

Development of Antimicrobial Film Based on Pectin-ZnO Bio-nanocomposites

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In recent years, antimicrobial packaging has attracted much attention from the food industries thanks to the increase in consumer demand for preservative-free products. Moreover, there has been a growing interest in developing biodegradable packaging materials to replace petroleum based polymers. As a by-product of fruit processing industries, biopolymer pectin is both inexpensive and abundantly available, which is thus an excellent candidate for applying to eco-friendly biodegradable packaging. Unfortunately, films prepared with pure pectin did not provide satisfactory functionality due to lack of mechanical properties and water resistance. The main goal of this study is to enhance functional packaging properties of pectin film prepared by blending pectin with zinc oxide nanoparticles (ZnO-NPs). Pectin/ZnO bio-nanocomposite films were fabricated at 4 levels of ZnO-NPs, i.e., 0.5, 1.0, 2.0 and 5.0% (w/w) with the addition of glycerol (10%, w/w) as plasticizer. The effects of ZnO-NPs incorporation on improving the mechanical properties and water resistance of the films were investigated.

Zinc oxide (ZnO) nanoparticles were successfully incorporated into pectin films by preparing pectin-ZnO-NPs solution and casting method. The presence of ZnO-NPs inside pectin films was observed clearly by using SEM and confirmed with FTIR spectra analysis. The improvement in tensile strength could be achieved with ZnO-NPs incorporation without obvious loss in elasticity. Thus, the addition of glycerol as plasticizer was not an important or necessary factor for preparing pectin-ZnO nanocomposite films. Water absorption of pectin-ZnO nanocomposite films was lower than that of pure pectin film, indicating that the improvement in water resistance had been achieved. Converse effect was observed by using glycerol as plasticizer which increased water absorption of the films. Antimicrobial activity of pectin-ZnO nanocomposite films was proved in the absence of mold after exposing them at 97% RH and room temperature for 14 days, whereas the growth of mold had been observed in pectin films after 5 days of exposure. The application of pectin-ZnO nanocomposite as edible coating of strawberry could inhibit the mold decay until two weeks with the storage at 5°C, a week longer than that of control. In terms of color analysis, the significant change in film opacity was only found in the film formed by using 5% of ZnO. Results suggested that it would be favorable to prepare antimicrobial film by using ZnO-NPs at the amount of 2% (w/w) without plasticizer in the future work.

Study on acid resistance in *E.coli*

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Foodborne pathogens like *Escherichia coli* O157: H7, is the leading causes of infectious gastrointestinal diseases, and death in severe conditions. During its journey, orally acquired food pathogen has to transit through the extremely acidic gastric compartment and cope with the extreme acidic stress (pH < 2.5). Therefore, the ability to endure the combined effect of HCl in gastric juice and of short chain fatty acids produced by the intestinal microbiota, is crucial for successful colonization of the gastrointestinal tract. Different food pathogen has have evolved different strategies to overcome acid stress. The glutamate-dependent acid resistance (GDAR) system is the most potent acid resistance system. The regulation of GDAR system is remarkably complex involving multiple regulatory circuits.

The MnmE protein, a GTPase involved in a tRNA modification, has been implicated in the regulation of the GDAR system. In the present study, to understand the role of MnmE and MnmG, another tRNA modifying enzyme, in the regulation of the glutamate decarboxylase gene (*gadA*), we constructed $\Delta mnmG$ and $\Delta mnmE$ deletion mutants of *E. coli* O157: H7 and *gadA-lacZ* translational fusions in K-12 strains. In O157:H7 strains, both the *mnmG* and *mnmE* deletion mutants were defective in the GDAR mechanism and lost their acid resistance at pH 2.0. The results of acid challenge test were further confirmed by western blot analysis. It was obvious that expression of the GadA protein was completely suppressed in the *mnmG* and *mnmE* deletion mutants, confirming that these tRNA modifying genes serve as regulators for GadA expression. Gene fusion studies in K-12 strains implicated that *mnmE* and *mnmG* are not directly involved in translational regulation of GadA production. However, overexpression of the *gadE* gene, encoding an essential transcription factor for *gadA*, in the $\Delta mnmG$ and $\Delta mnmE$ mutants could not restore acid resistance ability of mutants. Therefore, to get further insight into the translational regulation of *gadA* gene, more studies are required.

Inhibitory Effect of Chinese Fermented Soypaste and Catechin-rich Foodstuffs on Renin-angiotensin System

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Hypertension is a major public health problem associating with the incidence of various cardiovascular diseases. Renin-angiotensin system (RAS) plays a crucial physiological role in regulating blood pressure of human body, and renin (EC 3.4.23.15) and angiotensin I-converting enzyme (ACE, EC 3.4.15.1) are two key enzymes for maintaining the operation of this system. The control of RAS, such as by inhibiting the activities of renin and ACE, has been well established as an effective therapy to the treatment of hypertension.

ACE inhibitory activities of aqueous extracts of Chinese commercial soypaste products were investigated in this work. Six samples from northern part of China showed potent ACE inhibitory activities with IC_{50} values less than 40.0 $\mu\text{g/ml}$. In order to identify the active components, ACE inhibitors in the sample with the strongest activity were purified by the means of ultrafiltration, solid-phase extraction and gradient RP-HPLC. According to spectroscopic methods, a compound (M328.1) was separated as $C_{15}H_{21}NO_7$. It was supposed to be a conjugate of phenylalanine and glucose generated by Maillard reaction, providing support on the contribution of Maillard reaction products to the ACE inhibitory activity of the sample. Results further indicated that the total inhibition by the soypaste sample on ACE activity occurred from the combined function of various bioactive substances, such as Maillaid reaction products, peptides and even large molecules as well.

Concerning the exploration of natural occurring renin inhibitors in foodstuffs, renin inhibitory effect of catechin-related compounds, including catechin (C), epicatechin (EC), epicatechin gallate (ECg), epigallocatechin (EGC), epigallocatechin gallate (EGCg) and gallic acid, was investigated first in this work. EGCg was found to possess the strongest activity ($IC_{50} = 44.53 \mu\text{M}$) and act in an uncompetitive manner. Gallated catechins exerted higher inhibition than the ungallated forms, indicating that the galloyl moiety might be a favorable structure for these compounds to exert renin inhibitory activity.

Renin inhibitory activities of catechin-rich tea products as well as cocoa and coffee were further evaluated. Water extracts from oolong and black tea possessed effect with IC_{50} values of 20.31 and 17.27 $\mu\text{g/ml}$, in line with the relatively higher total phenolic contents of 16.71 and 20.34 g/100 g dried weight (gallic acid equivalent), respectively. By spectroscopic methods, four compounds, i.e., theasinensin B, theasinensin C, strictinin and M412.03, were identified from black tea extract, with IC_{50} values of 19.33, 40.21, 311.09 and 50.16 μM , respectively. Whereas catechins constituted the main bioactive compounds in green tea, they didn't play an essential role in contributing to the renin inhibitory activities of tea products. Results indicated another potential pathway and provided further support on tea consumption as an approach to helping control hypertension.

This work suggested that Chinese soypaste could be a good source of ACE inhibitors and fermented tea products contained abundant renin inhibitors against renin-angiotensin system, which was favorable for developing functional foods or food ingredients with antihypertensive effects.

Optimization of one-pot enzymatic synthesis of inositol from maltoheptaose and dextrin through response surface methodology

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Inositol and its derivatives were important in signal transduction, stress response, cell wall biogenesis and other pathways in a broad spectrum of species including animals, plants, fungi and bacteria. Now the cost of inositol production is very high. To efficiently produce the inositol from abundantly available sugars, in current study, for the first time, inositol was synthesized in one-pot reaction using four hyperthermostable enzymes system from the maltoheptaose and cheap material dextrin. The four enzymes are all involved in the inositol pathway in bacteria including α -glucan phosphorylase (TM1168), inositol monophosphatase (TM1415), Inositol 1-phosphate synthase (TM1419) from hyperthermophilic bacterium *Thermotoga maritima*, phosphoglucomutase/phosphomannomutase (PGM/PMM TK1108) from *Thermococcus kodakaraensis*, all these 4 enzymes were over expressed in *Escherichia coli*. And the recombinant protein was purified to homogeneity by Ni-NTA column.

To establish the reaction system and get the optimal condition of inositol synthesis, heat stable inositol 1-phosphate synthase (IPS, TM1419) was subjected to be characterized. In a series of assays, recombinant IPS exhibited maximal activity of at pH 8, 90°C. It was quite stable after exposure to 60°C for 1 day. Under the optimal conditions, The K_m and k_{cat} values of TM1419 were 1.76 mM and 0.042 s⁻¹, respectively. The catalytic efficiency, k_{cat}/K_m , was 0.024 s⁻¹mM⁻¹, similar to that of other IPS which was among 0.12 to 0.233 s⁻¹mM⁻¹. Mg²⁺ and Mn²⁺ enhanced the activity of TM1419 significantly, indicating it belongs to class II aldolase enzymes. The reaction system was also optimized by response surface methodology with 78mM maltoheptaose. The TK1108, TM1415 and TM1419 were turned out to be the 3 main factors in this system by one order selection- the first order fractional factorial design 2⁶⁻². Central composite design (CCD) with 3 independent variables, TK1108 (x_1) and TM1419 (x_2), TM1415 (x_3) was applied to obtain the maximal inositol production. After CCD optimization, the predicted maximum inositol yield was 167 mmol/L with corresponding TK1108 0.373 mg/ml and TM1419 0.38 mg/ml, TM1415 0.175 mg/ml. The optimal one pot enzymatic reaction for synthesizing inositol predicted by the model (per milliliter) was: 50 mM PBS (pH 8.0) TK1108 0.373 mg/ml, TM1419 0.81 mg/ml, TM1415 0.172 mg/ml, TM1168 0.84 mg/ml, NAD⁺ 0.1 mM, G 1,6P 0.5 μ g/ml, Mg²⁺ 20 mM. The 155 mM inositol was obtained in the optimal condition, at the same level as predicted production. Also 115.2 mM inositol was obtained under this optimal system from 165 mg/ml cheap material Dextrin within in 8h.

FORMULATION AND CHARACTERIZATION OF MICRO/ NANODISPERSIONS ENCAPSULATING FUNCTIONAL FOOD COMPONENTS

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Functional foods have been growing rapidly in food industry. The important key of their application is to deliver functional compounds to the target in the human body. Colloidal delivery systems have been widely used in foods, pharmaceuticals and cosmetics. The primary aim of this work was to formulate micro/nanoemulsions containing baicalein and lemongrass oil which are the local products from agriculture in Thailand. Baicalein possesses multiple medicinal activities like anti-circulatory failure, anti-cancer, anti-HIV, and antioxidant, while lemongrass oil has shown a reduction of spasmodic affection and gastric irritability as well as its characteristic scent. The baicalein-loaded oil-in-water (O/W) emulsions were successfully formulated using a high-pressure homogenization method. Our results demonstrated that O/W emulsions encapsulating baicalein with a Sauter mean diameter ($d_{3,2}$) as small as 300 nm were obtained. Their $d_{3,2}$ value decreased with increasing homogenization pressure from 20 MPa to 150 MPa. Physical stability in terms of the variation of the $d_{3,2}$ value remains unchanged during 30 days of storage. Chemical stability of baicalein during storage was also investigated by HPLC. Microchannel (MC) emulsification was performed to prepare lemongrass oil-in-water emulsions. Uniformly sized lemongrass oil droplets with a number-average droplet diameter of 23 μ m and a coefficient of variation of <4% were stably generated from hydrophilically treated silicon MC arrays. Mixtures of lemongrass oil and vegetable oil at different weight ratios were also successfully used for preparing monodisperse O/W emulsions.