Effect of starter culture on the structure development and acidification process of set yogurt
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ABSTRACT
The objective of the present work was to evaluate the effect of different commercial starter cultures on the rheological properties of yogurt during gelation. Yogurt samples were prepared using pasteurized and homogenized bovine milk (fat content 3.5%). Ten different commercially available starter cultures were used. Two of the starter cultures were probiotic. The process of structure formation during yogurt making was monitored using a custom made dynamic U-tube rheometer of novel design. The storage ($G'$) and the loss ($G''$) moduli, strain, tan\text{δ}, incubation temperature and pH was recorded as a function of time. The starter culture type significantly affected the structure development and the acidification process of yogurt. The times elapsed before the commencing of the storage modulus rise, for the pH value of the samples to reach 4.6 and for the attainment of the maximum tan\text{δ} value, were influenced by the type of starter culture. The pH value at the starting point of gelation, the pH value at maximum tan\text{δ}, as well as the storage modulus value at pH 4.6, were affected by the starter culture type as well. Principal component analysis revealed that the starter cultures could be classified into 4 distinct groups and that every group was characterized by different rheological properties.

Keywords: yogurt gelation; yogurt rheology; starter culture

INTRODUCTION
Yogurt is a product of milk fermentation with thermophilic homofermentative lactid acid bacteria \textit{Streptococcus thermophilus} and \textit{Lactobacilus delbrueckii} ssp. \textit{bulgaricus}. Due to its organoleptic properties and its high nutritional value, it is a product widely consumed around the world. Yogurt is a three-dimensional protein network consisting of a casein framework in which serum, fat globules and bacterial cells are entrapped [1]. Yogurt gel formation is considered to be of primary importance with reference to the quality of the final product [2]. The influence of factors such as total solids content, type of starter culture, heat treatment and cooling conditions on the rheological properties of yogurt has been studied by several authors using different rheological methods [3][4][5][6][7].

The aim of the study is to determine the effect of using different starter cultures on the gelation process using a custom made dynamic rheometer.

MATERIALS & METHODS
The yogurt samples were prepared using pasteurized and homogenized bovine milk (fat content 3.5%) bought from the local store. Ten different commercially available starter cultures manufactured by Chr. Hansen were used, with two of them being probiotic (ABY-1 and ABY-2), while eight were non-probiotic (YC-350, YC-370, YC-380, YC-381, YC-X11, YF-3331, YF-L811, CH-1). Milk was heat treated at 80ºC for 10 minutes and then it was cooled down to 42ºC, before it was inoculated with each of the starter cultures. Approximately 30 ml of inoculated milk were placed inside the measuring unit of the U-tube rheometer, where they were incubated at 42ºC until pH would drop to ~4.0.

The U-tube rheometer was based on the U-tube technique of Saunders and Ward (1953) [8], in which the rigidity modulus of a gel can be determined by applying a known pressure to a sample and measuring the resulting deformation. It consists of two identical limbs where the sample is loaded. Connected to each side of the U-tube there is an enclosed air chamber whose pressure is measured with transducers. Connected to the air chamber on one side a reciprocating piston creates a driving pressure, resulting in a sample deformation, and consequently to a pressure increment in the air chamber on the other side of the U-tube [9]. From these pressure measurements the rheological characteristics of the sample can be determined [2].
At regular time intervals (5 min) measurements of storage modulus $G'$, loss modulus $G''$, strain, $\tan\delta$, incubation temperature and pH were measured, calculated and automatically recorded via the rheometer’s microprocessor control unit and the data were displayed and stored in a PC which was connected to the rheometer. The pH measurements were carried out by inserting a combined pH electrode into a quantity of the above described sample contained in a beaker which was placed into a custom made incubation chamber which was kept at the same temperature as the rheometer’s measuring unit. The experiment was replicated twice.

From the curves of $G'$, $\tan\delta$ and pH as a function of time, the following variables were determined: 1) pH value at gelation start, 2) pH value when $\tan\delta$ was maximized, 3) time elapsed until gelation started, 4) time elapsed until the yogurt samples reached pH value of 4.6, 5) time elapsed until the maximum $\tan\delta$ value was attained, 6) the maximum $\tan\delta$ value, 7) $G'$ value when the samples reached pH value of 4.6, 8) $G'$ value when $\tan\delta$ was maximized and 9) the maximum $G'$ value.

Principal Component Analysis (PCA) was used to determine correlations between variables and also for grouping the starter cultures according to the respective gelation and acidification profiles of the yogurt samples. One-way Analysis of Variance (ANOVA) tests were performed in order to perform comparisons between groups determined by PCA. Statistical significance was declared at $p<0.05$. Statistical analyses were performed with Minitab 15.

**RESULTS & DISCUSSION**

The structure development and acidification process was recorded for each of the starter cultures used. An example can be seen in figures 1 and 2, respectively. The reproducibility achieved between the two replications of the experiment was better than 2%.

Storage modulus $G'$ and loss modulus $G''$ values were highly correlated throughout the gelation process (Pearson's $r>0.95$ for all starter cultures). Therefore, $G''$ was removed as a variable from the multivariate analyses that followed, in order to remove redundancy from the models.

According to the yogurts' gelation and acidification profiles (fig. 3), PCA showed that four distinct groups of starter cultures can be formed.

Figure 4 provides a global view of the effect of the 10 different starter cultures on the examined variables. Variables with longer arrows are more important in producing effects while those with the same direction show positive correlation and those with inverse direction show negative correlation. The intensity of this correlation increases as the angle between the variables diminishes and two variables with an angle of 90° are not correlated at all. For example, it is apparent that time elapsed until gelation started and time elapsed until the samples reached their maximum $\tan\delta$ value were highly correlated.

By examining figures 3 and 4 the defining characteristics of each group can be established. The first group, consisting of the ABY-1 and ABY-2 (probiotic starter cultures) is characterized by increased times until the gelation process started and also until the samples reached their maximum $\tan\delta$ values. It is also characterized by very small $G'$ values when $\tan\delta$ was maximized compared to the other groups. The second group, consisting of cultures YC-380 and CH-1, was characterized by increased pH values, when the gelation process started and also when $\tan\delta$ was maximized. The third group, consisting of starter cultures YF-L811, YC-381, YF-3331 and YC-370 was characterized by increased $G'$ values when $\tan\delta$ was maximized and when pH dropped to 4.6. The fourth group, consisting of starter cultures YC-350 and YC-X11 was characterized by increased maximum $\tan\delta$ values compared to other groups as well as small pH values when the gelation process started.

The results of one-way ANOVA tests for the groups determined by PCA are shown in table 1, along with mean ± standard deviation values for each variable and for each group. As indicated by the respective $p$-values, all variables were proven to be statistically significant. The defining characteristics of each group, as determined by the PCA, were confirmed by the ANOVA tests.
Figure 1: Gelation process of yogurt using the YC-350 starter culture incubated at 42°C.

Figure 2: Acidification curve for the incubation of yogurt using the YC-350 starter culture.
The gelation and acidification processes of yogurt are affected by the starter culture characteristics specified by the starter culture's manufacturer. Selection criteria for lactic acid bacteria include acidification rate, aroma, flavour and texture characteristics [10]. Several studies refer to the effect of lactic acid bacteria producing exopolysaccharides on the rheological properties of yogurt, which are often used to increase the viscosity of yogurt products [11][12][13][10]. These types of starter cultures are termed texturing starters [14].
Increased $G'$ values of groups 3 and 4 can possibly be attributed to the distinctive fermentation characteristics of starter cultures and their ability to produce increased amounts of exopolysaccharides. Short acidification times for the same groups can be attributed to the faster acidification rates of the specific cultures.

Table 1: Mean values ± standard deviation for starter culture groups as defined by PCA and one-way ANOVA.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time elapsed until gelation start</td>
<td>186.3 ± 27.50</td>
<td>164.8 ± 5.50</td>
<td>111.6 ± 6.61</td>
<td>126.0 ± 23.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time elapsed until pH 4.6</td>
<td>297.5 ± 23.27</td>
<td>331.0 ± 41.80</td>
<td>242.5 ± 25.12</td>
<td>242.3 ± 16.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time elapsed until max tanδ</td>
<td>200.0 ± 17.32</td>
<td>193.5 ± 7.23</td>
<td>139.1 ± 8.80</td>
<td>154.8 ± 25.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH at gelation start</td>
<td>5.93 ± 0.0096</td>
<td>6.12 ± 0.0988</td>
<td>5.96 ± 0.0757</td>
<td>5.77 ± 0.0866</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH at max tanδ</td>
<td>5.54 ± 0.0532</td>
<td>5.84 ± 0.2769</td>
<td>5.47 ± 0.1078</td>
<td>5.30 ± 0.0925</td>
<td>0.001</td>
</tr>
<tr>
<td>Max tanδ</td>
<td>0.495 ± 0.0100</td>
<td>0.413 ± 0.0900</td>
<td>0.484 ± 0.0233</td>
<td>0.498 ± 0.0206</td>
<td>0.038</td>
</tr>
<tr>
<td>$G'$ at pH 4.6</td>
<td>195.0 ± 11.28</td>
<td>173.3 ± 33.21</td>
<td>245.1 ± 32.7</td>
<td>233.7 ± 5.95</td>
<td>0.002</td>
</tr>
<tr>
<td>$G'$ at max tanδ</td>
<td>38.1 ± 7.20</td>
<td>41.2 ± 2.16</td>
<td>46.5 ± 4.30</td>
<td>43.4 ± 2.29</td>
<td>0.040</td>
</tr>
<tr>
<td>$G'$ max</td>
<td>206.8 ± 12.95</td>
<td>174.1 ± 35.75</td>
<td>269.3 ± 44.27</td>
<td>291.3 ± 41.64</td>
<td>0.001</td>
</tr>
</tbody>
</table>

CONCLUSION

The starter culture has a significant effect on the gelation and acidification processes. The variables that are affected are the times elapsed before the commencing of the storage modulus rise, for the pH value of the samples to reach 4.6 and for the attainment of the maximum tanδ value as well as the pH value at the starting point of gelation, the pH value at maximum tanδ and the storage modulus value at pH 4.6.

REFERENCES

