Encapsulation and Bio-Composite Films Prepared by Alginate Incorporated with Jamun (*Syzygium cumini*) Polyphenols

By

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Encapsulation and Bio-Composite Films Prepared by Alginate Incorporated with Jamun (*Syzygium cumini*) Polyphenols

ABSTRACT

Jamun or Jambolan (*Syzygium cumini*), which belongs to Myrtaceae family is one of the most important medically plants. The fruits, seeds, bark and leaves were reported as a traditional medicine for diarrhea, hypoglycemic and antibacterial effects. *S. cumini* consumption has different health benefits such as advantageous effects on pro-apoptotic and antiproliferative against breast cancer cells.

Additionally, its extracts were exploited in the treatment of diabetes in India as well as many other countries. Polyphenols present in the *S. cumini* plants act as useful antioxidants that obstruct the oxidative deterioration of oils and fats. Moreover, the extracts of seeds and fruits have been reported as a valuable source of antimicrobial agents against a lot of harmful bacteria and fungal strains. The natural and medical bioactive compounds like polyphenols could be grafted on biopolymers in form of encapsulation or coating techniques to enhance the control release, shelf life, stability and to provide protection against unsuitable environmental conditions. Thus, the objectives of the present studies were to optimize the extraction of the bioactive components from the seeds and fruits of *S. cumini* and testing its ability to encapsulate inside basic polymer named alginate mixed with various polymers types. On the other hand, fabrication of alginate films incorporated with *S. cumini* seeds extracts to maintain the quality and prolong the shelf life of bread slices during storage. To achieve these aims the following steps were performed;

Firstly, the optimal extraction conditions of polyphenols from *S. cumini* seeds were determined by response surface methodology (RSM). The antioxidant activity and inhibition on α-amylase and pancreatic lipase of extracted polyphenols were investigated. As results, the optimal extraction conditions in the ultrasonic extraction process which maximized total polyphenols content, minimized the IC_{50} values of α-amylase and pancreatic lipase were determined as follows: extraction time 60 min, ethanol concentration 63% and solvent/solid ratio 44 mL g^{-1}. The main phenolic compounds in partially purified fraction of *S. cumini* seeds were catechin, epicatechin, kaempferol, gallic, 5-caffeoylquinic, caffeic and ferulic

acids. In addition, the partially purified fraction inhibited 87.66 ± 5.55 and 86.61 ± 3.15% of α-amylase and pancreatic lipase, respectively. The results suggested that *Syzygium cumini* seeds could be explored as a natural antioxidant and could be used as a source of highly antidiabetic and anti-obesity bioactive compounds.

Secondly, high-speed counter-current chromatography (HSCCC) was utilized as an effective procedure for isolation of targeted three anthocyanins di-glucosides from *S. cumini* pulp by using an optimized biphasic successful combination of different solvents. The resulted fractions described by HPLC/ESI-MS to be delphinidin 3,5-diglucoside (DDG), petunidin 3,5-diglucoside (PDG) and malvidin 3,5-diglucoside (MDG). A weight of 150 mg of sample yielded 7.53, 22.68 and 39.09 mg for DDG, PDG and MDG, respectively. It was stated that the target three anthocyanins possessed strong antioxidant activities. Furthermore, MDG exhibited definite advantages for inhibition of nitric oxide release and proinflammatory mediators like IL-6, IL-1β and TNF-α in LPS-induced RAW264.7 macrophages. The results propose that HSCCC can be utilized to separate highly antioxidative and anti-inflammatory natural components from *S. cumini* pulp.

Thirdly, calcium alginate was used to encapsulate *S. cumini* seed polyphenolic extract to maintain its stability and functionality. Calcium chloride was used as a cross linking agent to get the final form of calcium alginate capsules. Different ratios from sodium alginate and calcium chloride were used to optimize encapsulation method. Two different methods for drying capsules were conducted; freeze-dried (FD.C) and vacuum dried (D.C). Encapsulation Efficiency for FD.C and D.C were 75.96 ± 0.68 and 70.20 % ± 0.59, respectively. The scanning electron microscopy (SEM) photographs and the mechanical properties indicated that the polysaccharide networks of D.C were strongly firm to prevent release of TPC in gastric phase and some delay of TPC in intestinal phase. FD.C showed fragile polysaccharide networks however, prevented the release of TPC in gastric phase and exhibited more release of TPC than D.C in intestinal phase. The microencapsulation process improved the thermal stability of the extract with 171.97 °C and 180.36 °C for FD.C and D.C, respectively.

Additionally, an efficient method based on encapsulation efficiency of *S. cumini* anthocyanin extract (ANC.E) was established by using a stable concentration from (ANC.E) and sodium alginate (SA) as a first step to interact with different concentrations from maltodextrin (MD), gum Arabic (GA) and chitosan (CH) as second carriers for the second step. Freeze-drying was used to fabricate particles from different gelling optimized blends. The encapsulation efficiency, Fourier transform-infrared spectroscopy (FT-IR), thermal

behavior, morphological structure, physical properties, ANC.E degradation and color properties during storage days greatly influenced. The optimum encapsulation efficiency 92.4% was achieved by SA (1.5% w/v) and CH (0.8% w/v) in ANC.E (278.21 mg/100mL) solution named SA.CH particles. The SA.CH particles surface were like-sheet particles and were unique in FT-IR analysis that showed new peaks and shifting in wave numbers. Additionally, the SA (1.5% w/v) and GA (0.8% w/v) in ANC.E (278.21 mg/100mL) solution named SA.GA particles besides the SA.CH particles decreased the ANC.E degradation rate to 3.57 and 3.96 %, respectively, compared with other tested particles. Moreover, they showed non-significant differences in color properties during the storage days. Therefore, the obtained results provided a potential approach to utilize ANC.E as colorants or healthy ingredients in functional food.

Finally, a novel biodegradable sodium alginate/gum Arabic (SG) films were obtained. The influence of *S. cumini* seeds extract (SCSE) incorporation in SG films on morphological structure, polymer interaction, thermal behavior, antioxidant activity and physical properties were investigated. Moreover, the fabricated films were tested as a coating material to extend the shelf life of the bread slices. Scanning Electron Microscopy micrographs showed heterogeneous and rough surface after incorporation by SCSE. Possible cross-linked interaction between alginate and gum Arabic with physical interaction between SCSE and SG films were assessed by FT-IR. Although, the addition of SCSE into SG films declined the thermal stability, elongation at break (EB), tensile strength (TS) and moisture content, films with better opacity, solubility and water vapor permeability were obtained. The SG-SCSE films showed obvious inhibitory effect on the growth of *Aspergillus niger* and *A. flavus* without non-significant differences in bread texture in comparison with fresh bread.

Generally, the findings of the current study showed that, *S. cumini* seeds as a by-product which accumulate annually with a large amount can be used to produce natural extracts that work as enzymes inhibitors for treatment of diabetic and obesity. On the other hand, it is possible to isolate compounds from *S. cumini* fruits with a high purity and good scavenging activity against free radicals to act as a standard and anti-inflammatory activity. Additionally, sodium alginate was an ideal matrix to interact with other polymers with the help of calcium ions to produce microcapsules for carrying of *S. cumini* bioactive compounds to control the release of extracts or prolong the shelf life of sensitive components like anthocyanins. Furthermore, sodium alginate in combination with gum Arabic can be utilized to produce a

novel active bio-composite films incorporated with seeds extracts that used to retard the yeasts, molds growth and staling in bread slices with preservation of its quality.

KEYWORDS: *Syzygium cumini*; Optimization; Polyphenols; Anthocyanins; Sodium alginate; Encapsulation; SG-films; Bread slices