Short Communication: Utilization of Sheep's Milk Cheese Whey in the Manufacture of an Alkylphenol Flavor Concentrate

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ABSTRACT

The recovery of species-related conjugated sheep-like flavored alkylphenols from Manchego-type cheese whey by ultrafiltration was investigated. Concentrations of conjugated alkylphenols were similar in the various fractions of whey permeate collected during ultrafiltration, and this was interpreted as a reflection of their high water solubility. About 49 and 62% of conjugated 3- and 4-ethylphenols and p- and m-cresols in sheep's milk cheese whey, respectively, were recovered in the permeate after ultrafiltration with a volume concentration factor of 5.4. Cheese whey retentate correspondingly contained 38 and 28% of conjugated 3- and 4-ethylphenols and p- and m-cresols from the original whey, respectively. Permeate fractions from sheep's milk cheese whey were combined, concentrated by vacuum evaporation, and lactose was partially removed by crystallization and filtration to obtain an aqueous sheep-like flavor precursor concentrate.

(**Key words:** flavor concentrate, alkylphenol, cheese whey)

Abbreviation key: UF concentrate = conjugated alkylphenol concentrate.

Whey from cheese manufacture is a by-product that is available to the food and pharmaceutical industries. Cheese whey is used by separating it into its components, including protein and lactose (Mann, 1984a, b). Acid and sweet whey solids are used as food or feed ingredients for their functional and nutritional properties (Carić, 1994). Ultrafiltration is commonly used to fractionate whey and whey protein concentrates of varying percentages of protein for use in bakery, dairy, and meat products, beverages, infant formula; many other products including lactose are usual products from the processing (Morr, 1992). Permeate fraction from the ultrafiltration of whey is of somewhat limited value due to its low solids content (6%), which is com-

prised mainly of lactose. As a result, whey permeate is utilized for production of lactose, animal feed, and variety of fermentation processes (Coton, 1980; Keller, 1990).

Current whey utilization industries are based on whey from cows' milk cheese manufacture. However, wheys from ewes' and goats' milk cheeses are not widely used because of differences in composition and flavor, inconsistent supply, and insufficient information about their functional properties compared to whey from cows' milk cheeses (Casper et al., 1998). Therefore, wheys from ewes' and goats' milk cheeses are often landspread, which results in an economic loss for the producers and creates a pollution problem for the environment (Marwaha and Kennedy, 1988; Casper et al., 1998).

Species-related alkylphenols are important flavor compounds in dairy products that are largely present as conjugates in ruminant milks (Lopez and Lindsay, 1993). Because the polar conjugated alkylphenols partition readily into the aqueous phase of milk (Lopez and Lindsay, 1993; Zeng, 1997), they would be expected to be associated favorably with the whey resulting from cheese making. Therefore, whey represents a source for these alkylphenol conjugates as flavor precursor compounds. Because the proteinaceous components of whey are largely excluded from whey permeate by ultrafiltration of whey, whey permeate provides an especially attractive raw material for alkylphenol conjugates. The objective of this study was to investigate sheep's milk cheese whey for the preparation of sheep-like flavored alkylphenol flavor concentrates for use in dairy products.

Ultrafiltration was carried out using a spiral-wound polysulfone membrane with a 10,000 molecular weight cut off (PTI Advanced Filtration, Inc., San Diego, CA) in a benchtop ultrafiltration unit (Triclover Unit, Inc., Kenosha, WI) with 10-L capacity. Whey from experimental Manchego-type cheese manufactured from sheep's milk was ultrafiltered by recycling of the retentate to the feed container to achieve concentration. Ultrafiltration was carried out using a 6.5 L-batch until a volume concentration factor of 5.4 was achieved. Five fractions (1 L each) from the permeate stream were collected during ultrafiltration. Retentate remaining in

Received August 22, 2004.

Accepted September 16, 2004.

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Table 1. Concentrations of conjugated alkylphenols in permeate during batch ultrafiltration of sheep's milk Manchego-type cheese whey.

Sample	Concentration ¹	
	3- and 4-ethylphenols ²	p- and m-cresols ²
	(ng/mL)	
Whey	62	727
Permeate fractions ³		
1	24	408
2	38	571
3	39	551
4	43	610
5	45	627
Retentate ⁴	129	1097
Concentrate ⁵	219	2857

¹Average of duplicate samples, CV: $\pm 10\%$.²Compounds coeluted.³Fractions of permeate taken during ultrafiltration while the retentate was recycled.⁴Retentate obtained at a volume concentration factor of 5.4.⁵Permeate fractions were combined and concentrated by vacuum evaporation; lactose was removed by filtration.

the unit was collected after the addition of 1 L of distilled water to the membrane to increase the recovery. Additionally, permeate fractions (400 mL each) from the ultrafiltration of the Manchego-type cheese whey were combined for preparation of a conjugated alkylphenol concentrate (**UF concentrate**). Combined permeate fractions with a total volume of 2 L were concentrated in batches in 2-L round bottom flasks at 40°C using a rotary vacuum evaporator (Harper, 1992) until a volume of 200 mL remained. This UF concentrate was stirred with a magnetic stirring bar for 2 h, and then held at 4°C for 12 h for lactose crystallization to occur. Lactose crystals were removed by vacuum filtration using Whatman No. 1 filter paper. The UF concentrate was then filtered through 2 membrane filters (Nalgene 150-mL analytical filter unit, Fisher Scientific, Itasca, IL), one with a pore size of 0.45 μm to remove bulk microorganisms, and another with a pore size of 0.2 μm to sterilize the solution (Brock, 1983). The UF concentrate was held at -23°C in a freezer until used.

Concentrations of conjugated alkylphenols in the whey, permeate, retentate, and UF concentrate were measured (Kilic, 1999). Before the analyses, the whey, retentate, and UF concentrate samples were diluted with double-distilled water to achieve alkylphenol conjugate concentrations similar to those in the permeate.

The concentrations of alkylphenol conjugates in the first fraction were notably lower than those in the other fractions collected (Table 1). In general, concentrations of 3- and 4-ethylphenols and p- and m-cresols were similar in the permeate fractions although small increases were observed as the ultrafiltration process pro-

Table 2. Recoveries of species-related alkylphenols in fractions of whey after processing.

Whey fraction	Recovery	
	3- and 4-ethylphenols ¹	p- and m-cresols ¹
	(%))	
Permeate	49	62
Retentate ²	38	28

¹Compounds coeluted.²Retentate obtained at a volume concentration factor of 5.4.

gressed to completion. The same trend has been observed for lactose concentrations during ultrafiltration of whey from cows' milk cheese (Glover, 1971; McDonough et al., 1971).

Although conjugated alkylphenols are dissociable into ionic components, they are also water soluble, and would be expected to be as mobile as water through ultrafiltration membranes. However, results (Table 2) indicated that notable amounts of conjugated alkylphenols remained in the retentate after reaching a volume concentration factor of 5.4. Farrell et al. (1987) found that p-nitrophenyl phosphate, p-nitrophenyl sulfate, and p-nitrophenyl β -glucuronide were hydrophobically bound to β -lactoglobulin A, B, and C genetic variants of cow's milk, possibly through associations with tryptophan and phenylalanine moieties. Similarly, alkylphenol conjugates could bind to proteins by either hydrophobic or electrostatic mechanisms.

There was also a possibility that negatively charged conjugated alkylphenols were repelled by the negative charges on the ultrafiltration membrane. The membrane used in the unit was made of polysulfone with diphenylsulfone repeating units $[(\text{C}_6\text{H}_5)_2\text{SO}_2]$, which carries a negative charge (Carić, 1994). Additionally, adsorption of molecules onto the membrane may occur during filtration due to the interactions between the membrane material and whey constituents via electrostatic and van der Waals forces (Brock, 1983), and this phenomenon might have affected the complete passage of conjugates into the permeate stream. Thus, use of a membrane fabricated with a different material might improve the recovery of alkylphenol conjugates in whey permeate.

Removal of conjugated alkylphenols in the permeate during ultrafiltration of whey was characterized using whey obtained from sheep's milk Manchego-type cheese. Conjugated alkylphenols were continuously removed in sequential permeate fractions as water was removed, reflecting their water solubility. The total accountable recoveries of conjugated alkylphenols from whey obtained from sheep's milk Manchego-type cheese during ultrafiltration process at a volume concentration

factor of 5.4 were about 87 and 90% for 3- and 4-ethylphenols and p- and m-cresols, respectively, which indicated about 10% loss in the ultrafiltration unit. Some of the conjugates remained in the retentate, possibly due to adsorption by whey proteins. A UF concentrate of alkylphenol conjugates was obtained from the whey permeate after partial removal of lactose. The flavor precursor concentrate reflected 56 and 49% recoveries from permeate and 27 and 30% recoveries from the original whey for 3- and 4-ethylphenols and p- and m-cresols, respectively. The concentrate could be used to prepare a flavor concentrate for enhancement of sheep species-related flavors in dairy products. Further research is needed for optimization of the procedures to increase recovery of conjugates in the permeate on an industrial scale, including evaluation of different types of ultrafiltration membrane materials.

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