Physicochemical characteristics of soy probiotic yoghurt with inulin addition during the refrigerated storage

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Abstract
The main objectives of this study were (1) to produce control cow’s yoghurt that will serve as basis of comparison for produced cow’s and soy yoghurts with added probiotic cultures and inulin as prebiotic, (2) to determine trend of change of pH values in yoghurt during the fermentation, (3) to investigate the changes of some physicochemical properties of experimental designed yoghurts during the refrigerated storage. The drop in pH during fermentation was faster in the cow’s milk than in soymilk. During the storage at 4 °C the pH value of the fermented products remained mostly unchanged compared to control sample under same condition of storage. No great differences are noticed among the viscosities and water holding capacity (WHC) after 1st and 20th day of storage. Only after 10th day of storage were noted increasing in WHC in all of produced yoghurt sample, except fermented cow’s milk with 3% inulin addition. Statistical analysis of experimental results showed no difference in syneresis among observed samples and control sample. This research proves that there are serious confirmations those supporting develop of new soy yoghurt formulation. Stability during storage is important characteristic what is proved observing physicochemical characteristics of produced samples of soy yoghurt.

Key words: cow’s milk, soymilk, yoghurt, probiotic, inulin, acidity, syneresis, viscosity

Introduction

Yoghurt is dairy product obtained by lactic acid fermentation as an action of starter culture contained of Lactobacillus delbrueckii ssp. bulgaricus and Streptococcus thermophilus, and with addition of probiotics, it becomes powerful functional food.

Probiotics are defined as “live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance” [1]. Lactic acid bacteria including lactobacilli and bifidobacteria are the most common bacterial species considered as potential probiotics [2]. Recently, it was shown that new probiotic strains with antimicrobial effects can be isolated from plants and animals [3,4]. Probiotics have been used for the treatment of various types of illness and expressed many nutritional benefits [5,6,7]. Fermented foods that have potential probiotic properties are produced worldwide from a variety of food substrates [8].

Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria. According to ROBERFROID (2007) [9] definition, a prebiotic is “a selectively fermented ingredient that allows specific changes, both in the composition and/or activity of the gastrointestinal microflora that confers benefits upon host well-being and health.” The frequently used prebiotic is inulin, a non-digestible fructan that offers a unique combination of interesting nutritional properties and important technological benefits [10-12]. Inulin have the ability to be a fat substitute, bulking agent, low-calorie sweetener, and texture modifier
When added to yoghurt, therefore potentially altering the sensory perception of the product [13].

When prebiotics are combined with probiotics, their relationship is classified as symbiotic. This combination can improve the survival rate of the probiotics and provide additional health benefits to the host [14]. Thus together probiotics and prebiotics have health promoting effects as well as develop of products assortment.

Due to cow’s milk allergy and requiring of vegetarian aliment also, interest in soy yoghurt has increased. Soymilk and fermented soymilk products considered as a suitable economical substitutes for cow’s milk and an ideal nutritional supplement for lactose-intolerant population [15,16]. Furthermore, physiological effects of fermented soymilks are greater than those of unfermented ones [17,18].

Soymilk is aqueous extract of soybean and because of generally dislike flavor, often described as “beany”, is not widely accepted by consumers. Soy yoghurt is a fermented soymilk made with a mixed starter culture consisting of *Streptococcus thermophylus* and *Lactobacillus delbureckii* ssp. Fermenting soymilk with lactic acid bacteria considerably increases its health value. Because of greater antioxidative actions [19], they are considered healthier than pure soymilk.

Soymilk contains raffinose, stachyose, pentanal, n-hexanal and phytoestrogens. Some strains of *Bifidobacterium* are able to reduce the concentration of raffinose and stachyose, eliminating the potential cause of flatulence, and also decrease the levels of pentanal and n-hexanal responsible for the beany flavour [20]. Bifidobacteria have α-galactosidase activity [21] that allows them to utilize sugars such as raffinose and stachyose, and sufficient proteolytic activity to support growth in soymilk [22].

The main objectives of this study were (1) to produce control cow’s yoghurt that will serve as basis of comparison for produced cow’s and soy yoghurts with added probiotic cultures and inulin as prebiotic, (2) to determine trend of change of pH values in yoghurt during the fermentation, (3) to investigate the changes of some physicochemical properties of experimental designed yoghurts during the refrigerated storage.

**Materials and Methods**

**Milk and Inulin**

Homogenised cow’s milk (2.5% fat, 3.4% proteins, 4.6% lactose), obtained from „MEGGLE“, d.o.o. Meggle Mljekara (Bihać, Bosnia and Herzegovina) and soymilk (2.2% fat, 3.6% proteins, 2.0% carbohydrate) obtained from “ALNATURA” (EU, Germany) were used for the production of yoghurt samples. The initial pH of the cow’s milk was 6.69 (±0.02) and pH of the soymilk was 6.87 (±0.01). Physical, chemical and microbiological characteristics of milk samples were entirely in accordance with the pertinent standards.

Inulin (Fibruline® Instant Cosucra Groupe Warcoing S.A., Belgium) contained min 90 % inulin, max 10 % fructose, glucose and sucrose, max 0,3% ash.

**Cultures**

Mixed probiotic starter culture DriSet BIOFLORA ABY 424: 70% w/w *Streptococcus thermophilus*, 10% w/w *Lactobacillus bulgaricus*, 10% w/w *Lactobacillus acidophilus*, 10% w/w *Bifidobacterium* ssp. (Vivolac Culture Corporation, Indiana, USA) was applied to achieve a concentration of 0.0025% in manufacturing yoghurt samples.

**Yoghurt Manufacturing**

Cow’s milk and soymilk were heated to 55°C and inulin was added in three concentrations: 0.5% w/w, 1.5% w/w and 3% w/w. The milk was cooled to 37°C, inoculated 8078...
with the chosen yoghurt starter and incubated at the same temperature until pH 4.5 to 4.6 was reached. Fermentations were stopped by rapid cooling to 20°C and the samples of fermented milk were placed in a cold storage at 5°C ±1. Each trial was repeated three times.

Experimental design and code of products are presented in Table 1. As the control samples were considered cow’s yoghurt sample without any addition.

Table 1. Codes of different yoghurt mixes present in this study

<table>
<thead>
<tr>
<th>Code</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control cow’s yoghurt</td>
</tr>
<tr>
<td>CY0.5IN</td>
<td>Cow’s yoghurt with 0.5% inulin (5 g kg⁻¹ IN)</td>
</tr>
<tr>
<td>CY1.5IN</td>
<td>Cow’s yoghurt with 1.5% inulin (15 g kg⁻¹ IN)</td>
</tr>
<tr>
<td>CY3IN</td>
<td>Cow’s yoghurt with 3% inulin (30 g kg⁻¹ IN)</td>
</tr>
<tr>
<td>SY</td>
<td>Soy yoghurt without any addition</td>
</tr>
<tr>
<td>SY0.5IN</td>
<td>Soy yoghurt with 0.5% inulin (5 g kg⁻¹ IN)</td>
</tr>
<tr>
<td>SY1.5IN</td>
<td>Soy yoghurt with 1.5% inulin (15 g kg⁻¹ IN)</td>
</tr>
<tr>
<td>SY3IN</td>
<td>Soy yoghurt with 3% inulin (30 g kg⁻¹ IN)</td>
</tr>
</tbody>
</table>

*CY- cow’s yoghurt, IN-inulin, SY-soy yoghurt

Methods of Analysis

After manufacturing, yoghurt samples were analyzed by measuring pH value, titratable acidity, macro-components (dry and ash), viscosity, water holding capacity (WHC) and syneresis. pH was measured using pH 510/mV Meter (Eutech Instruments, England), and pH during fermentation and over 20 days of storage. Acidity in °OSh was determined according to Soxhlet-Henkel method [23].

Macro-components, in all samples of yoghurt, were determined using standard methods: dry matter content after drying at 105°C and ash after mineralization at 550°C. All the methods applied are described in the handbook [23].

Viscosity was measured using a Brookfield DV-E viscosimeter (Brookfield Engineering Laboratories, Stoughton, MA, USA). The viscometer was operated at 20 rpm (spindle #4). Each result was recorded in mPa·s after a 30 s rotation, during 3 min.

Other measured physical properties are syneresis (in cm³ of whey separated by filtration of the 50 cm³ sample at room temperature for 3 h) [24], and water holding capacity (WHC).

Water-holding capacity of yoghurt was determined using a procedure by GUZMAN-GONZALEZ & al. [25]. 20g of yoghurt (Y) was centrifugated for 30 min at 1250xg and 20°C (h = 4.8 cm). The whey expelled (WE) was removed and weighed.

The water-holding capacity (WHC) was determined as:

\[ \text{WHC} = \frac{100 \times (Y - WE)}{Y} \]

The measurements of physicochemical properties of manufactured yoghurts were performed on 1st, 10th and 20th day of storage. The average value of 3 measurements was taken for further analysis. Values of different tests were expressed as the mean ± standard deviation (x ± SD). All data were subjected to one way ANOVA and means were compared by the Holm-Sidak test (SigmaPlot 11.0, Systat Software, Inc. USA). The level of significance was set at \( P < 0.05 \).
Results and Discussion

Fermentation of Cow’s Milk and Soy Milk

The efficiency of starter culture in different milk was monitored by decrease in pH value during fermentation. Fermentation was stopped near pH 4.5 in all of samples, but the samples reached mentioned pH in different periods (Figure 1). The drop in pH during fermentation was faster in the cow’s milk than in soymilk what is in agreement with previous results [26]. pH value of cow’s milk supplemented with 0.5% and 3% inulin were similar to pH value of control sample and this is in agreement with conclusion of BOŽANIĆ & al. (2001) [27]. Mentioned authors reported no significance in change of inulin addition on fermentation time of milk. Otherwise, cow’s milk with 1.5% inulin had shortest time of fermentation (250 min) compared to all of tasted samples in this performed experimental design and could be interesting percent of inulin supplementation for further research.

As far as soymilk is considered, it has the longer time of fermentation compared to cow’s milk. The shortest time of fermentation of soymilk had samples with 1.5% and 3% added inulin. No difference in time of fermentation for soymilk and soymilk with 0.5% added inulin.

Required time for fermentation is in connection to supplements and applied starters also [28]. In soymilk some lactic acid bacteria (LAB) grow slowly or poorly [29] and to improve the growth of probiotic bacteria soymilk needs to be supplemented with various prebiotics [20]. Previously, it was reported that the strain of Lactobacillus delbrueckii spp. bulgaricus is faster than other lactobacilli in producing lactic acid and then recommended it to be used for fermenting soymilk with added inulin [30-32]. Many studies indicate that soymilk is a good substrate for probiotic bacteria [29,33] and good base for fermentation process [34].

Characteristics of the Obtained Probiotic Yoghurts during the Storage

Content of Macro-components
Content of dry matter and ash in yoghurts products was determined and presented in Table 2. The differences in dry matter and ash content were analyzed by post-hoc Holm-Sidak test. The tests have shown that the compared quantities differed significantly in most of the cases.

The samples of soy probiotic yoghurts had lower level of dry matter comparing to similar experiment designed cow’s yoghurts. In both case increasing of percent of added inulin increased dry matter content. Inulin additions in quantity of 1.5 or 3% significantly increase ($P \leq 0.05$) dry matter content compared to samples without any addition or with lowest inulin addition (0.5%). Samples of soy yoghurt with 3% inulin had similar dry matter content as cow’s yoghurt with no added inulin, actually control samples.

As is obvious from Table 2, content of ash increased with percents of added inulin in samples of cow’s yoghurt, but without statistical significance ($P \geq 0.05$), while in samples of soy yoghurt increased of ash is significantly higher ($P \leq 0.05$) compared to soy yoghurt with no added inulin.

Table 2. Macro-components of probiotics yoghurt (mean±SD)

<table>
<thead>
<tr>
<th>Samples$^a$</th>
<th>Macro-components (%, w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry</td>
</tr>
<tr>
<td>Control</td>
<td>9.658±0.019$^b$</td>
</tr>
<tr>
<td>CY0.5IN</td>
<td>9.847±0.079$^b$</td>
</tr>
<tr>
<td>CY1.5IN</td>
<td>10.456±0.057$^c$</td>
</tr>
<tr>
<td>CY3IN</td>
<td>11.189±0.148$^d$</td>
</tr>
<tr>
<td>SY</td>
<td>7.357±0.171$^e$</td>
</tr>
<tr>
<td>SY0.5IN</td>
<td>7.582±0.028$^e$</td>
</tr>
<tr>
<td>SY1.5IN</td>
<td>8.244±0.170$^f$</td>
</tr>
<tr>
<td>SY3IN</td>
<td>9.238±0.119$^g$</td>
</tr>
</tbody>
</table>

$^a$ Labels of products according to Table 1

$^{b,c,d,e,f,g}$ Means in a column with different letters differ significantly ($P \leq 0.05$ Holm-Sidak test.)

$pH$ Value and Acidity in $^oSH$

During the storage at $4^\circ$ C the pH value of the fermented products remained mostly unchanged compared to control sample under same condition of storage. Statistical difference was occurred only in the sample of soy yoghurt with 1.5% added inulin after 20$^{th}$ day of storage (Table 3). This stability in pH values was presumably due to lower activity of starter culture during refrigerated storage.

Despite the pH remained stable during refrigerated storage, titratable acidity fluctuated (Table 3). Samples of cow’s yoghurts with all of designed inulin combination showed statistical difference ($P \leq 0.05$) regarding post hoc Holm-Sidak test, and soy yoghurt with no inulin addition compared to rest of samples.
Table 3. pH value and acidity (°SH) of probiotic yoghurt made of cow’s milk and soymilk fortified with inulin (0.5%, 1.5% and 3%) during 20 day of storage (mean±SD)

<table>
<thead>
<tr>
<th>Samples</th>
<th>pH value Days of storage</th>
<th>Acidity (°SH) Days of storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Controla</td>
<td>4.47±0.02</td>
<td>4.38±0.04</td>
</tr>
<tr>
<td>CY0.5IN</td>
<td>4.41±0.07</td>
<td>4.42±0.07</td>
</tr>
<tr>
<td>CY1.5IN</td>
<td>4.37±0.07</td>
<td>4.28±0.17</td>
</tr>
<tr>
<td>CY3IN</td>
<td>4.45±0.01</td>
<td>4.41±0.01</td>
</tr>
<tr>
<td>SY</td>
<td>4.54±0.01</td>
<td>4.52±0.02</td>
</tr>
<tr>
<td>SY0.5IN</td>
<td>4.51±0.04</td>
<td>4.50±0.09</td>
</tr>
<tr>
<td>SY1.5IN</td>
<td>4.55±0.01b</td>
<td>4.51±0.04b</td>
</tr>
<tr>
<td>SY3IN</td>
<td>4.55±0.05</td>
<td>4.50±0.09</td>
</tr>
</tbody>
</table>

a Labels of products according to Table 1
bc Means in a row (particularly for results of pH value, and particularly for results of acidity) with different letters differ significantly ($P \leq 0.05$ Holm-Sidak test.),

**Physical Characteristics**

Physical characteristics of produced cow’s and soy yoghurts were observed as viscosity, syneresis and water holding capacity.

Gel strength and viscosity are important quality indicators related to consistency and mouth feel of fermented dairy products [35] and stability of viscosity during the storage is important qualitative characteristic of yoghurt. According to RAWSON & MARSHALL (1997) [36], *Streptococcus thermophilus* have important role in the production of exocellular texturing agents called exopolysaccharides that might interact with the protein content of milk and increase the viscosity and rheological quality of products.

Viscosity of yoghurt sample during period of storage is presented in Figures 2.A,B,C. Produced yoghurt samples from cow milk had viscosity in range: 139 - 233 mPas, while the soy yoghurt had lower viscosity in range: 117 – 159 mPas.

No great differences are noticed among the viscosities after 1st and 20th day of storage. After first day (Figure 2.A) only soy yoghurt samples with 0.5% added inulin had significantly difference form respective control according post-hoc Holm-Sidak test ($P \leq 0.05$). After 10th day only cow’s yoghurt samples with 0.5% added inulin had no significantly difference from control ($P \leq 0.05$), while all of samples in this experimental design (Table 1) were significantly difference from control ($P \leq 0.05$). After 20th day of storage the manufactured samples showed more stability and among samples only soy yoghurt with 1.5% inulin had statistical difference from control ($P \leq 0.05$). All of statistical differenced samples showed decreasing of viscosity after inulin addition which is in agreement with finding of several authors [28,38].
Physicochemical characteristics of soy probiotic yoghurt with inulin addition during the refrigerated storage

Results of syneresis and water holding capacity (WHC) are presented in Figure 2.D,E,F. Produced yoghurt samples from cow’s milk had water holding capacity (WHC) in range 39 - 49%, while the soy yoghurt had WHC in range 46.5 – 53%. Despite well-known fact that inulin has a high water holding capacity [37], no statistical difference were found in WHC of produced fermented cow's and soy milk after 1st and 20th day of storage comparing to control sample. Only after 10th day of storage were noted increasing in WHC in all of produced yoghurt sample, except fermented cow's milk with 3% inulin addition.

Produced yoghurt samples from cow’s milk had syneresis in range 26 – 30%, while the soy yoghurt had syneresis in range 24.7- 28.6%.

Statistical analysis of experimental results showed no difference in syneresis among observed samples and control sample. This finding is opposite to reported results of BOŽANIĆ & al. (2002) [38] that increasing in inulin concentration decrease syneresis. However results of our study are pointing the stability of produced soy yoghurt as a very important characteristic during the refrigerated storage.
Conclusions

Fermented dairy products are generally considered to be one of the most suitable substrates to transfer an adequate number of probiotic bacteria to the consumer. This research proves that there are serious confirmations those supporting develop of new soy yoghurt formulation. The addition of inulin and application of probiotic culture have important role in design of various functional products including soy yoghurt. Stability during storage is important characteristics what is proved observing physicochemical characteristics of produced samples of soy yoghurt.

References

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