Microbiological and rheological studies on Portuguese kefir grains*

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Summary The native bacteria and yeasts present in Portuguese kefir grains stored under four distinct sets of environmental conditions have been isolated and identified on the basis of morphology and biochemical tests. The microbial population of the kefir grains as a whole has been characterized in terms of rates of biomass production and formation of lactic acid and ethanol. The rheological properties of the purified polysaccharide (kefiran) produced by the microflora of the grains and accumulated therein were studied in a low water activity solvent and as a component of a binary gel containing either κ-carrageenan or xanthan gum.

Keywords Biochemical parameter, dairy foods, gum blend behaviour, kefiran, mechanical spectra, storage and loss moduli.

Introduction

Kefir is a fermented beverage which has its ancient origins in the Caucasian mountains. This light alcoholic beverage is prepared by inoculation of raw milk with existing kefir grains (which can be stored in dried form). Kefir grains exhibit an irregular surface and they consist of a gel matrix in which yeasts and bacteria are imbedded and live symbiotically.

The microflora of kefir grains, which depends on their origin (Ottogalli *et al.*, 1973), storage conditions and handling pattern (Zourari & Anifantakis, 1980), consists predominantly of *Saccharomyces delbrueckii* and *Lactobacillus kefir* (previously included in *L. brevis*) (La Reviere *et al.*, 1967; Kandler & Kunath, 1983), although several other varieties can be found, e.g. bacterial species of such genera as *Streptococcus*, *Leuconostoc* and *Acinetobacter* (Rosi, 1978b; Rosi & Rossi, 1978), and mould species such as *Geotrichum candidum* (Kosikowski, 1977). At least one quarter of the dry matter of the kefir grains consists of a capsular polysaccharide usually denominated kefiran (La Reviere *et al.*, 1967). Kefiran is considered to be a result of the metabolism of such capsular bacteria as *Lactobacillus kefis* (La Reviere *et al.*, 1967; Yokoi *et al.*, 1990), a homofermentative, atypical Streptobacterium (Rosi & Rossi, 1978), *Lactobacillus kefiranofaciens* (Toba *et al.*, 1987; Fujisawa *et al.*, 1988), and a new LAB strain tentatively named KPB-167B (Yokoi *et al.*, 1991). Kefiran consists of approximately equal proportions of galactose and glucose residues (Kooiman, 1968). Rheological studies have shown that kefiran itself has a very low viscosity in solution and is unable to form rigid gels in the absence of ethanol (Mukai *et al.*, 1990, 1991). Although the structural characteristics and hydrodynamic prop-

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erties of kefiran raise some restrictions on its commercial use as a food thickening or gelling agent, some useful applications as a food additive might be devised. However, to date little work has been devoted to the rheological properties of this polysaccharide.

The purpose of the research reported in this communication was to gain insight into the microbiology of Portuguese kefir grains in terms of identification of their microflora and characterization of the biomass growth and product formation rates thereof, and to compare the results thus obtained with results available in the literature pertaining to kefir grains from other countries. Several rheological studies were also conducted on the polysaccharide extracted from the kefir grains in attempts to improve its potential applications as a gelling agent for foods.

Materials and methods

Microbial strains

The kefir grains (0.7 to 1.4 g fresh, ~10% dry matter) were obtained from private sources in Portugal. Propagation and activation was performed by consecutive immersion (c. 10–12% w/v) in fresh batches of sterilized cow's milk at room temperature three times a week.

The pure strains of *Lactobacillus brevis* NCFB 1749, *Lactobacillus kefir* NCFB 2753 and *Lactococcus lactis* subsp. *lactis* NCFB 604 were obtained from the National Collection of Food Bacteria (UK).

Microbiological analyses

Grains subject to four distinct environmental conditions were employed in these experiments, viz. (i) original grains, (ii) after propagation at home with daily change of milk, (iii) after storage for 3 months at room temperature (20–25°C), and (iv) after refrigerated storage (4°C) for 3 months.

For each experiment, 10 g of kefir grains (previously washed with sterile water) were homogenized with 90 g of Ringer solution in a Stomacher LAB-Blender 400™ (Seward Medical, UK) for 15 min. The homogenate, after appropriate decimal dilution, was spread on different media, and incubated under different conditions depending on the microorganism under study: (a) for *Lactobacillus* sp., the medium used was MRS (LabM, UK) supplemented with 3% (w/v) ethanol and 0.5% (w/v) cycloheximide (Sigma, USA) and incubation took place anaerobically (via Gas Pack BBL™ from Becton Dickinson, USA) at 30°C for 5 days; (b) for *Streptococci*, the medium used was M17 Agar (Merck, Germany) with 0.5% (w/v) cycloheximide, with aerobic incubation at 30°C for 2 days; (c) for yeasts, the medium used was MEA (Merck) incubated, aerobically, during 5 days at 25°C; (d) for acetic acid bacteria, the medium used was WLNA (Oxoid, UK) with 0.5% (w/v) cycloheximide, with aerobic incubation at 25°C for 21 days. Selected colonies were purified prior to identification. All isolated strains were stored in the presence of 30% glycerol at −80°C.

Identification of microoganisms

For biochemical profiles of lactic acid bacteria basal MRS media without meat extract and glucose were used according to Bergey (1986). The inoculated medium was added to the galleries of API 50™ (Bio Mérieux, France). The additional tests for streptococci and lactobacilli were examined according to the schemes of Harrigan & McCance (1976). For lactobacilli the genus was further confirmed by SDS-PAGE of the disrupted cells following preliminary growth under standard conditions according to the method of Laemmli (1970) modified by Kiredjian *et al.* (1986) using chemicals from BioRad (USA); numerical analysis of the protein electrophoregram was performed as described by Kersters & De Ley (1975).

In the case of yeasts, physiological and biochemical characteristics were tested according to Barnett *et al.* (1990) and identification was based on the Yeast Identification PC Program from the same authors.

Capsule production

For this purpose, two solid media were used, namely KPL, which was prepared as described by Toba *et al.* (1986) using lactic acid, glucose, galactose, and Tween 80™ (Merck) together with

milk whey and white table wine, and a new medium prepared with milk permeate (obtained by ultrafiltration), added with 10% (w/v) lactose and 3% (v/v) ethanol. Both solid media were supplemented with 0.5% (w/v) cycloheximide, inoculated with the homogeneous extracts of kefir grains, and incubated at 30°C for 5 days under anaerobic conditions.

Fresh permeate (without agar) was, after filter-sterilization (Nucleopore, USA), employed as the broth medium. After pouring 15 ml of broth into screw-capped tubes, each tube was inoculated with some of the lactobacilli isolated from the colonies in MRS added with 3% (v/v) ethanol and incubated at 30°C with continuous shaking (100 r.p.m.) for 6 days.

Growth rate

Nine wet grains of activated kefir, with similar and representative appearance, were selected and washed with sterile, distilled water. They were dried to constant weight at 25°C. Each grain was then submerged in 80 ml of sterile milk in a different beaker, and the beakers were incubated at 22°C under gentle shaking conditions. For biomass assays the grains were removed, each one at a different time, and dried to constant weight at 25°C. Assays for DL-lactic acid and ethanol by enzymic methods (Boehringer Manheim, Germany) of the supernatant milk of the corresponding beaker were performed at the six sampling times after the (previously determined) lag period of 3 days.

Extraction of polysaccharide from the kefir grains

For the extraction operation, 50 g of grains, previously washed with sterile water, were kept submerged in 100 ml water at 80°C for 1 h with uniform stirring (100 r.p.m.). After this period, the mixture was cooled and centrifuged at 12 000 g for 20 min. The procedure was repeated once again with the remaining sediment. The polysaccharides dissolved in the supernatant were precipitated out by addition of an equal volume of cold ethanol. The precipitate was redissolved in 100 ml of water and a second purification step was performed using isopropanol in place of ethanol following the procedure of Da Silva & Gonçalves (1990).

Determination of intrinsic viscosities

Several aqueous solutions of kefiran were prepared so as to give relative viscosities from about to 1.2 to 2.1. Viscosity was measured at 25.0 ± 0.1°C with a Cannon-Fenske capillary viscometer (Hipex, Portugal). The intrinsic viscosity, $[\eta]$, was obtained by combined application of the Huggins and Kraemer extrapolations (Morris & Ross-Murphy, 1981).

Preparation of the sucrose/kefiran systems

The kefiran ($[\eta] = 0.29\ m^3\ kg^{-1}$) was previously dispersed in water at room temperature under moderate shaking for 1 h, heated at 90°C for 30 min, and then centrifuged for 1 h at 28 000 g. The aqueous dispersion of kefiran was heated in a paraffin bath to 105°C for 3 min, and then sucrose (a water activity modifier) was added under continuous stirring. The heating was maintained for another 7 min, water lost being replaced with fresh deionized water, and the sample was transferred to the plate of the instrument at the desired temperature.

Preparation of the binary solutions of kefiran and κ-carrageenan or xanthan

The kefiran and κ-carrageenan samples were first dispersed in water under moderate agitation for 1 h, at room temperature, and then heated at 90°C for 30 min with stirring. The binary systems were prepared by mixing, at 90°C, the solutions of kefiran and κ-carrageenan at the 1:4 (w/w) ratio for a final 1% (w/w) total concentration of polymer. The hot mixture was maintained at 90°C for 15 min, and then cooled to 55°C. In these experiments, the only potassium ions present were those accounted for by the salt form of the native κ-carrageenan.

In the case of the kefiran/xanthan mixture, both the kefiran and xanthan were first dispersed in a 0.1 M solution of sodium chloride under moderate agitation for 60 min at room temperature, and then heated to 90°C for 30 min with continuous stirring. The binary systems were prepared by

mixing, at 90°C, the solutions of kefiran and xanthan at the 1:1 (w/w) ratio for a final 1% (w/w) total polymer concentration, and then maintained at 90°C for 30 min under continuous stirring.

Rheological measurements

Dynamic rheological measurements were performed using a Carri-Med CS-50 (UK) controlled-stress rheometer with a cone-plate device (radius 25 mm, cone-plate angle 4°) or a parallel plate geometry (radius 60 mm, gap 4 mm) with radial grooves in order to avoid gel slippage. The strain amplitude was fixed at 3%. After the hot sample was transferred to the rheometer plate, the exposed surface of the sample was covered with a thin layer of low viscosity paraffin oil to prevent evaporation of solvent.

Three types of experiments were then performed: cure experiments, mechanical spectra, and cooling/heating cycles. For the cure experiments, the kinetics of gel formation at different temperatures was monitored by measuring the storage (G') and the loss (G'') moduli at a frequency of 6.3 rad s^{-1}. Mechanical spectra were recorded in a constant strain mode over the frequency range 0.063 to 63 rad s^{-1}. For the cooling/heating cycles, the storage modulus, G', and the loss modulus, G'', were obtained from temperature sweep experiments at 6.3 rad s^{-1}, by cooling the systems from 55 to 5°C and then reheating to 55°C at the rate of 18°C h^{-1} (kefiran/κ-carrageenan); in the case of kefiran/xanthan, the mixture was cooled from 70 to 25°C and then reheated to 70°C at the rate of 60°C h^{-1}.

Results and discussion

Microbiological characterization

The proportions of the different groups of microorganisms (bacteria such as lactobacilli and streptococci, and yeasts) found in the grains are depicted in Fig. 1. The grains did not reveal the presence of acetic acid bacteria as reported by Rosi (1978b). While lactobacilli dominate the microflora of the grain, yeasts were apparently the least affected by environmental conditions. It is interesting to note the similarities of the microbial counts of grains 1 and 4, confirming that storage of wet grains at 4°C is a good method for the preservation of the grains as generally accepted (Kosikowski, 1977). This result is independent of the observed increase in weight of the refrigerated grains, which is consistent with the results obtained by Toba *et al.* (1990).

After screening bacteria showing different colony morphologies on MRS, two colonies (denoted hereafter as Isolate I and Isolate II) were further characterized. Table 1 shows sugar fermentation profiles of these two strains, and a comparison with those of reference strains. In a

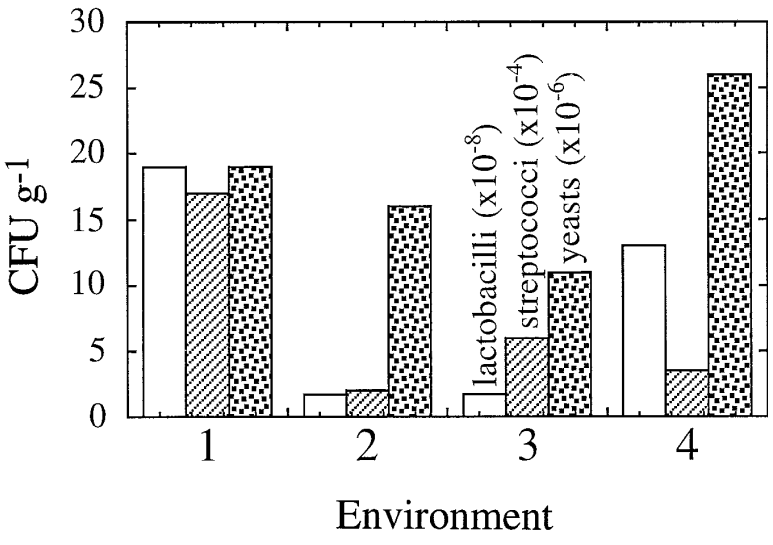

Figure 1 Number of Colony Forming Units (CFU) per unit mass of kefir grain for (1) original grains, (2) after propagation at home under typical conditions, (3) after storage for three months at room temperature, and (4) after refrigerated storage (4°C) for three months.

Table 1 Results of the tests performed on the microbial isolates

Test	Lactobacilli				Streptococci		Yeasts	
	Isolate I	Isolate II	*L. brevis*	*L. kefir*	Isolate III	*Lact. lactis*	Isolate IV	*Sacch. unisporus*
amygdalin	−	−	−	−	+	+		
L-arabinose	+	+	+	+	−	−	−	−
erythreitol	−	−	−	−	−	−	−	−
celobiose	−	−	−	−	+	+	−	−
esculin	−	−	−	−	+	+		
fructose	+	+	+	+	+	+		
galactose	+	+	+	+	+	+	+	+
glucosamine							−	−
glucose	+	+	+	+	+	+	+	+
gluconate	+	+	+	+	+	−	−	−
glucoronate							−	−
glycerol	−	−	−	−	−	−	−	−
inositol	−	−	−	−	−	−	−	
2-keto-D-gluconate	−	−	−	−	−	−	−	−
lactate							−	−
lactose	+	+	+	+	+	+	−	−
maltose	+	+	+	+	+	+	−	−
mannitol	−	−	−	−	−	−	−	−
mannose	−	−	−	−	+	+		
melezitose	−	−	−	−	−	−	−	−
melibiose	+	+	+	+	−	−	−	−
Me-α-D-glucosamine							−	−
raffinose	−	+	−	−	−	−	−	−
rhamnose	−	−	−	−	−	−	−	−
ribose	+	+	+	+	+	+	−	−
sorbose							−	−
salicin	−	−	−	−	+	+		
sorbitol	−	−	−	−	−	−		
sucrose	−	−	+	−	+	+	−	−
trehalose	−	−	+	−	+	+	−	−
xylose	−	−	+	−	+	+	−	−
NH_3 from Arg	+	+	+	+				
heterofermentative	+	+	+	+				
growth @ 10°C					+	+		
growth @ 40°C					+	+		
growth @ 45°C	+	+	+	+	−	−		
growth @ 4% NaCl					+	+		
growth @ 6.5% NaCl					−	−		
growth @ pH 9.2					+	+		
growth @ pH 9.6					−	−		
0.01% cycloheximide							+	+
0.1% cycloheximide							+	+
acetic acid production							−	−
L-lysine							+	+

similar fashion, one colony from M17 (denoted hereafter as Isolate III) and one colony from MEA (denoted hereafter as Isolate IV) were further characterized, and the results thereof are also included in Table 1. As shown in this table, all strains of lactobacilli detected are heterofermentative and produce ammonia from arginine. Inspection of Table 1 also indicates that the two strains of *Lactobacillus* tested differ in only one type of sugar fermented, raffinose (which has a

positive result for Isolate II). When compared with reference strains of lactobacilli commonly found in kefir grains, it can be observed that *L. kefir* is the most similar to the isolated strains. Confirmation of the above results was achieved via generation of electrophoretic patterns of the whole protein inventory of the two different isolates and reference strains. The associated dendograms have shown 94.1% and 89.0% homology with *L. kefir* for Isolates I and II, respectively; these results are in agreement with the conclusions of Kandler & Kunath (1983). Different results were obtained by other authors, e.g. *L. brevis* (now included in *L. kefir*) was isolated by Rosi & Rossi (1978), whereas Ottogalli *et al.* (1973) described *L. brevis* and *L. acidophilus* for grains from Bulgaria, Yugoslavia and Russia, and Molska *et al.* (1983) isolated *L. casei* from Polish and Norwegian grains.

With respect to lactococci, Isolate III was identified as *Lactococcus lactis* on the basis of the sugar fermentation characteristics and other growth conditions (Bergey, 1986). From the great degree of homology with the corresponding reference strain as apparent in Table 1, the isolated strains could be identified as *Lactococcus lactis* subsp. *lactis*. This result is similar to that obtained by Ottogalli *et al.* (1973) (i.e. *Str. lactis* subsp. *lactis*), but different from that by Rosi & Rossi (1978) who isolated *Str. durans*.

The isolated yeasts (Isolate IV) were identified as *Saccharomyces unisporus* or, synonymously, *Saccharomyces delbrueckii*, which is the yeast most frequently isolated from kefir grains (La Reviere *et al.*, 1963). The identification was based on analysis of the results pertaining to the utilization of several sugars (see Table 1) as well as additional tests of growth in 0.1% (w/v) cycloheximide, growth on L-lysine, and acetic acid production, and employed the PC Program written by Barnett *et al.* (1990) as an identification aid.

Although the results obtained for Portuguese grains in terms of yeast content compare well with those by Rosi (1978a) and Ohara *et al.* (1977), they are quite different from the results available for Russian (i.e. *Sacch. lactis*), Yugoslavian (i.e. *Candida tenuis*, *Saccharomyces lactis*, and *Sacch. carlbergensis*) and Bulgarian grains (i.e. *Cand. pseudotropicalis*) by Ottogalli *et al.* (1973), and those reported by Kosikowski (1977) (i.e. *Sacch. kefir* and *Torula kefir*).

Capsule production

All lactobacilli isolated from kefir grains proved to be encapsulated. Although capsule production in solid media was not successful, the isolates identified as *L. kefir* incubated in milk permeate broth for 6 days produced capsules surrounding their rod-shaped body which could be easily visualized via Indian ink staining. Further characterization of the capsular polysaccharide was not pursued (because it would have required sophisticated analytical techniques that were not available at our premises at that time); however, our result is expected in view of the conclusions by La Reviere *et al.* (1967), viz. that *L. kefir* (formerly *L. brevis*) was most probably responsible for producing kefiran, the material of the capsules.

Growth rate

It took almost 3 days before the dry grains submerged in milk started their exponential growth (see Fig. 2). (This lag phase was expected in view of the commonly accepted idea that microorganisms in dried form take a considerable time to readapt their metabolic system to biomass production.) The exponential curve corresponded to a specific growth rate of $\mu = 0.002475\ h^{-1}$ (doubling time of 16.8 days). Although our result is not of the same order of magnitude of that of Rozhkova (1984), viz. doubling time of 24 h, it should be emphasized that the two sets of data are difficult to compare in view of the different experimental conditions employed.

The rates of production of lactic acid and ethanol were followed along with the biomass increase, and the results obtained suggest that both products are growth-associated (see Fig. 2); although it is apparent that the concentration of lactic acid and the biomass concentration reach maxima after the maximum concentration of ethanol is attained, the time delay in question cannot be considered to be significant given the intrinsic variability of the data presented.

Rheological characterization of the kefiran/sucrose systems

The results of the gel cure experiments performed on the kefiran/sucrose systems (1% kefiran, 60% sucrose) at some ageing temperatures are depicted

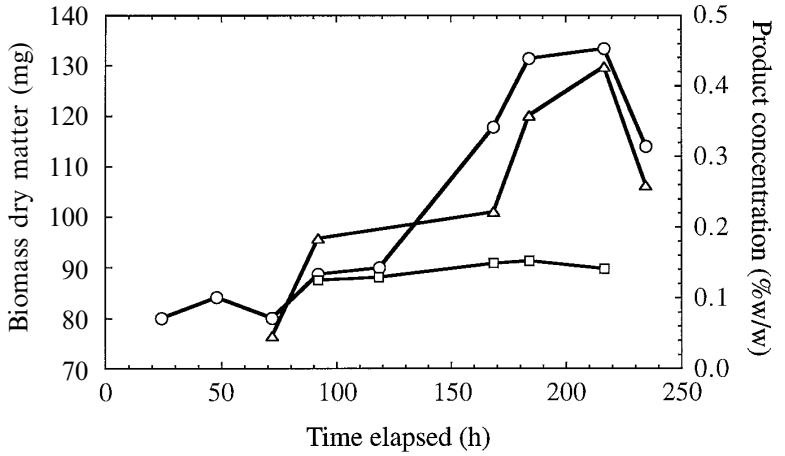

Figure 2 Growth data (○——○) for individual kefir grains each in 80 ml of milk at 22°C under gentle shaking conditions superimposed on the curves of release of ethanol (□——□) and lactic acid (△——△).

in Fig. 3. The general behaviour is typical of a biopolymer gel, with both moduli increasing as a result of the increasing junction zones density, and with the elastic component (storage modulus, G′) rising rapidly at first and then more slowly as the apparent plateau is approached. Note the small difference between the dynamic moduli, which is a characteristic of weak gels. The time elapsed from the moment when the sample reaches the temperature under study until the cross-over of the storage (G′) and loss (G″) moduli can be considered as the gel time.

The viscoelastic behaviour of the kefiran/sucrose gels changes with the ageing temperature to which the system has been exposed (Fig. 4). The behaviour is characteristic of a weak biopolymer gel with low enough density of junction zones to allow some molecular rearrangements to take place within the network over the time scale under consideration.

Rheological characterization of the kefiran/κ-carrageenan binary systems

The effect of addition of kefiran on the thermal behaviour of κ-carrageenan systems is shown in

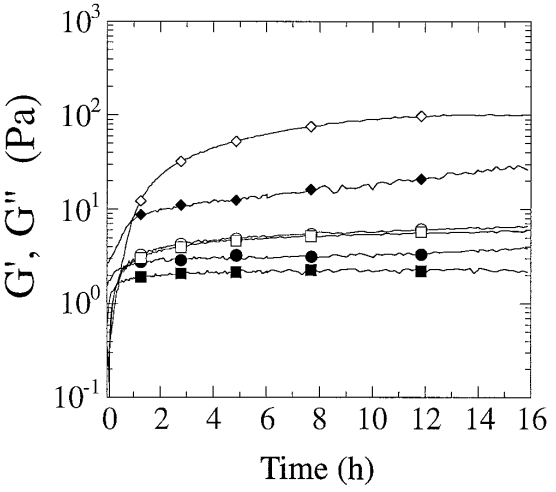

Figure 3 Storage modulus, G′ (open symbols), and loss modulus, G″ (filled symbols), as a function of time for a 1% kefiran sol/gel (60% (w/w) sucrose) at different ageing temperatures: 10°C (◇ or ◆), 20°C (□ or ■), and 30°C (○ or ●). (For clarity, only some of the experimental points obtained are shown.)

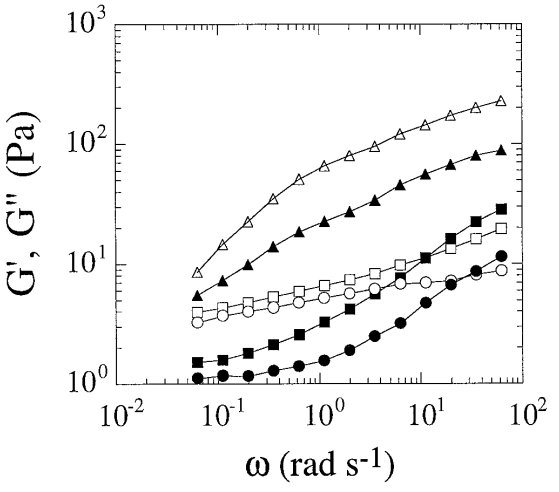

Figure 4 Mechanical spectra for a 1% kefiran gel (60% (w/w) sucrose) measured at 16 h ageing time, for different ageing temperatures: 10°C (△ or ▲), 20°C (□ or ■), and 30°C (○ or ●). Open symbols denote the storage modulus, G′, whereas filled symbols denote the loss modulus, G″.

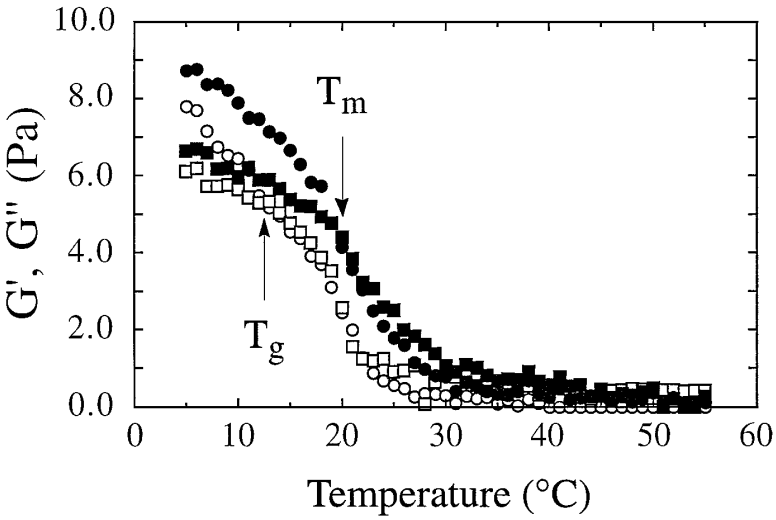

Figure 5 Evolution of G′ (○ for cooling and ● for heating) and G″ (for □ cooling and ■ for heating) as functions of temperature for 1:4 kefiran/κ-carrageenan at 1% total polymer concentration for the frequency of 6.3 rad s^{-1}. Tg: gelation temperature; T_m: melting temperature.

Fig. 5 as the evolution of G′ and G″ versus temperature for the 1:4 kefiran/κ-carrageenan blend at 1% final polymer concentration. The mixing ratio of 1:4 (kefiran/κ-carrageenan) was used because it allows a direct comparison of the data found for the kefiran/κ-carrageenan mixture with those of 1% 1:4 guar/κ-carrageenan previously described by Fernandes *et al.* (1992). A hysteresis is clearly exhibited via the gelation temperature (T_g) of c. 13°C and the melting temperature (T_m) of c. 21°C, assuming that the sol–gel transition corresponds to the cross-over of G′ and G″ (Cuvelier *et al.*, 1990; Lin *et al.*, 1991). During maturation of the kefiran/κ-carrageenan system at 15°C, G′ increased rapidly at first and then more slowly approaching an apparent plateau. The cross-over of G′-G″ occurred after 20 min. The mechanical spectra exhibited by the 1:4 kefiran/κ-carrageenan blend at 15°C are displayed in Fig. 6. The fact that G′ > G″ throughout the frequency range coupled with the fact that both G′ and G″ are quite dependent upon frequency confirms that the system abides by the usual definition of a weak gel (Clark & Ross-Murphy, 1987).

The data obtained for 1:4 kefiran/κ-carrageenan blend can be discussed by reference to the values of T_g, T_m and G′ at 5°C encompassing

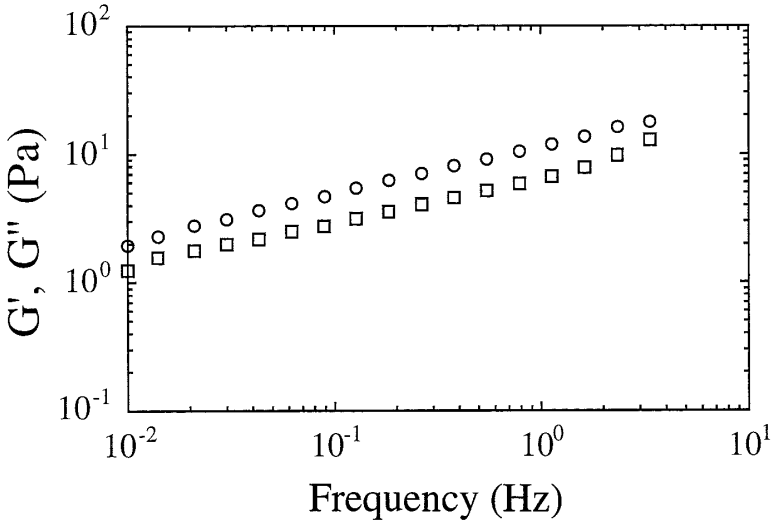

Figure 6 Frequency dependence of the viscoelastic moduli, G′ (○) and G″ (□), for 1:4 kefiran/κ-carrageenan at 1% total polymer concentration at the temperature of 15°C.

Table 2 Parameters related to the sol–gel transition of the binary system constituted by kefiran and κ-carrageenan (κ-car)

System	T_g (°C)	T_m (°C)	G′ at 5°C (Pa)
0.8% κ-car	5	16	6
1% κ-car	24	32	54
1% 1:4 kefiran/κ-car	13	21	9
1% 1:4 guar/κ-car	10	25	20

T_g: gelation temperature.
T_m: melting temperature.
G′: storage modulus at the frequency of 6.3 rad n^{-1}.

κ-carrageenan alone at the concentrations of 0.8% and 1% (Table 2). The first result expected from the addition of kefiran to κ-carrageenan would be a dilution effect since there is a lowering of the κ-carrageenan concentration down to 0.8% in the 1:4 mixed system at 1%. In fact, as apparent from inspection of Table 2, T_g, T_m and G′ for the mixture correspond to an intermediate behaviour between a κ-carrageenan concentration of 0.8% and 1%. This means that kefiran does not exhibit synergistic effects when mixed with κ-carrageenan similar to those described for mixtures of locust bean gum/κ-carrageenan (Fernandes *et al.*, 1991); however, kefiran plays a role in the gelation of the system. This behaviour should be explained by a stabilizing effect of kefiran in the system which is originated by an increase in the actual concentration of κ-carrageenan due to volume exclusion, which results in a higher actual concentration in the κ-carrageenan-enriched phase. This interpretation is consistent with the results described for the 1:4 guar gum at 1% (see Table 2) (Fernandes *et al.*, 1992, 1993). The difference between G′ values displayed by the two mixed systems (kefiran/κ-carrageenan and guar gum/κ-carrageenan) at 5°C should be ascribed to the lower intrinsic viscosity of the kefiran sample ($[\eta]_{water}$ = 0.29 m^3 kg^{-1}) in contrast to that of guar gum (1.2 m^3 kg^{-1}).

Rheological characterization of the kefiran/xanthan binary systems

The evolution of G′ and G″ during the cooling and heating cycles of the 0.5% and 1% xanthan gum ($[\eta]_{0.1\ M\ NaCl}$ = 3.3 m^3 kg^{-1}), 1% 1:1 kefiran/xanthan gum and 1% 1:1 guar gum/xanthan gum systems are depicted in Figs. 7 and 8. The G′ modulus for 0.5% and 1% xanthan gum increases strongly as the temperature is decreased from 70 to 25°C. The G′ vs. temperature profiles for cooling and heating were virtually the same. The G″ vs. temperature profile was essentially flat in this temperature range, and again the heating and cooling cycles gave identical results. In the presence of salt (0.1 M NaCl) the xanthan molecules should be present in the system in an ordered state (Milas & Rinaudo, 1986); hence, the G′ and G″ vs. temperature profiles of the ordered xanthan molecules can be associated with the degree of macromolecular association which increases

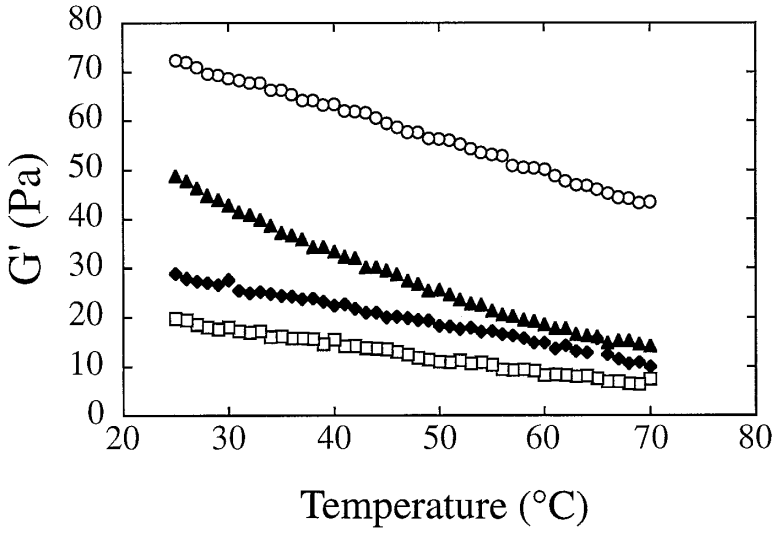

Figure 7 Evolution of G′ as a function of temperature at the frequency of 6.3 rad s^{-1} for xanthan at the concentrations of 0.5% (□) and 1% (○), and binary mixtures of xanthan with guar (▲) and with kefiran (◆).

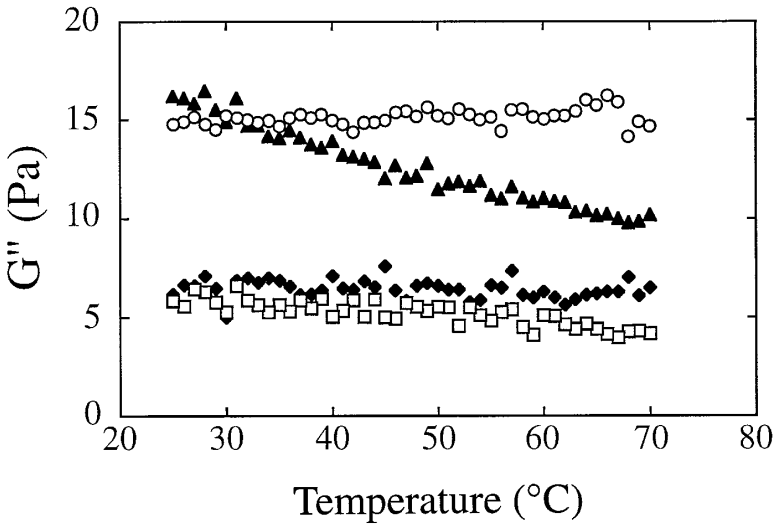

Figure 8 Evolution of G″ as a function of temperature at the frequency of 6.3 rad s^{-1} for xanthan at the concentrations of 0.5% (□) and 1% (○), and binary mixtures of xanthan with guar (▲) and with kefiran (◆).

with decreasing temperature. As in the case of xanthan alone, both 1% 1:1 kefiran/xanthan or guar/xanthan mixtures displayed an increase of G′ as the temperature dropped from 70 to 25°C. The G″ vs. temperature profile was flat for the 1% 1:1 kefiran/xanthan mixture, whereas in the case of 1% 1:1 guar/xanthan blend a slight increase of G″ was observed with the decrease of temperature. (The G′ vs. temperature profiles of both blends for either cooling or heating were again essentially the same.) In general, the G′ and G″ vs. temperature profiles of both kefiran or guar/xanthan mixtures displayed a thermorheological behaviour which is intermediate between those of 0.5% and 1% xanthan cooling-heating cycles. In the present experimental conditions (0.1 M NaCl), no significant interactions should take place between guar and xanthan (Lopes *et al.*, 1992). Therefore, the difference between the G′ and G″ vs. temperature profiles observed for kefiran or guar/xanthan mixtures and those for 0.5% and 1% xanthan should be ascribed to a lack of interactions between kefiran (or guar) and xanthan. Consequently, the differences described between the G′ and G″ vs. temperature profiles of the two mixtures (kefiran or guar/xanthan) and those of xanthan alone should reflect the lower intrinsic viscosities of kefiran and guar when compared with that of the xanthan gum sample. This fact can also be explained by a dilution effect since there is a lowering of the xanthan concentration down to 0.5% in the 1:1 mixed systems at 1% total polymer concentration. Kefiran/xanthan mixtures displayed similar trends to those of the guar/xanthan blend; however, the G′ and G″ vs. temperature profiles are lower than in the case of the guar/xanthan blend. By the same token, the lower G′ and G″ values described for the kefiran/xanthan mixture when compared with that of guar/xanthan can also be explained by the lower hydrodynamic volume occupied by the kefiran molecules in solution compared with the guar molecules (a fact that can be accounted for by the different values of the intrinsic viscosity of guar and kefiran samples).

Conclusions

The results reported in this communication pertaining to Portuguese native kefir grains are in general agreement with those obtained for kefir grains from other countries in respect of the microbial flora, especially lactobacilli and lactococci.

Kefiran can form weak gels in conditions of low water activity with potential practical applications. The effect of kefiran on the thermal behaviour of κ-carrageenan or xanthan systems shows no synergistic effects. The thermorheological profiles of kefiran/xanthan or κ-carrageenan mixtures are comparable to those described for guar/xanthan or κ-carrageenan mixed systems. The studies on the viscoelastic characteristics of biopolymers as a function of temperature allows

an assessment of their performances as ingredients in food systems which undergo thermal treatment.

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