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Effects of fermentation conditions and homogenization pressure on the rheological properties of Kefir

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ABSTRACT

In this study, the effects of fermentation conditions (temperature and time) as well as homogenization pressure on the rheology and EPS production in Kefir made from bovine whole milk were investigated. Results showed that the rheological characteristics and EPS production are affected significantly ($p < 0.05$) by the fermentation temperature but not by the incubation time. Furthermore, the homogenization pressure was found to influence significantly ($p < 0.05$) the rheology but not the production of EPS in Kefir.

1. Introduction

Fermented milks or beverages made with co-cultures of lactic acid bacteria and yeasts are widely produced in many countries in the region between Eastern Europe and Mongolia (Kurmann, Rašić, & Kroger, 1992). Some typical examples are Acidophilin, Acidophilus yeast beverage or milk, as well as Kefir and Koumiss. The latter product is traditionally made from mare's and/or yak's milk, whilst Kefir is mainly produced from bovine milk (Wszolek, Tamime, Muir, & Barclay, 2001). Kefir can be produced by fermenting milk with commercial freeze-dried Kefir starter cultures or traditional Kefir grains as well as the product obtained after the removal of grains. Kefir grains contain a wide and varying microflora, including lactic acid bacteria, acetic acid bacteria, yeasts and moulds (Pintado, Da Silva, Fernandes, Malcata & Hogg, 1996).

Gel formation is the most important physical property of fermented milk products. According to Tamime and Robinson (1989), rheological characteristics of milk gel are governed by milk composition, dry matter content, heating, homogenization, incubation temperature, cooling, storage time.

Studies on the rheological properties of Kefir are very scarce (Paraskevopoulou et al., 2003; Wszolek et al., 2001) and researches on the effects of processing conditions on Kefir quality are quite inexistent. The objective of this work was to make a contribution to the knowledge of Kefir quality in terms of rheology and EPS production, with a particular focus on the effects of fermentation time and temperature as well as homogenization pressure.

2. Materials and methods

2.1. Milk types and Kefir starter culture

A commercial freeze-dried Kefir starter culture (coded BE010) was purchased from "Wilderness Family Naturals" (Silver Bay, Minnesota, USA). According to the supplier, this starter culture contains a mixture of *Lactococcus lactis* subsp. lactis, *L. lactis* subsp. cremoris, *L. lactis* subsp. diacetylactis, *Leuconostoc mesenteroides* subsp. cremoris, *Lactobacillus kefyr*, *Kluyveromyces marxianus* var. marxianus and *Saccharomyces unisporus* (www.wildernessfamilynaturals.com). Skimmed milk powder (Guangming brand) and cow's raw milk were obtained from local supermarket and farm, respectively (Wuxi, China).

2.2. Preparation of Kefir working-culture

Sterile reconstituted skimmed milk was prepared by reconstituting approximately 12 g of skimmed milk powder in 100 ml of distilled water (Garrote, Abraham, & De Antoni, 1997), storing overnight at 4 °C to allow full rehydration, sterilizing at 121 °C for 2 min (Ozer, Robinson, Grandison, & Bell, 1998) and cooling down to about 25 °C. Reactivation was carried out by inoculating the sterile reconstituted skimmed milk with the commercial freeze-dried

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culture at 0.2 mg/100 ml, incubating at 22 °C for 24 h (Beshkova, Simova, Frengova, Simov & Dimitrov, 2003) and ripening at 4 °C for 24 h. Kefir working-culture was prepared by inoculating the sterile reconstituted skimmed milk with the reactivated culture at 3 ml/100 ml, fermenting at 22 °C for 24 h (Beshkova et al., 2003) and maturing at 4 °C for at least one day before further use.

2.3. Kefir manufacture

Kefir was prepared as described by Tamime, Muir, and Wszolek (1999) with some modifications. Bovine farm milk was divided into three batches which were pre-heated to 60 °C, homogenized at 15 MPa (Tamime & Robinson, 1999) and pasteurized at 92 °C for 15 min (Beshkova, Simova, Simov, Frengova, & Spasov, 2002) using a water-bath. The pasteurized milk was first cooled down to about 25 °C and then inoculated with the working-culture at 3 ml/100 ml. The effect of fermentation time was investigated by incubating milk samples in a thermostatically controlled incubator at 22 °C (Beshkova et al., 2003) for various periods of time (18, 24 and 30 h). The impact of fermentation temperature was evaluated by incubating milk samples for 18 h at different temperatures (20, 24 and 28 °C). The influence of homogenization pressure was studied by dividing bovine farm milk into four batches and pre-heating milk samples to 60 °C. Only one batch was pasteurized directly without homogenization, while the other three batches were homogenized prior to pasteurization. Homogenization was carried out using APV-1000 homogenizer (APV Co., U.K.) in a single-stage (15, 17, 19 and 21 MPa). Milk samples were pasteurized, cooled down, and inoculated as mentioned above. Fermentation took place in a thermostatically controlled incubator at 24 °C for 18 h.

2.4. Rheological analysis

Dynamic rheological measurements were carried out using an AR 1000 rheometer (TA Instruments Ltd., Surrey, England) with a 1 mm-gap parallel-plate sensor. A portion of sample was carefully removed and placed on the bottom plate of the rheometer. The top plate was slowly lowered until the gap was 1 mm and excess sample was removed from the edges of the plate. Following the method of Rimada and Abraham (2006), two types of measurements were done: a strain sweep at constant frequency (1 Hz) to determine the linear viscoelasticity range and a frequency sweep from 0.0368 to 10.0 Hz at the same deformation (1%), within the linear range. The elastic modulus (G') and the viscous modulus (G'') were recorded as a function of frequency. The complex dynamic viscosity (η^*) was measured according to Haque, Richardson, and Morris (2001) by varying the frequency at a fixed strain of 1%. Kefir samples were prepared in triplicate for each batch and were analyzed individually.

2.5. Determination of EPS production

The EPS production was determined following the method of Purwandari, Shah, and Vasiljevic (2007). Results were expressed as the amount of crude EPS per gram of Kefir.

2.6. Statistical analysis

Results were treated using the analysis of variance (ANOVA) procedure of the SAS System for Windows V8 (SAS, 1999). Kefir samples were prepared in triplicate for each batch and were analyzed individually. Data were expressed as mean values ± Standard Deviation (SD). The Duncan's Multiple-Range Test (DMRT) was used to compare means with 5% as significance level.

3. Results and discussion

3.1. Effect of fermentation time on Kefir rheology and EPS production

Fig. 1 shows the changes of storage modulus (G') and loss modulus (G'') as well as complex viscosity (η^*) values with fermentation time (1 Hz frequency and 1% strain). It is interesting to note that G', G'' and η^* values increased gradually with the fermentation time. In fact, Kefir samples incubated at 22°C for 18 and 30°h exhibited the lowest (269.40 Pa, 78.01 Pa and 38.36 Pa.s) and highest (548.76 Pa, 153.13 Pa and 77.92 Pa.s) values of G', G'' and η^*, respectively. Although Kefir samples were significantly different ($p < 0.05$) in terms of G', G'' and η^*, however no significant difference ($p > 0.05$) could be found among Kefir samples incubated at different time periods when other rheological characteristics such as tan δ and G^* were taken into consideration (data not shown). In addition, the production of EPS seemed to increase in function of incubation time, with the lowest (421.66 μg/g) and highest (586.66 μg/g) amounts of EPS being observed for Kefir samples fermented at 22 °C for 18 and 24°h, respectively (Table 1). Nevertheless, Kefir samples were not significantly different from each other ($p^\circ > ^\circ 0.05$).

The increase of G', G'' and η^* during fermentation time can be attributed to the increase of metabolic activity of lactic acid bacteria, which promote the association of casein micelles by lowering pH. Certain strains of lactic acid bacteria make a further contribution to the physical structure of fermented milks (Rohm & Kovac, 1994; Vlahopoulou & Bell, 1993) by producing extracellular polysaccharides (EPS). According to Renard, van de Velde & Visschers (2006), the polysaccharides synthesized by lactic acid bacteria during fermentation increase the viscosity of the serum phase, bind hydration water and reduce consequently the water flow in the matrix space. In this study, the decrease in EPS production recorded after 30 h may be due to the presence of glycohydrolases capable of hydrolyzing EPS and liberating monomers. This observation coincides with that of Pham, Dupont, Roy, Lapointe, and Cerning (2000) as well as Lin & Chang Chien, (2007).

3.2. Effect of fermentation temperature on Kefir rheology and EPS production

Fig. 2 illustrates the evolution of storage modulus (G'), loss modulus (G'') and complex viscosity (η^*) in function of incubation temperature at 1 Hz frequency and 1% strain. The values of G' and η^* increased gradually with fermentation temperature while those of G'' showed a slight increment. Indeed, the lowest (199.46 Pa, 58.54 Pa and 28.43 Pa.s) and highest (790.00 Pa, 223.43 Pa and

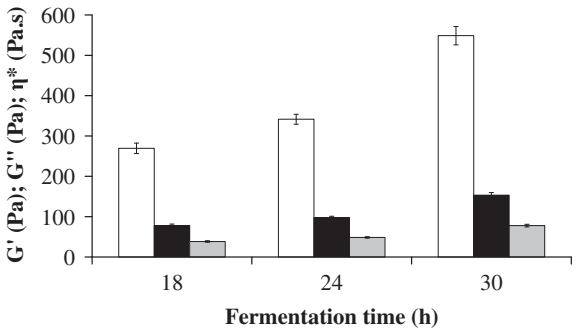

Fig. 1. Changes of G' (□), G'' (■) and η^* (▨) at 1 Hz and 1% strain as a function of fermentation time in Kefir prepared from milk homogenized at 15 MPa and incubated at 22 °C. Each experiment was repeated three times and values are means ± SD.