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RESEARCH ARTICLE

STRUCTURE OF HORSESHOE TYPE MICROCRYSTAL FIBER OF DEPROTEINIZED KEFIRAN DETECTED BY FTIR, X-RD AND AFM WITH ANTIBACTERIAL PROPERTIES

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ABSTRACT

Kefir contains lactic acid bacteria and yeasts. These microorganisms are confined in kefiran, a branched glucogalactan with antimicrobial, anti-tumor and anti-fungal properties .The purpose of this study was to analyze the structure of the polysaccharide in raw kefiran. Kefiran was produced from raw milk with. 5.0% fat and 10 grams of kefir grain. The polysaccharides were extracted by ethanol and then protein was separated by ammonium sulfate. Four different bands in the purified protein were revealed by gel electrophoresis. Separated proteins were heat resistant with anti-microbial properties. Structural analysis by means of FTIR, AFM and X-RD showed that by removing the antimicrobial protein, the remained polysaccharide formed zigzag-shaped micro-crystals of horseshoe with antibacterial characteristics. Enhancement of the resistance of bacteria to antibiotics, make this antibacterial polysaccharide useful in the production of polymer and drug delivery. To the best of our knowledge, the horse's micro-crystal fiber of deproteinized kefiran with antibacterial activities is reported for the first time.

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INTRODUCTION

Ever-increasing demand of natural polymers for various industrial applications in recent years has led to an interest in polysaccharide. Living organisms can synthesize a variable series of polymers (Gallego *et al*., 2008; Lanza *et al*., 2000; Enikeev, 2012). Many microorganisms including lactic acid bacteria, algae, fungi and plants have the capability to synthesize extracellular polysaccharides in the form of soluble or insoluble. Microbial polysaccharides are used in the food, pharmaceutical and chemical industries. They also have applications in bioflocculants, bioabsorbents, heavy metal removal agents and drug delivery agents. Examples of microbial polysaccharides that are used in industry include dextran, xanthan, gellan, pullulan, fermentative glucans and bacterial alginates (Lanza *et al*., 2000). Lactic acid bacteria that excrete polysaccharide with high molecular weight have been extensively studied in the recent decade. These reagents have several physical characteristics that make them suitable for usage due to the viscosity, sustainability, gellation feature and emulsifying factors. Lactobacillus kefiranofaciens that is separated from kefir grains produce an exopolysaccharide called kefiran (Lanza *et al*., 2000). Kefir is a microbial symbiont mixture which produces jelly-like grains. Kefir contains lactic acid bacteria (*Lactobacillus, Lactococcus, Leuconostoc, Acetobacter and Streptococcus*) and yeasts (*Kluyveromyces, Torula, Candida, Saccharomyces*). Both bacteria and yeasts are encompassed by a polysaccharide-protein matrix. Kefir polysaccharide referred to as kefiran is a branched glucogalactan soluble in water that has antibacterial, antifungal and antitumor properties (Enikeev, 2012). It seems that it acts against the virulence factors of *Salmonella, Helicobacter, Shigella, Escherichia coli* and *Bacillus cereus*. Moreover, kefiran is an anti-inflammatory agent (Rodrigues *et al*., 2005). Anti-microbial and anti-inflammatory features of kefir are used as a remedy for patients affected with one or several strains of resistant microorganism. It also regulates the gut immune system, by protecting the epithelial cells against the virulence factors of *Bacillus.cereus* and induces dehydrogenase activity of mitochondria. Thus, kefiran is used as an additive for fermented food products too (Rodrigues *et al*., 2005; Wang *et al*., 2008; Ghasemlou *et al*., 2012; Medrano *et al*., 2008). The yellowish white kefir has a weak acidic taste. The yeast within the kefir produces a little CO_2. It has a mild sour taste due to the mixture of organic acids. Helpful effects of fermented milk are attributed to the presence of kefir's

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micro flora and their metabolic products. Consumption of kefir increases digestion of lactose. In addition, polysaccharides are used as emulsifier and gellation factors. In fact, application of microorganisms for producing polymers by means of fermentation has several advantages such as high yields, reducing production time and complex separation methods (Wang *et al*., 2008; Ghasemlou *et al*., 2012). Microbial polysaccharides are among natural polymers that some of their characteristics are their biological degradation, biocompatibility, cost-effectiveness, availability, and similarity to extracellular matrix, their capability of inherent cellular reaction and connectivity to cell (Manuela *et al*., 2013; Malafaya *et al*., 2007). The purpose of this study was to investigate the antibacterial properties of polysaccharide, protein and crude kefiran along with analysis of their structures and detection of horseshoe type micro-crystal fiber of deproteinized kefiran

1.2. Materials and Methods

1.2.1. Extraction of kefiran polysaccharide from kefir grains

10 ml pasteurized milk containing 0.5% fat was heated and cooled down at room temperature and then 10 g of kefir grains was added and kept for 48 hours at room temperature. After incubation, kefir grains were separated from the fermented product by a plastic sieve, then the solvent collected and divided in two parts (Piermaria *et al.*, 2008). Samples were kept in sterilized distilled water at 50 ºC and 100 ºC respectively for an hour in order to inactivating hydrolyzing enzymes and solving the polysaccharide. Then samples were centrifuged at 1180g in 20 ºC for 15 minutes to remove all the cells from the sample. The supernatant contains polysaccharide, precipitated by adding two ethanol volumes in 96 % cold ethanol and kept 24 hours at -20 ºC. Then samples were centrifuged at 1180 g for 15 minutes at 4 ºC. The pellet was solved in hot distilled water. This step was repeated twice, and finally the obtained sample was lyophilized after solving the sediment in hot distilled water (Piermariaa *et al*, 2009; Rimada and Abraham, 2003).

1.2.2. Detection of peptide in kefiran

For evaluation of the protein, Bradford assay was used to estimate the amount of protein. Protein concentration for treatment in 50 ºC and 100ºC was determined (Bradford, 1976).

1.2.3. Detection of polysaccharide in kefiran

For evaluation of polysaccharide, anthrone assay was used to estimate the amount of polysaccharide. Polysaccharide concentration for treatment in 50 ºC and 100ºC was determined.

1.2.4. Purification of protein in kefiran

The alcohol was removed from the extracted sample. Then mixed polysaccharide and protein were dried, weighted and solved in distilled water equal to 10 mg/ml. It was not possible to achieve saturation by using 60% concentration of ammonium sulfate, so it was tried with 80% of ammonium sulfate. After reaching saturation, the sample was centrifuged at 14462 g for an hour at 4 ºC. The collected sediment was dissolved in 100 µl of phosphate buffer saline, 1M. Chloroform/ methanol were added at the ratio of 2/1 to the sample. It was kept at 4 ºC and 14462 g for an hour. Then the white sediment was separated and solved in distilled water (Harrison, 1993). Pure protein was used for further investigations.

1.2.5. Sodium Dodecyl Sulphate-PolyAcrylamide Gel (SDS-PAGE)

The molecular weight of protein was determined by SDS PAGE on a 12% polyacrylamide gel. Polyacrylamide concentration in the stacking gel and separating gel were 5% and 20%, respectively. Electrophoresis was conducted at a constant voltage of 70 V for 2 hours (Sambrook and Russel, 2001). Then, the gel was fixed by treating 20% iso-propanol and 10% acetic acid for 2 hours followed by staining with Coomassie Brilliant Blue G-250 .The protein standard (Fermentas Unstained Protein Molecular weight marker (SM0431) with range molecular marker (13.6-116 KDa) was used.

1.2.6. Antimicrobial assay

2.6.1. Microbial strains

Staphylococcus aureus (*S. aureus*) (ATCC 25923), *Pseudomonas aeruginosa (P. aeruginosa*)(ATCC 27853), *Escherichia coli (E. coli)* (ATCC 25922), *Rizoctonia* and *Pseudomonas sp*. isolated in Microbiology Laboratory of Isfahan University

2.6.2. Antibacterial activity assay

2.6.2.1. Detection of the antimicrobial assay by disk diffusion

Blank disks were impregnated with 50 µl of kefir, purified kefiran extraction and purified peptide and applied in the form of disk diffusion to test anti-microbial properties of these extracts. According to the procedure of the National Committee for Clinical Laboratory Standards, cell suspension of 3×10^8 CFU/ml (using McFarland turbidity standard solutions) from each organisms were grown in Muller Hinton Agar medium. The microorganisms were *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa (P. aeruginosa), Escherichia coli (E. coli)* and *Rizoctonia* and *Pseudomonas sp*. isolated in Microbiology Laboratory of Isfahan University. Plates were kept at 37 ºC for 24 hours and zone of inhibition was studied (Ghasemlou *et al*, 2011).

2.6.2.2. Determination of minimum inhibition concentration

The minimum inhibitory concentration (MIC) values were studied using broth microdilution method according to standard methods (National Committee for Clinical Laboratory Standards, 1983). Sterile 96-well microplates were used for the assay (0.34 ml volume, orange scientific). Bacterial strains were cultured overnight at 37 ºC in Trypton Soy Broth (TSB). Dilution series of the crude form of kefiran 1, 10^{-1}, 10^{-2}, 10^{-3},

10^{-4}, 10^{-5}, mg/ml were prepared. 50 µl of each dilution were transferred into 96-well microtitre plates of TSB, then 50 µl of standardized microorganism suspensions including 10^{8} colony forming units per ml (cfu/ml) (according to Mc Farland turbidity standards) was added and followed by transferring 100 µl of TSB and assays were done in a volume of 200 µl. A well, consisting of TSB, and microorganisms, was used as the growth control. After incubation at 37 ºC for 24 hours, the first well, without turbidity, was selected as MIC (mg/ml). The microorganism growth inhibition was evaluated by measuring absorbance at 630 nm, using ELISA reader (StatFax-2100, Awareness technology Inc, USA). Experiments with 3 replicates were performed

2.6.3. FTIR of crude and purified polysaccharide

Crude and purified polysaccharides were studied by using FTIR technique. Major structural groups of purified exopolysaccharide were identified by FTIR (Enikeev, 2012; Ghasemlou *et al*., 2011; Piermaria *et al*., 2011). The Fourier transform-infrared spectra were recorded on a Bruker Vector 22 instrument (Germany) in the region of 4000–400 cm−1, at a resolution of 4 cm−1 and processed by Bruker OPUS software. Polysaccharide and protein dried at room temperature were used to analysis by FTIR

2.6.4. Light microscopy

Evaluation of crystallization of polysaccharide and deproteinized polysaccharide was carried out by light microscopy. Deproteinized polysaccharide and polysaccharide of kefiran were dried on slide glass and then observed under light microscope.

2.6.5. AFM

The surface morphology of kefiran was studied by using atomic force microscopy (AFM) (Dualscope/Rasterscope C26, DME, Denmark) with a 200 × 200 µm scan size and a 10µm vertical range. Dried samples were used as dot form on the slide glass. The resulting data for each sample was transformed into a 3D image (Ghasemlou *et al*., 2011). The images were obtained from the central area of each surface. The images of different zones were examined and analyzed offline by Dualscope/Rasterscope SPM software (Version 2.4.2.1) to calculate the roughness value.

2.6.6. X- Ray diffraction

2 grams purified polysaccharide was analyzed by X-ray diffraction in an X-ray diffractometer, Bruker, D8 Advance, Germany, with wavelength, 1.5406(CuKα); voltage, 40 kV; and current, 40 mA.

2.7. RESULTS

2.7.1. Detection of peptide and polysaccharide in kefiran

Protein concentration for treatment in 50 ºC and 100 ºC was determined as 41.57 and 55.68µg/ml respectively, by comparing standard curve drawing by bovine serum albumin (Bradford, 1976). Polysaccharide concentration for treatment in 50 ºC and 100ºC was determined as 796µg/ml and 364µg/ml by comparing standard curve drawing by glucose.

2.7.2. Anti-microbial activities

The crude form of kefiran and purified polysaccharide showed anti-microbial characteristics against *Rizoctonia*. This property was more active in crude form of kefiran (Fig.1a). The same results were obtained with *Pseudomonas sp*. isolated in Microbiology Laboratory of Isfahan University as well as *S. aureus* (Fig.1b). In another experiment, purified polysaccharide, the crude kefiran and protein showed a weak anti-microbial activity against *Klebsiella*, *E. coli* and *P. aeruginosa*. The in vitro antibacterial activities of kefiran were assessed by the microdilution method against *S.aureus* and *E.coli*. Antibacterial activities were expressed as MIC. Optical density (OD) was decreased from 0.4 to 0.1 in dilution of 10^{-3} to 10^{-1} mg/ml crude kefiran in growth of *S.aureus* (Fig. 2). The MIC $_{(50)}$ of kefiran was within concentration range 0.01 mg/ml in growth of *S.aureus*. Optical density (OD) was decreased from 1 to 0.2 in dilution of crude form of kefiran from 10^{-2} to 10^{-1} mg/ml in growth of *E.coli* (Fig. 3). The $MIC_{(50)}$ of kefiran was within concentration range 0.01 mg/ml in growth of *E.coli*.

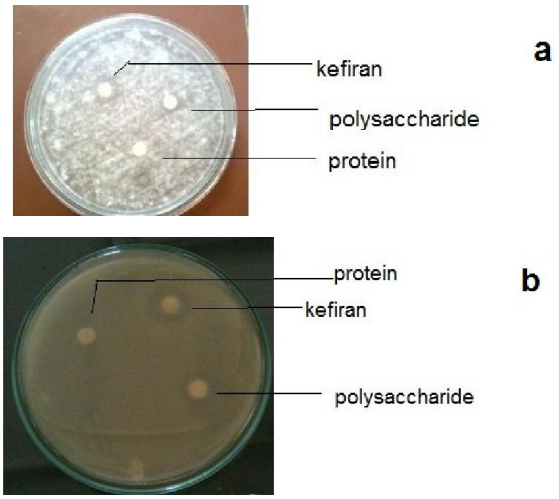

Figure 1. Antimicrobial properties of kefiran , polysaccharide and protein were analyzed by disc diffusion. The crude form of kefiran and purified polysaccharide showed anti-microbial characteristics against *Rizoctonia* (Fig 1a). This property was higher in crude form of kefiran. The same results were obtained *Pseudomonas sp*. isolated in Microbiology Laboratory of Isfahan University as well as *S. aureus* (Fig 1b)

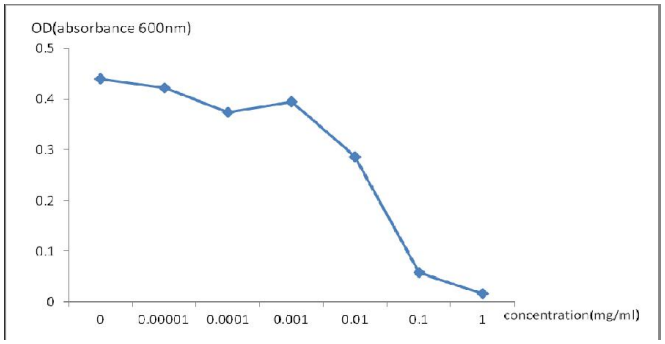

Figure 2. The efficacy of different concentration of kefiran with maximum growth of *S.aureus*. The $MIC_{(50)}$ of kefiran was within concentration range 0.1 mg/ml in growth of *S.aureus*

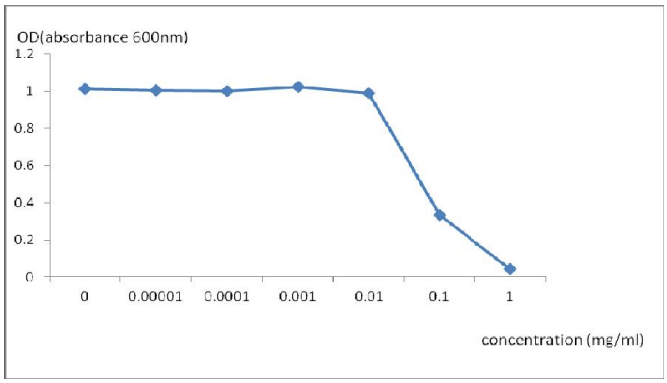

Figure 3. The efficacy of different concentration of kefiran with maximum growth of *E.coli*. The $MIC_{(50)}$ of kefiran was within concentration range 0.1 mg/ml in growth of *E.coli*

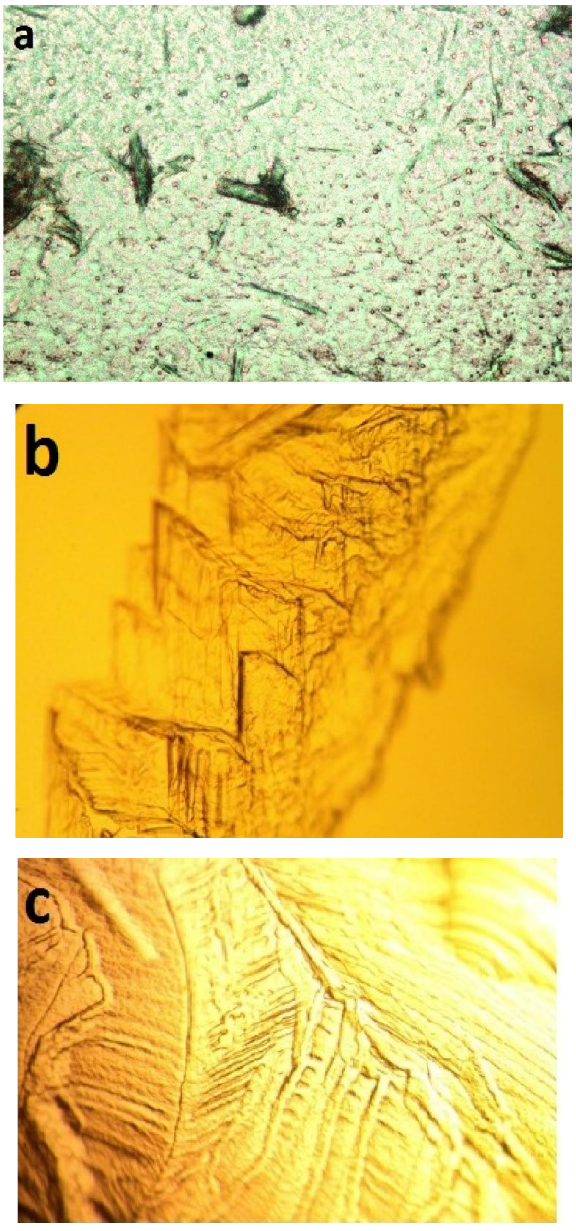

Figure 4. Crystallization of unpurified polysaccharide under light microscopy (a), Crystallization of purified polysaccharide under light microscope (b and c) which had regular and dense shape of crystal but unpurified polysaccharide had irregular and subtle shape of crystal

2.7.3. Microscopy study of crystals

Crystals which were made from purified (deprotein kefiran) and unpurified polysaccharide were shown by microscope showed that purified polysaccharide had regular and dense shape of crystal but unpurified polysaccharide had irregular and non-condensing shape of crystal (Fig.4). These data result that the reason of irregular crystal is existence of protein in unpurified polysaccharide.

2.7.4. SDS-PAGE

kefiran was tested by SDS-PAGE to check for extracted peptides and to estimate the molecular weight of extracted peptides. In this study, by SDS-PAGE four different bands were seen i.e. 14.4, 18.4, 21 and 25 KDa comparing with protein standard (Fig. 5). These data showed that there are at least 4 different types of peptides in kefiran that probably one or more of them might have the antimicrobial properties.

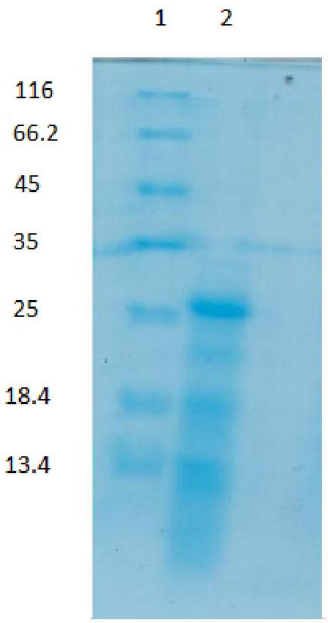

Figure 5. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of unpurified polysaccharide from kefiran after staining with Coomassie Brilliant Blue G-250. Fermentas Unstained Protein Molecular weight marker (SM0431) (line 1), purified protein of kefiran showed 4 bands i.e 14.4, 18.4, 21.5, 37 KDa (line 2)

2.7.5. AFM

Atomic Force Microscopy (AFM) is a helpful tool to study the structure of polymers and is used to achieve qualitative and quantitative information in biopolymers assembly with nanometer dimensions that are often not accessible through other techniques (Kacurakova *et al*., 2000). By AFM, horseshoe type microcrystal fiber of deproteinized kefiran (purified polysaccharide) was detected. Also AFM in this study revealed that regular, dense and strong crystals of kefiran polysaccharide are shaping in normal condition (Fig. 6).

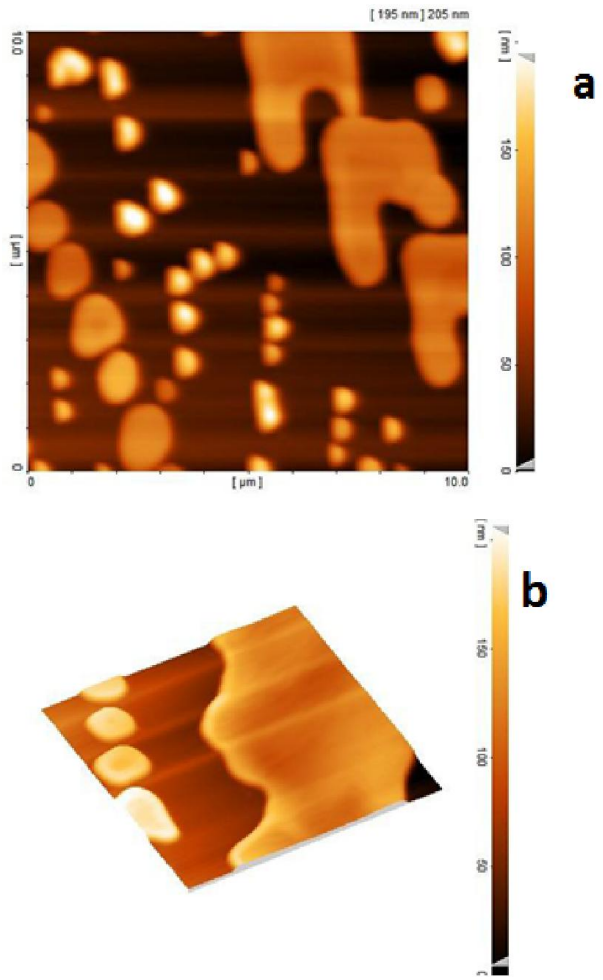

Figure 6. Crystallization 3D image of deproteinized kefiran was shown by AFM. Horseshoe type microcrystal fiber of deproteinized kefiran was detected (a), the process of crystallization of deproteinized kefiran was shown (b)

2.7.6. FTIR

Fourier-Transformed-Infrared Spectroscopy is a useful tool for showing structural changes in biopolymers. Purified polysaccharide had peaks of 3224, 3036, 2846, 2053, 1414, 1092 and 615. Regarding different formed peaks, 3224 peak is related to hydroxyl groups (piermariaa *et al*., 2009; Baimark *et al*., 2009; Bergo and Sorbal, 2007; Bertuzzi *et al*., 2007; Brancaleon *et al*., 2001; Gracía *et al*., 2009; Gu and Wu, 2007); 3036 and 2846 peaks are related to O-H group; 2053 peak is associated with triple bands; 1414 peak is related to C-H and C-O-C group; 1092 belongs to C-C and C-O-C (Bertuzzi *et al*., 2007; Brancaleon *et al*., 2001; Gracía *et al*., 2009) and 615 relates to the C-CL group (Table 1).

Unpurified polysaccharide had peaks equal to 3415, 2982, 2928, 2516, 2857, 1949, 1592, 1318, 1457, 1241, 1043, 825, 621 and 543. However, 3415 peak is related to N-H amide groups; and 2982 and 2516 are associated with hydroxyl groups (Infrared Spectroscopy IR Absorptions for Representative, jpkc.huanghuai.edu.cn/include/htmleditor/uploadfile/20130309153033372009. pdf) (Table 1) (Fig. 7). In the present study unpurified polysaccharide showed peaks related to the bands C=O, C=N, C=C, -CN. These data confirmed that the extracted unpurified polysaccharide consisted of proteins. However the concentration of protein in extraction was low. FTIR of protein revealed peaks equal to 3239,3049,2854,2065, 1656, 1425, 1105,974,901, 617 and 557. However, 3239.82 peak is related to amide group (N-H) stretch,3049.87 peak is associated with O-H carboxylic acid,2854.13 peak is related to C-H bonds, 1656.55 peak is related to amide group (C=O), 1425 peak is associated with C-H, 1105 peak related to C-O, 1105, 974 , 901, 617, 557 are related to C-C and C-N, C-H, C-O, C-H, C-CL and C-Br respectively (Infrared Spectroscopy IR Absorptions for Representative, jpkc.huanghuai.edu.cn/include/htmleditor/uploadfile/20130309153033372009. pdf) (Fig. 8).

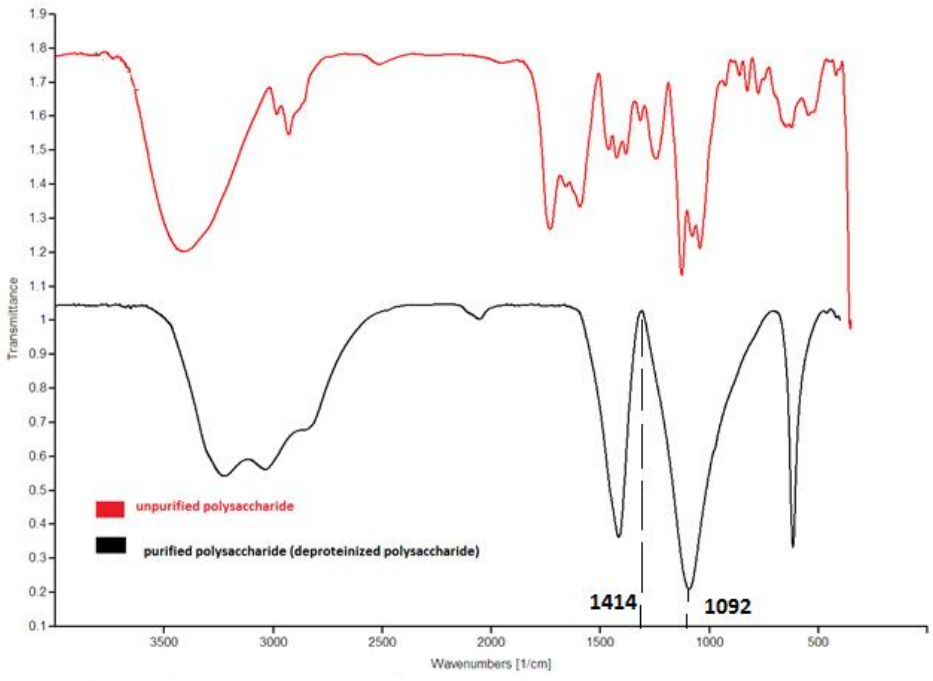

Figure 7. The changes in FTIR spectra of deproteinized polysaccharide of kefiran and crude polysaccharide of kefiran. Changes occurred through following: 1500-1200 cm^{-1} is a mixed region of fatty acid bending vibration, proteins and phosphate carrying compounds deleted except of 1414 cm^{-1} related to C-O-H in-plane bending in Carbohydrates (28) is predominant in deproteinized polysaccharide. In deproteinized polysaccharide, amide I and II was deleted (1500-1700 cm^{-1}). 1200-900 cm^{-1} contains polysaccharide. 1092 cm^{-1} is related to carbohydrate region that is predominant in deproteinized polysaccharide

In FTIR, 3000-2800 cm^{-1} spectral region is fatty acid region, 1700-1500 cm^{-1} contains the amide I and II bands of proteins and peptides, 1500-1200 cm^{-1} is a mixed region of fatty acid bending vibration, proteins and phosphate carrying compounds deleted except of 1414 cm^{-1} that is related to C-O-H in-plane bending in Carbohydrates (Davis and Mauer, 2010) that is predominant in deproteinized polysaccharide, 1200-900 cm^{-1} contains polysaccharide. In deproteinized polysaccharide, amide I and II were deleted (1500-1700 cm^{-1}). 1092 cm^{-1} is related to carbohydrate region that is predominant in deproteinized polysaccharide (Fig.7).

(2Θ) and peak around 40º (2Θ). All of the diffractograms show a crystalline structure. In this study, purified (deproteinized) kefiran had high- intensity diffraction peaks at around 20 º(2 Θ) and 40 º(2 Θ).

DISCUSSION

Our results showed that *S.aureus* was more susceptible to antimicrobial activity of kefiran than *E.coli*. The growth rate of *E.coli* was decreased in 10^{-1} dilution of kefiran (Fig. 3).

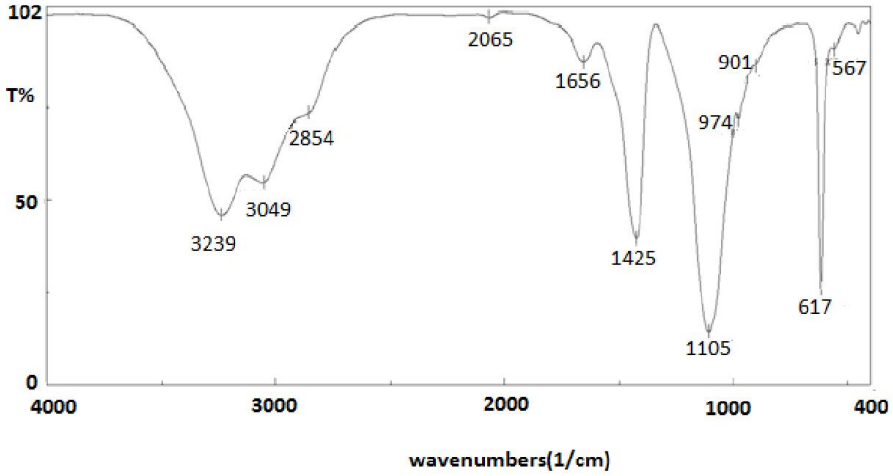

Figure 8. FTIR of protein separated from crude kefiran

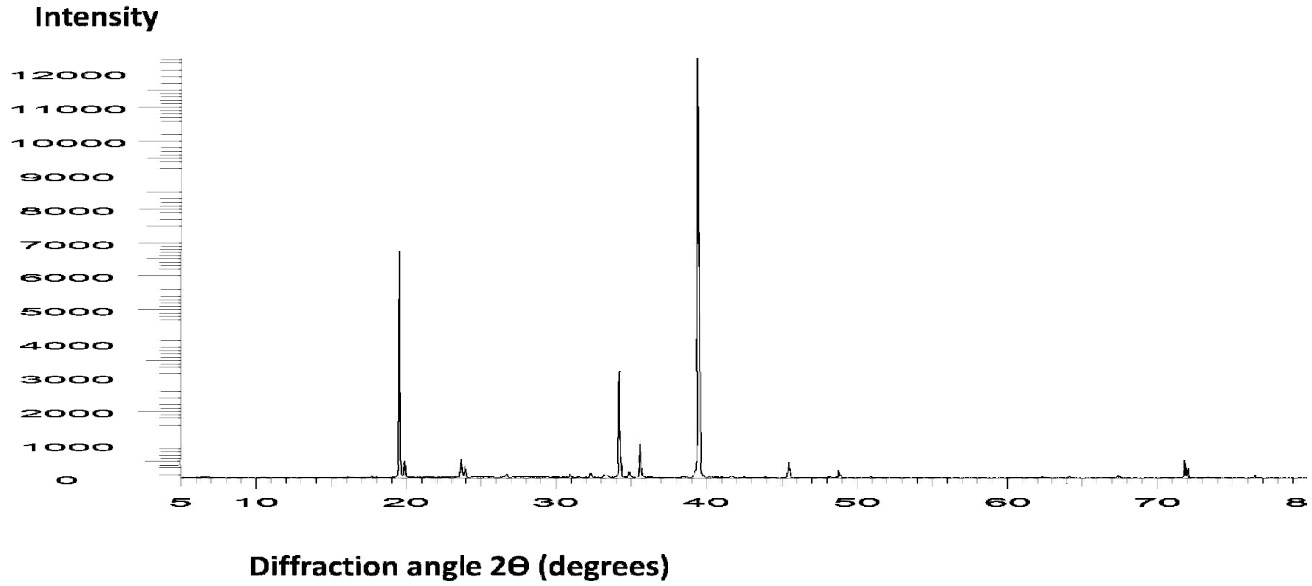

Figure 9. XRD pattern of deproteinized polysaccharide showed a flat and high- intensity peak around 20º (2Θ) and 40º (2Θ). This pattern shows a crystalline structure

2.7.7. X-RD

X-ray diffraction (XRD) analyses were performed to determine the 2Θ crystalline structure of the kefiran. The results obtained from these analyses are presented in Figure 9. XRD pattern of deproteinized polysaccharide showed a flat peak around 20º

Rodrigues *et al*. (2005), a range of bacteria consisting of *S. pyogenes, S. aureus, Streptococcus salivarius, Candida albicans, Salmonella. typhimurium, P. aeruginosa* and *E. coli* were evaluated for sensitivity to kefiran. Rodrigues *et al* (2005), proved that *S. pyogenes* is the most sensitive bacteria to kefiran. *S. aureus, Streptococcus salivarius, Candida*

albicans, Salmonella. typhimurium showed lower sensitivity to this product. *P. aeruginosa* and *E. coli* showed the least sensitivity to kefiran. In this study, the antimicrobial effect of kefiran to *S.aureus, Ecoli, P.aeruginosa*, isolated *Pseudomonas* and *Rizoctonia* were studied. The rate of sensitivity of *P, aeruginosa*, *Ecoli* and *S.aureus* are in accordance with the work done by Rodrigues *et al.* (2005). It seems that the reason of the sensitivity of isolated *Pseudomonas* to kefiran could be due to the presence porins in the outer membrane of these bacteria which is not seen in other bacteria. Resistance of gram negative bacteria to kefiran might be related to the outer membrane structure of the bacteria. In another study, Medrano *et al*. (2007) showed the protective effect of kefiran against structural cell damages produced by some virulence factors of *Bacillus. cereus* strain B10502 (Medrano *et al*., 2008).

Wang *et al*. in 2008 investigated FTIR in exopolysaccharide *L.kefiranofaciens* ZW3. Exopolysaccharides ZW3 showed more complicated models of peaks from 1200-2950 cm-1. Polysaccharides include a significant number of hydroxyl groups that have an extensive absorption band over the wave length 3000 cm^{-1} (Enikeev, 2012). Their results demonstrated that specific wave lengths identified C-O, C-H and OH bands that are more consistent with the results of this study. Also according to Wang *et al*, a weak peak of methyl group C-H in 2924 cm^{-1} is more consistent with a peak equal to 2928 related to unpurified polysaccharide that there isn`t in purified polysaccharide. The C-O-C, C-O in 1200-1000 cm-1 is related to the presence of carbohydrates and also Wang *et al*. suggested that there are C-N bands and peptide amines and one peak in 1378 cm-1 belongs to C=O from COO and C-O from COO. In the present study unpurified polysaccharide showed peaks related to bands C=O, C=N, C=C, -CN too and this is in consistent with Wang *et al*'s results. The compatibility of the results showed that there is some impurity with polysaccharide. Glucose FTIR was performed by Kacuralova *et al*. It showed peaks in 1149, 1106, 1079, 1035 and 994. Also, Galactose FTIR showed peaks in 1148, 1078, 1060 and 1040 peaks (Kacurakova, 2000). These data illustrate that there are some proteins in the primary polysaccharide with antimicrobial characteristics. In our study, these proteins were separated from polysaccharide with antibacterial activity. To the best of our knowledge, it is the horseshoe type micro-crystal fiber of deproteinized kefiran.

Ghasemlou *et al*. reported that the plasticized-kefiran films had only one peak that is low-intensity diffraction peak at around 19–20 º (2 Θ) (Sambrook and Russel, 2001). The low-intensity diffraction and irregular crystalline as their reports could due to the lipid components as plasticizers that can separate chains from each other and lack of the deproteinized polysaccharide.

2.8. Conclusion

Kefiran, a useful microbial product which is commonly applied in industry, was used in this study. Kefiran is a branched glucogalactan with antimicrobial, antitumor and anti fungal properties. By FTIR and SDS-PAGE, it was shown that polysaccharide in kefiran containing small heat-stable peptides. This study showed horse shoe type microcrystal fiber of the deproteinized kefiran structure by AFM and X-RD that not only polysaccharide but also the peptides which are along with kefiran have potential antimicrobial activities. Considering the resistance of bacteria to antibiotics, detection and production of antimicrobial peptides from kefiran might have an important revolution in production of new anti bacterial drugs. Although crude form of kefiran is more active than polysaccharide and proteins separately, but proteins can use as drug and can inject to cure illnesses.

Acknowledgments

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REFERENCES

Baimark, Y., Srihanam, P. and Srisuwan, Y. 2009. Preparation of flexible silk fibroin films plasticized with glucose. *Asian J. Mater. Sci.*, 1: 29-35.

Bergo, P. and Sobral, P. J. A. 2007. Effects of plasticizer on physical properties of pigskin gelatin films. *Food Hydrocolloids.*, 21(8): 1285-1289.

Bertuzzi, M. A., Armada, M. and Gottifredi, J. C. 2007. Physicochemical characterization of starch based films. *Journal of Food Engineering.*, 82(1): 17-25.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*., 722: 48-54.

Brancaleon, L., Bamberg, M. P., Sakamaki, T. and Kollias, N. 2001. Attenuated total reflection–Fourier transform infrared spectroscopy as a possible method to investigate biophysical parameters of stratum corneum in vivo. *Journal of Investigative Dermatology*, 116(3): 380-386.

Davis R, Mauer LJ. 2010. Fourier transform infrared (FT-IR) spectroscopy: a rapid tool for detection and analysis of food borne pathogenic bacteria. *Cur. Res. Tech and Edu*., 2: 1582–1594.

Enikeev, R. 2012. Development of a new method for determination of exopolysaccharide quantity in fermented milk products and its application in technology of kefir production. *Food Chem*., 134:2437-2441.

Gallego, D., Ferrell, N., Sun, Y. and Hansford, D. J. 2008. Multilayer micromolding of degradable polymer tissue engineering scaffolds. *Materials Science and Engineering, C*, *28*(3): 353-358.

García, M.A., Pinotti, A., Martino, M.N., Zaritzky, N.E. 2009. Composite edible films and coatings. In: Embuscado M, Huber K (eds) Edible films and coatings for food applications Thattanchavady: *Springer Science*, pp.169-210

Ghasemlou, M., Khodaiyan, F. and Oromiehie, A. 2011. Rheological and structural characterisation of film-forming solutions and biodegradable edible film made from kefiran as affected by various plasticizer types. *Int J Biol Macromol*., 49:814-821.

Ghasemlou, M., Khodaiyan, F., Jahanbin, K., Gharibzahedi, S. M. T. and Taheri, S. 2012. Structural investigation and response surface optimization for improvement of kefiran production yield from a low-cost culture medium. *Food Chem*., 133: 383-389.

Gu, W. and Wu, P. 2007. FT-IR and 2D-IR spectroscopic studies on the effect of ions on the phase separation behavior of PVME aqueous solution. *Anal. Sci*., 23:823-827.

Harrison Roger, G. 1993. Protein Purification Process Engineering, New York: Marcel Dekker, (vol. 18),115-208.

Infrared Spectroscopy IR Absorptions for Representative Available from: jpkc.huanghuai.edu.cn/include/htmleditor/uploadfile/20130309153033372009. pdf. [Accessed August 31, 2014].

Kacurakova, M., Capek, P., Sasinkova, V., Wellner, N. and Ebringerova, A. 2000. FT-IR study of plant cell wall model compounds: pectic. *Carbohyd. Polym.*, 43: 195-203.

Lanza, R.P., Langer, R.S. and Vacanti, J. 2000. Principles of tissue engineering, 2nd ed, San Diego: CA., Academic Press.

Malafaya, P.B., Silva, G.A. and Reis, R.L. 2007. Natural – origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv. Drug. Deliv. Rev*., 59:207–233.

Medrano, M., Fernando Pérez, P. and Graciela Abraham, A. 2008. Kefiran antagonizes cytopathic effects of Bacillus cereus extracellular factors. *Int. J. Food Microbiol*., 122: 1–7

National Committee for Clinical Laboratory Standards. 1983. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. NCCLS.

Piermaria, J. A., de la Canal, M. L. and Abraham, A. G. 2008. Gelling properties of kefiran, a food-grade polysaccharide obtained from kefir grain. *Food Hydrocoll*., 22: 1520-1527.

Piermaria, J., Bosch, A., Pinotti, A., Yantorno, O., Garcia, M. A. and Abraham, A. G. 2011. 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 Hydrocoll*., 25:1261-1269.

Piermariaa, J.A., Pionttia, A., Garciaa, M.A. and Abraham, A.G. 2009. Films based on kefiran, an exopolysaccharide obtained from kefir grain: development and characterization. *Food Hydrocoll*., 236: 84-90

Rimada, P.S. and Abraham, A.G. 2003. Comparative study of different methodologies to determine the exopolysaccharide produced by kefir grains in milk and whey. Le. Lait., 83:79-87.

Rodrigues, K. L., Caputo, L. R. G., Carvalho, J. C. T., Evangelista, J. and Schneedorf, J. 2005. Antimicrobial and healing activity of kefir and kefiran extract. *Int. J. Antimicrob. Ag*., 25:404-408.

Sambrook, J. and russel, D. W. 2001. Molecular cloning : A laboratory manual. 2001

Wang, Y., Ahmed, Z., Feng, W. And Song, S. Li. C. 2008. Physicochemical properties of exopolysaccharide produced by *Lactobacillus kefiranofaciens* ZW3 isolated from Tibet kefir. *Int. J. Biol. Macromol*., 43:283-288.

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