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Viscoelastic Properties of Kefir as Affected by Milk Protein Addition and Starter Culture Type

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Abstract

The effect of Sodium Caseinates (SCN) and Whey Proteins Concentrates (WPC) addition, as well as the starter culture type (kefir grains and commercial starter culture) on the viscoelastic properties of kefir samples was evaluated. The kefir samples were prepared from homogenized and pasteurized full fat bovine milk with or without the addition of SCN or WPC at 2% (w/w) concentration. According to the results, SCN increased the fermentation time of kefir samples when compared to control samples (samples without SCN or WPC addition), while WPC decreased it. The elasticity of the protein matrix was increased with SCN or WPC addition, however, the effect of SCN was more pronounced to than that of WPC. SCN contributed to the elasticity of the samples by the formation of strong as well as weak chemical bonds, while WPC participated to protein interactions that were characterized as weak ones. The commercial starter culture resulted in lower fermentation time and increased viscoelastic properties of the kefir samples when compared to kefir grains.

Keywords: kefir, sodium caseinates, milk protein concentrates, starter culture type, viscoelastic properties, creep-recovery test

1. Introduction

Kefir is a fermented dairy product that is considered to be a natural probiotic due to its beneficial health effects besides its nutritional status. Kefir grains, a mass of bacteria (lactic and acetic acid bacteria) and yeasts held together within a protein-polysaccharide matrix, comprise the traditional starter culture of kefir (Farnworth, 2005). The microflora of grains varies considerably depending mainly on grains origin and fermenting and storage conditions, resulting in products with variable properties. The use of cultures containing freeze-dried bacteria and yeasts, instead of kefir grains, can impart product's standard quality with desirable characteristic.

Texture enhancement and water-holding capacity improvement of fermented milk products, like yoghurt, is accomplished by the addition of milk protein preparations such as Whey Protein Concentrates (WPC) or Sodium Caseinates (SCN) (Lucey, 2004; Tamime & Robinson, 2007). Increasing milk protein concentration results in the formation of increased number of protein-protein interactions and thus increased elasticity of the gel (Sodini, Remeuf, Haddad, & Corrieu, 2004). Additionally, the nature (denatured or native) and the whey protein to casein ratio determine the magnitude of the effect on the rheological behavior of the final product (Remeuf, Mohammed, Sodini, & Tissier, 2003; Sodini et al., 2004; Amatayakul, Halmos, Sherkat, & Shah, 2006).

Natural food products with significant health attributes are currently occupying a substantial share of the food market and their potential is increasing rapidly. A functional dairy product, like kefir, with nutritional and favorable organoleptic characteristics (Farnworth, 2005) is thought to gain increased acceptance by the consumers. The desirability of a food product depends not only on its nutritional and health properties but also on its sensory characteristic, which to a great extent are related to its rheological behavior. Rheological properties of kefir are affected mainly by the chemical composition of the milk used for its preparation, the type and the amount of the starter culture, the fermentation conditions (temperature, agitation, incubation time) and the ripening of the product following production (rate of cooling, storage temperature) (Farnworth, 2005).

Most research studies published about the rheological behavior of kefir, concerned the effect of the kefir grains quantity (Garrote, Abraham, & De Antoni, 1998; Irigoyen, Arama, Castiella, Torre, & Ibanez, 2005), the storage time (Irigoyen et al, 2005; Sady, Domagala, Najgebauer-Lejko, & Grega, 2009), the heat treatment (Dimitreli, Gregoriou, Kalantzidis, & Antoniou, 2013) and the addition of whey proteins (Sady et al., 2009; Dimitreli et al., 2013), caseinates (Dimitreli & Antoniou, 2011) and honey (Dogan, 2010) on the viscosity of the final product. The effect of fermentation conditions (temperature and time) and homogenization pressure on the viscoelastic behavior of kefir was studied by Bensmira, Nsabimana and Jiang (2010). Sady et al. (2009) evaluated the effect of whey proteins addition and storage time on the texture (evaluated by Texture Profile Analysis and "back extrusion" test) of set-type kefir samples. According to their results, WPC did not have a significant effect on the textural properties of the samples. Wszolek, Tamime, Muir and Barclay (2001) studied the firmness, adhesiveness and gumminess of set-type kefir samples made with different milk types (bovine, caprine and ovine) and starter cultures (kefir grains and two freeze-dried kefir starter cultures). The authors reported that milk type had greater influence on the textural characteristics of the products. Particularly, starter culture type affected only the adhesiveness of the kefir samples.

The aim of the present work was to study the effect of milk proteins addition and starter culture type (a household kefir grain culture and a commercial starter culture) on the viscoelastic properties of kefir determined by dynamic analysis and creep-recovery test. The creep-recovery test has already been used for the evaluation of the rheological properties of stirred-type yoghurt (Petridis, Dimitreli, Chrysalidou, & Akakiadou, 2013; Dimitreli, Petridis, Akakiadou, & Chrysalidou, 2014).

2. Materials and Methods

2.1 Materials

Full-fat (3.5% w/v) pasteurised and homogenised bovine milk was used for the production of the kefir samples, while skimmed (0.3% w/v) Ultra High Temperature (UHT) bovine milk was used for the activation of the kefir grains. The pasteurised and the UHT milk were purchased from a local dairy (Thessaloniki, Greece). Kefir samples were prepared using a household kefir grain culture and commercial Direct Vat Set (DVS) cultures (Hansen A/S, Denmark). The commercial starter cultures consisted of a blend of mesophilic-thermophilic lactic acid bacteria (XPL-1), suitable to enhance the texture of kefir (increased production of exopolysaccharides from the bacteria), and a yeast culture (*Kluyveromyces marxianus*) that hydrolyses lactose and produces large amounts of CO_2 (LAF-4). Sodium Caseinates (SCN) (MIPRODAN 30; Arla Food Ingredients, Viby J., Denmark) and Whey Protein Concentrates (WPC) (Hellenic Protein S.A., Athens, Greece) were used for the enrichment of milk with proteins. The composition (% w/w) of SCN and WPC was moisture ≤ 6.0, proteins 88.0-93.5, fat 1.5, ash 4.0, lactose 0.3 and moisture ≤ 5.0, proteins 80.0, fat 3.5, ash 3.0, lactose 10.4, respectively.

2.2 Preparation of Kefir Samples

The SCN and WPC were added to the milk at a concentration of 2% (w/w) and dissolved in a shaking water-bath (Grant GLS400, Grant Instruments Ltd, Cambridge, G.B.) under continuous stirring for 20 min at 35°C. Protein supplements addition was made before the heat treatment of the milk (85°C for 15 min). The kefir samples that were prepared without SCN or WPC addition are mentioned as the control samples. The samples with SCN or WPC addition are referred to the text as SCN or WPC, respectively. Prior to fermentation process, the kefir grains were activated by successive cultures in UHT milk at 24ºC using a grain inoculum ratio of 3% (w/w) and until a pH of 4.4 was achieved within 24 h of incubation. Following activation, the kefir grains were inoculated at a ratio of 3% (w/w) in heat-treated milk, with or without the addition of milk proteins, and incubated at 25°C (Cooled Incubator Series 8000, Termaks AS, Bergen, Norway) until the pH value reached 4.4. The commercial starter cultures were directly inoculated into the heat-treated milk (with or without the addition of SCN or WPC) (according to the instructions of the manufacturer) and incubated at 30°C to a final pH value of 4.4. The optimal temperature for the kefir grains (increased lactic acid production and increased apparent viscosity) is 25°C (Dimitreli & Antoniou, 2011), while for the commercial starter cultures is 30°C (Hansen A/S, Denmark). The samples that were prepared with kefir grains or commercial starter culture are referred to the text as kefir grains or commercial starter culture, respectively. The kefir samples were incubated into sterilised glass containers that had a maximum capacity of 250 mL. At the end of the fermentation time, the kefir samples were mixed gently (20 times) and stored for 24 h before testing. Kefir samples were produced in duplicate.

2.3 Determination of pH

The pH values of kefir samples were determined by the use of a laboratory pH-meter (GP353 ATC, EDT Instruments, Kent U.K.).

2.4 Rheological Measurements

The viscoelastic properties of kefir samples were evaluated by the use of dynamic analysis and creep-recovery test. A DMA rheometer (Bohlin C-VOR 150, Malvern Instruments Ltd, Worcestershire, UK) was equipped with a 40 mm diameter serrated plate so as no slip effects will occur during the measurements. The samples were placed in specially made aluminum sample containers (height 10 mm and diameter 40 mm) that had also serrated surface. The upper plate was lowered so as to get in touch with the surface of the sample (approximately 1500 μm). Temperature during measurements was set at 25°C, using a Peltier plate system (−30 to + 180°C).

2.4.1 Dynamic Analysis

An amplitude test was first applied to determine the linear viscoelastic region of the samples, followed by a frequency sweep ranging from 0.01 to 10 Hz at a strain deformation of 1.104×10^{-5}. The elastic modulus (G') and the loss tangent (tan δ) of the samples were determined.

2.4.2 Creep-Recovery Test

A stress of 0.1 Pa, within the linear viscoelastic region (previously determined), was applied to the kefir samples for 180 s, followed by a recovery time of 100 s. From the creep-recovery curves the instantaneous compliance, the retarded compliance and the Newtonian viscosity at zero shear rate (η_0) were determined. The creep compliance data were finally expressed as elasticity (the reverse of compliance) that is instantaneous elasticity (G_g) and retarded elasticity (G_R).

2.5 Statistical Analysis

The two-way ANOVA was applied to the experimental data so as to study the effect of milk proteins addition and starter culture type on the fermentation time and the viscoelastic properties of the kefir samples. The Tukey multiple comparison test was used so as to determine whether statistically significant differences occurred among means. The statistical analysis was performed by the use of the statistical software Minitab 16.0.

3. Results and Discussion

3.1 Fermentation Time

According to ANOVA, the incubation time was significantly affected by milk proteins addition ($p<0.001$) and starter culture type ($p<0.001$). As it can be seen in Figure 1, samples made with SCN exhibited the highest fermentation time, followed by the control samples, while the samples with WPC addition exhibited the lowest fermentation time. The Tukey test showed that fermentation time increased in the following order: WPC < Control < SCN. The increase in fermentation time with SCN addition can be attributed to the increased buffering capacity of the caseins (Salaün, Mietton, & Gaucheron, 2005), resulting in the production of more lactic acid so as to reduce the pH of the samples to the favorable value. The reduced fermentation time with WPC addition is due to the increased availability of nutrients deriving from WPC, resulting in increased cell growth and thus increased lactose hydrolysis rate. According to Tamime and Robinson (2007), WPC are a good source of peptides and amino acids that might improve the viability of the microorganisms of the starter cultures.

As it concerns starter culture type, kefir grains exhibited the highest incubation time. According to Tukey test, fermentation time increased in the following order: commercial starter culture < kefir grains. The main reason for the reduced fermentation time of the commercial starter culture might be the uniform distribution of the microorganisms into the milk that results in direct outset of fermentation. In contrast, kefir grains are reposing at the bottom of the containers, which in turns causes difficulties as it concerns the steady access of nutrients to the microorganisms resulting in increased fermentation time.

3.2 Viscoelastic Properties

The G' and tan δ were significantly affected by milk proteins addition ($p<0.001$) and starter culture type ($p<0.001$) (Figures 2 and 3, respectively). Particularly, samples with increased values of G' exhibited decreased values of tan δ (increased solid-like behavior). According to Tukey test, G' increased in the following order: Control < WPC < SCN, while tan δ decreased in the following order: Control > WPC > SCN. As it can be seen in Figure 2, SCN exhibited the highest elasticity, followed by WPC. The addition of SCN into the milk system increases the number of the bonds being formed and the size of the aggregates that comprise the continuous network of the acid milk gels. This results in increased resistance to the application of stress and thus increased elasticity of the protein matrix. According to Walstra, Wouters and Geurts (2006), a power-law relation occurs between casein concentration and consistency of the casein-containing systems. The increase in G' with WPC addition is due to the heat denaturation of the whey proteins and the formation of complexes between denatured whey proteins, as well as between denatured whey proteins and κ-casein (Lucey, Munro, & Singh, 1998). This cross-linking or bridging increases the number of the bonds and the size of the aggregates being formed and thus increases the elasticity of the protein matrix. The pronounced effect of SCN when compared to WPC might be attributed to the existence of caseins in complexes (casein micelles) and to their participation into the structure of the fat globule membrane (in homogenized milk). Similar results, as it concerns the efficiency of SCN and WPC in increasing the elasticity of the protein matrix, have been reported for yoghurt samples prepared with buffalo milk (Dimitreli et al., 2014) and glucono-δ-lactone acidified milk gels (Dimitreli, Exarhopoulos, Goulas, Antoniou, & Raphaelides, 2016).

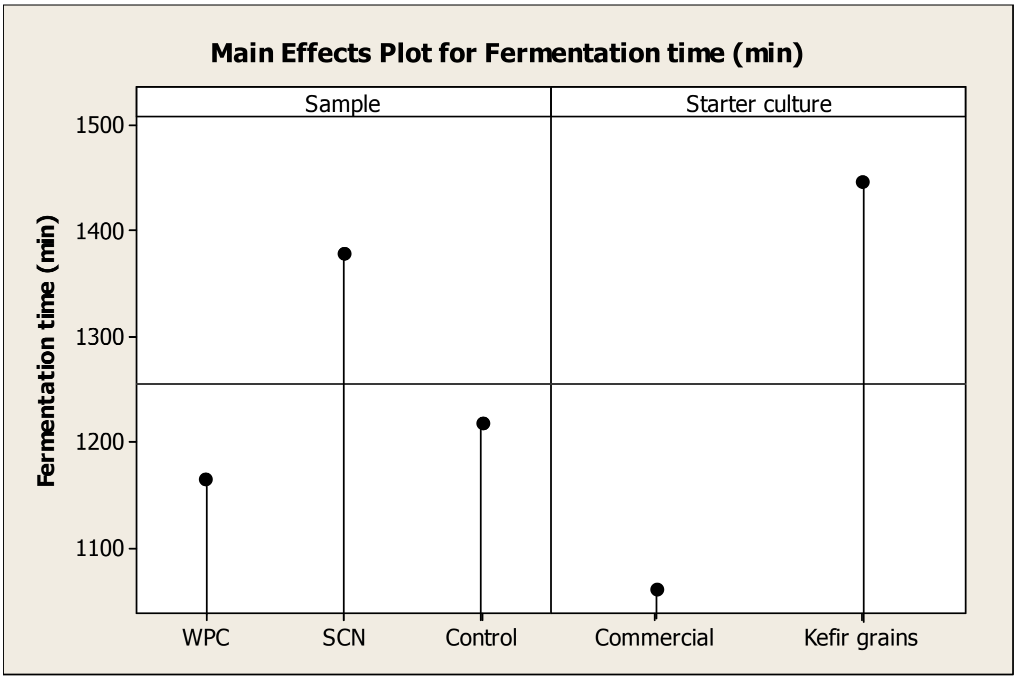

Figure 1. Effect of milk proteins addition and starter culture-type on the fermentation time of kefir samples. SCN: Sodium Caseinates, WPC: Whey Protein Concentrates

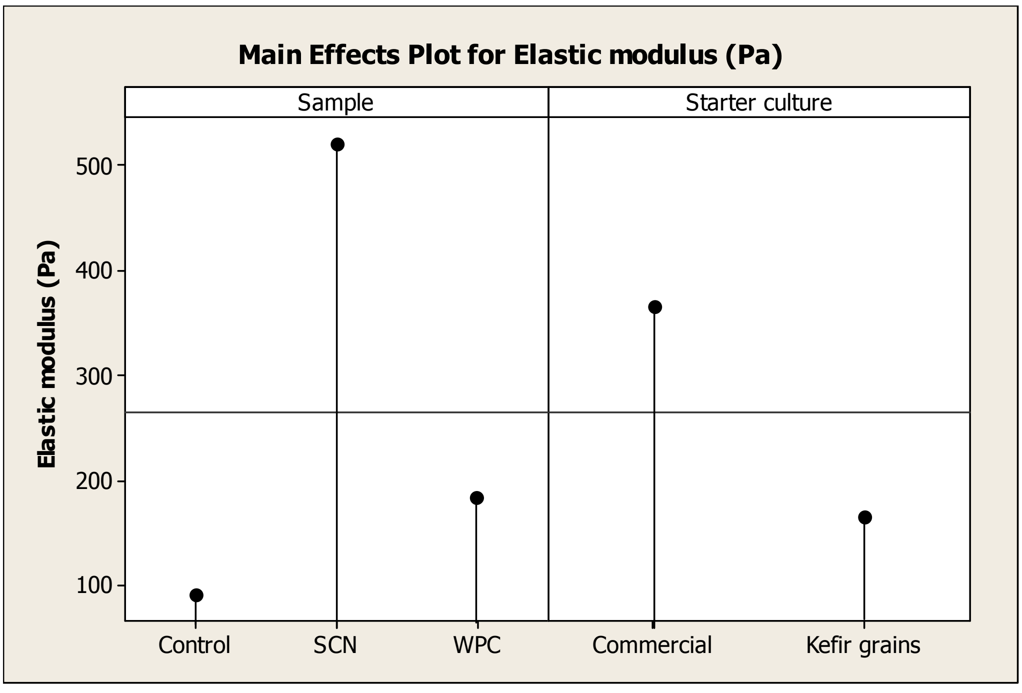

Figure 2. Effect of milk proteins addition and starter culture-type on the elastic modulus of kefir samples. SCN: Sodium Caseinates, WPC: Whey Protein Concentrates

The commercial starter culture resulted in increased elasticity and reduced liquid-like behavior when compared to kefir grains. According to Tukey test, G' increased in the following order: kefir grains < commercial starter culture, while tan δ decreased in the following order: kefir grains > commercial starter culture. This might be attributed to the increased production of exopolysaccharides from the lactic acid bacteria of the commercial starter culture, resulting in increased interactions between polysaccharides and milk proteins, which in turns enhanced the elasticity of the protein matrix (Duboc & Mollet, 2001).

According to two-way ANOVA, G_g was significantly affected by milk proteins addition ($p<0.001$), starter culture type ($p<0.001$), as well as by their interaction ($p<0.001$) (Figure 4). The G_g corresponds to the strong bonds of the matrix that either they have been deformed without breaking or they broke and reformed instantly by the removal of stress. As it can be seen, only the samples with SCN addition that were prepared with the commercial starter culture as well as the kefir grains, and the control sample made with the commercial starter culture exhibited G_g values. This means that caseins result in the formation of strong chemical bonds and that their cross-linking with whey proteins interferes that bonding. The control sample prepared with the commercial starter culture also exhibited strong chemical bonds that might be attributed to the interactions between the polysaccharides produced from the lactic acid bacteria and the caseins pre-existing in milk.

As it can be seen in Figure 5, G_R was significantly affected by milk proteins addition ($p<0.001$) and starter culture type ($p<0.001$). According to Tukey test, G_R increased in the following order: Control < WPC < SCN. The G_R corresponds to the weak bonds that have been destroyed during the application of the stress and have been reformed again in other places during recovery. The presence of caseins into the system increases the number of the bonds being formed some of which might be weak ones. WPC also increase the number of the weak bonds of the protein matrix, when compared to control samples, due to their interactions with other denatured whey proteins or caseins.

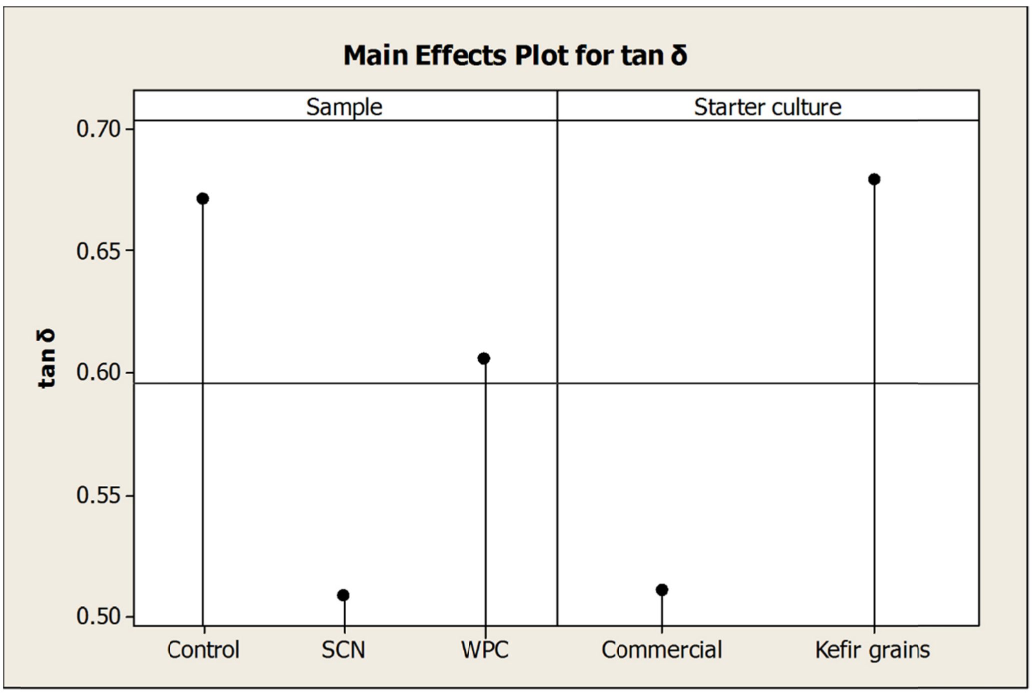

Figure 3. Effect of milk proteins addition and starter culture-type on the tan δ of kefir samples. SCN: Sodium Caseinates, WPC: Whey Protein Concentrates.

According to the results from the creep test, SCN contributed to the elasticity of the protein matrix by the formation of both strong and weak chemical bonds, while WPC participated to protein interactions that were characterized as weak ones. The results from the creep-recovery test are in good agreement with those derived from the dynamic analysis as it concerns the pronounced effect of SCN when compared to WPC.

The kefir samples prepared with kefir grains exhibited the lowest values of G_R. Particularly, the Tukey test showed that G_R increased in the following order: kefir grains < commercial starter culture. The increased concentration of the polysaccharides produced during fermentation of the kefir samples prepared with the commercial starter culture might be responsible for the formation of increased number of weak bonds between polysaccharides and milk proteins.

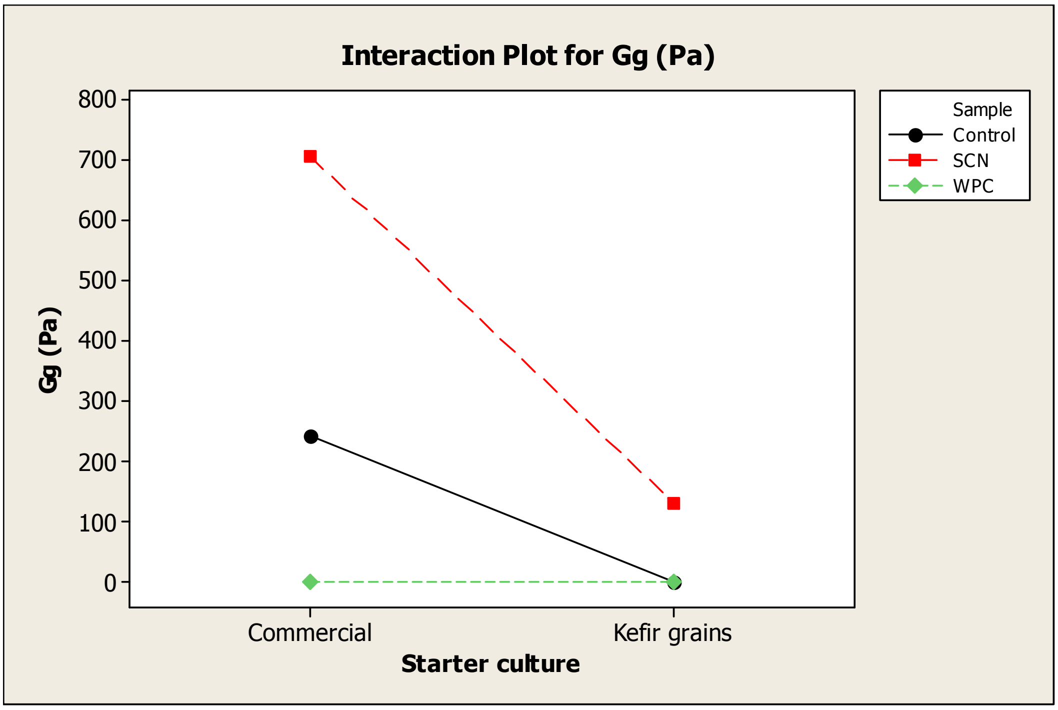

Figure 4. Effect of milk proteins addition and starter culture-type on the instantaneous elasticity (G_g) of kefir samples. SCN: Sodium Caseinates, WPC: Whey Protein Concentrates

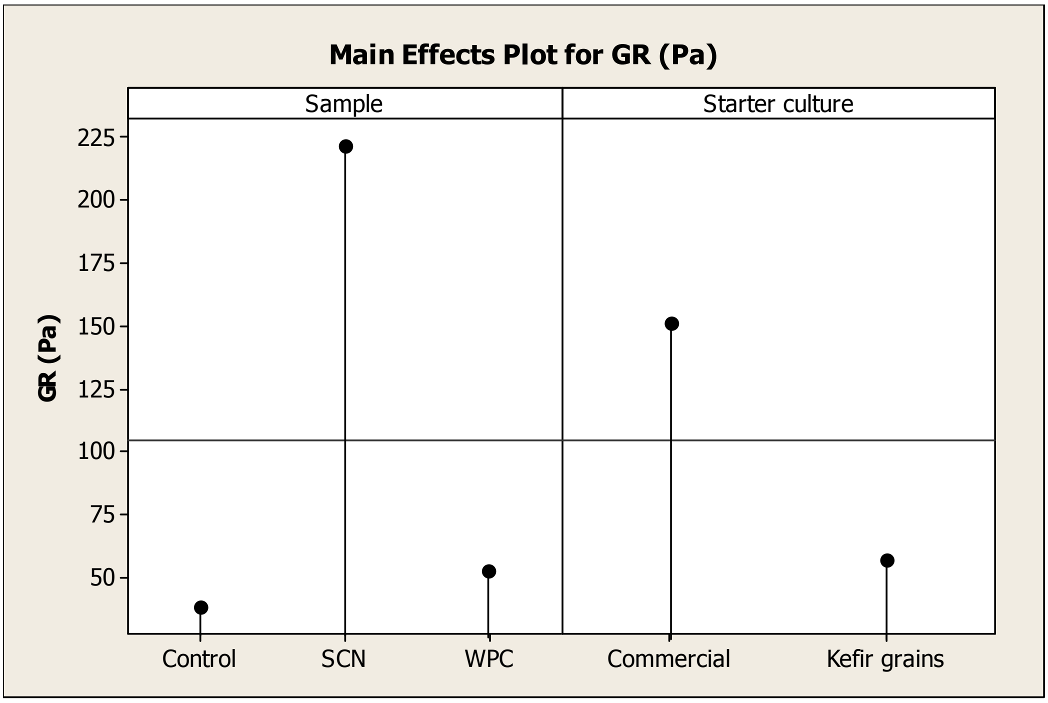

Figure 5. Effect of milk proteins addition and starter culture-type on the retarded elasticity (G_R) of kefir samples. SCN: Sodium Caseinates, WPC: Whey Protein Concentrates

The η_0 was significantly affected by milk proteins addition ($p<0.001$) and starter culture type ($p<0.001$) (Figure 6). According to Tukey test, η_0 increased in the following order: Control < WPC < SCN. Samples with increased elasticity exhibited increased values of η_0. When the number of protein interactions is increased, the protein matrix that is being formed becomes more continuous resulting in increased consistency.

The increased production of polysaccharides from the commercial starter culture increases the protein-polysaccharide interactions resulting also in increased density of the protein matrix. According to Tukey test, η_0 increased in the following order: kefir grains < commercial starter culture.

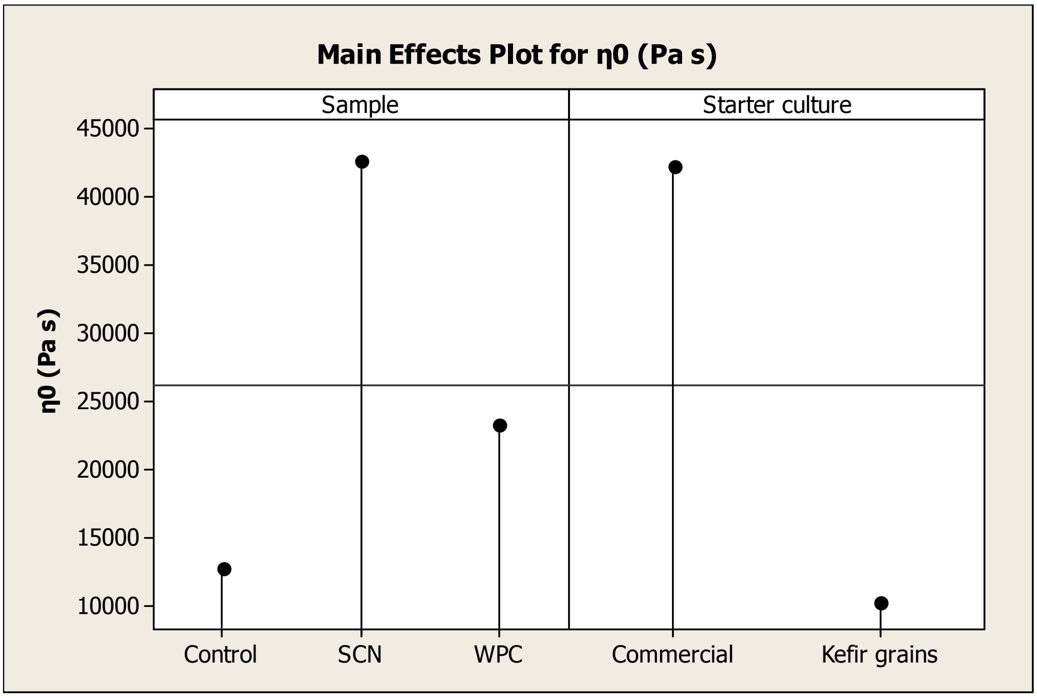

Figure 6. Effect of milk proteins addition and starter culture-type on the Newtonian viscosity at zero shear rate (η_0) of kefir samples. SCN: Sodium Caseinates, WPC: Whey Protein Concentrates

4. Conclusion

The addition of SCN increased the fermentation time of kefir samples when compared to control samples, while WPC decreased it. The commercial starter culture exhibited lower fermentation time than kefir grains. SCN and WPC increased the elasticity of the protein matrix. However, the effect of SCN was more pronounced to than that of WPC. SCN contributed to the elasticity of the samples by the formation of strong as well as weak chemical bonds, while WPC participated to protein interactions that were characterized as weak ones. The commercial starter culture resulted in increased viscoelastic properties of the kefir samples when compared to kefir grains.

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