UNIVERSITI PUTRA MALAYSIA

EFFECTS OF MIXING AND CONJUGATION PROCESS WITH PEA PROTEIN ISOLATE ON FUNCTIONAL PROPERTIES OF PECTIN IN EMULSIONS

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By

SAHAR TAMNAK

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirement for the Degree of Master of Science

August 2015

DEDICATION

I would like to dedicate my thesis to my beloved family.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement of the degree of Master of Science

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Chairman: **Associate Professor Seyed Hamed Mirhosseini, PhD**
Faculty: **Food Science and Technology**

Although pectin considered as a heteropolysaccharide gum, which is used as a gelling agent, thickening agent, and stabilizer in many food products. However, it cannot be used as a proper emulsifier due to the lack of hydrophobic moieties and thus having lower tendency to adsorb at the oil droplets surface. The main goal of this study was to improve the emulsifying activity of pectin in both single and double emulsions. A full factorial design was applied to investigate the effects of pectin to protein ratio (i.e. 1:1, 2:1, 3:1, 1:2 and 1:0 w/w) and conjugation time (i.e. 0, 6, 27 and 48 h) on the functional properties of pectin. In this study, pea protein isolate was selected for the conjugation with pectin. It was hypothesized that the mixing and/or conjugation of pectin (with the hydrophilic structure) and pea protein isolate (with the hydrophobic structure) could result in the formation of a hybrid polymer with the amphiphilic structure. It could provide stronger emulsifying properties than the native pectin or pea protein isolate only.

The most desirable conjugation condition was chosen after testing different conjugated hybrid polymers. Therefore, all mixed and conjugated hybrid polymers were subjected to various physicochemical tests. The main analytical assays were solubility, moisture content, morphology, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), apparent viscosity, emulsion stability, Z-potential and average particles size and distribution. In this study, functional properties of different mixed and conjugated polymers were compared to native pectin as a control. After one-month storage of single emulsions at 4 °C, a sample containing conjugated polymer (ratio 3:1) had the highest stability (83.33%) and smallest droplets size (D [3, 2], 0.287 μm). Further, all single emulsions stabilized with mixed and conjugated pectin-protein polymers had higher Z-potential ranging from –33.57 to –49.97 mV than the samples containing native pectin (–26.97 mV). This confirmed that both mixing and conjugation of pectin with pea protein isolate let to improve its interfacial emulsifying activity. During conjugation, the long incubation (48 h) resulted in a strong covalent linkage between pectin and pea protein isolate, thereby making a hybrid polymer with lower solubility than pectin. The results showed that the conjugated polymer (3:1), which was incubated for 48 h at 60 °C,

provided the most desirable functional properties among all single, mixed and conjugated polymers. Therefore, this compound was selected as the most desirable polymer for further comparison study.

Finally, the application of conjugated polymer (pectin: protein, 3:1) as a replacer for Tween 80 in the water-in-oil-in-water double emulsion was investigated. Therefore, the efficiency of different concentrations (i.e. 0.5%, 1% and 2% w/w) of the target emulsifiers with high hydrophilic-lipophilic balance (HLB) (i.e. Tween 80, native pectin and conjugated pectin) on the stability and characteristics of double ($W_1/O/W_2$) emulsions was tested. On the other hand, the effect of different concentrations (i.e. 2% and 5% w/w) of a low HLB emulsifier (i.e. Polyglycerol polyricinoleate, PGPR) on stability and release rate of the marker from different double ($W_1/O/W_2$) emulsions was investigated. In fact, encapsulation capability of various emulsifiers was compared by measuring release content of marker (i.e. edible dye Tartrazine) from the inner aqueous phase (W_1) to the outer aqueous phase (W_2) of the double emulsion.

The current study revealed that all single (O/W) and double ($W_1/O/W_2$) emulsions exhibited a non-Newtonian pseudoplastic flow behavior. Samples containing mixed pectin-pea protein isolate and native pectin showed the highest apparent viscosity (~6 mPa.s at a medium shear rate of 60 s^{-1}) among all prepared single emulsions. The present study showed that the double emulsions containing 2% conjugated pectin and 2% PGPR had proper encapsulation efficiency (81.26%) and encapsulation stability (37.05%). The fresh double emulsion stabilized with conjugated pectin had smaller droplets size (D [3, 2], 0.345-1.526 μm) than the other double emulsions containing native pectin (D [3, 2], 2.546-5.368 μm) and Tween 80 (D [3, 2], 1.480-1.642 μm). The present study revealed that the conjugated pectin-pea protein isolate at ratio 3:1 could be used as a proper replacer for Tween 80 in stabilizing double ($W_1/O/W_2$) emulsions. The single emulsions prepared in this study may be exploited in the food industry as a suitable replacement of traditional soybean-stabilized food emulsions, especially in the manufacture of hypoallergenic foods for people allergic to soybean proteins as well as in smoothies, infant formulas, fruit juices, yogurt drinks. The double emulsions produced in this study can have several food applications such as in the formulation of reduced fat-food products (by replacing some of the volumes of the oil droplets with entrapped water drops) and as vehicles for encapsulation and delivery of hydrophilic nutrients. For example to fortify foods with water-soluble vitamins or minerals. Besides, in order to improve the fat content of meat (in quantitative and qualitative terms) fat globules can be replaced by a double ($W_1/O/W_2$) emulsion prepared by different oils having healthy effects (as lipid phase).

Abstrak tesis yang dikemukeken kepada Senat Universiti Putra Malaysia sebegai memenuhi keperluan untuk ijazah Master Sains

KESAN PENCAMPURAN DAN PROSES DENGAN CONJUGATION PEA PROTEIN MENGASINGKAN ON HARTANAH BERFUNGSI PEKTIN IN EMULSI

Oleh

SAHAR TAMNAK

Ogos 2015

Pengerusi: **Profesor Madya Seyed Hamed Mirhosseini, PhD**
Fakulti: **Sains dan Teknologi Makanan**

Walaupun pektin dianggap sebagai getah heteropolisakarida, yang digunakan sebagai agen penggelan, agen penebalan dan penstabil dalam kebanyakkan produk makanan, namun, pektin tidak boleh digunakan sebagai pengemulsi yang betul kerana kekurangan *moieties* hidrofobik yang menyebabkan ianya mempunyai kecenderungan yang lebih rendah untuk menjerap di permukaan titisan minyak. Matlamat utama kajian ini adalah untuk meningkatkan aktiviti pengemulsian pektin dalam emulsi tunggal dan penggandaan dua. Reka bentuk faktorial penuh (Full factorial design) telah diaplikasikan untuk mengkaji kesan pektin kepada nisbah protein (iaitu 1:1, 2:1, 3:1, 1:2 dan 1:0 w/w) dan masa pengkojugatan (iaitu 0, 6, 27 dan 48 jam) terhadap sifat-sifat fungsi pektin. Dalam kajian ini, protein kacang pencilan telah dipilih untuk dikonjugatkan dengan pektin. Ini telah dihipotesiskan bahawa pencampuran dan / atau konjugatan pektin (dengan struktur hidrofilik) dan protein kacang pencilan (dengan struktur hidrofobik) boleh mengakibatkan pembentukan polimer hibrid dengan struktur amphiphilic. Ia boleh menghasilkan ciri-ciri pengemulsi yang kuat daripada pektin asli atau protein kacang pencilan sahaja.

Keadaan konjugatan yang terbaik telah dipilih selepas menguji polimer hibrid konjugatan yang berbeza. Oleh itu, semua polimer hibrid campuran dan konjugatan diuji dengan pelbagai ujian fiziokimia. Analisis utama adalah kelarutan, kandungan lembapan, morfologi, natrium sulfat dodecyl-gel elektroforesis (SDS-PAGE), kelikatan ketara, kestabilan emulsi, Z-potensi dan purata saiz zarah dan taburan. Dalam kajian ini, ciri-ciri berfungsi polimer campuran dan konjugatan berbeza dibandingkan dengan pektin asli sebagai kawalan. Selepas penyimpanan selama sebulan, emulsi tunggal pada 4 °C iaitu sampel yang mengandungi polimer konjugatan (nisbah 3:1) mempunyai kestabilan yang paling tinggi (83.33%) dan saiz titisan paling kecil (D [3, 2], 0.287 µm). Di samping itu, semua emulsi tunggal yang distabilkan dengan campuran dan konjugatan polimer pektin-protein mempunyai Z-potensi yang lebih tinggi iaitu daripada kadar -33.57 hingga -49.97 mV daripada sampel yang mengandungi pektin asli (-26.97 mV). Ini mengesahkan bahawa kedua-dua campuran dan konjugatan pektin dengan protein kacang pencilan membantu

meningkatkan aktiviti pengemulsian antara muka. Semasa pengkojugatan, pengeraman yang panjang (48 jam) menyebabkan hubungan kovalen yang kuat antara pektin dan kacang protein pencilan, seterusnya menjadikan polimer hibrid mempunyai kelarutan yang rendah berbanding pektin. Keputusan menunjukkan polimer konjugatan (3:1), yang telah dieram selama 48 jam pada suhu 60 °C, menghasilkan sifat-sifat berfungsi yang terbaik di kalangan semua polimer tunggal, campuran dan konjugatan. Oleh itu, kompoun ini telah dipilih sebagai polimer yang terbaik untuk kajian perbandingan selanjutnya.

Akhir sekali ialah aplikasi penggunaan polimer konjugatan (pektin: protein, 3:1) sebagai pengganti kepada Tween 80 dalam air-dalam-minyak-dalam-air penggandaan dua emulsi telah dikaji. Oleh itu, kecekapan kepekatan yang berbeza (iaitu 0.5%, 1% dan 2% w/w) daripada pengemulsi sasaran dengan hydrophilic-lipophilic (HLB) (iaitu Tween 80, pektin asli dan pektin konjugatan) yang tinggi terhadap kestabilan dan sifat-sifat penggandaan dua elmulsi telah diuji. Sebaliknya, kesan kepekatan yang berbeza (iaitu 2% dan 5% w/w) daripada pengemulsi HLB rendah (iaitu Polyglycerol polyricinoleate, PGPR) terhadap kestabilan dan pelepasan kadar penanda daripada pebezaan penggandaan emulsi ($W_1/O/W_2$) telah dikaji. Malah, keupayaan pengkapsulan daripada pelbagai pengemulsi telah dibandingkan dengan mengukur kandungan pembebasan penanda (iaitu pewarna makan Tartrazin) dari fasa berair (W_1) hingga fasa luar berair (W_2) daripada emulsi penggandaan dua.

Kajian ini mendapati kesemua emulsi tunggal (O/W) dan penggandaan dua ($W_1/O/W_2$) menghasilkan sifat bukan Newtonian aliran pseudoplastik. Sampel yang mengandungi campuran pencilan protein pektin-kacang dan pektin asli menunjukkan kelikatan ketara yang paling tinggi (~ 6 mPa.s pada kadar ricih medium 60 s^{-1}) di kalangan semua emulsi tunggal yang disediakan. Kajian ini menunjukkan bahawa emulsi penggandaan dua yang mengandungi 2% pektin berkonjugatan pektin dan 2% PGPR mempunyai kecekapan pengkapsulan (81.26%) dan penstabilan kapsul (37.05%) yang betul. Pengemulsian penggandaan dua yang segar yang distabilkan dengan pektin konjugatan mempunyai saiz titisan yang lebih kecil (D [3, 2], 0.345-1.526 μm) daripada emulsi penggandaan dua lain yang mengandungi pektin asli (D [3, 2], 2.546-5.368 μm) dan Tween 80 (D [3, 2], 1.480-1.642 μm). Kajian ini juga mendapati bahawa penkonjugatan pektin-protein-kacang pencilan dengan nisbah 3:1 boleh digunakan sebagai pengganti yang sesuai untuk Tween 80 dalam menstabilkan emulsi penggandaan dua ($W_1/O/W_2$) emulsi. Emulsi tunggal yang disediakan dalam kajian ini boleh dieksploitasi dalam industri makanan sebagai pengganti yang sesuai untuk emulsi makanan kacang soya stabil tradisional, khususnya dalam pembuatan makanan hypoallergenic untuk pengguna yang mempunyai alahan protein kacang soya selain di dalam smoothie, formula bayi, jus buah-buahan dan minuman yogurt. Pengemulsi penggandaan dua yang dihasilkan dalam kajian ini boleh mempunyai beberapa aplikasi makanan seperti produk makanan kurang lemak (dengan menggantikan beberapa titisan minyak dengan titisan air yang terperangkap) dan sebagai kenderaan untuk pengkapsulan dan penghantaran nutrien hidrofilik. Sebagai contoh dengan memperkayakan makanan dengan vitamin larut air atau mineral. Selain itu, sebagai usaha untuk meningkatkan kandungan lemak daging (dari segi kuantitatif dan kualitatif) globul lemak boleh digantikan dengan penggadaan dua emulsi ($W_1/O/W_2$) yang disediakan dengan pelbagai jenis minyak yang mempunyai kesan yang sihat (sebagai fasa lipid).

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This Thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the supervisory committee were as follows:

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ANOVA	Analysis of variance
COOH	Carboxyl group
DA	Degree of meyhyl acetylation
DE	Degree of meyhyl estrification
D-GalA	D-galacturonic acid
DLS	Dynamic light scattering
E_E	Encapsulation efficiency
e.g.	Exempli gratia
E_S	Encapsulation stability
GLM	General linear model
g	Gram
g	Gravitational acceleration
et al	Et alibi
h	Time (hour)
H^+	Hydrogen ion
HLB	Hydrophilic-lipophilic balance
i.e.	That is
kDa	Kilodalton
kV	kilovolt
L-Rha	L-rhamnose
M	Molar
min	Minute
mg/mL	Milligram per milliliter
mL	Milliliter
mm	Millimeter
mV	Millivolt
nm	Nanometer
OH	Hydroxyl group
O/W	Oil-in-water
O/W/O	Oil-in-water-in-oil
PGPR	Polyglycerol polyricinoleate
pH	Power of hydrogen ion
pKa	Acid dissociation constant
Ip	Isoelectric point
PPI	Pea protein isolate
RH	Relative humidity
R-SO_3	Sulfur trioxide
Ry	Recovery yield
s^{-1}	Time (per second)
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SEM	Scanning electron microscopy
UK	United Kingdom
USA	United States of America
V	Volt
Vs.	Versus
W/O	Water-in-oil

CHAPTER 1

INTRODUCTION

Hydrocolloids are large heterogeneous hydrophilic polymers from natural sources (i.e. animal, plant and microbial sources). They simply interact with water due to the presence of many hydroxyl groups in their molecular structure. Hydrocolloids have many applications in food industries as a stabilizer, emulsifier, thickener, texture modifier and coating and gelling agent (Phillips & Williams, 2000; Viebke, Al-Assaf, & Phillips, 2014). They are immensely useful in extending the shelf-life of emulsion systems. In food industry, the most well-known hydrocolloids include gum Arabic (Acacia senegal), modified starch, pectin, modified cellulose, and galactomannans (Dickinson, 2003; Garti & Reichman, 1993). The use of gums in food applications is a noteworthy work because they are low-cost, readily available, harmless and green nature. They are mainly extracted from fruit peels (e.g. citrus fruits: apples, oranges, and grapefruit) and seeds (e.g. creeping fig seeds) or grains flours (e.g. rice and maize). In the viewpoint of availability, plant sources are capable of being renewed.

Pectin is a linear polysaccharide gum from plant sources such as citrus peel (25-35% on a dry basis) and apple pomace (10-15% on a dry basis). The source and extraction condition play a significant role in the molecular structure and functional properties of pectin. Pectin composed of D-galacturonic acid linked in chains by α - (1→4) glycoside linkages. In addition to D-galacturonic acid, some other sugars like rhamnose, arabinan, galactan, or arabinogalactan are also present in the molecular structure of pectin. It is one of the most valuable by-products initially extracted from the agricultural biomass waste of food industries. Pectin has many applications in food, cosmetic and pharmaceutical products. It acts as a gelling agent, water binder, stabilizer, texture and rheology modifier, and biodegradable surfactant (Abbaszadeh, 2008). Oil-in-water (O/W) emulsions can be stabilized by using viscous polysaccharide gums (e.g. maltodextrins and pectin) (Shepherd, Robertson, & Ofman, 2000). However, they are mostly hydrophilic polymers and they do not act as suitable emulsifiers. This phenomenon is mainly because of the lack of hydrophobic zones in their molecular structure. Consequently, oil droplets will become larger time to time due to the flocculation and coalescence, thus inducing inefficient emulsifying function for most of polysaccharide gums (Huang, Kakuda, & Cui, 2001). In many cases, the concentration of viscous polysaccharides (like pectin) play a significant role in the emulsion stability (Mirhosseini, Tan, Aghlara, Hamid, Yusof, & Chern, 2008a). Pectin is mainly used to increase the viscosity of the continuous aqueous phase, thereby making very stable emulsion (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002). Consequently, a high level of energy is required for pumping and transportation of such high viscous solution containing high amount of pectin (Rao, 2014). On the other hand, the application of low amount of pectin results in flocculation and phase separation in the emulsion system (Huang et al., 2001).

The functional properties of pectin depend on the degree of methylation/esterification. In most cases, high methoxyl pectin is used as a stabilizer to retard the sedimentation and phase separation in the emulsion system. Furthermore, several factors such as pH, heat treatment, and ionic strength also considerably affect the functional characteristics of pectin (Jensen, Rolin, & Ipsen, 2010; Kim & Wicker, 2011). One of the disadvantages of pectin is that the pectin-stabilized emulsions do not show very high stability during storage (less than one or two months). It seems that the low absorption capability of pectin (to the oil droplets surface) causes such small emulsifying activity.

There is a possibility to improve the functional properties of polysaccharide gums through chemical, physical and enzymatic modifications. However, they have their own advantages and disadvantages. Conjugation process via Maillard reaction is one of the accepted green methods applied for modification of functional characteristics of protein and polysaccharide gums (Al-Hakkak & Al-Hakkak, 2010). In fact, through this environmentally-friendly reaction, the linkage is formed between the ε-amino-groups of protein and the reducing end carbonyl groups of polysaccharide gum (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012). In most cases, the newly conjugated hybrid polymer has better emulsifying activity than the individual polymers.-It must be noted that the effectiveness of conjugation process depends on many factors such as its experimental condition, type, content, and ratio of protein to polysaccharide gum.

The effect of conjugation with pea protein isolate (PPI) on functional characteristics of pectin, and the application of this conjugated hybrid polymer in oil-in-water (O/W) single and water-in-oil-in-water (W/O/W) double emulsions have not been reported yet. The main goal of the present study was to improve the emulsifying activity and functional characteristics of native pectin through conjugation with pea protein isolate (PPI). The use of synthetic emulsifiers (e.g. PGPR and Tween) is not recommended due to their side effect on human health (Bouyer et al., 2012). Furthermore, the high amounts of such synthetic surfactants are required to stabilize the double (W/O/W) emulsions. In the food industry, there are some restrictions on type and concentration of applied emulsifiers especially low molecular weight emulsifiers (e.g. PGPR and Tween 80) in producing double emulsions. Therefore, there is a huge demand for a natural suitable emulsifier in order to produce desirable double emulsion in the food systems.

In this study, the possibility of replacement of Tween 80 with the conjugated pectin in double (W/O/W) emulsion was investigated. Also, the emulsifying activity and functional properties of conjugated pea protein isolate-pectin were compared with that of native pectin. The following objectives were considered to approach the main goal:

1. To investigate the effect of different mixing and conjugation conditions on particle morphology, and emulsifying activity and other functional characteristics of pectin conjugated with pea protein isolate (PPI).

2. To examine the emulsifying activity and other functional properties of conjugated pectin as a replacer for Tween 80 in stabilizing double (W/O/W) emulsions.

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