Title: Chlorination alternatives for soft white wheat cake flour

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Executive summary
The Idaho and Oregon Wheat Commissions and the Washington Grain Commission provided funding to the Wheat Marketing Center (WMC) to evaluate chlorination alternatives for soft white wheat (SWH) cake flours on cake quality of batter- (i.e., layer) and foam-style (i.e., sponge) cakes. A summary follows along with results in Appendix A.

Introduction:
Chlorination has long been used in some markets to improve the functionality of soft wheat flours intended for cakes. In addition to brightening the overall flour color, the chlorination process alters starch and protein characteristics of a flour, generally rendering the protein non-functional and the starch more susceptible to swelling. This not only improves batter viscosity, thereby allowing the batter to retain more and smaller gas cells for light aerated textures, but also allows for a whiter, more delicate crumb grain that is desirable in cakes.

However, the European Union (EU) has phased out chlorination treatments due to safety issues associated with the use of chlorine gas. EU consumers are also increasingly critical of any food processing treatments that are considered potentially detrimental to nutritional quality. Although there is no evidence that chlorination of flour introduces any risk to consumers, consumer demand for “clean label” products has driven the industry to reconsider any treatments or ingredients that have the perception of being unnecessary or unnatural. This trend has spread to other countries, including the United States and Canada, and is increasingly being voiced in several important wheat export markets such as the Philippines.

During technical visits, Filipino millers have requested assistance with chlorination alternatives for soft white wheat cake flours in response to requests from their bakery customers. Visiting customer teams from South America have raised similar issues. This pattern of requests demonstrates a demand in export markets for help with chlorination alternatives. This project was designed to supply technical assistance to buyers of U.S. wheat.
Milling companies in the EU have replaced chlorination with various heat treatment strategies.

- Elevated temperature/time combinations can be applied to flour to alter gluten and starch characteristics.
- Although heat treatment does not typically result in the same end product quality, some heat treatment variants come very close and EU consumers have been willing to accept the quality of cakes made from these flours.

Another alternative is replacing chlorine gas with ozone ($O_3$), which has fewer associated safety issues.

- Ozone is currently used as a strategy to reduce the use of chemicals for pest/microbial control of stored grains, where the propensity of ozone to break down into oxygen ($O_2$) and an oxygen radical destroys microbial and insect populations during grain storage.
- It is possible to utilize this natural breakdown of ozone to alter starch and protein qualities in cake flours along with an associated bleaching effect that cannot be replicated in heat treated flours.
- Current literature suggests that cake quality from ozonated flours is better than that obtained from chlorinated flours, although odor can be an issue at excessive ozonation levels.

One final option is to replace chlorination with ozonation or heat treatment and an ingredient system that can be added at the mill or bakery.

- Emulsifiers are a common ingredient used to improve cake volume, symmetry, crumb grain, and texture as well as ensure a more consistent product. It is standard practice in commercial bakeries to use emulsifiers for these reasons, and it is a logical improving ingredient that may overcome any minor deficiencies in non-chlorinated flours.
- Further work on the impact of emulsifiers on cake quality is planned at WMC for FY 2021/22.

Objectives:

- Assess chlorination alternatives for soft white wheat cake flours on cake quality of layer and sponge cakes
- Determine appropriate flour quality tests for evaluating cake baking potential of flours treated with chlorination alternatives

Summary of Results
The results of this project confirm that Japanese sponge cakes are, by design, already optimized with untreated soft white wheat flour. As expected, chlorination and ozonation did not improve cake quality. Cakes that are formulated with a lower amount of sugar (by weight) than flour do not require or benefit from the improving effects of chlorination.

- Japanese Sponge Cakes Formulated with 0% Emulsifier
  - Few differences were observed between treated flours and the untreated control in the baseline formula.
  - Treated flours offered no advantage in this type of cake in the absence of emulsifiers.

- Japanese Sponge Cakes Formulated with 0.75% Emulsifier
Texture trended toward significant softening with the addition of 0.75% emulsifiers, essentially bringing all treated flours slightly closer to the untreated control in terms of texture. However, the addition of emulsifiers did not significantly improve overall cake quality for any of the flours, including the untreated flour.

- This does not account for potential improvements in production consistency due to emulsifier use.

Treated flours offered no advantage in this type of cake, even in the presence of emulsifiers.

Layer cakes present a more significant challenge as the amount of sugar in the formula is much higher than the amount of flour. This significantly alters starch pasting properties, and flour treatment (e.g., chlorination) and/or formulation adjustments are necessary to restore maximum peak viscosity (as measured by the RVA in this study) for proper volume and setting during baking. Chlorination is known to alter starch surface properties, resulting in greater RVA peak viscosity, greater cake volumes, and better crumb structure.

Ozonation has been reported as a potential alternative to chlorination, but this study demonstrates that it does not offer the same advantages as chlorination. Additionally, the use of emulsifiers in this study worsened the quality of layer cakes made with ozonated flours, with evidence of cake collapse and firmer textures for all ozonated flours. This is significant as emulsifiers are a standard formula ingredient in layer cakes and are often used to improve the volume and texture of these cakes.

- Layer Cake Formulated with 0% Emulsifier
  - Chlorinated flour bake performance was overall significantly better than ozonated and untreated flours.
  - The cake volume and height results had a positive relationship with the 50% w/w sucrose RVA peak viscosity and batter viscosity. The increase in viscosity may indicate the extent of starch swelling and gelatinization in the presence of sugar, with greater peak viscosities allowing for better volume and setting during baking.

- Layer Cake Formulated with 0.5% Emulsifier
  - Chlorinated cake flour produced significantly superior layer cakes compared to the ozonated and untreated flour. The addition of emulsifiers did not alter that finding.
  - Ozonated flours with emulsifiers showed evidence of collapse after baking, even though the overall volumes were improved.
  - Only the chlorinated and untreated cake flour exhibited the typically softer texture associated with emulsifier use.

Overall, the study shows that ozonation is not a viable pathway to chlorination replacement, even with the use of standard formulation ingredients to improve cake quality.

Although it was part of the study design to include a heat treated soft white cake flour for comparison as a chlorination alternative, COVID lab restrictions prevented sourcing of heat treated soft white cake flour from the same base flour. While we were able to secure and test a heat treated flour from another source, we have refrained from reporting the data as it is not a scientifically valid comparison. The heat treatment did show some promise as a chlorination alternative in our testing, however, and we will
pursue heat treatment of a portion of our remaining base flour for further testing as COVID restrictions allow. Results from any subsequent heat treatment trial will be reported in an addendum to this report.

Key findings:

- All flour samples evaluated had characteristics consistent with those for cake flour and were acceptable for cake products.

- Overall, the SRC values were similar between the sample cake flours. They all had very low lactic acid SRC values, indicating low gluten functionality.
  - The SRC results showed that untreated flour had a greater lactic acid value compared to chlorinated and ozonated SWH flours, which indicated greater gluten functionality. The ozone and chlorination treatments likely decreased the gluten functionality of the flour.
  - The chlorinated flour had slightly elevated water and sodium carbonate SRC values, which indicated that chlorination may affect the swelling properties of starch granules to a greater extent compared to ozone and untreated flours.
  - The lower sucrose SRC of chlorinated flour compared to the ozone and untreated flour may indicate an interaction with arabinoxylans not observed with the ozone treatment.

- The Rapid Visco Analyzer (RVA) peak viscosity results for flour in a water mixture did not appear to be practically different between flour samples.
  - Although the temperature profile was closer to the internal temperature of a cake during baking, the results were of limited use for predicting cake quality.

- RVA peak viscosity for flour in a 50% weight/weight (w/w) sucrose solution enhanced differences in peak viscosity and may provide a more useful way to evaluate cake flour quality.
  - The chlorinated flour sample had significantly greater peak viscosity compared to the untreated and ozonated flour.
  - The results were more predictive of cake quality, especially layer cake quality, when the solvent included sucrose and the temperature profile followed internal cake temperatures during baking.
    - This is a method that should be pursued for further validation and eventual proposal (if validated) to standards organizations such as ISO, ICC, and/or AACC.

- There were slight but not significant differences in Japanese sponge cake quality between untreated, chlorinated and ozonated flours for the baseline bake with 0% emulsifier.
  - Cake volume and height were not significantly different among the sample cake flours.
  - Untreated flour had the softest texture while all other flours were similar.
  - Untreated flour had the worst external quality score with all other flours being similar.
  - Chlorinated flour had the best crumb grain followed by untreated flour and lastly the ozonated flour.
  - Untreated flour had the greatest overall quality score. The ozonated and chlorinated flours were similar.

- There were no significant differences in Japanese sponge cake quality between untreated, chlorinated and ozonated flours with 0.75% emulsifier.
Sponge cakes formulated with 0.75% emulsifier resulted in incrementally greater cake height, softer crumb texture, improved crumb grain scores (smaller, more uniform, thinner cell walls), and overall greater quality scores for the cakes produced with chlorinated and ozonated flours compared to the baseline 0% emulsifier bake.

Sponge cakes baked with untreated flour did not show significant improvements in cake height and texture compared to the 0% emulsifier bake.

Sponge cake quality is not significantly improved with flour treatment or the use of emulsifiers.

This does not account for the potential for better consistency in cake quality or e.g., slower staling for untreated flour with the use of an emulsifier.

There were significant differences observed between chlorinated and ozonated flours for layer cakes made with 0% emulsifier.

Volume and Height
- Chlorinated flour had greater cake volume and height than all other SWH flours, followed by the 20 min ozonation, 30 min ozonation, 10 min ozonation, and untreated flour.

Viscosity
- The cake volume and height results had a positive relationship with the 50% w/w sucrose RVA peak viscosity and batter viscosity. The increase in viscosity may indicate the extent of starch swelling and gelatinization in the presence of sugar, with greater peak viscosities allowing for better volume and setting during baking.

Texture
- The chlorinated flour had the firmest texture of the SWH flours in agreement with previous reports, and untreated had the softest, which matches the results from the Japanese sponge cake.

Internal score
- Chlorinated cake had the best crumb grain score followed by the 20 min and 30 min ozonation treatments.
- The untreated sample had slightly worse cell uniformity, size, and grain than the other treatments.
- The differences in internal crumb score and internal non-instrumental texture could likely be masked by adding additional ingredients such as emulsifiers.

Off-odors
- An off odor could be detected in the 10 min, 20 min, and 30 min ozonated flour.
- An off-odor could be detected in layer cake produced with 10 min, 20 min, and 30 min ozonated flour.

Layer cakes formulated with 0.5% emulsifier resulted in improved cakes produced with chlorinated and untreated flours and worse cakes produced with ozonated flour compared to the baseline 0% emulsifier bake.

Volume and Height
For the layer cake baked with chlorinated flour, the addition of 0.5% emulsifier resulted in a significant increase in volume and an incremental increase in cake height compared to the 0% emulsifier bake.

Chlorinated flour had greater cake volume and height than all other SWH flours, followed by the 20 min ozonation, 10 min ozonation, untreated flour and 30 min ozonation treatment.

The emulsifier allowed for greater expansion in the oven for each cake compared to the baseline formula. After cooling, the cakes produced with ozonated flours collapsed, resulting in cakes with less height compared to the baseline bake.

The chemical changes imparted by chlorination on flour allowed for the layer cake to maintain its structure and take advantage of the improving characteristics of the 0.5% emulsifier addition.

- This is likely a reflection of the starch swelling capacity of chlorinated flour, as evidenced by its greater sucrose RVA peak viscosity.
- Although reports show ozonation to influence flour lipid functionality, it does not appear to improve starch functionality to the same extent as chlorination.

### Viscosity

- The cake volume and height results did not have as direct of a relationship with the 50% w/w sucrose RVA peak viscosity and batter viscosity. This is likely due to the collapse of the cakes produced with ozonated flour. The layer cake formulated with chlorinated flour and 0.5% emulsifier still displayed a strong relationship between its 50% w/w sucrose RVA peak viscosity and cake quality.
- The addition of 0.5% emulsifier resulted in increased batter viscosity (smaller Bostwick Consistometer reading) compared to the baseline formulation for all flours.
  - This is due to the emulsifier aiding in the incorporation of more air cells in the batter.
- The emulsifier aided in the incorporation of more air cells that were more finely dispersed in the batter, resulting in greater volume and finer crumb structures.

### Texture

- The layer cakes produced with ozonated flours collapsed upon cooling, resulting in firmer textures compared to the 0% added emulsifier bake.
- The chlorinated and untreated flours produced cakes with the softest texture and were softer compared to their 0% emulsifier formulation counterpart as expected.

### Internal score

- Chlorinated cake had the best crumb grain score followed by the 10 min, 30 min, and 20 min ozonation and untreated treatments.
- The chlorinated, 10 min ozonation, and untreated flours had improved crumb grain compared to the 0% emulsifier bake.
- The internal crumb score and internal non-instrumental texture values were improved by the emulsifier for some of the flour treatments, but they did not completely mask differences noted in the baseline 0% emulsifier bake.

### Off-odors

- An off-odor could be detected in 10 min, 20 min, and 30 min ozonated flour.
An off-odor could be detected in layer cake produced with 10 min, 20 min, and 30 min ozonated flour.

- Off-flavors
  - An off-flavor could be tasted in layer cake at 10 min, 20 min, and 30 min of ozonation.
  - The addition of emulsifiers would provide a path to improving layer cake quality. Emulsifiers would be expected to increase batter viscosity by incorporating additional air into the batter resulting in greater volume, tenderness, and finer grain.

Conclusions
The results confirm that Japanese sponge cakes are, by design, already optimized with untreated soft white wheat flour. Chlorination and ozonation do not improve cake quality, as expected. Cakes that are formulated with a lower amount of sugar (by weight) than flour do not require or benefit from the improving effects of chlorination.

Layer cakes present a more significant challenge as the amount of sugar in the formula is much higher than the amount of flour. This significantly alters starch pasting properties, and flour treatment (e.g., chlorination) and/or formulation adjustments are necessary to restore maximum peak viscosity (as measured by the RVA in this study) for proper volume and setting during baking. Chlorination is known to alter starch surface properties, resulting in greater RVA peak viscosity, greater cake volumes, and better crumb structure. Ozonation has been reported as a potential alternative to chlorination, but this study demonstrates that it does not offer the same advantages as chlorination. Additionally, the use of emulsifiers in this study worsened the quality of layer cakes made with ozonated flours, with evidence of cake collapse and firmer textures for all ozonated flours. This is significant as emulsifiers are a standard formula ingredient in layer cakes and are often used to improve the volume and texture of these cakes.

Overall, the study shows that ozonation is not a viable pathway to chlorination replacement, even with the use of standard formulation ingredients to improve cake quality.

Although it was part of the study design to include a heat treated soft white cake flour for comparison as a chlorination alternative, COVID lab restrictions prevented sourcing of heat treated soft white cake flour from the same base flour. While we were able to secure and test a heat treated flour from another source, we have refrained from reporting the data as it is not a scientifically valid comparison. The heat treatment did show some promise as a chlorination alternative in our testing, however, and we will pursue heat treatment of a portion of our remaining base flour for further testing as COVID restrictions allow. Results from any subsequent heat treatment trial will be reported in an addendum to this report. Additional formulation strategies to improve cake quality will be explored in FY 2021/2022 if heat treated flour appears to provide a performance advantage.

Finally, the Wheat Marketing Center thanks the wheat and grain commissions of Idaho, Oregon and Washington for their support for this research project. Questions and comments are welcome and may be directed to Dr. Jayne Bock at jbock@wmcinc.org.
Appendix A

Experimental Design:

Samples:

SWH wheat: 8.5 – 9.5% protein content
Chlorinated control flour

Treatments:

Heat treatment (Not possible; see above)
Ozone treatment (10, 20, and 30 min treatments with a flow rate of 0.06 L/min)
Ingredient systems (emulsifiers)

Procedures:

1. Base soft white wheat cake flour and chlorinated soft white wheat cake flour (from same base flour) provided by a commercial mill
2. Collaborated with partners to produce ozonated flours at specified conditions
3. Flour analyses including moisture, protein, ash, falling number, pH, SRC, and RVA in water or a 50% w/w sucrose system
   a. Water RVA with a temperature profile mimicking internal cake temperature during baking
   b. 50% w/w sucrose RVA with a temperature profile mimicking internal cake temperature during baking
4. Cake making – Approximately 165 cakes were baked as follows:
   a. A preliminary series of layer cakes were baked to determine the optimum formula water
      i. 3 formula water levels with 6 cakes baked at each level
   b. A preliminary series of layer cakes were baked to determine the appropriate shortening system
      i. 2 shortening systems were tested with 6 cakes baked for each system
   c. A preliminary series of sponge cakes were baked to determine the optimum emulsifier addition level
      i. 3 emulsifier levels with 3 cakes baked at each level
   d. A preliminary series of layer cakes were baked to determine the optimum emulsifier addition level
      i. 3 emulsifier levels with 6 cakes baked at each level
   e. Control sponge and layer cakes were baked with a baseline formula to assess quality characteristics of each flour
      i. 3 sponge cakes were baked for each flour
      ii. 6 layer cakes were baked for each flour
   f. Sponge and layer cakes were baked with the addition of their respective emulsifier addition level to assess the quality improvement effect for each flour
      i. 3 sponge cakes were baked for each flour
      ii. 6 layer cakes were baked for each flour
5. Cake evaluation
   a. Batter viscosity, batter pH, moisture, cake volume, cake height, cake symmetry, crumb color, crumb texture, external/internal cake score
Table 1. Flour Characteristics for Chlorinated, Ozonated, and Untreated Flour.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Moisture (%)</th>
<th>Ash (%)</th>
<th>Protein (%)</th>
<th>Falling Number (sec.)</th>
<th>Flour pH</th>
<th>RVA Water Peak Viscosity (RVU)</th>
<th>RVA Sucrose Peak Viscosity (RVU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>11.63±0.02</td>
<td>0.401±0.002</td>
<td>8.06±0.04</td>
<td>399±13</td>
<td>4.72±0.01</td>
<td>196.8±2.1ab</td>
<td>345.5±5.6ab</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>10.15±0.01</td>
<td>0.398±0.003</td>
<td>8.00±0.07</td>
<td>402±14</td>
<td>5.62±0.02</td>
<td>188.3±4.7a</td>
<td>283.6±4.6bc</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>9.75±0.03</td>
<td>0.398±0.002</td>
<td>8.11±0.13</td>
<td>483±25</td>
<td>5.46±0.01</td>
<td>202.6±0.1b</td>
<td>303.0±4.3d</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>10.02±0.02</td>
<td>0.400±0.001</td>
<td>8.09±0.04</td>
<td>446±6</td>
<td>5.53±0.01</td>
<td>196.9±0.6ab</td>
<td>300.2±1.6bd</td>
</tr>
<tr>
<td>Untreated</td>
<td>13.70±0.06</td>
<td>0.369±0.008</td>
<td>8.26±0.02</td>
<td>333±21</td>
<td>5.78±0.01</td>
<td>188.6±5.0a</td>
<td>282.1±4.5c</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significantly different at P < 0.05.

1A 50% w/w sucrose solution was used.

Table 2. Solvent Retention Capacity (SRC) values for Chlorinated, Ozonated, and Untreated Flour.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Water SRC (%)</th>
<th>Lactic Acid SRC (%)</th>
<th>Sodium Carbonate SRC (%)</th>
<th>Sucrose SRC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>57.9±0.4a</td>
<td>74.2±0.8a</td>
<td>74.8±0.1a</td>
<td>90.6±1.4a</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>55.7±0.1bc</td>
<td>73.0±0.3ab</td>
<td>72.0±0.3b</td>
<td>94.9±1.2b</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>57.4±0.5a</td>
<td>72.3±0.5b</td>
<td>72.1±0.5b</td>
<td>93.3±0.8ab</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>56.6±0.7abc</td>
<td>72.2±0.7b</td>
<td>72.3±0.6b</td>
<td>93.3±1.2ab</td>
</tr>
<tr>
<td>Untreated</td>
<td>55.5±1.1c</td>
<td>77.4±0.4c</td>
<td>72.6±0.4b</td>
<td>94.7±0.3b</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significantly different at P < 0.05.
### Table 3. Characteristics of Japanese Sponge Cake Formulated with 0% Emulsifier.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Moisture (%)</th>
<th>Weight (g)</th>
<th>Volume (mL)</th>
<th>Height (mm)</th>
<th>Compression Force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>29.0±0.3</td>
<td>300.1±1.7</td>
<td>1155±25</td>
<td>82±2</td>
<td>428±16</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>28.7±0.1</td>
<td>301.0±0.8</td>
<td>1147±21</td>
<td>81±2</td>
<td>408±41</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>29.7±0.2</td>
<td>300.8±0.7</td>
<td>1164±5</td>
<td>83±1</td>
<td>412±18</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>29.2±0.4</td>
<td>301.3±0.5</td>
<td>1162±11</td>
<td>82±1</td>
<td>422±26</td>
</tr>
<tr>
<td>Untreated</td>
<td>31.0±0.2</td>
<td>297.9±0.2</td>
<td>1150±12</td>
<td>81±2</td>
<td>345±21</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significantly different at P < 0.05.

### Table 4. Quality Evaluation of Japanese Sponge Cake Formulated with 0% Emulsifier.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>External Factors</th>
<th>Crumb Grain</th>
<th>Texture</th>
<th>Overall Quality Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>13±0</td>
<td>20±0</td>
<td>10±2</td>
<td>43±2</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>13±0</td>
<td>18±0</td>
<td>12±5</td>
<td>43±5</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>13±0</td>
<td>18±0</td>
<td>13±2</td>
<td>44±2</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>13±0</td>
<td>18±0</td>
<td>12±3</td>
<td>43±5</td>
</tr>
<tr>
<td>Untreated</td>
<td>12±0</td>
<td>19±0</td>
<td>19±2</td>
<td>50±2</td>
</tr>
</tbody>
</table>

### Table 5. Characteristics of Japanese Sponge Cake Formulated with 0.75% Emulsifier.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Moisture (%)</th>
<th>Weight (g)</th>
<th>Volume (mL)</th>
<th>Height (mm)</th>
<th>Compression Force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>35.2±0.6</td>
<td>298.1±0.3</td>
<td>1185±16</td>
<td>84±1</td>
<td>375±33</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>34.7±0.1</td>
<td>298.3±1.2</td>
<td>1164±6</td>
<td>83±0</td>
<td>398±19</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>35.8±0.1</td>
<td>299.4±0.7</td>
<td>1163±25</td>
<td>83±2</td>
<td>382±24</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>36.4±0.5</td>
<td>298.9±0.5</td>
<td>1166±12</td>
<td>83±1</td>
<td>384±16</td>
</tr>
<tr>
<td>Untreated</td>
<td>34.4±0.2</td>
<td>297.3±0.1</td>
<td>1149±13</td>
<td>82±1</td>
<td>349±4</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significantly different at P < 0.05.
Table 6. Quality Evaluation of Japanese Sponge Cake Formulated with 0.75% Emulsifier.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>External Factors</th>
<th>Crumb Grain</th>
<th>Texture</th>
<th>Overall Quality Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>13±0</td>
<td>21±0</td>
<td>13±6</td>
<td>47±6</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>13±0</td>
<td>20±0</td>
<td>8±3</td>
<td>41±3</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>13±0</td>
<td>21±0</td>
<td>12±5</td>
<td>46±5</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>13±0</td>
<td>21±0</td>
<td>12±3</td>
<td>46±3</td>
</tr>
<tr>
<td>Untreated</td>
<td>12±0</td>
<td>19±0</td>
<td>18±0</td>
<td>49±0</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significantly different at P < 0.05.

Table 7. Characteristics of Layer Cake Formulated with 0% Emulsifier.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Bostwick Consistometer (cm)</th>
<th>Batter pH</th>
<th>Moisture (%)</th>
<th>Weight (g)</th>
<th>Volume (mL)</th>
<th>Height (mm)</th>
<th>Compression Force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>5.9±0.4³</td>
<td>6.89±0.04³</td>
<td>29.51±0.4³</td>
<td>389.7±1.4³</td>
<td>835±6³</td>
<td>48±1³</td>
<td>776±16³</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>9.5±0.3³</td>
<td>7.00±0.06³</td>
<td>29.74±0.3³</td>
<td>390.6±4.0³</td>
<td>790±1³bc</td>
<td>46±1³bc</td>
<td>690±1³</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>8.2±0.2³</td>
<td>6.92±0.06³</td>
<td>29.66±0.37³</td>
<td>390.7±1.0³</td>
<td>814±11²b</td>
<td>47±0³</td>
<td>687±11³</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>8.3±0.2³</td>
<td>6.89±0.01³</td>
<td>29.75±0.16³</td>
<td>393.0±1.3³</td>
<td>805±1³bc</td>
<td>47±0³</td>
<td>709±25³b</td>
</tr>
<tr>
<td>Untreated</td>
<td>10.6±0.2³b</td>
<td>7.04±0.01³</td>
<td>27.94±0.25³</td>
<td>393.1±1.9³</td>
<td>777±13³c</td>
<td>45±0³b</td>
<td>673±35³b</td>
</tr>
</tbody>
</table>

Table 8. Quality Evaluation of Layer Cake Formulated with 0% Emulsifier.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Uniformity</th>
<th>Size</th>
<th>Thickness of Walls</th>
<th>Grain</th>
<th>Moistness</th>
<th>Tenderness</th>
<th>Crumb Color</th>
<th>Flavor</th>
<th>Overall Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>10±0</td>
<td>10±0</td>
<td>10±0</td>
<td>16±0</td>
<td>10±0</td>
<td>22±0</td>
<td>10±0</td>
<td>10±0</td>
<td>98±0</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>8±3</td>
<td>7±1</td>
<td>6±0</td>
<td>12±3</td>
<td>10±0</td>
<td>22±0</td>
<td>8±0</td>
<td>0±0</td>
<td>73±7</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>10±0</td>
<td>8±0</td>
<td>7±1</td>
<td>14±0</td>
<td>10±0</td>
<td>22±0</td>
<td>8±0</td>
<td>0±0</td>
<td>79±1</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>10±0</td>
<td>8±0</td>
<td>7±1</td>
<td>14±0</td>
<td>10±0</td>
<td>22±0</td>
<td>8±0</td>
<td>0±0</td>
<td>79±1</td>
</tr>
<tr>
<td>Untreated</td>
<td>6±0</td>
<td>5±1</td>
<td>2±0</td>
<td>10±0</td>
<td>10±0</td>
<td>22±0</td>
<td>6±0</td>
<td>10±0</td>
<td>71±1</td>
</tr>
</tbody>
</table>
Table 9. Characteristics of Layer Cake Formulated with 0.5% Emulsifier.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Bostwick Consistometer (cm)</th>
<th>Batter pH</th>
<th>Moisture (%)</th>
<th>Weight (g)</th>
<th>Volume (mL)</th>
<th>Height (mm)</th>
<th>Compression Force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>3.4±0.1^a</td>
<td>6.83±0.03^a</td>
<td>31.2±0.3^a</td>
<td>396.0±0.2^a</td>
<td>894±9^a</td>
<td>49±0^a</td>
<td>691±6^ad</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>4.5±0.2^a</td>
<td>6.93±0.11^a</td>
<td>30.4±0.2^b</td>
<td>396.9±0.7^a</td>
<td>822±1^b</td>
<td>45±0^b</td>
<td>745±47^ab</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>3.6±0.5^a</td>
<td>6.90±0.06^a</td>
<td>30.4±0.2^b</td>
<td>393.8±2.9^a</td>
<td>825±0^b</td>
<td>45±0^b</td>
<td>838±36^bc</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>3.4±0.1^a</td>
<td>6.87±0.01^a</td>
<td>31.2±0.1^a</td>
<td>395.6±3.0^a</td>
<td>808±1^b</td>
<td>44±0^b</td>
<td>925±16^c</td>
</tr>
<tr>
<td>Untreated</td>
<td>6.5±0.6^b</td>
<td>6.86±0.01^a</td>
<td>30.6±0.2^ab</td>
<td>395.4±0.2^a</td>
<td>815±4^b</td>
<td>45±0^b</td>
<td>625±16^d</td>
</tr>
</tbody>
</table>

Means with different letters in the same column are significantly different at P < 0.05.

Table 10. Quality Evaluation of Layer Cake Formulated with 0.5% Emulsifier.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Uniformity</th>
<th>Size</th>
<th>Thickness of Walls</th>
<th>Grain</th>
<th>Moistness</th>
<th>Tenderness</th>
<th>Crumb Color</th>
<th>Flavor</th>
<th>Overall Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated</td>
<td>10±0</td>
<td>10±0</td>
<td>10±0</td>
<td>16±0</td>
<td>10±0</td>
<td>24±0</td>
<td>10±0</td>
<td>10±0</td>
<td>100±0</td>
</tr>
<tr>
<td>10 min ozonated</td>
<td>10±0</td>
<td>8±0</td>
<td>8±0</td>
<td>14±0</td>
<td>10±0</td>
<td>22±0</td>
<td>8±0</td>
<td>0±0</td>
<td>80±0</td>
</tr>
<tr>
<td>20 min ozonated</td>
<td>8±0</td>
<td>8±0</td>
<td>8±0</td>
<td>13±1</td>
<td>6±0</td>
<td>20±0</td>
<td>8±0</td>
<td>0±0</td>
<td>71±1</td>
</tr>
<tr>
<td>30 min ozonated</td>
<td>8±0</td>
<td>10±0</td>
<td>10±0</td>
<td>14±0</td>
<td>6±0</td>
<td>20±0</td>
<td>8±0</td>
<td>0±0</td>
<td>76±0</td>
</tr>
<tr>
<td>Untreated</td>
<td>8±0</td>
<td>6±0</td>
<td>6±0</td>
<td>10±0</td>
<td>10±0</td>
<td>22±0</td>
<td>6±0</td>
<td>10±0</td>
<td>78±0</td>
</tr>
</tbody>
</table>
Figure 1. Japanese Sponge Cake Baseline Bake from: Top L to R: Chlorinated, 10 min Ozonation, 20 min Ozonation, 30 min Ozonation, Untreated.
Figure 2. Japanese Sponge Cake with 0.75% Emulsifier from: Top L to R: Chlorinated, 10 min Ozonation, 20 min Ozonation, 30 min Ozonation, Untreated.
Figure 3. Layer Cake Baseline Bake from: Top L to R: Chlorinated, 10 min Ozonation, 20 min Ozonation, 30 min Ozonation, Untreated.
Figure 4. Layer Cake with 0.5% Emulsifier from: Top L to R: Chlorinated, 10 min Ozonation, 20 min Ozonation, 30 min Ozonation, Untreated.