Factors Influencing Tenacity of Dried Milk Films Exposed to High Humidity

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ABSTRACT

Rinsability of milk films on stainless steel was impaired by exposure to 100% relative humidity (RH). Rinsability was determined by automated Lowry protein tests of detergent used to remove films. Residue of milk films was 1% of the initial soil load when dried on stainless steel plates without humidification, but was 6.35% of the initial load after drying (30 min), humidification (15 min) and redrying (30 min) at 37 C. Three successive exposures to 100% RH for 7.5 min at 37 C. each followed by 30 min of drying, yielded a residue of nearly 30% of the initial soil load. Exposure at 37 C produced the maximum amount of residue on plates. Experimental temperatures ranged from 0 to 75 C. Temperature of milk applied to plates was of little importance. Raw milk formed more tenacious film than skim milk or major components of milk. Milk produced during colder months yielded less soluble films than milk produced during warmer months. Lowering of milk pH to 5.7, adding soluble calcium, and aging milk at 0 C increased residues. Chelation of soluble calcium with EDTA or dissociation of milk protein with sodium dodecyl sulfate decreased soil residue levels. Exposure of instantized nonfat dry milk to the high humidity treatment decreased its solubility more than tenfold.

High relative humidity (RH) may cause formation of tenacious residue on stainless steel. Such a residue was observed when plates were left standing overnight under humid conditions in an experimental spray unit (2). The tenacious film that had formed on these plates resisted spray-rinsing with water at 35 C for 30 sec. The role of high RH in increasing the tenacity of milk films has not been explained, but the role of moisture in development of washable fatty soil in fabrics has been reported (18). The latter was attributed to possible polymerization of unsaturated oils. Aging effects on tristearin have also been observed on glass (4) and on stainless steel (9). The former report attributed aging to removal of a layer of moisture from the surface of glass and the latter to the transition of the fast-removed species to a slowly removed one.

High humidity exists in milk processing equipment such as pipelines, tanks, pasteurizers and separators. If such conditions cause buildup of tenacious soil, energy and detergent requirements will be increased. The efficacy of halogenated sanitizers may also be affected. Thus cleaning and sanitizing can be impaired.

The objectives of this research were to quantitate the effects of high RH on the tenacity of milk films and to determine effects of the following variables on film tenacity: time, temperature and number of exposures to high RH, age of milk, fractions of milk, pH, season of the year, and selected additives. Protein was chosen as the indicator of tenacious soil because it is an important and representative constituent of milk soil and is likely to be denatured in high humidity. Tenacious soil was that milk film which resisted rinsing with water and was removed by washing with a specially formulated detergent.

MATERIALS AND METHODS

Stainless steel plates

Plates used in the study were made of 16-gauge stainless steel, type 304, with a No. 4 finish. They measured 10 × 10 cm and 1.5-mm thick. Each had a hole, 2.5 mm in diameter, centered 5 mm from one edge.

Milk

Raw grade A milk was obtained fresh for each replication and stored at 0 ± 1 C until ready for use.

Soiling procedure

Clean plates and glass slides were immersed in cold (5-7 C) milk for 60 sec and then stacked vertically and separated in the incubator to air-dry (10-20% RH) at 37 C for 30 min.

Detergent solution

The detergent solution contained 3.75 g/l of a powdered formulation of the following components:

<table>
<thead>
<tr>
<th>Compound</th>
<th>g/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium tripolyphosphate (purified, granular)</td>
<td>35</td>
</tr>
<tr>
<td>Sodium metasilicate (technical, granular)</td>
<td>25</td>
</tr>
<tr>
<td>Sodium hydroxide (electrolytic pellets, anhydrous)</td>
<td>17</td>
</tr>
<tr>
<td>Sodium carbonate (Certified, ACS, anhydrous)</td>
<td>20</td>
</tr>
<tr>
<td>Triton CF-10</td>
<td>3</td>
</tr>
</tbody>
</table>

Fresh detergent solution was prepared daily with distilled water. This formulation possessed enough detergency to remove practically all protein. In preliminary experiments, no protein was detected in fresh detergent solution used to rewash plates.

Washing plates — vessels and procedure

Washing vessels were made of translucent plastic 3-mm thick and were glued together with silicone caulk. Internal dimensions were 3-cm width, 11-cm length and 15-cm height. With slotted plastic at the sides, each held five stainless steel plates, 4 to 5 mm apart, in a vertical plane. Two 1-cm blocks at the bottom raised the plates to allow a magnetic
stirring bar to rotate (300 rpm). For ease of placement and removal, plates were hooked by wires to a bar.

Rinsing and washing were accomplished in the vessel at 25 ± 2 C by submerging five plates in 350 ml of distilled water and detergent solution, respectively, and stirring for 15 min. After plates were rinsed, they were rinsed twice with distilled water to remove solubilized protein. Rinse solution was discarded unless specified, but protein content of detergent solutions was determined with each set of plates.

**Determining concentrations of protein**

A standard curve was prepared with pooled instantized nonfat dry milk (NDM) dissolved in detergent solution. The NDM had an average nitrogen content of 5.59% (35.6% protein) as determined in triplicate tests (5). NDM was carefully weighed, transferred to 1000-ml volumetric flasks, dissolved in detergent solution, and made to volume. Solutions contained 5, 10, 25, 50, 100, 150, 250, 300, and 350 μg of protein/ml. Absorbances were obtained by the automated Lowry test (3, 13), for each concentration. Average values from two replicate experiments were plotted as a standard curve, which was then used to determine protein concentrations in detergent solutions used to wash plates. The amount of protein removed per unit area (μg/cm²) was calculated from the concentration of protein, the volume of detergent, and the area of the plates.

**High humidity chamber**

The high humidity chamber was a thermostatically regulated incubator with a circulating fan. High humidity (100%) was produced with a vaporizer and monitored with a portable psychrometer.

**Design and analysis of experiments**

Experiments were duplicated. Each treatment involved 75 soiled stainless steel plates which were randomly divided into groups of 25 and handled as follows unless specified otherwise:

- **Unrinsed controls.** Plates in subgroups of five were washed in detergent solution immediately after they were soiled.
- **Exposed.** Soiled plates were incubated at 10 to 20% RH and 37 C for 112.5 min. Subgroups of five plates were then rinsed and washed.
- **Exposed.** Soiled plates were exposed to 100% RH at 37 C for 7.5 min, then dried at 10 to 20% RH and 37 C for 30 min, except in the experiment on “Temperature of exposure.” Plates were exposed to these conditions three successive times, for a total of 112.5 min, except in the experiment on “Single exposure—various times.” Subgroups of five were then rinsed and washed.

Tenacious milk soil was designated as that film which resisted rinsing and was collected by washing rinsed plates with detergent solution. Quantities of protein recovered in detergent solutions used to reincubate subgroups of five plates were then rinsed and washed.

**Combined and individual effects of high humidity and drying.** The 75 soiled plates used in each replication of this experiment were randomly divided as follows: (a) Combined exposure to 100% RH and drying. Plates were exposed three times to 100% RH at 37 C and dried 30 min at 37 C after each exposure. (b) Exposure to 100% RH only. Plates were exposed to 100% RH continuously for 22.5 min (7.5 min x three exposures) at 37 C, then dried for 30 min at 37 C. (c) Drying only. Plates were continuously dried for 90 min at 37 C.

**Additives.** The following were added to milk before plates were soiled: calcium chloride (hydrate, crystals, Certified ACS)-0.05, 0.1, 0.2, and 0.4 M; sodium dodecyl sulfate (SDS) (powder) - 0.1, 0.2, 0.4, 0.8, and 1% ethylenediamine tetraacetic acid (EDTA) (Certified ACS) - 0.2, 0.4, 0.8, and 1.6 g/l (w/v). Each solution was stirred for 15 min and cooled to 5-7 C before plates were soiled.

**pH.** The pH of 11 of milk at about 1 C was either decreased to 5.7 with 0.1 N HCl or increased to 7.7 with 0.1 N NaOH. The pH meter used for measurements was equipped with a combination electrode. Solutions were allowed to equilibrate 30 min before plates were soiled.

**Age of milk.** Sixteen liters of fresh commingled milk were stored at 5-7 C. Commencing on the day of milk collection, and every second day thereafter for 16 days, 1 l of milk was used to soil plates.

**Temperature of milk.** Plates were soilied in aliquots of commingled milk adjusted to 0, 10, 20, and 40 C (± 1 C).

**Components of milk.** From commingled milk 1 l was used to soil plates and 6 l were separated with a cream separator. The resulting skim milk and cream were used to soil plates. The remaining skim milk was cooled to 5 C and acidified to pH 4.6 with 0.1 N HCl. The precipitated casein was collected on cheese cloth. Fine particles were collected on Whatman No. 40 filter paper in a Büchner funnel. The precipitate was washed three times in excess distilled water to remove whey. The casein was resuspended in a volume of NaOH solution, at pH 11.0, sufficient to equal the original volume of skim milk. Both the casein and the whey were adjusted to pH 6.7, then cooled to 5-7 C. After the plates were soiled, casein was reprecipitated by acidification to pH 4.6. The curd was filtered out, washed as before and drained. Reprecipitated casein was then added back to whey, and the pH was adjusted to 11.0 for resolubilization. Before this recombined skim milk was used to soil plates, its pH was adjusted to 6.7, and its temperature was adjusted to 5-7 C. The cream was then added to the recombined skim milk to produce recombined whole milk, which was stirred vigorously with a magnetic stirrer for 30 min in an ice bath. The pH was adjusted to 6.7 and plates were soilied immediately.

**Nonfat dry milk (NDM) versus raw milk.** Raw milk and reconstituted instant NDM (10%) were each used to soil 75 plates.

**Solubility index of exposed instant nonfat dry milk.** In each of five replications 10 g of instant NDM were weighed into each of two large petri dishes (140 mm x 20 mm). The sample in one dish was exposed three times to 100% RH with drying, as previously; the other was incubated at 37 C for 112.5 min. Contents of each dish were mixed after each cycle of the high humidity-drying treatment. Dishes were sealed with masking tape and kept at room temperature until samples were used. Solubility indexes were determined according to the method of the American Dry Milk Institute(I). Briefly, samples were stirred in water under specified conditions and particles which failed to dissolve were centrifuged out. Insoluble material was quantitated in graduated conical centrifuge tubes.

**Seasonal variations.** Data obtained in analyses of fresh raw milk during the cold and warm months were compiled for comparisons.

**RESULTS AND DISCUSSION**

**Single exposure—various times.** Milk dried onto stainless steel plates became more tenacious when exposed to high humidity (Table 1). Less than 1% of the initial soil load on unexposed plates remained after rinsing, and the amount of residue did not change with the length of time they were held dry. Even after exposure of plates to high humidity for only 3.75 min, the residue on them was nearly three times as
much as that on unrinsed controls. Residue increased by four times when exposure time was doubled (7.5 min) and by nearly seven times when exposure time was quadrupled (15 min). Doubling time of exposure again (30 min) failed to increase residue recovered.

**Triple exposure--various times**

Exposure of films to high humidity three times, followed immediately by drying, further enhanced formation of tenacious films (Table 2). Each exposure duration yielded significantly different results. Maximum residue, nearly 30% of the initial residue, occurred when plates were exposed for 7.5 min. This quantity was more than five times greater than the maximum quantity recovered after a single-exposure (6.25% - 15 min).

**Individual effects of high humidity versus drying**

Data in Table 3 reveal that both high humidity and drying are required to form much tenacious film. On continuous exposure to high RH without drying, only 2.31% of the total soil remained after rinsing. After continuous drying, only 3.25% of the original soil remained. Although significant differences occurred between treatment means, the difference between the combined high humidity/drying treatment and either single factor was substantially greater than the difference between the means of the two individual factors. Summed residues from individual treatments were less than 25% of the tenacious residue obtained when they acted together. The high humidity treatment yielded slightly lower residue than the drying treatment because of the washing effect of prolonged exposure to the moisture-laden atmosphere.

Drying may have altered the surface properties of casein micelles such that aggregation was favored upon humidification. When dry film is moistened with water, fluidity is somewhat restored due to mobility of hydrated polypeptide chains. Thus their reactive groups may form adhesive and cohesive bonds, both of which types are

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**TABLE 1.** Mean quantities of protein recovered as affected by time of exposure of milk films to high humidity in a single treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of exposure to 100% RH (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.75</td>
</tr>
<tr>
<td>Unexposed</td>
<td>0.35</td>
</tr>
<tr>
<td>Exposed</td>
<td>0.96d</td>
</tr>
<tr>
<td>Unrinsed</td>
<td>2.97c</td>
</tr>
</tbody>
</table>

1n = 10 sets of 5 plates each.

**TABLE 2.** Mean quantities of protein recovered as affected by exposure of milk films to high humidity in three successive treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of exposure to 100% RH (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.75</td>
</tr>
<tr>
<td>Unexposed</td>
<td>0.34</td>
</tr>
<tr>
<td>Exposed</td>
<td>0.98e</td>
</tr>
<tr>
<td>Unrinsed</td>
<td>3.36</td>
</tr>
</tbody>
</table>

**TABLE 3.** Mean quantities of protein recovered from rinsed plates exposed to high humidity, to drying, and to combined high humidity and drying.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>High humidity only</th>
<th>Drying only</th>
<th>High humidity and drying</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treated</td>
<td>µg/cm²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>µg/cm²</td>
<td>0.81</td>
<td>1.12</td>
<td>8.68</td>
</tr>
<tr>
<td>% of unrinsed</td>
<td>2.31</td>
<td>3.25</td>
<td>23.84</td>
</tr>
<tr>
<td>µg/cm²</td>
<td>35.11</td>
<td>34.48</td>
<td>36.41</td>
</tr>
</tbody>
</table>

1n = 10 sets of 5 plates each.

**Temperature of exposure**

On unrinsed control plates, the amount of soil deposited increased with temperature from 31.5 to 34.3 µg/cm². The quantity of residue on unexposed plates was unaffected by temperature of incubation through 37 C (Fig. 1), but significant increases in residue occurred as temperature was raised to 50, 63 and 75 C. Considerably more soil was left on surfaces of exposed samples than unexposed samples, except at 75 C. Humidification caused a sharp rise in soil residues above 20 C. Quantities of residue peaked near 37 C then declined and leveled off between 50 and 60 C. A small, though significant, rise in soil residues was observed at 75 C. Thus protein became insoluble in high humidity at about 37 C, and the solubility of unexposed films decreased at high temperatures.
Effects of additives on formation of tenacious films

**Calcium chloride.** Amounts of tenacious residue on unexposed, exposed, and unrinsed control plates increased with additions of CaCl₂ (Table 4). The amount of residue on exposed plates paralleled that obtained for unexposed plates, but was 15% to 25% higher at concentrations of CaCl₂ below 0.4 M. When 0.4 M CaCl₂ was added, only small quantities of soil on both exposed and unexposed plates were rinsed off. Thus, regardless of humidity, soil became quite tenacious when high quantities of calcium were added. This experiment confirms the importance of calcium in formation of tenacious films and emphasizes the value of soft water in cleaning dairy equipment. Residual calcium on equipment would not only enhance formation of milkstone but also increase tenacity of proteinacious milk films upon exposure to high humidity.

**Sodium dodecyl sulfate (SDS).** SDS was added to milk to dissociate protein. Addition of 0.1% (equivalent to 0.003 M) SDS significantly (P < 0.05) decreased the amount of residual soil, but increasing the concentration had little additional effect (Table 5). This detergent practically cancelled the effects of high humidity, bringing soil levels on exposed plates after rinsing near those on unexposed plates. Thus, little tenacious film was formed if protein was dissociated, even though there was a pronounced increase in amounts of soil deposited on plates on dipping.

SDS interacts with proteins and causes dissociation of high-molecular weight aggregates into monomers (14). Cheeseman (10) suggested that the detergent binds with hydrophobic regions of casein to form complexes. When complexes form, the insolubilizing effect of high humidity on protein becomes insignificant. Reduced size of the casein plus its presumed complexation with SDS increased rinsability of the milk film.

SDS lowered the surface tension of milk such that it wet plate surfaces better than did milk without SDS. This improvement in wettability increased with detergent concentration in milk at least up to 1%, as exhibited by the increasing soil load on control plates.

**Ethylenediamine tetraacetic acid (EDTA).** Unexposed samples did not react significantly to EDTA in concentrations up to 800 mg/l, but residue increased considerably when 1600 mg/l were added (Fig. 2). Residue on exposed plates was lowered significantly by 200 to 800 mg/l of EDTA, but more than three times as much was left on exposed plates with 1600 mg/l as on exposed plates without EDTA and unexposed plates with equal EDTA.

We had already observed that added soluble calcium increased tenacity of residue. Total calcium in milk is estimated to be 30 mM (1200 mg Ca++) of which about 20 mM is colloidal, 10 mM is soluble and 3 mM is

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**TABLE 4. Mean quantities of protein recovered from plates soiled with milk containing added calcium chloride (CaCl₂).**

<table>
<thead>
<tr>
<th>Concentration CaCl₂ in milk (molar)</th>
<th>Treatment</th>
<th>Unexposed²</th>
<th>% of unriased</th>
<th>Exposed²</th>
<th>% of unriased</th>
<th>Unriased³</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.35</td>
<td>1.22b</td>
<td>17.28</td>
<td>7.978</td>
<td>28.60v</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>12.18</td>
<td>28.35f</td>
<td>17.96</td>
<td>41.65e</td>
<td>43.12w</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>18.80</td>
<td>29.13f</td>
<td>29.72</td>
<td>46.05d</td>
<td>64.54x</td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>66.26</td>
<td>65.51c</td>
<td>92.40</td>
<td>91.35b</td>
<td>101.15y</td>
<td></td>
</tr>
<tr>
<td>0.40</td>
<td>116.00</td>
<td>100.00a</td>
<td>116.00</td>
<td>100.00a</td>
<td>116.00z</td>
<td></td>
</tr>
</tbody>
</table>

²Where superscripts differ, means are significantly different (P < 0.05), Duncan (11).
³Where superscripts differ, means are significantly different (P < 0.05), Duncan (11).

**TABLE 5. Mean quantities of protein recovered from plates soiled with milk containing varying amounts of sodium dodecyl sulfate (SDS).**

<table>
<thead>
<tr>
<th>Concentration of SDS in milk (%)</th>
<th>Treatment</th>
<th>Unexposed²</th>
<th>% of unriased</th>
<th>Exposed²</th>
<th>% of unriased</th>
<th>Unriased³</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.35</td>
<td>1.22b</td>
<td>1.72</td>
<td>6.12a</td>
<td>28.11x</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.35</td>
<td>1.12bc</td>
<td>0.35</td>
<td>1.12bc</td>
<td>31.36w</td>
<td></td>
</tr>
<tr>
<td>0.2</td>
<td>0.34</td>
<td>0.86d</td>
<td>0.89d</td>
<td>0.89d</td>
<td>39.45x</td>
<td></td>
</tr>
<tr>
<td>0.4</td>
<td>0.34</td>
<td>1.04c</td>
<td>0.42</td>
<td>1.28b</td>
<td>32.73y</td>
<td></td>
</tr>
<tr>
<td>0.8</td>
<td>0.34</td>
<td>0.83d</td>
<td>0.39</td>
<td>0.92ed</td>
<td>41.13z</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.34</td>
<td>0.83d</td>
<td>0.35</td>
<td>0.85d</td>
<td>41.41z</td>
<td></td>
</tr>
</tbody>
</table>

Where superscripts differ, means are significantly different (P < 0.05), Duncan (11).
Unexposed 2

ethylenediamine tetraacetic acid

Figure 2.

Unexposed

2

Figure 2. Percentages of protein recovered from exposed and unexposed plates soiled with milk containing varying amounts of ethylenediamine tetraacetic acid (EDTA). Points with different letters (a-e) differed significantly in protein recovered (P < 0.05).

ionized (15). EDTA binds Ca⁺⁺ in a 1:1 basis (19). As ionized and soluble calcium were progressively bound with 200 to 400 mg/l of EDTA, the reaction to high humidity decreased. However, quantities of EDTA sufficient to bind, as well, colloidal calcium (800 mg Ca⁺⁺/l) increased the response to high humidity. Addition of 1600 mg/l caused the protein to become much more sensitive to high humidity, and quantities of residue approached the large amount normally deposited when there was no additive. The mechanism of this latter effect is unexplained.

**pH**

More tenacious residue formed in high RH with acidic (pH 5.7) and alkaline (pH 7.7) milks than in milk of pH 6.7, the normal pH (Table 6). Higher residue was obtained with pH 5.7 than with pH 6.7 and pH 7.7. The effect of low pH was probably related to decreases in hydration and in the colloidal calcium of the micelles.

Adjusting pH from 6.7 to 7.7 could have caused increased adhesiveness of caseinate. Viscosity increases as micelles swell when the pH of milk is raised (7). Also, more calcium bridges may have been formed because of the increasing availability of nonprotonated protein species (H₂NCHRCOO⁻) as pH was increased. Caution must be observed in the interpretation of these results because differences between treatment means, though statistically significant, were small.

**Age of milk**

Generally, as milk aged, soil deposition on dipped unrinsed plates increased, and the film became more susceptible to effects of high humidity. There was no significant change in the percentage of the initial protein left on unexposed plates as milk aged up to 14 days (Fig. 3). The significant (P < 0.05) increase observed after 16 days indicated that deteriorated milk was more tenacious even without exposure to high humidity. The amount of soil that remained on unexposed plates at 16 days, 10.68% of initial soil, approximated that from 4- or 6-day old milk on plates exposed to high humidity.

**Temperature of milk**

Although differences between treatments were small, milk deposited at 10°C rinsed readily (P < 0.05) than

**TABLE 6.** Mean quantities of protein recovered from plates soiled with milk of varying pH.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH of milk</th>
<th>6.7</th>
<th>6.7</th>
<th>7.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unexposed¹</td>
<td>µg/cm²</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
</tr>
<tr>
<td>% of unrinsed</td>
<td>µg/cm²</td>
<td>0.97d</td>
<td>0.94d</td>
<td>1.02d</td>
</tr>
<tr>
<td>Exposed²</td>
<td>µg/cm²</td>
<td>2.45</td>
<td>1.79</td>
<td>1.89</td>
</tr>
<tr>
<td>% of unrinsed</td>
<td>µg/cm²</td>
<td>6.92a</td>
<td>4.95c</td>
<td>5.60b</td>
</tr>
<tr>
<td>Unrinsed³</td>
<td>µg/cm²</td>
<td>35.42</td>
<td>36.05</td>
<td>33.73</td>
</tr>
</tbody>
</table>

¹n = 10 sets of 5 plates each.

²Where superscripts differ, means are significantly different (P < 0.05), Duncan (71).

³With exposed samples, age of milk became important on the 6th day, when a sharp rise in residue was observed (Fig. 3). Increases continued until the 14th day, but a slight decrease was noted on the 16th day.

Although pH was not measured, slight precipitation of protein was observed on the 14th day; therefore, developed acidity may have increased the sensitivity of protein to high RH. The slight decrease in residue on exposed plates at 16 days suggests partial proteolysis, hence higher protein solubility. However, unexposed samples exhibited a sharp rise in soil level at 16 days, indicating that the solubility of proteins in the milk had decreased. Proteolytic effect was not as evident in unexposed samples because protein coagulation predominated over proteolysis. Increasing adherence of residue to stainless steel as milk aged was observed by Berridge and Scurlock (8), who suggested that developed acidity was the likely cause.

**Figure 3.** Percentages of protein recovered from exposed and unexposed plates soiled with milk of increasing age. Points with different letters (a-i) were significantly different (P < 0.05).

With exposed samples, age of milk became important on the 6th day, when a sharp rise in residue was observed (Fig. 3). Increases continued until the 14th day, but a slight decrease was noted on the 16th day.
that deposited at 0, 20 or 40°C, and protein recovered was significantly higher (P < 0.05) with each successive increase in temperature, ranging from 19.5 to 33 μg/cm². Therefore, actual quantities left on plates after rinsing were highest on plates exposed to milk at 40°C (1.58 μg/cm² at 0°C vs. 2.45 μg/cm² at 40°C). Increasing temperature decreased viscosity which probably allowed quicker formation of adsorption films (17).

Reaction of separated components of milk

Generally, as components were removed from milk, less protein remained on exposed plates after rinsing (Fig. 4). More than 12% of the soil remained on plates when whole milk was used, but this amount decreased to a low of 1.5% with casein solution. When separated components were recombined as with casein plus whey (recombined skim milk) and skim milk plus cream (recombined whole milk), the amount of soil left on plates increased to 8.82% and 7.21%, respectively. Although these levels were still considerably lower than the 12.55% initially found for whole milk, the trend toward greater tenacity of soil as components were recombined was clear. There was no difference in the amount of soil remaining on exposed and unexposed plates when whey was used for soiling, and little difference was observed in the experiment with casein solution. Calcium was limited in the casein because it had been acid-precipitated, and calcium is necessary for development of stickiness. Calcium was largely in the whey, but whey lacked casein, which was later shown to be involved in formation of tenacious film.

Fat contributed to formation of tenacious film. Less residue was recovered from exposed and rinsed plates soiled with skim milk than with cream, despite the lesser amount of protein and lactose in cream than in skim milk. The influence of fat was also evident in comparisons of amounts of residue on rinsed, exposed plates soiled with whole milk and skim milk. Fat, being hydrophobic, protects the film surface against rinsing. However, phospholipids are highly surface active and may also have played a role by affecting deposition.

Limited decreases in response to high humidity observed in recombined skim and whole milks were expected because there was up to 10% dilution during isolation, separation and recombination. However, the significantly higher levels of soil obtained from plates soiled with recombined casein and whey than from plates soiled with skim milk was difficult to explain. The only likely explanation was related to the significantly thinner initial film when recombined skim milk was used (24.6 μg/cm²) than when skim milk (43.5 μg/cm²) was used. With the thinner film, more efficient moisture transfer would have been achieved. It is hypothesized that moisture penetration of thicker films is hindered, thereby decreasing the aggregative insolubilizing effect of high humidity.

Reaction of nonfat dry milk and raw milk to high humidity

More than 27% of the initial soil remained on plates exposed to high RH when raw milk was used to soil plates, but less than 11% was left from NDM. Both had practically the same soil load initially, 34.13 μg/cm² for NDM and 33.57 μg/cm² for raw milk.

The larger residue of whole milk than of NDM can be partly attributed to the hydrophobic nature of fat. However, fat in the whole milk did not cause formation of tenacious milk film without exposure to high humidity. This was evidenced by the small amount of residue on all unexposed plates soiled with each type of milk. Thus the conclusion is reinforced that proteins and salts are largely responsible for adhesion of milk upon exposure to high humidity.

Denaturation of whey protein and insolubilization of some calcium during heat-processing of NDM could have impaired its response to high humidity.

Solubility of instant nonfat dry milk exposed to high humidity

The solubility index of instant NDM increased from 0.35 to 5.74 ml (n = 5) on exposure to high humidity. The considerable decrease in solubility caused by exposure of NDM samples to high humidity indicated extensive changes in the casein. Hall and Hedrick (12) attributed the insolubility of NDM to the inability of the casein, when denatured, to form a stable dispersion when recombined with water.

Dry film was present near the top of each tube containing exposed samples (B, Fig. 5) and absent in tubes with unexposed milk (A, Fig. 5). This film failed to rinse from tubes without vigorous brushing.

Seasonal variation

Season of the year apparently affects formation of tenacious residue on plates exposed to high humidity. Representative data obtained during winter and summer months are listed in Table 7. About three times as much residue appeared on exposed plates during the cold months of November through February than appeared from May through August. This might have been related to higher protein expected in milk during the cold months than during the warm months (6). However, quantities of protein on unrinse control plates were as high in May and June as in the winter months. This result strongly suggests that the important factor was
TABLE 7. Mean percentages of protein remaining on unexposed and exposed plates and amount (µg/cm²) of protein deposited on unrinised plates during winter and summer months (n = 5).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Winter</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nov</td>
<td>Dec</td>
</tr>
<tr>
<td>Unexposed</td>
<td>0.96</td>
<td>1.01</td>
</tr>
<tr>
<td>Exposed</td>
<td>29.70</td>
<td>27.64</td>
</tr>
<tr>
<td>Unrinised</td>
<td>35.35</td>
<td>33.57</td>
</tr>
</tbody>
</table>

1Percentage of respective control.
2In µg/cm².

soil than skim milk or 10% NDM.

9. Season of the year. More tenacious residue formed during the winter than during the summer.

REFERENCES