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Short communication: Effect of active food packaging materials on fluid milk quality and shelf life

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ABSTRACT

Active packaging, in which active agents are embedded into or on the surface of food packaging materials, can enhance the nutritive value, economics, and stability of food, as well as enable in-package processing. In one embodiment of active food packaging, lactase was covalently immobilized onto packaging films for in-package lactose hydrolysis. In prior work, lactase was covalently bound to low-density polyethylene using polyethyleneimine and glutaraldehyde cross-linkers to form the packaging film. Because of the potential contaminants of proteases, lipases, and spoilage organisms in typical enzyme preparations, the goal of the current work was to determine the effect of immobilized-lactase active packaging technology on unanticipated side effects, such as shortened shelf-life and reduced product quality. Results suggested no evidence of lipase or protease activity on the active packaging films, indicating that such active packaging films could enable in-package lactose hydrolysis without adversely affecting product quality in terms of dairy protein or lipid stability. Storage stability studies indicated that lactase did not migrate from the film over a 49-d period, and that dry storage resulted in 13.41% retained activity, whereas wet storage conditions enabled retention of 62.52% activity. Results of a standard plate count indicated that the film modification reagents introduced minor microbial contamination; however, the microbial population remained under the 20,000 cfu/mL limit through the manufacturer's suggested 14-d storage period for all film samples. This suggests that commercially produced immobilized lactase active packaging should use purified cross-linkers and enzymes. Characterization of unanticipated effects of active packaging on food quality reported here is important in demonstrating the commercial potential of such technologies.

Key words: lactase, immobilized enzyme, active packaging, milk quality

Short Communication

Lactose intolerance can produce uncomfortable intestinal symptoms, which can be prevented by consuming lactose-free products (Vesa et al., 2000; Swallow, 2003; Matthews et al., 2005). Traditionally lactose-free fluid milk is produced by the addition of β-galactosidase to fluid pasteurized milk in a batch operation that requires a secondary heat treatment to deactivate the enzyme and to inactivate any microbial or enzymatic contamination from the enzyme preparation. This method results in a characteristic cooked milk flavor, which can be dissatisfying to consumers (Chapman et al., 2001; Adhikari et al., 2010). Immobilized enzyme reactors have been investigated as a continuous processing alternative (Albayrak, 2002; Husain, 2010; Freitas et al., 2011). Despite the potential for reuse and recovery of the immobilized enzymes, fouling and stability concerns have limited their commercial application.

Active packaging, in which an active component is embedded into or onto a packaging material with the goal of improving the safety, economics, and shelf life of packaged foods, may offer an alternative means to produce lactose-free fluid milk products (Vermeiren et al., 1999; Fernández et al., 2008). Developments in active packaging with immobilized enzymes for dairy products are aimed at reducing the negative sensory characteristics of batch processing (Goddard et al., 2007; Mahoney et al., 2013). One such active package was created by layer-by-layer deposition of lactase, in which lactase is covalently immobilized onto a UV functionalized low-density polyethylene (**LDPE**) surface between layers of repeated depositions of polyethyleneimine (**PEI**) and glutaraldehyde (**GL**) cross-linking layers (Wong et al., 2013).

Some researchers have evaluated the intended effects of active packaging in terms of product quality, consistency, and consumer acceptance in dairy products, whereas separate comprehensive fluid milk quality studies have evaluated bacterial counts, sensory acceptance, and shelf-life of the final product (Hansen and Arora, 1990; Grosová et al., 2008; Martin et al., 2012). However, reports on novel active packaging technologies typically do not evaluate their potential

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for unanticipated adverse effects on dairy quality. As additional steps are required in the manufacture of active packaging materials, potential for the introduction of microbial and enzymatic contaminants (e.g., lipases and proteases from the enzyme preparations) exists, which can directly affect the quality and shelf stability of the packaged food products. Therefore, the overall goal of this work was to evaluate the effect of an immobilized lactase active packaging material on the quality and shelf stability of fluid milk. Ultra-high temperature pasteurized (UHT) skim milk was subjected to storage studies in contact with the active packaging material, and was evaluated for protease and lipase activity, as well as microbial growth by SPC. Migration studies and activity studies were also performed.

Additive-free LDPE pellets were purchased from Scientific Polymer Products (Ontario, NY). Anhydrous potassium phosphate dibasic, anhydrous potassium phosphate monobasic, anhydrous sodium bicarbonate, anhydrous sodium carbonate, anhydrous sodium acetate trihydrate, glacial acetic acid, hydrochloric acid, sodium hydroxide, acetone (99.8%), isopropanol (99.9%), methanol (99.9%), peptone, and polytetrafluoroethylene filter units (0.2 μm) were purchased from Fisher Scientific (Fairlawn, NJ). The 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride was purchased from Proteo-Chem (Denver, CO). N-Hydroxysuccinimide, sodium phosphate tribasic dodecahydrate (98%), ortho-nitrophenol, para-nitrophenyl acetate (**pNPA**), and 2-(*N*-morpholino) ethanesulfonic acid (**MES**) were purchased from Acros Organics (Geel, Antwerp, Belgium). Glutaraldehyde (25%) was purchased from Alfa Aesar (Ward Hill, MA). Protazyme OL tablets were purchased from Megazyme International (Bray, Ireland). Aerobic Count Plate Petrifilms were purchased from 3M (Two Harbors, MN). Ortho-nitrophenol-β-D-galactopyranoside (**ONPG**), bicinchoninic acid (**BCA**) assay reagents, and BSA were purchased from Thermo Scientific (Rockford, IL). Branched PEI (molecular weight = 25 kDa) and para-nitrophenol were purchased from Sigma-Aldrich (St. Louis, MO). Amicon Ultra (50k molecular weight cut-off) centrifugal filter devices were purchased from Millipore Ireland (Carrigtwohill, County Cork, Ireland). Syringe filters were purchased from Whatman (Florham Park, NJ). Hershey's UHT skim milk was purchased from Diversified Foods Inc. (Metairie, LA). Dried lactase preparation from *Aspergillus oryzae* was donated by Enzyme Development Corporation (New York, NY).

The lactase was purified in a 0.1 *M*, pH 5.0 acetate buffer and filtered through a 0.2-μm polytetrafluoroethylene syringe filter before centrifugal filtration (50k molecular weight cut-off) at 5,015 × *g* for 30 min at 20°C. Lactase purification was performed to remove contaminating enzymes and microorganisms from the enzyme preparation, and to create a stock solution for ease of film preparation. Lactase trapped in the membrane filter was flushed out with 0.1 *M*, pH 5.0 sodium acetate buffer and stored at 4°C for further use. The purified solution is heretofore referred to as the free lactase enzyme solution.

The immobilized lactase active packaging films analyzed in this study were prepared using a layer-by-layer lactase immobilization method on functionalized polyethylene as previously reported for the development of multilayer films (Wong et al., 2013). Briefly, acetone and isopropanol cleaned films (2 × 1 cm) pressed from LDPE pellets on a Carver Laboratory Press (Fred S. Carver Inc., Summit, NJ) to 294 ± 17 μm were exposed to 28 mW/cm^2 of UV light at 254 nm to generate carboxylic acid groups to which branched PEI was bound using water-soluble cabodiimide chemistry to produce LDPE-PEI films. Purified lactase (**LAC**) was covalently linked to the amine-functionalized polyethylene using reductive animation chemistry through a GL cross-linker (LDPE-GL films). The resulting film represented a single multilayer (LDPE-LAC films). All quality and stability tests described herein were done on individual multilayer films.

The total protein content of the free lactase enzyme solution and LDPE-LAC was quantified using an altered BCA assay for film surfaces against buffer and LDPE controls. An aliquot of 10 μL of free lactase enzyme solution was reacted in 2 mL of BCA working reagent for 30 min at 37°C to determine free enzyme protein content, whereas each (2 × 1 cm) sample film was shaken in 3 mL of BCA working reagent for 1 h at 60°C (Mahoney et al., 2013). Samples were read at 562 nm on a Synergy 2 with Gen5 Software (BioTek Instruments Inc., Winooski, VT) and compared against a standard curve of known concentrations of BSA. Protein migration was measured by quantifying and comparing the total protein content of blank MES buffer, MES film storage buffer, LDPE films, and LDPE-LAC films following the methods for free enzyme and film content BCA assays, respectively.

Lactase activity was determined using the ONPG assay as outlined in the Foods Chemical Codex for *A. oryzae* (Institute of Medicine (US) Committee on Food Chemicals Codex, 2003). Assay conditions followed conditions for free lactase from *A. oryzae* with an optimum activity at 50°C and pH 5.0. The lactase activity was calculated using an experimentally determined extinction coefficient (ε) of 4.05 μmol/mL produced by a standard curve of *o*-nitrophenol (99%) and 1% (wt/vol) sodium carbonate aqueous solution (Wong et al., 2013). The resulting activity calculations in acid lactase units