JAP&T
TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2013, 19(2), 157-159

Journal of Agroalimentary Processes and Technologies

FTIR spectroscopic characterization of a new biofilm obtained from kefiran

Carmen Pop[1*], Sorin Apostu[1], Ancuţa M. Rotar[1], Cristina Anamaria Semeniuc[1], Marianne Sindic[2], Nicolas Mabon[2]

[1]*University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Mănăştur St., 40037 Cluj-Napoca, Romania*
[2]*University of Liege, Agro-Bio Tech Gembloux, Passage of Deportees, 25030 Gembloux, Belgium*

Received: 24 April 2013; Accepted: 08 May 2013

Abstract

The aim of this study was to investigate the structure and the film-forming capacity of the polysaccharide kefiran (extracted from kefir grains) using FTIR spectroscopy. Different forms of the polysaccharide (kefiran solution, frozen kefiran and kefiran pellet) were subjected to infrared measurements. Solutions of kefiran with different concentrations of glycerol (5, 7.5, 10 or 15% w/w) were prepared. The film-forming solutions of kefiran and the characteristics of the resulted biofilms were tested. The biofilm obtained from kefiran solution with 7.5% w/w glycerol had the best characteristics (transparency and elasticity).

FTIR spectroscopy results showed that the structural integrity of kefiran was maintained in biofilms, which highlights the relationship between chemical composition and future applications of the polysaccharide.

Keywords: kefiran, kefir grains, biofilm, FTIR

1. Introduction

FTIR spectroscopy is fast, simple and non-destructive, no sample preparation is required and no waste material is produced during a test [10]. Based on the vibration of functional groups and highly polar bonds of the components that are sensitive to IR radiation, FTIR technique is often used to determine the authentication of edible oil [11].

Kefiran, a water-soluble polysaccharide, is produced in kefir grains which consist of a complex population of lactic acid bacteria and yeasts firmly embedded [15].

These Kefir grains have a varying and complex microbial composition that includes species of yeasts, lactic acid bacteria (LAB), acetic acid bacteria (AAB) and mycelial fungi [16].

Among the lactic acid bacteria identified, the majority of species belonging to the genus Lactobacillus (*Lb.acidophilus, Lb.viridescens, Lb.gasseri, Lb.helveticus, Lb.kefiranofaciens, Lb.brevis, Lb.fermentum, Lb.kefiri, Lb.casei, Lb.parakefiri,* and *Lb.lactis* subsp. *lactis* [1].

Kefiran is used as a thickener, stabilizer, emulsifier, fat substitute or gelling agent, also shows antitumor activity [2,3] and can form edible transparent films at a concentration as low as 10 g/kg [12].

2. Materials and Methods

Extraction of kefiran from kefir grains. An aqueous solution of 10% (w/v) kefiran was obtained based on the protocols described by Rimada and Abraham (2003), Piermaria *et al.* (2008) and Ghasemlou *et al.* (2011) [7-9, 12, 14].

Corresponding author: *e-mail:* lazar_rody@yahoo.com

The kefiran solution was centrifuged (5000xg for 10 min at 4°C), using a Sorvall RC-5B centrifuge (Thermo Fischer Scientific, Asheville, North Carolina, USA) and the pellet was collected.

Preparation of the biofilm. Glycerol (Sigma-Aldrich, St. Louis, MO, USA) was used as plasticizer in contents of 5, 7.5, 10 or 15 % (w/w) with respect to the kefiran extracted from kefir grains. Biofilms were prepared by casting 17 g of the prepared solutions into micro-weighing dishes (65mm Dia. x 15mm H) and drying at 45°C for 18 h.

The FTIR spectra were recorded in the range of 4000-500 cm^{-1} using a Shimadzu spectrometer. Five replicates were taken for each sample. The spectra were processed using an IR solution Software Overview (Shimadzu) and Origin 8 SR1 Software (OriginLab. Corporation, Northampton, USA).

3. Results and Discussion

Transparency and elasticity are two important characteristics that influence the biofilm quality and applicability. The biofilm obtained from solution of kefiran with 7.5% (w/w) glycerol showed the best characteristics.

IR spectra of different forms of the polysaccharide (kefiran solution, frozen kefiran and kefiran pellet) are shown in Figure 1, and of biofilms in Figure 2. IR spectra fingerprint regions are: (1) 3700 - 3310 cm^{-1}; (2) 3000 - 2800 for biofilms; 2400 - 2300 cm^{-1} for the kefiran solution, the frozen kefiran and the kefiran pellet; (3) 1650 - 1630 cm^{-1} and (4) 1200 - 800 cm^{-1}; these results were compared with the data from journal articles [7,13].

The first region (1) 3700 - 3310 cm^{-1} is assigned to water and hydroxyl groups in the kefiran. The O-H stretching vibrations give rise to absorption bands with maximum intensity.

In the second region (2) 2400 - 2300 cm^{1}, the peaks correspond to CO_2, near 2350 cm^{-1}, the IR bands at lower wavenumber are drastically decreased.

CO_2, a linear molecule that does not have a permanent electric dipole, nevertheless produces an IR spectrum because the two C=O bonds can stretch in an asymmetric fashion and also bend to produce changes in the dipole moment [4-6].

The bands in the region of 3000 - 2800 cm^{-1} were attributed to the symmetric and anti-symmetric C-H stretching modes in the methyl (CH_3) and methylene (CH_2) functional groups.

The bands in the region 1630 - 1655 cm^{-1} were assigned to the OH bending mode of water molecules.

The last region (4) 1200 - 800 cm^{--1} is typical 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 [7]. The absorption peaks at 1035, 1080 and 1153 cm^{-1} indicate a pyranose form of sugar [17].

In this last region, the peaks detected and associated to the vibration model indicate the presence of glucose and galactose (present in the pure kefiran structure). These results suggest that kefiran consists of α- and β- forms of pyranose.

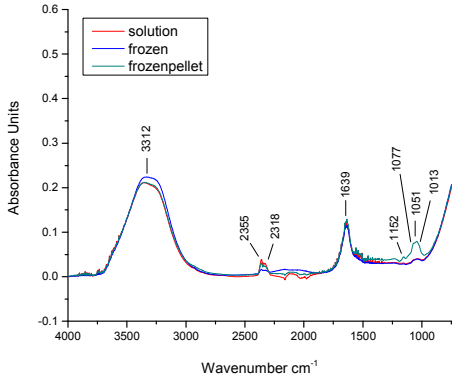

Figure 1. FTIR spectra (4000 -1000 cm^{-1}) of different forms of the polysaccharide (kefiran solution, frozen kefiran and kefiran pellet)

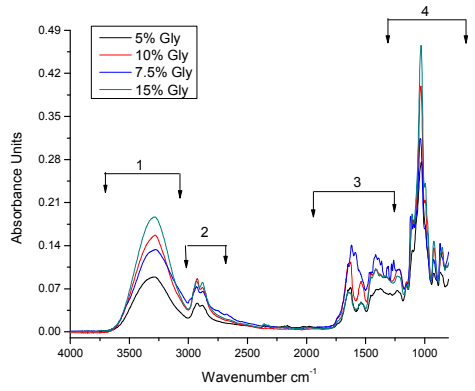

Figure 2. FTIR spectra (4000 -1000 cm^{-1}) of biofilms plasticized with 5, 7.5, 10 and 15% (w/w) glycerol

Any difference between IR spectra of the kefiran solution, the frozen kefiran and the kefiran pellet was noticed; water molecules are present in all samples because the frozen kefiran and kefiran pellet were not dried. The kefiran pellet and frozen kefiran had the same concentration of molecules.

Kefiran biofilms spectra showed quite similar general features; the differences are mainly given by the intensity of bands as result of different glycerol concentration of samples (Figure 2).

4. Conclusion

The structure of kefiran was stable during freezing (purification process) and biofilm formation. The chemical composition is an important feature for the bioactive property and future applications of the polysaccharide.

Compliance with Ethics Requirements

Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human and/or animal subjects (if exists) respect the specific regulations and standards.

References

1. Apostu Sorin, Ancuţa M. Rotar, Carmen R. Pop "*Microbiologia produselor alimentare*" vol. 3. Lucrări practice, ediţia a III-a, revăzută, Publishing House: Risoprint, Cluj-Napoca, 2012, ISBN 978-973-53-0886-5, 227-228 p.
2. Cerqueira, M. A., Bartolomeu, W. S. S, Teixeira, J. A., Vicente, A. A., Effect of glycerol and corn oil on physicochemical properties of polysaccharide films - A comparative study. *Food Hydrocolloids*, **2012**, *27*, 175-184.
3. Cheirsilp, B., Shimizu, H., Shioya, S., Enhanced kefiran production by mixed culture of *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae. Journal of Biotechnology*, 2003, 100, 43-53.
4. Cheirsilp, B., Shimizu, H., Shioya, S., Kinetic modeling of kefiran production in mixed culture of *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae*. Process Biochemistry **2007**, *42*, 570-579.
5. Cooke, J., *Spectroscopy in Inorganic Chemistry* (Theory), 2005
6. De Vuyst, L., Degeest, B.,Heteropolysaccharides from lactic acid bacteria. *FEMS Microbiology Reviews.*, **1999**, *23*, 153-177.
7. Ghasemlou, M., Khodaiyan, F., Oromiehie, A., Yarmand, M. A., Characterization of edible emulsified films with low affinity to water based on kefiran and oleic acid. *International Journal of Biological Macromolecules*, **2011**, *49*, 378-384.
8. Ghasemloua, M., Khodaiyan, F., Oromiehie, A., Physical, mechanical, barrier, and thermal properties of polyol-plasticized biodegradable edible film made from kefiran. *Carbohydrate Polymers*, **2011**, *84*, 477-483.
9. Karbowiak, T., Ferret, E., Debeaufort, F., Voilley, A., Cayot, P., Investigation of water transfer across thin layer biopolymer films by infrared spectroscopy. *Journal of Membrane Science*, **2011**, *370*, 82-90.
10. Kelly, J. F. D., Downey, G., Detection of sugar adulterants in apple juice using fourier transform infrared spectroscopy and chemometrics. *Journal of Agriculture and Food Chemistry*, **2005**, 53, 3281-3286.
11. Muik, B., Lendl, B., Molina-Diaz, A., Valcarcel, M., Ayora-Cañada, M. J., Two-Dimensional correlation spectroscopy and multivariate curve resolution for the study of lipid oxidation in edible oils monitored by FTIR and FT-Raman spectroscopy. *Analytica Chimica Acta,* **2007**, *593*, 54-67.
12. Piermaria J. A., Abraham A. G. (2008). Gelling properties of kefiran, a food- grade polysaccharide obtained from kefir grain. *Food Hydrocolloids*, **2008**, *22*, 1520-1527.
13. Piermaria, J., Bosch, A., Pinotti, A., Yantorno, O., Garcia, A. A., Abraham, A. G., Kefiran films plasticized with sugars and polyols: water vapor barrier and mechanical properties in relation to their microstructure analyzed by ATR/FT-IR spectroscopy. *Food Hydrocolloids*, **2011**, *25*, 1261-1269.
14. Rimada P. S., Abraham, A. G., Comparative study of different methodologies to determine the exopolysaccharide produced by kefir grains in milk and whey. *Lait.*, **2003**, *83*, 79-87.
15. Taniguchi M, Nomura M, Itaya T and Tanaka T (2001) Kefiran productionby Lactobacillus kefiranofaciens under the culture conditions established by mimicking the existence and activities of yeast in kefir grains. *Food Science and Technology Research*, **2001**, *7*, 333–337.
16. Witthuhn, R. C., Schoeman, T., Britz T. J., *Characterisation of the microbial population at different stages of Kefir*, 2005