Monitoring a Biotechnological Process in a Biofermentor to Obtain a Dairy Product Using Brewer’s Yeasts

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Abstract. Dairy products may go beyond the borders of nutritional value and become functional products if the fermentation process is performed in the best available manner. Our study monitors the process parameters and their variation during the fermentation time of milk incubated with a complex mixture of microorganisms formed by: a lactic culture, kefir-yeast culture and brewer’s yeasts sampled from the secondary fermentation of beer. The study was realized using a Tryton- Pierre Guerin Technologies biofermentor. The results were correlated with the development dynamics of lactic bacteria and yeasts population during 24 hr of fermentation. The pH level variation is typical for kefir-like products. The oxygen content during fermentation is a competitive factor for the two species of microorganisms featuring in our research: Lactococcus lactis and Saccharomyces cerevisiae. In this situation the semi-anaerobic environment insures the fastest rate of acidification for milk and the Saccharomyces cerevisiae yeasts did not display a fermentation metabolism but maintain their viability.

Keywords: fermented milk product, Saccharomyces cerevisiae, process parameters.

INTRODUCTION

Fermented milk products are very popular all over the world, due to both their pleasant flavor and the potential to preserve and even improve good health (Farnworth, 2004). The correlation between the consumption of yogurt and kefir and the overall health of the digestive, circulatory, and immune systems is among the main reasons for which consumers all over the world have become increasingly interested in these foods (Adolfsson, 2004). On the other hand, yeasts, due to their complex and harmonious chemical composition, are capable to insure the right amount of nutrients with high energetic and nutritional value, and in certain case a certain therapeutic value (Ferreira et al., 2010). Brewer’s yeast obtained as a byproduct of beer fermentation, due to the strict hygienic conditions maintained in breweries and to the use of malt worth, which insures good nutrition for the yeast, is the best functional yeast which can be used (Mussatto, 2009). Research has shown that the malt wort biosynthesizes greater quantities of vitamins and biologically active substances than other culture environments (Talbott et al., 2007). From the nutritional point of view, brewer’s yeast possesses valuable concentrations of proteins and vitamins (Webb, 2006).

Through the usage of select starter cultures whose metabolism can be coordinated through the size of the inoculators and the setting of physical and chemical activity conditions, one guarantees reproducible processes which result in the manufacturing of...
products whose features can satisfy both the quality requirements and the preferences of consumers (Costin et al., 2005).

Fermentation is not solely a means to create, maintain or improve storage of a product. Fermentation also has a strong impact on the quality and desirability of a product. During fermentation, the primary nutrients contained in milk, such as the superior protein compounds, calcium, phosphorus, and B vitamins remain available and increase the nutritional value of the fermented milk product (Stepaniak, 2003). Moreover, fermented milk products may go beyond the borders of nutritional value and become functional products if the fermentation process is performed in the best available manner. Flavour, viscosity, and chemical and microbial features may vary depending on the size of the inoculators added to milk, of agitation or the lack thereof during fermentation and of other such conditions (Tzanetakis, 1999).

The aim of the study was to monitories the manufacturing process of a dairy product obtained with lactic culture, kefir yeast and brewer’s yeast. Based on the phenotypes and the manufacturing features of the microorganisms in question, it was important to know the process parameters which influence the biotechnological process, namely the development dynamic of the competitive microorganisms and the formation of lactic acid.

MATERIALS AND METHODS

We used 1.8% (w/w) skimed, pasteurized milk, cooled at 30˚C which was inoculated with an inoculum formed by a mixture of a bacterial starter culture and two yeast cultures, as follows:

1. A FD-DVS CHN-22 mesophilic culture containing the following species: *Lactococcus lactis* ssp.cremoris, *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp lactis biovar diacetylactis, *Leuconostoc mesenteroides* subsp. *cremoris* (provided by Chr. Hansen)
2. A culture of kefir yeasts - LAF 3 (*Debaryomyces hansenii*) (provided by Chr. Hansen)
3. A brewer’s yeast (*Saccharomyces cerevisiae*) suspension sampled from the secondary fermentation of beer, with a cellular viability of 96% (provided by a local brewer).

Both of the starter cultures were freeze-dried powders and Direct Vat Set (DVS), while the brewer’s yeast was sludge. The density of the microbial cultures was $10^{10}$ colony forming units per mL (cfu/mL) and the volumetric ratio, expressed in mL, between milk: lactic culture: kefir yeast: brewer yeast was 1000:1:2:0.5. The density of microorganisms was determined by direct counting in the Thoma Chamber, while the viability of brewer’s yeast was determined by differential coloration.

A Tryton- Pierre Guerin Technologies biofermentor, with a maximum capacity of 2L and a useful capacity of 1.5 L was used. The biotechnological process was supervised by a digital control unit (*Tryton-sytem*) which realized the adjustment of the process parameters (temperature-time) in order to respect all the manufacturing stages of the dairy product (Fig. 1). So, after inoculation the sample was incubated at 29-30˚C for 12 hr, pre-cooled at 18-20˚C for 1hr and cooled again at 4-6˚C for 10 hr. The *Tryton-system* is an open system and allows the process management in batch fermentation, feed-batch fermentation or continuous fermentation.

The biofermentor has fermentation temperature setting and control equipments, a pH sensor, a dissolved oxygen sensor, and a gas analyzer for CO$_2$, and O$_2$. These equipments facilitate the supervision, control, and acquisition of process data.
In order to monitor the density and viability of lactic bacteria and brewer’s yeasts, a tryptone-water (Difco) mixture (1 g/L) was used to prepare the dilutions for the microbiological analysis. Lactic acid bacteria were counted in M17 medium (Difco), a selective medium for lactococci (Terzaghi & Sandine, 1975), at pH 7.2 ± 0.2, after incubation under anaerobic conditions, 5% (v/v) CO$_2$, at 30°C for 18-24 hr. Yeasts were enumerated in WLN (Wallerstein Laboratories Nutrient medium) at pH 5.5 ± 0.3 in a selective medium for *Saccharomyces cerevisiae* yeast, after incubation at 25°C for 48 hr.

All data were processed statistically, in order to identify significant differences. The variance analysis (ANOVA, using the software GraphPad Prism 5.00) was applied, considering a confidence interval of 95% (p<0.05) as threshold of significance.

### RESULTS AND DISCUSSION

After analyzing the parameters gathered by the process data acquisition system, we found the following parameters to be significant for our research: time, pH, and the concentration of dissolved oxygen in milk. The data was analyzed statistically, using value t, and we found very significant differences (***) p<0.0001 for the samples in question (t=15.5; t=18.9).

The manufacturing process of the fermented product may be split in two stages: the actual fermentation at 30°C for 12 hours and the post-fermentation stage, at a temperature of 4-6°C, for an additional 10-12 h. Based on the variation of the process parameters (pH and dissolved oxygen), we decided to analyze the milk product from the microbiological point of view. See Tab. 1 for the microbiological results.

#### Tab. 1

<table>
<thead>
<tr>
<th>Sampling after</th>
<th><em>Lactococcus lactis</em></th>
<th><em>Saccharomyces cerevisiae</em></th>
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<tbody>
<tr>
<td></td>
<td>X</td>
<td>s</td>
</tr>
<tr>
<td>12h of fermentation</td>
<td>1.2 x 10$^8$</td>
<td>0.5</td>
</tr>
<tr>
<td>24h of fermentation</td>
<td>4.6 x 10$^6$</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>9 x 10$^8$</td>
<td>0.6</td>
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<tr>
<td></td>
<td>3 x 10$ ^5$</td>
<td>0.5</td>
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Note: Data represent the means ±SD of three independent experiments
Fig. 2 and 3 represent plates with colonies of microorganisms enumerated on selective medium for each of them: WLN medium for *Saccharomyces cerevisiae* yeast and M 17 for *Lactococcus lactis*.

![Fig. 2 Plates with colonies of Saccharomyces cerevisiae (WLN medium)](image1)

![Fig. 3 Plates with colonies of Lactococcus lactis (M 17 medium)](image2)

As we can see in figure 4, the initial pH of the milk was 6.44; 12 hours after its insemination with the inoculator, the pH fell to a value of 4.56, namely a fall rate of 0.156/h. Yet another 12 hours later, the pH fell to 4.49, which represents a fall rate of 0.0058/h.

![Fig. 4 The acidification curve at 30°C](image3)
In the first 12 hours after insemination, the pH fell abruptly by almost two pH units; the period coincides with the maximum amount of lactococcus in the sample \((10^6 \text{ cfu/mL})\). During the next 12 hours, the fall of the pH level slowed down, likely due to the fall in the numbers of lactococcus \((\text{up to } 10^6 \text{ cfu/mL})\). The pH level variation is typical for kefir-like products (García Fontan et al., 2005). The pH level after 24 hours of fermentation was 4.49, which may be the reason behind the slight fall in the number of \textit{Saccharomyces cerevisiae} yeasts.

The dissolved oxygen percentage fell by approximately 63.57% after the first hour after insemination. After another hour, the percentage fell by 87.96%, and after the fourth hour, 90.58% of the initial dissolved oxygen level found in the milk had been consumed (Fig. 5).

![Fig. 5 Oxygen’s consumption variation](image)

With respect to the influence of the dissolved oxygen on the fermentation process, we must note that we used desaerated milk, with a very low content of dissolved oxygen, which can be considered a semi-anaerobic environment for microbial development. The oxygen content during fermentation is a competitive factor for the two species of microorganisms featuring in our research: \textit{Lactococcus lactis} and \textit{Saccharomyces cerevisiae}. Lactococcus is a micro-aerophilic microorganism; it is a known fact that levels of dissolved oxygen in milk can be used to control the kinetics of acidification in the manufacture of dairy products (Viljoen, B.C., 2001). Low levels of dissolved oxygen are correlated with a better acidification of the milk (Yuguchi, 1989). This also explains the fall of the pH level in milk as early as the first half hour after insemination. The semi-anaerobic environment we mentioned above insures the fastest rate of acidification for milk.

On the other hand, \textit{Saccharomyces cerevisiae} yeasts tend to adapt to environmental conditions when it comes to the oxygen content (Spickett et al., 2000). However, if the percentage of dissolved oxygen is very low, yeasts will use it to maintain their viability.

When the two species are co-cultivated, as in our research, there is a dramatic fall (by 90.58%) in the dissolved oxygen level during the first 12 hours of the fermentation process, as the oxygen is most likely consumed by the yeasts together with the glucose resulted from the split of lactose by the \(\beta\)-galactosidase of the lactic bacteria. In these conditions, the \textit{Saccharomyces cerevisiae} yeasts did not display a fermentation metabolism. The lack of alcoholic fermentation is also sustained by the lack of any significant variation in the content of carbon dioxide being picked up by the biofermentor’s sensor; the organoleptic analysis of the end product also failed to find trace levels of alcohol.
CONCLUSIONS

In a Tryton- Pierre Guerin Technologies biofermentor equipped with data’s aquisition sistem, we monitornies the process parameters of the biotechnological process in order to obtain a dairy product with brewer’s yeasts Saccharomyces cerevisiae. 1.8% skimmed milk was inoculated with a complex mixture formed by: lactic starter culture/ kefir-yeast starter culture and brewer’s yeast in ratio: 1000:1:2:0.5.

In the first 12 hours after insemination, the pH fell abruptly by almost two pH units; the period coincides with the maximum amount of lactococcus in the sample (10^8 cfu/mL). During the next 12 hours, the fall of the pH level slowed down, likely due to the fall in the numbers of lactococcus (up to 10^6 cfu/mL). The oxygen content during fermentation is a competitive factor for the two species of microorganisms featuring in our research: (Lactococcus lactis and Saccharomyces cerevisiae); there is a dramatic fall (by 90.58%) in the dissolved oxygen level during the first 12 hours of the fermentation process. In these conditions, the Saccharomyces cerevisiae yeasts did not display a fermentation metabolism.

REFERENCES