



Rheological, texture and sensory properties of kefir with high performance and native inulin

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ABSTRACT

Rheological, textural, and sensory properties of kefir with inulin were analysed. Besides, the effect of skim milk powder (SMP) and milk fat substitution was examined by high performance (HP) and native inulin. One-third of SMP was substituted by native (IQ) or HP (TEX1, HPX) inulin. Hardness of kefir with IQ inulin was lower than that with HP inulins or pure SMP. Analysis of cohesiveness and adhesiveness showed that kefir with inulin had higher firmness. Rheological analysis revealed that all manufactured kefir had higher storage than loss modulus, and exhibited thixotropic and shear thinning behaviour. There were no significant differences in odour and consistency between kefir with and without inulin. Only in case of whole milk powder (WMP) kefir, panellists noticed the difference in flavour, although kefir based on SMP had the same scores. Substitution of SMP by inulin allowed for reducing 15% and 37% of caloric value in comparison with SMP and WMP kefir, respectively.

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1. Introduction

Kefir is a fermented beverage, which traditionally is prepared by inoculation of raw milk with kefir grains (Dogan, 2011). Kefir grains consist of different species of yeasts, lactic acid bacteria, acetic acid bacteria, and mycelial fungi in a protein and polysaccharide matrix (Witthuhn et al., 2005). The microbes present in the kefir grains live symbiotically, however the population composition may differ if the grains depend on origins, methods and substrates (Grønnevik et al., 2011). Currently, direct-to-vat inoculation (DVI) of the milk base dominates in dairy industry because of time-saving and hygienic reasons.

Kefir originates from the Caucasian mountains (Assadi et al., 2000). It spread through the centuries in the Balkans and Eastern Europe. Nowadays, annual worldwide production of cultured milk products, such as sour cream, kefir etc., is estimated for about six million tons of the total market (www.chr-hansen.com). What is worth noting, yoghurt has been excluded from this statistics.

Some results indicate that kefir and its constituents have pro-healthy properties that positively affect immune and gastrointestinal system (Ertekin and Guzel-Seydim, 2010). In certain studies it helped with lactose intolerance (Hertzler and Clancy, 2003), affected the cholesterol metabolism (Tamai et al., 1996), revealed therapeutic activity against colon carcinogens (Kroger, 1993) was the source of some vitamins (Van Wyk et al., 2011), and delayed

breast cancer development (de Moreno de LeBlanc et al., 2006). Besides, kefir cultures can be applied to promote food safety by inhibiting coliforms and numerous pathogens (Van Wyk et al., 2011).

Many studies aimed to analyse the changes in kefir microbial population during storage and to determine factors which allow for controlling microbiological stability of kefir grains (Assadi et al., 2000; Beshkova et al., 2002; Grønnevik et al., 2011; Irigoyen et al., 2005; Witthuhn et al., 2005). Studies on the rheological textural and sensorial properties of kefir with inulin are very scarce. Beside other additives, Tratnik et al. (2006) as well as Ertekin and Guzel-Seydim (2010) studied the effect of 2% inulin addition on kefir properties. Generally the supplemented kefir samples had no worse and sometimes better sensory scores than the control. The applied inulin did not significantly affect the chemical or microbial properties, or the formation of flavour compounds. Kefir with inulin had higher apparent viscosity values than that produced from non-fat milk.

Inulin addition to food products can have beneficial effects on the consumer's health. Inulin–polyfructan containing fructose polymers of various length with β (2–1) glycosidic bonds, terminated generally by a single glucose unit (Ronkart et al., 2006) – is a dietary fiber which is not digested in gastrointestinal tract (Izzo and Franck, 1998). Consumption of 15–20 g inulin per day contributes to counteracting the constipation (Den Hond et al., 2000; Kleessen et al., 1997). An increase in defecation frequency decreases the risk of colon cancer (Schneeman, 1999). Additionally, inulin beneficially affects the host by selectively stimulating growth and/or activity of one or a number of health-promoting colon

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bacteria such as *Bifidobacterium longum* or *Lactobacillus acidophilus* (Gomes and Malcata, 1999; Roberfroid et al., 1998). Some studies indicate that inulin lowers the level of cholesterol in blood plasma (Roberfroid, 2005) and there are plenty of studies confirming that inulin consumption increases calcium, magnesium and iron absorption (Bosscher et al., 2003). Calcium increased absorption enhances bone mineralization, which is notably important in osteoporosis prevention (Bosscher et al., 2006). It should be mentioned that European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies concluded that a cause and effect relationship cannot be established between the consumption of inulin-type fructans and the health claims related to gut microbiota and changes in bowel function, calcium absorption and bone mineral density as well as blood cholesterol concentrations (EFSA, 2011).

Till now, only the effect of native inulin, with degree of polymerisation (DP) of 10, on rheological and sensory properties of kefir has been analysed (Tratnik et al., 2006; Ertekin and Guzel-Seydim, 2011). To the best of our knowledge there are no studies regarding high performance inulins (with DP equal or higher than 23) addition to kefir. Moreover, in the recent studies neither Texture Profile Analysis (TPA) nor oscillatory studies were applied for the kefir with inulin. Bearing in mind so many beneficial effects of inulin on the consumer's health and serious lacks in studies concerning kefir with this pro-healthy ingredient, the aim of this study was analysis of the effect of substitution of skim milk powder and milk fat by high performance and native inulin on rheological, textural and sensory properties of kefir.

2. Materials and methods

2.1. Materials

Inulin Frutafit® TEX! and IQ were kindly delivered by Sensus Operations C.V. (Roosendaal, The Netherlands). Inulin Beneo™ HPX was purchased from Orafiti (Oreyle, Belgium). All inulins were extracted from chicory roots. Average DP of HPX and TEX! inulin is ≥ 23 , while that for IQ inulin is ≥ 10 (producer's data). Skim milk powder (SMP) (SM Gostyń, Gostyń, Poland) and whole milk powder (WMP) (OSM Krasnystaw, Krasnystaw, Poland) were purchased from a local supermarket. Protein, lactose, and fat contents were 35.7%, 51.2%, and 1.25% in SMP and 26%, 38% and 26% in WMP, respectively (producer's data). DVS kefir culture was donated by Danisco Biolacta Sp. z o.o. (Olsztyn, Poland).

2.2. Kefir manufacture

Composition of the samples is given in Table 1. Inulin and/or milk powders were dispersed at 23 °C with moderate mixing using magnetic stirrer. The dispersions were stirred for 60 min. and subsequently were homogenized by laboratory homogenizer H 500 (Pol-EkoAparatura, Wodzisław Śląski, Poland). Homogenization lasted for 1 min at rotational speed 10000 min⁻¹. Afterwards, the solutions were poured into flasks, the flasks were sealed with aluminium foil and pasteurised in water bath at 80 °C for 30 min. Sub-

Table 1
Composition of kefir^A.

	Milk powder (%) w/w	Inulin (%) w/w	Fat content (%) w/w
WMP	16	–	4.2
SMP	16	–	0.2
SMP + TEX ^B	12	4	0.2
SMP + HPX	12	4	0.2
SMP + IQ	12	4	0.2

^A Fat content was calculated on the basis producer's data.

^B The sample contains inulin TEX!.

sequently, the solutions were cooled in a tap water to 23 °C and DVS kefir culture was added at a level of 0.003% (w/w). Next, the solutions were poured into plastic cylindrical containers of 35 mm inner diameter and the lids were twisted on to prevent evaporation. The containers were stored for 24 h at 23 °C in a thermostatic cabinet and then analyzed. The samples for sensory analysis were kept for another 48 h at 8 °C. The kefir were manufactured in three independent trials.

2.3. pH

pH was measured before inoculation and right after 24 h of fermentation using pH meter CP-401 (Elmetron Sp. J., Zabrze, Poland).

2.4. Rheometry

Rheological measurements were conducted using Haake RS 300 rheometer (Haake, Karlsruhe, Germany) equipped with cone-and-plate geometry (60 mm diameter, 2° angle). When the sample was placed on the plate, the lift moved and the cone took the measuring position (0.105 mm gap between the cone apex and the plate). Temperature control was maintained by a Haake DC30 circulator water bath (Haake, Karlsruhe, Germany). All measurements were carried out at 23 °C. The apparent viscosity was measured at 20 s⁻¹ shear rate for 120 s. For the analytical purposes, average value was calculated from 90th, 105th and 120th second of the measurement (Glibowski et al., 2008). In a shear stress vs. shear rate measurement shear rate was changed every 2 min from 0.1 to 100 (s⁻¹) linearly in eight steps and from 100 to 0.1 s⁻¹ also linearly in eight steps. Strain sweeps were conducted at a frequency of 0.1, 1.0 and 10 Hz. Frequency sweeps (0.1–100 Hz) were conducted at the strain corresponded to the maximum found within the linear viscoelastic region of the studied material. For analytical purposes values of G' and G'' obtained at 1 Hz were taken (Table 3). All rheological data were collected and calculated by Haake Rheo-win software version 3.61.0004 (Haake, Karlsruhe, Germany).

2.5. Texture analysis

The hardness, adhesiveness and cohesiveness were analysed according to TPA method modified by Bonczar et al. (2002). Sam-

Table 2
pH of kefir before and after 24 h fermentation^A.

	Before inoculation	After 24 h fermentation
SMP	6.26 ^a	4.53 ^b
SMP + HPX	6.34 ^a	4.47 ^b
SMP + TEX	6.34 ^a	4.47 ^b
SMP + IQ	6.33 ^a	4.47 ^b
WMP	6.24 ^a	4.45 ^b

^A Values are means. Means with different superscript letters are significantly different, $p \leq 0.05$.

Table 3
Rheological parameters of SMP, WMP and kefir produced with addition of different inulins^A.

	Apparent viscosity (Pa s)	G' (Pa)	G'' (Pa)
SMP	16.19 ^b ± 2.46	838 ^a ± 216	231 ^a ± 56
SMP + HPX	19.89 ^a ± 1.53	752 ^a ± 95	225 ^a ± 21
SMP + TEX	17.53 ^b ± 0.66	668 ^a ± 40	195 ^a ± 13
SMP + IQ	16.98 ^b ± 3.10	710 ^a ± 81	207 ^a ± 24
WMP	15.79 ^b ± 0.51	603 ^a ± 49	159 ^a ± 13

^A Values are means ± standard deviation. Means in the same column with different superscript letters are significantly different, $p \leq 0.05$.

ples were double punched, with a 30 s rest period between, by a cylindrical probe (1 cm diameter) with the crosshead speed 1 mm s^{-1} at 15 mm depth, using a TA-XT2i texture analyser (Stable Microsystems, Goaldming, England). Hardness was defined as a maximal peak value recorded after the first immersion into the sample, adhesiveness as a product of the force necessary for emerging the probe from the sample and time, and cohesiveness as ratio of positive force area during the second immersion to that during the first immersion area. The analysis was performed without removing the samples from the containers.

2.6. Sensory evaluation

Sensory evaluation was conducted using 40 untrained panellists (25 women and 15 men, age 24–36). Panellists examined and tasted the samples and recorded their perceptions by making marks on a ten-score grades. They examined flavour (0 – bad, 10 – very good), odour (0 – undetectable, 10 – very intensive), and consistency (0 – liquid, 10 – very thick).

2.7. Statistical analysis

The texture and rheological measurements were completed in three independent trials. Each analysis was performed in duplicate. All data were analysed by the Statistical Analysis System (SAS Enterprise Guide 3.0.3.414) using the ANOVA procedure for analysis of variance and Student–Newman–Keuls *t*-test for ranking the means.

3. Results and discussion

Since maintaining the texture can be a problem in commercial manufacture of fermented dairy products and keeping the quality of the final product manufacturers relies on increasing milk solids (Dogan, 2011), we intentionally manufactured kefir with relatively high dry mass content (Table 1). Usually the basic raw material in kefir manufacture is milk with fat content between 0.5% and 6% (Bylund, 1995), and dry mass at level 8–13% (Ertekin and Guzel-Seydim, 2010). In this study it was planned to analyse rheological, textural and sensory properties of model kefir with constant dry mass content. Besides, the applied composition simultaneously allowed substitution of four percent points of dry mass and fat in relation to SMP and WMP kefir, respectively (Table 1).

Three different inulin powders were applied in this study, one native (IQ) and two high performance, or in other words, long chain inulins (TEX! and HPX). The difference between TEX! and HPX inulin powders is that inulin TEX! powder has amorphous, whilst HPX crystal structure (Glibowski and Pikus, 2011). The differences in crystallographic structure of inulin powders result from thermal parameters during inulin powder production (Ronkart et al., 2007). Practical meaning of these differences is that inulin HPX is much easier dissolved, whilst inulin TEX! forms hard clumps during fast addition to water at ambient temperatures (Glibowski and Pikus, 2011). Both powders are excellent dissolved at temperatures above 60 °C.

3.1. pH

Usually fermentation is carried out till kefir reaches pH 4.4–4.6. Most frequently, it takes 16–24 h (Ertekin and Guzel-Seydim, 2010; Simona et al., 2002; Wszolek et al., 2001; Dogan, 2011). In this study, we decided to carry out fermentation for 24 h to examine how inulin presence can influence on pH changes. The twenty-four hour fermentation time was reported by other authors as the most crucial for pH decreasing during kefir production (Fontan

et al., 2006). pH significantly ($p \leq 0.05$) decreased after 24 h fermentation (Table 2). All kefir revealed very similar pH values. Typical pH of kefir made in dairy factory is between 4.3 and 4.4 (Bylund, 1995), and the measured values in this study are generally in accordance with those recorded by other authors. Dogan (2011) observed 4.65 pH for kefir made from whole milk and for kefir with addition of different amounts of honey between 4.04 and 4.64. Tratnik et al. (2006) reported pH 4.34–4.37 for whole milk kefir made with 2% addition of inulin, SMP and whey protein concentrates. Ertekin and Guzel-Seydim (2010) studying the effect of 2% inulin and protein-based fat replacer on kefir physicochemical and sensory properties measured pH of the kefir between 4.29 and 4.40. Tratnik et al. (2006) as well as Ertekin and Guzel-Seydim (2010) applied native inulin but apparently, as it is seen in our studies, neither high performance nor native inulin did affect the pH changes in kefir significantly.

Although some yeasts naturally present in kefir (*Kluyveromyces fragilis*, *Saccharomyces lactis*, *Candida kefir*) produce enzymes able to hydrolyse bonds present in inulin moieties (Szambelan and Nowak, 2006) and lactic acid bacteria as well as yeasts could metabolise fructose and glucose, simplifying, into lactic acid and ethanol respectively, we are not able to state, which sugar fermentation caused pH decrease. Further researches are necessary to answer this question. In kefir made from milk, lactose is the sugar that is metabolised firstly and not completely within the first 24 h of fermentation (Magalhaes et al., 2011) and perhaps yeasts, the activity of which is rather small in the first weeks (Grønnevik et al., 2011), did not metabolised inulin at all.

3.2. Textural properties

Hardness of SMP + IQ and WMP kefir was significantly ($p \leq 0.05$) lower than the others (Fig. 1). Kefir made on the WMP basis was richer in fat. This could have an influence on the recorded results. Bonczar et al. (2002) studying yoghurts made from skim, 4.5% to 6.5% ewe's milk showed that hardness of yoghurts decreased as the fat content increased. The lower hardness of SMP + IQ kefir can be a consequence of inulin IQ degree of polymerization. Both TEX! and HPX inulins are composed of long chain inulins (average DP ≥ 23) and are able to enhance kefir gel structure in contrary to native IQ inulin with an average DP of 10. Chiavaro et al. (2007) studying gelling properties of IQ and TEX! inulins reported significantly lower hardness in gels made from IQ inulin.

Adhesiveness of kefir with inulin did not significantly differ, however SMP and WMP kefir had considerably ($p \leq 0.05$) higher and lower values respectively (Fig. 1). The presence of fat could lower the amount of work necessary to overcome the attractive forces between kefir and the probe surface (Chiavaro et al., 2007), perhaps as an effect of lubricant properties of milk fat. Bonczar et al. (2002) noticed that yoghurts made from 4.5% to 6.5% ewe's milk were less adhesive in comparison with skim milk ones.

Data concerning instrumentally measured cohesiveness according to Bonczar et al. (2002) method show the ability for rebuilding the structure by the sample. In the case of TPA when the probe is immersed twice in the product the values of cohesiveness theoretically include between 0 and 1. “Zero” means that the sample does not rebuild its structure at all, while “1” means that the structure is completely recovered between first and second stroke, as it is in the case of liquids (Glibowski, 2007). Since there are frictions between the sample and the walls of the probe, zero value is practically never achieved. The greatest value of cohesiveness was shown by SMP kefir, while significantly ($p \leq 0.05$) lower cohesiveness – by kefir with inulin (Fig. 1). The least cohesive was kefir with TEX! inulin, which suggests its greatest firmness.

3.3. Rheological properties

The presence of relatively high amounts of inulin did not change rheological properties of the studied kefir. They revealed thixotropic properties (Fig. 2), which is typical behaviour both for kefir (Ertekin and Guzel-Seydim, 2010) and lactic beverages (Penna et al., 2001; Debon et al., 2010). The flow curves with characteristic hysteresis loops show that the examined kefir are shear thinning materials (Fig. 3). This type of rheological behaviour is common for

the samples with three-dimensional structure that is destroyed under the shear forces (Steffe, 1996).

Analysis of apparent viscosity revealed small differences between examined kefir (Table 3). Only SMP + HPX kefir had significantly ($p \leq 0.05$) higher apparent viscosity. The applied shear rate (20 s^{-1}) is considered as close to shearing forces present in the mouth during consumption, although some authors indicate that oral perception of solution viscosity correlated well with viscosity measurements at 10 s^{-1} (Khouryieh et al., 2007) and others that shear rate below 50 s^{-1} is considered to represent the in-mouth shear as perceived on the palate (Meyer et al., 2011). Perhaps the similarity of shear rate and oral perception was one of the reasons that panellists pointed at SMP + HPX kefir as very similar ($p \leq 0.05$) in flavour to WMP.

Kefirs were also analysed by means of the small strain deformation technique. Storage modulus (G') is related to elastic properties and loss modulus (G'') is associated with viscous properties of the analysed material (Steffe, 1996). Fig. 4a shows strain sweeps for

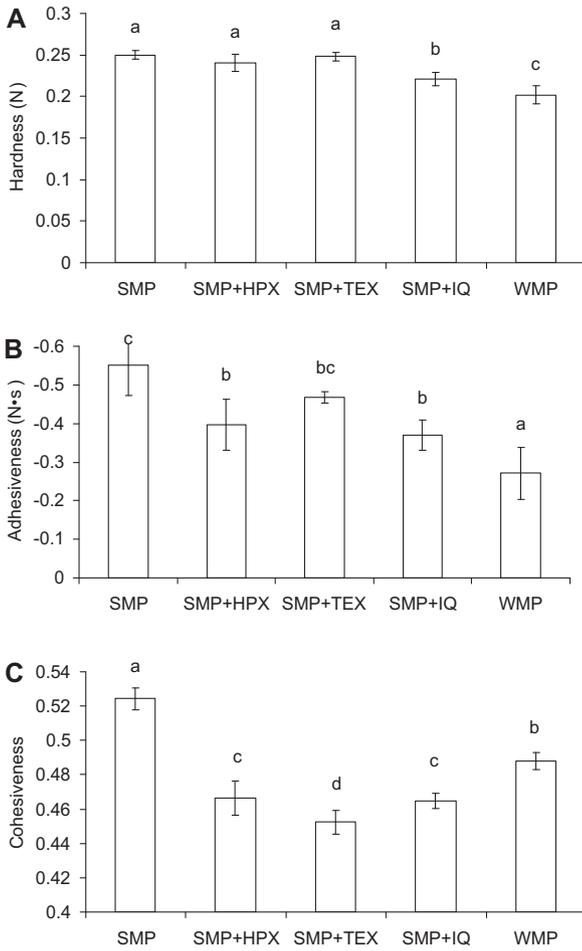


Fig. 1. The texture features (\pm S.D.) of SMP, WMP and kefir produced with addition of different inulins. (A) hardness, (B) adhesiveness, and (C) cohesiveness. Values are means \pm standard deviation. Means in the same graph with different letters are significantly different, $p \leq 0.05$.

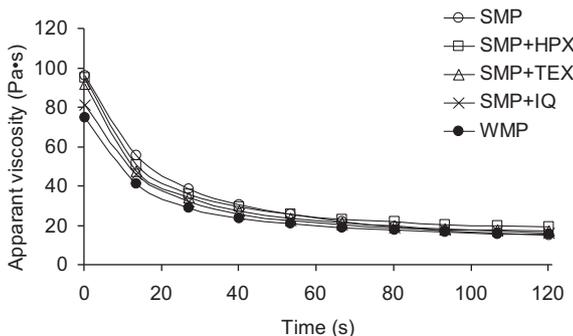


Fig. 2. Apparent viscosity of SMP, WMP and kefir produced with addition of different inulins.

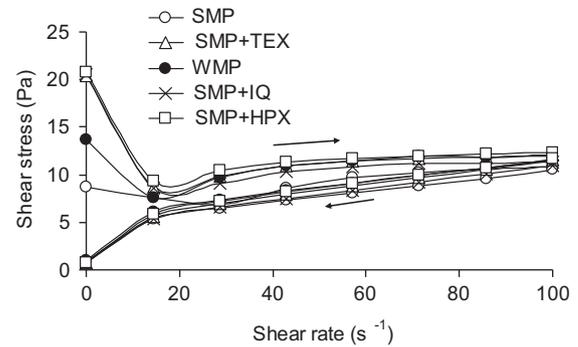


Fig. 3. Flow curves of SMP, WMP and kefir produced with addition of different inulins.

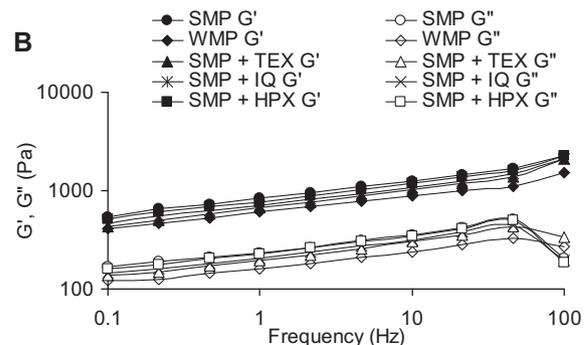
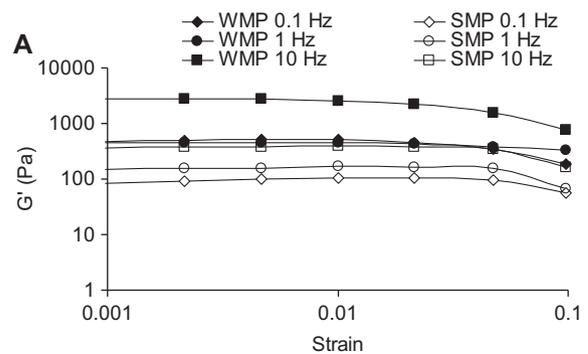


Fig. 4. (A) strain sweeps for SMP and WMP kefir expressed by storage modulus (G'). (B) frequency sweeps for SMP, WMP and kefir produced with addition of different inulins.

SMP and WMP kefir. For the clarity of this figure, we put only the results of storage modulus measured for SMP and WMP kefir. The results for G' values of the kefir with inulin were very similar. The tendencies noticed for storage modulus were also seen in the case of loss modulus (data not shown). The values of storage modulus decreased considerably for the samples after exceeding 0.01 strain (Fig. 4a). This tendency probably results from weak bounds stabilizing structure of kefir. The results obtained from this analysis showed that the studied kefir exhibited higher G' than G'' values, which proved more elastic behaviour (Fig. 4b). This type of behaviour is typical for fermented dairy beverages, especially made by set-type method (Guggisberg et al., 2007, 2011). Notice that there are no significant ($p \leq 0.05$) differences between storage and loss moduli for the studied kefir (Table 3). Ertekin and Guzel-Seydim (2010) fortified kefir made from skim milk with 2% native inulin and noticed significant viscosity increase and similar to viscosity of the whole milk kefir. Tratnik et al. (2006), by 2% addition of native inulin to whole milk, achieved 20% apparent viscosity increase in comparison to kefir without inulin. However, this increase was recorded after one day of storage. Apparent viscosity analysis after 5 and 10 day of storage revealed practical lack of differences between inulin fortified and pure whole milk kefir.

3.4. Sensory evaluation

Fig. 5 shows the results of sensory evaluation of the examined kefir. The panel group (40 untrained people) was intentionally constructed in this way to obtain evaluation very similar to consumers of commercial kefir who mostly are not experienced sensory experts. The results showed no significant ($p \leq 0.05$) differences in odour and consistency evaluation. However, a statistical panellist was able to distinguish between the flavour of WMP and SMP kefir. Regardless of the evaluated feature (flavour, odour, and consistency), kefir with inulin did not differ statistically ($p \leq 0.05$) from SMP kefir. No significant difference was affirmed for the flavour of SMP + TEX and WMP kefir. Native inulin with an average DP 10 was applied as an additive to kefir by Tratnik et al. (2006) and Ertekin and Guzel-Seydim (2010). Ertekin and Guzel-Seydim (2010) found that odour and taste scores of kefir samples with or without inulin were not different. Tratnik et al. (2006) reported that kefir with inulin addition had slightly worse taste whereas consistency and odour were very similar to the whole milk kefir without inulin, especially after 5 and 10 days of storage.

The general lack of significant differences in sensory evaluation found in our study, especially between SMP and kefir with inulins, needs to be emphasizing because the amount of inulin in the examined kefir was one-third of their dry mass (Table 1). From this data, it results that inulin can be successfully applied as a pro-healthy dry mass substitute in kefir production.

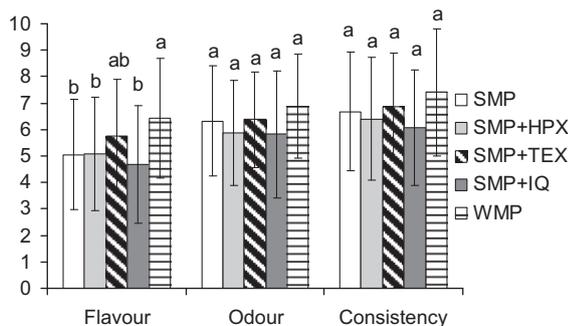


Fig. 5. The sensory features (\pm S.D.) of SMP, WMP and kefir produced with addition of different inulins. Values are means \pm standard deviation. Means with different letters are significantly different, $p \leq 0.05$.

Table 4

Composition and calorificity^A of kefir made on the basis of whole milk powder, skim milk powder, and skim milk powder mixed with inulin.

	WMP		SMP		SMP + Inulin	
	(%)	(kJ)	(%)	(kJ)	(%)	(kJ)
Fat	4.2	153.9	0.2	7.4	0.2	5.6
Protein	4.2	70.7	5.7	97.1	4.3	72.8
Lactose	6.1	103.4	8.2	139.3	6.1	104.4
Inulin	0.0	0.0	0.0	0.0	4.0	32.0
Total calorificity	–	328.0	–	243.8	–	214.8

^A Caloric values for fat, protein, lactose and inulin (37, 17, 17 and 8.0 kJg⁻¹, respectively) were calculated according to Council directive 90/496/EEC (1990).

As it was mentioned earlier, inulin, as a dietary fiber, is not digested in human gastrointestinal tract (Izzo and Franck, 1998). However, its caloric value is not equal to zero. Microorganisms present in the colon metabolise inulin and produce a number of products, mainly organic acids, that are utilized by the host. Hence caloric value of inulin was estimated as 8.0 kJ (2.0 kcal) (Roberfroid, 1999). According to this fact, inulin can be applied as a compound reducing caloric value of foods. In case of the studied model kefir, substitution of SMP by inulin allowed reducing 12% and 35% of caloric value in comparison with SMP and WMP kefir, respectively (Table 4).

The obtained results showed that only inulin TEX! can be applied as a fat substitute (Fig. 4). Since there are two ways in kefir manufacturing, set-type and stirred method, and commercial kefir are mostly manufactured using the second one, further studies are necessary to confirm our results.

4. Conclusions

The study showed that both native and high performance inulins can be successfully applied as a skim milk powder substitute. Substitution of milk fat did not succeed as generally showed rheological and textural studies. Kefir with different inulins revealed similar rheological and sensory properties. Little differences were noticed in texture analysis, where kefir with native inulin had lower hardness probably because of lower inulin degree of polymerization. Kefir with inulin exhibited higher firmness as less adhesive and more cohesive than kefir made only from skim milk powder. Viscoelastic properties appeared to be typical for kefir; they were more elastic than viscous. Rotational rheometry revealed that the studied kefir exhibited thixotropic and shear thinning behaviour. Substitution of SMP by inulin allowed for reducing 12% and 35% of caloric value in comparison with SMP and WMP kefir, respectively.

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