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Article

Studies on Mitigating Lipid Oxidation Reactions in a Value-Added Dairy Product Using a Standardized Cranberry Extract

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Abstract: A standardized whole cranberry extract (WCE) was used to stabilize a model sunflower-casein emulsion prototype for future formulation activities with a fresh cream cheese product. The WCE contained total organic acids (20% w/w) and polyphenols (5%), the latter consisting of total anthocyanins (10%, w/w) and proanthocyanidins (12% w/w). Antioxidant capacity of the WCE was determined by ORAC, (hydrophilic ORAC = 348.31 ± 33.45 μmol of Trolox equivalents/g; lipophilic ORAC = 11.02 ± 0.85 μmol of Trolox equivalents/g). WCE was effective at stabilizing the model emulsion at a level of 0.375% (w/w), yielding a final pH of 5.6. Generation of initial lipid peroxidation products, hexanal and pentanal was inhibited by 92.4% ± 3.9% and 66.6% ± 5.3% (n = 3), respectively, when emulsions containing WCE were incubated at 50 °C for 90 h. This information was useful for formulating a fresh cream cheese product containing WCE to produce value-added potential and good self-life. The standardized WCE gave a final pH of 5.6 for the cheese premix and also significantly ($P < 0.05$) lowered both the PV and CD after 28 and 21 days at 4 °C storage, respectively, compared to untreated control. We conclude that there are important functional role(s) for cranberry constituents when presented as a standardized ingredient for producing value-added, stable fresh dairy products.

Keywords: cranberry; antioxidant activity; lipid peroxidation; fresh dairy products

1. Introduction

Fresh dairy products, such as yogurt and cream cheese are foods that are being constantly innovated with regard to new flavors, texture and the addition of value-added components, such as natural antioxidants, omega-3 fatty acids, probiotics and soluble fibers [1,2]. The fat content of fresh dairy products ranges between 1%–30%, thus some products are highly susceptible to peroxidation reactions which lowers shelf-life, if the product is not packaged or stored at optimal conditions [3,4]. Indeed, fresh dairy products, require a combination of protective systems to successfully limit lipid peroxidation [5–7]. A limited number of natural antioxidants, such as ascorbic acid, tocopherols are added to fat-containing foods to prevent rancidity and extend shelf-life through a variety of different mechanisms [8]. Moreover, synthetic food additives, such as BHA, BHT, and TBHQ, are chain-breaking antioxidants, which impede lipid oxidative processes after intercepting the chain-carrying radicals and possess some carry-over effect [9].

There is high demand from the consumer for food ingredients to contain natural constituents, the result being that the food industry has to look for effective alternatives to the synthetic stabilizers presently in use. Soft fruits, such as berries contain a diverse mixture of polyphenols that in addition to being an excellent source of natural antioxidants, will also contribute to both color and flavor [10–13]. Polyphenols found in these fruits possess effective free radical scavenging capacity in the ABTS and ORAC assays, mainly through an electron delocalization mechanism around the aromatic nucleus [8,12]. Polyphenols also have a propensity to sequester potentially reactive free iron and copper prooxidants which arise during food processing [14].

Former studies have provided evidence that the consumption of cranberry as a whole fruit offers potentially greater health benefits than simply consuming individual phytochemical constituents [14–16]. Cranberries (*Vaccinium macrocarpon*) are rich in phenolic acids, anthocyanins, flavonol glycosides, as well as proanthocyanidins [8,17–19]. Anthocyanins are water soluble pigments that are present just beneath the skin and is the source of the red color common to the cranberry fruit. In addition, whole cranberries contain procyanidins, an abundant form of proanthocyanidin which also exists in the skin or peel of the fruit [17]. Flavonols and related glycosides have been identified in fresh and spray-dried cranberry fruit, and both exhibit antioxidant properties [20,21]. On a fresh weight basis, cranberries contain approximately 1 g/kg phenolic acids, mainly as hydroxylated derivatives of benzoic and cinnamic acids [22]. The predominant anthocyanins found in cranberries are 3-*O*-galactosides and 3-*O*-arabinosides of cyanidin and peonidin [21]. Glycosides of quercetin, kaempferol, and myricetin are also present in cranberries and possess characteristic affinity to scavenge the DPPH radical [23].

In most cases, isolating different cranberry constituents is a timely and costly practice and may not be practical depending on the desired application. Nevertheless, studies have sought to elucidate the relative contributions of individual phenolic constituents for a specific value-added function. Isolated compounds from cranberry have been studied for possible contributions to reduce the risk of cardiovascular diseases [24–26]. Enriched fractions of cranberry anthocyanins and phenolic acids reduced intracellular H_2O_2-induced damage and inhibited oxidation of cell membrane fatty acids [27]. In another study, a total cranberry extract was compared against its phytochemical constituents for anti-proliferative effects against human tumor cell lines [28]. This study found that a fraction containing all classes of polyphenols most effectively enhanced anti-proliferative activity relative to

the individual phytochemicals, thereby suggesting synergistic or additive anti-proliferative interactions of the anthocyanins, proanthocyanidins, and flavonol glycosides within the cranberry extract.

Cranberries, which are an especially acidic soft fruit, may also be well-suited for use in fresh dairy products that require an acidic matrix during production. The composition of cranberry constituents is therefore potentially useful for fresh dairy product formulation. In the present study, a model emulsion, was initially used to test the functional antioxidant activity of a standardized WCE under thermally accelerated conditions. This was done due to the fact that the onset of oxidation in fresh dairy products is a relatively slow process that occurs over the course of days or even weeks of storage. The effectiveness of the WCE was compared to the synthetic antioxidant BHT by monitoring the temporal development of lipid oxidation products (hexanal and pentanal). This information was used in a subsequent study to test the WCE as an ingredient in the formulation of a value-added fresh cream cheese product.

2. Results

2.1. Characterization and Standardization of WCE

2.1.1. Phenolic, Anthocyanin, Proanthocyanidin and Organic Acid Composition

The average yield of WCE obtained from 100 g of whole frozen cranberries was 9.64 ± 0.75 g. Phenolic and organic acid composition of the WCE is summarized in Table 1.

Table 1. Total phenolics, organic acids, and antioxidant activity of freeze-dried whole cranberry extract (WCE).

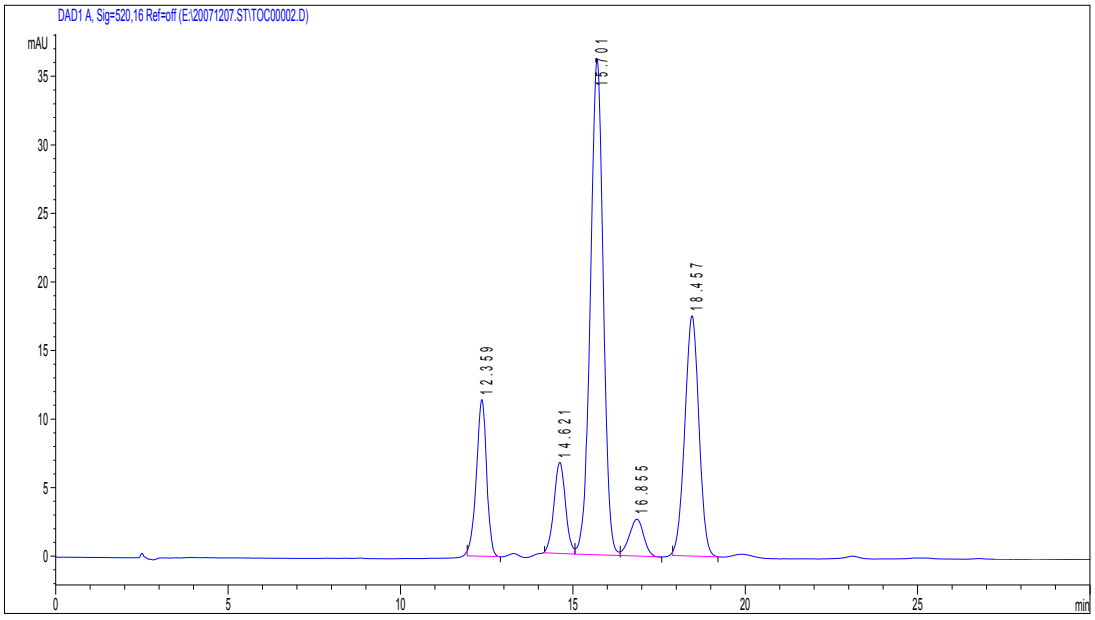
Component	
Phenolics (mg/g)	42.16 ± 4.21
Anthocyanins (mg/g)	4.09 ± 0.20
Proanthocyanidins (mg/g)	5.41 ± 0.56
Organic acids	
Quinic (mg/g)	64.30 ±1.61
Malic (mg/g)	59.11 ± 0.57
Citric (mg/g)	86.85 ± 11.94
Ascorbic (mg/g)	6.17 ± 0.01
Antioxidant activity [1]	
H-ORAC (μmol TE/g)	348.31 ± 33.45
L-ORAC (μmol TE/g)	11.02 ± 0.85

[1] TE = Trolox equivalents; H-ORAC = Hydrophillic Oxygen Radical Absorbance Capacity; L-ORAC = Lipophillic Oxygen Radical Absorbance Capacity.

A yield of 9.6% whole cranberry ethanol extract was obtained from three independent batches and blended to consist of 42.16 ± 4.21 mg/g total phenolics, with anthocyanin content being 4.09 ± 0.20 mg/g WCE, or 10%–12% of total phenolics. Five distinct anthocyanins present in the WCE were identified as cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-galactoside, and peonidin-3-arabinoside (Figure 1). The proanthocyanidin content of the WCE was 5.41 ± 0.56 mg/g making up 12%–13% of the total phenolics. The pH of a 10% (w/v) aqueous WCE solution was 2.84 ± 0.06, with

titratable acidity ranging from 8%–9% (w/w). Citric acid (86.95 ± 11.94 mg/g WCE) was the most abundant organic acid present followed by quinic (64.30 ± 1.61 mg/g WCE) and malic (59.11 ± 0.57 mg/g WCE) acids, respectively. The WCE was also found to contain a small amount of ascorbic acid (6.17 ± 0.01 mg/g).

Figure 1. HPLC chromatogram at 520 nm for 60% methanol fraction (F1) obtained from the whole cranberry extract (WCE). Anthocyanins identified from retention times for cyanidin galactoside (12.3 min); cyanidin arabinoside (14.6 min); peonidin galactoside (15.7 min); peonidin arabinoside, (18.5 min) according to previous studies [21].



2.1.2. Antioxidant Activity

The total antioxidant capacity of the WCE determined in both hydrophilic and lipophillic ORAC assays is presented in Table 1. H-ORAC antioxidant activity was 348.31 ± 33.45 µmol of Trolox equivalents (TE)/g WCE or more than 30 fold the activity observed in the L-ORAC assay (11.02 ± 0.85 µmol of TE/g WCE).

2.2. Emulsion Model

2.2.1. Emulsion Stability

Treatment of the sunflower-casein emulsion with WCE to yield a final pH 5.3 induced emulsion destabilization as indexed by the observed reduced homogeneity of the particle size distribution (Figure 2). These emulsions showed visual signs of fat coalescence and droplet aggregation at the surface. Reducing the WCE concentration to produce a final yield of 5.6 resulted in no significant differences in the average droplet size between control emulsions and those containing WCE (Figure 2, Table 2).

Figure 2. Particle size analysis for emulsions immediately after formation by high-pressure homogenization for top peak = control emulsion, (pH = 6.7); intermediate peak = emulsion containing 0.5% (w/w with aqueous phase) cranberry extract (pH = 5.6), and bottom peak = emulsion containing 1.0% (w/w with aqueous phase) cranberry extract (pH = 5.3).

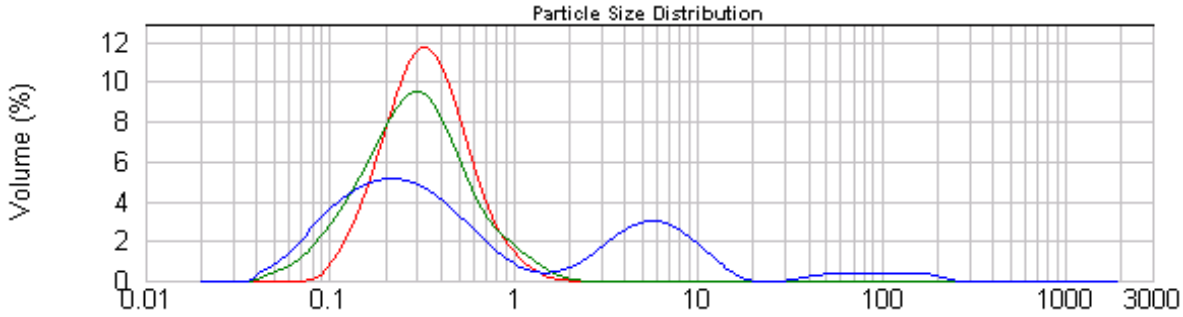


Table 2. Emulsion droplet size obtained for emulsions treated with whole cranberry extract (WCE).

Emulsion	pH	Paricle size (μm ± SD)
Control	6.8	0.261 ± 0.024a
T1	5.6	0.284 ± 0.024a
T2	5.3	0.363 ± 0.032b

Values for particle size are means ± SD (n = 3). Values within column followed by different superscripts were significantly different ($P < 0.05$).

2.2.2. Lipid Oxidation in Emulsions

Lipid oxidation experiments were first conducted on the model emulsion supplemented with 0.375% w/w WCE, to yield a final working pH of 5.6. The generation of conjugated dienes (CD) in the emulsion held at 50 °C over a 90 h incubation period is shown in Figure 3. Incorporation of WCE significantly extended the lag time (15.09 ± 1.32 h) prior to propagation of primary lipid oxidation products ($P < 0.05$), compared to the control emulsion (7.95 ± 0.80 h).

This effect was compared to similar emulsions that were formulated to contain 100 ppm BHT, and were characterized by a 30.01 ± 1.71 h lag phase. The inhibition of CD in model emulsions containing 100 ppm BHT was 29.1% ± 1.32% (Figure 4) and significantly greater ($P < 0.05$) than the inhibition for emulsions containing WCE, (8.54% ± 2.54%).

The incorporation of WCE or BHT into the sunflower oil-casein emulsion reduced the formation of hexanal (Figure 4) and pentanal (Figure 5) over the 90 h incubation period. Similar to the results obtained with CD, the affinity of the WCE to prevent lipid peroxidation was particularly evident with a prolonged lag phase for hexanal and pentanal peroxidation products.

The presence of WCE in the sunflower oil-casein emulsion resulted in a total of 92.4% ± 3.9% inhibition of hexanal and 66.6% ± 5.3% inhibition for pentanal generation (Figure 6). A similar effectiveness to reduce secondary products of lipid oxidation was observed with 100 ppm BHT, which reduced hexanal by 90.7% ± 2.6% and pentanal by 73.6% ± 3.4% respectively (Figure 6).

Figure 3. Production of conjugated dienes in sunflower o/w emulsions during oxidation in the dark at 50 °C for control emulsion (■), emulsion containing whole cranberry extract (WCE) (○), and emulsion containing 100 ppm BHT (◊). Each point represents a duplicate measurement.

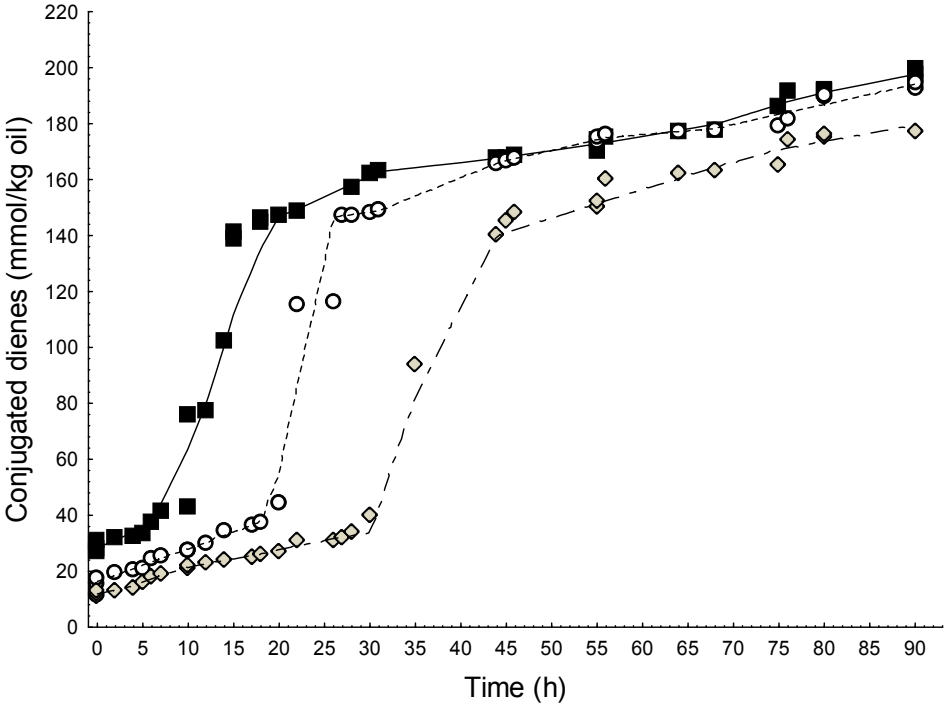


Figure 4. Production of hexanal in sunflower o/w emulsions during oxidation in the dark at 50 °C for control emulsion (■), emulsion containing whole cranberry extract (WCE) (○), and emulsion containing 100 ppm BHT (◊). Each point represents a duplicate measurement.

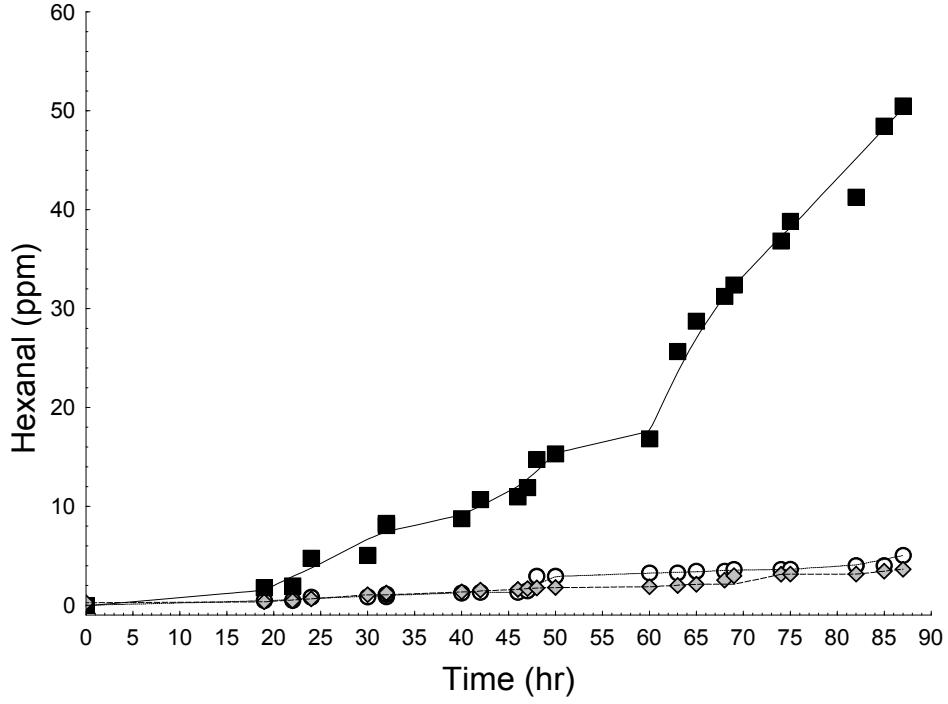


Figure 5. Production of pentanal in sunflower o/w emulsions during oxidation in the dark at 50 °C for control emulsion (■), emulsion containing whole cranberry extract (WCE) (○), and emulsion containing 100 ppm butylated hydroxytoluene (BHT) (◊). Each point represents a duplicate measurement.

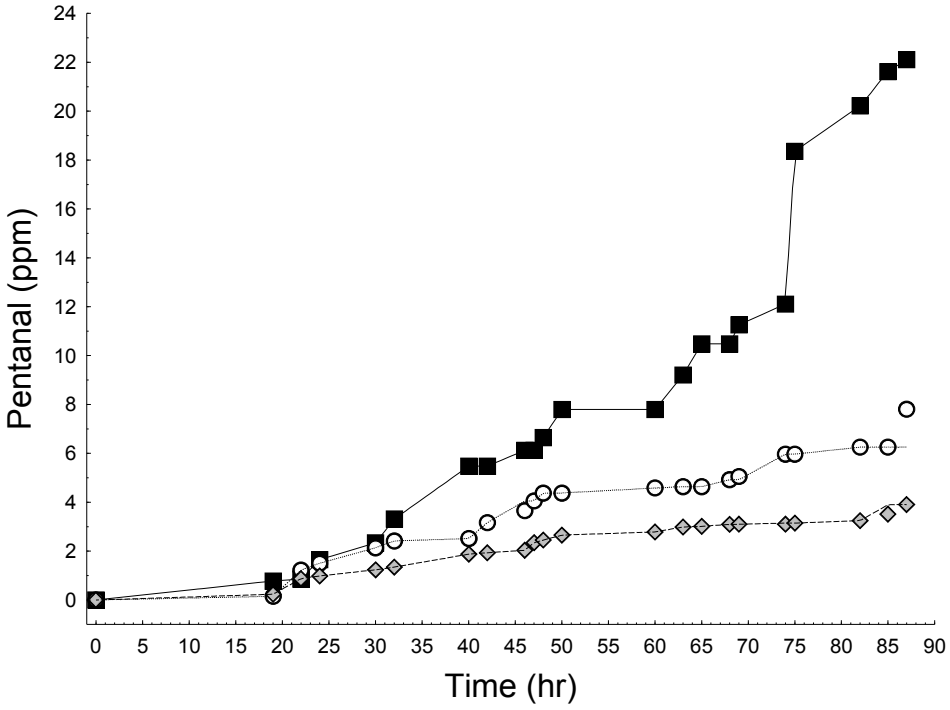
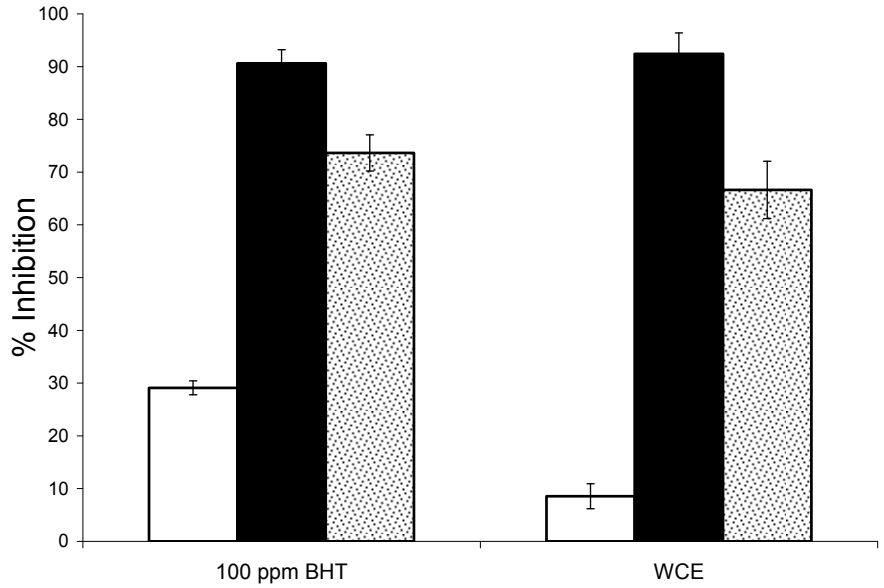


Figure 6. Antioxidant activity of BHT (100 ppm), and whole cranberry extract (WCE) in 25% sunflower o/w emulsions (pH 5.6) incubated at 50 °C for 90 h. Bars represent % inhibition ± SD of conjugated dienes (□), hexanal (■) and pentanal (▒), as calculated using area under the curve.



2.2.3. Effect of WCE in Fresh Cream Cheese Product

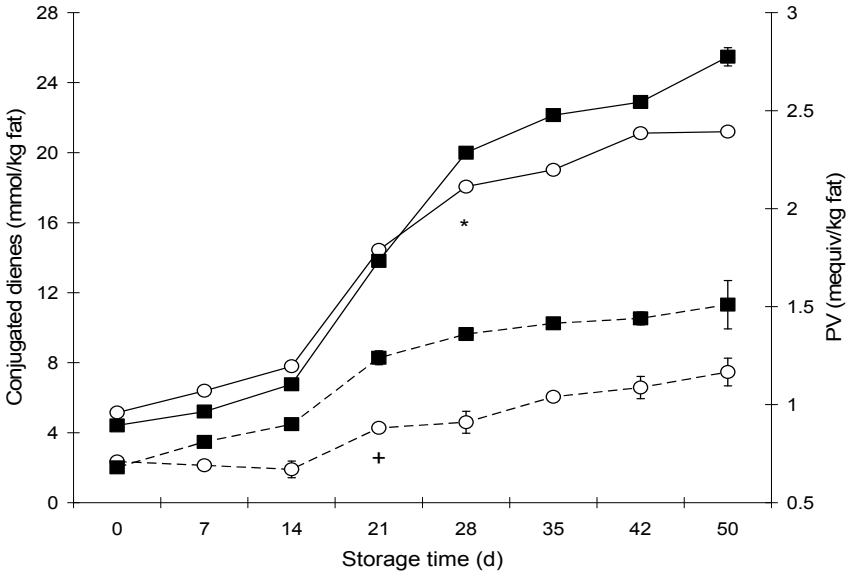
WCE was incorporated into the cheese premix to reach a pH of 5.6, prior to the addition of starter culture.

CD concentrations were significantly lower ($P < 0.05$) in cream cheese products containing WCE after 28 days of storage at 4 °C (Figure 7). The peroxide value was also significantly lower in cream cheese product containing WCE after 21 days of storage at 4 °C (Figure 7).

3. Discussion

Preparation of the WCE was carried out using ethanol due to the direct food application for the extract. The yield of total phenolics present in the final WCE recovered from frozen cranberries was correlated to the level of total soluble solids generally found in cranberries, (e.g., ranging from 9%–12% (w/w)) [29,30]. Whole cranberries have been found to contain between 190 and 533 mg/kg anthocyanins [30], while proanthocyanidin content in mature cranberries varies (e.g., 40–120 m.100 g, [11] depending on the variety, and maturation of the fruit. The total yield of anthocyanins and pro-anthocyanidins present in the standardized WCE are within the expected range for both phytochemicals present in whole cranberry fruit [31].

Figure 7. Level of conjugated dienes (—) and peroxide value (---) for control (■) and treatment (○) cream cheese stored for 50 days at 4 °C. * + shows time where scores significantly changed ($P < 0.05$) between treatment and control.



The naturally acidic property of the WCE made it useful in the production of a fresh cream cheese product. The manufacture of fresh dairy products requires a slow acid production, which is most commonly accomplished with the use of starter cultures, commercial acidulants, or a combination of both. Citric acid, which was the most abundant organic acid in the WCE, is a commonly used acidifying agent and flavor modifier in cultured milk, butter and some natural cheeses. Commercial acidulants are commonly employed in dairy processing to add flavor, provide protection from

microbial growth, and reduce processing time, all of which translates to improved efficiencies in the dairy industry. In our study, the acidity property of the WCE was effective at producing the required low pH for the cheese premix, while not adversely affecting starter culture curd formation.

Peroxy-radical scavenging activity was also observed with the WCE in both hydrophilic and lipophilic conditions, as demonstrated using ORAC. The antioxidant capacity of the WCE can be attributed to a diverse amount of phenolics, present in the WCE, in particular, anthocyanins and proanthocyanidins. Due to the complexity of the lipid oxidation reaction in emulsion systems, there are reported benefits to having numerous antioxidant components which can reduce lipid oxidation through diverse mechanisms [12]. Polar antioxidants, such as the anthocyanins present in cranberries, have a propensity to reduce lipid oxidation in emulsions by chelating metal ions in the aqueous phase, thereby reducing the prooxidant contact with unsaturated lipids [10,14]. The relatively non-polar phenolics present in the WCE are in turn effective antioxidants in oil-in-water emulsions, due to the affinity to position at the interface where oxidation reactions occur [8,32]. It is well known that increasing the concentration of antioxidants in the lipid phase enhances the antioxidant activity in oil-in-water emulsions [33]. In our study, the presence of WCE in the model emulsion effectively prolonged the lag time before CD production was observed, although the relative affinity to prevent peroxidation was comparatively less than that observed with BHT treatment. However, our findings also showed that the WCE components inhibited secondary peroxidation products, namely hexanal and pentanal to an extent comparable to BHT. The suppression of hexanal and pentanal development was an important finding in our model emulsion due to the fact that these volatile compounds contribute to characteristic flavors and odors often associated with oxidative rancidity. These findings demonstrated an additional use for WCE as a stabilizer, which can protect against both primary and secondary oxidation products in food emulsion systems that are susceptible to oxidative deterioration reactions.

We extended the findings of WCE to inhibit lipid peroxidation in the model emulsion to similar experiments conducted in a formulated fresh cream cheese product. The stabilizing effect of the WCE was confirmed in the cream cheese with the observation that both the CD and PV measures of lipid oxidation were significantly lowered in cream cheeses after 28 and 21 days storage, respectively, at 4 °C. Analysis of headspace volatiles was complicated by the presence of many other volatiles generated over the course of cheese production and storage. However, it was established that the rate of development of primary lipid oxidation products was reduced by the incorporation of WCE into the cream cheese formulation. The oxidized flavor that can occur in milk and dairy products is generally caused by autoxidation of unsaturated fatty acids associated with the phospholipid fraction present in the milk fat globule membrane [34,35]. During the homogenization process, these phospholipids are important for the stabilization of the fat phase by positioning at the interface between the aqueous and fat phases. The positioning of the phospholipids leaves the associated unsaturated fatty acids more susceptible to oxidative deterioration, since oxidation is known to take place at the surface of the lipid droplet in the emulsion. We expect that in order for the WCE phenolics to provide oxidative stability in the fresh cream cheese product, an affinity to scavenge free radicals formed at the lipid droplet surface is required. This can be facilitated in our formulation since polyphenols present in food emulsions can partition between hydrophilic and lipophilic phases, as well as being involved in protein-polyphenol complexes that can occur as a consequence of homogenization. Further work is

required to ascertain the interaction between cream cheese constituents and polyphenols in the WCE in reducing the occurrence of lipid oxidation.

4. Materials and Methods

4.1. Materials

Frozen cranberries were purchased from a local supermarket (Vancouver, Canada). Technical grade ethanol used for extracting bioactive components from cranberries was obtained from Commercial Alcohols Canada (Toronto, Canada). Gallic acid standard and ferrous sulfate heptahydrate for measurement of total phenolics were obtained from Sigma-Aldrich Canada Ltd. (Oakville, Canada). For the oxygen radical absorbance capacity (ORAC) assay for antioxidant activity, AAPH (2,2′-azobis (2-amidinopropane) dihydrochloride) was obtained from Wako Chemicals USA (Richmond, VA, USA); Trolox and 2′,7′-dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma–Aldrich Canada Ltd. Phosphoric acid (HPLC grade), methanol (HPLC grade), acetonitrile (HPLC grade), formic acid and hydrochloric acid were obtained from Fisher Scientific (Nepean, Canada). Quinic, malic, citric and ascorbic acid used as standards for HPLC analysis were obtained from Sigma–Aldrich Canada Ltd. (Oakville, Canada). Sephadex LH-20 was purchased from Amersham Biosciences (Uppsala, Sweden) and Zorbax C-18 columns obtained from Agilent Technologies (Mississauga, Canada). Sunflower seed oil (from *Helianthus annuus*), sodium caseinate and butylated hydroxytoluene (BHT) were obtained from Sigma–Aldrich Canada Ltd. Hexanal, pentanal and 4-heptanone were obtained from Fluka (Buchs, Switzerland). *Iso*-octane and 2-propanol (ACS grade) for measurement of conjugated dienes (CD) were purchased from Fisher Scientific. Pasteurized, non-homogenized milk and full-fat cream were obtained from local supermarkets. Freeze-dried cultures (*Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *cremoris*) for cheese-making were obtained from Danisco Canada Ltd. (Scarborough, Canada).

4.2. Preparation and Characterization of a Whole Cranberry Extract (WCE)

4.2.1. Preparation of WCE

Frozen cranberries (100 g) were blended 1:1 (w/v) with 80% (v/v) ethanol for 5 min using a Waring blender. The slurry was transferred to an Erlenmeyer flask, and extracted for approximately 4 h at 21 °C in an orbital shaker operating at 400 rpm (Innova 4000, New Brunswick Scientific, NJ, USA). The slurry was then filtered through a Buchner funnel using Whatman filter paper (No. 1). The filter cake was rinsed twice with 25 mL of 80% ethanol, and then transferred to a new Erlenmeyer flask where it was re-extracted overnight with 150 mL of 80% ethanol in the orbital shaker. The filter cake was extracted for a third time with 80% ethanol for 1 h and all filtrates were pooled together. The ethanol in the pooled filtrate was removed under vacuum at 35 °C. The residue was then freeze-dried, yielding crude whole cranberry extract and kept in a vacuum pack at 4 °C. This procedure was carried out for three separate batches of cranberries. The three batches of extracts were mixed together in equal parts to generate a WCE ready for compositional analysis.

4.2.2. Phytochemcial Composition of WCE

Total phenolic content of WCE was measured using the Folin-Ciocalteu assay [36] and total anthocyanins measured using the pH differential method [37]. Identification of major anthocyanins and proanthocyanidins in WCE was performed according to the procedure of Porter *et al.* [18], with modifications. The WCE was dissolved in methanol (10% w/v) and loaded onto a 60-g Sephadex LH-20 column (i.d. = 2.5 cm, length = 50.0 cm) equilibrated with 20% (v/v) methanol. A 20% aqueous methanol (v/v) eluted at 1.5 mL/min was used to collect non-phenolic cranberry constituents such as organic acids. Subsequently, 500 mL of 60% aqueous methanol (v/v) was used to elute cranberry flavanols and anthocyanins and recovered in fraction 1 (F1). Finally, 100% methanol was used for the elution of proanthocyanidins and non-polar phenolics; this was collected in fraction 2 (F2). Fractions were concentrated under vacuum by rotary evaporation at 35 °C and subsequently lyophilized. Freeze-dried powders F1 and F2 were re-dissolved in 100% methanol and filtered through a 0.45 μM nylon membrane-filter. The presence of proanthocyanidins in F2 was confirmed by the acid-butanol assay [38]. HPLC analyses for the separation of individual anthocyanins and proanthocyanidins was performed using an Agilent 1100 HPLC system (Agilent Technology 1100 series, Palo Alto, Canada), equipped with quaternary pumps, autosampler and a diode array detector. Separation of phenolics was performed using a Zorbax RX-C18 column (5 μm, 4.6 mm × 250 mm) at 30 °C. Mobile phases constituted of 2% formic acid in 100% methanol (A) and 2% formic acid water (B) at a flow rate of 1 mL/min. The gradient condition started with 23% A, linearly increased to 24.3% A at 15 min, then to 50% A at 20 min and 55% A at 25 min.

4.2.3. WCE Antioxidant Activity Capacity

The hydrophilic oxygen radical absorption capacity assay (H-ORAC) was followed as previously described [39]. Briefly, cranberry extracts and a range of Trolox standards were appropriately diluted in phosphate buffer (50 mM, pH 7.0) and added in triplicate to a 96-well plate (Nunc, Fluorescent microplate). To this was added 60 μL of 20 nM fluorescein, and plates were incubated at 37 °C for 15 min. The peroxyl radical initiator, AAPH, was added to a final concentration of 12 mM and fluorescence (Ex = 485 nm, Em = 527 nm) was continuously taken for 60 min (Fluoroskan Ascent FL, Labsystems). In the lipophilic ORAC assay (L-ORAC), the lipophilic components of the cranberry extract were extracted using hexane followed by a further dilution in 7% randomly methylated cyclodextrin (RMCD) in 50% acetone. Solutions were subsequently shaken at 400 rpm for one hour protected from exposure to light. Phosphate buffer (75 mM, pH 7.0) was added up to 90 μL in the microplate, and to this was added 10 μL of the lipid soluble component (dissolved in 7% RMCD), to give a final mixture volume of 100 μL. Trolox standard and fluorescein were maintained consistent to that used for the H-ORAC assay. The final concentration of AAPH however, was adjusted to 18 mM rather than 12 mM and the measurement time was extended to 100 min instead of 60 min. The blank constituted of 90 μL of phosphate buffer and 10 μL of 7% RMCD. Data transformation for both H-ORAC and L-ORAC was performed as previously described by Davalos *et al.*, [40]. The ORAC values were expressed as μmol Trolox equivalents (TE)/g extract.

4.3. Lipid Oxidation in Model Emulsion

4.3.1. Model Emulsion Preparation

The aqueous phase for emulsions was prepared by dissolving 10 g of sodium caseinate in water at 30 °C. For all emulsions, 150 g of aqueous phase was prepared. Sunflower oil (50 g) was then added, and emulsions formed by passing 5 times through a two-stage high-pressure homogenizer operating at 40 and 400 bar in the first and second stage, respectively. Treatments (T1 and T2) were prepared by incorporating 0.75 and 1.5 g of WCE, respectively, into the aqueous phase prior to homogenization, which were quantities predetermined to yield emulsions with a final pH of 5.6 and 5.3. For BHT treatment, 0.02 grams of BHT was incorporated into the aqueous phase so that the final concentration of BHT in emulsions was 100 ppm. A summary of all treatments is shown in Table 3. All emulsions were prepared in triplicate.

Table 3. Final composition and pH of control emulsions and emulsions containing BHT and whole cranberry extract (WCE) [1].

Treatment	Na-Cas (% w/w)	Sunflower oil (% w/w)	WCE (% w/w)	pH
Control	2	25	-	6.80
BHT (100 ppm)	2	25	-	6.80
T1	2	25	0.375	5.61
T2	2	25	0.75	5.33

[1] Na-Cas = sodium caseinate; WCE = Whole cranberry extract; BHT = β-hydroxytolulene.

4.3.2. Emulsion Stability Test

Emulsion stability was determined by measuring the droplet particle size distribution using a Malvem Mastersizer 2000 (Malvem Instruments Ltd, c/o Pharmacy Department, University of British Columbia), where the volume-surface average diameter ($d_{3,2}$) of particles was measured within 1 h of preparation. A non-uniform particle size distribution was interpreted as the destabilization of the emulsion. Measurements were repeated in triplicate.

4.3.3. Accelerated Lipid Oxidation Test

Aliquots (2.5 mL) of emulsions were distributed in 22.4-mL headspace vials sealed with polytetrafluoroethylene/silicon septa and aluminum crimp seals. An accelerated oxidation test was carried out by placing the emulsions in an orbital shaker in the dark at 50 °C. Emulsions remained physically stable at this temperature for at least 1 week. Vials were sampled in duplicate over a 90 h incubation period for measurement of hexanal, penatanal and conjugated dienes.

4.3.4. Measurement of Headspace Hexanal and Pentanal by Static Gas Chromatography

Vials containing emulsions were thermostated at 60 °C for 10 min in a Tekmar 7000 headspace autosampler. After 3 min pressurization with helium as the carrier gas, the headspace was injected during an interval of 0.10 min through the transfer line set at 115 °C to the gas chromatograph

(Hitachi). The gas chromatograph was fitted with a DB-5 column (J&W Scientific, Folsom, Canada) (30 m × 0.25 mm × 0.25 μm). The initial temperature of the oven was maintained at 35 °C for 2 min, then increased to 80 °C at a rate of 5 °C/min, and to 220 °C at a rate of 20 °C/min. The flame ionization detector (FID) was set at 250 °C. Hexanal and pentanal were identified by comparing the retention time of the peaks at with those of authentic reference compounds. Concentrations expressed in mg/kg of emulsion were determined from peak areas using a standard curve made from standards hexanal or pentanal added at varying concentrations to an untreated emulsion. Measurements were performed at least twice for each emulsion sample and averaged.

4.3.5. Measurement of Conjugated Dienes (CD)

CD were measured in the emulsions according to a modified version of the AOCS Official Method Ti:64 [41]. Emulsion samples (20 μL) were diluted to 10 mL with a mixture of isooctane/ 2-propanol (2:1 v/v) and vortexed for 1 min. The absorbance was measured at 232 nm by using a UV–Vis scanning spectrophotometer (Unicam Helios, Spectroncic Unicam EMEA, Cambridge, UK). A filtration through Macherey-Nagel filters (25 mm, pore 0.2 μm) was applied just before the measurement to remove protein from the sample and thereby diminish its spectrum interference in this region. The absorbance was determined at 232 nm using a path length of 1 cm. The amount of CD (mmol/kg oil) in the oxidizing emulsions was calculated on the basis of linoleic acid which has a molar absorptivity (ε) of 27,000.

4.3.6. Measurement of Inhibition of Lipid Oxidation.

The inhibition of headspace hexanal, pentanal or CD was calculated by measuring the areas under the curve (AUC) over the incubation period using statistical software (GraphPad Prism 5, La Jolla, Canada) for treatments and controls, using the following equation:

$$\% \text{ Inhibition} = [1 - (AUC_t/AUC_c)] \times 100$$

Where: AUC_t is area under the curve for the treatment, and AUC_c is the area under the control.

4.4. Lipid Oxidation in a Model Cream Cheese

4.4.1. Formulation of Cream Cheese Product

Pasteurized skim milk and cream (36% fat) were combined to give a cheese premix with 11% fat, which was used to produce both control and experimental cheeses. For cheeses containing WCE, to 988.2 grams of cheese premix was added 11.8 grams of WCE. This cheese premix was homogenized, and had a final pH of 5.6. All cheese premixes were inoculated with starter culture, and gelation took place until a final pH of 4.6 was reached. The cheese curd was whisked, and drained at room temperature through a muslin bag for 12 h. The final fat content was 30% (w/w), moisture content was less than 60% (w/w) and the salt (0.5% (w/w) for both control and experimental cheeses. Cream cheese samples (20 g) were incubated at 4 °C in thermo-formed plastic cups and covered using polypropylene wrap.

4.4.2. Determination of Lipid Oxidation in Cream Cheese

Lipid oxidation was monitored in cream cheese samples by following the formation of CD and measuring the peroxide value (PV) in the lipid fraction of the cheese. Lipids were extracted from cream cheese according to a previous method [42]. CD were measured in the separated lipids according to a the AOCS Official Method Ti:64[41]. The lag time was measured as the intercept between the baseline and the tangent of the CD curve at the onset of the propagation phase. PV of the product was tested using procedures from AOAC Official Methods 965.33: Fats and Oils [43].

4.5. Statistical Analysis

For analysis of particle size distribution, emulsions were prepared in triplicate for control, T1 and T2 and measurements were repeated three times for each emulsion. Statistical differences were determined using the Student's t test with the significance level set at $P < 0.05$. For lipid oxidation experiments, emulsions were prepared in duplicate, and measurement of CD, hexanal and pentanal were performed in repeated measures over the time course of the study. Careful attention was given to obtain sufficient data points for determination of the lag phase and propagation phase of oxidation. Cream cheeses were prepared in triplicate for both treatment and control. Statistical analysis was performed by two-way ANOVA (GraphPad Prism 5) with a significance level set at $P < 0.05$, followed by the Bonferrini t test for pairwise comparison ($P < 0.05$).

5. Conclusions

The synergies and interactions between cranberry components present in a standardized WCE has been shown herein to contribute to functional roles that involve incorporating a phenolic-rich extract in a food system that can be classified as an acidified protein-stabilized emulsion [44]. Future investigations are required to determine the acceptance of products with regard to color and flavor, as well the economic viability of incorporating WCE into other innovative value-added fresh dairy products.

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