Evaluation of whey, milk, and delactosed permeates as salt substitutes

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ABSTRACT

Whey and milk permeates are by-products of high-protein dairy powder manufacture. Previous work has shown that these permeates contribute to salty taste without contributing significantly to sodium content. The objective of this study was to explore the sensory characteristics and compositional analysis of permeates from different milk and whey streams and a low-sodium product application made from them. Skim milk, Cheddar, cottage, and Mozzarella cheese whey permeates were manufactured in triplicate, and delactosed whey permeate was obtained in triplicate. Composition (protein, fat, solids, minerals) was conducted on permeates. Organic acid composition was determined using HPLC. Volatile compounds were extracted from permeates by solid phase microextraction with gas chromatography-mass spectrometry. A trained sensory panel documented sensory attributes of permeates and cream of broccoli soups with and without salt or permeates followed by consumer acceptance testing ($n = 105$) on the soups. Cottage cheese whey permeate contained a higher lactic acid content than other permeates, which has been shown to contribute to a higher salty taste. Cottage cheese whey permeate also contained potato or brothy and caramel flavors and sour and salty tastes, whereas delactosed whey permeate had high intensities of cardboard and beefy or brothy flavors and salty taste. Milk, Cheddar, and Mozzarella cheese whey permeates were characterized by sweet taste and cooked milky flavor. Permeates with higher cardboard flavor had higher levels of aldehydes. All permeates contributed to salty taste and to salty taste perception in soups; although the control soup with added salt was perceived as saltier and was preferred by consumers over permeate soups. Soup with permeate from cottage cheese was the least liked of all soups, likely due to its sour taste. All

other permeate soups scored at parity for liking. These results demonstrate the potential for milk, whey, and delactosed permeates from different whey streams to be used as salt substitutes in product applications.

Key words: permeate, salt substitute, acid whey

INTRODUCTION

It is well known that the current American diet contains too much salt. The recommended daily allowance for sodium is 1,500 to 2,400 mg/d, but Americans typically consume an average of 3,000 mg/d, most of which comes from packaged, processed food items (USDA, 2010; Kim et al., 2012). High sodium intake levels have been linked to hypertension in adults. Hypertension, or high blood pressure, affects almost 50 million people in the United States. Hypertension is a state where the blood pressure in the adult human body reaches and maintains a blood pressure level above 140/90 mmHg. Hypertension places people at higher risk for cardiovascular disease. A reduced-sodium diet can lower the blood pressure of adults (Sacks et al., 2001; FDA, 2010); however, studies have shown that many people do not understand all of the health risks associated with a high-salt diet, and that many are resistant to change their sodium intake if it negatively affects food flavor (Kim et al., 2012).

The food industry has developed many approaches to reduce sodium intake; however, this has proven challenging because the primary driving forces behind the purchase intent and liking of foods are flavor and texture, both of which heavily correspond to salt content (Yates and Drake, 2007; Childs and Drake, 2009; Kim et al., 2012). The most common approach is by partial replacement of sodium chloride with potassium chloride or another halide salt; however, these salts are often perceived as bitter and metallic (Murphy et al., 1981; Sinopoli and Lawless, 2012). Because of its bitter taste, KCl is often blended with NaCl, which can reduce the bitter taste of the KCl (Breslin and Beauchamp, 1995; Monteiro et al., 2015). Another approach is through the use of flavor enhancers, which increase the salty

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taste perception of product applications. Studies have demonstrated that flavor enhancers that increase salty taste perception include nucleotides, such as monosodium and monocalcium glutamates, NPN compounds, and lactic, orotic, and citric acids (Yamaguchi and Takahashi, 1981; Roininen et al., 1996; Ball et al., 2002; Salles et al., 2002; Kang et al., 2007; Frankowski et al., 2014).

Due to the increased use of whey proteins in the food industry, whey permeate, also known as modified whey, deproteinized whey, or dairy product solids, is in abundance. Permeate, the by-product of whey protein manufacture, contains approximately 65 to 85% lactose, 8 to 20% ash or minerals, 3 to 8% protein (mostly NPN), and no more than 1.5% fat (Beucler et al., 2005; USDEC, 2011b). The production of permeate increased by 17% per year between 2005 and 2009 (Gerdes, 2011). Currently, whey permeate is mostly used in fermentation membrane bioreactors to produce lactic acid or ethanol (Mehaia and Cheryan, 1986; Gabardo et al., 2014), sprayed on fields as fertilizer, or fed to animals (Scott and Krishnapillai, 2015; Woyengo et al., 2015); however, permeate can also be used in many food applications. Permeate works well in baked goods due to its high lactose content, which is used in the Maillard reaction to produce a brown color and caramelized flavor. Permeate is also used in confectionary icings and coatings, and dry mixes for dips and sauces as a bulking agent or milk solids replacer. Research has also demonstrated the use of Cheddar whey permeate as a salt substitute because of its high mineral and lactic acid content (Frankowski et al., 2014).

Frankowski et al. (2014) analyzed the individual components of Cheddar whey permeate and delactosed permeates (**DLC**) from different suppliers to determine components of whey permeates that contributed to salty taste. Permeate and DLC compositions differed due to variations in processing parameters and storage. The main contributors to salty taste in permeates and DLC included NaCl, KCl, lactic acid, and orotic acid, though the components that contributed the most salty taste, other than sodium, were lactic acid and KCl. Salty taste in DLC was higher than standard permeate due to minerals and organic acids concentrated during the lactose removal process. Frankowski et al. (2014) also reported flavors in permeates consistent with other whey and milk products such as cooked or milky, beefy or brothy, diacetyl, cardboard, and graham cracker. These flavors have been sourced to volatile compound components in the whey or milk (Carunchia Whets-tine et al., 2003, 2005; Drake et al., 2003; Gallardo-Escamilla et al., 2005; Wright et al., 2009; Campbell et al., 2011b). Frankowski et al. (2014) hypothesized that

permeates made from acid whey would be saltier than those made from sweet whey due to the higher organic acid composition in acid whey. The objective of the current study was to compare composition and sensory differences of permeates from 4 different sources; milk, Cheddar, Mozzarella, and cottage cheese wheys, as well as DLC. Our study will help to determine appropriate ingredient applications for permeates from different whey sources.

MATERIALS AND METHODS

Experimental Design

Permeates were manufactured across 2 d. On d 1, two permeates were manufactured, and on d 2 the other 2 permeates were manufactured. One lot of milk was used for each replicate of all permeates. The order in which permeates were made on which day was randomized across all 3 replicates. Approximately 190 L of milk was used for each replicate of each permeate. Cheddar, Mozzarella, and cottage whey were made using standard manufacturing procedures (Campbell et al., 2011b; McAuliffe et al., 1999). Wheys and skim milk were pasteurized, fat separated, UF, diafiltered, and then concentrated to 10 to 12% solids (wt/wt) using reverse osmosis (**RO**) and spray-dried. Liquid DLC (37% solids) from Cheddar whey used in our study was obtained from a commercial supplier from 3 different production days. Following analytical tests, Cheddar, Mozzarella, cottage, milk, and DLC permeates were evaluated with a salt and a no-salt control in a cream of broccoli soup application.

Permeate Production

Mozzarella and Cheddar wheys were produced in a similar manner. Raw whole bovine milk was obtained from the North Carolina State University Dairy Research and Education Unit (Raleigh, NC). Milk was HTST pasteurized at 72°C for 16 s using a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC). Milk was then heated to 31°C (Cheddar) or 35°C (Mozzarella) and inoculated with mesophilic starter culture (Choozit MA 11, Danisco, New Century, NJ) at a rate of 50 Danisco culture units (**DCU**)/454 kg of milk (Cheddar) or thermophilic starter cultures (Choozit LH100 and TA 61, Danisco) at rates of 20 DCU/454 kg of milk for LH100 and 50 DCU/454 kg of milk for TA61 (Mozzarella). Calcium chloride was then added at 0.39 mL/kg of milk (50% wt/vol, Dairy Connection Inc., Madison, WI). The milk was ripened for 60 min under constant agitation. Milk

was then coagulated for 30 min with double strength recombinant rennet (Dairy Connection Inc.), which was added at a rate of 0.09 mL/kg of milk. The coagulum was cut into approximately 2.5-cm cubes and allowed to rest for 5 min, followed by gradual heating to 39°C (Cheddar) or 42°C (Mozzarella) over the course of 30 min with gentle agitation. Whey was then drained through a sieve and the whey cream was removed with a hot bowl centrifugal separator (Model SI600E, Agri-Lac, Miami, FL). For cottage cheese whey manufacture, pasteurized skim milk was heated to 37°C and inoculated with thermophilic starter culture (F-DVS Fresco 1000–21, Chr. Hansen, Milwaukee, WI) at a rate of 5 g/25 L of milk. After 30 min, double strength recombinant rennet (Dairy Connection Inc.) was added at a rate of 0.2 mL/100 L of milk. The milk was then incubated for approximately 4 to 5 h until a pH of 4.6 was reached. Afterward, the curd was cut into 2.5-cm cubes and allowed to rest for 10 to 15 min. The curd was then heated gradually to 55°C over 90 min. Subsequently, the whey was drained through a sieve and the whey cream was removed with a separator.

Raw bovine skim milk was also obtained from the North Carolina State University Dairy Research and Education Unit. After pasteurization, milk was heated to 50°C using a water bath. Wheys and skim milk were then UF using a pilot scale UF system (Model Lab 46, Filtration Engineering, Champlin, MN) using 4 spiral-wound UF membranes (Snyder Filtration, Vacaville, CA.; nominal cutoff = 10,000 Da, surface area = 5.0 m²). Deionized water was added for diafiltration at 40% of the original weight of the milk or whey. Before processing, the UF system was cleared of the soak solution (0.54% vol/vol, Hydriosoak, Hydrite Chemical, Brookfield, WI) using water until neutral pH was attained. Water was then drained from the system in preparation for the addition of whey or milk. Approximately 170 kg of whey/milk was added to the system for processing. The operating inlet pressure was 210 kPa and the outlet retentate pressure was 140 kPa. Whey/milk was UF and the permeate was collected in a holding tank. After UF, permeates were pumped using a centrifugal pump to the RO system. The RO system used a high-pressure pump (model A96084762P10324US193, Grundfos Pumps Corporation, Olathe, KS) and two 10-cm outer diameter RO thin-film composite filter column membranes (model RO-SYN2–4040, Snyder Filtration, Vacaville, CA). Permeates were concentrated approximately 2 times up to 10 to 12% solids (wt/wt). Total percent solids was analyzed using the Smart System 5 moisture/solids analyzer (CEM, Matthews, NC). Concentrated permeates were spray-dried (model Lab 1, Anhydro Inc., Soeberg, Denmark). Inlet temperature

was 200°C and outlet temperature was 95°C. Spray-dried samples were collected in mylar bags (TF-4000, Impak Corp., Central City, SD) and stored at –80°C until analysis.

Proximate Analysis

Proximate analyses were conducted in triplicate. Total nitrogen and NPN were analyzed by Kjeldahl (AOAC International, 2000; method numbers 991.20; 33.2.11 and 991.21; 33.2.12). True protein nitrogen for each whey was determined by subtracting NPN from total N. True protein values were obtained by multiplying true protein nitrogen values by a conversion factor of N × 6.38 (Barbano and Lynch, 1992). Fat content was determined using Mojonnier fat extraction (AOAC International, 2000; method number 989.05), and TS was determined using air oven drying (AOAC International, 2000; method number 990.20). The pH was measured on all permeates at 10% solids (wt/vol) using a pH meter (model VWR Symphony SB70P, Radnor, PA). Sodium, potassium, calcium, and magnesium were determined by inductively coupled plasma spectrophotometry by the North Carolina State University Analytical Services Laboratory (Raleigh, NC).

Organic Acids and Lactose

Organic acids and lactose were extracted using a method modified from Upreti et al. (2006). Permeates were analyzed at 10% solids. One hundred microliters of each sample was extracted in 900 µL of 0.013 *N* H₂SO₄ (Sigma Aldrich, St. Louis, MO), vortexed, and centrifuged at 8,000 × *g* at room temperature (21°C) for 5 min. The top layer was removed and filtered using 0.45-µm nylon syringe filters (VWR International, West Chester, PA). Twenty microliters of each sample was injected on the HPLC (Waters 1525 Binary Pump, Waters, Milford, MA) using an autosampler (Waters 2707 Autosampler) onto the column (0.8 mL/min, 300 × 7.8 ion exclusion, 55°C; Bio-Rad Labs, Richmond, CA). Each replicate of each permeate was injected in duplicate. The temperature of the injector was 4°C and a photodiode array detector (Waters 2998) was used for organic acids and a refractive index detector (Waters 2414 Refractive Index Detector, 30°C) was used for lactose. The maxima used for organic acid calculation was 254 and 285 nm due to citric and orotic acids coeluting at 254 nm. Citric acid was not detected at 285 nm. A standard curve was created for each organic acid and lactose. Organic acid standards were obtained from Thermo Fisher Scientific Inc. (Pittsburgh, PA) and lactose standard was obtained from Sigma Aldrich.

Descriptive Sensory Analysis of Permeates

Descriptive analysis was performed on all permeates (10% solids, wt/vol). Powders (manufactured permeates) were rehydrated to 10% (wt/vol) solids with deionized water. Liquid DLC (37% wt/vol solids,) was diluted to 10% (wt/vol) solids with deionized water. A trained panel of individuals ($n = 8$, 6 females, 2 males, age = 22–49 yr) with >100 h of experience using an established dairy sensory lexicon and the Spectrum method (Meilgaard et al., 2007; Frankowski et al., 2014) evaluated the permeates. Permeates were dispensed into lidded soufflé cups and evaluated at 15°C. Each panelist evaluated each sample replicate in duplicate. Unsalted crackers and room-temperature deionized water were used for palate cleansing. Paper ballots were used for data collection.

Volatile Compound Analysis

For each permeate, headspace solid-phase microextraction (SPME) with GC-MS was used to extract and separate selected volatile compounds. Methods were taken and modified from Liaw et al. (2010) and Campbell et al. (2011b). Injections were made on an Agilent 7820A with a 5975 inert mass selective detector with a ZB-5 MS column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m; Phenomenex, Torrance, CA) using a CTC Analytics CombiPal Autosampler (CTC Analytics, Alexandria, VA). Three replicates of volatile analysis samples were prepared by adding 5 mL of liquid permeate (10% solids) to 20 mL of amber SPME vials (MicroLiter Analytical Supplies Inc., Suwanee, GA) along with the addition of internal standard (10 μ L of 81 mg/kg 2-methyl-3-heptanone in methanol; Sigma Aldrich) and 0.5 g of NaCl to enhance volatile compound extraction. Samples were equilibrated for 25 min at 40°C with 4-s pulses at 250 rpm agitation. Analyses were all done using the same DVB/Carboxen/PDMS 1-cm SPME fiber (Supelco, Bellefonte, PA) for each replicate of the study. The fiber was exposed at a depth of 3.1 cm for 40 min in the sample vials before being retracted and then injected at 5 cm for 5 min into the GC inlet. Based on previous studies, selected volatile compounds were searched for using single ion monitoring (Karagül-Yüceer et al., 2003; Gallardo-Escamilla et al., 2005; Campbell et al., 2011b, Frankowski et al., 2014). Simultaneously, a mass collection of all volatiles was also evaluated by scanning from 35 to 350 m/z .

The GC method increased the oven temperature from an initial 40°C to 250°C after 3 min at a rate of 10°C/min and held the temperature for 5 min. The SPME fibers were introduced into the split/splitless injector at 250°C. A constant flow rate of 1 mL/min was used

and a 1-min purge time was set. The MS transfer line was maintained at a temperature of 250°C with the quadrupole at 150°C and source at 250°C. The NIST (2005) library of spectra and comparison of spectra of authentic standards injected under identical conditions were used for compound identification. Relative concentrations of each compound were calculated using the calculated recovery of the internal standard concentration. Retention indices were calculated using an alkane series (Sigma Aldrich; Vandendool and Kratz, 1963).

Cream of Broccoli Soup Preparation

Cream of broccoli soups used for consumer acceptance testing were prepared using a formula from the Dairy Research Institute website on permeate usage (DRI, 2015). Cream of broccoli soup was chosen for this experiment due to the high sodium levels typically found in prepared soups (DRI, 2015). The formula consisted of 50.2% low-sodium chicken broth, 16.9% whole milk, 8.3% heavy cream, 8.2% broccoli, 4.1% unsalted butter, 4.1% diced onion, 3.0% all-purpose flour, and 5.24% permeate. All percentages calculated on a weight-by-weight basis. All ingredients except permeates were purchased at a local grocery store (in Raleigh, NC). Milk and cream used in all formulas were from the same production date. For the DLC sample, the formula was adjusted to include the same amount of permeate solids as the permeate formula (wt/wt). For the salt control, the formula was adjusted to include 0.55% salt addition. For the no-salt control, the formula omitted the addition of both salt and permeate. Soups were prepared in one large batch (approximately 42 L) on a stove top and subdivided into smaller batches before the milk, cream, and permeate or salt were added to each batch. Soups were then maintained at 80°C in crock pots with lids. Temperature probes were used to confirm consistent temperature. Soups were dispensed for sensory testing (consumer or trained panel) as needed so that each consumer or panelist received soups at consistent temperatures.

Descriptive Sensory Analysis of Soup

Descriptive sensory analysis was performed on cream of broccoli soups made with the addition of Cheddar, Mozzarella, cottage, milk, and DLC permeates, as well as salt and no-salt controls. A trained panel of individuals ($n = 8$, 6 women, 2 men, age = 22–49 yr) with >100 h of experience using an established sensory lexicon and the Spectrum method (Meilgaard et al., 2007; Frankowski et al., 2014) evaluated the soups. Soups were dispensed into lidded 120-mL styrofoam cups, each labeled with a 3-digit blinding code and

evaluated at 80°C. Each panelist evaluated each sample replicate in duplicate. Paper ballots were used for data collection.

Consumer Acceptance Testing of Soup

Consumer acceptance testing was also performed on cream of broccoli soups made with the addition of Cheddar, Mozzarella, cottage, milk, and DLC permeates, as well as salt and no-salt controls. Consumer acceptance testing was approved (exempt) by the University Institutional Review Board for Human Subjects. Participants ($n = 105$) were self-proclaimed soup consumers who were gathered using fliers and university email listservs. Consumers were served approximately 75 mL of each soup at 80°C in 125-mL styrofoam cups labeled with 3-digit blinding codes (PFS Sales Co., Raleigh, NC). The temperature of each soup was taken periodically to ensure consistency between samples. The order of presentation for each of the samples was randomized and balanced among all 7 soups. Consumers were provided with unsalted crackers and ambient temperature deionized water as palate cleansers between samples. Consumers first gave informed consent to participate in the panel. Consumers then scored all products for appearance liking, overall liking, overall flavor liking, thickness liking, and salty taste liking using a 9-point hedonic liking scale where 9 = like extremely and 1 = dislike extremely. Consumers then answered a series of 5-point just about right (**JAR**) questions regarding the salty taste, thickness, broccoli flavor, and milky or creamy flavor where 1 = not nearly salty enough, much too thin, much too weak, and much too weak, respectively, 2 = not salty enough, too thin, too weak, and too weak, respectively, 3 = JAR, and 4 = too salty, too thick, too strong, and too strong, respectively, and 5 = much too salty, much too thick, much too strong, and much too strong, respectively. A 3-min rest was enforced between each soup. After all samples were tasted, consumers were asked a series of demographic questions about themselves including sex, age, income, and level of education. Consumers received a food treat for participation. Compusense Five version 5.6 (Compusense, Guelph, Canada) was used for data collection.

Statistical Analysis

One-way ANOVA with means separation was conducted on proximate analysis, sensory, and volatile compounds. Principal component analysis was also conducted on sensory and volatile data to visualize differences. Consumer panel JAR scores were evaluated using chi-square. Statistical analyses were conducted

with XLSTAT (Version 2013.5.03, Addinsoft, New York, NY).

RESULTS AND DISCUSSION

Proximate analysis results were consistent with previous studies and reported values (Frankowski et al., 2014; USDEC, 2011a,b). Moisture concentration was not different between manufactured permeate samples ($1.3 \pm 0.54\%$, $\pm$ SD, wt/wt; $P > 0.05$). True protein content was also not different between manufactured permeate samples ($0.003 \pm 0.003\%$, wt/wt; $P > 0.05$) though NPN values were higher in cottage permeate than other manufactured permeates (3.6 vs. $2.5 \pm 0.04\%$, wt/wt; $P < 0.05$). The DLC permeate contained $37.3 \pm 0.14\%$ (wt/wt) solids, $0.01 \pm 0.001\%$ (wt/wt) true protein, and $2.5 \pm 0.2\%$ (wt/wt) NPN. Higher NPN values have been reported in low-pH wheys, such as cottage cheese whey, due to proteolysis caused by lactic culture during the longer fermentation times of these wheys, which led to higher soluble nitrogen values found in the whey (Hill et al., 1985). Potassium, magnesium, and sodium concentrations were consistent between manufactured permeates, but calcium was higher in cottage whey permeate than the other permeates ($P < 0.05$; Table 1). This difference was due to the method of setting of the curd for the cottage whey. In sweet wheys, rennet is used, which causes calcium caseinates to form and bind the calcium. In the acid-precipitated cottage whey, ionized calcium is formed which favors the whey rather than binding to the curd (Wong et al., 1978; Hill et al., 1985). The DLC permeate was higher in NPN, true protein, and all minerals except calcium by 2 to 3 times ($P < 0.05$). This is because the proportion of mineral solids to the rest of the solids in DLC permeates is increased as lactose is removed, consistent with previous results (Frankowski et al., 2014). The pH was not different between Cheddar and Mozzarella permeates (6.2 ± 0.02 ; $P > 0.05$), whereas pH of the milk, cottage, and DLC permeates were 6.4 ± 0.02 , 4.8 ± 0.03 , and 5.4 ± 0.05 , respectively.

Table 1. Mineral composition of permeates (% wt/wt)

Permeate	% Ca	% K	% Mg	% Na
Cottage	2.09 ^a	2.51 ^b	0.19 ^{ab}	0.66 ^b
Cheddar	0.50 ^c	2.43 ^{bc}	0.13 ^c	0.65 ^{bc}
Milk	0.40 ^d	2.35 ^c	0.12 ^d	0.62 ^c
Mozzarella	0.48 ^c	2.51 ^b	0.13 ^c	0.66 ^b
Delactosed permeates	0.84 ^b	7.54 ^a	0.27 ^a	1.85 ^a

^{a-d}Means within a column that do not share a common letter are significantly different ($P < 0.05$).

The lactose concentration of DLC was the lowest of all permeates due to the mechanical removal of lactose from this permeate (Figure 1). The Cheddar, Mozzarella, and milk permeates were higher in lactose than cottage permeate ($P < 0.05$; Figure 1). The long fermentation time required to produce the cottage whey allows for the lactic acid bacteria to convert more of the lactose into lactic acid, thus lowering the lactose content. This result also explains the higher lactic acid concentration in the cottage permeate compared with the other permeates ($P < 0.05$). The DLC permeate also undergoes several processing steps to remove lactose, which allows sufficient time for the bacteria to convert lactose. Few differences were observed among citric and orotic acid results among all permeates with the largest difference observed in the DLC permeate ($P > 0.05$; Figure 2). Differences documented were likely a result of different starter cultures used or different rates of starter culture metabolism due to the cheese make procedures which would produce organic acids at different rates (Kandler, 1983). The low citric acid levels in DLC permeate were likely caused by the extra time required to remove the lactose in this permeate, which provided time for bacteria to use the citric acid for metabolism (Llano et al., 2008).

Trained panel profiling on permeates demonstrated that each permeate had distinct flavors, characteristic of the whey sources that they were made from or from milk in the case of milk permeate (Frankowski et al., 2014; Figure 3, Table 2). The DLC permeate was the saltiest of the permeates, followed by cottage ($P < 0.05$), and both of the permeates also had sour and umami tastes. Though all permeates were salty, the higher salty taste in the DLC and cottage permeates was attributed to their mineral and acid compositions, as the sodium, potassium, calcium, and lactic acid concentrations all contribute to salty taste (Lawless et al., 2003; Frankowski et al., 2014). Studies have also shown that the sweet taste of lactose suppresses salty taste; therefore, the salty taste of the cottage and DLC permeates were expected to be higher than the Cheddar, Mozzarella, and milk permeates due to their lower lactose concentrations (Pangborn, 1959; Frankowski et al., 2014). The sour taste of the cottage and DLC permeates was due to the much lower pH of the cottage permeate and the high levels of lactic acid present in these 2 permeates. Cheddar, milk, and Mozzarella permeates were characterized by sweet aromatic flavors and sweet tastes, with low levels of cardboard flavor in the Cheddar and Mozzarella permeates. Consistent with previous studies on fluid whey, no cardboard flavor was detected in cottage and milk permeates, whereas Mozzarella permeate had lower cardboard flavor as compared with Cheddar. This result has been attributed to

Figure 1. Lactose concentration of whey permeates. DLC = delactosed permeates; Mozz = Mozzarella. Means that do not share a common letter (a–c) are significantly different ($P < 0.05$).

the starter culture used in the cheese manufacture procedure (Campbell et al., 2011a,b). Milk permeate did not contain any added starter culture and Mozzarella and cottage used thermophilic starter cultures. Wheys produced without starter culture have been shown to exhibit lower cardboard flavors than wheys made with starter culture, and wheys made from thermophilic starter cultures have been documented as having lower levels of lipid oxidation compounds than those from mesophilic starter culture (Campbell et al., 2011a,b). In addition to salty, sour, and umami tastes, cottage permeate was also characterized by potato brothy and caramel flavors, possibly due to the higher heat profile during cheese manufacture. Vitamin or brothy flavor was documented in the DLC permeates. Frankowski et al. (2014) previously evaluated delactosed permeates and reported similar results.

Figure 2. Organic acid concentration of whey permeates. DLC = delactosed permeates; Mozz = Mozzarella. Bars that do not share a common letter (a–d) within each grouping are significantly different ($P < 0.05$).

Table 2. Descriptive analysis results of permeates (10% solids wt/vol)¹

Sample	Aroma intensity	Sweet aromatic	Cardboard	Potato brothy	Beefy brothy	Caramel	Sweet	Sour	Salty	Umami
Cottage	2.2 ^a	1.0 ^c	ND ²	0.5	ND	1.7	ND	3.5 ^a	3.3 ^b	3.2 ^a
Cheddar	1.8 ^b	1.7 ^{ab}	1.0 ^b	ND	ND	ND	3.1 ^{ab}	ND	1.8 ^{cd}	1.1 ^{bc}
Milk	1.0 ^c	1.9 ^a	ND	ND	ND	ND	3.2 ^a	ND	1.3 ^d	0.6 ^c
Mozzarella	2.0 ^{ab}	1.5 ^{abc}	0.6 ^c	ND	ND	ND	2.7 ^b	ND	1.9 ^c	1.1 ^{bc}
Delactosed permeates	2.0 ^{ab}	1.8 ^a	2.4 ^a	ND	2.8	ND	2.0 ^c	2.4 ^b	7.4 ^a	3.0 ^a

^{a-d}Means within a column that do not share a common letter are significantly different ($P < 0.05$).

¹Attributes were scored using a 0 to 15 point universal Spectrum intensity scale (Meilgaard et al., 2007).

²ND = not detected.

Volatile composition of permeates was consistent with sensory results (Table 3, Figure 4) and characteristic of those found in fluid whey and whey products (Carunchia Whetstone et al., 2003, 2005; Karagül-Yüceer et al., 2003; Gallardo-Escamilla et al., 2005; Frankowski et al., 2014). All permeates contained lipid oxidation compounds which contribute to off flavors, DLC permeate contained the highest levels of aldehydes, hexanal,

octanal and decanal, which have been correlated with cardboard flavor ($P < 0.05$; Karagül-Yüceer et al., 2003; Gallardo-Escamilla et al., 2005; Liaw et al., 2010; Whitson et al., 2010; Campbell et al., 2011b). Aldehydes increase in liquid whey products with storage time and high-heat processing steps (Vazquez-Landaverde et al., 2005; Whitson et al., 2010; Liaw et al., 2011). The DLC permeate undergoes high-heat treatment and longer

Figure 3. Principal component analysis biplot of descriptive analysis of whey and milk permeates. Milk (milk permeate), Mozz (Mozzarella permeate), Ched (Cheddar permeate), Cottage (cottage permeate), and DLC (delactosed whey permeate). Color version available online.

Table 3. Relative abundance for selected volatile compounds in permeates ($\mu\text{g/kg}$)¹

Item	Cottage	Cheddar	Milk	Mozzarella	Delactosed permeates
Pentanal	0.745 ^a	0.107 ^{ab}	0.085 ^b	0.104 ^b	0.080 ^b
Hexanal	0.060 ^{ab}	0.131 ^a	0.011 ^b	0.098 ^{ab}	0.065 ^{ab}
Heptanal	0.086	0.105	0.049	0.051	0.035
Octanal	0.038 ^b	0.048 ^b	0.060 ^b	0.046 ^b	0.291 ^a
Nonanal	0.357	0.094	0.118	0.061	0.298
Decanal	0.148 ^b	0.180 ^b	0.064 ^b	0.259 ^b	0.561 ^a
3-Methyl butanal	0.261 ^b	3.579 ^a	0.528 ^b	0.047 ^b	0.027 ^b
DMS	0.071 ^{bc}	0.244 ^a	0.242 ^a	0.173 ^{ab}	0.014 ^c
DMDS	0.255 ^a	0.038 ^{bc}	0.034 ^{bc}	0.081 ^b	0.015 ^c
Methional	0.015 ^a	0.004 ^{bc}	0.001 ^c	0.002 ^c	0.008 ^b
Diacetyl	2.172 ^a	1.692 ^{ab}	0.259 ^b	0.072 ^b	0.261 ^b
Acetaldehyde	1.285 ^c	2.160 ^{bc}	2.449 ^{bc}	2.449 ^{bc}	4.164 ^a
Acetic acid	0.555 ^b	0.202 ^b	0.004 ^b	0.639 ^b	5.588 ^a
1-Octen-3-one	0.007 ^c	0.011 ^{bc}	0.029 ^{abc}	0.050 ^{ab}	0.058 ^a
2-Methyl-3-furanthiol	0.002 ^{ab}	0.002 ^a	0.001 ^c	0.001 ^{bc}	0.001 ^{abc}
4-Octanone	0.060	0.054	0.056	0.079	ND
Benzene-acetaldehyde	1.290 ^a	0.046 ^b	0.020 ^b	0.081 ^b	0.180 ^b
2-Pentanone	1.453 ^a	0.704 ^{ab}	0.590 ^{ab}	0.773 ^{ab}	0.006 ^b
E,Z-2,6-nonadienal	0.011 ^{bc}	0.029 ^a	0.042 ^a	0.015 ^b	0.003 ^c
Benzaldehyde	1.067 ^a	0.097 ^b	0.009 ^b	0.129 ^b	0.070 ^b

^{a-c}Means within a column that do not share a common letter are significantly different ($P < 0.05$).

¹DMS = dimethyl sulfide; DMDS = dimethyl disulfide. ND = not detected.

processing time to remove the lactose, which likely caused the increased aldehyde load. Cheddar, milk, Mozzarella, and cottage permeates had lower levels of aldehydes, consistent with lower levels of cardboard off flavors. Cottage permeate contained higher levels of methional as compared with other permeates ($P < 0.05$), consistent with the low but distinct potato flavor of this permeate (Carunchia Whetstone et al., 2005; Liaw et al., 2011).

Trained panel profiling was performed to characterize the flavor and basic taste profiles of the cream of broccoli soups made with the addition of each of the permeate varieties and a salt control and a no-salt control (Table 4). Soups made with permeate had an approximately 60% decrease in sodium content. This varied slightly between samples based on sodium content of the permeates. Sensory results showed that each soup had a distinct flavor profile determined by the permeate added. All soups had similar aroma intensities and equal levels of vegetable or brothy and milky or creamy flavors ($P > 0.05$); therefore, the differences noted in the soups were attributed to differences in their basic taste profiles. The sweet taste of the Cheddar, Mozzarella, and milk permeate soups was increased due to the addition of these permeates which contain high levels of lactose. The sour taste was much higher in the cottage permeate soup than the other permeate soups ($P < 0.05$), which was also expected because of the sour taste of this permeate which carried over into the soup. The sour taste of the Cheddar, Mozzarella, milk, and DLC permeate soups was lower than the no-salt control

($P < 0.05$). This was likely due to the sweet taste of these permeates, suppressing the natural, slightly sour taste of the soup (Pangborn, 1959; Frankowski et al., 2014). The umami taste was higher in the Cheddar, Mozzarella, cottage, and milk permeate soups than the 2 control and DLC soups ($P < 0.05$). The salt control was the saltiest of all of the soups, followed by the cottage and DLC permeates, though all of the permeate soups were saltier than the no-salt control ($P < 0.05$). A low but distinct metallic or bitter taste in the cottage and DLC soups was also noted, which carried over from the permeates.

Consumer acceptance testing was conducted with 105 consumers on the cream of broccoli soups. A salt control and a no-salt control were also evaluated with the permeate soups to serve as baselines for comparison. Not surprisingly, consumers scored the salt control the highest for overall liking, flavor liking, thickness liking, and salty taste liking ($P < 0.05$; Table 5). This soup was likely the most similar to cream of broccoli soup consumers had previously tasted, and may be why consumers liked it the most. The higher thickness liking of this soup is likely a result of the increased overall liking of the soup (halo effect) rather than an actual difference in thickness as the formulas were the same between the salt and the no-salt control with the exception of the added salt. The Cheddar, Mozzarella, and milk permeate soups scored higher than the no-salt control, which scored at parity with DLC, and were liked more than the cottage permeate soup. Consumers also scored the cottage permeate soup lowest in salty taste liking. The

Figure 4. Principal component analysis biplot of volatile analysis of whey and milk permeates. Milk (milk permeate), Mozz (Mozzarella permeate), Cheddar (Cheddar permeate), Cottage (cottage permeate) and DLC (delactosed whey permeate). Color version available online.

low scores given to the cottage permeate soup were likely due to the overall negative perception that consumers had for this soup rather than an actual lack of salty taste. Trained panel results documented that this soup was saltier than the other permeate soups, which also scored higher than the cottage permeate soup in salty taste liking. Studies have shown that disconfirmed consumer expectations for products negatively affect

consumer perceptions of that product (Cardello et al., 1985; Cardello and Sawyer, 1992). Our hypothesis was that because the cottage permeate soup was sour and consumers did not expect that a creamy soup would be sour, they did not like the soup. Because the cottage whey permeate was saltier (by trained panel) than other sweet whey and milk permeates, another more congruent soup flavor with the accompanying sour taste

Table 4. Descriptive analysis results of cream of broccoli soups made with and without permeate addition¹

Sample	Aromatic intensity	Vegetable complex	Milky/creamy	Sweet	Sour	Salty	Umami
Salt control	2.1 ^a	2.0 ^a	3.0 ^a	2.0 ^b	1.2 ^{bc}	6.1 ^a	2.3 ^b
No-salt control	2.2 ^a	2.0 ^a	3.2 ^a	2.0 ^b	1.3 ^b	3.0 ^d	1.5 ^c
Cottage	2.2 ^a	2.2 ^a	2.7 ^a	2.0 ^b	3.0 ^a	4.6 ^b	3.5 ^a
Cheddar	2.0 ^a	2.2 ^a	3.1 ^a	3.5 ^a	0.8 ^c	4.0 ^c	3.3 ^a
Milk	2.0 ^a	2.0 ^a	3.0 ^a	3.6 ^a	0.8 ^c	3.7 ^c	3.2 ^{ab}
Mozzarella	2.1 ^a	2.3 ^a	3.3 ^a	3.0 ^{ab}	1.0 ^{bc}	4.0 ^c	3.5 ^a
Delactosed permeates	2.0 ^a	2.2 ^a	3.0 ^a	2.0 ^b	0.5 ^c	4.3 ^{bc}	1.5 ^c

^{a-d}Means within a column that do not share a common letter are significantly different ($P < 0.05$).

¹Attributes were scored using a 0 to 15 point universal intensity scale (Meilgaard et al., 2007).

Table 5. Consumer-liking scores for cream of broccoli soups with and without added salt or permeate¹

Attribute	Salt	No salt	Cottage	Cheddar	Milk	Mozzarella	Delactosed permeates
Appearance liking	5.7 ^{ab}	5.7 ^{ab}	6.0 ^a	5.5 ^{ab}	5.4 ^b	5.8 ^{ab}	5.8 ^a
Overall liking	6.1 ^a	5.3 ^c	4.2 ^d	5.6 ^b	5.5 ^b	5.6 ^b	5.4 ^{bc}
Flavor liking	6.4 ^a	5.3 ^c	4.1 ^d	5.6 ^b	5.6 ^b	5.8 ^b	5.5 ^{bc}
Thickness liking	5.0 ^a	4.2 ^b	4.2 ^b	4.3 ^b	4.1 ^b	4.3 ^b	4.2 ^b
Salty taste liking	5.8 ^a	5.0 ^{bc}	4.6 ^c	5.4 ^{ab}	5.3 ^b	5.1 ^b	5.1 ^b

^{a-d}Means within a row that do not share a common letter are significantly different ($P < 0.05$).

¹Data represent 105 consumers. Liking attributes are scored on a 9-point hedonic scale, where 1 = dislike extremely and 9 = like extremely.

(e.g., cheese or broccoli cheese) may be more acceptable. The sweet taste of the Cheddar, Mozzarella, and milk permeate soups identified by trained panelists also likely had an effect on the overall liking of these soups. Panelists likely did not expect that level of sweetness in these soups and this may have decreased the overall liking of these soups.

Consumers also evaluated the soups for salty taste, broccoli flavor, thickness, and milky or creamy flavor on a 5-point JAR scale, which indicated whether these attributes were too high, JAR, or too low for each soup (Table 6). The JAR scores indicated that some consumers considered the salt control and cottage permeate soups to be too salty (33.3 and 25.7%, respectively), but a larger portion of consumers considered the cottage permeate to be not salty enough (41.0%; $P < 0.05$). This indicates that consumers may have been confused about the salty taste of the cottage permeate soup due to the masking of the other flavors or tastes (e.g., sour taste) of the soup and were unsure of how to score it. A higher number of panelists scored the no-salt control soup as being not salty enough than the Cheddar, Mozzarella, milk, and cottage permeate soups (57.1 vs. 41.0, 45.7, 42.9, and 41.0%, respectively), further

confirming that consumers perceived saltiness in these permeate soups was higher than in the no-salt control ($P < 0.05$). The percentage of consumers that scored the Cheddar, Mozzarella, and milk permeate soups as JAR in salty taste was not different from the salt control ($P > 0.05$), further evidence that the addition of permeate increased the acceptability of the salty taste of this product application. Consumers also evaluated the broccoli flavor of the soups using the same scale. The results showed that fewer panelists considered the broccoli flavor to be too weak for the Cheddar, Mozzarella, and milk permeate soups than the no-salt control. All soups were at parity with one another for the thickness JAR question, indicating that all samples were consistent in perceived thickness ($P > 0.05$). A higher number of consumers scored the cottage, DLC, and, to a lesser extent, no-salt control samples as being too weak for milky or creamy flavor compared with the Cheddar, milk, Mozzarella, and salt control samples ($P < 0.05$). This result may be due to the lack of sweet taste in these soups, especially in the cottage and DLC varieties, which consumers may also perceive as lack of milky or creamy flavor or may simply be a sensory horns effect (product not liked so other attributes are

Table 6. Just about right (JAR) scores for cream of broccoli soups with and without added salt or permeate¹

Attribute	Level (%)	Salt	No Salt	Cottage	Cheddar	Milk	Mozzarella	Delactosed permeates
Salty taste	Not salty enough	8 ^c	57 ^a	41 ^b	41 ^b	43 ^b	46 ^b	56 ^a
	JAR	59 ^a	41 ^{bc}	33 ^c	51 ^{ab}	49 ^{ab}	51 ^{ab}	39 ^{bc}
	Too salty	33 ^a	2 ^c	26 ^a	9 ^b	9 ^b	4 ^{bc}	5 ^{bc}
Broccoli flavor	Too weak	28 ^c	51 ^a	42 ^{ab}	31 ^{bc}	31 ^{bc}	36 ^{bc}	39 ^{abc}
	JAR	70 ^a	46 ^{cd}	33 ^d	59 ^{abc}	60 ^{ab}	52 ^{bc}	55 ^{bc}
	Too strong	3 ^c	3 ^c	25 ^a	11 ^b	9 ^{bc}	11 ^b	7 ^{bc}
Thickness	Too thin	69 ^a	73 ^a	75 ^a	71 ^a	77 ^a	74 ^a	71 ^a
	JAR	31 ^a	27 ^a	24 ^a	29 ^a	23 ^a	26 ^a	26 ^a
	Too thick	1 ^a	0	1 ^a	0	0	0	3 ^a
Milky/creamy flavor	Too weak	24 ^c	38 ^{ab}	51 ^a	32 ^{bc}	30 ^{bc}	34 ^{bc}	50 ^a
	JAR	68 ^a	51 ^b	21 ^c	54 ^b	54 ^b	53 ^b	43 ^{bc}
	Too strong	9 ^b	11 ^{ab}	18 ^a	13 ^{ab}	15 ^{ab}	12 ^{ab}	7 ^b

^{a-d}Means within a row that do not share a common letter are significantly different ($P < 0.05$).

¹Data represent 105 consumers. JAR scales are scored on a 5-point scale where too little = 1 or 2, just about right = 3 and too much = 4 or 5. Percentage of consumers that selected these options is presented.

also not liked) from consumers not liking these soups as much as the control.

CONCLUSIONS

Permeates from different sources were viable options for use as salt substitutes. All permeates were salty, but the cottage permeate was the saltiest of the non-reduced lactose permeates due to its higher lactic acid and lower lactose concentrations as compared with the other permeates. The cottage permeate also elicited sour taste and potato/brothy flavor. Milk, Mozzarella, and Cheddar permeates elicited sweet taste and cooked milky flavor. The DLC permeate elicited the highest levels of salty taste due to the high proportions of minerals and organic acids caused by the removal of lactose. The DLC permeate also elicited the highest cardboard flavor of the permeates, attributed to the high aldehyde content in this permeate. As expected, permeates contributed specific flavors and tastes into the soups depending on the characteristics of the permeates; therefore, special consideration must be taken when choosing the permeate source for a particular application. The milk, Mozzarella, and Cheddar permeates contributed a milky flavor and sweet and salty tastes to the soup. The cottage permeate contributed the highest level of salty taste of the nonreduced lactose permeates by trained panel results; however, this permeate also contributed a sour taste, which consumers disliked with this soup and consumers did not report increased salty taste, possibly due to its unexpected (and disliked) sour taste. This permeate would work well in a product application where sour taste is also desired or expected. Consumers preferred the salt control soup over the permeate soups; however, the addition of Cheddar, Mozzarella, and milk permeates increased the overall liking and salty taste scores over the no-salt control and provided a significant decrease in the sodium content of the soups over the salt control. Because of the high salty taste profiles of the cottage and DLC permeates, they are best suited for use as salt substitutes in product applications. Future work on these permeates should focus on optimizing processing parameters to reduce or minimize cardboard flavor and perhaps reduce sour taste of cottage permeate or to identify ideal applications for flavoring systems to enhance liking of reduced-sodium permeate applications.

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