color properties of yogurt were carried out according to the previous method described by (Rui *et al.*, 2011; Zahir *et al.*, 2020). An analysis of texture was performed using a texture analyzer (TMS-Pro, Food Technology Corporation, [ruix1] [zs2] USA). A stainless-steel cutter was used to take cylindrical samples (diameter 2.5 cm, height 1.5 cm) from the central part. We used a 50 N load cell at a speed of 5.0 mm s⁻¹. In the pre-test, the compression speed of the probe was set to 1.0 mm s⁻¹. The hardness, springiness, cohesiveness and gumminess were measured. Data were analyzed using ANOVA (SAS 9.1, SAS Institute INC).

RESULTS AND DISCUSSION

Change in pH during storage

Based on the result, there was a projected pH difference after 28-day storage in all samples as shown in (Fig 1). In this study, the fermentation was terminated when the pH reached 4.60 and the results showed that the CBWFY

sample reached pH 4.60 significantly earlier than the control sample (Fig 1). In all samples, pH decreased slightly by 0.8 to 0.95 units over the 21-day storage period which was more apparent during the first 14 days of conservation. This could be due to the increased activity of bacterial cultures in the presence of CBW, particularly for cow milk (CM) fortified with cranberry and black bean whey. A slight increase was observed for CBWFY and subsequent reduction was noticed for all samples at 28 days of storage, which was greater for CM fortified with cranberry bean whey followed by black, kidney and white and control samples. A similar finding was reported by (García Pérez *et al.*, 2005). This might be due to the high bacterial metabolic activity and subsequent increase of lactic acid. After 28 days of cold storage, all samples reached pH values (ranging from 3.65 to 3.88) and the acidity 130°T-150°T despite their evolution along storage. Consistent with earlier research conducted by (Chen *et al.*, 2018).

Acidification by *L. bulgaricus*

The acidity of CBWFY after production and 28-day storage in the refrigerator is shown in (Fig 2). Compared to the control, a high acidification rate was observed for CBWFY ($P < 0.05$). At day 0, the acidity in CM fortified with black bean whey was significantly lower than in three other control samples ($P < 0.05$). On day 1, the CM fortified with cranberry bean whey had the highest acidity, followed by black, white and kidney samples and among all varieties, the three were significantly higher in comparison to the control and the same value was obtained for kidney bean whey ($P < 0.05$). At day 21 of the storage period, the rate of acidification was still higher for CM fortified with cranberry bean whey in comparison with other bean whey and control samples ($P < 0.05$). At 28 days, the acidity reduction was observed for all samples (including control samples) and CM fortified

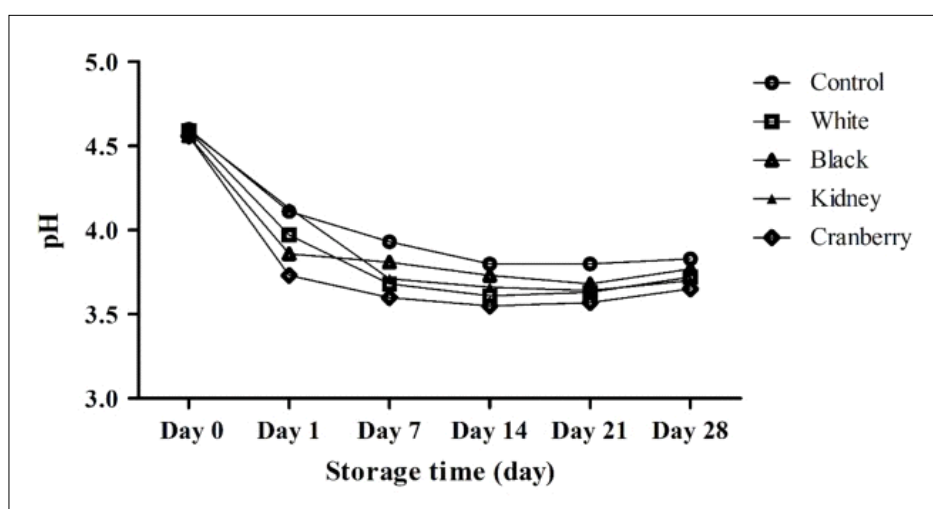


Fig 1: pH trend in CBWFY and control after production and during 28 days of storage at 4°C, white (□), black (△), kidney (▲) cranberry (◆) and control (○).

with black bean whey sample demonstrated the highest reduction in comparison with other treated samples. The acidity of yogurt can be affected by the metabolic activity of the organisms in yogurt during storage; the high acidity value demonstrates a high metabolic activity of microorganisms. Therefore, the results showed that higher availability of nutrients, especially lactose and essential amino acids, results in rapid acid production by LAB probably due to a more rapid transformation of lactose into lactic acid (Ramirez-Santiago *et al.*, 2010). According to the previous investigation, pulse ingredients can enhance the acidity value of yogurt in the presence of probiotic bacteria. This could explain the greater acid production observed in the CBW FY. Compared to other varieties, cranberry bean whey possessed greater acid production among all varieties of CBW FY which is probably due to richer complex carbohydrates, minerals and vitamins. Our results were

similar to the findings of (Zare *et al.*, 2013). To understand changes in microbial growth during acidification, the viable counts were measured at the production and during storage.

Bacterial growth and survival

As a functional food or the strains involved, it is proposed that 10^9 CFU/g per portion of a probiotic culture be the minimum concentration in fermented food products. To preserve these numbers, it is important to follow the probiotic viability during manufacture and storage (Zare *et al.*, 2013). On day 0, viable counts of *L. bulgaricus* for all samples varied from log 8.82 to log 8.97 CFU/mL and it decreased over the 28-day storage period by between 0.16 and 0.42 log CFU/mL (Fig 3). These observed rates were higher than the minimum level of effective and active cultures ($7 \log \text{CFU mL}^{-1}$) as proposed by FDA rules (Chen *et al.*, 2018). Compared to the control, all varieties of CBW FY showed high levels of

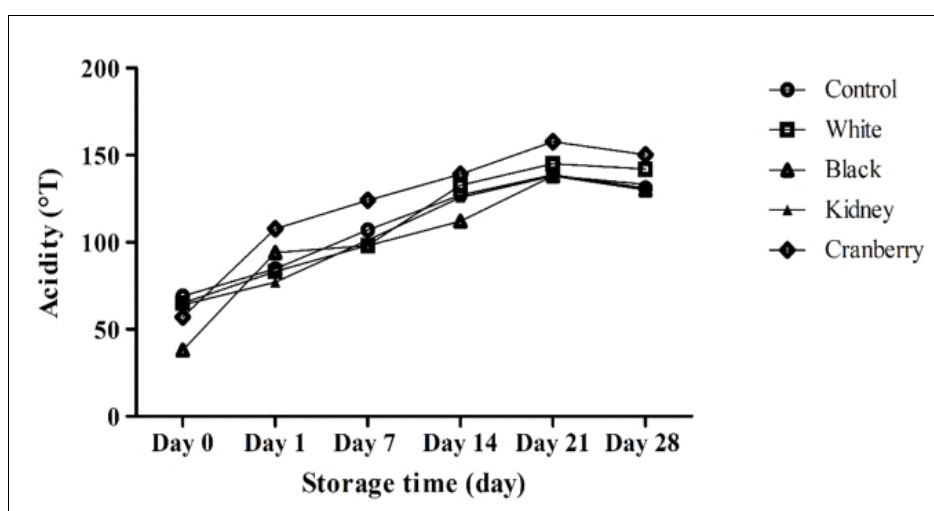


Fig 2: Effect of CBW supplementation on acid production in CBW FY by *L. bulgaricus* immediately after the fermentation and during 28 days of storage at 4°C, white (□), black (△), kidney (▲) cranberry (◇) and control (○).

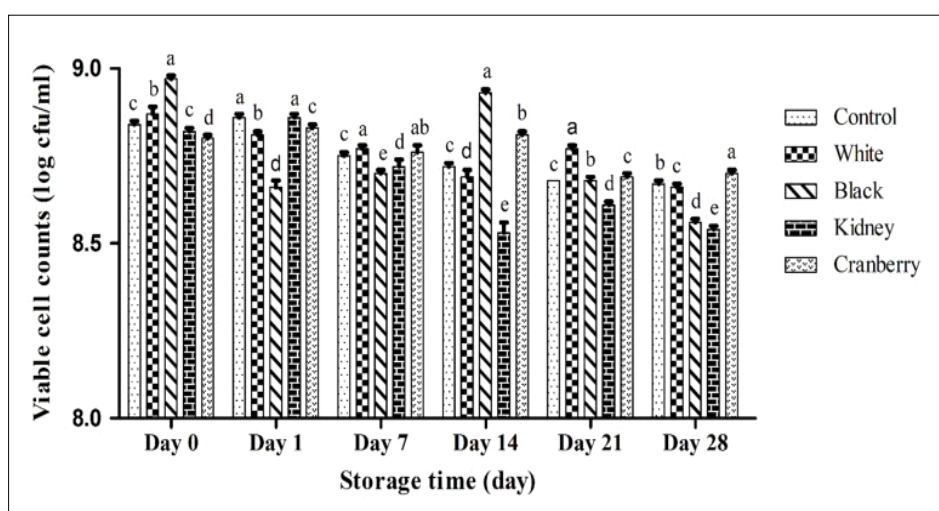


Fig 3: Effect of CBW supplementation on viable cell counts (CFU/mL) in CBW FY immediately after the fermentation and during 28 days of storage at 4°C.

L. bulgaricus in the freshly prepared products and during 28-day cold storage, which was greater in CM fortified with black bean whey (log 8.97 CFU/mL). During the storage for 28 days, the viable cell counts of LAB in yogurt fortified with cranberry bean whey were significantly ($P<0.05$) greater followed by black and white bean whey and the lowest value was obtained for CM fortified with kidney bean whey. However, the viability of the probiotic bacteria *L. bulgaricus* significantly ($P<0.05$) reduced among all the samples regardless of the CBWFY, resulting in maintaining a stable microbial count of 8.70 logs CFU/mL at 28 days (Fig 3). This could explain the higher growth observed in the CBWFY, particularly for CM fortified with black bean whey at 0 days due to low pH value and higher acidity. Compared to other varieties, cranberry bean whey possessed greater LAB counts probably due to richer complex carbohydrates, minerals and vitamins. This result can be attributed to the functional capability of CBWFY in stimulating growth, as likewise reported by (Bakr, 2013). Similarly, a 3% addition of pea flour enhanced the microbial populations after production and 28 days of storage (Zare *et al.*, 2013). Chickpea flour stimulated the growth of microbial populations and maintained higher counts over the 21-day cold storage, due to the presence of minerals and raffinose oligosaccharides (Chen *et al.*, 2018), which is consistent with our study.

Color determination

The color of the fortified products should ideally remain unchanged after production and during storage. Fig 4 (a, b and c) shows differences in the color (a, b and L values) of the CBWFY and control samples at 0 and 28 days after production. On the 0-day, the lowest "a" value was observed in the CM fortified with black bean whey and the highest "b" value was observed in the control sample. Significant ($P<0.05$) lower "L" values were observed for all samples of CBWFY compared to the control. Furthermore, the "a" and "b" values were significantly ($P<0.05$) different among all CBWFY. After 28 days, the "L" value was highest in the control sample in comparison with all other samples ($P<0.05$). The "a" value was lower in CBWFY samples except for CM fortified with white bean whey and cranberry, whereas the "b" value was highest in the control sample ($P<0.05$). The color measurements indicate that immediately after production the CBWFY had lower lightness, more yellowness and less greenness hue in comparison with the control sample. After 28 days, the lightness of the CBWFY samples was decreased and a slightly yellowness hue was developed (Fig 4). These results are in agreement with the results obtained by (Ozcan and Kurtuldu, 2014). As differences in color between the CBWFY and control samples were visually realizable, further studies may be required to optimize color will be useful to any product development aimed at commercialization of this type of product.

Syneresis

Syneresis is the separation of whey from yogurt without using any external force and is a very important characteristic that should be considered, especially during product storage. The syneresis of CBWFY and control samples immediately after production and after 1 and 28 days of cold storage is illustrated in (Fig 5). Syneresis was higher in CBWFY compared with control yogurt on days 1, 7, 14 and 21, whereas control yogurt showed higher syneresis ($P<0.05$) than CBWFY as storage prolonged. As for titratable acidity, the values of all yogurts were higher on days 1, 7, 14 and 21, thus it is responsible for higher syneresis. Immediately after production, the highest syneresis was obtained for CM fortified with black bean whey followed by kidney and cranberry, but an even lower value was obtained for CM fortified with white bean whey compared to the control. On day 1, the highest syneresis was observed in the CM fortified with white bean whey followed by black, cranberry and kidney and significantly lowest in the control samples ($P<0.05$). After 1 and 21 days of storage, the volume of whey separated from the gel increased in all samples in comparison with what was observed after day 0 of production. At day 7, among all varieties of CBWFY, the least syneresis was found in the CM fortified with cranberry followed by kidney, black and white and in comparison, to CBWFY, the lowest syneresis was observed in the control sample ($P<0.05$). After 28 days of cold storage, the amount of syneresis in the CBWFY decreased and the lowest syneresis was found in the CM fortified with kidney bean whey, followed by cranberry, black and white, but in comparison to days 1, 7, 14 and 21 the liquid separated from control samples increased ($P<0.05$). As a result, among all varieties of CBWFY, the CM fortified with kidney bean whey showed low syneresis through whole cold storage except for days 0 and 7 and the highest whey separation was obtained for CM fortified with white bean whey. This is because of low acidity and log CFU/mL values for kidney bean whey and high values for white bean whey. The results, clearly showed that CBW fortification had no significant effect on the gel stability of the fermented milk systems in comparison to the control sample during 28 days of cold storage, except for CM fortified with kidney bean whey which showed better gel stability than others. The analysis showed that a higher percentage of whey is present in yogurt samples during cold storage, probably because CBW damaged the connectivity of the protein gel network. Our result is parallel to (Codinã *et al.*, 2016). The superior change in syneresis in CBWFY samples after 1 and 21 days is potentially due to the higher decrease in pH, as acid production was highest in the CBWFY products during storage (Fig 5). The greater acidification causes more initial water separation from the gel and the syneresis increased.

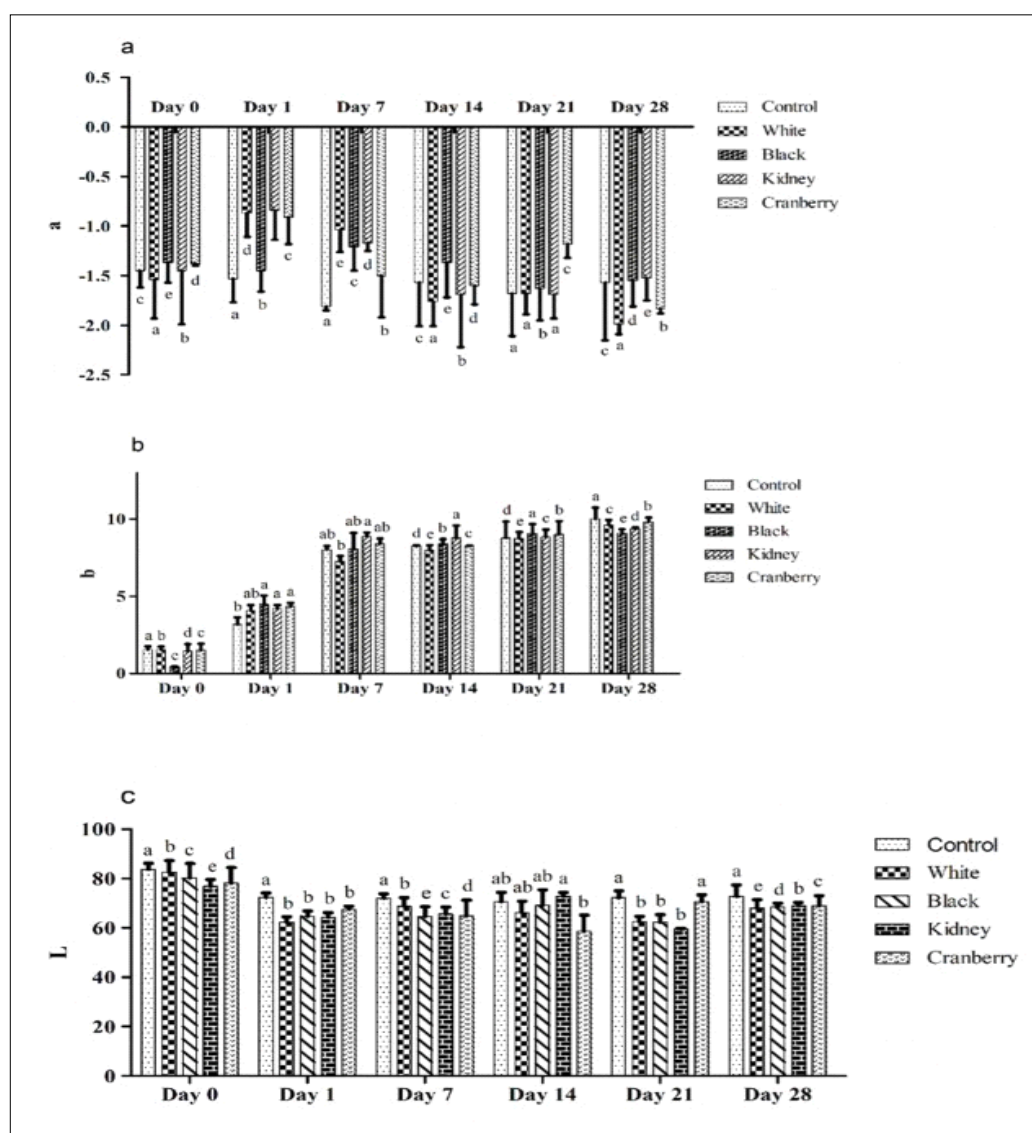


Fig 4: Color profile CBWFY and control sample after production and 28-day storage.

Therefore, syneresis is directly related to acidification during storage. Moreover, previous studies have also reported that the high-fat content of yogurt is related to lower syneresis amounts (Ozturkoglu-Budak *et al.*, 2016), which is in agreement with our findings.

Texture profile analysis

Hardness

The hardness, defined as the amount of force needed to achieve a given extent of deformation, is a commonly appraised parameter in determining yogurt texture (Mousavi *et al.*, 2019). The hardness values of the samples were in the range of 15.66 (g) to 26.15 (g). Storage time had less effect on the hardness of all samples, whereas CBW fortification had no significant effect on hardness ($P < 0.05$). In comparison to the control, no significant difference was observed for samples CBWFY through the

entire storage period, except at 0-day CM fortified with white and black showed lower value in comparison to other varieties and control. Among all varieties of CBWFY, the hardness of CM fortified with white bean whey was high followed by black, cranberry and kidney (Table 1). The results indicated that the storage time of CBWFY had no significant effect on yogurt's hardness. This is probably due to a loose gel system (due to brittle protein gel) and a high amount of syneresis, especially for CM fortified with black extract which showed high syneresis at day 0. However, no significant changes in hardness in CBWFY can be explained by an increase in its moisture content, which is due to fortification with CBW. Comparing all varieties, the hardness for CM fortified with white bean whey was higher probably due to tight and rigid molecular structure and interaction with cow milk finally resulting in a firm protein gel. As a result, a loose network structure with low hardness was formed (Table 1).

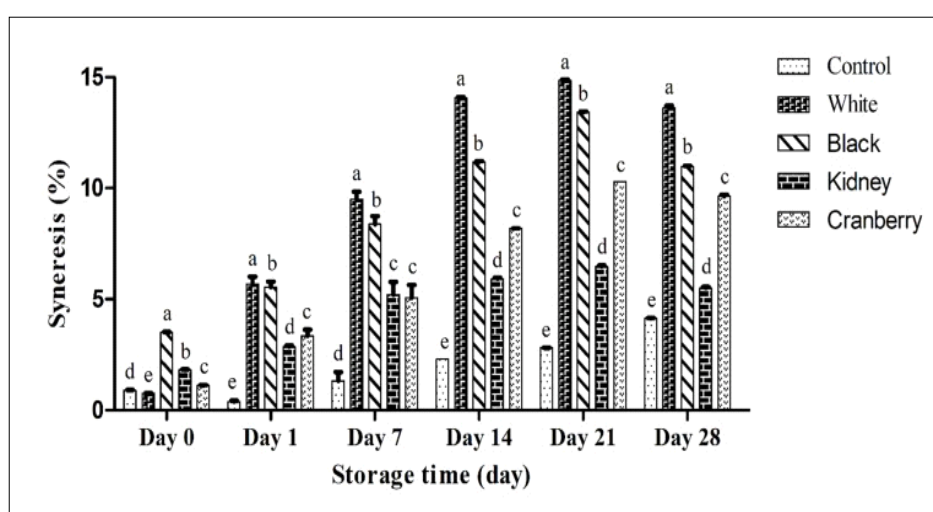


Fig 5: Syneresis in CBW FY and control during 28- days of cold storage.

Table 1: Texture profile analysis of CBW FY and control after production and during 28 days of storage at 4°C.

	Medium	Day 0	Day 1	Day 7	Day 14	Day 21	Day 28
Hardness (g)	White	15.66±0.41Bc	23.23±0.44Aab	20.73±1.18Ab	23.48±1.48Aa	21.20±1.53Aab	23.75±2.34Aa
	Black	16.14±0.41Bb	21.46±2.19Aa	21.41±1.02Aa	23.21±0.41Aa	21.51±0.88Aa	21.95±1.29Aa
	Kidney	19.98±1.77Aab	21.97±1.31Aa	19.03±1.56Ab	21.47±0.47Aa	21.98±1.29Aa	20.49±0.00Aab
	Cranberry	20.97±0.41Abc	19.25±2.23Acd	18.69±0.59Ad	21.30±0.67Ab	23.45±0.00Aa	21.78±0.41Aab
	Control	21.24 ±1.29Ac	24.24±0.76Aab	21.75±1.18Abc	26.15±0.31Aa	23.48±1.48Abc	22.49±2.28Abc
Cohesiveness	White	0.56±0.03Aa	0.36±0.02Bb	0.42±0.04Ab	0.38±0.01Bbc	0.35±0.01Bc	0.40±0.04Bbc
	Black	0.55±0.05Aa	0.42±0.07Ab	0.43±0.02Ab	0.38±0.01Bb	0.39±0.01Ab	0.40±0.03Bb
	Kidney	0.46±0.02Ba	0.43±0.03Aab	0.46±0.05Aa	0.40±0.01Ab	0.43±0.00Aab	0.41±0.02Ab
	Cranberry	0.45±0.02Ba	0.50±0.07Aa	0.48±0.06Aa	0.42±0.04Aa	0.44±0.06Aa	0.48±0.11Aa
	Control	0.42±0.05Bab	0.34±0.01Bc	0.33±0.03Bd	0.45±0.01Aa	0.39±0.03Aab	0.37±0.05Bb
Springiness (mm)	White	0.13±0.02Be	0.26±0.03Abc	0.21±0.06Bcd	0.31±0.03ab	0.19±0.02Bd	0.34±0.01Aa
	Black	0.13±0.02Bc	0.20±0.04Ab	0.22±0.01Bb	0.30±0.02Aa	0.23±0.01Ab	0.32±0.01Aa
	Kidney	0.23±0.04Aab	0.17±0.04Bbc	0.18±0.05Cbc	0.28±0.01Ba	0.21±0.03Bbc	0.22±0.02Bab
	Cranberry	0.25±0.01Aa	0.18±0.02Bab	0.17±0.02Cb	0.22±0.09Bab	0.17±0.01Bb	0.22±0.01Bab
	Control	0.24±0.03Ad	0.28±0.02Ac	0.32±0.03Aa	0.36±0.01Aa	0.29±0.04Ad	0.37±0.08Aa
Gumminess (g)	White	8.73±0.12Ab	8.37±0.24Bb	8.83±0.59Aab	9.04±0.58Aab	7.58±0.39Bc	9.51±0.06Aa
	Black	8.94±0.58Aab	8.84±0.60Aab	9.17±0.00Aa	8.77±0.41Aab	8.29±0.12Bb	9.38±0.10Aa
	Kidney	9.28±1.06Aa	9.42±1.14Aa	8.49±0.59Ba	8.63±0.41Aa	9.51±0.65Aa	8.36±0.35Ba
	Cranberry	9.41±0.56Aa	8.78±0.72Aa	8.83±1.18Aa	8.70±0.39Aa	9.62±0.12Aa	9.45±1.18Aa
	Control	8.77±0.54Aab	8.32±0.24Bab	7.82±0.59Bb	9.79±0.10Aa	8.97±0.20Aab	8.32±1.93Bab

Therefore, our result is not similar to those of Mousavi *et al.* (2019).

Cohesiveness

Cohesiveness is the power of internal bonds to combine the structure of a product (Azari-Anpar *et al.*, 2017). The cohesiveness of yogurt samples ranged from 0.33 to 0.56 (Table 1). CBW FY showed a higher value of cohesiveness in comparison to control. Among all varieties of CBW FY, the cohesiveness of cranberry bean whey was high followed by kidney, black and white. It seems that the protein matrix had an important effect on cohesiveness. Therefore, the addition of CBW resulted in a cohesiveness increment of the final product. In addition, results indicated that the

cohesiveness of yogurt increased with CBW concentration and storage time had a significant effect on the cohesiveness of stored yogurts and decreased during cold storage ($P<0.05$). This is probably due to the storage time diminishing the strength of the internal bounds of yogurts resulting in high whey separation and low values for cohesiveness. Our results were similar to those (Seçkin and Baladura, 2012).

Gumminess

Gumminess has an unpleasant influence on food product's appearance and texture and is linked to foods with low hardness (Mousavi *et al.*, 2019). Our results showed that the gumminess of the CBW FY and control showed no

differences (Table 1). Regarding all varieties of CBW FY, CM fortified with cranberry extract showed high value followed by kidney, black and white. The storage time had no significant influence on gumminess ($P < 0.05$). Thus, the result suggested that CM supplementation with 25% CBW showed less effect on gumminess during the storage period, due to low values for hardness of all samples.

Springiness

The springiness of samples ranged from 0.13 to 0.37(mm). Our finding showed that the storage time had a significant effect on yogurt springiness and it increased throughout the storage period ($P < 0.05$). In comparison to the control, the CBW FY had low values throughout the storage period (Table 1). Among all varieties of CBW FY, the CM fortified with cranberry bean whey followed by kidney, black and white demonstrated a higher value of springiness, indicating that it returned not easily to its original shape after the deforming force was removed. Comparing days 0 and 28 for CBW FY, there was a trend for extension in all analyzed texture parameters, except for cohesiveness, which decreased throughout the storage period ($P < 0.05$). However, CBW FY had similar textural properties (hardness, gumminess, cohesiveness and springiness) as control yogurt.

CONCLUSION

In this study, CBW FY and control samples were stored for 28 days at 4°C and microbial, physicochemical and textural properties were investigated. The results demonstrated that CBW FY storage resulted in a significantly faster lowering of the pH and higher viable counts of *L. bulgaricus* in comparison with the control sample. The color of the CBW FY samples was slightly altered in comparison with the control immediately after production and during storage. CBW FY had the highest syneresis initially and decreased after 28 days of storage in comparison to the control. Therefore, sensory reception is critical to consumer acceptance of stored novel products.

Conflict of interest

We have no conflicts of interest to disclose. All authors declare that they have no conflicts of interest.

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