Two Methods for Highlighting the Influence of Sugars Type on Fermentation Processes Produced by Staphylococcus Carnosus and Lactobacillus Curvatus

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Abstract. Purpose of this paper is to establish the influence of different types of sugars on fermentation processes found in the meat industry. In the analysis used a mixture of bacteria (Lactobacillus curvatus and Staphylococcus carnosus), and the types of sugars were used lactose, lactulose, sucrose and two types of glucose syrup. To highlight the speed with which the fermentation process takes place, the variation in pH for the environment and the amount of energy released during biotechnological processes was measured.

Keywords: meat, fermentation, DSC, Lactobacillus curvatus, Staphylococcus carnosus.

INTRODUCTION

Modern biotechnology refers to various scientific techniques used to produce specific desired traits in plants, animals or microorganisms through the use of genetic knowledge (Gaspar, 2011, Moise, 2011). Currently, fermented meat product are made by using starters culture, usually from both Lactobacilli (eg Lactobacillus curvatus) and Micrococcaceae (eg Staphylococcus carnosus) because this combination ensures rapid acidulation and optimal flavor development (Demeyer, Toldra, 2004). These bacteria are able to metabolize a wide range of sugar types to generate lactic acid through either homo or heterofermentative pathways. The accumulation of lactic acid produces a pH drop towards acidic values (Toldra, 2008).

The speed at which bacteria ferment the sugar presented and the amount of acid formed varies from sugar to sugar. Generally speaking the further the chemical structure of sugar is from that of glucose, and closer it is to that of starch the less acid will be produced.

As sources of sugars for the microorganisms were used: lactose, lactulose, sucrose and two types of glucose syrup with different degrees of polymerization. Between these sugars, lactulose has a special role, which can be considered as a prebiotic, because is able to stimulate healthy intestinal microflora (De Souza Oliveira et. al., 2011)

To highlight the effects of fermentation processes by carbohydrates used, besides determining the pH can be used the thermal analysis knowing that the transformation of substrate fermentation process is exothermic process as well as growth of bacteria (Kaletunç, 2009).

MATERIALS AND METHODS

The research was carried out using bacterial cultures SUPREMIA FERMENT F-FU 2 (SC SUPREMIA GROUP SRL), a lyophilized mixture of Lactobacillus curvatus and
Staphylococcus carnosus, with max. $4.0 \times 10^9$ CFU/g. Carbohydrates that can be used according to technical specifications are listed below (Tab. 1)
Tab. 1

<table>
<thead>
<tr>
<th>Sugar fermentation</th>
<th>Staphylococcus carnosus</th>
<th>Lactobacillus curvatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Sugars used were: lactose, lactulose, sucrose and glucose syrup with different degree of polymerization submitted in tab. 2

Tab. 2

<table>
<thead>
<tr>
<th>Glucose syrup</th>
<th>DP1 (Dextrose)</th>
<th>DP2 (Maltose)</th>
<th>DP3 (Maltotriose)</th>
<th>DP4+ (4 or more glucose units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>15</td>
<td>13</td>
<td>10</td>
<td>62</td>
</tr>
<tr>
<td>Type II</td>
<td>30</td>
<td>30</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>

Porcine Biceps femoris were removed from the carcass at 1 day postmortem and stored into a hermetic package at 4°C until analysis.

For pH analysis SR ISO 2917 : 2007 method and a pH-meter from Thermo Scientific with food industry specific electrodes for meat, semi-frozen products applications FC 230 Series from Hanna Instruments were used.

A TA Instruments, differential scanning calorimeter (DSC), model SDT Q600, with computer-assisted data acquisition was used for all thermal analysis.

Determinations were made on medium with or without meat, to highlight the pH variation better, knowing that the meat has a buffer capacity (Kyla-Puhju et al., 2004)

In the first case, without meat, with 50% sugars solution was mixed with a suspension of bacteria in 2:1 ratio, just before performing the analysis.

In meat samples, this was finely ground in advance, and then was use the following proportion meat-sugars-suspension of bacteria = 10-1-0,5

After mixing all samples were thermostat to 24°C

Samples for DSC analysis were between 10 to 15 mg and were used Tzero ® aluminum pans and crimp sealing. Isothermal curve was recorded for a temperature of 26°C using an empty Tzero ® aluminum pans in crimp seal mode

All results are submitted with a blank that do not add sugars

RESULTS AND DISCUSSION

Initially was monitored pH decrease, in the absence of meat, due to its buffer capacity and the results are submitted the Fig. 1 and Fig. 2.

Lactose (galactose-glucose) and lactulose (galactose-fructose) are two disaccharides and both contain galactose. The results were expressed as percentage of initial pH of each medium to be able to compare data. Unexpectedly, as can be seen the Fig. 1, more pronounced decrease in pH occurs for lactulose, especially in the first 2 hours. Subsequently the pH difference between the two disaccharides decreases.
But unlike previous dates, thermal analysis results (Fig. 2) indicate that bacteria prefer glucose over fructose.

**Fig. 1.** Variation over time of pH expressed as percentage relative to baseline for lactose, lactulose and control.

Following the slope of the three curves (Tab. 3) for different time intervals it is noticed that, slope increases gradually and the highest values are for lactose, but close to those obtained for lactulose.

**Fig. 2** Isothermal calorimetric curves obtained scanning for lactose, lactulose and control (at 26°C).
Rate of fermentation for expressed as slope of isothermal calorimetric curves obtained scanning for lactose, lactulose and control (at 26°C)

<table>
<thead>
<tr>
<th></th>
<th>Rate of fermentation (mW/g/min) after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 minutes</td>
</tr>
<tr>
<td>Control</td>
<td>-0.09849</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.4788</td>
</tr>
<tr>
<td>Lactulose</td>
<td>0.3486</td>
</tr>
</tbody>
</table>

An interesting result is obtained for sugar which is also a disaccharides, but unlike the previous ones does not contain glalactose. Under these conditions it can be said that through thermal analysis, it can identify bacteria in culture preferentially used glucose, fructose and finally then galactose.

This preference for glucose and sugars of bacteria with small molecules as is applies also for the two types of glucose syrups

Glucose syrup type II has a higher content of glucose (DP1 = 30) and maltose (DP2 = 30), have initially a smaller slope of isothermal calorimetric curves, but then approaches that of sucrose.
The interpretation of these results should take into account the lag phase. Different authors have different opinions about the length of this phase, which is dependent on environmental compositions (Szczepaniak 2011, Verluyten, 2004).

Thus, for the meat samples fermentation was longer than for thermal analysis (Fig.4). Note that in the first around 5 hours, the variation of pH is low for all added sugars, then pH decreases according to the preference for carbohydrates in bacteria culture medium.

![Graph showing pH variation over time for different samples](image)

**Fig. 4. Variation over time of pH for samples containing meat**

**CONCLUSIONS**

Carbohydrate choice must be made taking into account the culture of bacteria used and the duration of fermentation knowing that initially are transformed monosaccharides, and then carbohydrates with large molecules. Also it can be concluded that thermal analysis by, Differential scanning calorimetry scan can give satisfactory results which are confirmate by classical analysis for determining the pH variation over time.

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