Changes in Bacterial Cell and Spore Counts of Reduced-Fat Egg Products As Influenced by Pasteurization and Spray Drying

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ABSTRACT

Microbial counts of several laboratory-prepared defatted egg products and ingredients were determined. Commercial full-fat egg yolks (EY) were defatted with hexane, air-dried overnight, mixed with water, homogenized, pasteurized (60°C, 5 min), and spray-dried to yield a defatted egg yolk product (DEY). Egg products for scrambling (EPS) were formulated with DEY and other ingredients, processed as above, and held wet (EPS-W) or spray-dried (EPS-SD). On a dry matter basis, the log counts in colony forming units per g (log CFU/g) of EY for aerobic bacteria, yeasts and molds, and aerobic mesophilic sporeformers were low at 1.3, 1.0 and 0, respectively. For DEY these counts were 6.0, 2.4, and 4.3, respectively. These counts for DEY after pasteurization decreased by 98.6, 89.6, and 40.0%, and after spray drying decreased by 99.3, 96.1, and 83.5%, respectively, compared to the pre-pasteurization counts. For EPS-W, pasteurization reduced aerobic bacteria, yeasts and molds, and coliforms by 82.0, 86.7, and 98.7%, but did not reduce aerobic mesophilic sporeformers. Compared to prepasteurization counts, for EPS-SD the aerobic bacteria, yeasts and molds, and aerobic mesophilic sporeformer counts after pasteurization decreased by 99.7, 91.9, 99.3, and 50.0%, while after spray drying the count reductions were 99.9, 98.9, 99.9, and 85.8%, respectively. Microbial counts of finished products were below guidelines set by the U.S.D.A. for egg products. No Salmonella were detected in any of the ingredients or prototype products at any stage of processing. The combination treatment of pasteurization followed by spray drying significantly reduced the spore counts of DEY and EPS-SD, compared to pre-pasteurization counts.

Use of eggs and egg products in the food industry is vast. Eggs are generally used in mixes, bakery foods, noodles, mayonnaise and salad dressings, candies, ice cream, pet foods, etc. (7). Processed eggs are often used as ingredients in ready-to-eat foods and can be vectors for transmission of some serotypes of Salmonella. This potential foodborne hazard has been greatly reduced in the U.S. by installing careful controls in egg production and processing facilities (7,16).

During recent years, the quality of egg products has improved due to regulations and quality control procedures established by the Food and Drug Administration (FDA), the U.S. Department of Agriculture (U.S.D.A.), and the food industry. The Egg Products Inspection Act (EPIA) was passed in December 1970, and required mandatory continuous inspection of all egg product plants in the U.S. The EPIA also requires the use of wholesome raw materials and processing procedures, e.g. pasteurization, that will assure production of wholesome products that are free of Salmonella (16).

Pasteurization of liquid whole eggs and liquid yolk to reduce bacterial pathogens was first practiced by the egg-product industry in the 1930s. Pasteurization of egg products in the U.S. became virtually mandatory on June 1, 1966, with the purpose of ensuring against the presence of any pathogenic bacteria (7,11). Many studies have been done concerning the effects of various time/temperature pasteurization treatments on the microbiology and functionality of eggs and egg products (8,9,10,11,17,25,28,29,31). The U.S.D.A. requires that liquid whole eggs be heat-pasteurized to at least 60°C and held for no less than 3.5 min (11).

Generally, less than 1% of the bacteria of raw egg products survive pastureization (5,28,30). The principal genera found in pasteurized egg and egg products include Alcaligenes, Flavobacterium, Bacillus, Proteus, Pseudomonas, Escherichia, Staphylococcus, coryneform bacteria, and fecal streptococci (5,12,17,18,20,23,30). Salmonella has also been reported to survive in unpasteurized spray-dried whole eggs (21,22).

Users of eggs have demanded dried egg products having good functional properties and high quality. Such products must meet strict chemical, physical, functional, and microbiological specifications that include standards for coliforms, yeasts and molds, and Salmonella (6). Destruction of bacterial vegetative cells in dried egg products seems to be related to the prior state of the bacteria.

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Organisms being subjected to a wet heat treatment before drying are more susceptible to heat treatment than those in a dried state (6). Thus a combination of pasteurization of liquid egg products before drying and heat treatment of the finished products is practiced to help ensure low bacterial populations. However, there appears to be little published about the bacterial spore content of dried egg products (7,16).

Consumer concerns about dietary cholesterol intakes (19), presence of this fat in egg yolks (13), and a general lack of inexpensive, easier-to-use convenience forms of low-cholesterol egg products available directly to the consumer, has led to a general decline in the consumption of eggs and egg products. The low-cholesterol egg products commercially available generally have all the egg yolk removed. Research in our laboratories has focused on developing solvent extraction methods to remove fat and cholesterol from egg yolks, which can then be added back to egg products. The objective of this study was to examine the changes caused in microbiological content of defatted egg yolks and of egg products fabricated from these yolks after pasteurization and spray drying.

**MATERIALS AND METHODS**

**Sample preparations**

*Fresh egg whites (FEW).* Commercial Grade A large-size shell eggs were cracked at room temperature, the whites manually separated from yolks with a strainer, and the whites used immediately.

*Egg yolks (EY).* Commercially spray-dried, full-fat egg yolk solids (EY) were obtained from Monark Egg Corp. (Kansas City, MO). The EY were placed in a polypropylene container. The container was flushed with nitrogen, sealed with a lid, and stored at -18°C until needed. The EY served as the starting material from which all other products were prepared.

*Defatted egg yolks (DEY).* For convenience in the subsequent drying step, 100-g samples of EY were slurried with 300-ml amounts of hexane (27) in a beaker on a magnetic stir plate for 30 min at room temperature. The slurry was allowed to sit at room temperature for an additional 30 min before being filtered through Whatman #1 filter paper. The residue was rinsed twice with 100-ml amounts of fresh hexane before being air-dried as a thin film at 25°C for 14-16 h at room temperature in a laboratory fume hood, which was the best facility available for that purpose. The resulting defatted egg yolk product (DEY) was placed in polyethylene bags and stored at -18°C until needed. This product contained 56% less cholesterol and 66% less fat than the EY (data not shown).

*Spray-dried DEY (DEY-SD).* A composite of three 100-g samples of DEY, prepared as above, was mixed with 1,250 ml of water (to provide a 19.4% solids solution) and homogenized in a food processor for 5 min at room temperature. The mixture was then pasteurized to give an internal temperature of 60°C for 5 min under continuous stirring with a hand mixer in a stainless-steel pitcher which was immersed in a steam-jacketed kettle of hot water (68-70°C). After pasteurization, the yolks were immediately spray-dried in an Anhydro laboratory spray dryer (APV Anhydro, Inc., Attleboro Falls, MA) using a centrifugal atomizer with an inlet temperature of 150°C and an outlet temperature of 68-70°C.

**Egg product for scrambling-wet form (EPS-W).** An egg formulation for preparing scrambled eggs was developed by mixing and homogenizing (in a food processor for 1 min) 49.0 g of DEY-SD, 11.5 g of water, 119.2 g of liquid skim milk, 4.9 g of instant N-oil™ (as a lipid replacer, National Starch and Chemical Co., Bridgewater, NJ), and 1.6 g of Alcolec®lecithin (as an emulsifier, American Lecithin Co., Inc., Woodside, NY). Fresh egg whites (FEW, 395.9 g) were added separately and the entire formulation was pasteurized at 60°C for 5 min as above and then analyzed fresh. The EPS-W product was then held frozen until used for baking tests.

**EPS-spray-dried form, EPS-SD.** The same formulation and processing as for the EPS-W were used, except that an additional 1.000 ml of water was added to each 892 g of EPS-W mixture before pasteurization to aid in spray drying. After pasteurization, the product was spray dried as above.

**Chemical assays**

Total solids (TS) of egg products were determined by drying the samples for 6 h in a vacuum oven according to AOAC method 17.007 (2). Pre- and post-pasteurized DEY in a liquid form contained an average of 17.0% TS while post-spray-dried DEY contained an average of 96.1% TS (data not shown).

To convert microbial counts for the liquid samples from a count/ml basis to a count/g dried product basis, triplelicate 100-ml samples of each liquid product were taken, dried in a vacuum oven, weighed, and averaged. The following formula was used to convert microbial counts from counts/ml to counts/g:

\[
\text{count/g} = \frac{\text{count/ml}}{\text{g dry wt/ml}}
\]

**Microbiological assays**

Microbial counts were made on EY and EPS-W at the pre- and post-pasteurized stages, and on DEY and EPS-SD at the pre- and post-pasteurized and post-spray-dried stages using recommended methods (7). Due to limitations on amounts of the different products available, two replications were done on EY, three on EPS-W, six on DEY, and eight on EPS-SD. Microbial counts (7) were also done on skim milk (SM), lecithin, and fresh egg whites (FEW) used to formulate EPS products.

**Aerobic bacteria.** A 10-g (or 10-ml) sample was serially diluted with 0.1% sterile peptone solution. One-ml amounts of appropriate dilutions were dispensed into sterile petri dishes and duplicate pour plates prepared using Difco Plate Count Agar (PCA). Plates were incubated inverted at 30°C for 72 h before counting colonies using a Quebec®darkfield counter (AO Instrument Co., Buffalo, NY).

**Yeasts and molds.** Using the same dilution scheme as outlined for aerobic bacteria, duplicate pour plates were prepared using Difco Potato Dextrose Agar (PDA) acidified with sterile 10% tartaric acid to pH 3.5 (14). Plates were incubated inverted at 25°C for 5-7 d before counting colonies.

**Coliform bacteria.** The same dilution scheme as outlined for aerobic bacteria was used for coliform determinations. Standard procedures (15) were followed for determination of coliform bacteria and for confirmation of *E. coli* using the 5-tube MPN (Most Probable Number) procedure (4).

**Aerobic mesophile sporeformers.** Sporeformers were determined (26) using a modified method. A 10-g (or 10-ml) sample was diluted 10-fold with 90 ml of 0.1% sterile peptone solution and shaken 25 times. Volumes - 10 ml or 1 ml - of the diluted sample were pipetted into a set of two 500-ml Erlenmeyer flasks, with each flask containing 100-ml of tempered sterile Difco...
Tryptone Glucose Extract (TGE) Agar. The flasks were gently agitated to disperse the sample throughout the medium and occasional agitation. At the end of the 30 min heating period, the flasks were removed from the water bath and cooled rapidly in a 80°C; the samples were held in the water bath for 30 min with 48°C water bath for a period not exceeding 10 min. The total volume from each flask was poured into a separate set of five container of cold tap water. The flasks were then transferred to a room temperature (ca. 25°C) for 1 h. Samples were then resuscitated by adding it aseptically to a screw-cap bottle containing 90 ml of sterile lactose broth. The bottle was shaken 25 times, allowed to sit for 1 min, and then shaken 25 times again. Lids were tightened and bottles allowed to remain at room temperature (ca. 25°C) for 1 h. Samples were then resuspended by inverting bottles several times, caps were loosened, and bottles incubated at 35°C for 24 h. Subsequent isolation and identification procedures recommended by FDA for Salmonella determination. Due to limitations on amounts of products available, 10 g (or 10-ml) samples were taken rather than the 25-g (or 25-ml) samples usually specified (J). Each sample was pre-enriched by adding it aseptically to a screw-cap bottle containing 90 ml of sterile lactose broth. The bottle was shaken 25 times, allowed to sit for 1 min, and then shaken 25 times again. Lids were tightened and bottles allowed to remain at room temperature (ca. 25°C) for 1 h. Samples were then resuspended by inverting bottles several times, caps were loosened, and bottles incubated at 35°C for 24 h. Subsequent isolation and identification procedures recommended by FDA for Salmonella were then used (J).

**Statistical analyses**

Means of main and interactive effects were separated by the Least Significant Difference (LSD) test (24) at the 1% level. An analysis of variance (ANOVA) was conducted on microbial count data for the defatted egg yolks (DEY) and the spray-dried egg product for scrambling (EPS-SD) to determine differences due to treatments, product type, or product x treatment interactions.

**RESULTS AND DISCUSSION**

The microbial counts of the ingredients used to formulate the EPS products are summarized in Table 1. Counts were low for all ingredients except DEY, which compared to EY, apparently gained appreciable numbers of aerobic bacteria and aerobic mesophilic sporeformers during preparation or during the 14-16 h air-drying treatment in a chemical fume hood after hexane extraction.

For the defatted egg yolk product, DEY, counts were low for all ingredients except DEY, which compared to EY, apparently gained appreciable numbers of aerobic bacteria and aerobic mesophilic sporeformers during preparation or during the 14-16 h air-drying treatment in a chemical fume hood after hexane extraction.

For the spray-dried egg product, EPS-SD, the number of samples per code assayed were: DEY, 6; EPS-W, 3; and EPS-SD, 8.

**TABLE 1. Microbial counts of ingredients used for egg product for scrambling (EPS) products.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Aerobic bacteria</th>
<th>Yeasts and molds</th>
<th>Coliforms</th>
<th>E. coli</th>
<th>Aerobic mesophilic sporeformers</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEW</td>
<td>1.4²</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>EY</td>
<td>1.3</td>
<td>1.0</td>
<td>&lt;0.2</td>
<td>&lt;0.2</td>
<td>0</td>
</tr>
<tr>
<td>DEY</td>
<td>6.0</td>
<td>2.4</td>
<td>520</td>
<td>130</td>
<td>4.3</td>
</tr>
<tr>
<td>SM</td>
<td>2.1</td>
<td>0</td>
<td>&lt;1.0</td>
<td>ND</td>
<td>2.5</td>
</tr>
<tr>
<td>Lecithin</td>
<td>1.6</td>
<td>1.6</td>
<td>&lt;1.0</td>
<td>ND</td>
<td>1.6</td>
</tr>
</tbody>
</table>

¹Ingredient abbreviations: FEW, fresh egg whites; EY, commercially spray-dried full-fat egg yolks; DEY, hexane-defatted, air-dried egg yolks; SM, commercial liquid pasteurized skim milk.

²Values are means of two samples, each done induplicate.

³ND, not determined.

**TABLE 2. Effects of pasteurization and spray drying on microbial counts of defatted egg products for scrambling.**

<table>
<thead>
<tr>
<th>Product</th>
<th>Treatment</th>
<th>Aerobic Bacteria</th>
<th>Yeasts and molds</th>
<th>Coliforms</th>
<th>E. coli</th>
<th>Aerobic mesophilic sporeformers</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEY</td>
<td>Pre-past</td>
<td>6.0 x</td>
<td>2.4 x</td>
<td>0</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Post-past</td>
<td>4.1 y</td>
<td>1.5 y</td>
<td>89.6</td>
<td>2.3 y</td>
<td>99.6</td>
</tr>
<tr>
<td></td>
<td>Spray-dried</td>
<td>3.8 y</td>
<td>1.0 y</td>
<td>96.1</td>
<td>&lt;0.2 y</td>
<td>&lt;1.0 y</td>
</tr>
<tr>
<td>EPS-W</td>
<td>Pre-past</td>
<td>4.0 x</td>
<td>2.3 x</td>
<td>780 x</td>
<td>1.0 y</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>Post-past</td>
<td>3.3 y</td>
<td>1.5 y</td>
<td>86.7</td>
<td>&lt;10 y</td>
<td>1.0 y</td>
</tr>
<tr>
<td>EPS-SD</td>
<td>Pre-past</td>
<td>5.6 x</td>
<td>3.0 x</td>
<td>290 x</td>
<td>3.8 x</td>
<td>2.4 x</td>
</tr>
<tr>
<td></td>
<td>Spray-dried</td>
<td>3.0 y</td>
<td>1.5 y</td>
<td>91.9</td>
<td>&lt;2.0 y</td>
<td>99.3</td>
</tr>
<tr>
<td></td>
<td>Spray-dried</td>
<td>1.8 y</td>
<td>1.0 y</td>
<td>98.9</td>
<td>&lt;0.2 y</td>
<td>99.9</td>
</tr>
</tbody>
</table>

⁴Means for microbial counts are expressed on a dry matter basis. Microbial counts were subjected to ANOVA and treatment means in vertical columns within product type followed by different letters were significantly different (P<0.01) by the Least Significant Difference (LSD) test.

⁵Product codes: defatted egg yolk, DEY; egg product for scrambling-wet form, EPS-W; egg product for scrambling-spray-dried form, EPS-SD. The number of samples per code assayed were: DEY, 6; EPS-W, 3; and EPS-SD, 8.

⁶Treatment codes: pre-pasteurization, Pre-past; post-pasteurization, Post-past; and post-spray drying, Spray-dried.

²Percent reduction, calculated using numerical values.
Pasteurization of the mixture for the dry form of EPS (EPS-SD) caused significant reductions of about 99, 92, and 99%, respectively, in the counts of aerobic bacteria, yeasts and molds, and coliforms (Table 2), but did not cause significant reductions in counts of E. coli or aerobic mesophilic sporeformers. However, the combination treatment of pasteurization followed by spray-drying did not significantly reduce the counts of the latter two bacterial groups, compared to the pre-pasteurization counts. Compared to the post-pasteurization counts, the post-spray drying counts were significantly lower for the yeast and mold counts but not for the other microbial counts (Table 2). No Salmonella were detected in any of the samples of egg ingredients or egg products tested during any stage of processing (data not shown).

The analysis of variance (ANOVA) for the main and interactive effects for the products of DEY and EPS-SD indicated that treatment effect was significant (p<0.01) for the reduction of all the microorganisms tested (data not shown). There were no significant reductions in microbial counts between DEY and EPS-SD, except for aerobic mesophilic sporeformers which remained significantly higher at p<0.01 for DEY than for EPS-SD throughout treatments. There were no significant product x treatment interactive effects for DEY and EPS-SD, indicating that treatment effects were the same for these two products (data not shown).

All of the laboratory-prepared products, DEY, EPS-W, and EPS-SD, after pasteurization or spray drying had bacterial cell counts below the maximum levels permitted by the U.S.D.A. (7) for egg products of 25,000 (= log 4.4) aerobic bacteria, 10 coliforms, and 10 yeasts and molds per g.

There appears to be little published about the bacterial spore counts of, or standards/guidelines for, dried egg products (7,12,16). The commercially feasible pasteurization temperature/time treatments of 60 to 65°C for 3.5 to 5 min needed to conserve egg functionality properties are not considered adequate to kill aerobic mesophilic sporeformers. It is, therefore, interesting, that the combination of pasteurization followed by spray drying of EPS did not appear to give some small but significant reductions in EPS-SD counts of aerobic mesophilic sporeformers, which control tests (Table 1) indicate came from DEY rather than any of the other ingredients used to formulate the EPS-SD product.

These lowered aerobic mesophilic sporeformer counts in EPS-SD may have been caused in part by the indigenous lysozyme present in the egg white added to EPS. The EPS-W did not receive the spray dry treatment, which may account for the lack of reduction in the count of aerobic mesophilic sporeformers seen for this product (Table 2). Lysozyme, present as a contaminant in several commercial crystalline egg albumin samples, was reported to be bactericidal for some Bacillus spores (3).

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REFERENCES
TABLE 2. Concentration and percentage retention of elements in canned chick peas (wet weight basis).

<table>
<thead>
<tr>
<th>Element</th>
<th>Dry (mg/100 g)</th>
<th>Soaked (mg/100 g)</th>
<th>Blanched (mg/100 g)</th>
<th>Canned (mg/100 g)</th>
<th>Retention (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>116 ± 5</td>
<td>59.9 ± 2.7</td>
<td>53.4 ± 3.4</td>
<td>33.0 ± 2.8</td>
<td>28</td>
</tr>
<tr>
<td>Chloride</td>
<td>25.2 ± 7.8</td>
<td>6.96 ± 1.53</td>
<td>7.42 ± 2.22</td>
<td>602 ± 19</td>
<td>N.A.b</td>
</tr>
<tr>
<td>Copper</td>
<td>3.82 ± 2.00</td>
<td>0.89 ± 1.01</td>
<td>0.42 ± 0.37</td>
<td>0.16 ± 0.05</td>
<td>4</td>
</tr>
<tr>
<td>Iron</td>
<td>2.60 ± 0.96</td>
<td>2.45 ± 0.52</td>
<td>2.55 ± 0.19</td>
<td>1.16 ± 0.10</td>
<td>45</td>
</tr>
<tr>
<td>Magnesium</td>
<td>118 ± 5</td>
<td>62.1 ± 1.6</td>
<td>56.8 ± 3.4</td>
<td>26.5 ± 1.1</td>
<td>22</td>
</tr>
<tr>
<td>Manganese</td>
<td>2.24 ± 0.05</td>
<td>1.12 ± 0.06</td>
<td>0.99 ± 0.05</td>
<td>0.55 ± 0.02</td>
<td>25</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>140 ± 15</td>
<td>73.5 ± 8.0</td>
<td>75.9 ± 6.1</td>
<td>45.4 ± 5.4</td>
<td>32</td>
</tr>
<tr>
<td>Potassium</td>
<td>1,100 ± 21</td>
<td>515 ± 10</td>
<td>438 ± 6</td>
<td>188 ± 4</td>
<td>17</td>
</tr>
<tr>
<td>Sodium</td>
<td>19.7 ± 1.8</td>
<td>11.9 ± 1.2</td>
<td>10.2 ± 1.1</td>
<td>361 ± 10</td>
<td>N.A.b</td>
</tr>
<tr>
<td>Zinc</td>
<td>5.44 ± 1.25</td>
<td>2.06 ± 0.59</td>
<td>1.67 ± 0.18</td>
<td>0.79 ± 0.04</td>
<td>15</td>
</tr>
</tbody>
</table>

aRetention of elements in canned chick peas, on the basis of element concentration in dry chick peas being 100%. Retention = dry X 100.

bNaCl was added during canning process.

filling medium, and their concentrations varied depending on the concentration of the medium used.

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REFERENCES


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in blended eggs before and after pasteurization. Poultry Sci. 46:1321.