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Microbiological Assessment of Yoghurt Enriched with Flakes from Barley Grain and Malt Extract during Shelf-Life

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Abstract**—**The effect of flakes from biologically activated hull-less barley grain and malt extract on microbiological safety of yoghurt was studied.

Pasteurized milk, freeze-dried yoghurt culture YF-L811 (Chr. Hansen, Denmark), flakes from biologically activated hull-less barley grain (Latvia) and malt extract (Ilgezeem, Latvia) were used for experiments. Yoghurt samples with flakes from biologically activated hull-less barley grain and malt extract were analyzed for total plate count of mesophylic aerobic and facultative anaerobic microorganisms, as well yeasts and moulds population during shelf-life.

Results showed that the changes of pH and titratable acidity affected the concentration of added malt extract. The lowest pH and the highest titratable acidity were determined in samples YFBG5% ME4% and YFBG5% ME6% on the 14th day. The total plate count decreased in all yoghurt samples except sample YFBG5% ME6%, where was determined the increase of microorganisms from 7th till 14th day. The adding of flakes from biologically activated hull-less barley grain in yoghurt samples caused the higher initial content of yeasts and moulds comparing with control. The growth of yeasts and moulds during shelf-life provided the added malt extract in yoghurt samples. Yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract from a microbiological perspective is safe product.

Keywords**—**Microbiological assessment, yeasts, moulds, barley grain, malt extract, yoghurt.

I. Introduction

YOGHURT is one of the popular fermented milk products known for thousands of years [1]. Being nutritionally rich in protein, calcium, riboflavin, vitamin B_6 and vitamin B_{12}, yoghurt is considered to have more nutritional benefits than milk [1]. The consumption of yoghurt is associated with overall diet quality [2].

Hygiene and sanitation procedures during manufacturing and selection of good quality raw materials are essential in controlling microbial contaminations in food [3]. The deterioration of yoghurt is the result of changes in its physical, chemical and organoleptic characteristics, making it unacceptable for human consumption [4]. The spoilage of yoghurt is mainly due to the development of yeasts and moulds [4]. Contamination by yeasts is one of the main limiting factors for the stability and the commercial value of yoghurts [5]. The rapid growth of yeasts results in excessive gas formation, appearance of yeasty flavor and odor as well loss of texture quality [6], [3]. Spoilage becomes evident when the yeast population reaches 10^5-10^6 cells per gram [7], [8]. The growth of yeasts in yoghurt is related to the ability of these species to grow at refrigeration temperatures, to ferment lactose and sucrose, and to produce lipolytic and proteolytic enzymes that hydrolyze milk fat and protein [3]. When yoghurt is produced under conditions of good manufacturing practice, the final product should contain less than 1 yeast cell per gram [3]. The addition of sugar, honey, fruits and cereal preparations, acting as fermentable growth substrates, further enhances the growing capabilities of yeasts, making the yoghurt prone to yeast spoilage [6], [9].

Moulds growth on fermented dairy products is a common and recurring problem, where mycotoxins produced by moulds are penicillic acid, patulin, ochratoxin A and citrinin [10]. Good hygiene practice is very important to fight mould spoilage, because moulds are present in air, water and soil [11].

Due to the global importance of barley and its by-products in the diet it is important to know and to control the microbiota occurring in this cereal, because contamination of barley by moulds and mycotoxins results in quality and nutritional losses and represents a significant hazard to the food chain [12]. Therefore the aim of research was to evaluate the microbiological safety of yoghurts enriched with flakes from biologically activated hull-less barley grain and malt extract during shelf-life.

II. Materials and Methods

Pasteurized milk with fat content 2.5% and the yoghurt culture YF-L811, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Chr.Hansen, Denmark), were used for experiments. Yoghurt culture was stored in freezer at -18^{o}C and used directly for milk fermentation.

Flakes from biologically activated hull-less barley grain (Latvia) were added to milk in concentration of 5% and malt extract (Ilgezeem, Latvia) in different concentrations (2%, 4% and 6%). Milk samples with flakes from biologically activated hull-less barley grain and malt extract were inoculated with yoghurt culture and fermented at 43±1^{o}C for 4 hours. After

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fermentation the maturation of yoghurt samples was done at 5±1°C for 24 hours. All yoghurt samples were stored at 5±1°C for 14 days.

Five yoghurt samples were analyzed (Table I). The control sample was prepared without the flakes from biologically activated hull-less barley grain and malt extract for comparing results.

TABLE I
YOGHURT SAMPLES DESCRIPTION

Code	Sample
Control	Yoghurt without flakes from biologically activated hull-less barley grain and malt extract
YFBG5%	Yoghurt enriched with 5% of flakes from biologically activated hull-less barley grain
YFBG5% ME2%	Yoghurt enriched with 5% of flakes from biologically activated hull-less barley grain and 2% of malt extract
YFBG5% ME4%	Yoghurt enriched with 5% of flakes from biologically activated hull-less barley grain and 4% of malt extract
YFBG5% ME6%	Yoghurt enriched with 5% of flakes from biologically activated hull-less barley grain and 6% of malt extract

pH of yoghurt samples was determined using pH-meter WTW series inoLAB pH 720. Titratable acidity of yoghurt samples was determined by tritation following the LVS ISO 6092:2003 using phenolphthalein as an indicator. The measurements of pH and titratable acidity were carried out on the 1st, 3rd, 5th, 7th and 14th day.

Plate counting method was used for microbial detection. The samples for investigation in two reiterations were taken on the 1st, 7th and 14th day. Total plate count of mesophylic aerobic and facultative anaerobic microorganisms was investigated on Nutrition agar (dilutions 1:1000; 1:10000) in conformity standard method LVS EN ISO 4833:2003. Yeast plate count was investigated on Malt extract agar (dilutions 1:100; 1:1000) in conformity standard method ISO 21257 – 2:2008. Counting of colonies formed and calculating the number of CFUs was accomplished by automatic colony counter Acolyte.

Morphological properties of microorganisms were determined, coloring was performed according to Gram's method, Catalyse test and identification up to species by means of API biochemical test systems, for identification of LAB cultures API CH 50 test was used while ID 32 C for yeasts.

III. RESULTS AND DISCUSSION

pH of all yoghurt samples slightly decreased during storage period (Fig. 1). The pH value during 14 days storage changed from 4.35 to 4.27 for control, from 4.54 to 4.28 for yoghurt enriched with flakes from biologically activated hull-less barley grain and from 4.52 to 4.20 for yoghurts enriched with flakes from biologically activated hull-less barley grain and malt extract. pH of all yoghurt samples during storage period conformed with optimal pH value of yoghurt (4.0-4.4) mentioned in literature [13]. pH values changes affected the concentration of added malt extract. The lowest pH values were determined in samples YFBG5% ME4% and YFBG5% ME6% on the 14th day, respectively 4.22 and 4.20.

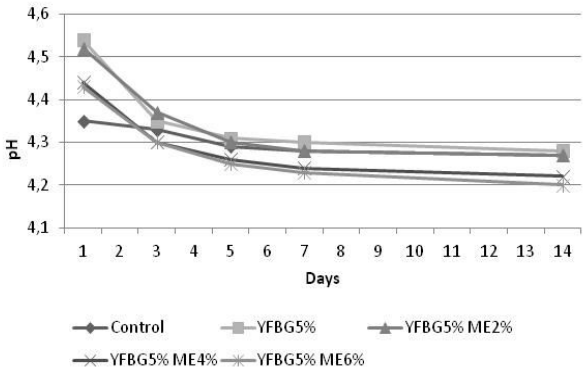

Fig. 1 pH changes of analyzed yoghurt samples during shelf-life

Evaluating the data for titratable acidity there was determined gradual increase of titratable acidity in all yoghurt samples during shelf-life (Fig. 2).

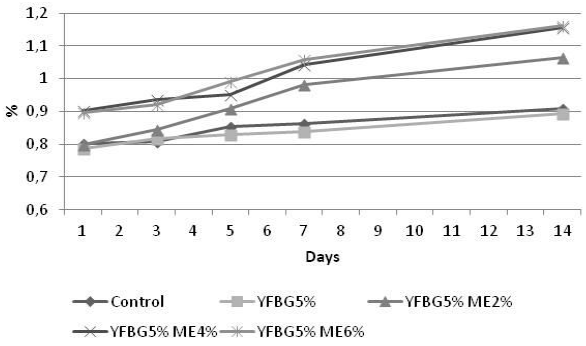

Fig. 2 Titratable acidity changes of analyzed yoghurt samples during shelf-life

The changes obtained for titratable acidity were for control from 0.802 to 0.910%, for yoghurt enriched with flakes from biologically activated hull-less barley grain from 0.787 to 0.892% and for yoghurts enriched with flakes from biologically activated hull-less barley grain and malt extract from 0.799 to 1.160% during storage period. There were observed similar tendencies with pH that increase of titratable acidity in yoghurt samples influenced the concentration of added malt extract. The highest values of titratable acidity were determined in samples YFBG5% ME4% and YFBG5% ME6% on the 14th day, respectively 1.156% and 1.160%.

Hygiene requirements for yoghurts can be divided to the requirements of the raw material, requirements of the starter culture and sanitation during production. Pasteurized milk is a nutrient medium so its composition and cultural characteristics must allow cultural microorganisms for other suitable production conditions, rapid development and full quality.

Apart from the undesirable, pathogen organisms the total plate count includes the desirable microorganisms such as

lactic acid bacteria in fermented foods (yoghurt or cheeses). Therefore the count of aerobic, mesophilic organisms can be used to determine the hygienic status of these foods. A high total plate count generally points to a poor microbial quality of a non-fermented product. For specific details, further tests will have to be done (http://www.florin-ag.ch/).

The effect of added flakes from biologically activated hull-less barley grain and malt extract on total plate count in yoghurt samples during shelf-life is shown in Fig. 3.

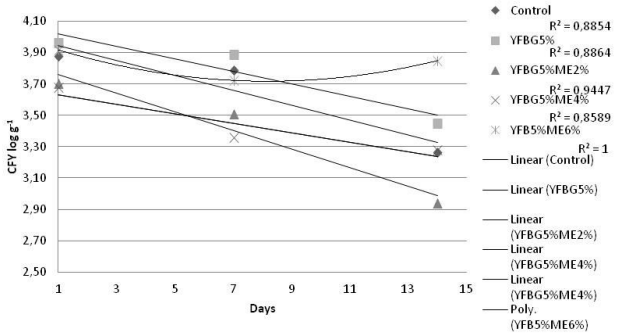

Fig. 3 Total plate count of analyzed yoghurt samples during shelf-life

Microorganisms do not take place exponentially, but it depends on the nutrient consumption and production of toxic metabolites in the culture medium. If there accumulate a certain amount of unwanted metabolites, the microorganisms slow down or stop growth.

The results showed that the total plate count decreased in all yoghurt samples except sample YFBG5% ME6%. Many microorganisms can grow at a decreasing pH, but for many it is unsuitable environment for reproduction. The other reason for decreasing of total plate count can be explain that moulds during the storage time start to produce mycotoxins, which do not develop microorganisms in the yoghurt. In sample YFBG5% ME6% the total plate count grew from 7th till 14th day. The increasing of microorganisms could be explained by concentration of added malt extract, which was as nutriment media.

Cultured milk products (fermented milk, sour cream, yoghurt, drinking yoghurt, cottage cheese, cream cheese, etc.) are ideal media for the propagation of yeasts, as they exhibit a low pH of 4.0 – 6.0, which is optimal for yeast growth. The approximate decimal doubling time in fruit yoghurt without shaking for *Saccharomyces cerevisiae* is 5 hours (30°C), 10 hours (20°C), 62 hours (10°C) and 84 hours (4°C) [14].

Yeasts and moulds may cause spoilage of yoghurt during storage period, because low pH insignificant affects their growth in yoghurt. Yeasts and moulds can tolerate acidic environment as well low temperatures are convenient for theirs. The contamination can be caused by using contaminated yoghurt micro flora, using older starter culture or contaminated flavor component. The result their metabolic activity is change in taste and flatulence of yoghurt, i.e., bubbles in yoghurt and bloated consumer packaging. The changes of yeasts and moulds population in yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract during shelf-life are showed in Fig. 4.

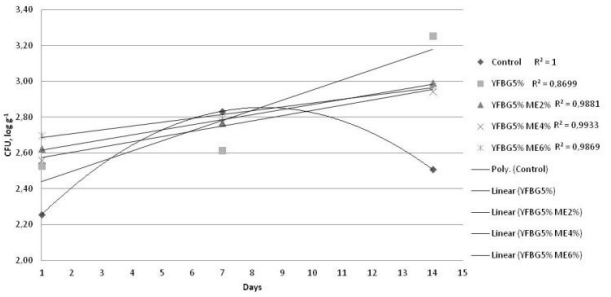

Fig. 4 Dynamic of yeasts and moulds in analyzed yoghurt samples during shelf-life

The results showed that the lowest amount of yeasts (2.26 log CFU g^{-1}) was determined in control sample. It could be explained that there was not contamination with flakes from biologically activated hull-less barley grain, because the others yoghurt samples enriched with flakes from biologically activated hull-less barley grain presented the higher initial content of yeasts and moulds.

During storage period the population of yeasts and moulds grew progressively in yoghurt samples enriched with flakes from biologically activated hull-less barley grain and malt extract. The growth of yeasts and moulds provided the added malt extract. Similar conclusion was obtained in research of yoghurt with fruits, where the addition of fruits into the yoghurts reinforced the spoilage of yeasts because additional sources of contamination and fermentable substrates are provided [4]. The increase of yeasts and moulds population in control sample was stopped on the 7th day with insufficient nutritive, therefore the amount of yeasts and moulds progressively decreased in control sample during storage time from 7th till 14th day.

Yeasts cells in the yoghurt samples were identified using API test. One of yeasts spp. was identified as *Saccharomyces cerevisiae.* This is typically yeasts type from cereals, which incurred in experimental yoghurt samples from added flakes from biologically activated hull-less barley grain. The colonies on a macroscopic level are cream colored to pale pink, with the majority of colonies being smooth with a mucoid appearance. The results of API test showed, that this yeasts should be from the family *Rhodoturula* ssp. In the experimental yoghurt samples was found two typically moulds from grain micro flora – *Penicillium* spp. and *Aspergillus* spp.

Microorganisms of *Enterobacteriacea* spp. are not found and identified in analyzed yoghurt samples. Yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract fermented at 43^{o}C for 4h with the titratable acidity from 0.787 to 1.160% and pH value less than 4.6 from a microbiological perspective is safe product.

IV. Conclusion

1. The changes of pH and titratable acidity affected the concentration of added malt extract. The lowest pH and the highest titratable acidity were determined in samples YFBG5% ME4% and YFBG5% ME6% on the 14th day.
2. The total plate count decreased in all yoghurt samples except sample YFBG5% ME6%, where was determined the increase of microorganisms from 7th till 14th day.
3. The adding of flakes from biologically activated hull-less barley grain in yoghurt samples caused the higher initial content of yeasts and moulds comparing with control. The growth of yeasts and moulds during shelf-life provided the added malt extract in yoghurt samples.
4. Yoghurt enriched with flakes from biologically activated hull-less barley grain and malt extract from a microbiological perspective is safe product.

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