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Effect of kafirin-based films incorporating citral and quercetin on storage of fresh chicken fillets

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ABSTRACT

This study investigated mechanical and bioactive properties of kafirin films consisting of 2.5% citral (CK), 2% quercetin (QK) or 1.25% citral + 1% quercetin (CQK). Incorporation of citral reduced the maximum stress (σmax) and stiffness, accompanied by an increase in the strain at break (εb), and yield stress (σy) of the films. Compared to the plain kafirin films (PK) and QK, citral–containing films showed significant antimicrobial activity ($p < 0.05$) against the total viable count on chicken fillets stored at 2 ± 0.5 °C for 96 h. The final thiobarbituric acid reactive substances (TBARS) were lower for PK, CQK, and QK (0.18, 0.16 and 0.23 mg MDA/kg respectively), compared to unwrapped fillets and CK (0.41 and 0.59 mg MDA/kg respectively). These findings implied that quercetin and those polyphenolics likely co-extracted with kafirin possessed the ability to inhibit the development of lipid oxidative products. CIE L*a*b* colour values showed pronounced yellowness (b*) of wrapped samples. This colouration was attributed to the sorghum phytochemical co-extracted with the kafirin, and the intrinsic colour of citral, and quercetin. Kafirin edible films incorporating citral and quercetin demonstrated potential as a packaging material to improve food safety and quality of the chicken.

1. Introduction

In the global trade of food products, safety and quality are of paramount importance. Microbial growth and product oxidation are the primary causes of food safety and quality deterioration. Of particular interest are thermophilic *Campylobacter spp.* and psychrotrophic *Listeria monocytogenes*, which increase the risk of human infection in instances of undercooking or cross-contamination during refrigerated storage (Coma, 2008). Poultry is the leading source of microbial related food-borne infections, with 75% of the cases arising from bacterial species of *Campylobacter*, *Salmonella* or *Listeria* (Brown et al., 2014; Zhang, Wu, & Guo, 2016). Besides their frequent occurrence on poultry preparation sites, the risk of microbial related food-borne infections is compounded by the ability of these microbes to adhere to meat surfaces and preparation benches (Brown et al., 2014).

Recent studies have focused on combining packaging function with the control of surface contamination to improve meat product safety. Synthetic petroleum-based polymers have, for a long time, provided convenience and ease of use in different situations. However, questions have been raised about their continued use; because they are non-renewable and non-biodegradable, they increase the risk of environmental pollution (Marcos, Aymerich, Monfort, & Garriga, 2010; Vijayendra & Shamala, 2014) and some may produce carcinogenic compounds (Robertson, 2016). In recent years, there has been increasing interest in materials naturally produced by living organisms as substitutes for synthetic plastic packaging. Biopolymers, including proteins, polysaccharides, and lipids have been investigated in the formation of edible films and coatings since they are naturally biodegradable or may be consumed with the food (Bourtoom & Chinnan, 2008). Besides their functional role in providing mechanical support and gas and aroma barriers, edible films can be tailored for controlled delivery

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of other components. For instance, incorporating within them bioactive compounds such as antimicrobials and antioxidants, it may be possible to further increase the shelf life of food (Shojaee-Aliabadi et al., 2014).

Until now, edible films and coatings have not been developed enough to be able to replace conventional plastic packaging totally. Moreover, there is a growing need to ensure that the new bioactive and biodegradable materials help improve mechanical handling of food and lessen the migration of gas, volatiles, vapour and lipids in synergy with conventional packaging (Bourtoom & Chinnan, 2008). The use of protein-based materials is of particular interest due to their impressive gas barrier and mechanical properties. One kind of protein, kafirin a prolamin protein of sorghum (*Sorghum bicolor* L.), has received recent attention in the development of edible films and coatings because of its superior water, gas and lipid barrier properties, which are drawn from the hydrophobic nature of the protein (Belton, Delgadillo, Halford, & Shewry, 2006). Kafirin coatings have been found to decrease the rate of respiration of pears thereby delaying the onset of ripening and subsequently prolonging their shelf-life (Buchner, Kinnear, Crouch, Taylor, & Minnaar, 2011). Due to the low solubility in water and resistance to enzymatic digestion, kafirin-based biomaterials are also used for controlled delivery in food and pharmaceutical applications (Lau et al., 2015a). Kafirin nanoparticles have been reported to provide stability of encapsulated curcumin (Xiao, Li, & Huang, 2015) and prednisolone (Lau et al., 2015b) during oral delivery.

Citral is a monoterpene aldehyde extracted from the plant *Cymbopogon citratusis* (Choi, Decker, Henson, Popplewell, & McClements, 2010). It has been demonstrated to be effective in growth inhibition of a broad range of microorganisms (Seow, Yeo, Chung, & Yuk, 2014) and potentials to maintain food quality as observed during the storage of fresh fruits (Guerreiro, Gago, Faleiro, Miguel, & Antunes, 2015). The main active ingredients, $\alpha-$ and $\beta-$ citral have been reported to possess anti-inflammatory, fungistatic and antiseptic properties (Seow et al., 2014). Quercetin is a phenolic compound naturally occurring in plants, such as vegetables and fruits. It is reported to exhibit anti-obesity, antioxidant, antitumor and therapeutic activities (Azuma, Ippoushi, & Terao, 2010). A recent study by Boadi and Johnson (2014) demonstrated that quercetin at 1.0–3.5 μM was effective in suppressing protein oxidation, therefore minimizing the risk of age-related diseases in humans. Bioactive agents may not be applied directly to a food system due to possible evaporation losses, inactivation or rapid release into the food matrix (Xiao et al., 2015). Therefore, biopolymers have been investigated for encapsulation, delivery and their gradual release into during food storage. In our previous study, kafirin films incorporating citral and quercetin have demonstrated promising physicochemical, mechanical properties, and in-vitro antimicrobial activity against *Campylobacter jejuni*, *Listeria monocytogenes,* and *Pseudomonas fluorescens* (Giteru et al., 2015).

So far, however, application of these functionalities to enhance the shelf-life of a food product has not been documented. In addition, little attention has been paid to the significance of the effect of incorporating the bioactives on the required mechanical properties of edible films. Therefore, the objective of the study was to investigate the effects of incorporating the bioactives citral and quercetin on mechanical and techno-functional properties of kafirin films examined using stress-strain measurements, thickness, and moisture content. Efficacy of these films for prolonging the shelf life of chicken fillets during cold storage was also evaluated using microbial total plate count, colour and thiobarbituric acid reactive substances test for oxidation.

2. Experimental

2.1. Materials

White whole grain sorghum (*Sorghum bicolor* L) with protein, lipid, and ash content 9.9%, 1.3% and 1.5% (db.) respectively (Licata, 2012), was obtained from Queensland Department of Agriculture, Fisheries, and Forestry (DAFF) (Queensland, Australia). Citral (95%), 3, 7-dimethyl-2, 6-octadienal, (a mixture of geranial and neral), quercetin (98%), 3, 3′, 4′, 5, 7-pentahydroxyflavone were obtained from Sigma-Aldrich (Sydney, Australia). Mueller-Hinton agar plates were obtained from SA Analytical Lab Services (Adelaide, Australia) and bacteriological peptone was sourced from Oxoid Chemicals Ltd. (Adelaide, Australia). All media was prepared and maintained at 0–4 °C until required for analysis. All other reagents were analytical grade.

2.2. Kafirin extraction

Kafirin was extracted from whole grain sorghum flour using a modified 70% ethanol extraction procedure (Emmambux & Taylor, 2003). In brief, ground sorghum flour in batches of approximately 250 g was extracted using a mixture of 900 mL ethanol (70% w/w) in deionized water, 25 g kg^{-1} sodium metabisulphite and 17.5 g kg^{-1} (w/w) sodium hydroxide as a reducing agent. The mixture was heated and held at 70 °C with continuous stirring during 1 h. A supernatant containing solubilized protein was recovered by centrifugation (1000 × *g*; 23 °C; 5 min) (581OR, Eppendorf, North Ryde, Australia). After an overnight evaporation of the solvent at ambient conditions in a fume cupboard, the slurry was acidified to approximately pH 5.0 using 1 mol L^{-1} HCl to precipitate the protein. The wet mass of protein concentrate recovered by filtration under vacuum was freeze-dried (Christ Alpha 1–2/LD plus, John Morris Scientific, Sydney, Australia) and ground using a kitchen coffee grinder. The protein was defatted three times in 1 h cycles using n-hexane at a protein to solvent ratio of 1:10 (w/v), then air-dried and stored under refrigeration at 4 °C until required for use. Total lipid determination was carried out using Soxhlet extraction (AOAC official method 2003.05) on a Buchi Soxhlet Extraction Unit e-816 (Switzerland) from 1 g of kafirin powder using petroleum ether (AOAC., 2006). The protein content of the kafirin powder was determined in triplicate using an AOAC micro-Kjeldahl method 920.87 (N × 6.25) (Kjeltec 2100 distillation unit Tecator, Hillrod, Denmark) respectively (AOAC, 2011). Results were expressed as a percentage on a dry basis (db.); the yield (% kafirin in the original grains) and the protein purity (g protein kg^{-1} dry solids) were also calculated.

2.3. Preparation of kafirin film-forming solutions

Kafirin film forming solutions (KFFS) were prepared as described by Da Silva and Taylor (2005) with modification. Briefly, 13.8% (w/w) solution of kafirin was prepared by dissolving the powder in 80.6% (w/w) of 96% ethanol containing 5.6% (w/w) plasticizer (1:1:1 w/w polyethylene glycol 400: lactic acid: glycerol). The mixture was heated to 80 ± 2 °C on a magnetic stirrer and held for 10 min with stirring at 200 rpm. Ethanol was added to replace the lost solvent by subtracting the weight after heating from the initial weight of the flask plus the contents. The mixture was cooled and held at 40 ± 2 °C in a warm water bath, and four formulations were made from the film forming a solution with or without the addition of citral and quercetin.