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Evaluation the Effect of Milk Total Solids on the Relationship Between Growth and Activity of Starter Cultures and Quality of Concentrated Yoghurt

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Abstract: The effect of increasing milk total solids to 4 levels (14, 18, 23 and 27 %) on the growth of starter cultures and chemical properties of yoghurt was studied. All samples were analyzed for acidity and counts of starter bacteria during fermentation and for total solids, fat, protein and syneresis for final product. Sensory assessments were also conducted. Results showed that increasing of milk total solids from 14 to 27%, increased the time of lag phase for both of starter bacteria. The time of lag phase in acidity curve also increased with increasing milk total solids. The growth and activity of starter bacteria improved in the samples with higher amounts of total solids. Increasing milk total solids to 23% improved sensory acceptability and decreased syneresis significantly (" = 5%).

Key words: Yoghurt % concentrated yoghurt % starter cultures % acidification

INTRODUCTION

Yoghurt is a popular fermented milk product that has a special importance on the human health because of its perceived nutritional benefits. In general, the overall properties of yoghurt, such as acidity level, free fatty acid content, the production of aroma compounds (diacetyle, acetaldehyde, acetoin) as well as the sensory profile and nutritional value, are important traits of the product. These aspects are influenced by the chemical composition of the milk base, processing conditions, the activity of starter culture during the incubation period [1].

The lactic acid fermentation is one of the oldest methods Employed the preservation of milk, but even these relatively acidic products can still be prone to spoilage. However further improvement in keeping quality can sometimes be achieved by concentration [2].

Concentrated yoghurt has properties superior to those of regular yoghurt, with higher protein (2.5x) and mineral (1.5x) contents, very low lactose content and fat content which can be varied according to consumer demand. Concentrated yoghurt should have considerable market potential [3].

In general, starter bacteria growth is influenced by many factors such as chemical composition of the milk base, the amount of inoculum, milk temperature, incubation time and cooling time of the milk [4].

Chemical composition of the milk base (total solids and fat content) have significant effects on the activity of starter cultures. Ozer and Robinson [5] have studied the behavior of starter cultures in concentrated yoghurt produced by different methods. They discovered that production method and milk total solids content influenced the growth and activity of starter cultures.

A study on the effect of milk supplementation (whey, casein hydrolysate and milk protein) on the acidification and microbiological stability of fermented milks showed that acidifying activity was greatly improved with casein hydrolysate, with a reduction of the fermentation time by about 55% by comparision with the other supplementation [6].

Despite of the results above, Abd-El Salam and El-Alamy [7] showed that total solids content had no adverse effect on starter activity or coagulation time.

Increasing milk total solids from 16 to 23% had significant effect on decreasing rate of pH during fermentation [8]. The incubation time for pH of 4.6 was reached, in milk was shorter than this time in retentate (3 vs 5 h) [9].

Shaker *et al*. [10] studied the effect of milk fat content on the acid development during fermentation and reological properties of plain yoghurt. They indicated that increasing milk fat content, increased the initial pH of the samples and the rate of decreasing

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PH during incubation of high fat samples was lower than others.

In the previous works, we realized that increasing milk fat content, influenced the growth and activity of starter cultures in samples with 2 levels of TS (12 and 23%) [11, 12].

Hardi and Slacanac [13] reported that type of starter culture, milk fat content and addition of inulin, were influenced the coagulation kinetics of fermented milk products.

Chemical composition of the milk base especially total solids has the major effect on the acceptability of concentrated yoghurt. Concentrated yoghurt containing < 20% TS was assessed as "thin and tastless" and that with > 25% TS became gummy and bitter [14].

The objectives of this paper were to optimization of concentrated yoghurt production conditions via evaluation the effect of milk total solids on the growth of starter bacteria and their relationship with acidification activity.

MATERIALS AND METHODS

Pasteurized standardized milk (2.5% fat) was supplied from Toos plant. The starter culture (CH_1) was obtained from CHr. Hansen's laboratory, Denmark.

Preparation of the milk consists of three stages: Heat treatment (85°C for 5 minutes), separation of the fat (model D-470, westfalia separator) and concentration. A batch type single effect evaporator (T = 55°C, P =-0/8 bar) was used for concentration of the milk to 4 levels (14, 18, 23, 27%). In order to controlling TS of the milk during concentration, model OK-GYEM refractometer was used.

Preparation of the yoghurt samples was done according to Tamime and Robinson [14] advised method in 50gr containers. Inoculated milks was incubated at 45°C until pH of 1.6-1.7 or acidity was reached, then stored overnight at 4°C at which time the experiment was started [15].

Total solids levels of the samples and the titrable acidities were determined according to the British standard Institution method [16]. Fat content was determined by the Gerber method using Gerber butyrometer tubes as cited in the British standard Institution method, 1998 [17]. Total nitrogen was determined by the standard micro-kjeldahl procedure and the crude protein value obtained by multiplication by 6.38, degree of syneresis, expressed as proportion of free whey, was measured by Al-kadamany *et al*. [18]. A 20g sample of concentrated yoghurt was layered on 10-cm whatman paper (#2) that was fitted into a Buchner funnel and vacuum filtered for 10 min. the proportion of free whey was calculated as follows:

$$\text{Free whey (g/100 g)} = \frac{\text{Weight of initial sample - Weight of sample after filtration}}{\text{Weight of initial sample}}$$

Sensory characterization: The products were assessed by six judges using a sensory rating scale of 1-5 (unacceptable/excellent) [1].

Enumeration of yoghurt starter bacteria: Bromocresol Green Whey Agar (BGWA) developed by Yamani and Ibrahim [19] was used to quantify the yoghurt starters [19]. At intervals of 60 minutes, two samples removed from the incubator in order to measure the titratable acidity and total colony counts of each starter organism. Serial dilutions of the products were made in sterile peptone water (0.1%) to 10^{-8}, the pour plate technique was used for the enumeration. Plates were incubated in plastic bags at 43°C/48h. after incubation, the plates were removed from the bags. The two organisms were recognized on the basis of the morphology of their colonies: streptococcus thermophilus colonies were small (1-1.5mm diam), compact and regular. They were green and lenticular with entire edges, some times with white margins. Colonies of *Lactobacillus bulgaricus* were larger (2.5 mm diam) and mostly of light color with greenish centers. They were irregular in shape and in the form of a mass with twisted or fuzzy filament projections.

The statistical analysis of the result was completed using the Excel and MSTAT-C software programme and significantly different groups were detected by the Duncan test.

RESULTS AND DISCUSSION

Relationships between growth of starter bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) and increasing of acidity during fermentation of 14 and 18% TS samples are shown in Fig. 1 and 2.

While the growth of *streptococcus thermophilus* is in the lag phase, the acidity does not increase significantly. Production of lactic acid after 60 minutes became more noticeable and it continues to 300 minutes. In this time range *streptococcus thermophilus* is in logarithmic growth phase. The maximum growth of this bacteria (peak point of the curve) occur after 5 h when the acidity amount is maximum too. As shown in Fig. 1 the

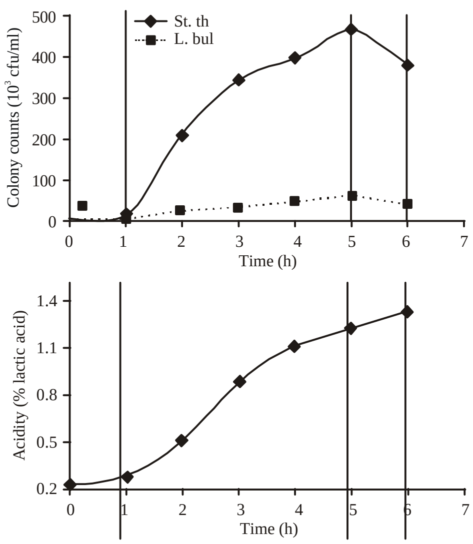

Fig. 1: Relationship between growth of starter bacteria and acid production for sample with 14% TS

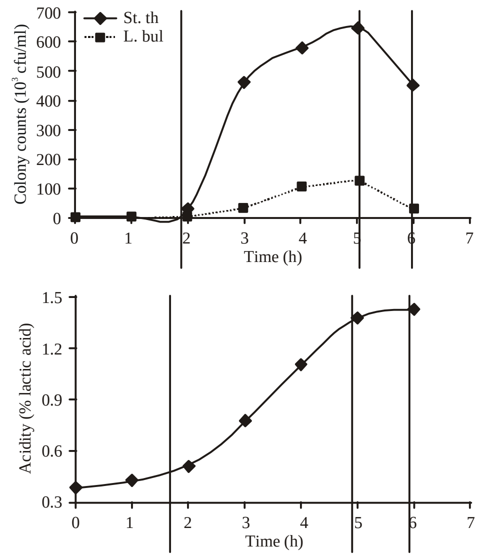

Fig. 3: Relationship between growth of starter bacteria and acid production for sample with 23% TS

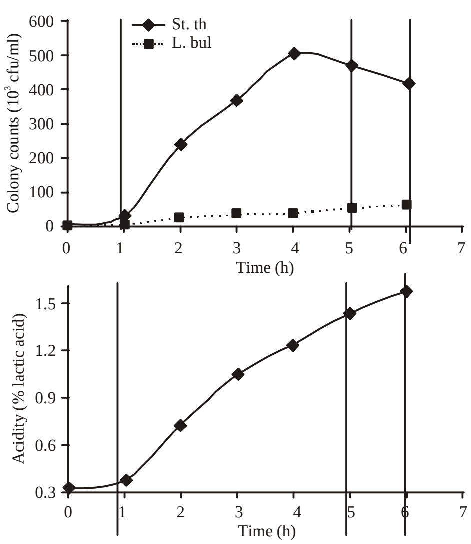

Fig. 2: Relationship between growth of starter bacteria and acid production for sample with 18% TS

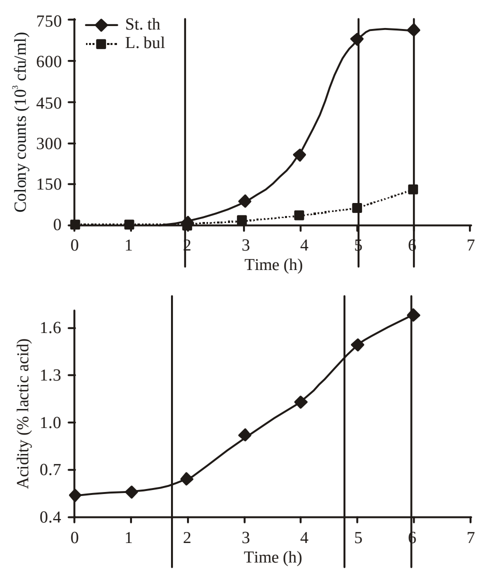

Fig. 4: Relationship between growth of starter bacteria and acid production for sample with 27% TS

growth of *streptococcus thermophilus* after 5 h fell in death phase for sample with 14% TS and acidity increasing become constant whereas for sample with 18% TS the survival of this organism kept in stationary phase and for this reason acidity amount continue to increase.

Regression coefficient between counts of *streptococcus thermophilus* and acidity at the time of sampling varied from 0.91-0.97 that confirmed the close relationship between *streptococcus thermophilus* and acidity.

The growth curves of *lactobacillus bulgaricus* for 14% and 18% TS are the same. A little difference observed in the time of peak point, that occured after 5 hours for 14% TS and after 6 hours for 18% TS.

With increasing milk total solids from 18% to 23% and 27%, the time of lag phase became longer (2-3x) for both of starter bacteria (Fig. 3 and 4). Despite of longer lag phase in these samples (23 and 27%) the time of log phase of *streptococcus thermophilus* became shorter than other samples where the time of maximum growth is the same for

Table 1: Protein and fat content of yoghurts with different total solids (g/100g)

Total solids	Protein	Fat
14	4.93	1.51
18	6.05	2.00
23	7.98	2.30
27	9.23	3.07

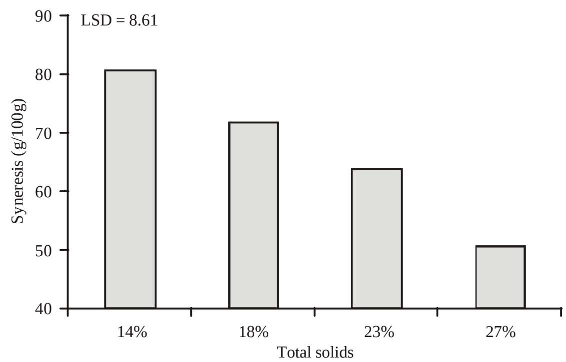

Fig. 5: Degree of syneresis for samples with different levels of total solids

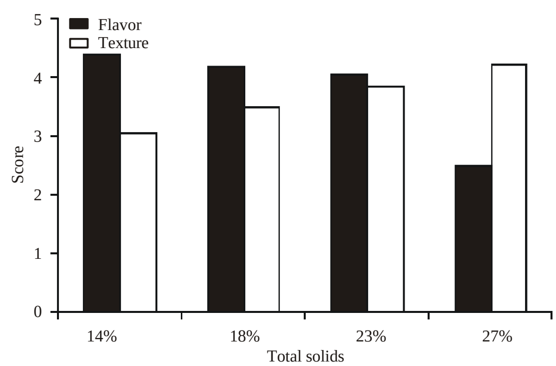

Fig. 6: Flavor and texture scores of the samples with different total solids

all samples (after 5 hours). Also counts of this organism in the samples with high total solids is higher than other samples.

A relationship between *streptococcus thermophilus* and acidity was also evident and regression coefficients between counts of *streptococcus thermophilus* and acidities at the time of sampling for 23 and 27% TS were 0.92 and 0.97 respectively.

Similarly, Ozer and Robinson [5] indicated that the cultures growing in milks with high total solids had shorter generation times (1.15-1.23h) compared with times of 2.13-2.20h for cultures in milks with 160 g/kg total solids. They also concluded that regression coefficients between counts of *streptococcus thermophilus* and pH at the time of sampling varied from 0.829 to 0.935 in all samples. In contrast, the counts of *lactobacillus bulgaricus* were not markedly affected by acidity (r^2 between 0.536 and 0.668) in milks with lower total solids. When the total solids of the milks was elevated to approximately 230 g/kg, however the effect of acidity on the growth of *lactobacillus bulgaricus* become relatively more important (r^2 between 0.79 and 0.845).

The maximum growth of *lactobacillus bulgaricus* in 27% TS happened 1hour later than other samples (6h vs 5 h). for this reason the acidity of that sample increased until the end of incubation.

Ozer and Robinson [5] showed that increasing of milk total solids from 16 to 23 g/100 g improved the growth of *lactobacillus bulgaricus* where counts of this organism after 240 minutes in 23% TS were highest.

Chemical composition of samples with different total solids content, is shown in Table 1. because of the higher proportion of fat and protein in the samples with higher total solids, concentrated yoghurt (23, 27% TS) has more nutritional values in comparison with regular yoghurt (14, 18% TS).

Total solids content of the yoghurt samples had significant effect on degree of syneresis (Fig. 5). Reduction of free water and increasing the proportion of solids contents, which occur during concentration, are two main factors decreased rates of wheying off in the samples with high total solids.

Similarly, shaker *et al*. [10] indicated that the increase in viscosity of yoghurt with highest fat content may be due to increase of total solids of the milk which has a significant effect on the firmness of yoghurt gel and decreasing degree of syneresis.

Sensory attributes: The mean score of flavor and texture of samples as affected by total solids content are shown in Fig. 6. No significant difference in flavor were recognized between 14, 18 and 23% TS (" = 5%). They all had high score but the acceptability of sample with 27% TS was significantly lower than other samples (" = 5%).

According to Tamine and Robinson [15], during fermentation, *lactobacillus bulgaricus* produces aroma compounds more than *streptococcus thermophilus*. With considering this fact, the lower acceptability of sample with 27% TS could be due to longer lag phase and uncompleted growth of *lactobacillus bulgaricus* which didn't provide enough time for aroma production in this sample. It is suggested that longer incubation time for samples with high TS could improve this property.

As shown in Fig. 6, texture acceptability increased with increasing total solids significantly (" = 5%). It could be due to the effect of high total solids contents on increasing firmness of the gel and decreasing degree of syneresis [20]. Improving effect of total solid on the growth of starter bacteria and exopolysaccharide production by these organisms during extended incubation time [21], could consider as other reason for higher texture scores of samples with high TS.

CONCLUSION

1. There is a close relationship between the growth of starter culture and acidity increasing patterns during fermentation. Because of the improving effect of high total solid on the growth of starter bacteria, lactic acid amounts are higher in samples with high TS.
2. Degree of syneresis decreased with increasing TS significantly.
3. Increasing milk TS more than 23% decreased flavor acceptability
4. Samples with higher TS had better textural properties than those with lower TS.

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