Influence of extraction conditions on characteristics of microbial polysaccharide kefiran isolated from kefir grains biomass

CARMEN R. POP – LIANA SALANȚĂ – ANCUȚA M. ROTAR – CRISTINA A. SEMENIUC – CARMEN SOCACIU – MARIANNE SINDIC

Summary
Kefiran is a water-soluble polysaccharide, which can be isolated from kefir grains biomass. Rheological behaviour and physico-chemical characteristics of kefiran solutions were influenced by the extraction parameters. Degradation of the polymer chain occurred at the highest temperature tested (100 °C). The intrinsic viscosity of kefiran solutions (0.1 g·ml^{-1}) varied between 8.24 mPa at 100 °C to 19.32 mPa at 80 °C. Regarding rheological properties, kefiran solutions had characteristics of a Newtonian behaviour in diluted solutions and pseudoplastic at higher concentrations. High-performance liquid chromatography analysis of monosaccharides revealed that kefiran is composed of glucose and galactose in a relative molar ratio of 0.94–1.1. Infrared spectra of kefiran suggested the structure of α- and β-configurations in pyranose-form carbohydrates, which indicated a purified structure of kefiran. The molecular weight of kefiran polymer was between 2.4×10^6 Da and 1.5×10^7 Da, the values of molecular weight depending on extraction conditions. This polysaccharide was found to have higher intrinsic viscosity and higher apparent viscosity in aqueous solutions, which brings a perspective for its use as thickening or gelling agent in food, or as a matrix in film-forming solutions.

Keywords
kefir grains; lactic acid bacteria; kefiran; rheological properties

Kefir is a dairy product, made from milk (cows', goats' or ewes', coconut, rice or soya). It contains a mixture of natural bacteria and yeasts, valuable vitamins and minerals, together with easily digestible complete proteins, which are beneficial to health [1, 2]. Kefir is made from gelatinous, white or yellow particles called "grains". This makes kefir unique, as no other milk culture forms grains. Kefir grains contain a mixture of bacteria and yeasts, including some strains of bacteria, e.g. *Lactobacillus*, *Leuconostoc*, *Acetobacter* species and *Streptococcus* genera. It also contains beneficial yeasts, such as *Saccharomyces kefir* and *Torula kefir* [3]. Regular use of kefir can help relieve intestinal disorders, promote bowel movement, reduce flatulence and create a healthier digestive system [4]. In addition, its cleansing effect on the whole body helps to establish a balanced inner ecosystem for optimum health and longevity. Kefir grains, used as a starter for Caucasian fermented milk, contain casein and complex carbohydrates, excreted by the microorganisms in the fermented product, as exopolysaccharides [4].

Among lactic acid bacteria, kefir grains are considered food-grade organisms, Generally Recognized as Safe (GRAS) [5], which produce exopolysaccharides that determine the consistency and rheology of fermented milk [6]. Exopolysaccharides are bioactive molecules, due to their role as prebiotics, but also immunomodulatory agents having a large diversity. These are of interest because interactions between the health and economic sectors are of a major concern of European governments, as health has become a top priority throughout all European Union member states' strategies [7].

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Kefiran is considered to be a metabolite of capsular bacteria such as *Lactobacillus kefir* [8], *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae* [9]. Kefiran is a slimmy-gel polysaccharide, whose viscosity and adhesive properties are due to its protein-amino acid-fat complex, as a stable biological mass. On scientific basis, the molecular structure of kefiran is not well understood, but it was proposed to be a branched hexa- or heptasaccharide with repeating units of D-glucose and D-galactose in an almost equal proportion [10, 11]. Each unit is composed of a regular pentasaccharide, to which one or two saccharide residues are randomly linked, a reason why kefiran is resistant to enzymatic attack [12]. The length and frequency of branching strongly affects the rheological properties since it affects the compactness of exopolysaccharides that have a high molecular mass of above 10^6 Da [13].

Kefiran has antibacterial and antitumor activities, modulates gut immune system and can also be used as a food-grade additive for fermented products since it improves rheological properties of chemically acidified skim milk [11]. Numerous applications in industrial sectors of microbial exopolysaccharide polymers were reported, due mainly to their rheological properties i.e. formation of viscous solutions even at low concentrations, good stability in a wide range of pH and temperatures [14]. The functionality, stable cost and good availability of polysaccharides, as biopolymer membranes and films, are pre-requisites for their use in different industries, together with tailored flow properties [15]. The knowledge of rheological properties of kefiran solution is important since they determine the processing conditions and are mainly responsible for the presence of different defects in the edible film structure obtained from this matrix.

Rheological behaviour of polysaccharide solutions and the influence of physical or chemical extraction factors on their viscosity may provide information on the biopolymer quality and the relations between microstructure and physical properties [13]. Rheological properties of the polysaccharide depend on features such as chemical composition and molecular size. Within this context, the aim of this study was to evaluate the effect of extractions conditions, such as temperature, the rheological properties of kefiran solutions, and to examine the characteristics by applying different methodologies such as Fourier-transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC) and high-performance size-exclusion chromatography (HPSEC). This is the first report on the influence of extraction conditions on rheological properties and molecular weight of an exopolysaccharide isolated from kefir grains. The kefir grains as by-products were used to obtain the polysaccharide kefiran.

MATERIAL AND METHODS

Starter culture

Kefir grains were originally purchased from the collection of the Walloon Agricultural Research Centre (CRA-W, Gembloux, Belgium). Their purity and composition was determined by NINANE et al. [16] and the microflora identified in the kefir grain included *Acetobacter* sp., *Kazachstania exigua, Lactobacillus kefiranofaciens* subsp. *kefirgranum, Lb. kefiri, Lb. parakefiri, Lactococcus lactis* subsp. *lactis* and *Leuconostoc mesenteroides*. Fresh active kefir grains, washed with sterile water, were inoculated in ultra-high temperature-treated skim milk and incubated at 28 °C for 24 h. The medium was exchanged daily or at the end of incubation, for new culture to maintain viability of the grains [17].

Isolation and purification of kefiran

Isolation and purification of kefiran were performed as was previously reported by ZAJŠEK et al. [11] and PIERMARIA et al. [18]. Kefiran was extracted from kefir grains, frozen before use for at least 24 h. To a weighed amount of kefir grains, hot water was added in a ratio 1 : 10 (w/w) at four different temperatures: 70 °C (T70), 80 °C (T80), 90 °C (T90) and 100 °C (T100), using different extraction times (from 1 min to 8 h) under stirring. The previous research [19] demonstrated that using temperature at 100 °C can denature the polymer structure. The temperature levels were established in order to solubilize the biomass of kefir grains. The mixture was then cooled and centrifuged at 10 000 × *g* for 10 min at 4 °C to remove microbial cells and proteins. The polysaccharide dissolved in the supernatant was purified by freezing at –20 °C overnight followed by slow thawing, avoiding the destruction of polysaccharide structure. After centrifugation at 5 000 × *g* for 10 min at 4 °C, the kefiran-rich pellets were dissolved in distilled water at 60 °C. The purification procedure was repeated twice, the kefiran solution obtained showed high purity. Proteins and microbial cells that remained after the first step of purification were removed by centrifugation. Lactic acid bacteria in kefiran solution were enumerated on de Man-Rogosa-Sharpe (MRS) agar (Oxoid, Basingstoke, United Kingdom) anaerobically incubated for 72 h at 37 °C using Anaerocult A system (Merck, Darmstadt, Germany). Yeasts were enu-

merated on yeast extract-peptone-dextrose (YPD) agar (Sigma-Aldrich, St. Louis, Missouri, USA) incubated for 24 h at 30 °C. The kefiran solutions with concentration ranking from 0.005 g·ml^{-1} to 0.2 g·ml^{-1} were obtained in the same way. These samples were prepared in this way to determine the rheological properties and identifying the optimal concentration of the kefiran solution, in order to establish the use in food industry. The samples were stored in a refrigerator at 4 °C before use. Kefiran solutions were allowed to acclimatize at room temperature for 2 h before analysis.

Rheological measurements of kefiran solutions

Viscosimetric measurements of kefiran solutions with concentrations ranking from 0.005 g·ml^{-1} to 0.2 g·ml^{-1} were determined by a Bohlin CV 120 High Resolution (Bohlin Instruments, Cirencester, United Kingdom) at 25 °C, a controlled-stress rheometer equipped with a water jacket-type temperature control unit. To measure the kefiran samples, C25 system (Bohlin Instruments) was used, with coaxial form: Cup (27.5 mm diameter and 50 mm height) and Bob (25 mm diameter and 37.5 mm height). The shear rate increased from 0.1 s^{-1} to 500 s^{-1} in 2 min, and then decreased from 500 s^{-1} to 0.1 s^{-1} in 2 min, the sweep direction was calculated in the up-down and the range logarithmic. The shear stress and apparent viscosity were recorded as a function of shear rate.

A Power-law fluid or the Ostwald-de Waele models were used for the description of the flow behaviour. This model relates the shear stress of a fluid to the shear rate, thus enabling the apparent viscosity to be calculated as the ratio between shear stress and shear rate as in Eq. (1).

$$\tau = k \cdot \gamma^n \quad (1)$$

where τ is the shear stress (in pascals), k is the consistency index (in pascals multiplied by seconds to exponent n), γ is the shear rate (in reciprocal seconds) and n is flow index, a dimensionless number that indicates the closeness to Newtonian flow. The parameter n is considered to be 1 for Newtonian fluids, higher than 1 for dilatant (the viscosity of a shear thickening fluid increases with increasing shear rate) and between 0 and 1 for pseudoplastic fluids (the viscosity of a shear thinning fluid decreases with increasing shear rate) [18, 20].

Physico-chemical characterization of the kefiran polysaccharide

Protein concentration

The purity of the purified kefiran samples was tested using the Protocol Quick Start (Bradford Protein Assay) from Bio-Rad (Hercules, California, USA), the protein standards used being bovine serum albumin (BSA) and bovine gammaglobulin (BGG).

Polysaccharide concentration

The polysaccharide concentration was determined by the general anthrone method (used for total carbohydrate quantification), by measuring the absorbance of the coloured complex at 620 nm in parallel with lactose solutions (from 10 μg·ml^{-1} to 100 μg·ml^{-1}) to make a calibration curve [21].

HPLC quantification of the kefiran monosaccharide composition

The kefiran solutions in a ratio 1:10 (w/w), which were extracted at four different temperatures: at 70 °C/100 min (T70), 80 °C/30 min (T80), 90 °C/20 min (T90) and 100 °C/5 min (T100) were hydrolysed with 0.2 mol·l^{-1} trifluoroacetic acid at 80 °C for 72 h according to the method described by Garna et al. [22], then all the samples were filtered through a microfilter of pore size of 0.45 mm (Millipore, Billerica, Massachusetts, USA). The monosaccharide profile was determined by HPLC on a Shimadzu system (Shimadzu, Tokyo, Japan) equipped with a LC–10AD pump, a DGU–14A degasser, a SIL-10AV VP autosampler, and detection by measuring the refractive index (RI; RID–10A device). The HPLC column was Altima Amino (Shimadzu), 250 mm × 4.6 mm, particle size 5 μm. For separations, the column was set at 30 °C using a CTO–10AS VP temperature controller (Shimadzu). The chromatographic separation was carried at a flow rate of 1.3 ml·min^{-1}, using a mixture acetonitrile:water 80:20 (v/v) as a mobile phase.

FTIR characterization of kefiran solutions

The spectral analysis of the four kefiran samples was performed by a FTIR spectrometer (IR Prestige 21, Shimadzu), using a single attenuated total reflection (ATR) unit at room temperature. Each spectrum was recorded from 4000 cm^{-1} to 500 cm^{-1} and was composed of an average of 64 separate scans [19]. The FTIR spectra were processed using OriginPro 8 SR1 Software (OriginLab; Northampton, Massachusetts, USA).

Determination of kefiran molecular weight by HPSEC

The kefiran samples were analysed by HPLC Alliance Waters 2690 system (Waters; Milford, Massachusetts, USA) equipped with refrac-

Tab. 1. Rheological parameters of kefiran solutions depending on concentration.

Kefiran solution [g·ml^{-1}]	η [mPa·s]	τ [Pa]	k_0 [Pa s^n]	N	R^2
0 (distilled water)	3.44	0.1915	2.6×10^{-4}	1.4162	0.9983
0.005	3.58	0.1657	6×10^{-4}	1.2857	0.9970
0.01	3.92	0.2171	1.33×10^{-4}	1.5540	0.9987
0.015	4.06	0.2240	0.6×10^{-4}	1.5880	0.9987
0.02	4.43	0.2486	0×10^{-4}	1.8151	0.999
0.035	4.71	0.2396	2.26×10^{-3}	1.5313	0.9981
0.05	4.81	0.1965	4.2×10^{-3}	1.9712	0.9981
0.1	11.08	0.1683	2.76×10^{-1}	0.8311	0.9994
0.15	16.48	0.0872	7.19×10^{-1}	0.742	0.9995
0.20	22.88	0.1293	2.31	0.5921	0.9994

η – apparent viscosity, τ – shear stress, k_0 – consistency index, n – flow behaviour index, R^2 – coefficient of determination.

tive index detector Waters 2410. The separation was performed at 40 °C on a TSK-Gel GMPWxl (Tosoh Bioscience; Grove, Ohio, USA) stainless steel column (30 mm × 7.5 mm). The column was eluted isocratically at a flow rate of 0.7 ml·min^{-1} with $NaNO_3$ (4.24 g·l^{-1}) + NaN_3 (500 mg·l^{-1}), vacuum-filtered through a microfilter of a pore size of 0.45 μm and degassed at 30 °C. A volume of 100 μl from each sample was injected and the run time was 30.0 min. Dextran polymers of molecular weight of 80 kDa, 270 kDa, 410 kDa and 670 kDa were used as reference compounds. The method was a modification of the protocol described by Ghasemlou et al. [1].

Statistical analysis

The ANOVA analysis of variance was used to compare the mean values, using SPSS 19.0 statistical software (IBM; Armonk, New York, USA). Tukey's HSD test was carried out with a confidence interval of 95% or 99%. Differences were considered to be significant at $p < 0.05$. The intensity of relationships between the kefiran viscosity and molecular weight values were determined with Pearson's correlation within 95% confidence interval.

RESULTS AND DISCUSSION

Rheological properties of kefiran solutions

The values of the parameters of kefiran solutions, obtained by applying the flow behaviour models, were dependent on the concentration as shown in Tab. 1. To establish the optimal concentration of kefiran solution for the extraction, ten kefiran solutions were tested. Results of rheological measurements showed that 0.1 g·ml^{-1} was the optimum concentration of kefiran in aqueous solution. This concentration was further used to characterize the polysaccharide.

The kefiran solution revealed characteristics of a non-Newtonian pseudoplastic fluid that shows concentration-dependent changes in viscosity; the longer the fluid undergoes shear stress, the lower is its viscosity. All kefiran solutions exhibited pseudoplastic behaviour. For comparison, Piermaria et al. [18] indicated that kefiran solution had a Newtonian behaviour at low concentrations (1 g·l^{-1}), but became pseudoplastic at higher concentrations. The intrinsic viscosity of kefiran was observed to be higher than the viscosity of dextran.

Our results show that kefiran solutions had Newtonian behaviour in diluted solutions. The same behaviour was observed for solutions extracted at a high temperature (100 °C). The pseudoplastic or reo-thinning behaviour was observed for the higher concentrated solutions. The pseudoplastic properties are important in helping to provide good sensory qualities, such as mouth feeling, flavour releasing and suspending properties of food products [23]. It could be observed that at a high concentration of 0.15 g·ml^{-1}, the kefiran solution showed the characteristics of a gel, in agreement with the data reported by Piermaria et al. [18]. Therefore, 0.1 g·ml^{-1} kefiran solution was used to determine apparent viscosity, which depends on the extraction temperature (Fig. 1) for the physical and structural characterization. Following the rheological measurements, the optimal duration of extraction for each temperature was established: for 70 °C – 100 min, for 80 °C – 30 min, for 90 °C – 20 min and for 100 °C – 5 min.

The highest value of apparent viscosity (19.33 mPa·s) was determined for the sample extracted at 80 °C for 30 min. Rimada and Abraham

[10] as well as PIERMARIA et al. [18] treated kefir grains in boiling water for 1 h with continuous stirring. PIERMARIA et al. [24] reported a value of 13.24 mPa·s for apparent viscosity, when kefiran solution was extracted at 100 °C for 60 min. PIERMARIA et al. [18] indicated that the intrinsic viscosity of kefiran was lower than that of some other polysaccharides used as food additives, such as locust bean gum or guar gum, but was higher than that of some dextrans.

The flow behaviour of kefiran solution depended on the extraction parameters (Fig. 2).

The results indicated the relationship between shear stress – shear rate and instantaneous viscosity – shear rate. The graphical representation indicated that the kefiran solution showed characteristics of a typical non-Newtonian pseudoplastic fluid behaviour. Also, the viscosity of each solution showed a high value at the low shear rate, and linearly decreased with the increasing shear rate [13, 25, 26]. It was observed that, with the increase in shear rate as well as temperature, viscosity of kefiran solution decreased indicating that aqueous solution of kefiran had shear-thinning behaviour. Further it was observed that, by

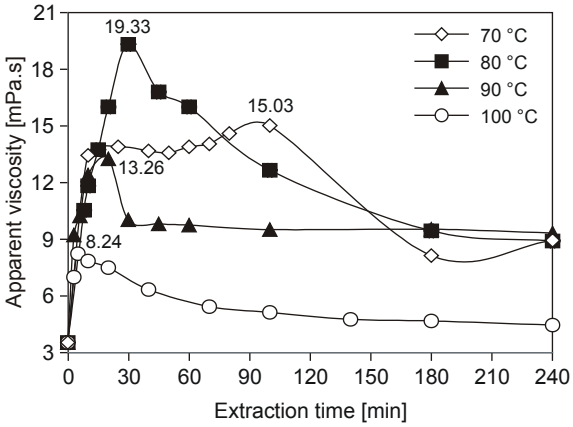

Fig. 1. Apparent viscosity of kefiran solutions depending on extraction conditions.

increasing the temperature of extraction, the viscosity of aqueous solution decreased significantly ($p < 0.001$). Polysaccharide solutions with high viscosity can prevent air bubbles to escape from the film and pores. On the other hand, solutions with low viscosity can lead to obtaining very thin films [24], which require more careful handling and are

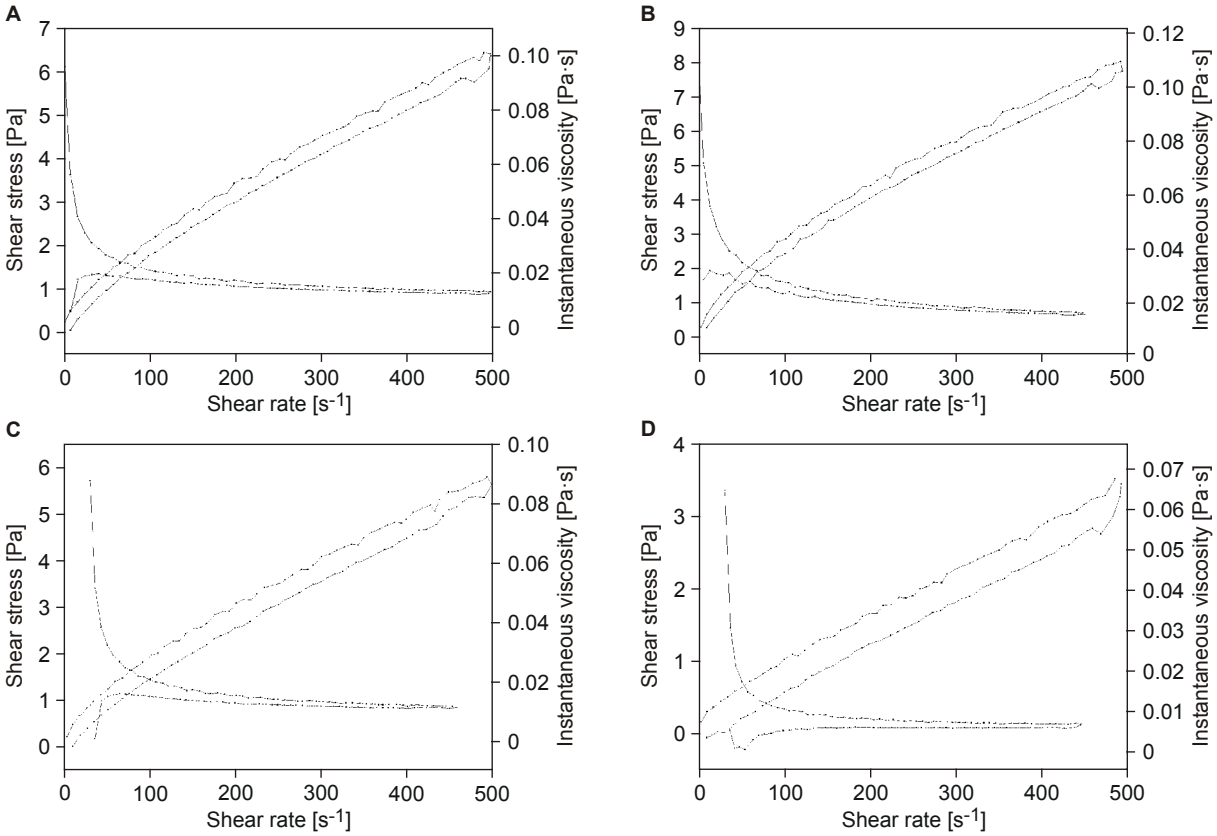

Fig. 2. Flow curves for kefiran solution measured at 25 °C.

Extraction conditions of kefiran: A – 70 °C for 100 min; B – 80 °C for 30 min; C – 90 °C for 20 min; D – 100 °C for 5 min.

practically less applicable due to worse mechanical properties.

Evaluation of kefiran composition

The kefiran solutions obtained by extraction at four different temperatures and durations, purified according to our procedure using two passages of purification by freezing from kefir grains, showed a high purity grade. As determined by colorimetry, the kefiran-rich sample contained only 0.0056 g·ml^{-1} protein expressed per dry matter, while Piermaria et al. [18] reported a concentration of 0.0001 g·ml^{-1} protein in kefiran solution, using the method by ethanol precipitation (the procedure was repeated three times). In the purified solution of kefiran, no viable microorganisms were detected (data not shown). Identical results were reported by Zajšek et al. [11]. The difference was not significant statistically ($p > 0.05$) for the polysaccharide concentration with extraction parameters (temperature and time). The total carbohydrates concentration of kefiran estimated by the phenol-sulphuric method were not dependent on the extraction parameters, the results varied between 0.985 ± 0.094 g·ml^{-1} for kefiran solution extracted at 100 °C and 0.99 ± 0.025 g·ml^{-1} for kefiran solution extracted at 80 °C. The latter temperature was optimal, as proven by rheological measurements.

HPLC determination of the kefiran monosaccharide composition

According to HPLC data (Fig. 3), monosaccharides separated from the kefiran solution of 0.1 g·ml^{-1} were represented by glucose and galactose, in a molar ratio of 0.94 : 1.1, as determined from peak areas and by comparison with calibrated mixtures of glucose and galactose. These data are in agreement with the values reported by La Riviére et al. [8], Kooiman [12] and Badel et al. [27], which said that the polysaccharide kefiran isolated from kefir grains contained, approximately in equal proportions, galactose and glucose residues. Liu et al. [28] reported that the polysaccharide isolated from soymilk kefir grains contained more glucose than galactose. Zajšek et al. [11] identified D-glucose and D-galactose residues within the kefiran samples in a ratio of 1 : 0.7 using capillary electrophoresis. Following the dynamics of kefiran hydrolysis to monosaccharides, maximum release was noticed after 72 h of hydrolysis with 0.2 mol·l^{-1} trifluoroacetic acid without any degradation (data not shown).

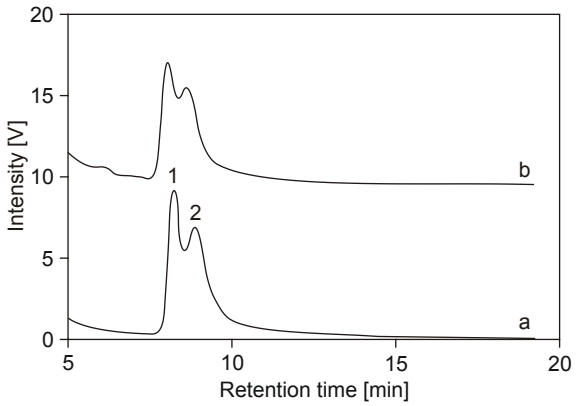

Fig. 3. HPLC chromatogram of monosaccharides identified in 0.1 g ml^{-1} kefiran solution.

a – Standard mix of glucose and galactose; b – Kefiran solution 0.1 g ml^{-1} extracted at 80 °C for 30 min; 1 – glucose with retention time 8.187 min, 2 – galactose with retention time 8.793 min.

Kefiran fingerprinting by FTIR spectroscopy

FTIR spectra of all purified kefiran solutions in concentration 0.1 g·ml^{-1} obtained by applying four different extraction conditions are given in Fig. 4. Four major absorption zones are identified: 3400–3310 cm^{-1}, 2933–2880 cm^{-1}, 1650–1413 cm^{-1} and 1200–852 cm^{-1}. The first region (3700–3310 cm^{-1}) is assigned to water and hydroxyl group in kefiran. In these spectra, the signals due to the O–H stretching vibration had a high intensity. The area of the bands close to those at approx. 2720 cm^{-1} and approx. 2900 cm^{-1} correspond to the C–H stretching vibration. The most relevant peak of 2925 cm^{-1} can be related with the symmetric and asymmetric stretching vibration of the aliphatic group (CH_2) [29]. At approx. 2850 cm^{-1} and 2933 cm^{-1}, the bands are attributed to C–H. The reduction in intensity of this peak indicated the disruption of kefiran solution structure due to the presence of absorbed water molecules, which masked the C–H bond of the carbohydrate rings and thus reduced the contribution of C–H absorbance bands in the spectra [30]. The disruption of the polymer chain was due to a high temperature of extraction. Villetti et al. [31] found by infrared spectroscopy that, at low temperatures, scission of the exocyclic groups of xanthan and methylcellulose occured and, at high temperatures, scission of strong links occured. The region 1650–1413 cm^{-1} is an area specific for water molecules, assigned to bending mode of O–H. The relative absorption intensities of the bands assigned to water molecules depended on quality of kefiran solution given by the extraction conditions. This information is important for future industrial applications.

The last zone, from 1200 cm^{-1} to 852 cm^{-1}, is distinct for each polysaccharide. This region is dominated by ring vibrations overlapped with stretching vibrations of (C–OH) side groups and (C–O–C) glycosidic band vibration [30]. The absorptions at 1035 cm^{-1}, 1080 cm^{-1} and 1153 cm^{-1} indicate a pyranose form of carbohydrates [32]. In this last region, the peaks detected and associated with the vibration mode indicated the presence of glucose and galactose, which are components of the purified kefiran structure. The results obtained suggest that kefiran is composed of α- and β-configurations of saccharides in pyranose form.

The specific infrared spectra of monosaccharides in the 1170–980 cm^{-1} region were sensitive to the axial and equatorial position of the (OH) groups, which could affect quite significantly the main band positions. KAČURÁKOVÁ et al. [33] reported that the main infrared band of galactose was 1106 cm^{-1} for C–O–C group, and the all-equatorial (OH) group showed in the infrared spectrum the lowest frequency maximum at 1035 cm^{-1}. One axial (OH) group either in position C-2 or C-4 in the case of galactose gave maxima at 1070 cm^{-1} and 1078 cm^{-1}, respectively. Spectra showed a peak around 900 cm^{-1}, which was the same pattern as reported by PIERMARIA et al. [30]. This indicated the vibration modes of glucose, galactose and β-linkage in the structure of purified kefiran.

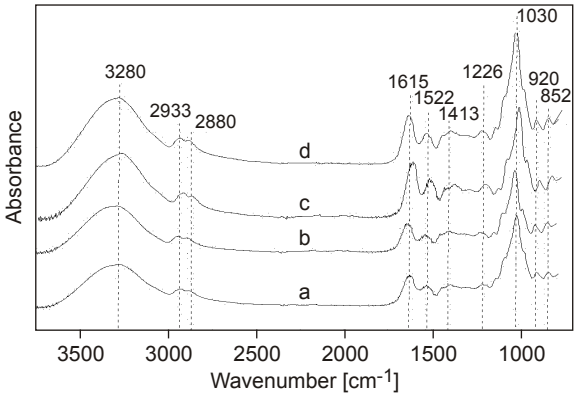

Fig. 4. ATR-FTIR spectra of kefiran extracted in different conditions.

A – 70 °C for 100 min; B – 80 °C for 30 min; C – 90 °C for 20 min; D – 100 °C for 5 min.

Size-exclusion profile and molecular mass of kefiran polymers

Using the HPSEC technique, a good precision in the molecular weight estimation in kefiran samples was achieved. Another interesting information could be deduced by correlating molecular weight and viscosity value of polysaccharide solutions. A very significant positive correlation ($R^2 = 0.903$, $p \leq 0.01$) was obtained between the high molecular weight, which indicated that the kefiran solution can be highly viscous with pseudoplastic character. This is discussed in later sections of this paper.

Tab. 2 shows the results of molecular weight estimation for eight kefiran solutions. The samples were chosen to establish the optimal parameters for the extraction process. The concentration of each sample was 0.1 $g \cdot ml^{-1}$. Correlation with the calibration curve of dextran standards indicated that the molecular weight of kefiran polymer ranged between 2.4×10^6 Da and 1.5×10^7 Da (Tab. 2). The values of molecular weight depended on conditions of the extraction of the polysaccharide from kefir grain biomass.

Different authors reported different molecular weights for kefiran obtained from *L. kefiranofaciens* and kefir grains extracted at 100 °C for at least 30 min. AHMED et al. [34] reported for kefiran the molecular weight of 5.5×10^4 Da. WANG and BI [35] reported the value of 1.5×10^5 Da, GHASEMLOU et al. [1] reported the value of 1.35×10^6 Da, and the other researchers

Tab. 2. Molecular weight of kefiran from samples extracted under different conditions.

Sample	Temperature [°C]	Time [min]	Retention time [min]	Molecular weight [Da]	Peak area [mAU min^{-1}]
T70.100	70	100	9.701	14071400	1148810
T80.30	80	30	9.666	15204500	1269110
T80.60	80	60	9.673	14970800	1175823
T80.100	80	100	9.7	14102600	1110266
T90.20	90	20	9.768	12132500	1181834
T100.5	100	5	9.968	7793800	800835
T100.60	100	60	10.251	4166600	640055
T100.100	100	100	10.482	2499100	384533

reported molecular weight of 10^7 Da [18].

In recent years, microbial exopolysaccharides have been studied extensively to determine their structure and molar-mass distribution by using size exclusion chromatography coupled on-line to multi-angle laser light scattering, differential viscometer detector and differential refractive index detector [36]. Kim et al. [37] estimated that the average molecular weight of pullulan ranged from 1.5×10^4 Da to 1.0×10^7 Da, depending on conditions of preparation. Santos et al. reported in their study [38] that the molecular weight of dextran was from 4×10^5 Da to 10×10^6 Da, which meant a very large polysaccharide, whose molecular weight depended on culture conditions. The most widely accepted molecular weight of dextran is between 3 kDa and 2000 kDa. The weight-average molecular weight for xanthan gum produced by *Xanthomonas campestris* pv. *campestris* was determined by Faria et al. [39] as 4.2×10^6 Da. Gellan gum, a linear polysaccharide produced by *Pseudomonas elodea*, has molecular weight from 2×10^5 Da to 3×10^5 Da [40].

As observed previously, kefiran is exhibited higher values of molecular mass, which was attributed to kefiran samples that did not undergo degradation. Degradation is mainly due to aggressive extraction conditions, temperatures above 80 °C and long-time maintenance at such temperatures.

CONCLUSIONS

Rheological properties of kefiran solution suggest that the extraction conditions have a major influence on quality of this polysaccharide. Viscous behaviour of kefiran indicated its potential to be used as an effective biothickener or biostabilizer in food industry. This is the first report on the influence of extraction conditions on rheological properties and molecular weight of an exopolysaccharide isolated from kefir grains. Viscosity of polysaccharides decreases with increasing temperature at which extraction is carried out. The results of structure elucidation indicated that kefiran contains glucose and galactose in a ratio of 0.94 : 1.1. FTIR spectra in the 1200 cm^{-1} ± 800 cm^{-1} region provided information on main polysaccharides present in the complicated systems of polysaccharide mixtures, as the distinctive band positions facilitated identification of structures and composition of polysaccharides. Results on molecular weight suggested that high treatment applied to the extraction of polysaccharide led to a polymer with a shorter chain. Temperature had dramatic effects on viscosity. However, industrial applicability of this polysaccharide depends on its ability to retain stable rheological characteristics and a high degree of structural integrity.

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