Quality Loss Estimation in Frozen Apples

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ABSTRACT
The current study focused on the analysis of quality loss during freezing and thawing of Royal Gala apples under three different freezing conditions namely, freezing in a static freezer (SF) at -18°C, freezing in an air blast freezer at -40°C (IF), and freezing in an air blast freezer at -72°C (FF). The frozen samples were thawed at room temperature (20±1°C) for 2 hours prior to quality loss tests. Two types of evaluation was done namely colour and solute diffusion tests. The solute diffusion test revealed that SF samples had higher quality loss than IF and FF samples which was supported by the colour test. The ΔE values were found to be 30.39±0.23, 25.74±0.21, 24.01±0.17 for SF, IF and FF samples respectively. The browning index (BI) value of SF was found to be 100.39 which indicated a major loss in static frozen samples which coincided with the hue and chroma value. The solute diffusion test proved to be a better method in estimating the quality loss in frozen-thawed Royal Gala apple samples.

Keywords: Apples, Colour, Solute diffusion test, Quality loss.

INTRODUCTION
The consumption of fruits have increased over the years, but preserving their quality and increasing their stability is a challenging task (Herremans et al., 2015, Vicent et al., 2017). In general, the fruits have higher water content which is the major cause for its shorter shelf life. Many methods exists in the preservation of fruits but freezing remains to be the best preservation method as it helps in maintaining original properties, similar to as the fresh one (Alvarez & Canet, 2000, Canet et al., 2006). Though freezing has higher advantages, some factors such as stress in cell volume, movement of water (dislocation), cracks developed during freezing and changes to the cellular structure on thawing proves to be disadvantageous (Cano et al., 1999). The quality loss of fruits and vegetables is accompanied by several chemical and biochemical deteriorative reactions that affects important characteristic parameters such as texture, flavor, colour, pH, vitamins and structural changes during and its subsequent storage (Cano et al., 1993, Cano et al., 1992, Cano et al., 1990, Füster et al., 1994, Lim et al., 2006, Murray et al.,1968, Murray et al., 1976, Oruna-Concha et al.,1997, Jha et al., 2018, Rastogi et al., 2002).


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There are direct and indirect methods to detect the quality loss in fruits and vegetables (Chassagne-Bercès et al., 2009, Lim et al., 2006 Mousavi et al., 2005, Mousavi et al., 2007, Parniakov et al., 2015, Jha et al., 2018), but colour which is the most important feature that satisfies human perception is a major indicator of quality changes during freezing and thawing (Francis, 1995). The colour changes in the fruits are mainly attributed to the carotenoids for orange, yellow and red fruits and the colour changes in the majority of the berries are due to the anthocyanins (Cano et al., 1990, Lim, et al 2006, Jha et al., 2018, Skrede et al., 1996). After freezing and thawing the color of the frozen fruit is prone undesirable changes due to chemical, biochemical and physiological modifications (Jha et al., 2018). One of the major reasons is the enzymatic browning that is caused due to the enzymes such as polyphenol oxidases in fruits such as apples (Chassagne-Bercès et al., 2009). Another method to detect the quality loss is the solute diffusion/gain measurement. The fruits and vegetables when treated with solutes such as sucrose, osmotic mass transfer takes place that causes alteration in the permeability of the cell membrane. The change in the cell membrane permeability increases the rate of mass transfer of solutes and water during the treatment. Various factors such as temperature and concentration of the solute, the size and the shape of the fruit/vegetable, solution to mass ratio and the speed of the agitation play a very important role in the diffusivity of the solute in the fruit (Rastogi et al., 2002). Thus this method can be considered to be a global method in determining the quality loss during freezing due to the fact that freezing causes ruptures in the cell membrane which facilitates the diffusion of solute in to the material. Since this method is not commonly used the impact of the method in determining the freeze damage is difficult (Jha et al., 2018).

Based on these findings, the current study focused on determining the quality loss during freezing and thawing in royalty gala apples. Two methods namely colour and sugar diffusivity test was carried out to analyse and differentiate the damages caused by three different freezing protocols viz., (i) Freezing in a static freezer (SF) at -18°C (ii) Freezing in an air blast freezer at -40°C (IF) (iii) Freezing in an air blast freezer at -72 °C (FF).

MATERIALS AND METHODS

2.1 Raw materials, Freezing and Thawing Protocol:
Royal Gala apples (Malus domestica 'gala') were purchased from a local market from Nantes, France and were stored at -4°C cold storage until frozen by the three conditions namely SF, slow freezing at -18°C in a static freezer (FCB400EA, Firstline, Denmark), IF, intermediate freezing at -40°C (Servathin, Poissy, France), FF (VC7018, Vötisch, Avionik Straubing Entwicklungs GmbH, Germany). After freezing, the samples were stored at -40°C static freezer until further experiments were conducted. The size of apples were varied as per the requirements of the experiments and is explained in the further sections. For thawing, zip lock pouches of size 18 cm x 20 cm (Elembal, France) was used. All the samples were thawed for a period of 2 hours at room temperature (20±1 °C) before performing the colour and sugar diffusion tests. The results were compared with the fresh unfrozen apple samples.

2.2 Analysis of sucrose gain by the apple cylinders
For this experiment, the samples were cut from middle parenchymal region using a cork borer (0.8 cm in diameter) and finally they were resized to 1 cm x 0.8 cm cylinders using a scalpel and then frozen and stored as per the protocol mentioned in the section 2.1. The sucrose gained by the apple cylinders can be a quality parameter in evaluating the quality loss in the apple subjected to different freezing processes. The experimental setup used for sucrose diffusivity test is shown in Figure 1. Six frozen apple cylinders of known weight were thawed first and installed placed in tea snap ball infuser. They were then put in a vessel containing sugar solution (20% of sucrose in distilled water (wt/wt)). The TSS
content of the sugar measured at the beginning of the test was \( \approx 16.30 \text{ Brix} \). An agitator (RW16 basic, IKA labortechnik, Germany) was used to ensure homogeneous concentration during 3 h of experiment at a temperature of 20\( \pm \)1\(^\circ\)C. The samples were removed from the sugar solution after every 30 min during the test period (till 3 h) and were placed in a hot air oven (maintained at 105 \( \pm \) 2 \(^\circ\)C) for 24 hours to determine the dry matter content of the samples. From the dry matter values, the amount of sugar gain (i.e. the difference between the initial dry matter of the respective batch and the dry matter after diffusion test) as a function of time was obtained. The TSS content of the osmotic solution measured at the beginning and the end of the test and was not significantly affected during the test (measured as \( \approx 16.30 \text{ Brix} \) before and after the test). The experiments were repeated in triplicates for each freezing conditions.

2.3 Color of the apple

The colour of the apple was measured using a portable and handheld chroma meter CR-400 (Konica Minolta, Inc. Japan) at 21\( \pm \)2\(^\circ\)C for fresh and immediately frozen-thawed samples. The cylindrical shaped sample used for measuring sugar diffusion, did not suit this study, as the size of the sample was comparatively smaller than the diameter of the light projection tube of the chroma meter. When small cylinders were analyzed, it was observed that the chroma meter was not only captured the details about the sample but also from the background, resulting in a false estimation of CIE colour parameters (i.e. L*, a*, b*). Hence, samples (polygonal shaped, 1.8\times1.6\times0.6 \text{ cm}^3 \text{ height} \times \text{breadth} \times \text{thickness}) covering the entire diameter of the light projection tube was chosen to study the freezing effect on colour parameters of apple.

The colour results were reported as L* value (corresponds to the lightness), a* value (corresponds to the green to red color axis (redness)) and b* value (corresponds to the blue to yellow axis (yellowness) (Chassagne-Berces et al., 2010). Further, \( \Delta E \) (total colour difference) (Equation. 1), chroma (Equation. 2), Hue (Equation. 3), browning index (BI) (Equation. 4), values were calculated using CIE color parameters (Barreiro, et al., 1997, Lopez et al.,1997, Maskan et al., 2001) and used to describe the colour changes associated with freezing-thawing process.

\[
\Delta E = [(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2]^{1/2} \quad \ldots (1)
\]

\[
\text{Chroma} = ((a^*)^2 + (b^*)^2)^{1/2} \quad \ldots (2)
\]

\[
\text{Hue angle} = tan^{-1} \frac{b^*}{a^*} \quad \ldots (3)
\]

\[
BI = \left( \frac{a^* - 0.31}{0.17} \right) * 100 \quad \ldots (4)
\]

2.4 Statistical Analysis:
The data obtained from the experiments such as colour and sugar diffusion test were subjected to one way ANOVA (Analysis of Variance). The level of statistical significance was determined at p<0.05.

RESULTS AND DISCUSSION

3.1 Quality loss estimation by Solute gain

From the preliminary studies, it was evident that an unstable phenomenon of gaining and losing solute occurred during the treatment period. At present, the mechanism for such trend of solute gain (during diffusion test) inside the apple cylinders is a bit complicated to explain. However, some interesting results related to solute diffusivity in the case of fresh and frozen-thawed sample are presented below. Fig. 2 presents the solid gain by the fresh and frozen-thawed sample during osmotic treatment of three hours. It can be seen that the fresh sample gained comparatively less solute than all frozen and thawed samples. This provides an evidence that ice formation and melting during the freezing-thawing process might have distorted
the cell structures and increased the permeability of cell membranes, ultimately resulting in a higher solute gain (Alizadeh et al., 2009). In fact, diffusion in intact cellular tissues (e.g. fruits and vegetables) occurs mainly in the intercellular spaces. When the pectocellulosic walls are disrupted, different path appears resulting in an increase in mass diffusivity. Furthermore, the damage of the disrupted pectocellulosic walls could explain the higher solid intake for damaged tissues. The solute uptake for all freezing condition was found to be inconsistent during the test period; the phenomenon of gaining and losing solute occurred during the treatment period for all conditions. Thus, fitting these data into diffusion model and obtaining mass transfer coefficient value that would allow easy comparison (between freezing protocols) was not possible. The inconsistency in the solute gain was observed mainly between 0.5 to 2 h for all freezing trials. The solute gain for each condition reached steady state after 2 h. In order to compare the freezing methods, the average value of solute gain after steady state was considered (average of data at 2, 2.5 and 3 h). It was observed that FF conditions resulted in significantly (p < 0.05) lower solute gain (4.71 ± 0.60%) during treatment period than SF (6.26 ± 0.51%) condition depicting better preservation of cellular structure and cell membranes integrity under FF condition. As evident in Fig. 2, SF process favored the formation larger ice crystal in the apples, and this would have caused higher dehydration of the cells, thus resulting in greater breakage of cell wall and higher solid intake (during osmotic treatment). The solute uptake by IF (5.63 ± 0.74%) sample was neither significantly different with FF sample nor with SF sample. Based on these results, it can be concluded that mass diffusivity methods can only detect a large difference in the quality or can discriminate the freezing protocols when the magnitude of the difference between freezing rates (offered by freezing conditions) is very high.

3.2. Colour as a parameter in determining quality loss
The color of a food sample is a characteristic key feature in determining the quality of a product. A considerable difference is seen in the color values of fresh and frozen-thawed apple samples with the three different freezing conditions. The L* value significantly decreased after freezing-thawing. The a* value significantly increased after freezing and thawing (p<0.05). The total color differences were also significant with respect to different freeze-Thaw conditions. The results were in agreement with the work done on apple-<i>Malus domestica</i> ‘Borkh’ by Alizadeh et al., 2009 (Chassagne-Berces et al., 2010).

The hue, chroma and browning index were calculated and tabulated in Table 3. There was no much variation in hue angle values before and after freezing. The hue angle indicated an orange-red hue (When Hue < 90º). The chroma value signifies the saturation of color and is proportional to the strength of the color. A very minute change was found between fresh and frozen-thawed samples. This indicates stability of yellow color in the apple cylinders. Another important color parameter was, BI which is an indicator of purity of brown color, which is an important parameter in enzymatic and non-enzymatic browning processes. The highest BI was recorded for SF (100.39) followed by IF (99.44) and FF (85.35) apple samples when compared with Fresh Samples (43.76). It was evident that there was a significant difference between the fresh and frozen-thawed samples and also between different Freezing conditions. The FF apple samples had lesser BI when is indicative of the fact that it had a slow degradation rate. The color differences after freezing and thawing was due to the polyphenolic compounds present in the apples and they are predominant in cascading the enzymatic browning reactions which is majorly responsible for the color change according to the investigations of (Mastrocola, Pittia, & Lerici) (Mastrocola et al., 1996) on apples.
3.3 Comparison of quality loss estimation using colour and solute diffusion test
From both the experiments, it is evident that the quality loss was higher in the SF samples when compared to IF and FF samples. The SF samples had suffered a greater quality loss due to the fact the slow freezing produces larger sized irregular shaped ice crystals that damages the cell wall material and collapses the internal structure. On the other hand, the structure could be well preserved for IF and FF samples which might be due to the uniform sized ice crystal formation during freezing. The apple cylinders when subjected to solute diffusivity test could better differentiate the quality loss as the steady gain in sucrose which was higher for SF samples is due to the heavier damage experienced by the SF samples during freezing than by IF and FF apple samples. The ΔE values were also in agreement with the fact that SF samples experienced higher quality loss.

Fig. 1: Experimental set up for sugar gain measurement

Fig. 2: Solid gain for fresh and frozen-thawed sample
Table 1: L*, a*, b* values of apple cylinders before and after freezing

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fresh Samples</th>
<th>Slow Freezing</th>
<th>Intermediate Freezing</th>
<th>Fast Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>67.01±0.68</td>
<td>41.03±0.43</td>
<td>44.51±0.21</td>
<td>47.33±0.79</td>
</tr>
<tr>
<td>a*</td>
<td>-7.65±0.45</td>
<td>6.58±0.26</td>
<td>5.26±0.28</td>
<td>5.63±0.31</td>
</tr>
<tr>
<td>b*</td>
<td>27.15±0.82</td>
<td>24.94±0.37</td>
<td>25.31±0.24</td>
<td>25.82±0.38</td>
</tr>
<tr>
<td>ΔE</td>
<td>-</td>
<td>30.39±0.23</td>
<td>25.74±0.21</td>
<td>24.01±0.17</td>
</tr>
</tbody>
</table>

Values with different letters within same row differ significantly (p<0.05)

Table 2: Chroma, Hue and BI values of apple cylinders under different Conditions

<table>
<thead>
<tr>
<th>Samples</th>
<th>Chroma</th>
<th>Hue</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh</td>
<td>29.38</td>
<td>57.23</td>
<td>43.76</td>
</tr>
<tr>
<td>SF</td>
<td>25.79</td>
<td>57.24</td>
<td>100.39</td>
</tr>
<tr>
<td>IF</td>
<td>26.04</td>
<td>57.28</td>
<td>94.44</td>
</tr>
<tr>
<td>FF</td>
<td>25.84</td>
<td>57.28</td>
<td>85.35</td>
</tr>
</tbody>
</table>

CONCLUSION
The study investigated the quality loss due to freezing and thawing in royal gala apples. It was found that the SF samples frozen at -18ºC in a static freezer had a greater quality loss when compared to IF samples and FF samples frozen at -40ºC and -72ºC in air blast freezers. Colour is a universal and direct parameter for determining the quality change in any product. The values of colour revealed that there was a change in colour for all the frozen and thawed samples but the change was higher in SF samples which can also be supported with chroma, hue and browning index values respectively. The solute diffusion test also revealed that more solute gain was experienced in SF samples when compared to IF and FF samples and it is the better method in detecting the quality loss during freezing and thawing but could be time consuming. Further the study could be investigated by the comparing various other methods such as texture analysis, driploss, microscopic analysis and storage studies to determine the efficacy of different freezing methods.

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