Short communication: The effect of liquid storage on the flavor of whey protein concentrate¹

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ABSTRACT

Unit operations in dried dairy ingredient manufacture significantly influence sensory properties and, consequently, their use and consumer acceptance in a variety of ingredient applications. In whey protein concentrate (WPC) manufacture, liquid can be stored as whey or WPC before spray drying. The objective of this study was to determine the effect of storage, composition, and bleaching on the flavor of spray-dried WPC80. Liquid whey was manufactured and subjected to the following treatments: bleached or unbleached and liquid whey or liquid WPC storage. The experiment was replicated 3 times and included a no-storage control. All liquid storage was performed at 4°C for 24 h. Flavor of the final spray-dried WPC80 was evaluated by a trained panel and volatile compound analyses. Storage of liquids increased cardboard flavor, decreased sweet aromatic flavor, and resulted in increased volatile lipid oxidation products. Bleaching altered the effect of liquid storage. Storage of unbleached liquid whey decreased sweet aromatic flavor and increased cardboard flavor and volatile lipid oxidation products compared with liquid WPC80 and no storage. In contrast, storage of bleached liquid WPC decreased sweet aromatic flavor and increased cardboard flavor and associated volatile lipid oxidation products compared with bleached liquid whey or no storage. These results confirm that liquid storage increases off-flavors in spray-dried protein but to a variable degree, depending on whether bleaching has been applied. If liquid storage is necessary, bleached WPC80 should be stored as liquid whey and unbleached WPC80 should be stored as liquid WPC to mitigate off-flavors.

Key words: whey protein, flavor, bleaching, storage

Short Communication

Due to increased consumer demand for foods with greater protein content, whey proteins have become much more than a by-product of cheese manufacture. In 2014, over 244,000 t of whey protein concentrate (34–89% protein; **WPC**) was produced in the United States, an 8.1% increase from 2013 (USDA, 2015). Whey proteins are commonly used because of their unique nutritional and functional properties. The successful use of whey protein ingredients requires that they do not negatively affect the flavor of their ingredient applications. Due to delicate flavors in whey protein ingredients, off-flavors can become prominent and negatively affect consumer acceptance of food applications (Childs et al., 2007; Wright et al., 2009; Evans et al., 2010; Oltman et al., 2015).

Many of the off-flavors in WPC arise from the various unit operations applied to fluid whey to concentrate proteins. In the United States, annatto (norbixin) is an approved color additive in Cheddar cheese and its usage is consistent with good manufacturing practice (US FDA, 2015). The majority of the colorant is retained in the cheese but approximately 10% of annatto remains in the whey (Smith et al., 2014). This colorant is then concentrated along with the protein during manufacture of WPC or whey protein isolate (**WPI**) and must be removed by bleaching. Unit operations such as cheese manufacture, bleaching, spray drying, agglomeration, and instantization have all been demonstrated to influence the flavor of WPC or WPI (Wright et al., 2009; Campbell et al., 2011; White et al., 2013; Park et al., 2014a,b; Smith et al., 2015).

During the manufacture of WPC or WPI, liquid product may be stored for extended periods. This can be due to the need to ship product from one facility to another or to spray-drying capacities not meeting production capacities. Whitson et al. (2011) investigated how storage of liquid WPI before spray drying affected the flavor of WPI and WPC80. They observed that lipid oxidation products and off-flavors increased in both spray-dried Mozzarella WPC80 and Cheddar

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Figure 1. Diagram of experimental design. All samples were spray dried under identical conditions. This experiment was replicated 3 times. DF = diafiltration; WPC80 = whey protein concentrate with 80% protein.

WPI with increased storage of liquid protein product (liquid WPC80 or WPI). Liaw et al. (2010) demonstrated that lipid oxidation increased with storage of fluid whey but did not investigate whether or how fluid whey storage affected the flavor of the resulting WPC. To our knowledge, the role of bleaching and fluid composition during storage on the flavor of WPC80 has not been investigated. The objective of this study was to determine the effect of bleaching and fluid composition across liquid storage on the flavor of spray-dried WPC80.

Approximately 235 kg of raw whole milk was obtained from the North Carolina State University Dairy Education Unit (Raleigh). The milk was pasteurized at 72°C for 16 s with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC). Liquid Cheddar whey was manufactured as described by Park et al. (2014b). The whey was defatted with a centrifugal separator (model SI600E, Agri-Lac, Miami, FL) and pasteurized at 72°C for 15 s. The following treatments were then applied to manufacture of spray-dried WPC80: hydrogen peroxide bleach or no bleach and 24-h fluid whey storage or no storage. Half of the pasteurized whey was bleached with 250 mg/kg hydrogen peroxide (35% wt/vol, VWR International, Westchester, PA) for 1 h at 50°C, after which 20 mg/kg catalase was added (FoodPro CAT, Danisco, New Century, NJ). The other half of the whey was held at

50°C for 1 h before UF. Then, bleached or unbleached whey was concentrated and spray dried or concentrated and held for 24 h before spray drying. Six treatments (WPC80) were manufactured; a summary of the experimental design is shown in Figure 1.

Wheys (47 kg each) were concentrated to WPC80 by UF/diafiltration (DF) with a UF system containing 10 polyethersulfone membrane cartridges (model P2B010V05, nominal cutoff = 10 kDa, surface area = 0.5 m², Millipore Inc., Billerica, MA) and a variable-speed peristaltic pump (model P2B010V05, Cole Parmer, Vernon Hills, IL), which was used to circulate the product in a batch process. Before UF, the membrane cartridges were cleaned as described by Park et al. (2014a). A concentration factor of 3× was achieved and then DF water was added to equal the original weight followed by concentration to a solids content of 15% (wt/wt) (12% protein, wt/wt) to produce liquid WPC80. Total solids were measured by a rapid moisture analyzer (Smart Trac II, CEM Corp., Matthews, NC) and protein was measured with a Sprint Rapid Protein Analyzer (CEM Corp.). Total time for each batch of UF/DF was approximately 1 h.

The liquid WPC80 produced from the no-storage controls and liquid whey storage treatments were spray dried immediately, whereas the liquid WPC80 storage treatments were spray dried after 24 h at 4°C (Figure 1). Spray drying was performed with a pilot-scale spray

dryer (model Lab 1, Anhydro Inc., Soeborg, Denmark) with an inlet temperature of 200°C and an outlet temperature of 90°C. The WPC80 powders were stored in Mylar bags (TF-4000, Impak Corp., Central City, SD) at -80°C until analyses were performed. The entire experiment was replicated 3 times with 3 different lots of milk.

Proximate analysis was performed on the WPC80 powders. Percent moisture was measured by vacuum oven drying (AOAC International, 2000; method 990.20; 33.2.44), fat content was measured by Mojonnier ether extraction (AOAC International, 2000; method 932.06; 33.5.08), and protein was measured using the Kjeldahl method (AOAC International, 2000; method 991.20; 33.2.11) with a nitrogen conversion factor of 6.38.

Descriptive sensory analysis was performed in compliance with the North Carolina State University Institutional Review Board for Human Subjects guidelines. The WPC80 powders were rehydrated to 10% solids (wt/vol) in deionized water. The solutions were dispensed into 3-digit-coded 60-mL soufflé cups (Solo Cup, Highland Park, IL), lidded, and tempered to 21°C. Flavor attributes were evaluated by a trained panel ($n = 8$) using an established dried dairy ingredient lexicon and scored using a 0 to 15 Spectrum intensity scale (Wright et al., 2009). Panelists were between the ages of 22 and 47 yr with >150 h of experience of descriptive analysis of dried dairy ingredients. Each sample was evaluated in duplicate by each panelist, and data were collected using Compusense Five, version 5.6 (Compusense, Guelph, ON, Canada).

Extraction of volatile compounds was performed with headspace-solid phase microextraction-GC-MS (HS-SPME-GC-MS) as described by Park et al. (2014a). The WPC80 powders were rehydrated to 10% solids (wt/vol) in HPLC-grade water along with 10% (wt/vol) sodium chloride (VWR International) and 10 μ L of internal standard solution (2-methyl-3-heptanone in water, 81 mg/kg; Sigma-Aldrich, St. Louis, MO). Five milliliters of WPC80 solution was added to 20-mL amber autosampler vials and sealed with a Teflon-sided silicone septum with screw cap (MicroLiter Analytical Supplies Inc., Suwannee, FL). A Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) was used to inject samples in an Agilent 7820A gas chromatograph with 5975 inert mass selective detector (Agilent Technologies Inc., Santa Clara, CA) and a ZB-5 MS column (30 m $\times$ 0.25 mm i.d. $\times$ 0.25 μ m; Phenomenex, Torrance, CA). The SPME fiber (1-cm divinylbenzene/carboxen/polydimethylsiloxane; Supelco, Bellefonte, PA) was exposed to each sample as described by Park et al. (2014a). The GC oven conditions were also as described by Park et al. (2014a). Compounds were identified using the NIST 2014 library of spectra and comparison of

spectra and retention time under identical conditions (NIST, 2014). Compounds of interest were quantified in selective ion monitoring mode by relative abundance using the internal standard.

Statistical analysis was performed with XL STAT 2015.1 (Addinsoft, New York, NY). Sensory and volatile data were analyzed by 2-way ANOVA (bleach $\times$ storage composition) with Fisher's least significant difference (LSD) test to determine differences among sample means.

The percentage moisture, fat, and protein in the WPC80 powders were $3.83 \pm 0.76\%$, $4.12 \pm 0.54\%$, and $81.5 \pm 0.89\%$, respectively, and no significant differences between treatments were observed ($P > 0.05$). The trained panel profiles are shown in Table 1. Interaction effects between bleaching and storage composition were significant ($P < 0.05$). Consistent with previous research (Croissant et al., 2009; Kang et al., 2012; Qiu et al., 2015), bleaching increased overall aroma intensity and cardboard and cabbage flavors, and decreased sweet aromatic flavor ($P < 0.05$). The effect of liquid composition during storage on the flavor of WPC80 was different between bleached and unbleached treatments. For unbleached treatments, storage of liquid whey increased cardboard and decreased sweet aromatic flavors compared with the no-storage control ($P < 0.05$), whereas liquid WPC80 storage did not differ in flavor from the no-storage control ($P > 0.05$). In contrast to the unbleached treatments, storage of liquid whey was not different in cardboard flavor intensity than the no-storage control ($P > 0.05$). Storage of bleached liquid WPC80 increased cardboard flavor compared with the no-storage control and the liquid whey storage treatments ($P < 0.05$).

Volatile compound results were consistent with sensory analysis (Table 2, Figure 2). Interaction effects were significant between bleaching and storage composition for all volatile compounds ($P < 0.05$). In agreement with the descriptive sensory analysis results, in general, volatile lipid oxidation products were increased in the bleached WPC80 treatments. In unbleached WPC80, storage of liquid whey increased lipid oxidation observed by increases in pentanal, hexanal, heptanal, octanal, nonanal, decanal, 3-methyl butanal, 1-octen-3-one, and 2-pentyl furan concentrations compared with the no-storage control and liquid WPC80 storage treatments ($P < 0.05$). In bleached WPC80, storage of liquid product increased hexanal, nonanal, 2-methyl butanal, dimethyl trisulfide (**DMTS**), 1-octen-3-one, and 2-pentyl furan concentrations compared with the no-storage control ($P < 0.05$). Storage of bleached liquid whey increased heptanal and dimethyl disulfide concentrations compared with the no-storage control and increased octanal concentration compared with

Table 1. Trained panel profiles of rehydrated whey protein concentrate (WPC)80 powders

Treatment	Aroma intensity	Sweet aromatic	Cardboard	Cabbage
Unbleached control (no storage)	2.5 ^{ab}	1.9 ^a	0.5 ^d	ND ¹
Unbleached liquid whey storage	2.1 ^b	1.0 ^b	1.2 ^c	ND
Unbleached liquid WPC80 storage	2.3 ^{ab}	1.4 ^{ab}	0.9 ^{cd}	ND
Bleached control (no storage)	2.8 ^a	ND	2.3 ^b	1.1 ^a
Bleached liquid whey storage	2.8 ^a	ND	2.2 ^b	1.2 ^a
Bleached liquid WPC80 storage	2.6 ^a	ND	2.9 ^a	1.1 ^a

^{a-d}Means in the same column followed by a different letter indicate significant differences ($P < 0.05$).

¹Not detected.

both no storage and liquid WPC80 storage ($P < 0.05$). Storage of liquid WPC80 increased pentanal, decanal, and DMTS concentrations compared with no storage and liquid whey storage ($P < 0.05$).

It is important to understand how unit operations affect cardboard flavor and lipid oxidation because they are the main contributors to decreased quality and shelf life (Wright et al., 2009; Whitson et al., 2010, 2011). In bleached WPC80, we observed increases of pentanal and DMTS, which have been demonstrated to contribute to cardboard flavor in whey protein ingredients (Whitson et al., 2010). Similar to our results, Whitson et al. (2011) observed an increase in cardboard flavor and lipid oxidation in Mozzarella WPC80 with increased liquid WPC80 storage but to a greater extent in liquid Cheddar WPI. This could be due to the chemical bleaching step that was applied to Cheddar whey to remove residual annatto colorant. The common bleaching agents hydrogen peroxide and benzoyl peroxide increase lipid oxidation in whey protein ingredients (Croissant et al., 2009; Smith et al., 2015). In our experiment, we observed similar results, where storage of bleached liquid WPC80 increased cardboard flavor and lipid oxidation in the spray-dried WPC80 except in WPC80 from stored unbleached liquid WPC80, where cardboard flavor and lipid oxidation did not increase

compared with the no-storage control. Bleached liquid WPC80 was higher in cardboard flavor than its no-storage control, whereas the unbleached liquid WPC80 was not different from its respective control. Another source of difference in flavor stability between liquid Mozzarella and Cheddar whey protein products is the starter culture. Campbell et al. (2011) observed that mesophilic starter cultures used in Cheddar cheese manufacture increased lipid oxidation in liquid whey compared with thermophilic starter culture used in Mozzarella cheese manufacture.

Whether or not the liquid whey was bleached had a large effect on the effect of storage composition. In unbleached WPC80, liquid whey storage increased lipid oxidation and cardboard flavor more than liquid WPC80 storage. This could be due to compositional differences in pro-oxidant minerals between liquid whey and liquid WPC80. The endogenous pro-oxidant transition metals copper and iron are known to significantly increase lipid oxidation in milk fat (O'Connor and O'Brien, 2006). The copper and iron contents in liquid sweet whey have been reported as 3.5 and 89 $\mu\text{g}/100\text{ g}$, respectively (Wong et al., 1978). Wong et al. (1978) reported that concentrations of copper and iron in fluid sweet whey increased with increased volume reduction with UF membranes. There may be 2 reasons they did

Table 2. Relative abundance ($\mu\text{g}/\text{kg}$) of selected volatile compounds in rehydrated whey protein concentrate (WPC)80

Volatile compound	Bleached control	Bleached liquid whey	Bleached liquid WPC80	Unbleached control	Unbleached liquid whey	Unbleached liquid WPC80
Pentanal	1.17 ^c	1.55 ^{bc}	2.33 ^a	0.606 ^d	1.81 ^b	0.504 ^d
Hexanal	54.7 ^b	73.2 ^a	76.2 ^a	14.3 ^c	44.7 ^b	20.3 ^c
Heptanal	2.49 ^{bc}	3.22 ^a	2.69 ^{ab}	0.719 ^d	1.85 ^c	0.636 ^d
Octanal	0.513 ^{bc}	0.877 ^a	0.586 ^b	0.143 ^d	0.400 ^c	0.069 ^d
Nonanal	2.95 ^c	4.19 ^a	3.69 ^{ab}	2.25 ^d	3.26 ^{bc}	1.23 ^c
Decanal	0.041 ^{cd}	0.052 ^c	0.090 ^b	0.037 ^{cd}	0.144 ^a	0.015 ^d
2-Methyl butanal	0.002 ^c	0.018 ^b	0.033 ^a	0.002 ^c	0.002 ^c	0.010 ^{bc}
3-Methyl butanal	0.211 ^b	0.308 ^b	0.220 ^b	0.286 ^b	0.555 ^a	0.186 ^b
Dimethyl disulfide	0.172 ^{bc}	0.379 ^a	0.296 ^{ab}	ND ¹	0.123 ^c	0.029 ^c
Dimethyl trisulfide	0.017 ^c	0.024 ^b	0.035 ^a	0.002 ^d	0.005 ^d	0.002 ^d
1-Octen-3-one	0.077 ^{cd}	0.170 ^b	0.159 ^b	0.070 ^d	0.271 ^a	0.104 ^c
2-Pentyl furan	1.73 ^d	8.16 ^b	3.42 ^c	1.91 ^d	10.5 ^a	2.85 ^c

^{a-e}Means in the same column followed by a different letter indicate significant differences ($P < 0.05$).

¹Not detected.

not see a decrease in iron and copper concentrations. First, they did not use DF to produce WPC80, which would have increased the removal of soluble minerals. Second, with increasing protein concentrations, the trace minerals that are bound to protein would also be concentrated. Thus, the pro-oxidant trace minerals would mainly be bound to protein and therefore less able to catalyze lipid oxidation (Sugiarto et al., 2010; Ueno et al., 2012). In the current study, DF was applied and, therefore, unbound minerals would be removed in the permeate. As such, liquid WPC80 would have decreased concentrations of unbound trace minerals compared with fluid whey. Unbleached liquid whey storage could also have increased cardboard flavor and lipid oxidation more than liquid WPC80 storage because the higher protein content in liquid WPC80 is an effective antioxidant. Tong et al. (2000) reported that the antioxidant activity of whey proteins increased with concentration. This was attributed to their sulfhydryl groups and their ability to chelate iron and scavenge free radicals.

In bleached WPC80, lipid oxidation and cardboard flavor increased with liquid WPC80 storage compared with liquid whey storage and the no-storage control. This could be due to the increased lipid concentration in

the fluid during storage. The reason that this difference was not observed in unbleached product could be that the antioxidant activity of the system in the bleached product was not high enough to slow lipid oxidation due to free radicals formed during bleaching. Fox et al. (2013) observed increased lipid oxidation products when bleaching was performed on liquid WPC80 compared with liquid whey, suggesting decreased oxidative stability of liquid WPC80. Bleaching by both hydrogen peroxide and benzoyl peroxide generates free radicals, which greatly accelerate lipid oxidation (Jervis et al., 2012; Jervis and Drake, 2013). The lipid concentration is roughly 10 times higher in liquid WPC80 than in fluid whey. The increased lipid concentration combined with the free radicals generated from bleaching could explain why spray-dried bleached WPC80 that included liquid WPC80 storage had increased lipid oxidation and cardboard flavors, whereas this was not observed in unbleached WPC80.

These results confirm the finding, from previous studies, that to mitigate off-flavors, WPC manufacturers should avoid liquid storage if possible. However, based on our results, if liquid storage is necessary, specific steps can be taken to mitigate off-flavors. An understanding of fluid composition and pretreatment

Figure 2. Principal component analysis (PCA) biplot of volatile compound relative abundance ($\mu\text{g/kg}$) in rehydrated whey protein concentrate (WPC)80 treatments. DMS = dimethyl disulfide; DMTS = dimethyl trisulfide; B = bleached, U = unbleached, Con = control. Color version available online.

during storage is critical in reducing lipid oxidation and off-flavor development in WPC. If liquid storage is necessary, manufacturers of bleached and unbleached product should store liquid whey and liquid WPC80, respectively.

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