UV Polymerization-Based Surface Modification Technique for the Production of Bioactive Packaging

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ABSTRACT: The term bio-active packaging refers to a packaging material that has been modified by the attachment or immobilization of bioactive components on the food contact surface. This article describes a novel, economical, and feasible technique for embedding bioactive components in energy curable food contact resins. While the technique is versatile and potentially applicable to any antimicrobial or bioactive compound; the proof of concept discussed in this article has focused on enzyme immobilization. Glucose oxidase (GOx) and catalase were used as representative enzymes. These oxidoreductases are very sensitive to inactivation by extrinsic factors and therefore present a challenging model for immobilization. Embedding of activity occurred via UV polymerization of commercial polymer coatings. The efficiency of immobilization and the performance of bioactive packaging were tested in both food simulants and actual food products. In both cases immobilization resulted in food contact surfaces with high retained enzyme activity as demonstrated by oxygen removal. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 107: 1647–1654, 2008

Key words: biological applications of polymers; enzymes; curing of polymers; photopolymerization

INTRODUCTION

Nonmigratory bioactive packaging or bioactive packaging is a class of polymers in which biological activity is attached to the polymer without subsequent migration into the food product.[1] Bioactive packaging is an important niche area in the field of active packaging because the active compound can interact with the components in the food to promote desirable changes without itself migrating into the food.

Covalent linkage is probably the best method to immobilize enzymes or other bioactive compounds on polymers. The chemical steps involved in covalent attachment, however, are complex and time consuming and are therefore not currently feasible for routine commercialization for food contact packaging. Enzymes are efficient biological catalysts commonly used as processing aids in the food industry to improve quality, performance, and/or appearance of food. Use of enzymes as active components in food packaging is not new.[2] Some researchers have developed advanced covalent techniques to incorporate enzymes in food packaging in the past decade.[3] Although a broad range of enzymatic reactions stemming from enzyme incorporation into packaging can be conceived, only a relatively small number have actually been commercially attempted.[4,5] Enzymes attached to the food contact surface can help create a small processing environment within the package which continues to benefit the food during transportation and storage.[1] For example, a thorough paper written by Soares and Hotchkiss in 1998 discusses in detail the covalent attachment of naringinase to packaging materials to reduce the bitterness of processed grapefruit juice. While elegant and effective, such a process is too complicated and costly, for in-line real-time application to polymer roll stock.

Glucose oxidase (GOx) is one of the most extensively used enzymes in the food and chemical industry.[2,6,7] GOx is a highly specific enzyme for the oxidation of β-D-glucose to glucono-1,5-lactone, which spontaneously hydrolyses nonenzymically to gluconic acid. Molecular oxygen is consumed in the process and hydrogen peroxide is released according to the following reaction scheme:

$$\text{D-glucose} + \text{oxygen} \longrightarrow \text{D-glucono-1,5-lactone} + \text{hydrogen peroxide} \quad (1)$$

GOx consumption of oxygen finds many uses as an oxygen scavenger to protect the appearance and nutritional quality of oxygen sensitive foods. Removal of glucose in foodstuffs by GOx has also been used to improve storage stability of foods prone to Maillard browning. Hydrogen peroxide formed as a product in eq. (1) even has broad bactericidal properties.

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GOx is often used in combination with catalase in food products when hydrogen peroxide can create unwanted quality changes. This combination is used in such applications as desugaring egg whites to prevent nonenzymatic browning before drying. GOx is used to reduce the head space oxygen in fruit juices, canned drinks, and other applications. Incorporating GOx by itself and in conjunction with catalase in packaging structures has been reported previously.[8]

UV polymerization

Polymerization of polymer inks and coatings with UV is often used in the packaging industry. Adaptation of this technology has been explored in a variety of applications especially in the fields of biosensing, biomedical engineering for drug delivery, and encapsulation of biological matrices and gels. Monomers are selected which are compatible with the conditions required for enzyme activity such as neutral functionality and ready hydration by aqueous solutions.[9,10] UV-polymerization offers a rapid and straightforward procedure for immobilization of enzymes in construction of biosensors. For example cholesterol biosensors were constructed with entrapped cholesterol oxidase within methacrylate and polypyrrole membranes.[11] GOx paste has also been immobilized using UV irradiation with hydrophilic monomer.[12]

In this study, the feasibility of binding biological active compounds in the form of oxygen scavenging GOx/catalase enzyme system directly to the food contact surface of packaging polymers using UV curable resins as an immobilization matrix has been studied. GOx/catalase for the deoxygenation of apple juice was used primarily as a model system to demonstrate the feasibility and efficiency of the technique as well as to develop the proof of concept. The ability of immobilized enzymes to achieve and maintain low oxygen conditions in glass vessels, barrier packaging, and PET containers in model systems and in commercial fruit juices was also examined.

MATERIALS AND METHODS

Materials

Acrylic monomers trimethylolpropane triacrylate (Polysciences, Warrington, PA) and methacrylated polybutadiene (Sartomer, Exton, PA) were used for enzyme embedding. The photoinitiator used was methyl benzoylformate (Rahn USA, Aurora, IL). The polymer support material such as low density polyethylene (LDPE) was obtained from Bemis (Neenah, WI). LDPE was corona-treated to increase the surface tension and to enhance wettability corona treatment unit was obtained from Electro Technik, (Chicago, IL). UV polymerization was carried out in a UV crosslinker (UVX-1000 by Fisher Scientific) that was modified to facilitate nitrogen purge during crosslinking. GOx G-6122 and catalase C-9322 were obtained from Sigma Aldrich (St. Louis, MO). Laboratory supplies and other chemicals were purchased from VWR (Batavia, IL), while frozen apple juice concentrate was obtained from a local grocery store.

UV polymerization

The intensity of UV light used in this study was $\sim 3400\ \mu W/cm^2$ with a cure time ranging between 1 and 2 min under nitrogen atmosphere. Figure 1 provides an overview of the different steps involved in the process. The time required for polymerization depends on several factors including the type of UV lamp used, the intensity of light, and the photoinitiator. Incorporating the active element in the cure mixture and subjecting to UV polymerization embeds the active element in the cured polymer matrix.

Preparation of the immobilized enzyme plaque

A 3 cm $\times$ 3 cm LDPE plaque was treated with corona discharge to obtain a surface tension of greater than 45 dyne/cm as measured by Marker Pens obtained from Diversified Enterprises (Claremont, NH). About 0.2 g of monomer was weighed on to the LDPE plaque along with 2% of the photoinitiator (wt/wt). The crude enzyme powder (5100 U/g) was added onto the above mixture at a concentration of 0.2 mg/mL ($\sim$ 20 mg of solid for every 100 mL of buffer or juice). Once all the components were added to the plaque, they were spread evenly throughout the 3 cm $\times$ 3 cm area using a spreader (Bio-Rad Hercules, CA) and UV polymerization was carried out as specified in Figure 1.

Choice of monomer and cure characteristic tests

The choice of monomer was based on the following characteristics: (a) fast cure time, (b) hydrophobic nature, (c) material flexibility after cure, (d) extent of crosslinking, and (e) viscosity in terms of spreadabil-

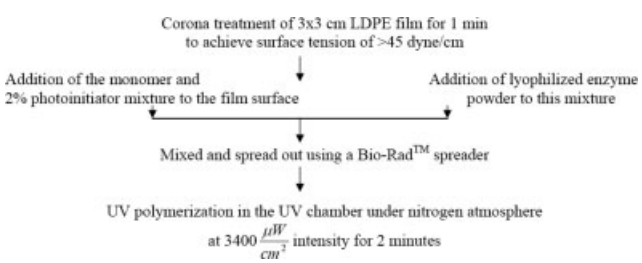

Figure 1 Block diagram of the UV polymerization process for the production of bioactive packaging.

ity. Trimethylolpropane triacrylate (TMPTA) was chosen among all the monomers tested as representing the best balance of these characteristics, and was used for all embedding experiments.

Tests to measure the characteristics of cure

Several tests were used to test the durability properties of UV cured TMPTA without the enzyme.

Permanganate staining test for proper curing

FDA's major concern is the migration of uncured monomer into foods. The crosslinked films (cured films) were tested with an aqueous 1% potassium permanganate ($KMnO_4$) solution to test for the presence of any unreacted double bonds. The intensity of stain color is directly proportional to the degree of unsaturation. The solution was applied to a cured sample so that a 1/2 square inch diameter was covered. The solution was allowed to remain on the surface for 5 min after which it was rinsed with water and evaluated by the presence or absence of color.[13]

Solvent resistance tests

Methyl ethyl ketone (MEK) is commonly used as a solvent to qualitatively measure the solvent resistance of the cured material in the coatings industry.[13] Although food systems are not as harsh as the solvents used in the industry, MEK test was used to evaluate the stability and solvent resistance of the cured polymer. For this test, a small portion of the cured film is rubbed with a swab soaked in MEK, and the number of rubs required to disintegrate the film was used as an indication of solvent resistance of the polymer.

Assessment of pH stability of crosslinked polymer

The optimum conditions for activity of GOx are well documented in literature.[14] However, in this study the stability of cured film to wide range of pH was considered essential to facilitate good immobilization. If the monomer with hydroxyl groups is involved in crosslinking then there is a possibility that these groups can get protonated below a certain pH. Protonation of the hydroxyl groups may result in loss of cure integrity. The cured film was tested for stability and integrity at pH from 2.5 to 6 using acetate buffer, pH was varied using titrimetric techniques. Stability and integrity were evaluated visually for stability against swelling and against disintegration or breakage of cured film.

Assessment of binding efficiency in terms of residual activity in wash solution

To assess the dislodging of embedded enzyme in contact with liquid, the plaque was washed in acetate buffer (100 m*M* acetate buffer at pH 5.1) and stirred for 2 h at 300 rpm at room temperature. The activity of the enzyme in the wash solution was used as an indication of the binding efficiency of the polymerization. In short, a fixed weight ($\sim$ 44 mg) of the enzyme was immobilized on a 3 cm $\times$ 3 cm plaque of LDPE and cured as per the method mentioned in Figure 1. Immediately after curing, the immobilized enzyme was washed with acetate buffer (220 mL for 44 mg of enzyme) by stirring at 300 rpm for 2 h at room temperature. Enzyme activity in the wash was assessed using a standard protocol based on the coupled reaction between GOx and horse radish peroxidase (HRP).[15] As listed in eq. (2) below, GOx-HRP reaction produces a reddish-brown solution, proportional to the activity of GOx used, which is measured spectrophotometrically (using Beckman-Coulter DU-800 UV–vis spectrophotometer) at 500 nm at 35°C.

$$\begin{aligned} &\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \\ &\qquad\qquad \text{D-Glucono-1,5-Lactone} + H_2O_2 \\ &H_2O_2 + \text{o-Dianisidine (reduced)} \xrightarrow{\text{POD}} \\ &\qquad\qquad \text{o-Dianisidine (oxidized)} \end{aligned} \quad (2)$$

Equation (2) showing the coupled reaction between GOx and HRP.[15] Here GOD represents Glucose Oxidase and POD represents Peroxidase.

The concentration of 0.2 mg/mL was specified by Sigma (for cat no. G-6766) to get a spectrophotometric reading within linear range of the Beer–Lambert's law.[15] The same concentration (0.2 mg/mL) of free enzyme in buffer was used as the positive control to the wash solution while the buffer without any enzyme was used as the negative control.

Assessment of availability of active sites of the bound enzyme

Effective attachment of the enzyme does not really indicate the availability of active sites on the polymer. An indirect method was used to assess the availability of active sites on the plaque. The availability of enzyme active sites was assessed immediately after enzyme immobilization; reaction cocktail, consisting of the substrates required for the GOx-HRP coupled reaction was added directly to the plaque and incubated at 35°C. Upon development of reddish-brown color, the reaction mixture from the plaque was transferred to polystyrene cuvettes and the absorbance was read spectrophotometrically at 500 nm at 35°C. As a control to this experiment, the same amount of free enzyme (as used for immobilization, about 10 mg) was added directly to the reac-

tion cocktail and the absorbance was determined spectrophotometrically. The percentage activity of the bound enzyme based on the absorbance of the free and bound enzyme at 500 nm at 35°C was calculated as per the following formula:

$$\%\text{Activity} = \frac{\text{Absorbance of immobilized enzyme}}{\text{Absorbance of free enzyme}} \times 100 \quad (3)$$

Evaluation of the effect of immobilized GOx in a food sample

A glass jacketed vessel connected to a circulating water bath was used as the reaction setup. Enzyme was immobilized on LDPE as described in previous subsection about preparation of immobilized enzyme plaque. Buffer systems or actual apple juice (reconstituted from frozen concentrate) was used as the media into which the immobilized enzyme plaque was introduced. As the GOx interacted with the D-glucose present in the sample, oxygen depletion occurred according to eq. (1). A Thermo Orion 810 A (Waltham, MA) oxygen analyzer (with a resolution of 0.01 mg/L and accuracy of ±1.0% full scale) was used to measure the concentration of oxygen in ppm every 10 min for 2 h or until the oxygen concentration reached a low steady level (close to 0 ppm). The experiments were conducted at 25°C (room temperature: typical storage temperature of shelf-stable juices) and 5°C (temperature of storage after opening the package). The same deoxygenation experiments as described above (for both buffer and juice media) were conducted on each of the following samples:

a. Treatment: 0.2 mg/mL of immobilized enzyme
b. Positive control: 0.2 mg/mL of free enzyme
c. Negative control: 0.2 mg/mL of deionized water instead of enzyme

Immobilization of GOx and catalase

When experimentation was carried out for longer than 20 h for treatment and positive control, there was around 6% rise in oxygen level (around 16 h), attributed to the forward inhibition of GOx by high levels of hydrogen peroxide. To breakdown this hydrogen peroxide, catalase was incorporated into the tests (both immobilized and free enzymes) at concentrations (0.2 mg/mL) and activities equal to GOx. It was observed that the oxygen levels remained as low as 0–0.2 ppm throughout the entire 5-day test.

Buffer as a food simulant

Most of the commercially available frozen concentrates are fortified to at least 100% daily value (DV) of Vitamin C. The nonenzymatic oxidation of ascorbic acid may also have a deoxygenating effect on some juices. To make sure the deoxygenation effect was only due to the enzyme and not due to ascorbic acid, experiments were conducted in citric acid buffer (100 m*M*) at pH 3.3, to simulate apple juice. β-D-glucose (6% by wt) was added to simulate the glucose in apple juice without adding ascorbic acid.

Testing in packaging material

Barrier packaging systems were examined for the ability of bound enzyme to remove oxygen to levels protective of food. Materials included pouches made from a flexible commercial film containing an EVOH barrier layer. The enzyme was immobilized as an active spot on the food contact layer in the package within a 3 cm × 3 cm area. To test if the rate of oxygen transmission was greater than the rate of oxygen removal, the treatment time was extended to overnight.

Since PET bottles are commonly used to package juices for both refrigerated and the shelf-stable storage, the ability of bound enzyme to remove oxygen in PET bottles was examined. However, an active spot on the bottle could not be made due to equipment limitations of the cure setup. Therefore, the immobilized enzyme was introduced as a 3 cm × 3 cm plaque in a PET bottle. Oxygen concentration was monitored as a function of time as described above.

Data representation

For the ease of understanding and comparison, for the treatments of apple juice or buffer with GOx and catalase in glass reaction vessel, and the experiments in packaging material (results corresponding to subsection Immobilization of GOx and Catalase to subsection Buffer as a Food Simulant) the deoxygenation value is represented as the half-life ($t_{1/2}$) in minutes. Half-life in this case, is defined as the time required for depletion of half of the initial oxygen concentration at experimental conditions. This response term is used to compare the positive and negative controls with treatments for each experiment.

Statistical analysis

All the experiments were performed in triplicate and the data reported here is the average of the triplicate values. For the assessment of enzyme efficiency (in apple juice or buffer system), Anova and Tukey's multiple comparison tests were performed for different treatments to evaluate if the treatment was significantly different from the positive and negative controls.

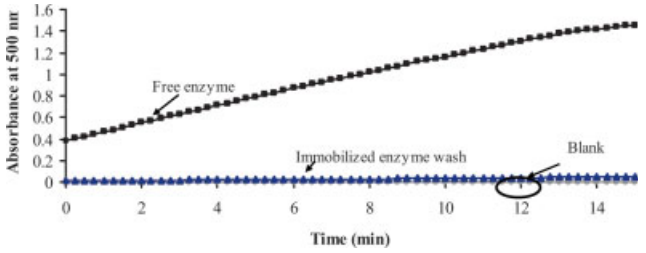

Figure 2 GOx immobilized in trimethylolpropane triacrylate matrix. Comparison of free enzyme solution with immobilized enzyme wash after stirring for 2 h at 300 rpm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

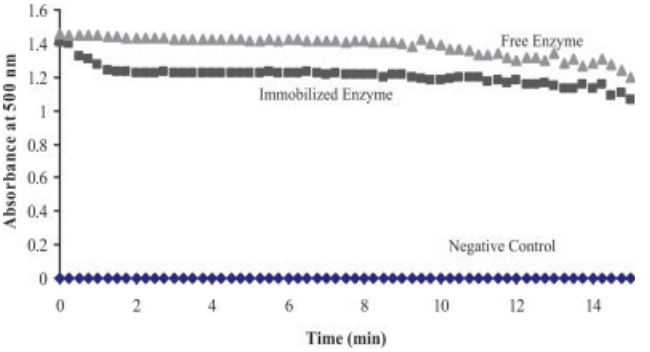

Figure 3 Available activity of GOx immobilized in trimethylol propane triacrylate matrix in comparison with free enzyme for incubation at 25° and stirring at 300 rpm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

RESULTS AND DISCUSSION

Cure characteristics

The permanganate test was conducted to assess the presence of uncrosslinked double bonds in the cured polymer. It was observed that using a cure time of 2 min (using the low intensity UV source), the permanganate staining test did not result in a colored stain indicating that the cure was complete without any detectible residual double bonds. Solvent resistance tests showed that the cure was intact for at least 50–65 rubs of MEK.[13] This indicates that the solvent resistance of the cured films was medium to high and authenticates the cure procedure and the setup. pH stability of cured film was evaluated using acetate buffer in the acidic range (pH of 2.5–6.0). It was observed that there was no visual disintegration of films at pH 2.5–6.0 indicating that the cure was stable to this pH range. Stability at lower pH indicates that the cured polymer matrix does not have any residual carbonyl groups or aldehyde groups that are susceptible to protonation.

Assessment of binding efficiency in terms of residual activity in wash solution

Activity of GOx in wash solution was used as an indication of the immobilization efficiency. The activity in the wash should be inversely proportional to the immobilization efficiency and also gives an estimation of dissolution effects of the bound enzyme mixture. Figure 2 shows the effect of washing for 2 h on GOx immobilized in TMPTA matrix. The activity of the wash solution after stirring for 2 h is very similar to that of the negative control (blank) ($P > 0.05$) and much lower than the activity of the free enzyme ($P < 0.05$). This suggests that the enzyme was effectively attached to the polymer surface. The same assessment of retained activity in washed plaques was conducted for enzyme immobilized in methacrylated polybutadiene (results not included). As with TMPTA the activity of wash solution after stirring for 2 h was very similar to the activity of negative control indicating that methacrylated polybutadiene is also a very effective matrix to attach the enzyme to the polymer.

Assessment of availability of active sites of the bound enzyme

Effective immobilization does not necessarily translate to the availability of active sites of the enzyme. Thus an indirect activity assay was conducted to assess the availability of active sites of the bound enzyme. Figure 3 shows the absorbance reading for the reaction cocktail directly added to the plaque and to the free enzyme. The graph shows that the activity of the free enzyme and the immobilized enzyme are not significantly different ($P < 0.05$), indicating that the % activity [calculated based on eq. (3)] of the immobilized enzyme is similar (89% ± 1.15% for three replicates) to the activity of the free enzyme.

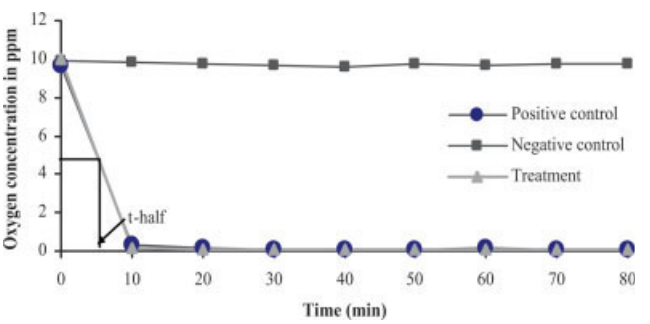

Figure 4 Treatment with immobilized GOx at 25°C for 220 mL apple juice from frozen concentrate with 300 rpm stirring. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

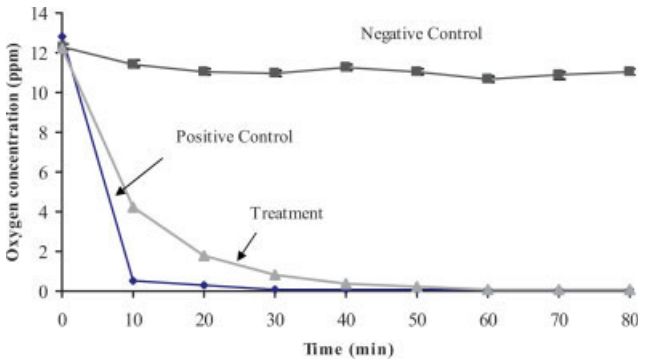

Figure 5 Treatment with immobilized GOx at 5°C for 220 mL apple juice from frozen concentrate with 300 rpm stirring. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Tests in food samples

The above results demonstrate that the immobilization works effectively in buffer systems however, to demonstrate that the performance would be similar in foods; the same tests were conducted in apple juice and food simulants. The depletion of oxygen over time was measured. Figures 4 and 5 show the comparison of activity of free and immobilized GOx at 25 and 5°C, respectively. The data shows that the oxygen depletion for the immobilized enzyme and the free enzyme are not significantly different at 25°C ($P > 0.05$). Figure 4 shows the half-life ($t_{1/2}$) in minutes for the treatments at 25°C. Table I gives a summary of the $t_{1/2}$ values for the different treatment conditions and the media tested. It can be noted from Table I that there is a decrease in the rate of oxygen depletion (almost two fold at 25°C and 1.36 times at 5°C) with immobilized enzyme in the presence of catalase than compared with the GOx alone. The reason for this reduced rate in oxygen depletion in the presence of catalase may be attributed to the fact that catalase also produces oxygen as the product of H_2O_2 breakdown and this lag would possibly explain the delay in attaining overall oxygen depletion. Figure 5 shows that there is a lag in oxygen depletion (based on the shape of the curve) at 5°C which can potentially be explained by the changes in diffusion of the substrate to the enzyme embedded within the polymer matrix. Specific diffusivities at different temperatures and the reason for this anomaly at lower temperatures are presently being investigated.

Immobilization of GOx and catalase

The deoxygenation experiments were conducted for >20 h by only using GOx without catalase resulted in a raise in oxygen concentration after ~16 h. This could be ascribed to the well known forward inhibition of GOx by hydrogen peroxide which can be resolved by the addition of catalase to the system.[16] The addition of catalase appeared to produce a slight lag in deoxygenation (similar to shape of treatment curve in Fig. 5, data not shown) however, the overall effectiveness of short term deoxygenation experiments to achieve oxygen concentration of 0 ppm (<5 hr) was not affected by catalase (Table I). Moreover, the problem with a rise in oxygen in longer experiments was eliminated using catalase in addition to GOx (data not shown).

Buffer as a simulant

To confirm that the deoxygenation effect was just due to the enzymes and was not due to the presence of ascorbic acid, the experiments were conducted in acetate buffer (at pH 3.3 with added D-glucose and no ascorbic acid). Comparing the results in Tables I and II it can be observed that there was a difference between the deoxygenation (*t*-half values) in juice and the buffer simulant. However, the fact that the deoxygenation still occurred in the absence of ascorbic acid suggests that there could be other potential components (minerals and other organic acids) in the juice which may also contribute to the deoxygenation effect. The main limitation of this experiment was that the buffer simulant could only simulate the ionic strength and pH of the apple juice and not all the other components to act as a true model for comparison. It was observed that the time required for deoxygenation in the presence of catalase is higher than that with GOx alone at 25°C and this was reversed at 5°C (Table II). The exact reasons for this remain unknown at present time.

TABLE I
Summary of *t*-Half Values for the Reconstituted Apple Juice Treated with GOx and Catalase in Glass Reaction Vessel at 25 and 5°C

Media	Temperature (°C)	Reaction vessel (material)	Enzyme mixture	Positive control (*t*-half in minutes)	Treatment (*t*-half in minutes)
Reconstituted apple juice	25	Glass	GOx	6.06 ± 0.14	6.52 ± 0.17
Reconstituted apple juice	25	Glass	GOx and catalase	6.02 ± 0.24	12 ± 013
Reconstituted apple juice	5	Glass	GOx	5.1 ± 0.026	11.45 ± 0.41
Reconstituted apple juice	5	Glass	GOx and catalase	5.6 ± 0.73	15.8 ± 0.21

TABLE II
Summary of *t*-Half Values in Citric Acid Buffer (100 m*M* at pH 3.3), Without Added Ascorbic Acid in Glass Reaction Vessel at 25 and 5°C

Media	Temperature (°C)	Reaction vessel (material)	Enzyme mixture	Positive control (*t*-half in minutes)	Treatment (*t*-half in minutes)
Buffer (Citric acid buffer 100 m*M* at pH 3.3)	25	Glass	GOx	5.4 ± 0.45	10.02 ± 0.338
Buffer (Citric acid buffer 100 m*M* at pH 3.3)	25	Glass	GOx and catalase	7.2 ± 0.32	36 ± 0.905
Buffer (Citric acid buffer 100 m*M* at pH 3.3)	5	Glass	GOx	5.4 ± 0.78	25 ± 0.13
Buffer (Citric acid buffer 100 m*M* at pH 3.3)	5	Glass	GOx and catalase	6 ± 0.32	15.2 ± 0.29

Testing in packaging material

To simulate the actual commercial application of this technology tests were conducted in actual packaging material such as PET bottles. Table III provides the effect of treatment in commercial PET bottles with just GOx and the combination of GOx and catalase. These results demonstrate the effectiveness of the immobilized enzyme system to remove oxygen levels in barrier material. However, experiments were also conducted in LDPE [with oxygen transmission rate of (840 cm^3 mil)/100 square inches/day atm at 23°C][17] to evaluate the effectiveness of the enzyme deoxygenation system in highly permeable material. The results (Table III) demonstrated that the enzyme system was also able to achieve low oxygen concentrations (0.2 ppm) in LDPE pouches. The experiments in LDPE pouches demonstrate that this technology with careful adaptation has the potential to supplement or replace barrier components of existing food packaging.

CONCLUSIONS

The results demonstrate the efficiency of the technology to attach GOx and catalase. It was observed that GOx can be effectively immobilized in the TMPTA polymer matrix by the use of UV polymerization. The % activity of the immobilized GOx is about 89% ±1.15% when compared with the activity of the free enzyme in soluble form. GOx and catalase were used as the model system for the deoxygenation of apple juice. It was observed that within the treatment time of 1 h the levels of oxygen concentration dropped to ~ 0 ppm in all the experiments. The addition of catalase was needed to maintain the low levels of oxygen in experiments carried for longer than 20 h. However, the presence of catalase increased the *t*-half values to an average of 2–2.5 times as compared with the experiments without catalase. The exact reason for this remains unknown however; a possible explanation could be owed to the oxygen produced as a product of H_2O_2 breakdown by catalase. At refrigerated temperature an opposite effect was observed in the experiments with catalase in buffer medium. That is, the *t*-half values for deoxygenation with GOx alone were 1.66 times higher than the experiments with GOx and catalase, the reasons for which remain unclear at this time. The experiments conducted in packaging material conclude that with modifications the technique could be used to enhance the barrier capabilities of high oxygen permeable material such as LDPE.

The technology presented here sets the stage for a novel surface activation technique in the field of active packaging. The data clearly demonstrates the feasibility and the effectiveness of the technology to immobilize GOx and catalase for the deoxygenation of apple juice and model systems. Though the GOx was only used as a model system for the proof of concept, the results help authenticate the potential of the methods for immobilization of sensitive and susceptible bioactive components. The types of bioactive compounds that can be attached to enhance the quality and safety of food products are almost endless. Using this rapid and versatile technique, active agents ranging from chemical antimicrobials to compounds of biological origin including enzymes and phytochemicals could be potentially commercialized.

TABLE III
Summary of *t*-Half Values for Reconstituted Apple Juice Treated with GOx and Catalase in PET Bottles and LDPE Bottles at 25°C

Media	Temperature (°C)	Reaction vessel (material)	Enzyme mixture	Positive control (*t*-half in minutes)	Treatment (*t*-half in minutes)
Reconstituted apple juice	25	PET bottle	GOx	6 ± 0.45	13 ± 0.46
Reconstituted apple juice	25	PET bottle	GOx and catalase	5 ± 0.34	10 ± 0.90
Reconstituted apple juice	25	LDPE pouch	GOx	6.06 ± 0.25	8.01 ± 0.36
Reconstituted apple juice	25	LDPE pouch	GOx and catalase	6.26 ± 0.32	8.5 ± 0.25

References

1. Steven, M. D.; Hotchkiss, J. H. Novel Food Packaging Techniques; Woodhead: Cambridge, England, 2003; pp 71–95.
2. Sarett, B. L.; Scott. D. U.S. Patent 2,765,233 (1956).
3. Soares, N. F.; Hotchkiss, J. H. J Food Sci 1998, 63, 61.
4. Brody, A. L.; Strupinsky, E. R.; Kline, L. R. Active Packaging for Food Applications: Oxygen Scavenger Systems; Technomic: Lancaster, PA, 2001; pp 53–65.
5. Brody, A. L.; Budny, J. A. Enzymes as Active Packaging Agents; Blackie Academic & Professional: Glasgow, 1995; pp 174–192.
6. Ohlmeyer, D. W. Food Technol 1957, 11, 503.
7. Baldwin, R. R.; Cambell, H. A.; Theissen, R.; Lorant, G. J. Food Technol 1953, 7, 275.
8. Andersson, M.; Andersson, T.; Adlercreutz, P.; Nielsen, T.; Hornsten, E. G. Biotechnol Bioeng 2002, 79, 37.
9. Madsen, F.; Peppas, N. A. Biomaterials 1999, 20, 1701.
10. Beginn, U.; Keinath, S.; Moller, M. Macromol Chem Phys 1998, 199, 2379.
11. Brahim, S.; Narinesingh, D.; Guiseppi-Elie, A. Anal Chim Acta 2001, 448, 27.
12. Rohm, I.; Kunnecke, W.; Bilitewski, U. Anal Chem 1995, 67, 2304.
13. Cork Industries. Testing the Cure of UV/E-Beam Curing. Available at: http://www.corkind.com/ttn/cttn_4_98_Testing_UV_Cure.pdf (1998).
14. Biozyme. Glucose Oxidase. Available at: http://www.biozyme.com/GLUCOSE_OXIDASE.html (2001).
15. Sigma Aldrich. Available at: http://www.sigmaaldrich.com/Area_of_interest/Biochemicals/Enzyme_Explorer (1996).
16. Brody, A. L.; Strupinsky, E. R.; Kline, L. R. Active Packaging for Food Applications; Technomic: Lancaster, PA, 2001; pp 40–43.
17. Robertson, G. L. Food Packaging Principles and Practice; Marcel Dekker: New York, 1993; p 53.