Division of Dockets Management
Food and Drug Administration
5630 Fishers Lane
Rockville, MD 20852

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Re: **Docket No. 2000P-0586 - Cheeses and Related Cheese Products; Proposal to Permit the Use of Ultrafiltered Milk**

The University of Wisconsin's Center for Dairy Research (CDR) submits these comments which are intended to be educational in nature and provide a more thorough understanding of present cheesemaking and milk filtration technology. CDR is a world-renown research, technical service and educational/training program within UW's Department of Food Science that encompasses the manufacture and use of dairy foods and dairy ingredients. We have attempted to give needed background information that provides an accurate, "state of the art" description of the practical nature of the proteins involved in cheesemaking from a compositional, nutritional, and cheese performance point of view. Due to the importance of developing a rule that is workable from a regulatory standpoint and an industry standpoint and because of the subtle complexities of the issues at hand, CDR university staff will volunteer to travel to FDA offices to provide an educational training and question and answer session for FDA staff charged with developing the Final Rule from the numerous comments that are sure to be received.

Background on Membrane Processes

Membrane filtration is a sieving process that separates components according to size. All membrane processes separate a feed material into two streams, retentate and permeate. The retentate stream does not cross the membrane, that is, it is retained by the membrane while the permeate stream crosses the membrane. The composition of the streams vary depending on the type of filtration employed. All membrane processes also use crossflow, that is, the feed stream flows parallel to the membrane under pressure thereby allowing the system to operate for an extended period of time without plugging or blocking of the membrane pores. A number of factors determine whether a component will cross the membrane but the size of the molecule and size of the membrane pores are two of the more important factors.

Membrane Classification System

Membrane separation processes are classified according to the size of the components separated and pressures used during processing. The general classification system is reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF). Some of these terms are often used interchangeably because there is much overlap between these systems and there exists no standard definitions.

Reverse osmosis membranes generally remove water. In the dairy industry, RO membranes are used to concentrate milk like an evaporator. Reverse osmosis membranes are rated according to their ability to reject salt (sodium chloride).

Nanofiltration membranes originally were referred to as "loose RO" membranes. Nanofiltration membranes remove very small molecules with a single charge. An example of such a component is sodium chloride. Nanofiltration membranes are essentially RO membranes that leak a bit of the material that RO membranes typically would retain. Nanofiltration membranes also can be rated by ability to reject salt as are RO membranes; however, the values for the NF membranes would indicate a reduced ability to reject salt compared to an RO membrane.

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Ultrafiltration membranes typically allow small molecular weight compounds (lactose, minerals, vitamins, smaller proteins) into the permeate while retaining large compounds such as large proteins and fats. Ultrafiltration membranes often are rated according to their molecular weight cutoff. The rating indicates the largest molecular weight compound that generally can cross into the permeate. An example of the range of molecular weight cutoffs available for ultrafiltration membranes is provided in Attachment 1.

During the early years of UF membrane use for milk separation it was noted that UF membranes often "leaked" protein (Barbano, 1988). Design improvements reduced the amount of protein crossing into the permeate. Microfiltration takes advantage of the loose UF concept to "leak" some of the smaller proteins into the permeate. Microfiltration membranes range from those that can separate specific proteins to membranes that permeate all proteins but retain lipids.

Although the membrane processes appear distinct, the classification system is somewhat arbitrary and varies with the membrane manufacturer. It is possible for one manufacturer to refer to a membrane with a given molecular weight cutoff as an UF membrane while another manufacturer could say their membrane with the same characteristics is a MF membrane. It is more appropriate to consider membrane classification systems as overlapping continuum. An example of several manufacturers' system for classifying UF and MF membranes is given in Attachment 2.

The absence of a concrete dividing line between UF and MF is especially apparent when considering membranes from two separate companies both used for the same purposes of separating casein from whey proteins from milk, defatting whey and concentrating milk (Attachment 3 a and b). Synder Filtration lists their element as an UF membrane with an 800,000 molecular weight cutoff while PTI Advanced Filtration describes their element as a MF membrane with a 0.3 or 0.5 micron rating.

The molecular weight cutoffs or pore sizes also are not absolute for membranes. Membranes have a range of pore sizes and the rating they are given often reflects a separation under very specific conditions with certain molecules. It is very possible to have compounds with higher molecular weights pass through the membrane or conversely, compounds with lower molecular weights may be retained contrary to the rating given a membrane.

The relationship between size of milk components and membrane separations is illustrated in a chart titled "Relative Milk Component Sizes in Comparison with Membrane Pore Size Ranges" given in Attachment 4 (Smith, 2001). The large areas of overlap among the membrane classifications and considerable overlap in the molecular weights of proteins present in milk are apparent in the chart. Therefore, for the purposes of these educational comments, we will use the term "filtered milk(s)".

Separation Process

During membrane filtration of milk with membranes having relatively smaller pores, some of the lactose, minerals and water will cross through the membrane and become the permeate stream. Casein and whey proteins because of their large size will not be able to pass through the membrane. The proteins along with the lactose and minerals that did not go into the permeate stream will become the retentate stream. The concentration of protein in the retentate stream will increase as more lactose and minerals are removed in the permeate stream. A diafiltration (DF) or washing step is required to reach protein concentrations greater than 65% in the final dried product. Diafiltration involves adding water to the retentate as it is being filtered to reduce product viscosity and further remove lactose and minerals.

The same principles apply to membrane filtration of milk with membranes having relatively larger pores. Some membranes will allow smaller proteins such as α-lactalbumin and β-lactoglobulin to cross into the permeate while retaining the larger casein molecules. The specific proteins that will cross into the permeate depend on the specific membrane and operating conditions used. A relatively small change in the operating conditions (for example feed velocity or pressure) can markedly change the ability of proteins to cross the membrane (Sachdeva, 1997). An example of the dependence of the separation on operating conditions is provided in Attachment 5.

Composition and Characteristics of Native Whey Proteins

Whey protein concentrates (WPC) are widely used in the food industry. A major problem with WPCs has been the inconsistent functionality and flavor (Banavara, 2003; Fuente, 2002; Morr, 1992). Consistency problems have largely been attributed to changes in whey proteins that occur during the cheese making process. Whey proteins removed before cheese making have improved and more consistent functionality thereby making them more suitable for high end nutraceutical applications (Anon, 2004; Bacher, 2000; Maubois, 1984; Vivekanand, 2004). Whey proteins produced by membrane filtration without going through the cheese making process are known as native whey proteins or ideal whey proteins and may command a higher price in the marketplace (Anon, 2002).

Attachment 6 gives a comparison of the characteristics of the ideal whey as compared to typical whey. The lack of starter culture bacteria is a very important benefit since their absence makes it much easier to control pH of the whey and thereby maintain product quality.

The composition of the ideal whey stream as produced by a membrane with relatively large pores as compared to typical whey is provided in Attachment 7. Depending on the exact membrane and operating conditions used there can be more or less casein present in the permeate that constitutes the ideal whey stream. Membranes that are more permeable to whey proteins tend to allow greater amounts of casein into the permeate stream. Small amounts of casein are evident in the ideal whey stream because of this permeation of casein in conjunction with whey proteins.

Composition of Milk with Reduced Whey Proteins

The composition of skim milk with a portion of the whey proteins removed is compared to skim milk processed by membranes with smaller pores, skim milk and casein in Attachment 8. The comparisons are on a solids basis. The products represent a continuum in concentrating the protein components of milk. Skim milk has all of the components of milk minus the water. The milk processed with membranes having smaller pores has an increased protein content and reduced lactose. The ash concentrations are similar for both skim milk and the smaller pore membrane processed milk. The milk processed by the larger pore membranes is very similar to the smaller pore membrane processed milk. The protein content is slightly lower and the lactose content slightly higher by comparison. The casein to whey protein ratio of the small pore membrane processed milk is 4.0 while the large pore membrane milk has a ratio of 9.68. On the other end of the continuum is casein as produced by traditional casein isolation practices. The composition of the casein product is very different from any of the other milks. Whey proteins and lactose are not present which makes a distinct difference between casein as an isolated protein product and any of the membrane processed milks which still contain casein, whey proteins, lactose and ash.

Current Status on Separation of Milk Proteins

The ability to separate/enrich milk into specific protein fractions through membranes has been known since the 1980's (Pierre, 1992). The extent to which milk protein composition can be altered is given in Attachments 9 and 10. Only recently have advances in membrane technology made some of these separations economically viable. It is now possible to selectively remove some or all of one protein such as α-lactalbumin, β-lactoglobulin, β-casein, κ-casein or α_s-casein (Anon, 2002; Maubois, 1984; Maubois and Ollivier, 1997).

An example of what is possible would be the removal of one specific casein, such as β-casein (Maubois, 1984; Maubois and Ollivier ,1992; Rosenberg, 1995; vanHekken, 2000). The cheese made from such milk would have a reduced tendency for bitterness and better melt properties that would benefit the consumer (Rosenberg, 1995).

It also would be possible to conceal that there were any alterations to the milk when a casein to whey protein ratio is used as the criteria for acceptability. During membrane processing a portion of the whey proteins also would be removed with the β-casein. It is possible to then replace some of the whey proteins such that the original casein to whey protein ratio is restored yet the composition of the milk is significantly altered. The biggest concern is a ratio that is LOWER than the original milk which would be detrimental to cheese production and performance. If a casein to whey protein ratio is needed, it would be more prudent to use language such that "the casein to whey protein ratio be at least that of the original milk, within limitations of filtration technologies employed".

The process of reducing the β-casein and β-lactoglobulin content of cow's milk to more closely replicate human milk through larger pore milk filtration was patented in 1992 (Woychik). The resulting milk was designed to be more similar to mother's milk in composition and therefore better for infants. The removal of specific proteins was considered a benefit for using the resulting milk as infant formula.

The technology for removing whey proteins through membrane processes was commercialized in Europe in the early 1980's (Anon, 2002). Not only have Europe and New Zealand been using larger pore milk filtration technology to produce milk with a reduced whey protein content for cheese manufacture, they are now taking it one step further. European and New Zealand processors are removing the lactoferrin from milk (Tamura, 2004) and then using the remaining milk to make milk powder (Hembry, 2005; Mann, 2005). It is possible for this milk powder with reduced lactoferrin concentrations to be imported into the United States and converted into cheese or Europe/New Zealand may make the milk/milk powder into cheese that then could be sold in the United States. Again, this puts US cheese makers at a competitive disadvantage compared to European and New Zealand processors who are able to either more efficiently convert milk with a reduce whey protein content into cheese or make cheese/milk powder with lactoferrin removed and sold as a high value ingredient while receiving full price for their cheese/milk powder.

> "Dairy companies, including Fonterra and Waikato-based Tatua, produce bovine lactoferrin from cows milk and sell it for about $500 a kilogram in Japan and Korea. About 10,000 tonnes of milk are used in the production of each tonne of lactoferrin, with leftover milk re-used to make basic milk powder." (Hembry, 2005)

Cheese Making and the Use of Membrane Filtered Milks

Cheese making is a partitioning and concentration process, whereby milk protein, specifically casein, is first clotted. The coagulum is then cut into smaller pieces called curd. The curd is composed of a continuous network of casein molecules which surround pools of fat. Almost all of the water in cheese and any component dissolved within the water (lactic acid, serum proteins, minerals, and salt) are trapped between the casein molecules. However, immediately after the coagulum is cut, the water phase (now also referred to as whey) begins to be expelled from the curd. This process continues through out the cheese making process. As a result > 95 % of the water from the milk is expelled from the curd. Consequently cheese contains less than 5 % of the components such as whey proteins (also called serum proteins), lactose, and calcium that were originally in the water phase of the milk.

The Impact of Filtration of Milk on the Water Phase Protein Content of Milk

A very small portion of the water in cheese can not act as a solvent. This means that the components of the water phase in cheese are very slightly more concentrated than in the water phase of milk. With this caveat and for purposes of this discussion the water in cheese will be considered equal in composition to the water phase of the milk from which it was made. Consequently changes in the composition of the water phase of milk will be reflected in similar changes to the composition of water phase of cheese. This is an important concept because of misconceptions about the negative impact that the use of membrane filtered milks may have on water phase components of cheese, specifically calcium and whey protein. A major misconception is that the membrane filtered milks will contain less whey protein and calcium, and by reference the cheese made with these milks will contain less whey protein and calcium than cheese made from non-filtered milks. This is not case when membrane filtered milks are used to supplement or to standardize milk for cheese making. In fact, the percent calcium and whey protein content of filtered milks are higher than in the milk from which they were derived and consequently they can be higher in cheese made from milk supplemented with filtered milks. To illustrate this point please refer to Attachments 11 and 12. Consequently, even though the use of some membranes remove a portion of water phase proteins from the filtered milk, the filtered milk will actually contain more water phase protein than the milk from which it was produced.

Composition of Large Pore Membrane Processed Skim Milk

Depending upon pore size of the filtration membrane the ratio of casein to protein in the water phase in filtered milk remains the same (small pore size) or increases (large pore size) compared to the milk from which it was made. What impact does this ratio have on cheese composition in regards to serum protein content? The answer is that cheese made from milk supplemented with filtered milks may have an equal content of water phase proteins as compared to cheese made from non-filtered milk.

There are two scenarios, (1) milk filtered with large pore sized membranes or (2) milk filtered with small pore sized membranes. The latter has previously been accepted by FDA as a ingredient for cheese making albeit only if it is produced at the facility where it will be used under the alternative make procedure provision and FDA has also stated that use of this milk will not adversely affect cheese quality nor will it defraud the consumer (Docket No. 2000P-0586). The issue is with the use of milk that has been filtered with large pore sized membranes since this process allows a portion of water phase proteins to pass through the membrane. It is reasoned that since a portion of the water phase proteins passes through the membrane, it effectively alters the ratio of casein to water phase protein (or whey protein) in the filtered milk and thus cheese made from this milk would be lower in serum protein. While the former statement is correct the latter statement is not.

These points are illustrated in Attachments 11 and 12. Composition of fluid skim milk that has been processed through a large pore size membrane (0.1 µm nominal pore diameter membrane, Nelson and Barbano, 2005) is given in Attachment 11. There is a higher percentage of serum proteins in the filtered skim milk (0.72%) than there is in the skim milk from which it was derived (0.53%). However, the casein to whey protein ratio is higher in the filtered milks than in the skim milk, 10.15 versus 4.71 respectively. In an earlier study (Neocleous, et al. 2002), cheese made with skim milk filtered with a 0.1 µm nominal pore diameter membrane and supplemented and standardized with cream had the same protein content as did cheese made from non-filtered milk even though the standardized milks had casein to whey protein ratios ranging from 4.38 (non-filtered milk) to 7.03 (filtered skim milk) (Attachment 13).

Currently filtered milks are used as a supplement to an existing milk supply to adjust the casein content. Adjustment of milk composition is also referred to as standardization. In the case of large pore size membranes, skim milk is processed rather than whole milk as fat can foul the membranes. Consequently, the filtered skim milk is used to standardize whole milk for use in cheeses that require milk with high casein to fat ratio to produce a cheese with a lower FDM (fat-in-dry-matter) than could be achieved with whole milk. Low moisture mozzarella is an example. The casein to fat ratio of the milk for this cheese is approximately 1.0, while the casein to fat ratio for Cheddar cheese is about 0.7. Of course cheese makers could remove cream to increase the casein to fat ratio but it may be more economical to use skim milk or filtered skim milk. Use of filtered skim milk precludes the removal of cream and is advantageous in that its use increases the casein content of the milk and much higher than if non-condensed skim milk were used. Consequently the use of membrane filtered milks will result in an increase in cheese yield and cheese plant productivity. This is especially true in the Midwest region where the milk supply can at times be deficient.

Effect on Cheese Serum Protein Content when Using Liquid Filtered Skim Milk to Standardize Whole Milk for the Manufacture of Low Moisture Part Skim Mozzarella

Composition of whole milk standardized with skim or filtered skim milk is given in Attachment 12. Computations for standardization were completed using standardization software of Kerrigan and Johnson (1984). The casein to whey protein ratio in the blend of whole milk (100 pounds) with filtered milk (12.35 pounds) is higher than in the blend of whole milk (100 pounds) with skim milk (36.1 pounds), 4.70 versus 4.35 respectively. However the serum protein content of the cheese is not significantly different (0.084g per serving compared to 0.078g, respectively). The reason for this is that the whey protein content of the filtered milk is higher than in the skim milk.

Effect on Cheddar Cheese Composition and the Nutritional Label when Cheddar Cheese is made from Liquid Milk Concentrated with a Large Pore Membrane

Neocleous et. al. (2002) demonstrated that Cheddar cheeses made from milk concentrated with 0.1 µm nominal size pore membrane had the same solids composition (protein and fat) as cheese made from non-concentrated milk but they retained slightly more calcium. This is depicted in Attachment 13. Nutritional labels were developed from the compositional data of Cheddar cheeses (from Neocleous et. al. 2002) and are given in Attachment 14. As the concentration factor of the milk increased, the casein to serum protein of the milk also increased from 4.38 in unfiltered milk to as high as 7.03 with filtered milks. An increase in the casein to serum protein ratio of the milk from 4.38 to 7.03 did not impact the nutritional label in regards to the protein content of the cheese. The nutritional label of cheeses made with milk with a higher casein to serum protein ratio (and higher calcium content) indicate that a serving of this cheese would provide 25% of the daily requirement for calcium rather than the 20% from cheese made from milk without concentration. However, the calcium content of any cheese is easily modified by employing traditionally used manufacturing procedures (preacidification or development of more acid prior to addition of rennet) to produce cheeses with identical calcium contents.

Effect on Cheese Performance when made from Liquid Filtered Milk Concentrated with a Large Pore Membrane

Govindasamy-Lucey et al. (2005) reported that pizza cheese made from whole milk standardized with skim milk filtered with a large pore size membrane was identical in respect to composition (including calcium content), sensory attributes (bitterness, off flavors, acidity, smoothness and firmness) and functional properties (melt) to cheese made from milk standardized by cream removal.

Potential Misconception on Use of Filtered Milk as Stated in Docket No. 2000P-0586.

There is potential for misconception with the use of filtered milk due to the language used in Docket No. 2000P-0586. It is stated in this document that filtered milks replace fluid milk as an ingredient for cheese making. Filtered milks are just different versions of the same fluid milk that would be used in cheese making. No fluid milk is being displaced. Filtered milks are an economical means of transporting fluid milk from the site of production (sometimes several hundreds of miles distant from site of use) to the site of use.

Use of small pore sized membrane filtered fluid milks in cheese making is accepted under FDA ruling as an alternative make procedure. The current FDA ruling on labeling requires that cheese makers put on the label that the cheese was made using membrane filtered milk unless the filtered milk is processed at the site in which it was used. This sets the situation that is biased in favor of the large milk volume cheese makers who have the capacity to filter milk, and establishes a competitive advantage over small volume milk cheese makers who cannot afford to purchase filtration equipment. Regardless of milk volume processed into cheese, cheese makers need the ability to supplement existing milk supplies on an equal basis. FDA's current policy has the potential to be detrimental for certain cheese makers solely based on size of their facility or their inability to finance or justify the purchase of filtration equipment. In addition, since the casein to whey protein ratio in the filtered milks does not result in a decrease in whey protein content of the cheese and is essentially unenforceable from a regulatory standpoint, casein to whey protein ratio of the liquid filtered milks should not be mandated.

Please contact me if CDR can assist FDA in any way, including our offer for an educational session at FDA offices.

Sincerely,

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