Using Fermentation to Create Environmentally Friendly Food Packaging: Testing Antioxidant Properties of Kefiran for Use in Plastic-Free Films

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INTRODUCTION

- Kefir grains are composed of multiple microorganisms that, when added to milk, produce a fermented beverage called kefir. This symbiotic culture of microbes include Lactobacilli, Lactic streptococci, yeast, and acetic acid bacteria bound together in a self-generated polysaccharide matrix [1].
- The bacterial polysaccharide that can be extracted from kefir grains is known as kefiran (Figure 1). This exopolysaccharide and its components are formed from the multitude of microorganisms and several species of Lactobacillus [2].
- The potential uses of a biopolymer formed from kefiran are many due to its antimicrobial, antibacterial, and anti-inflammatory properties among others.
- The film formed from the extracted and purified kefiran is biodegradable and can be used as a substitute for plastics in uses such as food packaging [2].
- The use of a biopolymer instead of a plastic material, such as those made from a petrochemical base, not only are better for the environment, but they also reduce the use of energy and economic cost [3].
- The objective of this research is to produce microbial polysaccharide films from kefir grains and to test the antioxidant properties of kefiran.

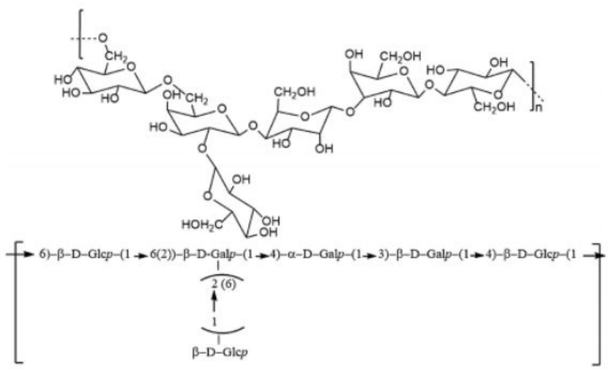


Figure 1: Chemical structure of the branched polysaccharide, kefiran

METHODS

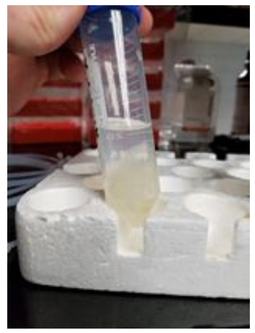
Kefiran Purification:

- Kefir grains were stirred in distilled water (1:10) at 90°C until dissolved, and then centrifuged at 10,000 rpm for 15 minutes at 20°C.
- An equal volume of ethanol was added to the supernatant and stored at -80°C overnight.
- This sample was centrifuged at 10,000 rpm for 15 minutes at 5° C to collect the precipitate.
- The above process is repeated twice, and the resulting precipitate (purified kefiran) was incubated and dried.

Methanolic Extraction:

Methanolic extracts of the active film were produced by using the method as described by Nwakaudu et al [4].

- Three differing concentrations (.25g/10mL, .5g/10mL, and 1.0g/10mL) of the active polymer films were cut into small pieces and mixed with absolute methanol.
- The mixtures were vigorously shaken in a vortex for 3 minutes and allowed to stand at room temperature for 24 hours and centrifuged at 2300 rpm for 10 min.
- Finally, the solutions were filtered using P4 Fisherbrand filter paper before spectrophotometric evaluation.



Antioxidant Testing using DPPH scavenging assay:

- The supernatant was analysed for DPPH radical scavenging activity: One mL aliquots of each methanol extract (n=9) was mixed with 2 mL of 0.1 mM DPPH in methanol. The mixtures were vigorously shaken in a vortex for 1 min and allowed to stand at room temperature in the dark for 30 min.
- Absorbances were measured at 517 nm using a Fisher Scientific UV- VIS spectrometer. The methanol was used as control and was mixed with 0.1 mM DPPH.
- Scavenging Activity for both antioxidant tests were calculated using the following equation:
- **Scavenging Activity (%) = [1-(Abs sample/Abs control)] x 100**

Antioxidant Testing using H_2O_2 scavenging assay:

The supernatant was analysed for H_2O_2 radical scavenging activity: 500 µL aliquots of each methanol extract (n=3) was mixed with 3 mL of 40 mM in methanol H_2O_2 in phosphate buffer (pH 7.4). The mixtures were incubated at 37°C for 10 minutes.

Absorbances were measured at 230 nm using a Fisher Scientific UV- VIS spectrometer. The phosphate buffer without H_2O_2 was used as control.

RESULTS

DPPH scavenging assay

- The results of the DPPH scavenging essay show that a concentration of .25g/10mL yields an average-of -three scavenging percentage of 1.25%, .5g/10ml yields 0.87%, and 1.0g/10ml yields 3.85%.

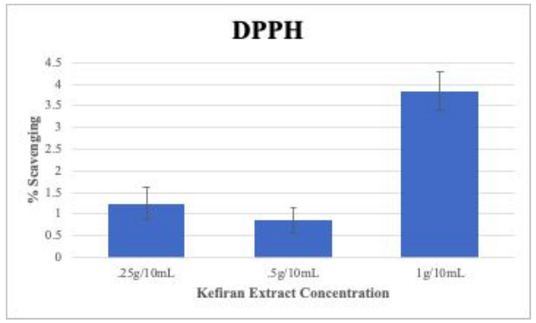


Figure 2: DPPH radical scavenging assay

H_2O_2 scavenging assay:

- The results of the Hydrogen Peroxide scavenging essay show that a concentration of .25g/10ml yields an average-of-nine scavenging percentage of 22.80%, .50g/10ml yields 7.78%, and 1.0g/10ml yields 17.21%.

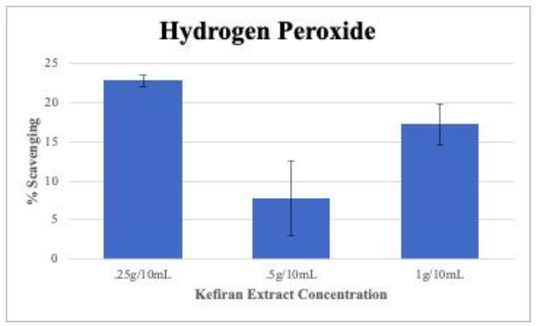


Figure 3: H_2O_2 radical scavenging assay

CONCLUSIONS

- Kefiran films are a promising biopolymer for active food packaging.
- These results conclude that the kefiran exopolysaccharide has antioxidant properties, which are important for its consideration as a substitute for plastic in food packaging.
- The ability of kefiran to scavenge DPPH radicals and hydroxyl radicals is important for food systems.

Future Research

Future research on kefiran optimization is needed to make the purification and extraction processes more efficient. In addition, the antimicrobial activity of of kefiran films needs investigation to fully understand its potential as a environmentally friendly food packaging material.

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