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Effect of incubation temperature and caseinates on the rheological behaviour of Kefir

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Abstract

The effect of incubation temperature and the addition of caseinates on the rheological behaviour of kefir was studied by using a pneumatic tube viscometer of novel design. The results indicated that the incubation time increased as the incubation temperature was reduced and the casein concentration was increased. Kefir samples incubated at 25°C showed the highest values of viscosity, while the samples incubated at 30°C exhibited the lowest viscosity. The addition of caseinates caused the viscosity of the samples to increase and their flow behaviour index values to decrease. Kefir samples incubated at 30°C exhibited the highest flow behaviour index values.

Keywords: kefir; apparent viscosity; flow behaviour index

1. Introduction

Kefir is a fermented dairy product, slightly carbonated that contains small quantities of alcohol. The starter culture used for its production is a mass of lactic acid bacteria, acetic acid bacteria and yeasts held together by a matrix of proteins and polysaccharides named kefir grains [1]. Kefir grains are gelatinous and irregular in shape, having a white or lightly yellow color [2]. The microorganisms into the grains exist in a relatively stable and specific balance in a complex symbiotic relationship. During fermentation these microorganisms are multiplying by producing lactic acid and other compounds that contribute to flavour and body (polysaccharides) of kefir [3]. The main polysaccharide found in kefir and kefir grains is kefiran, an extra-cellular-polysaccharide that plays a significant role in regulating rheological properties [4].

Rheological properties of kefir are of major importance, since they affect the quality of the final product as well as its acceptance by the consumers. The major factors affecting rheological properties of

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kefir are the chemical composition of the milk used for its production, the starter culture, the incubation temperature, the thermal processing of milk, etc [5]. Despite the importance of studying rheological properties of kefir, only a few reports are found in the literature regarding them [3; 6]. So far, most of the research done on kefir concerned its microbiology [7]. The objective of the present work is to evaluate the effect of incubation temperature and the addition of caseinates on the time needed for the pH to reach the 4.4 value and the rheological behaviour of kefir.

2. Materials & Methods

Manufacture of kefir samples: Kefir samples were prepared from homogenized and pasteurized full fat (3.5%) bovine milk with and without the addition of caseinates at varying concentrations (1%, 2% or 3%). Following thermal processing (85°C for 900 s) the milk was inoculated with kefir grains obtained from a household culture at a ratio of 3% (w/w) and incubated at 20°C, 25°C or 30°C until the pH dropped to 4.4. Caseinates were added to the milk before its thermal processing.

Rheological measurements: The apparent viscosity of the samples was determined using a capillary tube viscometer (TR-1 Rheometer, A.T.E.I., Thessaloniki, Greece). The instrument consists of a stainless steel cylindrical sample vessel (0.086 m height; 0.049 m internal diameter; maximum capacity of 0.05 L), an air chamber to which compressed air is supplied through an inlet valve and a pin shape stirrer consisting of four rods placed in a row. Four extra rods are attached to the bottom of the sample vessel in positions that they do not restrict the movement of the rods of the stirrer. Temperature control is achieved by means of a platinum resistance thermometer-proportional and an integral temperature controller [8]. A capillary tube with an inner diameter of 6.53×10^{-4} m and a length of 0.045 m was used for the rheological measurements. Kefir samples were introduced inside the sample vessel of the viscometer, under continuous stirring (0.83 rps), while the temperature was set to 20°C. To obtain the flow curves of the samples, the flow rate of the discharged fluid at the exit of the tube at varying pressures was determined.

Statistical analysis: Two-way ANOVA was applied to the experimental data, while the Tukey multiple comparison test determined whether statistically significant differences occurred among means. The statistical analysis of the experimental data was performed using Minitab 15.0 statistical software.

3. Results & Discussion

Table 1 shows the pH values of the samples as well as the time needed for the pH to reach 4.4. According to Anova, there is no significant difference to the pH values among the samples.

The time needed for the pH to reach the 4.4 value is significantly affected by both incubation temperature ($p<0.001$) and addition of caseinates ($p<0.001$).

Table 1. The pH values and the time needed for the pH to reach 4.4 of kefir samples

Incubation temperature (°C)	*Caseinates concentration (% w/w)*	*pH*	*Time ($s\times10^3$)*
20	0	4.42	73.50
	1	4.41	74.28
	2	4.42	82.20
	3	4.42	92.28
25	0	4.40	61.92
	1	4.41	65.34
	2	4.41	72.30
	3	4.42	72.84

Incubation temperature (°C)	*Caseinates concentration (% w/w)*	*pH*	*Time (s×10³)*
30	0	4.40	45.12
	1	4.41	47.70
	2	4.42	48.84
	3	4.41	50.28

Decreasing incubation temperature resulted in increased incubation time necessary for the pH to reach 4.4. The Tukey test showed that the incubation time required in order for the pH value to reach 4.4 is greater at 20°C than at 25°C or 30°C. Incubation temperature of 30°C caused the time needed for the pH to reach 4.4 to exhibit the lowest value. Similar results were reported for yogurt samples incubated at 37°C, 40°C, 43°C and 46°C [9]. According to the authors, the increase in the time needed for the pH to reach 4.4 with reducing incubation temperature can be attributed to the reduced activity of the bacteria.

The addition of caseinates in the milk used for kefir fermentations reduced the observed rate of pH reduction. According to Tukey test the incubation time of the kefir samples with and without caseinates increased in the following order: 0% caseinates = 1% caseinates < 2% caseinates = 3% caseinates. The increase in the time needed for the pH to reach 4.4 with increasing casein concentration can be attributed to the increased buffering capacity of the milk used for kefir production. Caseins have strong buffering capacity [10], resulting to increased incubation time for the pH to reach 4.4 when they are added to milk. Yogurt samples made from milk with a caseinate to whey protein ratio of 1/1 showed faster rate of acidification when compared with samples made from milk where the caseinate to whey protein ratio was 4/1 [11]. Furthermore, during acidification of kefir the precipitation of caseins results to the formation of larger clusters when caseinates are added. These clusters block the transport of lactose resulting to a nonhomogeneous fermentation environment with regards to lactose concentration and consequently to a reduced lactose hydrolysis rate and lactc acid formation [10].

According to the flow curves of the samples (Figures 1 and 2), kefir behaves as a pseudoplastic fluid with its viscosity reducing with increasing shear rate. Figure 1 shows the flow curves of the kefir samples incubated at 20°C, 25°C and 30°C with the addition of caseinates at 3% concentration. As it can be seen kefir sample incubated at 25°C showed the highest viscosity, followed by the sample incubated at 20°C, while the sample incubated at 30°C exhibited the lowest viscosity. Similar behaviour was exhibited by the kefir samples made without the addition of caseinates and with the addition of caseinates at 1% and 2% concentration.

As it can be seen in Figure 2, the addition of caseinates causes the viscosity of the kefir samples to increase. Kefir samples incubated at 20°C and 30°C exhibited similar behaviour.

The values of the flow behaviour index obtained from the flow curves of the samples are shown in Table 2. Kefir samples that exhibited increased viscosity showed also increased pseudoplastic behaviour (decreased values of the flow behaviour index). According to ANOVA, both incubation temperature ($p<0.001$) and addition of caseinates ($p<0.001$) affect significantly the flow behaviour index. Kefir samples incubated at 30°C exhibited the highest values when compared to samples incubated at 20°C or 25°C. No significant difference to the flow behaviour index values occurred among the samples incubated at 20°C and 25°C. The flow behaviour index of the samples decreased as the casein concentration was increased. According to Tukey test the flow behaviour index of the kefir samples is increased in the following order: 3% caseinates < 2% caseinates < 1% caseinates < 0% caseinates.

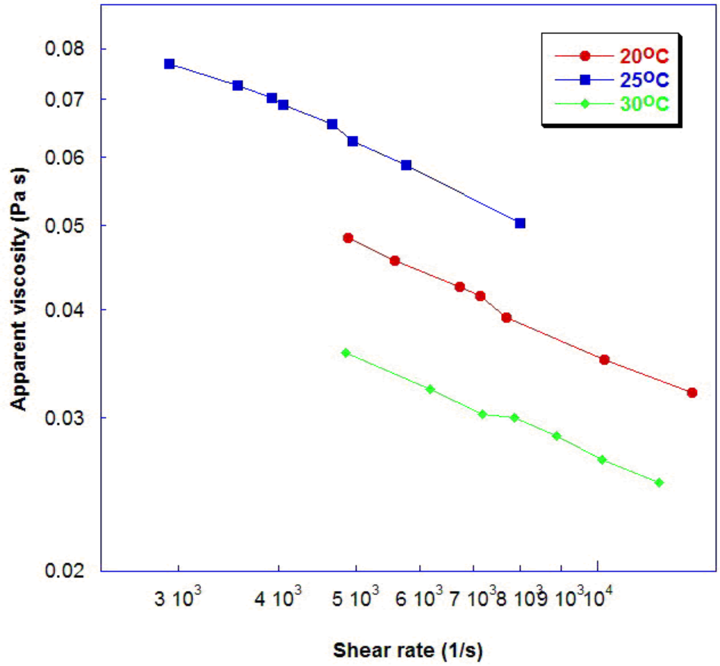

Fig. 1. Flow curves of kefir samples incubated at 20°C, 25°C and 30°C with the addition of caseinates at 3% concentration

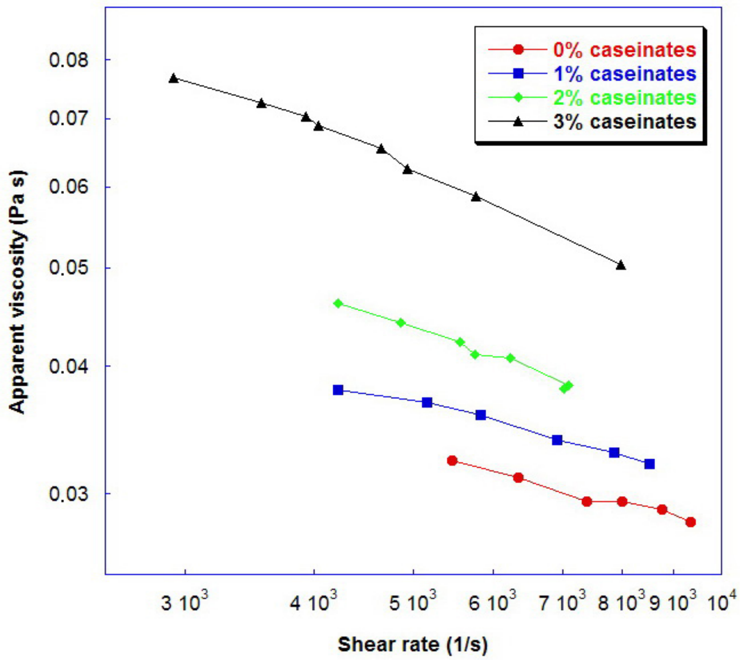

Fig. 2. Flow curves of kefir samples made with and without the addition of caseinates incubated at 25°C.

Table 2. Flow behaviour index of kefir samples

Incubation temperature (℃)	*Caseinates concentration (% w/w)*	*Flow behaviour index*
20	0	0.864
	1	0.757
	2	0.529
	3	0.504
25	0	0.748
	1	0.627
	2	0.531
	3	0.510
30	0	0.926
	1	0.788
	2	0.756
	3	0.545

The effect of incubation temperature on the viscosity and the pseudoplastic behaviour of kefir samples can be explained by the presence of the polysaccharide kefiran. Polysaccharides, due to their ability to bind water [11] and to interact with proteins, contribute to the increase of the viscosity and the pseudoplastic behaviour of the fermented dairy products. Furthermore, their production is closely related with the optimal growth conditions of the bacteria that produce them [12]. Thus according to the results of the present study the temperature incubation of 25°C enhances the production of the kefiran compared to 20°C or 30°C, contributing this way to increased viscosity of the kefir samples.

The addition of caseinates results in increased viscosity and pseudoplastic behaviour of the kefir samples. There is a power-law relation between the casein concentration and the consistency of the casein-containing systems [10]. The addition of caseins into the milk system increases the possibility of either bigger or more casein clusters being formed during fermentation resulting to increased consistency of the protein matrix.

4. Conclusion

The time needed for the pH to reach the 4.4 value increased as the incubation temperature was reduced and the casein concentration was increased. Kefir samples incubated at 25°C showed the highest values of viscosity, followed by the samples incubated at 20°C, while the samples incubated at 30°C exhibited the lowest viscosity. Increasing caseinate concentration caused the viscosity of the kefir samples to increase. Kefir samples incubated at 30°C exhibited the highest values of flow behaviour index. The addition of caseinates resulted in decreasing the flow behaviour index of the samples.

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