Research Note

Changes in Galactose and Lactic Acid Content of Sweet Whey during Storage

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ABSTRACT

Whey is often stored or transported for a period of time prior to processing. During this time period, galactose and lactic acid concentrations may accumulate, reducing the quality of spray-dried whey powders in regard to stickiness and agglomeration. This study evaluated industry samples of Cheddar and mozzarella cheese whey streams to determine how galactose and lactic acid concentrations changed with storage at appropriate (4°C) and abuse (37.8°C) temperatures. Samples stored at 4°C did not exhibit significant increases in levels of lactic acid or galactose. Mozzarella whey accumulated the greatest amount of galactose and lactic acid with storage at 37.8°C. Whey samples derived from cheese made from single strains of starter culture were also evaluated to determine each culture’s contribution to galactose and lactic acid production. Starter cultures evaluated included Streptococcus salivarius ssp. thermophilus, Lactobacillus helveticus, Lactobacillus delbrueckii ssp. bulgaricus, Lactococcus lactis ssp. cremoris, and Lactococcus lactis ssp. lactis. Whey derived from L. helveticus accumulated a significantly greater amount of lactic acid upon storage at 37.8°C as compared with the other cultures. Galactose accumulation was significantly decreased in whey from L. lactis ssp. lactis stored at 37.8°C in comparison with the other cultures. Results from this study indicate that proper storage conditions (4°C) for whey prevent accumulation of galactose and lactic acid while the extent of accumulation during storage at 37.8°C varies depending on the culture(s) used in cheese production.

Spray drying is a common technique used to produce whey powders. Under ideal conditions, this process will result in a fine, free-flowing powder. However, this is often not the case. During spray drying, whey rapidly changes from a liquid to a dry solid. Incomplete drying will lead to lower product yield and powder handling difficulties, including caking and agglomeration of the powder. Such a powder will cause fouling of drying equipment as it adheres to hot metal surfaces. Much research has been done to define the nature of this problem and develop solutions.

Whey is a complex product composed of greater than 90% water, with the solids portion usually having the following composition: lactose (70%), protein (14%), minerals (9%), fat (4%), and lactic acid (3%) (8). During the drying process, virtually all of the water is removed from the product and the remaining solid components dictate the physical properties of the resulting powder. With lactose constituting nearly three-fourths of whey powder, the properties of lactose dominate the behavior of whey powder (8). In addition to lactose, lactic acid also contributes to processing difficulties and the overall stickiness of whey (10). Proteins, minerals, and fat do not significantly influence the drying properties of whey.

The glass transition temperature of lactose strongly influences the temperature during spray drying at which the semidried fluid in the rubbery state will transition into a glassy, amorphous state. In turn, this temperature will influence the extent of fouling that may occur during spray drying. The glass transition temperature of lactose has been measured at 101°C (7). However, because whey powders are not composed of a single material, they are not expected to show a sharp glass transition at a single temperature (11). In fact, the presence of other components with correspondingly lower glass transition temperature values may decrease the range at which this glass transition occurs.

Hydrolysis of lactose in whey produces breakdown products of galactose and glucose, which have significantly lower glass transition temperatures, 30°C and 31°C, respectively (7). As these breakdown products accumulate in whey, the tendency for stickiness and agglomeration to occur in whey powders may be increased as well.

Whey has historically been perceived as a waste product and is in fact still often treated as such (8). Whey awaiting processing may not be pasteurized or stored at appropriate temperatures. Storage under abuse conditions can lead to a reduction in the quality of whey and the resulting dried whey products. Starter cultures remaining from cheese production may still actively metabolize whey components, namely lactose, throughout storage of whey under a certain temperature range. The primary purpose of a dairy starter culture is to produce lactic acid from lactose; therefore, lactic acid will accumulate in whey with storage (5). In addition to lactic acid, galactose and glucose are also being produced as lactose is hydrolyzed. When grown in

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the presence of excess lactose, certain cultures, e.g., *Streptococcus salivarius* ssp. *thermophilus*, are known to only ferment the glucose portion of the lactose molecule (16). Galactose is thus allowed to accumulate in the whey.

The objectives of this study were to determine differences in galactose and lactic acid accumulation in whey during storage with respect to (1) storage temperature; (2) cheese type from which the whey was derived, namely mozzarella and Cheddar cheeses; and (3) culture used in the cheese-making procedure. In making these comparisons, whey and cheese processors will be able to better understand what may contribute to variations in lactic acid and galactose levels in whey.

**MATERIALS AND METHODS**

**Collection of whey.** Commercial samples of Cheddar and mozzarella cheese whey were collected at the time of whey draining. Samples were provided by the Babcock Hall Dairy Plant and the Wisconsin Center for Dairy Research, both of Madison, Wis. Cheddar and mozzarella whey samples were collected from 5 separate days of cheese production. Cheddar cheese was produced from pasteurized whole milk according to the traditional Cheddar manufacturing procedure (12) using a DVS mesophilic blended culture (CH 970, Chr. Hansen, Milwaukee, Wis.). Mozzarella cheese was produced from pasteurized 2% milk following a traditional low-moisture mozzarella procedure (9) using a thermophilic bulk set culture (C257, Rhodia, Madison, Wis.).

Whey samples derived from single strains of starter culture were produced based on a procedure for cheese production in 1-liter beakers (14). The procedures were modified to resemble either a mozzarella or Cheddar cheese production schedule. Cheese-making trials were done in triplicate for each strain of starter culture.

For Cheddar cheese production, approximately 600 g of pasteurized whole milk was weighed into a 1-liter beaker and brought to 32.2°C in a water bath. Cultures used for Cheddar production included bulk starters of *Streptococcus salivarius* (14) or *Lactococcus lactis* ssp. *lactis* (D15) or *Lactococcus lactis* ssp. *cremoris* (D290) (Rhodia). The bulk starters were produced by inoculating 200 ml of skim milk that had been previously steam treated for 20 min with 0.2% of the frozen starter stored at 4°C with continued stirring. Whey at pH 6.10 (±0.02) was collected by draining through a funnel lined with cheese cloth.

After collection, the lots of whey were divided into two equal portions. Half of the whey was stored at 4°C for 24 h while the other half was held in a water bath at 37.8°C for 24 h, representing appropriate and abusive storage temperatures, respectively. For the industry survey, whey samples were analyzed for titratable acidity and galactose content initially and after 24 h of storage at 4°C with the samples stored at 37.8°C examined periodically during 24 h of storage. For the individual culture strain trials, wheys held at 37.8°C were analyzed for titratable acidity and galactose at 0, 8, 16, and 24 h, with refrigerated whey samples examined only after 0 and 24 h.

**Lactic acid and galactose analysis.** α-Galactose content was determined in duplicate using the Lactose/D-Galactose enzymatic test kit (Boehringer Mannheim, Germany) according to manufacturer’s instructions. In this procedure, samples were deproteinized using the Carréz clarification, and diluted either 4- or 10-fold before clarification based on the projected level of galactose in the sample. Aliquots of 0.1 to 0.5 ml were then used for the enzymatic analysis. Lactic acid content of the whey samples was measured in duplicate as titratable acidity according to Association of Official Analytical Chemists method 947.05 for titratable acidity of milk (1).

**Statistical analysis.** Results were analyzed using one-way ANOVA on Minitab statistical software (Release 11; Minitab, Inc., State College, Pa.). The level of significance was determined at \( P < 0.05 \).

RESULTS AND DISCUSSION

**Industry survey.** Storage of whey at elevated temperatures promotes the growth and continued metabolism of starter cultures present in whey. This in turn leads to a greater accumulation of galactose and lactic acid. When whey samples were stored at appropriate refrigeration temperatures (4°C), no significant increase in either galactose (Table 1) or titratable acidity (Table 2) was seen during 24 h of storage. Because refrigeration temperatures are not optimal for growth or activity of starter culture bacteria or other native flora in whey, products of metabolism are not produced (15).

Mozzarella and Cheddar cheese whey exhibited increases in titratable acidity when stored at 37.8°C. The titratable acidity of mozzarella whey increased to 0.64%

**TABLE 1. Galactose content (g/liter) of whey during 24 h of storage**

<table>
<thead>
<tr>
<th>Whey source</th>
<th>0 h</th>
<th>24 h at 4°C</th>
<th>24 h at 37.8°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar cheese</td>
<td>0.14 ± 0.18b</td>
<td>0.16 ± 0.18b</td>
<td>0.63 ± 0.18b</td>
</tr>
<tr>
<td>Mozzarella cheese</td>
<td>0.71 ± 0.18A</td>
<td>0.76 ± 0.18A</td>
<td>1.87 ± 0.18A</td>
</tr>
</tbody>
</table>

\( ^a \text{Mean} (n = 5) \text{within the same column without a common letter differ} (P < 0.05) \)
TABLE 2. Titratable acidity (%) of whey, expressed as lactic acid, during 24 h of storage

<table>
<thead>
<tr>
<th>Whey source</th>
<th>0 h</th>
<th>24 h at 4°C</th>
<th>24 h at 37.8°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar cheese</td>
<td>0.12</td>
<td>0.11</td>
<td>0.44</td>
</tr>
<tr>
<td>Mozzarella cheese</td>
<td>0.14</td>
<td>0.13</td>
<td>0.64</td>
</tr>
</tbody>
</table>

* There were no statistically significant (P > 0.05) differences in titratable acidity between whey sources within each storage treatment.

compared with Cheddar, which had a titratable acidity of 0.44% after 24 h of storage. The nature of this difference most likely relates to the differences in starter cultures used during cheese production and the cooking procedures used in cheese manufacture. Cheddar cheese typically uses a combination of mesophilic cultures, including L. lactis ssp. lactis and L. lactis ssp. cremoris. Mozzarella cheese, however, is typically made with a combination of the following thermophilic cultures: S. salivarius ssp. thermophilus, L. delbrueckii ssp. bulgaricus, and/or L. helveticus. Lactic acid is the major fermentation end product for all of these starter cultures (15). The extent to which end products are produced depends on the growth conditions. Given that thermophilic cultures are better suited than mesophilic cultures to grow at 37.8°C, whey from mozzarella cheese would be expected to produce greater levels of lactic acid during storage at elevated temperatures.

Galactose content in the mozzarella cheese whey was consistently higher than that in Cheddar. The initial galactose level in mozzarella whey was four to five times greater than that in Cheddar whey. Though both Cheddar and mozzarella whey increased significantly in galactose content during storage at 37.8°C, mozzarella whey accumulated three times as much galactose (1.87 g/liter) as Cheddar whey (0.63 g/liter). It is well established that galactose accumulates whenever S. salivarius ssp. thermophilus is grown in a lactose-containing media, such as whey or cheese (6). The presence of this starter culture in mozzarella may account for this elevated level. Studies by Tinson et al. (17), Hutkins et al. (5), and Thomas and Crow (16) showed that galactose accumulation and lactose utilization occur at similar rates when S. salivarius ssp. thermophilus is grown in complex media containing excess lactose. In addition, this occurrence took place with both Gal⁺ and Gal⁻ strains (4, 5, 16).

Individual culture strain trials. Results from whey samples generated using a single strain of starter culture are summarized in Tables 3 and 4. As found previously in the wheys, titratable acidity and galactose content did not change significantly during storage at 4°C. This further demonstrates how appropriate storage conditions for whey may be able to reduce levels of whey components that can make spray drying difficult and reduce the quality of the finished whey.

Increases in titratable acidity during storage at 37.8°C were similar, increasing on average from 0.12 to 0.37% for all cultures except when produced using L. helveticus. In L. helveticus-derived whey, titratable acidity significantly increased from 0.11 to 0.79%. L. helveticus is known to produce almost twice the concentration of lactic acid in milk compared with other common lactic acid bacteria, including S. salivarius ssp. thermophilus and L. delbrueckii ssp. bulgaricus (3, 13).

Initial levels of galactose present in the whey samples were similar, averaging about 0.12 g/liter for all cultures except S. salivarius ssp. thermophilus, which had an initial galactose level of 0.92 g/liter. As stated previously, this starter culture is well recognized for its preference to completely deplete lactose sources before utilizing galactose (5). Thus, galactose readily accumulates as lactose is utilized.

After 24 h of storage, galactose levels increased to 2.50 to 3.06 g/liter for all cultures except L. lactis ssp. lactis,

TABLE 4. Galactose content (g/liter) in whey derived from cheese made with single-strain starter cultures during 24 h of storage

<table>
<thead>
<tr>
<th>Culture type</th>
<th>0 h</th>
<th>24 h at 4°C</th>
<th>24 h at 37.8°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus salivarius</em> ssp. thermophilus</td>
<td>0.92 A*</td>
<td>1.06 A</td>
<td>3.06 A</td>
</tr>
<tr>
<td>Lactobacillus helveticus</td>
<td>0.13 B</td>
<td>0.14 B</td>
<td>2.57 A</td>
</tr>
<tr>
<td>Lactobacillus delbrueckii ssp. bulgaricus</td>
<td>0.19 B</td>
<td>0.22 B</td>
<td>2.92 A</td>
</tr>
<tr>
<td>Lactococcus lactis ssp. lactis</td>
<td>0.09 B</td>
<td>0.08 B</td>
<td>0.24 B</td>
</tr>
<tr>
<td>Lactococcus lactis ssp. cremoris</td>
<td>0.08 B</td>
<td>0.08 B</td>
<td>2.50 A</td>
</tr>
</tbody>
</table>

* Means (n = 3) within the same column without a common letter differ (P < 0.05).
which remained unchanged from its initial galactose level of 0.24 g/liter. This \emph{L. lactis} ssp. \emph{lactis} strain showed a significantly greater ability to utilize galactose than the other strains tested.

Results from this study indicate that proper storage conditions (4°C) for whey may prevent accumulation of galactose and lactic acid. Mozzarella whey streams present the greatest problem for controlling lactic acid and galactose levels when stored at abuse temperatures (37.8°C). Cultures commonly used for Cheddar and mozzarella production varied in their contribution to galactose and lactic acid at elevated storage temperatures. By selecting appropriate starter cultures, cheesemakers may be able to better control the quality of whey for spray drying.

**ACKNOWLEDGMENTS**

We are very grateful to John Jaeggi and Bill Hoesly of the Center for Dairy Research and Walter Brandli of the Babcock Hall Dairy Plant for providing whey samples. This research was supported in part by the College of Agriculture and Life Sciences (University of Wisconsin–Madison) and the Center for Dairy Research through funding from Dairy Management Inc.

**REFERENCES**