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Physicochemical and antimicrobial properties of citral and quercetin incorporated kafirin-based bioactive films

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ABSTRACT

The aim of this study was to determine the physicochemical and antimicrobial properties of kafirin-based bioactive films incorporating the plant essential oil citral and the polyphenol quercetin. The addition of quercetin and citral both imparted a yellowish colour to the films. The tensile strength of films significantly decreased and elongation at break increased when citral was incorporated, whereas addition of quercetin did not alter these two film parameters. The rate of water vapour transmission of the films decreased with citral incorporation but the water vapour permeability was not affected by either citral or quercetin incorporation. Furthermore, incorporation of citral and quercetin significantly lowered the oxygen permeability of the films. Film made of kafirin alone had antimicrobial activity against *Listeria monocytogenes*, however, films incorporating citral exhibited the highest antimicrobial activity against *Campylobacter jejuni*, *L. monocytogenes,* and *Pseudomonas fluorescens*. These results suggest that kafirin-based films incorporating citral and quercetin have potential as bioactive packaging to improve food safety and quality.

1. Introduction

During the past decade, there has been an increasing interest in the development and use of bio-based active films with demonstrated antimicrobial activities to improve food safety and reduce the use of chemical preservatives (Aider, 2010). These bio-based active films may also possess the abilities to avoid moisture loss or water absorption by the food matrix, oxygen penetration to the food material, aromas loss and solute transport (Dutta, Tripathi, Mehrotra, & Dutta, 2009), therefore providing further potential to maintain food quality during product transportation and storage. These films can be made from food grade biopolymers including proteins, lipids, and carbohydrates, which are edible and biodegradable (Coma, 2008). However, these biopolymers can be more expensive than current plastic films and the total cost of a food product is highly related to the packaging material cost. Therefore, the search for more economical packaging materials is a very important subject in the food industry (Aider, 2010). For instance, it is recommended that the use of biopolymers from underutilised materials or from agro-food by-products and waste would significantly reduce the cost of bioactive films (Campos, Gerschenson, & Flores, 2011).

Kafirin is a prolamin protein found in the grain of the cereal crop sorghum and accounts for about 60–80% of the total grain protein (Da Silva & Taylor, 2005). As kafirin is highly hydrophobic, it has low solubility in water, and is not easily enzymatically digestible after wet heat processing, it is an ideal biopolymer for coating and film manufacture, due to its potential to provide water and gas barrier properties (Taylor, Taylor, Dutton, & de Kock, 2005a). In addition, unlike some other predominant cereal crops such as rice and wheat, sorghum is tolerant to poor soil conditions, drought, and a variety of crop diseases, and is well-adapted to high temperatures; highlighting its potential as a sustainable crop for future agro-food security in the predicted challenge of climate change (Stefaniak & Rooney, 2013).

To enhance the bioactivity (e.g. antimicrobial and antioxidant activity) of bio-based films, plant essential oils and natural antioxidants (polyphenols) can be incorporated into the film. For example, the essential oils from oregano, rosemary and garlic have been incorporated into whey protein films (Seydim & Sarikus, 2006) and cinnamon essential oils in chitosan based films to improve the antimicrobial and physicochemical properties

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(Ojagh, Rezaei, Razavi, & Hosseini, 2010). The potential of cellulose-based packaging films containing cinnamaldehyde and eugenol for lowering the growth rate of *Pseudomonas aeruginosa* has also been documented (Sanla-Ead, Jangchud, Chonhenchob, & Suppakul, 2012). Current natural polymer-based packaging materials often have poor mechanical properties and low water resistance (Rhim & Ng, 2007), therefore the improved mechanical property, oxygen/water barrier properties, and antimicrobial activities reported for kafirin films incorporating polyphenols (tannins) (Emmambux, Stading, & Taylor, 2004), indicate the promise of kafirin-based films as active packaging for foods.

The incorporation of plant essential oil and polyphenols into bioactive films might be a feasible approach for improving their industrial applications. It has been reported that the essential oil citral is effective at inhibiting the growth of a wide range of microorganisms (Seow, Yeo, Chung, & Yuk, 2014) and the polyphenol quercetin is widely used as a potent antioxidant in breakfast cereals, dairy products, processed fruits, fats and oils (Harwood et al., 2007). The purpose of this study was to develop a bio-based active film using kafirin with the incorporation of citral and quercetin as natural antimicrobial and antioxidant agents. The potential of the films for food quality preservation was investigated by evaluating their antimicrobial properties against selected spoilage and pathogenic bacteria and their physicochemical strength and barrier properties.

2. Materials and methods

2.1. Materials

2.1.1. Sorghum flour and chemicals

Sorghum (*Sorghum bicolor*) grain variety Liberty (tannin free, grain with un-pigmented pericarp/testa) was obtained from Queensland Department of Agriculture, Fisheries and Forestry, Brisbane, Australia. The sorghum wholegrain was milled to flour with a ZM 200 Retch Mill (Retsch Gmbh & Co, Haan, Germany) using a 500 μm screen. All chemicals including citral and quercetin were purchased from Sigma–Aldrich (Sydney, Australia).

2.1.2. Microbiological test cultures and media

A common food spoilage organism *Pseudomonas fluorescens* A150 and 2 pathogenic bacteria strains, *Campylobacter jejuni* A625, *Listeria monocytogenes* A360 were provided by the School of Biomedical Sciences, Curtin University. Viability and purity of the cultures were maintained by sub-culturing single colonies fortnightly on appropriate media until the end of the experiments. *C. jejuni* was maintained at 42 °C under microaerophilic conditions on campylobacter blood agar base No. 2 (Skirrow selective supplement, SA Analytical Lab Services, Adelaide, Australia). *P. fluorescens* was maintained aerobically at 22 °C on brain heart infusion agar (SA Analytical Lab Services, Adelaide, Australia) and *L. monocytogenes* aerobically at 37 °C on tryptic soy agar (Becton, Dickinson and Company, Sydney, Australia).

2.2. Kafirin extraction

Kafirin was extracted using the method described by Taylor, Taylor, Dutton, and de Kock (2005b). Briefly, the sorghum flour (250 g) was extracted for 1 h with 900 mL aqueous ethanol (70% v/v) containing 4.38 g (≈0.5% w/w) sodium hydroxide and 6.25 g (≈0.4% w/w) sodium metabisulphite at 70 °C with continuous stirring. The kafirin containing supernatant was separated by centrifugation at 3000 rpm at 23 °C for 5 min. Kafirin precipitate was obtained through neutralization by adjusting the supernatant to pH 5.0 and was collected by vacuum filtration. The kafirin filter-cake was then freeze-dried (Christ Alpha 1-2/LD plus, John Morris Scientific, Sydney, Australia). The freeze-dried kafirin was then ground using a domestic coffee grinder and defatted three times with hexane at a protein to solvent ratio of 1:10 (w/w) and air-dried at ambient temperature. The isolated kafirin had 82.12 ± 1.68% protein (dry basis) as determined by the Kjeldahl method of AOAC 925.10 (AOAC., 2005) using a nitrogen to protein conversion factor of N × 6.25.

2.3. Film preparation

Kafirin film forming solution was prepared by dissolving the kafirin in ethanol as described by Buffo, Weller, and Gennadios (1997) with some modifications. Briefly, 30.88 g of kafirin was dissolved in 180.67 g of 96% ethanol containing 12.64 g of plasticizer (1:1:1 w/w polyethylene glycol 400: lactic acid: glycerol). The mixture was heated for 10 min at 80 ± 2 °C with rapid and continuous stirring. Four formulations were made from the film forming solution with or without the addition of citral and/or quercetin (Table 1).

Consideration for the 2.5% and 1.25% citral was based on a preliminary antimicrobial efficiency study carried out in this work. Two concentrations of quercetin (2% and 1%) were considered based on the study by Fujisawa and Kadoma (2006). All formulations were homogenised for 2 min at 6000 rpm at 23 °C using a homogenizer disperser mixer AD500S-P (IKA Works Inc., Wilmington, NC, USA).

Casting was carried out on glass sheets fabricated to form 320 × 290 × 3 mm (L × W × D) glass trays. The film forming solutions were placed on glass trays and evenly distributed using a spreading rod. The films were dried for 24 h using forced draft in an oven (Panasonic Biomedical, Leicestershire, UK) at 28 ± 2 °C and 33 ± 2% RH. After peeling from the casting wells the resulting free-standing films were conditioned in a desiccator at room temperature (23 ± 2 °C) and 52 ± 3% relative humidity (RH). Conditioned films were used for analysis of their physical and antimicrobial properties. The process of film-making and analysis was performed in triplicate.

2.4. Film thickness

The film thickness, taken as the average of eight random locations, was obtained using a digital caliper micrometer (Thorlabs, NJ, USA) according to the method of Mei and Zhao (2003). The film thickness was used in the calculations for the tensile and water vapour permeability (WVP) properties of the films.

2.5. Moisture content of the film

The moisture content of the films was determined according to AOAC 925.10 (AOAC, 2005). Samples of 30–40 cm^2 (300–400 mg) were placed on pre-dried aluminium dishes in triplicate followed by drying to a constant mass in a forced draft oven at 105 ± 2 °C for 24 h.

Table 1
Compositions of kafirin-based active films.

Treatment	Formulation (percentage of ingredient in formula)
Plain kafirin film (P)	Kafirin: 13.8%; plasticizer: 5.6% (1:1:1 w/w mixture of glycerol: polyethylene glycol 400:lactic acid); 96% ethanol: 80.6%
Citral kafirin film (C)	P + 2.5% citral
Quercetin kafirin film (Q)	P + 2% quercetin
Citral quercetin kafirin film (C + Q)	P + 1.25% citral + 1% quercetin