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Research Article

Improving Properties of Whey-Less Cheese Fortified with Acylated Anthocyanin from Purple Carrot Powder during the Storage Period

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Whey-less cheese (WLC) containing different concentrations (1% and 2%) of purple carrot powder (PCP) was produced, then its physical, chemical, and sensorial characteristics were investigated during a six-week storage period (SP). The PCP-2% sample showed the highest total phenolic content at the beginning of the SP (163.58 mg gallic acid/100 g) and achieved the highest overall acceptance (3.95). Total anthocyanin content and a^* value were statistically stable during the SP. The antioxidant activity for PCP-2% was enhanced. Fortified WLC samples showed less hardness compared to the control. The results indicated that fortification of WLC with PCP could improve properties, and due to the acylated anthocyanins provided by PCP, the color remained constant during the SP. Furthermore, bioactive components of milk are preserved in WLC. Also, it is more cost-efficient and environmentally friendly. In conclusion, WLC fortified with PCP can be suggested as a dairy product with significant improved properties.

1. Introduction

Functional foods are foods that in addition to being nutritional also improve health or reduce disease, and they are getting more popular [1]. Moreover, consumer knowledge of the effects of food on health is developing meaningfully. As a result, many labels have been advertising healthier meals, and manufacturing healthier foods is becoming more widespread [2].

Plants, plant mixes, and even extracted chemicals are frequently added to food to deliver their valuable benefits [3]. For instance, orange carrot powder was applied to cheese after milk incubation. The results indicated that vitamin A and β-carotene of the cheese samples increased, but the texture and overall acceptance decreased due to a deficit of fine carrot powder [4].

Each color in carrots represents a nutritious substance. For example, orange corresponds to β-carotene, and purple is for anthocyanins [5]. Anthocyanins, like other polyphenolic chemicals, are secondary metabolites that help plants defend themselves. They prevent oxidative damage to plant cell components by scavenging free radicals and thereby avoiding their harmful effects on nucleic acids, proteins, and lipids [6]. Purple carrot (*Daucus carota*) contains high amount of anthocyanins. In addition, the anthocyanin pigment in carrots is acylated with *p*-coumaric, ferulic, *p*-hydroxybenzoic, and sinapic acids (cinnamic acids). Consequently, they are more resistant to heat processing [7]. Color loss in anthocyanin is due to the reversible water addition to the flavylium ion (producing colorless hemiketal) and to a combination of irreversible routes typically resulting in cleavage of the C-ring. The color stability of diacylated anthocyanin is much higher than for non- or monoacylated anthocyanin. Indeed cinnamic acid residues can develop π-stacking interactions with the anthocyanin chromophore, thereby protecting it against water addition [8].

Cheese is one of the most important dairy products (20 million tons) in the world [9]; for instance, 37% of accessible milk in Europe was utilized for cheese production in 2018 (10 million tons of cheese out of 157 million tons milk) [10]. Cheese samples can be classified based on their origin, final pH, moisture, and other characteristics [11]. Whey-less cheese (WLC) is a soft cheese with a high whey protein/casein ratio (e.g., 60/40 or higher). WLC can maintain more than 65% of the moisture even though it has ideal firmness that prevents whey separation. Producing WLC is more cost-effective, environmentally friendly, and easy to formulate. Moreover, it preserves the whey and other bioactive components of milk in cheese [12]. Additionally, for health-promoting attributes, cheese has various nutraceutical compounds such as ɣ-aminobutyric acid, bioactive peptides, exopolysaccharides, lipids, bacteriocins, organic acids, and vitamins [13]. Whey proteins and bioactive components from milk are preserved in whey-less cheese [14]. Whey protein is a rich source of bioactive peptides which may play a role in the dietary management of chronic diseases [15]. Whey proteins have the highest nutritional quality of the dietary protein sources and even greater biological value than the milk protein casein plus it is rich in human-essential amino acids [16].

Enrichment of dairy products with fruits and vegetables has already resulted in more nutritious and palatable products. For instance, as this study tries to introduce a nutritious dairy product fortified with carrots, the addition of orange carrot juice (5% to 20%) to milk, before making yogurt, was studied. Accordingly, its sensorial properties showed that the addition of the orange carrot could increase sensorial acceptance (A. [17]). Since this study targeted to produce a palatable and healthier product similarly, [18] evaluated the characteristics of Gouda cheese containing plum blossom liquors. They designate that Gouda liquors supplemented can contribute additional nutrient value while the flavor and palatability were sustained [18]. In another study, the effects of different concentrations of grape extract on cheese-making were discussed. Grape extract similar to purple carrot supplied antioxidant activity (AA) and total phenolic content (TPC), but increased brittleness and decreased firmness [19]. Some studies focused on the bioavailability of bioactive compounds in dairy products. For instance, cheese enriched with green tea extract in a simulated gastrointestinal environment was studied. Green tea extract decreased the release of free fatty acids by 7% at the end of digestion and improved the hardness of the cheese, but did not affect the matrix degradation or proteolysis profile. Surprisingly, the digested form of enriched cheese showed 14% higher AA [20]. The current study used purple carrot as powder; similarly, some researchers utilized powder in formulation to enrich cheese samples. Grape pomace is used to make semihard cheese more nutritious. The physicochemical properties of the cheese samples were not substantially affected by the grape pomace powder [21].

The objective of this study was to fortify WLC with purple carrot powder (PCP) with favourable color and decent organoleptic characteristics that show more color stability during the storage period, moreover, to evaluate its TPC, total anthocyanin content (TAC), AA, organoleptic, and color attributes.

2. Materials and Methods

2.1. Chemicals. Sodium acetate, KCl, Folin-Ciocalteu reagent, gallic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl radical), BHT (butylated hydroxytoluene), neocuproine, $CuCl_2$, and ammonium acetate were purchased from Sigma–Aldrich (St. Louis, MO) and Merck (Darmstadt, Germany).

2.2. Purple Carrot Powder Preparation. November 2018 cultivated purple carrots were purchased from a local farm in Bandar-e-Lengeh city (Hormozgan province, south of Iran). The purple carrots were washed and blanched (15 minutes at 90°C). Blanched purple carrots were sliced (maximum 1 mm thickness) and dried by a cabin dryer (4 hours at 60°C). Dried slices of the purple carrots were grounded by a hammer mill (GM 200, Retsch, Duesseldorf, Germany) and sieved (mesh No. 120) to achieve a fine powder. Produced PCP was sealed in the plastic bags and stored at 4°C until used in WLC production.

2.3. Whey-Less Cheese Production. Whey-less cheese (WLC) was formulated according to Lashkari et al. [22] with some modifications. Figure 1 is presenting the flow chart of WLC fortified with PCP. Accordingly, for 100 g WLC, 61 g milk (3.1% fat) mixed with 20 g cream (68% fat) then 9.9 g milk protein concentrate (MPC), 3 g whey protein concentrate (WPC), and 5 g skim milk were added to achieve 12% protein, 16% fat, and 40% dry matter. After mixing and letting them be hydrated (60 minutes), three formulations of WLC were produced by adding different concentrations of PCP (0%, 1%, and 2% PCP), then the homogenization process with two steps (50-120 bar) (Model Homolab 2.20, FBF Italia, Parma, Italy) was applied. The pasteurization was accomplished at 80°C for 30 minutes [23]. After that, the starter culture (White daily 41, Chr. Hansen, Hørsholm, Denmark) was added. Next, at 35°C, the pH was reduced to 5.8, and the rennet (Chy-Max, Chr. Hansen, Hørsholm, Denmark) and salt were added. The formulation was packaged and incubated to reach the pH of 4.5. The samples were stored at 4°C for 6 weeks, and their physicochemical, textural, and organoleptic properties were evaluated every 2 weeks.

2.4. Sample Preparation. The preparation of the whey-less cheese (WLC) samples for phytochemical and antioxidant measurement has been done according to Lucera et al. [24] with some modifications. Briefly, WLC samples were crushed and mixed with ethanol 96% (2 : 1) and centrifuged at 5000g, 4°C for 5 minutes, then this process was repeated three times. The extracts were used for the TPC, TAC, and antioxidant measurements with the spectrophotometry method. Finally, the results are multiplied by respective dilutions.

2.5. Organoleptic Evaluation. The organoleptic evaluation was carried out according to Shahbazi et al. [25]. Twenty untrained panelists took part in the acceptance exam (between the ages of 22 and 38). The panelists evaluated the WLC samples containing different concentrations of

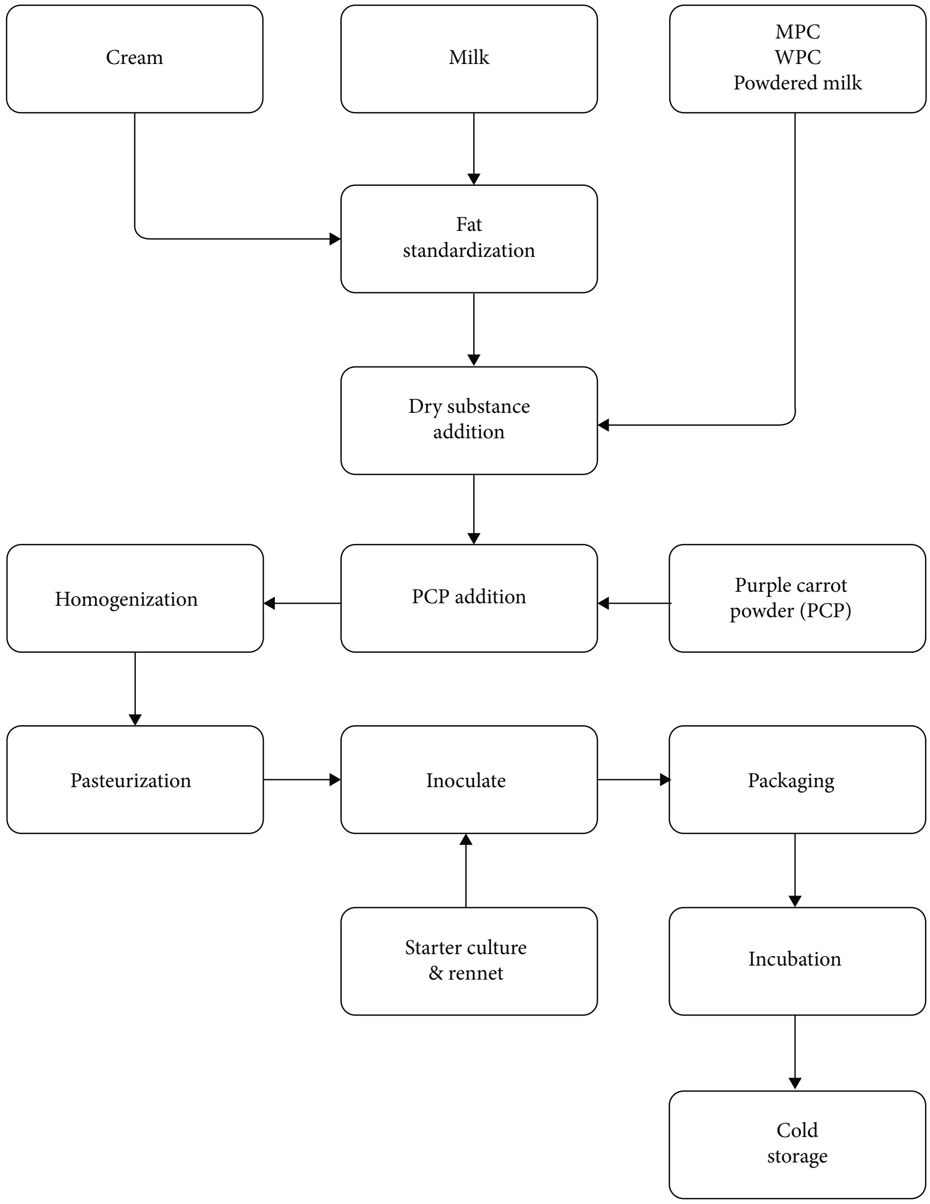

Figure 1: Flow chart of whey-less cheese fortified with purple carrot powder (PCP).

PCP on a five-point scale for odor, color, appearance, texture, and overall acceptance at the beginning of the storage period. Twenty grams of cheese (at 7°C) were served with water to keep the mouth clean between the samples as well as biscuits to assess the texture.

2.6. Color Attributes. Color attributes of WLC samples containing different concentrations of PCP were measured by taking photos in adjustment circumstances and achieving L^* a^* b^* with Adobe Photoshop software (Adobe Photoshop, version CC 2019, San Jose, CA) [26]. The adjustment circumstances were achieved by placing the samples into a box covered by black paper from the inside and taking shots with a single camera (Nikon, model Coolpix B700, Tokyo, Japan) from a hole provided above the box with fixed a distance for all samples. A 20-watt LED light is used to avoid fluctuation in light intensity. Also, the total color change (ΔE) and hue angle ($h\circ$) was calculated according to

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta b^*)^2 + (\Delta a^*)^2}, \tag{1}$$

$$h\circ = \arctan\left(\frac{b^*}{a^*}\right), \tag{2}$$

where L^* is lightness, b^* is yellowness-blueness, and a^* is redness-greenness of the samples.

2.7. Total Phenolic Content. TPC of whey-less cheese was estimated spectrophotometrically using Folin–Ciocalteu reagent [27]. Briefly, 200 μL prepared sample for that described previously was added to 1.3 mL deionized water followed by 500 μL Folin–Ciocalteu reagent. After 3 min, 500 μL sodium carbonate (10%, w/v) was added. Afterwards, the samples were left in the darkness at room temperature for 1 hour, vortexing every 10 minutes. Finally, the absorbance was measured at 760 nm (Cytation5, BioTek, Winooski, VT). The standard curve was established using various concentrations of gallic acid in water (0-0.5 mg gallic acid/100 g).

2.8. Total Anthocyanin Content. The TAC of whey-less cheese (WLC) samples were measured using the pH differential method [28]. Absorbances of the prepared samples that were described previously were measured by a spectrophotometer (Cytation5, BioTek, Winooski, VT); then, according to Equation (3) and Equation (4), their TAC was estimated, and the final TAC of WLC calculated.

$$A = \left(A_{\lambda_{\text{Max}}} + A_{\lambda_{700}}\right)_{\text{pH 1}} - \left(A_{\lambda_{\text{Max}}} + A_{\lambda_{700}}\right)_{\text{pH 4.5}}, \quad (3)$$

$$\text{cyanidin} - 3 - \text{O} - \text{glucoside}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{A \times \text{MV} \times \text{DF} \times 1000}{\varepsilon}, \quad (4)$$

where A is the absorbance of the sample, $A_{\lambda\text{Max}}$ is the absorbance at 510 nm, $A_{\lambda 700}$ is the absorbance at 700 nm, MV is the molecular mass of cyanidin-3-o-glucoside (449.2 g/mol), DF is dilution factor calculated according to the buffer and samples portion, and ε is molar extinction coefficient (26,900 L/cm/mg).

2.9. Antioxidant Activity. Radical scavenging activity (RSA) was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method [29]. A total of 300 μL of different concentrations of whey-less cheese (WLC) prepared samples described as preciously containing PCP in methanol (1, 10, 100, and 1000 mg/mL) were applied to 1200 μL of DPPH$^{\circ}$ (0.1 mM) methanolic solution. The mixtures were vigorously shaken before being allowed to sit at room temperature for 60 minutes in the darkness. The absorbance values were then compared to the blank at 517 nm. The IC_{50} values were calculated using plotted inhibition.

Cupric reducing antioxidant capacity (CUPRAC) was measured according to the method described by [26] with some modifications using a spectrophotometer (Cytation5, BioTek, Winooski, VT). So, 1 mL $CuCl_2$ solution (10^{-2} M), 1 mL neocuproine solution (7.5×10^{-3} M), 1 mL ammonium acetate buffer (1 M at pH 7), and 300 μL of the prepared sample described as previously were mixed. Then, the mixture was incubated at 50°C in a water bath. After 30 min, the absorbance was measured at 450 nm. The standard curve was prepared using different concentrations of vitamin C (0.1, 0.2, 0.5, and 1 mg/mL), and the results were expressed in terms of mg vitamin C/100 g.

2.10. Texture Profile Analysis (TPA). TPA of whey-less cheese (WLC) samples containing different concentrations of PCP was performed to calculate hardness, cohesiveness, and springiness by TA.TX2 (Stable Micro System, Godalming, UK) equipped with 40 mm probe, 1 mm/s speed penetrate 25% in the samples, and the probe returned to its original position. The second compression was similar to the first compression by a two-second delay. The samples were in 75 mm diameter and 30 mm height containers at 4°C [11].

2.11. Statistical Analysis. All experiments were performed in triplicate. To determine the significant differences ($P < 0.05$) among the means, a completely randomized design (CRD) was applied. To compare among the mean values, Duncan's multiple range test was used [30]. All statistical analyses have been performed using SAS (Statistical Analysis Software, version 9.4, SAS Institute Inc., Cary, NC).

3. Results and Discussion

3.1. Organoleptic Evaluation. Figure 2 shows the effect of PCP addition on the organoleptic characteristics of the whey-less cheese (WLC) samples. PCP addition positively affected the odor, color, and appearance of the WLC samples ($P < 0.05$). WLC sample containing 2% PCP had the highest odor, color, appearance, and texture score (3.8, 4.1, 3.9, and 4.05, respectively) in comparison with the control sample (3.55, 3.6, 3.6, and 3.85, respectively). It can be observed that the organoleptic scores of WLC samples supplemented with PCP were significantly ($P < 0.05$) higher than those of the control WLC sample. These results are mainly due to the positive effects of PCP supplementation on WLC. Similarly, El-Sayed and Ibrahim (2021) revealed that supplemented UF-soft cheese with red radish root nanopowder showed higher general acceptability in comparison with the control sample.

The WLC sample containing 2% PCP showed the highest (3.95) overall acceptance score, while there were no significant differences ($P < 0.05$) between the control and 1% PCP. It seems that the main reason for the lower overall acceptance score of the 1% PCP can be attributed to its lower color score. Similarly, Lashkari [31] reported that the cheese samples containing pomegranate juice had higher overall acceptance due to their higher red color.

3.2. Color. Color is the first way for consumer attraction, so it is determinative in marketing [32]. Table 1 shows the color attributes of the whey-less cheese (WLC) samples containing different concentrations of PCP. The highest L^* was observed for the control sample (88.00), while 2% PCP showed the lowest L^* (48.33). The L^* of the control and PCP-containing samples was statistically constant during the storage period, but all the samples revealed a slight insignificant decrease in the lightness at the end of the storage period. Similarly, Silva et al. [19] reported that due to the intermolecular and intramolecular reactions, the lightness of the fermented milk beverage containing different concentrations of blueberry juice was reduced.

As expected, PCP addition increased the a^* value significantly. The highest a^* value was observed for the WLC sample containing 2% PCP at the end of the storage period (31.67). The a^* did not change significantly ($P < 0.05$)

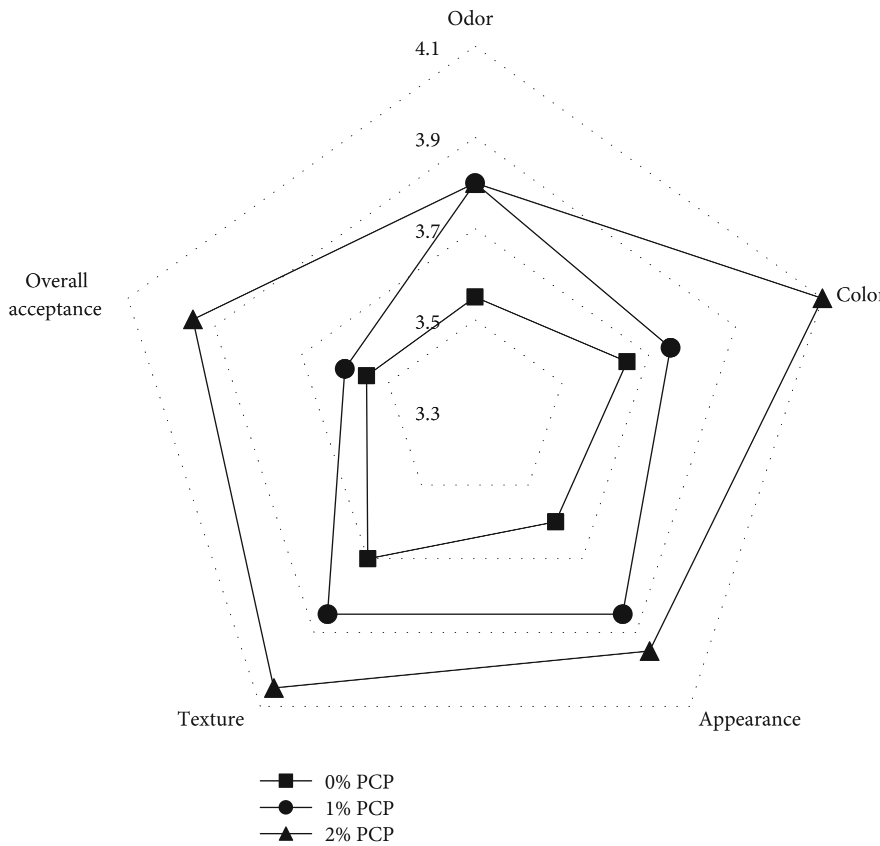

Figure 2: Organoleptic evaluation of whey-less cheese samples containing different concentrations of purple carrot powder (PCP) at the beginning of the storage period.

during the storage period. No significant loss was observed in the redness of the samples, which can be related to the presence of acylated anthocyanins in PCP. Also, Sadilova et al. [33] verified that the acylated anthocyanins may withstand the process better than nonacylated ones.

Although the b^* value of the control WLC sample was somewhat in the yellow zone (6.33) and the b^* value of the WLC samples containing PCP was somewhat in the blue area (-1.67 and -3.67 for 1% and 2% PCP, respectively), there were no significant differences ($P < 0.05$) among their b^* values. At the end of the storage period, the b^* values of all samples increased. According to the results, as the RSA decreased during the storage period, the b^* value of WLC samples raised. Similarly, Silva et al. [19] stated that antioxidant and prooxidant ratios in cheese fortified with blueberry were correlated to their b^* values during the 28 days of storage period.

For calculating the ΔE, all samples were compared to the control sample at the beginning of the storage period. There were no significant differences ($P < 0.05$) among the ΔE values of samples during the storage period. This result can be related to the insignificant changes in the a^* values as well as slight changes in L^* and b^* values during the storage period.

The a^* data of WLC samples obtained by the method described in Section 2.6 correlated with color acceptance of the WLC sample obtained by organoleptic evaluation described in Section 2.5. More a^* received higher acceptance. Consequently, this elucidated that the most aspect of fortified products for panelists was redness.

3.3. Total Phenolic Content. The TPC values of whey-less cheese (WLC) containing different concentrations of PCP are given in Table 2. The phenolic compounds detected in control cheeses were endogenous phenolic compounds in bovine milk [34]. The results showed that with the addition of different concentrations of PCP, the amounts of TPC were increased significantly, which is due to the high TPC of the PCP (4274.24 mg gallic acid/100 g). The TPC correlated with the TAC of the WLC sample except for the control sample which is due to the Folin–Ciocalteu method which affects not only by phenolic content but also by protein provided in cheese, and TAC does not have this issue. The PCP is a suitable source of phenolic compounds such as *p*-coumaric, ferulic, and sinapic acids [35, 36]. Because the source of the bioactive is a food that has traditionally been consumed, and no purification steps have been involved other than minimal drying, it is less likely that there would be regulatory restrictions on its use as a food ingredient [6]. At the beginning of the storage period, all samples indicated a considerable amount of the TPC ($P < 0.05$). At the end of the storage period, the TPC of 1% and 2% PCP samples decreased from 131 to 100 mg gallic acid/100 g and from 163 to 105 mg gallic acid/100 g, respectively. This result can be related to the

Table 1: Color attributes of whey-less cheese (WLC) samples containing different concentrations of purple carrot powder (PCP) during the storage period.

Color attribute	Time (week)	PCP concentration (%) 0	1	2
L^*	0	$88.00 \pm 1.00^{Aa**}$	62.00 ± 1.00^{Ab}	48.33 ± 1.53^{Ac}
	2	85.67 ± 0.58^{ABa}	58.00 ± 1.00^{ABb}	49.00 ± 1.00^{ABc}
	4	78.67 ± 0.58^{Ba}	59.00 ± 1.00^{Bb}	51.00 ± 0.00^{Bc}
	6	82.00 ± 0.00^{ABa}	60.67 ± 4.73^{ABb}	51.00 ± 6.08^{ABc}
a^*	0	-1.33 ± 0.58^{Ac}	21.33 ± 0.58^{Bb}	29.33 ± 1.53^{Ba}
	2	-1.33 ± 0.58^{Ac}	22.00 ± 2.00^{Ab}	29.33 ± 0.58^{Aa}
	4	-1.00 ± 0.00^{Ac}	23.33 ± 2.52^{Ab}	30.67 ± 0.58^{Aa}
	6	-1.00 ± 0.00^{Ac}	22.33 ± 2.89^{Ab}	31.67 ± 0.58^{Aa}
b^*	0	6.33 ± 0.58^{Ca}	-1.67 ± 1.53^{Bb}	-3.67 ± 1.15^{Bb}
	2	9.67 ± 0.58^{Ba}	1.00 ± 1.00^{Ab}	-3.00 ± 0.00^{Bb}
	4	9.00 ± 1.00^{Ba}	0.67 ± 0.58^{Ab}	-1.33 ± 0.58^{Bb}
	6	7.67 ± 0.58^{Aa}	2.33 ± 1.15^{Ab}	2.67 ± 0.58^{Ab}
h	0	-77.99 ± 5.60^{Ab}	-4.52 ± 4.14^{Ca}	-7.06 ± 1.82^{Ba}
	2	-82.21 ± 3.07^{Ab}	2.03 ± 2.12^{Ba}	-5.84 ± 0.11^{Ba}
	4	-83.61 ± 0.71^{Ab}	1.57 ± 1.38^{Ba}	-2.48 ± 1.05^{Ba}
	6	-82.54 ± 0.58^{Ab}	6.96 ± 2.72^{Aa}	4.82 ± 1.08^{Aa}
ΔE^{***}	0	—	35.40 ± 1.54^{Bb}	51.13 ± 2.71^{Ba}
	2	4.22 ± 1.33^{Cc}	38.37 ± 1.19^{ABb}	50.49 ± 1.76^{ABa}
	4	9.73 ± 0.67^{Ac}	38.48 ± 1.89^{Ab}	49.52 ± 1.32^{Aa}
	6	6.19 ± 1.03^{Bc}	36.36 ± 6.17^{ABb}	49.87 ± 5.25^{ABa}

**Mean ± SD ($n = 3$). In each column and for each property, means with different capital letters are significantly different ($P < 0.05$). In each row, means with different subscript letters are significantly different ($P < 0.05$). ***Reference sample for ΔE calculation was the WLC sample without PCP at the beginning of the storage period.

different chemical changes in the samples such as compounds produced in lipid oxidation [37], microbial activity [38], and enzymatic reactions [39]. Similar results were reported by Lashkari et al. [22] who studied the TPC of fortified cheese incorporated with pomegranate juice and stated that the TPC of the fortified cheese sample increased with the addition of pomegranate juice. Also, the TPC of the samples was significantly decreased during the storage period due to the interaction of phenolic compounds with proteins. In addition, Saito et al. [40] studied the effects of jabuticaba (*Myrciaria cauliflora*) peel extract addition on the TPC of petit-suisse cheese and reported that the TPC increased after the addition of jabuticaba peel extract. Also, during the storage period, the TPC was significantly ($P < 0.05$) decreased due to the presence of oxygen, temperature, light, and interactions with other food components. Furthermore, the changes in the TPC of the control sample during the storage period can be related to the other physical and chemical parameters modified during storage, such as moisture. Despite that it is not statistically significant, it can be numerically observed.

3.4. Total Anthocyanin Content. Table 2 shows TAC values of whey-less cheese (WLC) containing different concentrations of PCP. The control sample had the lowest TAC, while the sample containing 2% PCP had the highest TAC. This finding can be related to the high TAC of PCP (1059.86 mg C-3-G/L). Moreover, previous studies showed that PCP is a rich source of anthocyanin components such as cyanidin, pelargonidin, and peonidin [35, 36, 41]. Similar results were reported by Lashkari et al. [22], who studied the TAC of fortified cheese incorporated with pomegranate juice. Their results showed that the TAC of the fortified cheese sample increased by pomegranate juice addition. Kabakcı et al. [42] studied the effects of black carrot extract on the TAC of fortified kefir and reported that the TAC of the fortified kefir sample significantly increased after the addition of black carrot extract.

The TAC of WLC containing different concentrations of PCP was statistically constant during the 6 weeks of storage period. The PCP contains acylated anthocyanin, so the lack of significant changes in TAC during the storage period can be related to the acylated anthocyanin. These results showed that 85% of TAC endured the storage period. Malien-Aubert et al. [43] observed that 70% of the anthocyanin in the black carrot was acylated, which is more heat and process resistant. Additionally, Wallace and Giusti [44] studied the effect of acylated and nonacylated anthocyanins on TAC in an 8-week storage period of fortified yogurt. They showed that the TAC of purple carrot (as acylated anthocyanin) can tolerate considerably during the storage period in comparison with the TAC of *Berberis boliviana* (as

Table 2: Phytochemicals and antioxidant activity of whey-less cheese samples containing different concentrations of purple carrot powder (PCP) during the storage period.

Property	Time (week)	PCP concentration (%) 0	1	2
TPC* (mg gallic acid/100 g)	0	$89.43 \pm 1.97^{Ac**}$	131.21 ± 5.56^{Ab}	163.58 ± 13.53^{Aa}
	2	89.69 ± 4.98^{Ac}	119.84 ± 5.65^{Bb}	146.28 ± 9.34^{Ba}
	4	90.74 ± 3.52^{Ac}	105.86 ± 0.64^{Cb}	128.12 ± 4.10^{Ca}
	6	91.37 ± 5.50^{Ac}	100.44 ± 5.67^{Cb}	105.32 ± 11.49^{Da}
TAC (mg C3G/L)	0	0.17 ± 0.05^{Ac}	8.22 ± 2.47^{Ab}	22.84 ± 1.73^{Aa}
	2	0.16 ± 0.02^{Ac}	8.09 ± 0.23^{Ab}	20.92 ± 1.62^{Aa}
	4	0.15 ± 0.04^{Ac}	7.70 ± 0.61^{Ab}	20.41 ± 0.70^{Aa}
	6	0.15 ± 0.05^{Ac}	7.53 ± 0.75^{Ab}	19.52 ± 0.54^{Aa}
DPPH (IC_{50}; mg/mL)	0	39.32 ± 0.00^{Aa}	0.37 ± 0.00^{Db}	0.17 ± 0.00^{Dc}
	2	35.70 ± 0.00^{Ba}	0.39 ± 0.00^{Cb}	0.18 ± 0.00^{Cc}
	4	29.61 ± 0.00^{Ca}	0.41 ± 0.00^{Bb}	0.21 ± 0.00^{Bc}
	6	25.38 ± 0.00^{Da}	0.45 ± 0.00^{Ab}	0.29 ± 0.00^{Ac}
CUPRAC (mg vitamin C/100 g)	0	4.05 ± 4.38^{Cc}	66.55 ± 2.41^{Ab}	117.75 ± 1.11^{Aa}
	2	10.45 ± 2.21^{Bc}	60.77 ± 2.25^{Ab}	114.82 ± 1.46^{Aa}
	4	13.15 ± 2.57^{Ac}	51.39 ± 1.34^{Bb}	110.49 ± 1.74^{Aa}
	6	15.36 ± 3.39^{Ac}	40.99 ± 3.48^{Bb}	103.76 ± 4.07^{Ba}

*Total phenolic content (TPC), total anthocyanin content (TAC), cyanidin-3-glucoside (C3G), radical scavenging activity indicated with IC_{50} value, and cupric ion reducing antioxidant capacity (CUPRAC). **Mean ± SD ($n = 3$). In each column and for each property, means with different capital letters are significantly different ($P < 0.05$). In each row, means with different lowercase letters are significantly different ($P < 0.05$).

nonacylated anthocyanin). They stated that the proteins and other macromolecules in yogurt may interact with the acylated components of the anthocyanins and prevent the loss of TAC in yogurt during the storage period.

3.5. Antioxidant Activity. Table 2 shows the results of the inhibition of DPPH by whey-less cheese (WLC) containing different concentrations of PCP. The value of IC_{50} is in contrast to radical scavenging activity (RSA) which means higher IC_{50} means lower RSA. The IC_{50} of the control sample (39.32 mg/mL) was considerably higher than 1% and 2% (0.37 and 0.17 mg/mL, respectively) at the beginning of the storage period. The Higher RSA of samples containing PCP is due to their higher TPC and TAC. Similarly, Masmoudi et al. [45] showed that the addition of *Arbutus unedo* extract to soft cheese increased the RSA. RSA of the control sample can be related to its bioactive peptides and other antioxidant components [46]. While the TPC was decreased, the RSA of the samples containing PCP was significantly ($P < 0.05$) decreased during the storage period. In contrast, the RSA of the control sample showed a negligible increase which can be related to the production of bioactive peptides during the storage period [47].

Table 2 shows the CUPRAC value of WLC samples containing different concentrations of PCP. With the addition of PCP, the CUPRAC value of WLC samples increased considerably (66.55 and 117.75 mg vitamin C/100 g for 1% and 2% PCP, respectively) in comparison with the control sample (4.05 mg vitamin C/100 g). The results of the CUPRAC assay were similar to the results reported for RSA. The higher CUPRAC value of samples containing PCP was due to the higher TPC and TAC of the PCP. Similar to the RSA results, CUPRAC values of the samples containing PCP significantly ($P < 0.05$) decreased during the storage period, while the AA of the control sample increased significantly ($P < 0.05$) during the storage period. The rate of AA reduction of WLC containing PCP in CUPRAC values in comparison with the IC_{50} values was slower. According to [48], the IC_{50} value indicates the amount of free radical quenching in ethanol solution while the CUPRAC shows the capability of reducing Cu (II) to Cu (I) in aquatic solution. Consequently, the capability of reducing Cu (II) to Cu (I) in aquatic solution was affected less during the storage period which shows active antioxidant components that have AA properties in aquatic solution decay less during the storage period. Similar results were reported by Lashkari et al. [22], who studied the AA of fortified cheese incorporated with pomegranate juice. They reported that the RSA of the samples decreased significantly during the storage period due to the interaction with proteins, but the CUPRAC value was affected less during the storage period. The antioxidant activity of anthocyanins plays a key role in various health benefits such as anti-inflammatory, antiobesity, anticancer, and hypoglycaemic effects [49]. Overall, phytochemicals have the antioxidant capacity and may protect cells against oxidative damage caused by free radicals. Oxidative stress can cause oxidative damage to large biomolecules such as proteins, DNA, and lipids, resulting in an increased risk of cancer and cardiovascular disease. It is estimated that one-third of all cancer deaths in the United States could be avoided through appropriate dietary modification (Chun et al., 2005).

Table 3: Texture profile analysis of whey-less cheese samples containing different concentrations of purple carrot powder (PCP) during the storage period.

Property	Time (week)	PCP concentration (%)		
		0	1	2
Hardness (N)	0	35.60 ± 2.84^{Aa*}	29.89 ± 0.34^{Ba}	20.32 ± 2.14^{Ac}
	2	29.37 ± 2.43^{Ba}	27.93 ± 0.42^{Ab}	15.40 ± 3.03^{Bb}
	4	25.11 ± 1.89^{Ba}	26.00 ± 1.72^{Ba}	14.60 ± 3.00^{Bb}
	6	22.66 ± 1.44^{Ca}	19.77 ± 9.04^{Ca}	13.66 ± 0.99^{Bb}
Springiness (unit less)	0	0.88 ± 0.01^{Aa}	0.88 ± 0.02^{Aa}	0.87 ± 0.02^{Aa}
	2	0.86 ± 0.01^{Aa}	0.84 ± 0.02^{Aa}	0.82 ± 0.01^{Aa}
	4	0.75 ± 0.06^{Ba}	0.81 ± 0.09^{Ba}	0.79 ± 0.06^{Ba}
	6	0.70 ± 0.03^{Ca}	0.76 ± 0.02^{Ca}	0.72 ± 0.08^{Ca}
Cohesiveness (unit less)	0	0.58 ± 0.03^{ABa}	0.56 ± 0.02^{ABa}	0.51 ± 0.01^{ABa}
	2	0.49 ± 0.00^{Ba}	0.51 ± 0.02^{Ba}	0.50 ± 0.03^{Ba}
	4	0.53 ± 0.05^{ABa}	0.55 ± 0.04^{ABa}	0.57 ± 0.16^{ABa}
	6	0.59 ± 0.02^{Ba}	0.56 ± 0.11^{Ba}	0.55 ± 0.03^{Ba}

*Mean ± SD ($n = 3$). In each column and for each property, means with different capital letters are significantly different ($P < 0.05$). In each row, means with different lowercase letters are significantly different ($P < 0.05$).

3.6. Texture Profile Analysis. TPA results of the whey-less cheese (WLC) containing different concentrations of PCP are shown in Table 3. The control sample had the highest hardness (36.60 N), while PCP addition decreased the hardness, which may be due to the proteolysis by PCP. Similarly, soft cheese fortification with ginger extract weakened the microstructure and decreased the hardness (Abd El-Aziz, Mohamed, & Seleet, 2012). In contrast, Giroux et al. (2013) and Zhang, Yang, and Zhao (2011) showed that after the addition of green tea extract and blueberry, the hardness of the cheese samples increased. The interaction of phenolic compounds with casein seemed to strengthen the curd network and increased the cheese's firmness. Although the fortification of WLC soften the cheese, the organoleptic evaluation indicated that the fortified sample achieved higher acceptance.

At the end of the SP, all samples became softer, and the lowest hardness (13.66 N) was observed for the 2% PCP sample. Similarly, previous studies indicated a decrease in the hardness during the SP, which may be related to the destruction of the microstructure of the WLC containing PCP samples during the SP due to the enzymatic activity of PCP.

The cohesiveness and springiness of the WLC samples did not affect by PCP addition. Similarly, Zappia et al. (2020) stated that cohesiveness and springiness achieved by calculating the criteria change between the first and the second compressions in the TPA test. Consequently, it can be concluded that the WLC containing different concentrations of PCP has no significant effects on the cohesiveness and springiness. In contrast, the cohesiveness and springiness increased with the addition of ginger extract and red radish root nanopowder to the cheese samples significantly. The increase in dry matter content can lead to an increase in the cohesiveness and springiness due to the water-binding capacity of the added powder or extract (Abd El-Aziz et al., 2012; El-Sayed & Ibrahim, 2021). Moreover, Giroux et al. (2013) and Ibrahim, Mohamed, and Bahgaat (2019) reported that the reduction of the cohesiveness and springiness after the addition of the green tea extract to the cheese can be related to the alterations of the protein network.

4. Conclusion

Physicochemical and organoleptic properties of whey-less cheese (WLC) containing PCP have been studied during the 6 weeks of storage period. The results showed that the PCP fortification increased the bioactive compounds (TPC and TAC) that promote the health aspects of the WLC. Furthermore, due to the presence of acylated anthocyanins provided by PCP, no anthocyanin loss and change in a^* was observed during the storage period as well as more organoleptic acceptance for fortified WLC which are important achievements for the commercial aspects. In addition, WLC samples containing PCP showed enhanced AA (lower IC_{50} and higher CUPRAC values), and the fortification of WLC can be a good source of antioxidants to promote health criteria. Furthermore, WLC is more efficient, has less waste, and preserves the bioactive compounds of milk in cheese. This study alongside other investigations for fruits and vegetables containing anthocyanin application showed that fortified foods with these components can introduce a healthier diet. In conclusion, 2% PCP can enhance the health-promoting properties of WLC, while TAC and color remain constant during the storage period.

Abbreviations

PCP: Purple carrot powder
TPC: Total phenolic content
TAC: Total anthocyanin content
CUPRAC: Cupric reducing antioxidant capacity
AA: Antioxidant activity

RSA: Radical scavenging activity
WLC: Whey-less cheese.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Additional Points

Practical Applications. Due to the growing functional foods demand, manufacturing dairy products with additional functional properties is favorable. Acylated anthocyanins in purple carrot can play an important role as a health-promoting attribute. The solubility of anthocyanins in purple carrot powder (PCP) is challenging in the production of cheese containing PCP. Present work focused on producing whey-less cheese (WLC) enriched with PCP to evaluate its properties. Our study showed that the enrichment of WLC with the PCP not only improved the properties of WLC but also the color of the final product remained stable during the storage period. PCP enrichment improved radical scavenging activity, total phenolic content, and total anthocyanin content of the WLC. *Highlights*. Acylated anthocyanin from purple carrot powder was stable during the storage period. The color of fortified whey-less cheese was constant during the storage period. Palatability increased in whey-less cheese containing purple carrot power.

Ethical Approval

Ethics approval was not required for this research.

Conflicts of Interest

The authors declare no conflict of interest.

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