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The highlight of this journal is to give an opportunity for those researchers with high quality research articles presented at International Conferences such as the International Symposium on Biocontrol and Biotechnology, the International Conference of Biotechnology and Environment, and the International Symposium on Mathematical, Statistical and Computer Sciences organized by various countries, mainly in ASIA, to be published in Current Applied Science and Technology.

The journal is an open access peer-reviewed and double blinded journal using Online Journal System (OJS). Previously, articles were published in print on a regular basis (two issues per year) since 2001 and since 2010 onward the articles have been published both in print and electronic forms starting from volume 10. In 2017, the journal title has been changed from *KMITL Science and Technology Journal* to *Current Applied Science and Technology* (CAST) (e-ISSN 2586-9396) to be more identifiable to the international scientific community according to the suggestion of Thai-Journal Citation Index Centre. The journal has been published online only since volume 2 (July-December, 2017). In addition, the journal has attracted researchers from other countries more than 22% according to the data. The examples of countries where researchers have chosen to publish the articles in CAST are as follows: UK., U.S.A., Australia, Japan, Nigeria, Malaysia, Iraq, Egypt, etc. As of 2017, the number of citation is 203 from 49 countries. Because of more demands on publication in CAST, the editorial board has decided to publish online original academic research and review articles three issues per year (April, August and December) from 2018 onward.

Furthermore, the advisory board and editorial board comprises honorable and wellknown members from around the world in which 50% of editorial board members are from various countries like U.K., Norway, Japan, India, China, Singapore and Egypt. Only 25% of Thai editorial board members are from the publisher organization and 25% from other publisher organizations. Most of advisory and editorial board members have high H-index according to SCOPUS.

The journal is also committed to maintaining the high level of integrity in the content published and has a Conflict of Interest policy in place. The journal uses plagiarism detection software to screen the submissions. The journal has been working closely with Thai-Journal Citation Index Centre to ensure that the journal complies with international standard of SCOPUS.

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Instructions for Authors I

The Effect of Ti-doped on the Structure of Y134 and Y257 Superconductors

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Abstract

The powder of Y134, Y257 and Ti composite was synthesized by solid state reaction. The crystal structure of the powder was investigated by using powder X-ray diffraction. The raw data of XRD was characterized for phase separation of composition. The samples consist of a superconducting phase with orthorhombic structure and Pmmm space group and a non-superconducting phase that have various structures and space groups. The Ti doped affected the increasing *c* lattice parameters and superconducting phase. The impurity phase had no effect on increasing the *c* lattice superconducting phase. The *c* lattice parameters and superconducting phase was increased following the Ti-doped.

Keywords: Y134 superconductor, Y257 superconductor, titanium-doped

1. Introduction

During the past thirty years, many researchers have intensively carried out experiments on the properties of Y123 as synthesized by Chu and co-workers [1] In 1987 with the critical temperature at 93 K. The Y123 superconducting material was one of the materials chosen for candidate application such as magnetic bearing [2], flywheel energy storage [3] and microwave devices [4], etc. The properties of this material were high-current density and high critical magnetic fields [5]. The development of this material requires a crystal structure. The Y123 has the orthorhombic perovskite structure with two CuO₂ planes and one Cu-O chain [6]. The superconductivity occurs in the CuO₂ plane, but the Cu-O chain is non-superconducting and acts as charge reservoirs as show in Figure 1 [7]. After the discovery of Y123, The Y124 [8] and Y247 [9] have a critical

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temperature of 80 K and 40 K, respectively. The critical temperature values remain in the range of Y123. In 2009, Y358 superconductors were synthesized by conventional solid state reaction by Aliabadi *et al.* [10]. The Y358 was a new Y-based high-temperature superconductor with the highest critical temperature above 100 K. In 2010, Tavana and Akhavan [11] simulated the crystal structure of Y358. The Y123 and Y358 had similar crystal structures. The Y358 has five CuO_2 planes and three Cu-O chains and the lattice parameters $a=3.888 \text{ \AA}$, $b=3.823 \text{ \AA}$ and $c=31.013 \text{ \AA}$ as show in Figure 1.

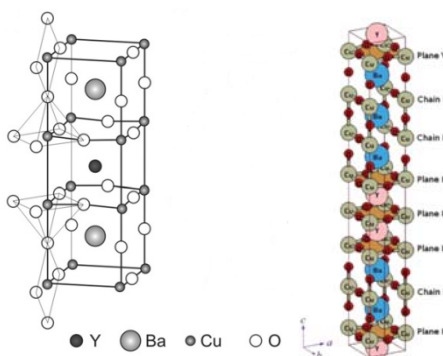


Figure 1. Crystal structures of $\text{YBa}_2\text{Cu}_3\text{O}_7$ and $\text{Y}_3\text{Ba}_5\text{Cu}_8\text{O}_{18}$

Increasing the CuO_2 planes and Cu-O chain essentially affects an increase in the critical temperature of Y-based superconductors. In 2010, Udomsamuthirun *et al.* [12] referred to the assumption that Yttrium-atoms plus Barium-atoms were equal to Cu-atoms used to synthesize the new YBaCuO superconductor by using solid state reaction. The critical temperature measurement was by standard four-probes method. The values of the critical temperature were close to Y123. In 2011, Sujinnapram *et al.* [13], characterized the crystal structure using the Reitveld refinement method [14]. Results revealed that, the lattice parameters of a and b are nearly equal in all samples, whereas the lattice parameter of c increases with Y-atoms. In 2015, Chainok *et al.* [15], synthesized the Y134 superconductor by conventional solid state reaction. The calcinations and sintering temperature was 950°C . The annealing temperature was 500°C . The lattice parameters were $a=3.80665 \text{ \AA}$, $b=3.88835 \text{ \AA}$ and $c=15.26554 \text{ \AA}$. In 2013, Kruachong [16] prepared a new Y257 superconductor by solid state reaction. The calcinations and sintering temperature was 950°C and annealing was 500°C with the lattice parameters of $a=3.81080 \text{ \AA}$, $b=3.85440 \text{ \AA}$ and $c=26.49670 \text{ \AA}$. The Y134 and Y257 were Y-based superconductors. However, the Y134 and Y257 superconductors have different Y-atoms. Y134 has one yttrium atom and Y257 has two Yttrium atoms in its formula. Both superconductors show different physical properties. However, the improved superconducting material must be substitute for the constitutional element [17]. The atom radius of element substitution should be smaller than Cu-atoms ($0.730 \text{ \AA}$). Therefore, the element used in this research was Ti with the atom radius as $0.605 \text{ \AA}$. The smaller atom radius improves the crystal structure.

This paper studies the effect of Ti doped on the crystal structure of Y134 and Y257 superconductors, The crystal structures of bulk Y134 and Y257 samples doped Ti with the various concentrations of 0, 0.05, 0.10, 0.15, 0.20, and 0.25, respectively. The space groups, lattice parameter of superconducting compound and non-superconducting compound, anisotropic parameters were carried out from Rietveld refinement method.

2. Materials and Methods

The two series of polycrystalline Y134 ($\text{YBa}_3\text{Cu}_4\text{Ti}_x\text{O}_{9-x}$) and Y257 ($\text{Y}_2\text{Ba}_5\text{Cu}_7\text{Ti}_x\text{O}_{15-x}$) with $x=0, 0.05, 0.10, 0.15, 0.20$ and 0.25 were prepared by the solid state reaction method from appropriate amounts of high-purity powder of Y_2O_3 , BaCO_3 , CuO and TiO_2 . The calcinations of mixture powder were twice operated at a temperature of 950°C for 24 hrs. The result of each series was powders. Finally, the powder was pressed into pellets and sintered at 950°C for 24 hrs, annealed at 500°C for 12 hrs in an oxygen atmosphere and cooled down to room temperature with a heating rate of $2^\circ\text{C}/\text{min}$. The pellets of the each series were then reground to powder. The structural analysis was done by Powder X-ray Diffraction method (Philips, Netherlands) with a scan range (2θ) of 5° - 90° . The X-ray source of Cu tube and X-ray generator at 40 kV and 30 mA is step time 3 sec/min with step size (2θ) at 0.05° and the wavelength of X-ray at $\lambda = 1.5406 \text{ \AA}$. The scan speed was $1^\circ/\text{min}$ at room temperature. The source of the X-ray was CuK_α radiation and Ni was a filter. The Rietveld method characterized raw data of XRD for determined space groups and the lattice parameter of composition, respectively.

3. Results and Discussion

The XRD pattern of the samples is shown in Figure 2. Pure Y134 and Y257 are represented in blue. The samples with doped Ti are shown in a different color. The spectrum of our samples was similar to the Y123 spectrum [18] at the 2θ at 32° . The impurity occurred because of the increasing of Ti concentration. The planes of superconducting the Y134 occurred at (hkl) as (0 0 3), (010) and (0 0 4) and at the 2θ at 17.414° , 22.852° and 23.289° , respectively. The (hkl) of Y257 appeared as (1 0 3), (1 1 1) and (0 0 6) and at the 2θ at 29.276° , 33.434° and 35.247° , respectively. All planes of the two samples show an orthorhombic structure.

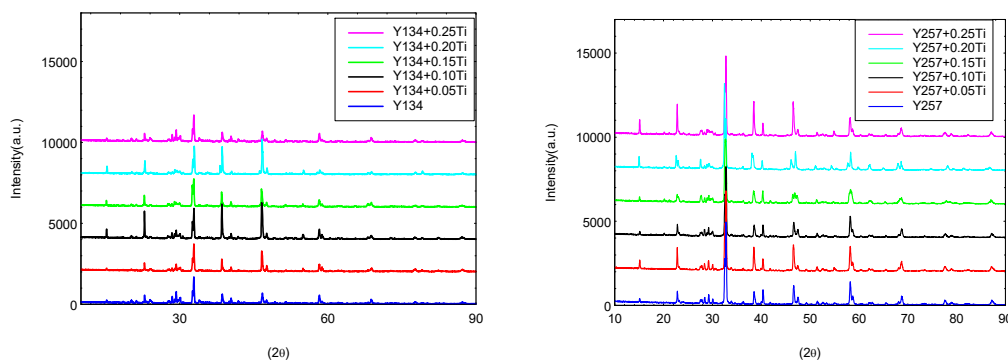


Figure 2. XRD pattern of Y134+Ti and Y257+Ti superconductors

The samples consist of two phases. The first was a superconducting phase with an orthorhombic structure and Pmmm space group symmetry and the second was a non-superconducting phase with various space groups as shown in Table 1. The Y134+0.25 and Y257+0.25 show the highest superconducting phase in Y134 and Y257. Thus, increasing the Ti concentration also increased the superconducting phase. The lattice parameters of the superconducting phase is shown in Table 2. The c lattice parameter of Y257 with Ti composite

was twice as long as Y134. Increasing the superconducting phase also causes a in longer c lattice parameter. The Y134 and Ti composite does not have the non-superconducting phase of Y211.

Table 1. The percentage of superconducting and non-superconducting compounds

Samples	Superconducting Compound	Non-superconducting Compounds		
		(Y ₂ BaCuO ₅), Pbnm	BaCuO ₂ , Im-3m	Ba ₂ Cu ₃ O ₆ , Pccm
Y134	60	-	20	20
Y257	70	30	-	-
Y134+0.05Ti	72	-	18	10
Y257+0.05Ti	76	24	-	-
Y134+0.10Ti	74	-	16	10
Y257+0.10Ti	80	20	-	-
Y134+0.15Ti	79	-	11	10
Y257+0.15Ti	85	-	10	5
Y134+0.20Ti	82	-	15	3
Y257+0.20Ti	90	-	5	5
Y134+0.25Ti	88	-	10	2
Y257+0.25Ti	95	-	5	-

Table 2. The lattice parameter of superconducting compounds

Samples	Lattice constant		
	a(Å)	b(Å)	c(Å)
Y134	3.80926	3.86889	15.13311
Y257	3.81974	3.88374	26.49670
Y134+0.05Ti	3.80924	3.86598	15.15486
Y257+0.05Ti	3.82896	3.88965	26.50630
Y134+0.10Ti	3.82172	3.88705	15.18759
Y257+0.10Ti	3.81917	3.88797	26.51320
Y134+0.15Ti	3.80939	3.88326	15.24488
Y257+0.15Ti	3.82412	3.87321	26.52132
Y134+0.20Ti	3.80918	3.88134	15.31448
Y257+0.20Ti	3.82432	3.88542	26.53212
Y134+0.25Ti	3.82451	3.88123	15.35231
Y257+0.25Ti	3.81596	3.86231	26.54321

In this study the physical properties and crystal structure of the high-temperature superconductor were investigated the impurity phase was present and important for analysis of the experiment data. The impurity phase was related to precursors of the materials. However, generally the impurities phase occurred during the heat treatment. Table 3 shows each of the impurities in the samples in 3 categories. The first impurity was Y211 (Y₂BaCuO₅). The Y211 influenced the microstructure of the superconducting properties of the samples. It is well known that the samples with a Y211 inclusion raised the critical current density (J_c) [19] and critical magnetic field [20]. However, Y211 inclusion exceeded 40 mol% [21] in the samples [22]. Additionally, the Y211 had a positive effect on the magnetic properties as a good pinning center [23]. One phase of the impurities appeared as a result of the samples in BaCuO₂ where the physical properties have not been often reported. The appearance of BaCuO₂ is highly

inhomogeneous to the surface of the samples. The oxygen content of BaCuO_2 varies between 1.8-2.5 in the chemical formula [24]. The variation of the oxygen content of BaCuO_2 changes following the Cu^{2+} [25] ion in the crystal structure. Small amounts of $\text{Ba}_2\text{Cu}_3\text{O}_6$ in the last phase of the impurity can be detected. The $\text{Ba}_2\text{Cu}_3\text{O}_6$ and BaCuO_2 that appear in the samples showed a defective crystal structure.

Table 3. The lattice parameter of non-superconducting compounds

Samples	Non-Superconducting Compounds								
	Y_2BaCuO_5 , Pbnm			BaCuO_2 , Im-3m			$\text{Ba}_2\text{Cu}_3\text{O}_6$, Pccm		
	a(Å)	b(Å)	c(Å)	a(Å)	b(Å)	c(Å)	a(Å)	b(Å)	c(Å)
Y134	-	-	-	18.31372	18.31372	18.31372	13.05722	20.63098	11.39764
Y257	7.25231	12.23212	5.54890	-	-	-	-	-	-
Y134+0.05Ti	-	-	-	18.30035	18.30035	18.30035	13.02788	20.63243	11.39855
Y257+0.05Ti	7.21762	12.07321	5.63210	-	-	-	-	-	-
Y134+0.10Ti	-	-	-	18.29048	18.29048	18.29048	13.00549	20.60827	11.37064
Y257+0.10Ti	7.24030	12.15212	5.65223	-	-	-	-	-	-
Y134+0.15Ti	-	-	-	18.29277	18.29277	18.29277	13.00968	20.61089	11.37079
Y257+0.15Ti	-	-	-	18.27230	18.27230	18.27230	13.02557	20.64878	11.39420
Y134+0.20Ti	-	-	-	18.35385	18.35385	18.35385	13.07883	20.68951	11.44247
Y257+0.20Ti	-	-	-	18.29818	18.29818	18.29818	13.01123	20.67820	11.48885
Y134+0.25Ti	-	-	-	18.23412	18.23412	18.23412	13.05234	20.68235	11.45685
Y257+0.25Ti	-	-	-	18.31958	18.31958	18.31958	13.02392	20.67834	11.40198

Y134 and Y257 with Ti composites were synthesized by using solid state reaction and used the temperature to powder calcined and sintered at 950 °C. The powders samples were investigated to study the crystal structure by using powder X-ray Diffraction. The characterized and lattice parameters of phases composition used the Reitveld refinement method. The samples divided into two phases, The superconducting phase had an orthorhombic structure and Pmmm symmetry. The non-superconducting phase had 3 categories. The Y211 had the orthorhombic structure with Pbnm space group. The BaCuO_2 had the cubic structure with Im-3m space group. The final phase of the non-superconducting sample was $\text{Ba}_2\text{Cu}_3\text{O}_6$ had an orthorhombic structure with Pccm space group. The effect of the Ti doped on the Y134 and Y257 showed a more superconducting-phase and longer c lattice parameter. The Y134 and Y134 with Ti composite does not have the impurity of Y211.

4. Conclusions

Y134 and Y257 with Ti composites were synthesized by using solid state reaction and used the temperature to powder calcined and sintered at 950°C. The powders samples were investigated to study the crystal structure by using powder X-ray Diffraction. The characterized and lattice parameters of phases composition used the Reitveld refinement method. The samples divided into two phases, The superconducting phase had on orthorhombic structure and Pmmm symmetry. The non-superconducting phase had 3 categories. The Y211 has an orthorhombic structure with Pbnm space group. The BaCuO_2 has a cubic structure with Im-3m space group. The final phase of the non-superconducting phase was $\text{Ba}_2\text{Cu}_3\text{O}_6$ which had an orthorhombic structure with Pbnm

space group. The BaCuO_2 has a cubic structure with Im-3m space group. The final phase of the non-superconducting phase was $\text{Ba}_2\text{Cu}_3\text{O}_6$ which had an orthorhombic structure with Pccm space group. The effect of the Ti doped on Y134 and Y257 showed the more superconducting-phase and the longer c lattice parameter, While Y134 and Y134 with Ti composite did not have the impurity of Y211.

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Relationship among Starch Digestibility, Antioxidant, and Physicochemical Properties of Several Rice Varieties using Principal Component Analysis

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Abstract

Seven rice samples were investigated for *in vitro* starch digestibility, total phenolic content (TPC), total anthocyanin content (TAC), antioxidant activities and physicochemical properties such as moisture, color, total starch (TS), digested starch (DS) and resistance starch (RS) contents. The relationship between these parameters and the samples was investigated by principal component analysis (PCA). All of the samples exhibited predicted glycemic index (pGI) 47.87 – 52.22, TS 65.69 – 80.60 g/100 g d.b., and DS 65.69 – 80.22 g/100 g d.b., ($p < 0.05$). RS content was generally lower than 1 g/100 g d.b. The TPC and TAC varied in the range of 0.15 – 2.25 mg gallic acid equivalent (GAE)/g and 0.51 – 68.70 mg cyanidin -3- glucoside (CG)/g, respectively ($p < 0.05$). The PC1 (50.51%) explained the digestion parameters with reference to polished-waxy rice (KorKhor 6 and Sanpatong) and low-amylose milled rice (KhaoDawkMali 105), which is opposite to yellowness and RS which refer to unpolished rice (Homnil, brown DawkMali, and red Jasmine). The PC2 (40.89%) explained the antioxidant properties with reference to unpolished-black rice (KumDoiSaket), which is opposite to lightness which refer to polished-waxy rice and low-amylose milled rice. The PCA plot clearly differentiated the dissimilarity between PC1 and PC2. The high RS and TPC improve strong antioxidant property and low starch digestion. It can be used a combination of these parameters to classify rice properties better than one individual parameter.

Keywords: rice varieties, starch digestibility, antioxidant properties, physicochemical properties, principal component analysis

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1. Introduction

Rice, being one of the primary dietary sources of carbohydrates, is a staple food for over half of the world's population [1–3]. It is a good source of thiamine, riboflavin, niacin, and dietary fiber [4]. The world's rice is produced and consumed in Asia, North and South America and Africa with thousands of varieties [4–5]. Specialty rice varieties, such as aromatic rice (Thai Jasmine rice), local rice varieties of different pigments (red/purple/ black) and glutinous rice (very low amylose content and superior processing quality), are recently being increasingly cultivated and consumed with the growing of its health benefits [5–6]. Several bioactive compounds are present in rice, such as anthocyanins: cyanidin-3-glucoside and peonidin-3-glucoside, anthocyanidin, quercetin, tocopherol, and phenolic acids: ferulic acid, 4-hydroxycinnamic acid, 4,7-dihydroxy vanillic acid, protocatechuic acid methyl ester, syringaldehyde, vanillin [7–9]. These compounds have shown antioxidant properties [10] and aldose reductase inhibitory activity [7], which are beneficial in oxidative stress and diabetes prevention, respectively.

The GI has been recommended as a helpful guideline as regards food choices, especially starchy food [11] because it has been reported that low GI foods improve blood glucose control [12–13] and increase insulin sensitivity [14]. Miller *et al.* [15] reported that the GI values of foods are grouped into low GI (< 55), medium GI (55–70), and high GI (> 70). Rice is known to have a relatively high glycemic response compared to other starchy foods [1]. Rice has been given a wide range of GI values, ranging from 54 to 121, with white bread as the reference [15]. The consumption of rice and diets with a high GI are associated with an increased risk of developing type 2 diabetes as the report of Indrasari *et al.* [13] and Hu *et al.* [16]. A better understanding on starch digestibility of rice grain varieties is important for diet-related health complications, especially type 2 diabetes. Differences in GI value of rice have been ascribed to various factor including physicochemical properties and food processing. Amylose content had an impact on the estimated GI value and resistant starch (RS) content [1, 17–19]. As the research of Hu *et al.* [17], they reported that the high amylose rice cultivar was obviously higher in RS content and lower in GI value. There is no published data available to help with understanding the effects of either RS or antioxidant capacity on starch digestibility of rice at this present. The aim of this work was to investigate the beneficial rice varieties which have high potential in resistant starch and antioxidant activity but low starch digestibility. This study covered these aspects in terms of the physicochemical properties and the antioxidant activities of seven different rice varieties. Starch digestibility as a digestogram and as mathematical model parameters was also described. The present study will consequently help to guideline the consumption of rice by increasing consumer awareness of the health benefits of rice grains, especially type 2 diabetes population. In this respect, it will be recommended for the selected properties in food industry application.

2. Materials and Methods

2.1 Rice preparation

Rice was harvested in 2013 and provided by Chiang Mai Rice Research Center, Chiang Mai, Thailand. Selected rice varieties used in this study are shown in Table 1. Rough rice grains were dehusked (C.L.P. Engineering Co., Ltd., Thailand), milled with an Ultra Centrifugal Mill (ZM200, Retsch, Germany) to achieve uniform powder (100–300 µm), vacuum packed in aluminum bag, and stored at –18°C until use.

Table 1. Selected rice samples used in this study

Cultivar	Process	Rice variety	Amylose content	Color	Abbreviation
<i>Oryza sativa</i> L.	Milled	KhaoDawkMali 105	15-17%	White	KDML
	Un-milled	DawkMali	18-20%	Brown	BHML
		Jasmine	17-22%	Red	RHML
		Homnil	19-25%	Purple	HN
<i>Oryza sativa</i> var. glutinosa	Milled	KorKhor 6	4-7%	White	RD6
	Un-milled	Sanpatong	5-8%	White	GST
		KumDoiSaket	2.5-5%	Black	KDSK

2.2 Physicochemical properties

Moisture content (%) of the rice sample was investigated by using a hot air oven (Binder R3 Controller, Binder GmbH, Germany) at 105°C [20]. Color quality was investigated on the rice sample by using a color measurement (Hunter Lab Color Quest XE, Color Global Co., Ltd, USA). The color data of ground rice sample were expressed as tri-stimulus parameters: L*, a* and b*. L* values indicate lightness (100=white and 0=black), while a* and b* values indicate redness–greenness and yellowness–blueness, respectively [21].

Total starch (TS), resistant starch (RS), and digested starch (DS) contents of the rice powder sample were determined using a glucose oxidase kit (K-RSTAR 08/11) from Megazyme (Megazyme International, Ireland) by the modified method of Englyst *et al.* [22], which is based on controlled enzymatic hydrolysis of starch and measurement of released glucose. RS refers to the starch that is not digested following 16 h of exposure to enzymes (pancreatic α -amylase and amyloglucosidase) at 37°C. The absorbance was measured at a wavelength of 510 nm using a Thermo Spectronic Biomate 5 UV/Vis spectrophotometer (Scintec Instruments, USA), and the glucose concentration was converted into starch content by applying the factor 0.9. The TS content in g/100 g d.b. was calculated by estimating the composition of DS and RS.

2.3 Starch digestion analysis

The time-course starch digestion was determined using a rapid *in vitro* digestibility assay based on glucometry [23]. Ground sample (0.5 g) was treated with artificial saliva containing porcine α -amylase (250 U/mL, E.C.3.2.1.1.) before the addition of pepsin (1 mg/mL, E.C.3.4.23.1.), and incubated at 37°C for 30 min in a reciprocating water bath (Mettler, Germany) at 85 rpm. The digesta was neutralized with 0.02 M NaOH before adjusting the pH to 6.0 (sodium acetate buffer) prior to the addition of pancreatin (2 mg/mL, Sigma-P1750) and amyloglucosidase (28 U/mL, E.C. 3.2.1.3.). The mixture was incubated for 3 h, during which the glucose concentration in the digesta was measured with an Accu-Check® Performa® glucometer at specific periods (0, 30, 60, 90, 120, 150, 180 min). The digested starch (DS) as g/100 g d.b. starch was calculated as, $(0.9 \times G_G \times Mw \times V) / (W \times S \times [100 - M])$, where G_G =glucometer reading (mM/L), V =volume of digesta (mL), Mw =molecular weight of glucose (180 g/mol), W =weight of sample (g), S =starch content of sample (g/100 g sample), M =moisture content of sample (g/100 g sample), and 0.9=stoichiometric constant for starch from the glucose content.

The digestogram (digested starch at a specific time period) of each sample was modeled using a modified first-order kinetic model as discussed in the method of Mahasukhonthachat *et al.* [24]. From the report of Goni *et al.* [25], single-point measurement of starch digestion at 90 min in the sample (H90) was also used to calculate the predicted glycemic index (pGI) as, $39.21 + (0.803 \times H90)$.

2.4 Total phenolic content (TPC)

Rice extract was prepared by the procedure of Abru *et al.* [26] in the following steps. Ground rice (10 g) and distilled water (200 mL) were mixed and heated at 100°C for 15 min. The rice solution was then separated by a centrifuge at 1,500 rpm for 10 min before antioxidant analysis. The total phenolic content was measured using the Folin–Ciocalteu method with slight modifications [9] where gallic acid (GA) (in the concentration range of 0–100 mg/mL) was used as the standard. A volume of 200 μ L of the sample solution or gallic acid standard (0–100 mg/L) was added into a test tube with 1.0 mL of diluted 10-fold Folin–Ciocalteu's reagent, and it was mixed thoroughly. The mixture was allowed to stand at room temperature for 5 min. Then, 800 μ L of 7.5% (w/v) Na_2CO_3 was added to the mixture and mixed gently. After leaving the sample at room temperature in darkness condition for 30 min for the stabilization of the blue color formed, the absorbance was measured by a UV/Vis spectrophotometer at 765 nm. The absorbance of each reference standard was plotted as a linear graph and the calculation was carried out to obtain a linear equation. The content of phenolic acid, evaluated using the obtained linear equation, was expressed as mg gallic acid equivalent (GAE)/ g of dried sample.

2.5 Total anthocyanin content (TAC)

The anthocyanin content was determined by the pH differential method [8]. The absorbance was measured at 500 nm and 700 nm for the elimination of interference from the background. The rice extract which was diluted with the potassium chloride buffer, pH 1.0, was left at rest for 15 min before measurement, whereas the rice extract diluting with the sodium acetate buffer pH 4.5 was measured after 5 min. The corresponding pure buffer was used as the reference sample in the spectrophotometer. The absorbance shift (ΔA) was formulated as, $(A_{500} - A_{700})_{\text{pH } 1.0} - (A_{500} - A_{700})_{\text{pH } 4.5}$. The total anthocyanin content (TAC) in mg of cyanidin-3-glucoside (CG)/g of dried sample was calculated as, $(\Delta A \times \text{Mw} \times \text{Df} \times 10^3) / (\text{Me} \times \text{L})$, where Mw=molecular weight of cyanidin-3-glucoside (449.2 g/mol), Df = dilution factor (10), Me = molar extinction coefficient of cyanidin-3-glucoside (29,600 L/mol cm), and L=width of cuvette (1 cm).

2.6 Antioxidant activities

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity: This activity was measured spectrophotometrically according to the DPPH assay [27]. The volume of 60 μ M of the DPPH solution in ethanol was prepared fresh daily. The DPPH radical stock solution (2000 μ L) was added to 200 μ L of the rice extract. The mixture was placed in a dark room (30°C) for 30 min and monitored at 517 nm of a UV/Vis spectrophotometer.

ABTS (2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) free radical scavenging activity: This activity was assessed by the method of Re *et al.* [28] with slight modifications. The ABTS radical cation (ABTS^+) was generated by making 1 portion of ABTS stock aqueous solution (7 mM) react with 1 portion of $\text{K}_2\text{S}_2\text{O}_8$ (2.45 mM) after incubation in darkness at room temperature for 12–16 h. The freshly prepared ABTS^+ solution was diluted with distilled water to obtain absorbance of 0.70 ± 0.05 verified by a spectrophotometer at 734 nm before use. The ABTS^+ solution (2,000 μ L) was added into the sample solution (200 μ L). The absorbance reading at 734 nm was recorded exactly 6 min after initial mixing and standing in darkness at room temperature using a UV/Vis spectrophotometer.

The decreasing in the absorbance of sample solution after incubation was measured and computed in the %inhibition of DPPH and ABTS radical scavenging activity as the following

equation, $[(AB-AS)/AB] \times 100$, where AB=absorbance of blank (no rice extract) and AS=absorbance of sample. Distilled water was used as a blank.

2.7 Statistical analysis

The results are presented as mean $\pm$ standard deviation from three replicate. The analysis of variance (ANOVA), significant differences among means using Duncan's multiple range test (DMRT), and the principal component analysis (PCA) were performed using SPSS (Version 16, SPSS Inc., Chicago, USA) at a 95% significance level.

3. Results and discussion

3.1 Physicochemical properties

As demonstrated in Table 2, the moisture contents of the seven rice samples were in the range of 9.35%–10.52% ($p < 0.05$). There were significant differences in the moisture content, L^* , a^* and b^* values ($p < 0.05$). The L^* value of the rice samples, which expresses as the brightness, varied between 26.40 and 52.82. The pigmented rice samples, KDSK, HN and RHML, exhibited much lower L^* values than the non-pigmented rice. The positive a^* values of redness were also high for all of the three rice varieties (1.58–4.67), $p < 0.05$. The b^* value of yellowness was the highest for KDSK. As far as this result is concerned, the different rice colors were dark purple, light purple, and red brown, respectively. The color of pigmented rice could be derived from the mixture of anthocyanins, which are located on a rice seed coat or aleurone layer [3, 7, 26]. The difference in color could depend on the form of anthocyanin content and rice genotype [7, 20]. As for the non-pigmented rice varieties, KDML and GST, they gave higher L^* values, nearer to BHML and RD6. The negative a^* values (redness) of KDML, GST and RD6 were also very low (–0.13 to –0.07), $p \geq 0.05$. The white rice genotypes had no the presence of proanthocyanidin which were typically observed in the red kernels [8].

The starch content is given in Table 3. The TS content ranged between 65.98% and 80.60% d.b. with slightly significant differences, except for KDSK, which stood out with a lower TS content (65.98% d.b.), $p < 0.05$. Generally, the RS content was very low (lower than 1% d.b.). As this result, the DS content was high in all of the rice samples and varied from 65.69% to 80.22% d.b., $p < 0.05$. In agreement with the study of Frei *et al.* [1] and Juliano [5], they described that TS and DS contents were high in all rice varieties. As far as this results are concerned, the RS content of the three waxy rice varieties (RD6, GST, and KDSK) which refer to glutinous and low-amylose rice varieties, were significantly lower than the RS content of the non-waxy rice varieties, except KDML 105 ($p < 0.05$). It is possible due to the compact linear structure of high- and intermediate-amylose in non-waxy rice was more resistant to starch digestion than the molecule of amylopectin in waxy rice [17, 29].

Table 2. Moisture content and color of different rice samples

Rice sample	MC (%)	L* value	a* value	b* value
KDML	9.46 ± 0.06 ^c	51.01 ± 1.43 ^a	-0.07 ± 0.02 ^c	4.36 ± 0.21 ^{bc}
BHML	10.44 ± 0.11 ^{ab}	48.20 ± 0.74 ^b	0.41 ± 0.03 ^d	7.92 ± 0.46 ^a
RHML	9.65 ± 0.11 ^{bc}	37.28 ± 0.83 ^d	4.67 ± 0.11 ^a	7.69 ± 0.11 ^a
HN	9.36 ± 0.16 ^c	30.46 ± 1.26 ^c	1.58 ± 0.07 ^c	4.34 ± 0.03 ^{bc}
RD6	10.52 ± 1.16 ^a	45.86 ± 1.49 ^c	-0.13 ± 0.03 ^c	4.13 ± 0.22 ^c
GST	9.61 ± 0.24 ^{bc}	52.82 ± 1.10 ^a	-0.11 ± 0.01 ^c	4.62 ± 0.14 ^b
KDSK	9.35 ± 0.17 ^c	26.40 ± 0.27 ^f	3.14 ± 0.04 ^b	2.28 ± 0.02 ^d

^{a-f} Values with different letters in the same column are significantly different (p<0.05).

Table 3. Total starch, digestive starch, and resistant starch content (g/100 g d.b.) of different rice samples

Rice sample	Total starch	Digestive starch	Resistant starch
KDML	80.60 ± 1.92 ^a	80.22 ± 1.96 ^a	0.38 ± 0.04 ^b
BHML	80.41 ± 0.59 ^a	79.64 ± 0.58 ^a	0.77 ± 0.01 ^a
RHML	79.19 ± 1.62 ^{ab}	78.39 ± 1.68 ^{ab}	0.80 ± 0.07 ^a
HN	76.59 ± 1.46 ^{ab}	75.81 ± 1.44 ^{ab}	0.78 ± 0.03 ^a
RD6	75.73 ± 1.27 ^b	75.14 ± 1.27 ^b	0.39 ± 0.00 ^b
GST	77.98 ± 3.11 ^{ab}	77.61 ± 3.13 ^{ab}	0.37 ± 0.02 ^b
KDSK	65.98 ± 0.48 ^c	65.69 ± 0.47 ^c	0.29 ± 0.01 ^c

^{a-b} Values with different letters in the same column are significantly different (p<0.05).

3.2 Starch digestibility

Seven rice samples were digested for different hydrolysis periods of time (0–180 min), and the glucometer was able to differentiate between the digestogram of the different samples. Figure 1 shows that all of the three glutinous rice varieties (KDSK, GST, RD6) and the milled white rice (KDML) had higher starch digestibility than the colored and non-waxy rice varieties (BHML, HN, RHML). The starch digestibility of the unmilled purple rice (HN) was the lowest in comparison to the other rice varieties. The finding also shows that the starch digestibility values of the unmilled red rice (RHML) and the unmilled rice (BHML) presented themselves into the second and third order, respectively.

The estimated parameters, D_0 , D_t , and k , from the starch hydrolysis model, H90 and pGI , are shown in Table 4. The results indicate substantial differences between the rice samples in all of the parameters. Our comparison of the results of the seven rice samples indicates that their pGI decreased in the following order: KDSK > RD6 > GST > KDML > BHML > RHML > HN. The pGI value of all the seven rice grains varied in the range of 47.87–52.22. All of the seven rice grains could be grouped into the category of low GI food material [19]. As this result, it can be concluded that the glutinous rice which is a low-amylose rice had higher pGI than the non-glutinous rice which is an intermediate-amylose rice. This was consistent with previous studies on rices [1, 17] and rice flours [19, 30]. The digestion rate (k) of the non-waxy and intermediate-amylose colored rice varieties (BHML, RHML, HN) presented the lowest value (0.013 min⁻¹) in comparison with the low-amylose rice and waxy rice varieties (RD6, 0.015 min⁻¹; GST, 0.015 min⁻¹; KDSK, 0.013 min⁻¹) and KDML (0.014 min⁻¹). The comparative k value of the selected rice samples within the group of the waxy rice, KDSK which was a colored waxy rice, showed lower k values than RD6 and GST which were a milled white waxy rice. In similar results, the k value of the rice samples within the

group of the non-waxy rice, the unpolished rice varieties (BHML, RHML, HN) exhibited lower k values than the milled white rice (KDML). The low pGI value of rice grains is mainly contributed by the high RS value and amylose content present in them [1, 17, 19, 30]. However, the RS content is not the only factor that affects starch digestibility. Many studies have reported that the starch digestibility of rice and rice flour are influenced by the genetic cultivar [17], the starch granule morphology [19], including other components such as the formation complexes between amylose and lipid [1], protein [23], and phytochemical compounds [31].

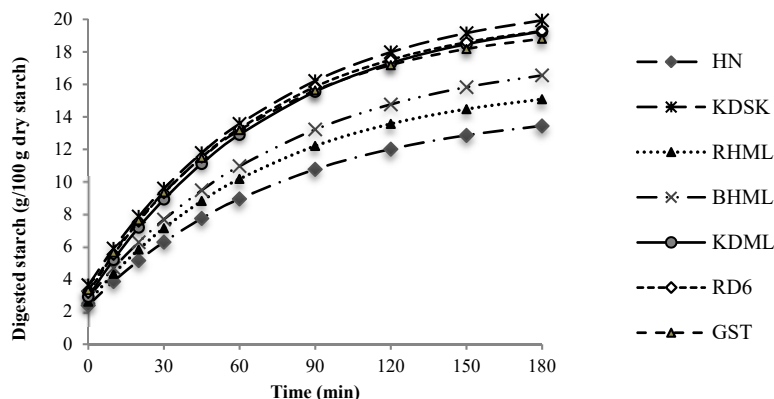


Figure 1. The starch digestograms of different rice sample. KDSK, KumDoiSaket; HN, Homnil; RHML, red Jasmine; BHML, brown DawkMali; KDML, KhaoDawkMali 105; RD6, glutinous rice KorKhor 6; GST, Sanpatong

Table 4. Model parameters and predicted glycemic index of different rice samples

Rice sample	D_0	D_t	k (min^{-1})	H90	HI	pGI
KDML	29.30	178.60	0.014	15.54	19.24	51.69
BHML	28.90	152.50	0.013	13.23	16.56	49.84
RHML	26.20	137.00	0.013	12.22	15.09	49.02
HN	24.00	123.00	0.013	10.78	13.45	47.87
RD6	31.80	173.80	0.015	15.84	19.28	51.93
GST	33.30	166.00	0.015	15.62	18.81	51.75
KDSK	36.40	178.70	0.014	16.21	19.94	52.22

D_0 = the digested starch at starting time (g/100 g d.b.), D_t = the digestion at infinite time (g/100 g d.b.), k = the rate constant (min^{-1}), H90 and HI (hydrolysis index) are the mean predicted digestibility starch at 90 and 180 min, respectively, using the modified first-order kinetic model of Mahasukhonthachat *et al.* [24], and the pGI using the equation of Goni *et al.* [25].

3.3 Antioxidant property

The phytochemicals, namely total phenolic content and anthocyanin content, that were studied in seven rice samples are reported in Table 5. The results showed that milled white rice and low-amylose rice varieties (KDML, RD6, GST) contained significant lower amounts of TPC (0.15–0.18 mg GAE/g) as compared to unmilled rice and colored rice varieties, BHML (0.56 mg GAE/g); RHML (1.51 mg GAE/g); and purple rice, HN and KDSK (1.81–2.25 mg GAE/g); $p < 0.05$. In similar results, TAC was found to be significantly high in purple rice (KDSK, HN, 10.83–68.70 mg CG/g)

as compared to red rice (RHML, 2.43 mg CG/g), brown rice (BHML, 0.91 mg CG/g) and milled white rice (KDML, RD6, GST, 0.51–0.71 mg CG/g); $p < 0.05$. From this result, it is evident that the group of the purple rice group had higher polyphenolic and anthocyanin content than the red, brown, and white rice groups, respectively, in agreement with the study of Pongjanta *et al.* [29]. It can be noticed that TAC had higher values in the pigmented rice than in the white rice [3, 8, 29, 31]. This is related to their color parameter, especially the a^* and b^* values [3, 20, 26]. The high amount of anthocyanin content has been found in dark purple rice, with the possible phytochemicals identified as cyanidin-3-glucoside and peonidin-3-glucoside [7, 9]. Moreover, TPC of the unpolished rice varieties had higher than those of the polished rice varieties. Previous works evidenced that phenolic compounds present in the kernel of rice [8].

The antioxidant activity of the seven rice water extracts was determined by DPPH $^{\cdot}$ and ABTS $^{+}$ assays, as presented in Table 5. There were significant differences in their antioxidant activity (in both the DPPH $^{\cdot}$ and the ABTS $^{+}$ assays); $p < 0.05$. The white rice water extracts (KDML, RD6, GST) had DPPH radical inhibition activity in the range of 1.40–5.76%, and the values were lower than those of the brown-colored rice (BHML, 23.32%), the red-colored rice (RHML, 49.54%), and the purple-colored rice (61.44–86.05%); $p < 0.05$, respectively. Similar results were observed in the ABTS $^{+}$ radical inhibition activity: the pigmented rice water extracts (KDSK, HN, RHML) had higher ABTS $^{+}$ radical inhibition activity than those of the brown rice and the white rice ($p < 0.05$). The first three high values of the rice water extracts were from the KDSK, HN and RHML varieties, and the values were 96.91%, 94.63% and 83.31%, respectively.

Table 5. Antioxidant property of different rice samples

Rice sample	TPC (mg GAE/g)	TAC (mg CG/g)	%Antioxidant activity*	
			DPPH	ABTS
KDML	0.18 $\pm$ 0.01 ^e	0.71 $\pm$ 0.18 ^d	1.40 $\pm$ 0.61 ^e	17.60 $\pm$ 1.28 ^d
BHML	0.56 $\pm$ 0.01 ^d	0.91 $\pm$ 0.06 ^d	23.32 $\pm$ 3.83 ^d	46.55 $\pm$ 1.45 ^c
RHML	1.51 $\pm$ 0.03 ^c	2.43 $\pm$ 0.05 ^c	49.54 $\pm$ 1.63 ^c	83.31 $\pm$ 3.83 ^b
HN	1.81 $\pm$ 0.05 ^b	10.83 $\pm$ 0.63 ^b	61.44 $\pm$ 4.46 ^b	94.63 $\pm$ 0.65 ^a
RD6	0.15 $\pm$ 0.01 ^e	0.51 $\pm$ 0.18 ^d	5.76 $\pm$ 2.26 ^e	18.55 $\pm$ 0.78 ^d
GST	0.17 $\pm$ 0.01 ^e	0.71 $\pm$ 0.18 ^d	2.64 $\pm$ 0.25 ^e	20.72 $\pm$ 1.97 ^d
KDSK	2.25 $\pm$ 0.02 ^a	68.70 $\pm$ 1.53 ^a	86.05 $\pm$ 0.61 ^a	96.91 $\pm$ 0.13 ^a

*It was performed at a concentration of 0.05 mg/mL of rice solution.

^{a-e}Values with different letters in the same column are significantly different ($p < 0.05$).

It is known from previous studies that pigmented rice varieties and grains are a good source of antioxidant compounds associated with pigmentation in rice and grains, and show extremely strong antioxidant capacity [8–9]. This was consistent with the studies on pigmented rice varieties [9, 20, 26, 31]. Rice is well known to contain phenolic and flavonoid compounds, especially the mixture of anthocyanins which have been found as a major active component for antioxidant property in rice [7]. Rice phenol compounds exist in free, esterified, and insoluble bound forms which can be released by water, base, acid, or enzymatic treatments prior to extraction, leading to higher phenolic contents, thus occurring in antioxidant activity [3, 8]. This finding summarizes that the pigmented and unmilled rice varieties were the most effective in anti-oxidative reactions, confirming in the pigmented rice had higher the DPPH $^{\cdot}$ and ABTS $^{+}$ scavenging activities than the milled white rice.

3.4 Principal component analysis (PCA)

PCA was applied to the data set of seven different rice samples after standardization (the mean of values for each variable is subtracted from each variable value and the result is divided by the standard deviation of values for each variable). After standardization, each parameter contributes equally to the data set variance and carries equal weight in the principal component (PC) calculation. The PCA plot of the seven rice samples based on their physicochemical properties, antioxidant capacity, and starch digestibility is presented in Figure 2. The sample score plot for PC1 vs PC2 is shown with 91.40% of the total variance explained. PC1 explained 50.51% of the total variance in the data set while PC2 explained 40.89% of the total variance. PC1 is generally better correlated with the variables than PC2. This is to be expected because PCs are extracted successively, with each one accounting for as much of the remaining variance as possible. Based on the relationships between their properties as studied in the present work, PC1 could be used to group the starch digestion parameters (D_0 , D_t , k , H90, HI, pGI) which are negatively related to the RS content with a highly significant correlation (with r ranging from -0.942 to -0.857). PC2 could be used to combine the antioxidant capacity, DPPH, ABTS, TPC and TAC, with a^* value which are opposite to the L^* value and the TS and DS contents. TPC, a^* value and antioxidant activity (both DPPH and ABTS) are clustered together on the top left-hand side of the loading plot ($r=0.809-0.993$). The TAC values correlate significantly negatively with the L^* and the b^* values based on their Pearson correlation coefficients ($r=-0.738$ and -0.617 , respectively). At the same time, TS and DS occupied a unique location at the very low position of the loading plot, with a strong positive correlation ($r=0.999$).

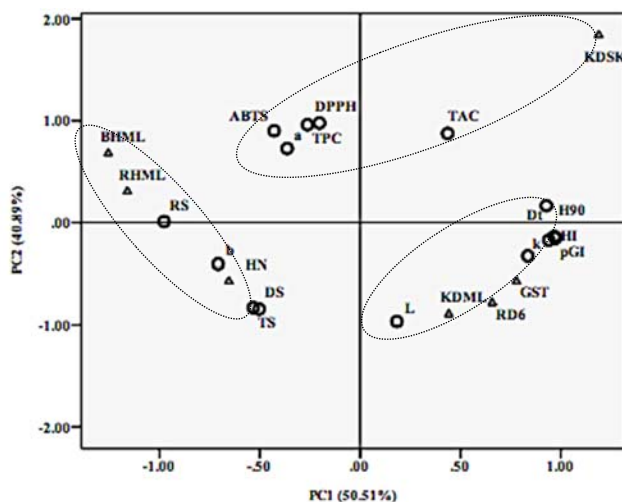


Figure 2. The principal component analysis (PCA) plots of seven rice samples and different properties (starch content, color, antioxidant property, and starch digestibility).

By using the PCA, it is possible to suggest reasons for the location of the rice varieties on the basis of their physicochemical properties, starch digestibility, and antioxidant properties. The location of KDML, RD6 and GST, which are the milled white rice and waxy white rice varieties, exhibited nearness on the right-hand side of the PC1 zero point. They confirmed their high starch digestibility (Table 4) but low RS content (Table 3), antioxidant capacity (Table 5) and a* value (Table 2). On the other hand, the intermediate-amylose content and pigmented rice varieties, HN, RHML and BHML were clearly at a differentiable distance away from the PC1 zero point to the left-hand side due to the RS content (Table 3) and the pigmentation (Table 2), resulting in their antioxidant capacity (Table 5). The high positive contribution on PCs of KDSK, which was the low-amylose waxy and purple-colored rice varieties showed that it had high TAC and starch digestibility as a negative RS property.

Therefore, it can be noticed that it was the antioxidant activity related to the polyphenol and anthocyanin contents that caused the colored rice pigmentation. This relationship of TPC, TAC, antioxidant properties (DPPH and ABTS) and color is consistent with other previous reports on various rice varieties [3, 9, 20, 29, 31]. They reported that the antioxidant properties were strongly correlated with total phenolic and anthocyanin contents in rice [3, 9, 20, 31]. As for the color parameters, L* and b* values were negatively correlated with TPC and TAC, and a* value was positively correlated with TPC [3, 20, 29]. Moreover, the starch digestibility for the waxy rice and low-amylose varieties were relatively high, especially for the waxy rice varieties, in agreement with the study of Hu *et al.* [17]. Similarly, the lower starch digestibility exhibited in the intermediate-amylose and relatively high RS rice varieties [17, 30]. Pongjanta *et al.* [29] reported that the predicted glycemic index had highly positive association with RS content and TPC. A combination of parameters is better than one individual parameter to classify rice properties. However, Thailand has many rice varieties from spread of cultivar areas, leading to their different properties. Therefore, it might be a useful data if further more investigation in other varieties was studied.

4. Conclusions

Seven rice samples were investigated in this study for their properties, especially color, antioxidant activity, and starch digestibility. The pigmented rice varieties had higher antioxidant capacity, in both the content and the activity, than that of the groups of non-pigmented rice varieties. The principal component analysis showed the relationship of their color, antioxidant capacity and starch digestibility with PC1 (50.51%) and PC2 (40.89%). The high resistant starch contained in the pigmented and non-waxy rice was shown to have strongly affected the reducing of starch digestibility, particularly the predicted glycemic index. This useful piece of information provides the knowledge to help consumers for the healthy rice consumption and the increasing of consumer awareness of the health benefits of rice varieties, especially in relation to the risk of type 2 diabetes. As this result, rice varieties which has high resistant starch and antioxidant capacity, Homnil, suitable for antidiabetes. Moreover, the alternative properties of rice varieties will be promoted for various value-added product development in food industry such as, ready-to-eat rice, ready-to-cook rice, rice noodle, instant rice, and protein-based flour bread and cookies in order to give the optimal properties.

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Effect of Modified Molasses Carbon Filler on Mechanical Properties of Natural Rubber Vulcanizates

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Abstract

In this research, a possibility study was carried out on the utilization molasses, inexpensive by-product of the refining of sugarcane into sugar, as alternative filler in rubber. Liquid molasses was converted to powder form under the heating process. Molasses carbon powder (MCP) was finally obtained. As a result of percentage of carbon content in MCP, it was evident that MCP containing 64.5 percent of carbon. Interestingly, the MCP was rich in carbon as well as carbon black (CB) reinforcing filler in rubber. In order to enhance the mechanical properties of MCP filled NR vulcanizates, MCP was modified with a silane coupling agent such as bis (3-triethoxysilylpropyl) tetrasulfide or Si69. Thus, the present work aims to investigate the role of silane coupling agent on properties of NR vulcanizates filled with MCP. Modified and unmodified MCP was incorporated into the NR with loadings of 30 phr (parts per hundred of rubber) and also compared with CB. All of NR filled with carbon fillers were examined in the term of cure characteristics, tensile properties, tear strength and swelling resistance. From the results, it was observed that the cure times were decreased with the incorporation of silane modified MCP. In addition, torque difference, tensile strength, tear strength and swelling resistance of NR vulcanizates were improved.

Keywords: natural rubber, molasses, carbon black, silane coupling agent, filler

1. Introduction

Natural Rubber (NR) is a high molecular weight natural polymer originally derived from latex found in *Hevea brasiliensis*. NR is a very versatile raw material mainly used as solid rubber and to a lesser extent as latex. Because NR possesses good mechanical properties such as high tensile strength, tear resistance, resilience and abrasion resistance, it is widely used in numerous industries especially in tire industry. Other applications include rubber gloves, anti-vibration mounts, seals, bridge-bearing, conveyor belts, gaskets, rolls, and pharmaceutical goods, for example, urinary drainage catheters [1-5]. Normally, NR is always compounded with additives to satisfy the given applications in terms of properties, cost and processability. Such compounding ingredients include filler, activator, accelerator, antioxidant, processing aids, vulcanizing agents [6-8]. In the case of filler, carbon black (CB) is the most important filler in rubber industry.

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Although CB is still well known among rubber technologists, the use of some other fillers from the natural resources and by-product as alternative reinforcing fillers in NR is an interesting and challenging for rubber industry [9-11].

Molasses is an end product in the sugar industry. Generally, molasses is widely used as raw materials to produce animal feed, ethyl alcohol and a growth medium for yeast production [12]. To add value of the molasses as an efficient low cost carbon source, viscous molasses was converted to powder form under the proper condition. Since the molasses carbon powder was rich in carbon as well as CB filler.

The present work aims to further investigate the improvement of the using carbon powder prepared from molasses by focusing on the modification of molasses carbon powder filler via silane coupling agent. The properties of NR filled with modified molasses carbon powder composites including cure characteristic, tensile properties, tear strength and swelling resistance were studied.

2. Materials and Methods

2.1 Preparation and characterization of molasses carbon powder

Molasses carbon powder (MCP) was prepared from original molasses. Firstly, molasses was dehydrated and carbonized in an electric furnace by setting the temperature at 400 °C for 5 hour. The black ash was obtained and was further milled to a powder form. Carbon containing in MCP was analyzed by CHN elemental analyzer. Then, X-ray fluorescence spectrometer (XRF) was used for determination other elements containing in MCP.

In this study, silane treatment used for modifying the MCP surfaces. The MCP was pre-treated by a silane solution. Silanes used in this study is bis (3-triethoxysilylpropyl) tetrasulfide (Si69 or TESPT). The loading of Si69 was varied from 0-6 wt%. The silane solution was prepared with ethanol before wetting on the MCP surfaces. For instance, for a silane content of 2.0 wt%, 2.0 g of the silane was mixed with 100 ml of ethanol, and then stirred for 30 min. The 100g of MCP were then added into the solution with a further 15 min stirring to ensure a uniform distribution of the silane on the MCP surfaces. The treated MCP was then dried at 100 °C for 12 h in an oven until a constant weight was reached.

In addition, Fourier Transform Infrared Spectrophotometer (FTIR) was also employed to identify the MCP functional groups. Finally, a scanning electron microscope (SEM) was used to observe the morphology of MCP particles.

2.2 Preparation of rubber composites

The loading of both MCP (with and without silane) and CB filled NR compounds was 30 phr. The formulation of compounds is given in Table 1. All ingredients were mixed with NR in 2- roll mill. The total mixing time was 10 min. Finally, sheets of NR compounds were obtained.

Table 1. Compounding formulation for natural rubber composites

Ingredient (phr)	Formulations	
	no filler	CB, 0% Si69 MCP, 2% Si69 MCP, 4% Si69 MCP, 6% Si69 MCP
Natural rubber (STR5L)	100.0	100.0
Zinc oxide	4.0	4.0
Stearic acid	2.0	2.0
6-PPD	4.0	4.0
filler	-	30.0
TBBS	2.25	2.25
Sulfur	0.75	0.75

2.3 Measurement of cure characteristics

Cure time and torque difference of rubber composites were determined at 150 °C with a moving die rheometer (MDR). The rubber compounds were further vulcanized at 150 °C with compression molding machine. Finally, vulcanized rubber sheets having a thickness of about 2 mm were obtained.

2.4 Measurement of mechanical properties

Vulcanized rubber sheets were used for tensile and tear testing according to ASTM D412 and D624, respectively. The measurements were carried out using an universal testing machine (Instron Model 3366) with a crosshead speed of 500 mm/min. At least, 5 specimens were tested for these properties and the average values were reported.

2.5 Measurement of swelling resistance

The vulcanizate was cut into rectangular shape with weight about 0.5 g. Then, the sample was immersed in 100 ml toluene for 7 days. The swollen sample was removed from the toluene and the excess toluene was blotted with a paper towel. Then, the swollen sample was accurately weighed. The swelling ratio (Q) was determined using Equation (1). The value of swelling ratio of each vulcanizate was the average of three specimens:

$$Q = (W_S - W_U) / W_U \quad (1)$$

where W_S : weight of swollen sample
 W_U : weight of unswollen sample

3. Results and Discussion

3.1 Characterization of molasses carbon powder (MCP)

Determination of some characteristics of MCP and CB used in this study was carried out. Results of percentage of elements and carbon containing in MCP and CB are shown in Tables 2 and 3. It is evident that MCP and CB mainly consist of carbon approximately 64.5% and 98.6%, respectively.

Table 2. Percentage of carbon determined by CHN elemental analyzer of MCP and CB

Filler	MCP	CB
Carbon (%)	64.5	98.6

Table 3. Percentage of elemental containing in MCP and CB analyzed by XRF

Elements (%)	Filler	
	MCP	CB
Carbon, Hydrogen, Oxygen	82.885	98.395
Potassium	7.459	0.006
Calcium	2.527	0.025
Iron	0.102	0.001
Sulfur	2.988	1.430
Phosphorus	-	0.120
Copper	0.003	0.001
Zinc	0.002	-
Manganese	0.015	-
Silicon	0.903	-
Chlorine	3.114	-

Additionally, FTIR spectra of MCP as depicted in Figure 1 shows the strong peaks at $3,500\text{ cm}^{-1}$ and $1,380\text{ cm}^{-1}$ indicating the presence of O-H and C-O bonds, respectively. In addition, MCP filler also presents peak in the region $1,600\text{-}1,700\text{ cm}^{-1}$. The peak can be assigned to the C=O and C=C. The presence of peaks is probably due to hydroxyl groups and some double conjugated bonds with carbonyl group on MCP surface [13, 14]. For modified MCP, FTIR technique was also used to monitor the change in the surface chemistry of MCP before and after the silane treatment. In addition, Figure 1 shows the comparison of FTIR results of MCP with and without surface treatment. Upon the treatment with Si69, slightly peaks at wave numbers of $2,900\text{-}2,800\text{ cm}^{-1}$ and broad peak at wave numbers $1,400\text{-}1,100\text{ cm}^{-1}$ are noticeable which are in the same region of the stretching vibration of the $-\text{CH}_2$, $-\text{CH}_3$ groups and C-O bond [15]. However, slightly peak of the stretching of alkyl group in Si69 silane at $2,900\text{-}2,800\text{ cm}^{-1}$ and broad peak of C-O bond at $1,400\text{-}1,100\text{ cm}^{-1}$ suggests the successful deposition of Si69 on the MCP surfaces. The SEM micrograph illustrated in Figure 2(a). It clearly shows large particle size of MCP when compared with CB as illustrated in Figure 2(b).

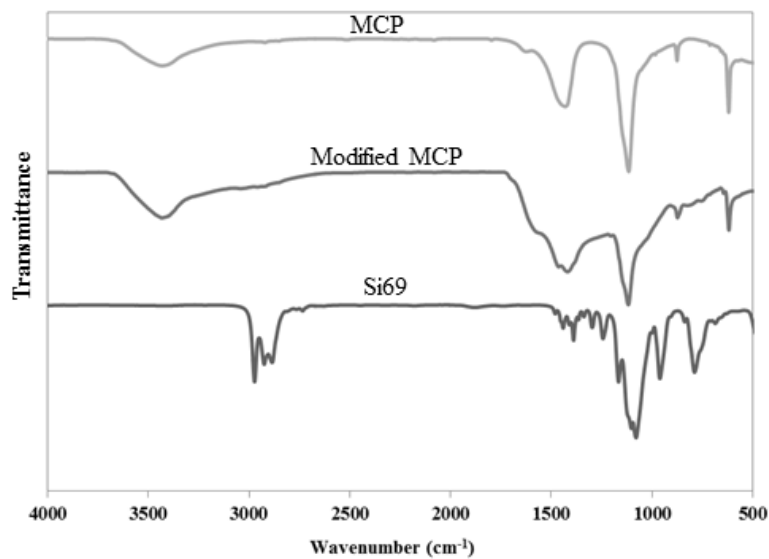


Figure 1. FTIR spectra of molasses carbon powder (MCP), modified molasses carbon powder (Modified MCP) and silane coupling agent (Si69)

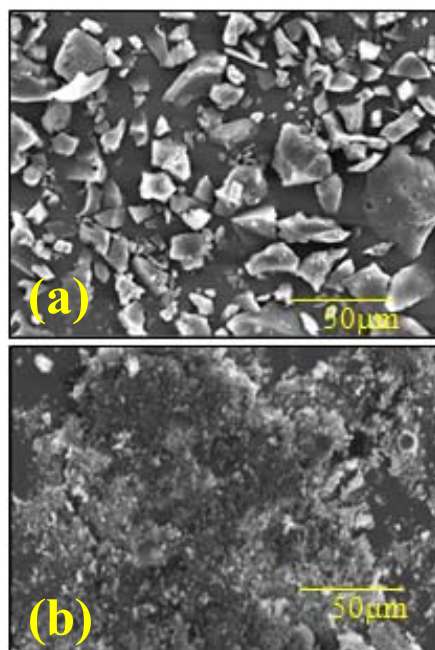


Figure 2. SEM micrographs of (a) molasses carbon powder (MCP) and (b) carbon black (CB)

Some cure characteristics which are cure time and torque difference of MCP, modified MCP and CB filled NR vulcanizates are shown in Figures 3 and 4, respectively. From the result of cure time result, it can be seen that the vulcanizates with the silane treatment MCP obviously show shorter cure times. This is due to the decreasing in adsorption of curing agent and accelerator molecules on MCP surface during vulcanization via Si69 treatment surface. For torque difference which is the difference between the maximum and minimum torques of the compound and related to crosslink density of the vulcanizates, it is noticed that the torque difference of the vulcanizates filled with both modified and unmodified MCP are improved when compared with no filler. However, the vulcanizates filled with 6% Si69 modified MCP give maximum torque difference which is similar to CB filler. It appears that the formation of rubber networks is increased when using modified MCP filler. This is due to (i) the reduction in the mobility of rubber chains by the presence of the fillers and (ii) Si69 can participate in sulfur vulcanization reaction giving rise to an increase in crosslink density and hence delta torque [16]. The structure of Si69 are depicted in Figure 5. It can be noticed that Si69 have sulfur which can promote curing and the rising in crosslink density.

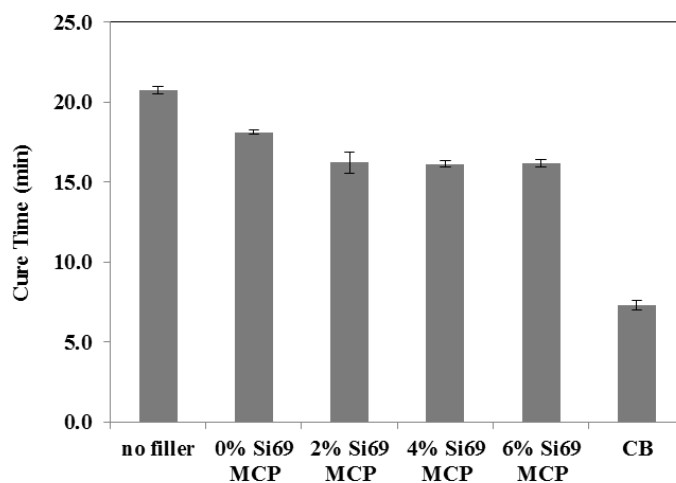


Figure 3. Cure time of unmodified MCP, modified MCP and CB filled NR vulcanizates

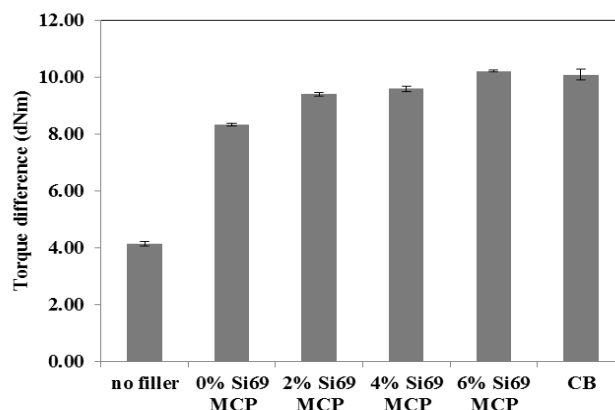


Figure 4. Torque difference of unmodified MCP, modified MCP and CB filled NR vulcanizates

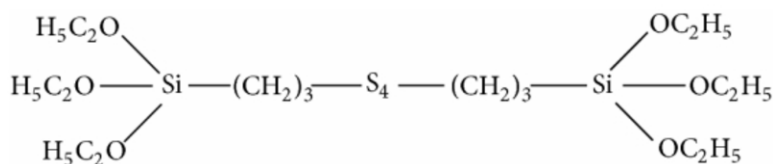


Figure 5. Structures of bis(3-triethoxysilylpropyl) tetrasulfide (Si69 or TESPT) [17]

Figure 6 illustrates tensile strength of the vulcanizates filled with MCP and compares with CB. From the result, it can be seen that the tensile strength of CB filled NR vulcanized shows higher than those filled with MCP. This is because the large particle size of MCP. In general, the smaller particle size of filler, the greater the tensile strength of the NR vulcanized. Another explanation is given to the carbon content of fillers. Filler with higher carbon content shows more effective reinforcement than those with lower carbon content, MCP. However, the tensile strength of NR filled with MCP slightly improves with modified MCP with Si69. This behaviour is probably due to the Si69 can improve the filler-rubber interaction leading to enhanced filler dispersion and Si69 can also assist sulfur vulcanization and increase the number of crosslinking [18, 19]. The elongation at break of MCP or CB filled NR vulcanizates are illustrated in Figure 7. It can be observed that the elongation at break of all modified MCP filled NR vulcanizates are no significant change when compared with unmodified MCP and CB filler. The modulus at 100 % strain of NR vulcanizates increases with incorporation all of fillers as presented in Figure 8. The enhancement in modulus at 100 % strain clearly displays with increasing silane loading in MCP. It is suggested that Si69 can promote sulfur and then increase in crosslink density. Normally, crosslink density which is related with the torque difference as shown in figure 4 which are chief factors for controlling modulus at 100 % strain [20-22].

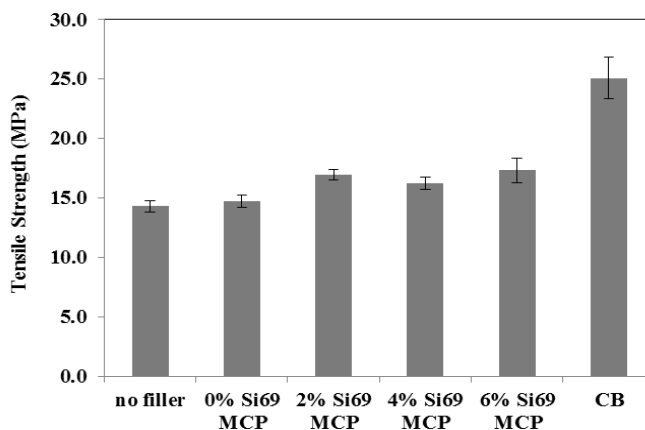


Figure 6. Tensile strength of unmodified MCP, modified MCP and CB filled NR vulcanizates

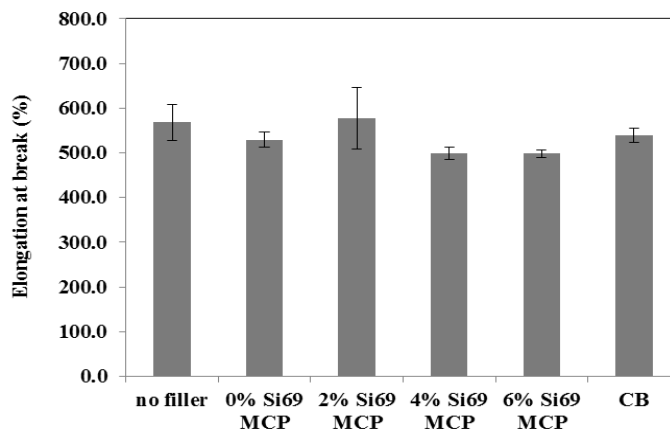


Figure 7. Elongation at break of unmodified MCP, modified MCP and CB filled NR vulcanizates

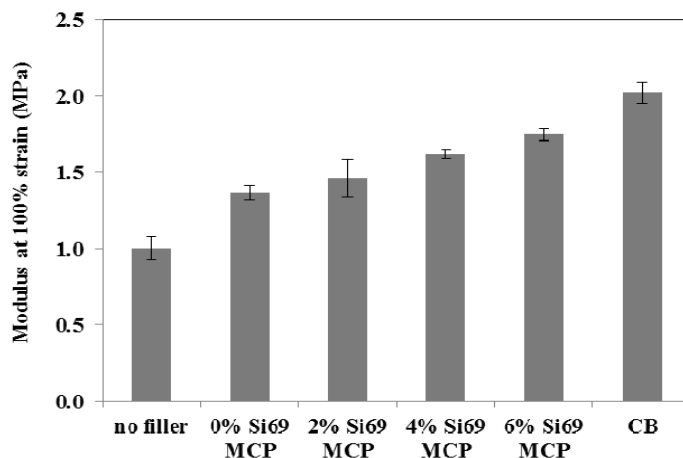


Figure 8. Modulus at 100% strain of unmodified MCP, modified MCP and CB filled NR vulcanizates

As illustrated in Figure 9, the result shows that tear strength increase with incorporation MCP with silane coupling agent. However, it should be noted that tear strength of vulcanizates having modified MCP is clearly lower than those filled with CB. As expected, this is probably because the low reinforcement ability of MCP as a result of large particle size and carbon content. The results of swelling resistance of MCP or CB filled NR vulcanizates is illustrated in Figure 10. Swelling resistance of the vulcanizates, which is inversely proportional of swelling ratio. It can be noticed that the swelling ratio decreases or swelling resistance enhances with incorporation all of filler. Compared with unmodified MCP, MCP modified with silane shows improvement in swelling resistance. This is probably due to the Si69 can improve the filler-rubber interaction and the increase in crosslink density. However, swelling resistance of NR filled with CB is still higher than those filled with modified MCP. CB is small particle size and carbon content than MCP [23].

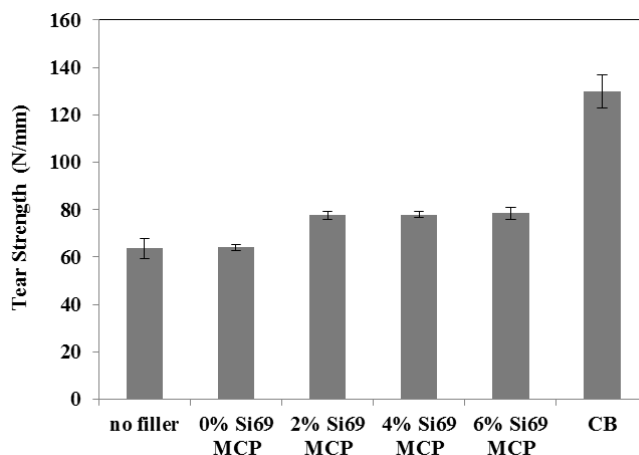


Figure 9. Tear strength of unmodified MCP, modified MCP and CB filled NR vulcanizates

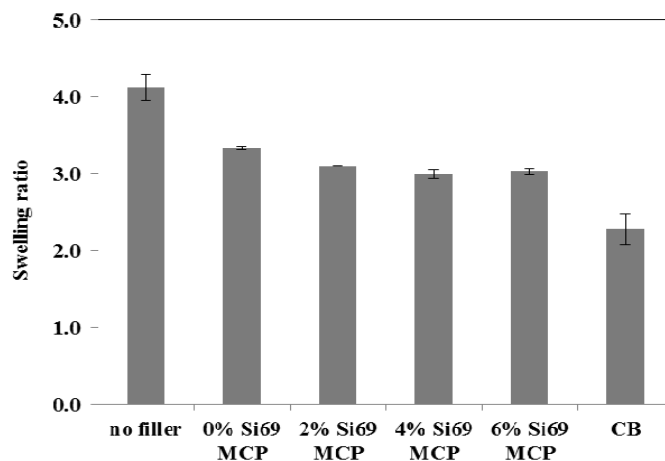


Figure 10. Swelling ratio of unmodified MCP, modified MCP and CB filled NR vulcanizates

4. Conclusions

Molasses which is the by-product can be converted to molasses carbon powder (MCP) under heating process for using as filler in natural rubber. In order to improve the properties of MCP filled NR vulcanizates, MCP was modified with a silane coupling agent namely bis (3-triethoxysilylpropyl) tetrasulfide or Si-69. From the results, it was observed that the cure times were decreased with the incorporation of silane modified MCP. In addition, torque difference, tensile strength, tear strength and swelling resistance of the NR vulcanizates were improved.

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Botanical Characteristics and Pectin Properties of *Canthium parvifolium* Roxb.

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Abstract

Canthium parvifolium Roxb. is a native plant found in the northern region of Thailand. *Canthium parvifolium* Roxb. has been found to have pectin in the fruits. Accordingly, the botanical characteristics of *C. parvifolium* and characterization of its pectin were evaluated. It was found that the habit of this plant is shrub with paired straight supra axillary thorns. Leaves are simple and opposite in arrangement with interpetiolar stipule. Young branches and lower surface of leaves are covered with pubescence. The inflorescence is axillary cymose. Calyx is green and limb is very short undulating 5-lobed with subglobe ovary portion. Corolla is tubular with 5 lobes, green to white or yellow in color. There are 5 stamens inserted at corolla throat. The ovary is inferior, 2-loculate with only 1 ovule each. The edible fruits are ovoid in shape and the skin is green or yellow, depending on maturity. The fruits of *C. parvifolium* were used as plant materials for pectin properties. The extracted pectin was designated as pectin with high degree of esterification due to its low moisture of 10.00% and the approximately 76.92% degree of esterification (DE) with methoxyl content around 7.06%. In addition, *C. parvifolium* yielded pectin around 38.00%. The chemical structure of pectin was studied through Fourier Transform Infrared (FTIR) spectroscopy and ¹H NMR spectroscopy. On the other hand, the molecular vibration was confirmed by using Density Functional Theory (DFT).

Keywords: *Canthium parvifolium*, botanical characteristics, pectin, density functional theory

1. Introduction

Canthium parvifolium Roxb. or “Nam Ma Khet” in the Thai vernacular [1], is one of the dicotyledonous plants belonging to the family Rubiaceae, order Gentianales [2]. *C. parvifolium* is a local plant which is widely distributed in the northern region of Thailand, e.g. Chiang Mai and Chiang Rai. In Chiang Rai Province, *C. parvifolium* is found in Wiang Chiang Rung District and Chiang Rai Rajabhat University. The fruits of this plant are very interesting because it could serve as another source of pectin [3].

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Pectin is a carbohydrate polymer compound that is found in plants. Pectin is a substrate of the enzyme pectinase which is found in plant cells between fibers at the top of the first layer cell wall. Pectin serves to anchor cells together. Pectin is the key component of parenchyma tissue which contains arabinose, galactose, and galacturonic acid [4]. It is a high-molecular weight compound with pectinic acid that could be served as a gelling agent when mixing it with food and water. Also, it is used as thickener and stabilizer in many types of food products, such as jam and jellies [5]. The gelling properties of pectin are known to be closely related to the degree of methylation (DM) and distribution of ester groups. Both rupture strength and gel threshold were found to be partly proportional to the free energy of hydrophobic interaction between the CH₃-groups in the model systems [6]. However, there are many factors which could affect the functionality of pectin, e.g. their composition, DM, solubility, pH, temperature and presence of soluble solids. Typically, the objective of commercial extraction of pectin is to obtain certain water-soluble and high molecular weight pectin [7]. There are different approaches to elucidate the pectin structure reported in the literature [8]. Nuclear Magnetic Resonance (NMR) was extensively to study pectin in food carbohydrates at a structural level [9]. However, the application of NMR as a quantitative analytical tool in carbohydrate determination is still limited despite the NMR's great capability [10]. DFT was used to study biology of some methylxanthines. According to the previous theoretical study [11], B3LYP was employed to calculate the gap between highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO). The result from the gap is representative for pharmaceutical properties of pectin. The result of this important properties of pectin is supported by the experimental study [12].

Moreover, the studies of silk retardant through plasma treatment, computational simulation, and vibration give a good agreement with FTIR in the work of Chaiwong and co-worker [13].

Thus, the objective of this research was to study the botanical characteristics of *Canthium parvifolium* Roxb. In addition, the physical and chemical properties of pectin in *C. parvifolium* such as methoxyl content, degree of esterification, equivalent weight and moisture were also evaluated. Lastly, we preliminary investigated the structural properties of pectin in *C. parvifolium* using the DFT as a tool for calculation together with the experimental approach.

2. Materials and Methods

2.1 Plant and extraction methods

The intact *C. parvifolium* plants were collected from Chiang Rai Rajabhat University, Chiang Rai Province, Thailand. The fruits of *C. parvifolium* were used as plant materials for pectin extraction. Fresh fruits were cut into small pieces and dried in an oven at 105 °C. Pectin was extracted from the ground *C. parvifolium* fruits with water (Ratio 25:1w/w) mainly to remove pigments. The material was filtered and the solids were treated with a solution (6% w/w) of sodium hexameta phosphate (SHMP) at 80 °C for 90 min. A pH 3.0 was obtained by adding HCl (1 M). After extraction, the pectin solution was filtered through a white filter cloth. Then, 95% acidified Alcohol and Acetone were utilized to precipitate the filtrate. To explain this step clearly, there is the persuasive detail: at the first, acidified alcohol was employed to remove impurities, and then the residue was dried by acetone. This process continues iteratively. 5 iterations were required to produce colorless filtrate.

The formed gel was allowed to precipitate at 25 °C for 24 h. After that, the pectin was dried at 35 °C for 24 h. The dry pectin was ground to pass through 60 mesh sieves for further study. The functional groups of *C. parvifolium* pectin were investigated by FTIR method with

Nicolet 6700 FTIR spectrophotometer (Bruker, Germany). The spectra were collected by averaging 64 scans at a resolution of 4 cm^{-1} from $400 - 4,000\text{ cm}^{-1}$. The ^1H NMR spectra were acquired on a Bruker 400 MHz spectrometer, operating at 400 MHz for proton. Spectra were collected at 298 K, with 32K complex points, using a 90° pulse length. Sixteen scans were acquired with a spectral width of 8278 Hz, an acquisition time of 3.95s.

2.2 Physio-chemical

C. parvifolium fruit was analyzed as dry matter content [14]. Crude protein, crude fat (ether extract), crude fiber, and ash content using the standard techniques [14]. All samples were analyzed in duplicate and the results were reported including the total energy (cal/g) with bomb calories.

2.3 Methoxyl content

Methoxyl content was determined by dissolving 0.5 g of pectin in a 250 mL Erlenmeyer flask with 100 mL deionized (DI) water and then adding 1 g of NaCl with 6 drops of phenol red for titration with 0.1 M NaOH until the indicator color changed (pH 7.5). This solution was used to determine the methoxyl content by adding 0.25 M NaOH 25 mL, 0.25 M HCl to be titrated with 0.1 M NaOH.

$$\% \text{ Mehtoxyl content} = (\text{NVE} \times 100) / 1000\text{S} \quad (1)$$

Where S is dry pectin weight, N is base normally used in the titration, V is the base volume in the titration and E is the equivalent weight of methoxy.

2.4 Degree of esterification

The %DE was determined by dissolving 0.5 g of pectin powder with 2 mL of ethanol into 100 mL of water. The mixture was stirred until the solution became homogenous. After that, 5 drops of phenolphthalein and 0.5 M NaOH (V1) was added for titration. After that, 0.5 M NaOH (10 mL) was added and stirred for 15 min. After that, 0.5 M HCl (10 mL) was added and stirred in until the solution was colorless. Finally, a phenolphthalein indicator was added followed by titration with 0.5 M NaOH (V2). The methoxyl content and %DE was calculated by the following equation.

$$\% \text{ DE} = (\text{NaOH volume1} / \text{NaOH volume 1+2}) \times 100 \quad (2)$$

2.5 Equivalent weight

Equivalent weight was determined by weighing 0.5 g of sample in a 250 mL conical flask and 5 mL ethanol was added. Then, 1 g of NaCl and 100 mL of distilled water was added. Finally, 6 drops of phenol red were added and titrated against 0.1 M (N) NaOH. The titration end point was indicated by purple color. This neutralized solution was stored for determination of methoxyl content. Equivalent weight was calculated by the following formula:

$$\text{Equivalent weight} = 1000\text{S} / \text{N.V.} \quad (3)$$

Where S is dry weight, N is normality concentration, and V is the quantity of NaOH.

2.6 Moisture

The moisture of extracted pectin was analyzed by weighing 0.5 g pectin into a crucible and content was weighed (W1). The whole crucible was baked at 105 °C for 9 h. The crucible was let cool and weighted (W2). The moisture content may be calculated as follow.

$$\% \text{ Moisture} = ((W1-W2)/0.5) \times 100 \quad (4)$$

2.7 Theoretical Study

The ground state optimization of a model compound of pectin was performed using DFT. Calculation of molecular structure in order to predict pectin properties was achieved using DFT with Beck-Lee Yang Parr (BLYP) functional of the Generalize Gradient Approximation (GGA). All simulations were performed under the conditions corresponding to 0 K. The analyzed effects of the structural features and molecular energy were achieved at the same level function. After that, the optimized structure was used to study the vibrational mode using a Dmol³ module in a Materials Studio (MS) [15] program package

3. Results and Discussion

3.1 Botanical characteristics of *C. parvifolium*

The botanical characteristics of *C. parvifolium* were shown in Figure 1. The plant appearance is shrub with paired straight supra axillary thorns. Leaves are simple and opposite in arrangement with interpetiolar stipule. Young branches and lower surface of leaves are covered with pubescence. The inflorescence is axillary cymose with a few to several flowers. Calyx is green and limb is very short undulating 5-lobed with subglobse ovary portion. Corolla is tubular with 5 lobes, green to white or yellow in color. There are 5 stamens inserted at corolla throat. The ovary is inferior, 2-loculate with only 1 ovule each. The edible fruits are ovoid in shape. The skin is green or yellow, depending on maturity. Moreover, it is interesting to note that the fruit of *C. parvifolium* is the one excellent source of pectin [3].

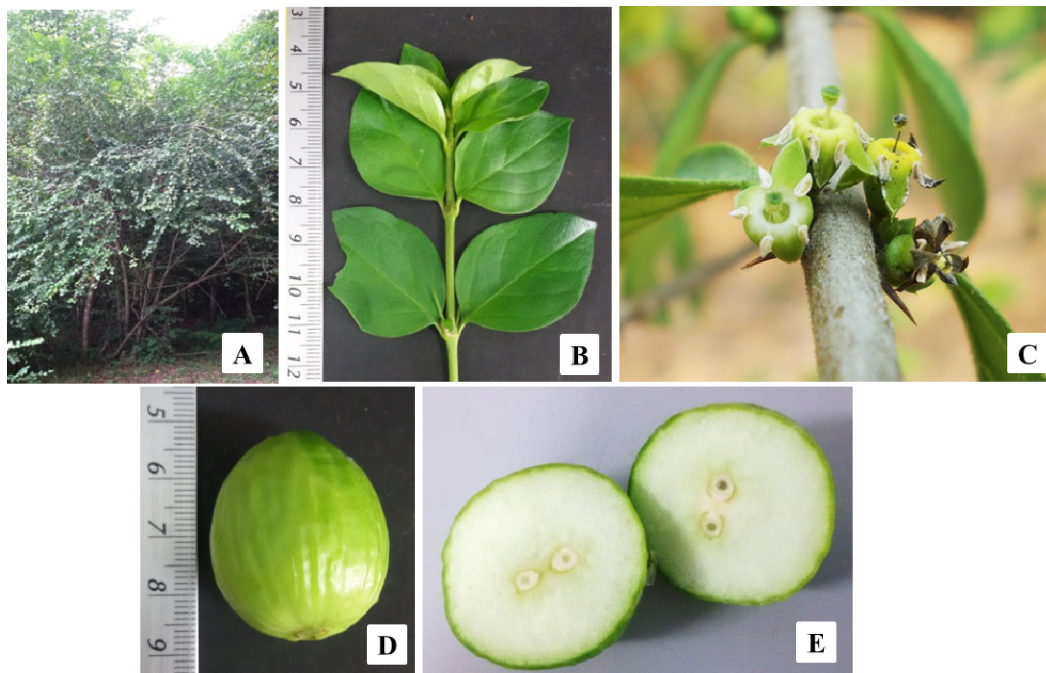


Figure 1. Botanical characteristics of *Canthium parvifolium* Roxb. (A) habit (B) leaves and stipule (C) flowers (D) young fruits and (E) cross section of young fruits

The results for physicochemical characteristics of *C. parvifolium* Roxb. fruit are displayed in Table 1. The ash content is at 0.57% dw. The protein content is 1.5%. The amount of fat is significantly low compared with apple pomace (0.69%). The dry matter is 19.65%. The crude fiber in *C. parvifolium* Roxb. is at 4.96% which is in the same range as of apple pomace. Finally, *C. parvifolium* Roxb. has a high total energy at 828 cal/g which is lower total energy than apple pomace.

Table 1. Proximate composition of *Canthium parvifolium* Roxb. fruit

Composition	Samples
Dry Matter (%)	19.65
Crude Fat (Ether Extract) (%)	0.69 (3.49-3.90)
Crude Fiber (%)	4.96 (4.7-48.72)
Crude Protein (%)	1.5 (4.45-5.67)
Ash Content (%)	0.57 (1.82)
Total Energy) cal/g)	828 (2,950)

Note:* The numbers in parentheses is a reference value from apple pomace [16]

3.2 Characterization and properties of *C. parvifolium* pectin

It was found that the appearance of dry extracted pectin is light brown color as shown in Figure 2.



Figure 2. The characteristic of *C. parvifolium* pectin.

The properties of extracted pectin were analyzed and the results are as shown in Table 2. All measurements were carried out in triplicate for each of the sample. The results are reported in mean values and standard deviations. The results revealed that pectin from *C. parvifolium* fruits has a high value of 7.06% methoxyl content which was higher than standard pectin and pharmaceutical pectin. Moreover, *C. parvifolium* pectin has high viscosity and gelation when compared with the reported DE of 76.92% [17]. Ptichkina *et al.* [18] reported that pectin with DE > 60% is suitable for use in food industry. The chemical characteristics of the pectin that influence gel qualities are DE, which is related to the rate of gel formation in food industry [19]. High DE means high gelation temperature when preparing gels by the usual procedure of mixing the hot ingredients and then solidifying by cooling. When the gel batch is cooled below the gelling temperature, gelation occurs after a delay, which is short with pectin of high DE and longer with pectin of lower DE [20].

The moisture of *C. parvifolium* pectin was 10%, which was quite high compared to the pharmaceutical grade pectin. The equivalent weight of this study was 181.81 which indicated the total content of free galacturonic acid. Therefore, it is associated with the % DE and methoxyl content. According to work of Rouse [21], the higher DE causes the decrease of free acid content of pectin that the equivalent weight also increases.

The extraction of pectin from *C. parvifolium* yielded 38.00 ± 0.875 g%, while it was preliminary studied by Kaewpo [3] following the method described by Rangana [22]. The results reported that the content of pectin in *C. parvifolium* was 48.37 mg/ml dry weight, which was 1.6 fold significantly higher than the content of pectin in apple (29.97 mg/ml dry weight). However, by Rangana method, the high percentage of moisture (21.55%) was found in the extracted pectin. Thus, this research presented an efficient protocol for the pectin extraction of *C. parvifolium*.

Table 2. The properties of *C. parvifolium* pectin compared with standard pectin and lab & pharmaceutical grade pectin

Properties	<i>C. parvifolium</i> Pectin*	Standard Pectin [17]	Lab&pharmaceutical grade pectin [23]
Moisture (%)	10.00 ± 0.20	-	4.81
Degree of Esterification (%)	76.92 ± 0.53	64.95	-
Equivalent weight	181.81 ± 0.35	-	-
Methoxyl content (%)	7.06 ± 0.44	> 2.50	6.29
yield (%)	38.00±0.87	-	-

Note: * Values showing the mean ± SD in a column.

3.3 Functional group of *C. parvifolium* pectin by FTIR method

The samples of pectin were subjected to FT-IR analysis. The bands relevant for the structural organization are presented in Figure 3. The spectra shows two intense pectin bands at 1,741 cm^{-1} and 1,641 cm^{-1} were assigned to the stretching C=O vibration of esters and the asymmetric stretching vibration of carboxylate anion $\nu_{\text{as}}(\text{COO}^-)$, respectively. The two functional groups are from carboxylic ester and protonated carboxylic acid groups. The region of 1,250 cm^{-1} – 950 cm^{-1} is contributed to glycosidic bonding ($\nu_{(\text{COC})}$, glycosidic bonding). Moreover, the spectra of pectin at 2,929 cm^{-1} was assigned to $\nu_{(\text{C-H})}$, while the peak shown up at 3,272 cm^{-1} was assigned to $\nu_{(\text{O-H})}$ of the carboxylic acid group.

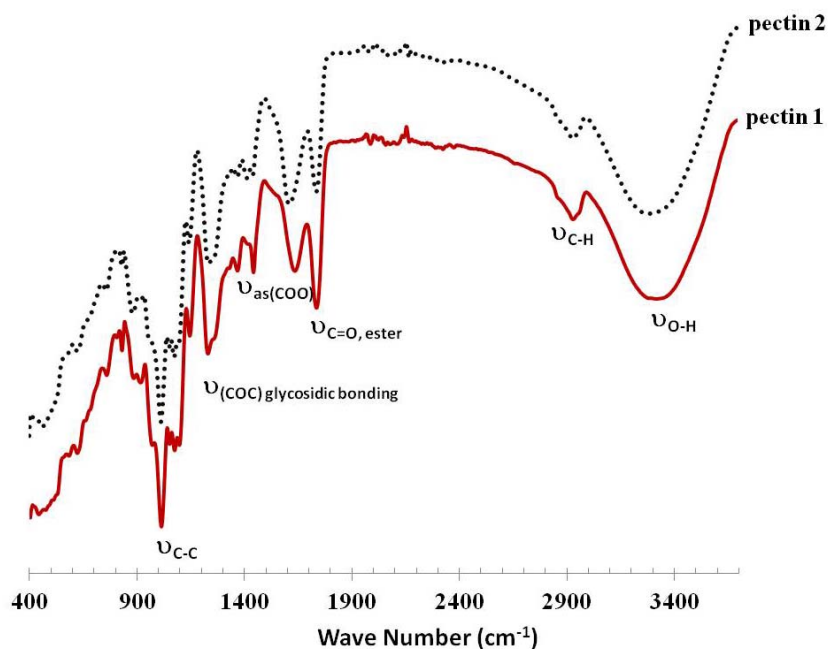


Figure 3. FT-IR spectrum for extracted pectin from *C. parvifolium*.

3.4 Vibrational results

The optimized structure of *C. parvifolium* pectin model compound was also studied. The ground state minimization of the pectin model compound was carried out using Dmol3 module in MS program with spin unrestricted and BLYP functional of GGA. A Fermi smearing of 0.005 Hartree was used to improve computational performance.

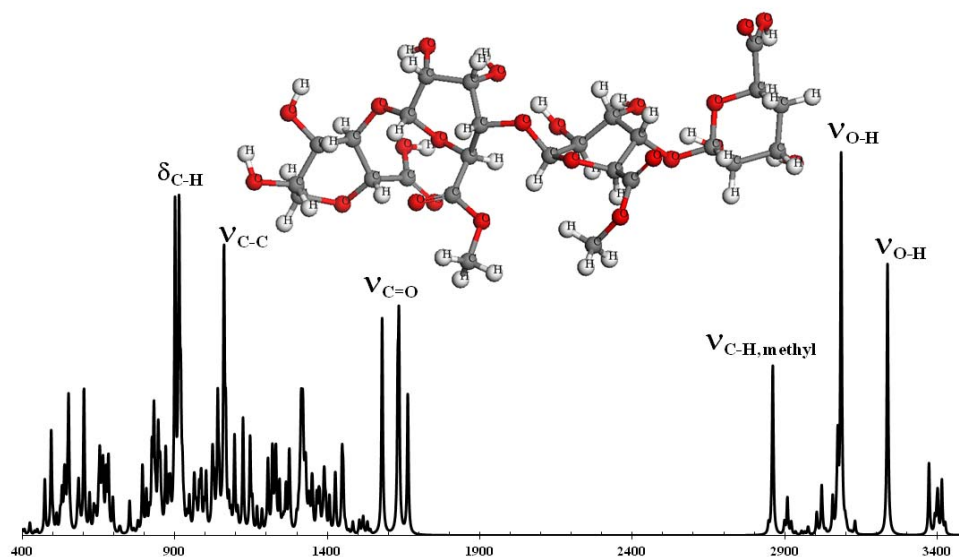


Figure 4. Vibrational spectra for pectin model compounds.

The vibration spectra of *C. parvifolium* pectin was presented in Figure 4. The highly coupled and conformational specific region between 1,100 and 800 cm^{-1} belongs to non-localized, highly coupled vibrations of polysaccharide backbones ($\delta_{\text{C-H}}$, 913 cm^{-1} and $\nu_{\text{C-C}}$, 1,060 cm^{-1}) [22]. Two intense bands at 1,634 and 1,579 cm^{-1} are assigned to $\nu_{\text{C=O}}$, which indicates its stretching vibration in ester and carboxylic acid. Additionally, the presence of $\nu_{\text{C-H, -CH}_3}$ peak at 2,859 cm^{-1} also indicates an existence of methyl ester group. The peaks at 3,054, 3,236 cm^{-1} suggest that there are stretching vibrations of O-H at carboxylic groups.

3.5 NMR Analysis

The structural analysis by IR is supported by NMR result. As illustrated in NMR (Figure 5), there is a board singlet peak at 1 ppm corresponding to methyl groups ($-\text{CH}_3$) in the structure. Moreover, NMR shows doublet peak at 3-5 ppm. This is related to hydrogen in hydroxyl group, which is coupled with a neighboring hydrogen atom. The quartet peak at approximately 3.5 ppm is from the ring protons.

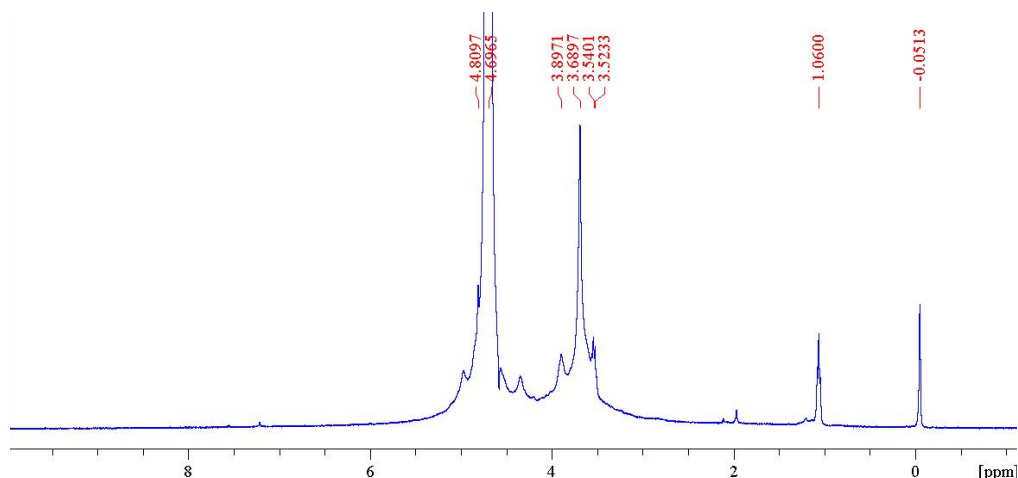


Figure 5. ¹H NMR spectrum of pectin sample. Experimental condition are reported under Materials and Methods.

4. Conclusions

This report presents the botanical characteristics of *C. parvifolium* and an efficient protocol for the pectin extraction from *C. parvifolium* fruits. The proximate composition of *Canthium parvifolium* Roxb. fruit was lower value compared with apple pomace. However, some properties of extracted pectin were clarified. It was found that the pectin extracted yield was $38.00 \pm 0.87\%$ w/w which is significant higher than apple pomace (3.5-14.32% w/w), the moisture, and equivalent weight of *C. parvifolium* pectin was 10% and 181.81, respectively. The pectin of *C. parvifolium* had % DE (76.92%) and methoxyl content (7.06%), revealing its potential for in gelation application. In light of FTIR spectrum, it is routine to scale the frequency axis by a small amount to align major bands with those experimentally observed. The co-alignment of calculated vibrational spectra and experimental bands via the same scaling, as seen here, constitutes good agreement. The structural analysis by IR and NMR confirm the expected pectin structure. As our preliminary results mentioned above, we would like to probe and predict gelling factors, such as pH and temperature of *C. parvifolium* pectin in our further work.

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Extraction and Characterization of Zein Protein from Corn for Controlled Drug Release

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Abstract

The aim of the present study was to analyze the effect of β -zein in a coating film and on the controlled drug release properties in pharmaceutical tablets. The zein protein was obtained by solvent extraction using acetic acid, ethanol and isopropanol with different concentrations from 70 to 90% v/v in each solvent. The structure of the extracted zein protein was analyzed using SDS-PAGE and FT-IR. Only the zein extracted using acetic acid had a low molecular weight (17 kDa), which was represented as β -zein. The extracted zein from 70% isopropanol and 70% acetic acid was then used to prepare coating solutions for coating tablets and compared with commercial zein. The film was coated on the tablets containing a model drug, namely theophylline or chlorpheniramine maleate, with the samples prepared using the wet granulation method. Investigation of the film elasticity indicated that the extracted zein from acetic acid had greater flexibility than the commercial zein. The dissolution profiles of the coated tablets from the extracted zeins were slower than that of the commercial zein as zero-order release profiles. The better elongation and flexibility could prevent penetration of the solvent into the core of the tablet and help retain the film function.

Keywords: zein, tablet coating, control release, solvent extraction, physicochemical property

1. Introduction

In recent years, there has been much attention in pharmaceutical development focused on controlled drug release for a better therapeutic outcome. To achieve this, various controlled-release-dosage materials have been developed for coating tablets [1, 2]. The main goals in designing a drug coating are drug protection in the stomach or from moisture during storage, controlled drug release, masking the taste, and reinforcing the tablet [3, 4]. Controlled drug release in a delivery system should also maintain a relatively constant therapeutic blood level of the drug during the desired release period

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[1,5]. Typically, coating materials involve the application of a sugar or polymer onto the tablet. Pharmaceutical coating technology has been shifting away from a sugar base to biopolymer base because of their hydrophobic and low water uptake properties. Many types of polymer have been used for drug coating, such as acrylic derivatives (polyethyl acrylate-co-methacrylate, poly (vinyl acetate)) and cellulose derivatives (ethyl cellulose) [3, 6, 7].

Zein is a natural polymer obtained as a by-product from industrial corn processing [8, 9, 10, 11]. The protein content in different varieties of corn is 6–12% on a dry basis and about 75% of endosperm tissue contains protein [8]. Zein was first extracted from whole corn or dry-milled corn (DMC) using a hydroalcoholic solvent. The total protein content of DMC was 6.8–8.0% of the milled corn. [12]. Zein is a major protein that is found in the endosperm of the corn kernel and it is a class of alcohol-soluble prolamine proteins [8, 13, 14]. Basically, zein is classified into several types based on its solubility in alcohol and sequence homology. Zein consists of three protein fractions: α -zein (75% to 85% of total zein), consisting of two polypeptides with an estimated molecular weight of about 19 kDa and 22 kDa, which is soluble in 95% ethanol; β -zein (10% to 15% of total zein), with a molecular weight of 17 kDa and is soluble in 60% ethanol; and γ -zein (5% to 10% of total zein), with a molecular weight of 27 kDa [8, 16, 17, 18, 19, 20]. Mostly, commercial zein has been used as an aqueous alcohol solution, extensively about 50–90% and containing only α -zein. [8]. In contrast, glacial acetic acid can fully solubilize zein (α -, β -, and γ -zein), resulting in zein comprising α -, β -, and γ -zein [15]. Zein is generally recognized as safe and is biodegradable and biocompatible. [14,21] Due to the alcoholic solubility properties of zein, its thermoplastic character and low water uptake, related to a high content of non-polar amino acids [8], it is useful for tablet coating. Film materials made from zein have relatively high barrier properties compared to other proteins. Films from zein have been applied for the controlled release of compounds in medicinal tablets, for protection of the tablet from moisture, and for masking the taste of bitter orally administered drugs [4]. King *et al.* [15] reported that zein isolation procedures produce zein comprising predominantly α -zein, without β - and γ -zein, which can produce films with elasticity for various applications, whereas zein containing β - and γ -zein content could produce a film only when cast from a solution of aqueous ethanol and perform high stress tolerance with brittle [9, 15]. To improve the film forming capability plasticizers such as polyethylene glycol 400 (PEG 400) and triethylene glycol (TEC) are added [9], which control drug release property effect.

The present work concerns developing coating film formulations using different compositions of the different zein structures (α - and α - + β -zein), with an aim for achieving an improvement in the controlled release of a drug in a medicine tablet model. The findings should also improve the value of the residue products from corn industry waste for use in pharmaceutical applications.

2. Materials and Methods

1. Materials

The corn kernels (strain, Suwan 5) used in this study were obtained from the National Corn and Sorghum Research Center, Pak Chong, Nakhon Ratchasima, Thailand. Commercial zein was purchased from Acros Organics (New Jersey, USA). The organic solvents purchased were ethanol (Chachoengsao, Thailand), isopropanol (J.T.Baker® Chemicals, Avantor's Proven Brands, USA), acetic acid (ACI Labscan, Thailand), and hexane (J.T.Baker® Chemicals, Avantor's Proven Brands, USA). The model drugs used were theophylline (Jilin Shulan Synthetic Pharmaceutical Co., Ltd., China) and chlorpheniramine malate (CPM) (Vankatara Chemicals Ltd., India). The plasticizer used was polyethylene glycol (PEG 400) and triethyl citrate (TEC) (T.C.Sathaporn Group Ltd., PART,

Thailand). Other chemicals used were hydrochloric acid (Qrec, New Zealand), sodium hydroxide (Ajax, Finechem, New Zealand), and sodium lauryl sulfate (T.C. Sathaporn Group Ltd., PART, Thailand).

2. Methods

2.1 Corn powder preparation

Zein protein was extracted from corn kernels. First, corn kernels (strain, Suwan 5) were ground and the sample was extracted with hexane to remove the oil. The extraction process produced a slurry contaminated with oil. The slurry was then filtered using an aspirator pump (EYELA, Aspirator A-1000S Pump, Shanghai, China) with a Whatman No. 1 filter. The defatted powder corn kernel was dried in a hood overnight and then ground.

2.2 Zein protein extraction process

Defatted powdered corn kernel was extracted for 45 min with three different organic solvents (ethanol, isopropanol, and acetic acid). The concentration in each solvent was 70%, 80%, and 90%. The mixture was stirred in a beaker using an overhead stirrer (IKA, RW 20 Digital, USA) and the beaker was covered with aluminium foil. Then, the slurry was filtered to separate the extract from the other components, and the extract containing the dissolved zein protein was vaporized in a rotary evaporator (EYELA, Tokyo Rikakikai Co., Ltd., Japan) to remove the organic solvent and obtain zein protein. The precipitate zein protein was cooled with cool distilled water (4–10 °C) and the acidity was adjusted to approximately pH 6.2. The zein protein was then washed with distilled water. Finally, the zein protein was dried, ground, and analysed for quality and quantity.

2.3 Characterization of the extracted zein protein

- Protein analysis

The moisture content of all the samples was determined by drying the samples at 105 °C for 2 h [22]. Protein content analysis was conducted using the Kjeldahl method [23]. The % yield was calculated as the amount of protein in the ground corn.

- Fourier transform infrared spectroscopy (FT-IR)

The infrared spectra of commercial zein and zein protein extracted from the corn kernel (strain, Suwan 5) in different organic solvents and at different concentrations were obtained using an FT-IR spectrometer (Bruker Tensor 27, USA) in the attenuated total reflection (ATR) mode. The protein sample was placed on the ATR crystal to cover the crystal surface. The FT-IR data were collected in the region 400–4000 cm⁻¹ [24].

- Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to classify the zein protein composition. The protein concentration of each sample was determined using the Bradford reagent [24]. SDS-PAGE was prepared using 12% separating gel and 4% stacking gel according to the Laemmli method. A prepared sample buffer (2X Laemmli buffer system, Biorad, USA) was diluted with deionized water and mixed with 2-mercaptoethanol. The protein samples were prepared by dissolving them in 2% sodium dodecyl sulfate using a sonicator; then they were mixed in with the sample buffer, heated in boiling water (96 °C) for 4 min, and then loaded into the sample well. Electrophoresis was conducted at 150 V and 35 mA for 50 min. The gels (Mini-Protean® Tetra Cell, Biorad, China) were stained with a Coomassie stain (Biorad, USA). A molecular weight standard of 10–250 kDa was used (Precision Plus Protein™ All Blue Standards, Biorad, USA) [13,25].

2.4 Determination of the physical properties of the extracted zein films

Zein films were obtained by dissolving 5% zein (two samples from the extraction experiment, with the first sample consisting of only α -zein and the second sample consisting of α -zein and β -zein) and 2% sodium dodecyl sulphate (w/w) into an 80% concentration of isopropanol, which was then mixed until it homogenized. Then, 20% plasticizer (PEG 400 or TEC) was added to the solution and stirred using an overhead stirrer for 30 min. After cooling, the films were formed in a Teflon tray (5 x 5 cm) and dried at 50 °C until the solvent had completely evaporated [9, 26].

The mechanical properties of the zein films containing the different amounts of extracted zein and different types of plasticizer were measured in the tensile and elongation modes using a Texture Analyzer (Lloyd Material Testing, Lloyd TA1, USA) with a 5 kg load cell. The speed was kept constant at 5 mm/sec and 2.5 cm distance. In order to measure the tensile properties, a film with a well-defined geometry was used. Film strips (5 cm long and 1 cm wide) were cut (adapted from [27]). The thickness of the film was measured by using a thickness gauge tester (Mititoyo Absolute, USA). All the extracted zein samples were compared with the film from a commercial zein sample. The results were reviewed according to two parameters: elongation (calculated using equation 1) and tensile strength (calculated using equation 2).

$$\text{Tensile strength} = (\text{load at break}) / (\text{original width}) (\text{original thickness}) \quad (1)$$

$$\text{Percent elongation} = (\text{elongation at rupture}) \times 100 / (\text{initial gauge length}) \quad (2)$$

2.5 Preparation of a coated tablet from zein

Two model drugs with different solubility, namely theophylline and chlorpheniramine maleate (CPM), were used. Core tablets containing a model drug were prepared by wet granulation. The model drug (100 mg) was added into each core tablet (300 mg), using lactose as a filler, magnesium stearate as a lubricant, and corn starch as a disintegrant. Core tablets with hardness higher than 7–8 kg were required

Zein protein (5% w/w or 10% w/w) with 2% w/w sodium lauryl sulphate (calculated from the polymer weight) was dispersed in 80% v/v isopropanol, and then a plasticizer polyethylene glycol 400 (PEG 400) was added to the solution at a concentration of 20% w/w (calculated from the polymer weight). The solution was mixed using a magnetic stirrer. The coated tablets were manually obtained using a dip-coating technique. The film coating solution was cast onto a Teflon tray and dried in a hood overnight, followed by drying at 0°C in a hot-air oven for 2 h.

2.6 In vitro drug control release properties

Drug release from the zein-coated tablets was studied using USP dissolution apparatus 2 (Varian VK7010 Dissolution Apparatus, USA). An amount (900 ml) of 0.1 N HCl or phosphate buffer pH 6.8 at 37±0.5 °C was used. The sample was determined using a UV-VIS spectrophotometer (Agilent Technologies, Varian Cary® 50 UV-Vis Spectrophotometer, USA) at 272 nm and 265 nm for theophylline and CPM, respectively. This experiment was conducted in triplicate.

2.7 Statistical analysis

Statistical analyses were performed using the SPSS software (version 19). Duncan's multiple range test ($P < 0.05$) was used to compare significant differences of the means of the % yield, protein content, and the mechanical properties in the zein film experiments.

3. Results and Discussion

3.1 Percentages of the yield and protein content

The zein extraction from corn was focused on two factors: the type of solvent and the concentration. Ethanol, isopropanol, and acetic acid at concentrations of 70%, 80%, and 90% were used to extract the zein from corn and its coproducts. The performance of zein extraction under each condition was determined based on the % yield and % protein. The results showed that isopropanol at 70% had the highest yield and protein content with amounts of 85.33% and 73.64%, respectively, followed by acetic acid at 90%, which had a yield and protein content of 66.23% and 69.87%, respectively, as seen from Table 1. Moreover, the protein yields of the extracted zein decreased with increasing the solvent concentration, because a high concentration of solvent came with high hydrolytic properties such that the protein was hydrolysed into corn as small molecules of amino acid. Thus, the optimum suitable condition was a low concentration of solvents [28]. Selling and Wood [29] obtained the same results after using acetic acid as the solvent for zein extraction and reported that acetic acid removed zein with a higher yield and more effectively compared with ethanol solvents, whereas the structure of the extracted zein from acetic acid and ethanol had similar structures based on SDS-PAGE results.

Table 1. % Yield and protein content of extracted zein protein by using different solvent and concentration

Properties	Ethanol (% , v/v)			Isopropanol (% , v/v)			Acetic acid (% , v/v)		
	70	80	90	70	80	90	70	80	90
Yield (%)	50.61±	52.90	20.88±	85.33±	48.40±	43.34±	64.15±	58.85±	66.23±
	1.3 ^{bc}	±3.1 ^{bc}	6.6 ^d	14.9 ^{ab}	4.2 ^{bc}	17.4 ^c	11.9 ^{ab}	3.6 ^{abc}	0.1 ^a
Protein (%)	56.65±	43.07	33.87±	73.64±	52.20±	43.14±	63.46±	72.11±	69.87±
	6.0 ^{bc}	±2.2 ^{dc}	13.7 ^c	0.6 ^{ab}	1.3 ^{cd}	4.15 ^{dc}	0.5 ^{abc}	17.9 ^{ab}	11.3 ^a

a – d Means± SD followed by difference letters in each column were significantly difference at $P < 0.05$

3.2 Structural analysis

Zein protein: Size analysis was carried out using two main methods: SDS-PAGE and size exclusion chromatography. The SDS-PAGE patterns (Figure 1) of the zein samples exhibited two major broad bands at 19–22 kDa and 23–24 kDa, indicating the presence of α -zein [30, 31]. A thinner band below the α -zein band was observed at 16–20 kDa, indicating the presence of β -zein [31]. Another light band above the β -zein appeared at 37 kDa, representing a zein dimer of γ -zein. Zein with ethanol and isopropanol (Lanes 1–6) showed similar patterns, with broad bands at 19–24 kDa representing α -zein. On the other hand, zein with acetic acid extraction (Lanes 7–9) showed a small

band beside the main bands at 17 kDa, which could have represented β -zein. Additionally, dimers were represented by thin bands at 37 kDa and 47–48 kDa. Zhu, Kale, and Cheryan [32] obtained the same results with extracted zein from whole corn with minor amounts of δ - and β -zein at low molecular weights compared with commercial zein, which only presented α zein. FT-IR was used to probe the zein-corn structure under different conditions of solvent extraction (Figure 2). The FT-IR spectra from 4000 to 400 cm^{-1} of corn zein using the different extraction solvents showed the typical protein sequence absorption bands, consisting of amide A ($3600\text{--}3100\text{ cm}^{-1}$), amide I ($1700\text{--}1600\text{ cm}^{-1}$), and amide II ($1575\text{--}1480\text{ cm}^{-1}$) [13, 33, 34]. Each sample's zein spectrum had a similar pattern to the commercial spectrum. According to Forato [33], β -zein could be detected by FT-IR by the presence of a peak at 1656 cm^{-1} . However, the quantity of β -zein was low, and the peak could not be clearly seen in FT-IR chart. The only difference between the sheet structures resulted from fatty acid contamination, as seen in the peak at $2980\text{--}2850\text{ cm}^{-1}$. This was found as a result of the high amount of fatty acid in zein from extraction using a high concentration of solvent [31]. Zein with acetic acid extraction showed such a pattern in its spectrum, because the structure of protein had already been hydrolyzed to small molecules of amino acid and fatty acid [11]. According to a study by King [15], glacial acetic can fully solubilize zein (α -, β -, and γ -zein). Thus, zein extracted with acetic acid could contain both α - and β -zein. Both extracted zein samples with 70% isopropanol (consisting of α -zein) and 70% acetic acid (consisting of α - and β -zein) were selected for further study of their film properties and drug control release properties.

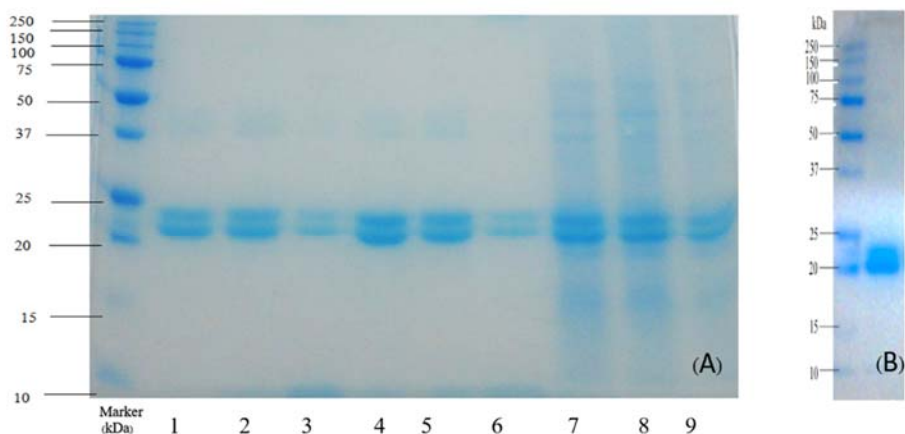


Figure 1. SDS-PAGE of (A) zein proteins extracted from corn kernel Suwan 5 by using ethanol (lane 1, 2, 3; isopropanol (lane 4, 5, 6) acetic acid (7, 8, 9) at concentration 70%, 80%, 90%, respectively and (B) commercial zein

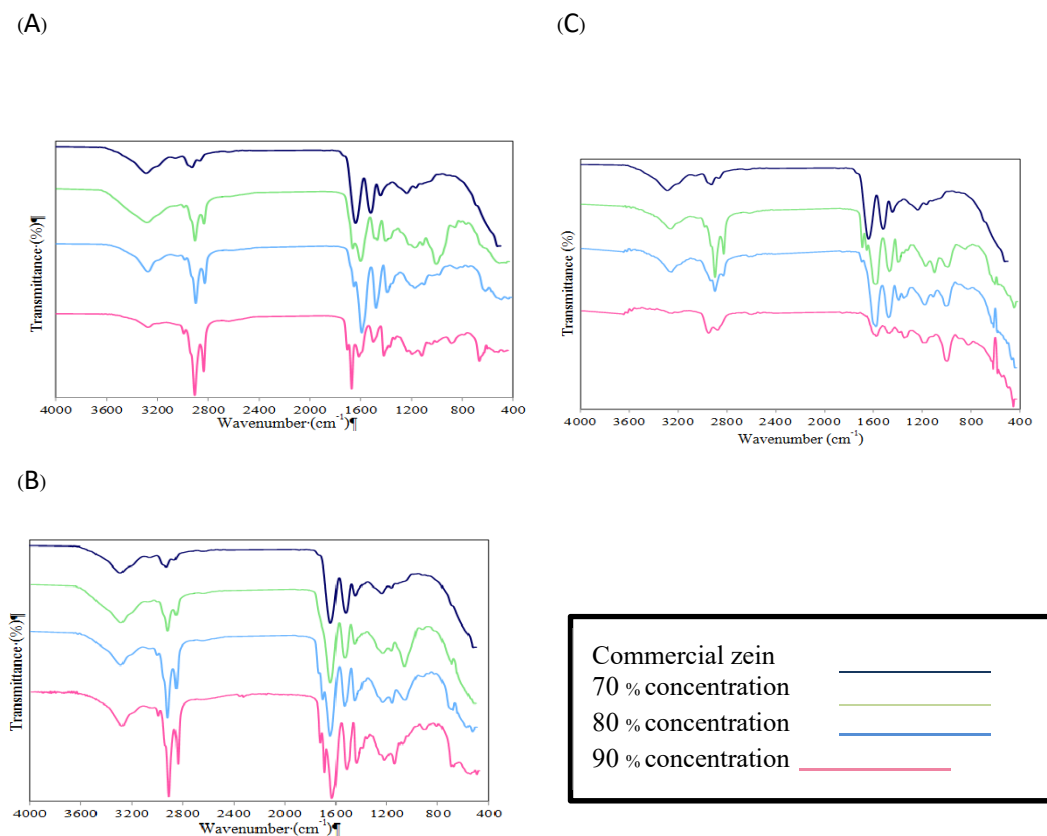


Figure 2. Fourier transform infrared spectra of the zein protein extracted by using (A) ethanol, (B) isopropanol and (C) acetic acid at concentration 70%, 80% and 90% comparing with commercial zein.

3.3 Mechanical properties of the zein films

Based on the study of the zein structure (SDS-PAGE and FT-IR), zein films were prepared using two types of extracted zein. The first consisted of only α -zein (70% isopropanol solvent) (zein 1), while the second consisted of α - and β -zein structures (70% acetic acid solvent) (zein 2). Basically, the moisture barrier properties and control drug release were the main features of the protein-based film used for the tablet film coating. However, the mechanical properties of biopolymers film are equally important to maintain the structural integrity of the tablet as well as providing protection. The tensile strength and elongation at failure and the modulus of the commercial and extracted zein films with/without plasticizers are shown in Table 2. Both the 10% and 20% concentrations of zein without a plasticizer had a low tensile strength of 0.49 N and 0.67 N, respectively, and elongation at 4.36% and 8.56%, respectively, which are quite low because of the brittle nature of the zein film free of plasticizer [27]. Plasticizers, such as TEC or PEG 400, are usually added to modify the mechanical properties of zein film because the plasticizer can embed itself between the polymer chains and reduce the intermolecular and intramolecular interactions of polymer molecules, thus lowering the glass transition temperature (T_g) of the film and improving the flexibility [14, 35]. Consequently, a comparison was then made between commercial zein film with added TEC and

PEG 400 for both levels of zein contents, and they showed significant differences in the % elongation at 7.4% and 35.2% in the 5% zein content and 7.84% and 38.52 % in the 10% zein content films, respectively. Basically, zein is a non-polar protein and contains a lot of hydrophobic amino acids, which causes a low vapor permeability in the zein structure [6]. Zein film added with PEG 400, which is highly polar and has a higher molecular weight than TEC, could reduce the number of hydrogen bonds and prolong the distance between zein molecules, resulting in the zein film structure being more flexible and moveable. Thus, PEG 400 was more suitable to apply in the tablet coating than TEC in our further study. Film coatings using extracted zein 1 and extracted zein 2 with added PEG 400 were compared based on their mechanical properties. The film with extracted zein 2 had a higher % elongation than the film with extracted zein 1 (144.83% and 84.4%, respectively), because the short chains of β -zein in extracted zein 2 increased the distance between the α -zein molecules. Thus, the film with extracted zein 2 was more flexible at the molecular level [35].

Table 2. Mechanical properties (tensile strength and elongation) of zein films

Type of zein	Plasticizers	Film thickness (mm)	Tensile strength (N)	Elongation (%)
5% Commercial zein	None	0.49±0.11 ^{bc}	10.1±4.4 ^a	4.36
	TEC	0.31±0.06 ^d	3.73±0.9 ^b	7.4
	PEG 400	0.41±0.10 ^{cd}	2.49±1.98 ^{bc}	35.2
10% Commercial zein	None	0.67±0.20 ^a	4.52±1.60 ^b	8.56
	TEC	0.56±0.18 ^{ab}	12.73±4.6 ^a	7.84
	PEG 400	0.57±0.13 ^{ab}	4.45±2.87 ^b	38.52
10% Extracted zein 1*	PEG 400	0.31±0.08 ^{cd}	2.60±2.11 ^{bc}	84.4
10% Extracted zein 2**	PEG 400	0.28±0.01 ^d	0.18±0.09 ^c	144.83

* Extracted zein 1 is zein from 70% isopropanol solvent

**Extracted zein 2 is zein from 70% acetic acid solvent

a – d Means± SD followed by difference letters in each column were significantly difference at P<0.05

3.4 Drug release from tablets coated with zein

The release of theophylline and CPM demonstrated different release kinetics. The release from the tablets without a coating was rapid, reaching 90% within 45 min (Figures 4 and 5). With commercial zein, the drug release was slow and the cumulative drug release was less than 5% after 30 min, except for the coated CPM tablets in 0.1 N HCl because of the high solubility of the weak basic drug in the acidic solution. The pulsatile release from the theophylline and CPM tablets coated with the commercial available zein are presented in Figures 4 and 5. The pulsatile release could be explained by the penetration of solvent into the dosage forms and as the drug release started after there had been sufficient water adsorption to break the films. Surprisingly, the drug release of the tablets coated with the extracted zein showed zero-order release profiles (Figures 4 and 5). A linear relationship of the drug release over time was achieved. Zero-order release profiles from coated tablets prepared by both extracted zeins occurred regardless of the model drug and the release medium used in the study. The solvent penetration into the core tablet retarded the drug release [1].

The low tensile strength and the greater elongation, which represent good mechanical properties of the extracted zein films, helped the films to remain intact. The extracted films could control drug release over a period of 4 h. However, the stability of the tablets was a concern. Batch-to-batch variation of the extracted zein might be further studied to ensure the stability of the coated tablets using extracted zein.

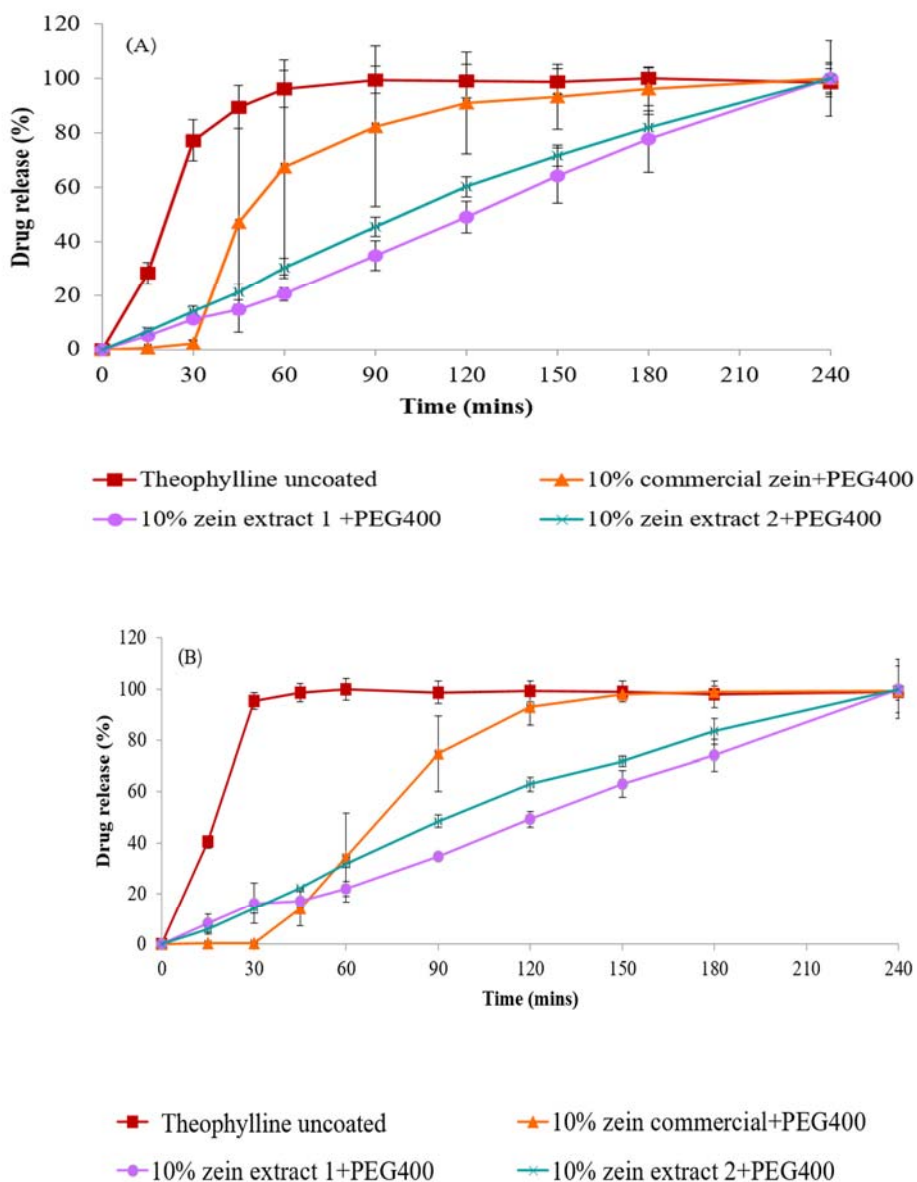


Figure 3. Dissolution profiles of tablets containing Theophylline; uncoated tablets, 10% commercial zein coating with PEG 400, 10% extracted zein 1 with PEG 400 and 10% extracted zein 2 with PEG 400; (A) 0.1 N HCl and (B) phosphate buffer pH 6.8

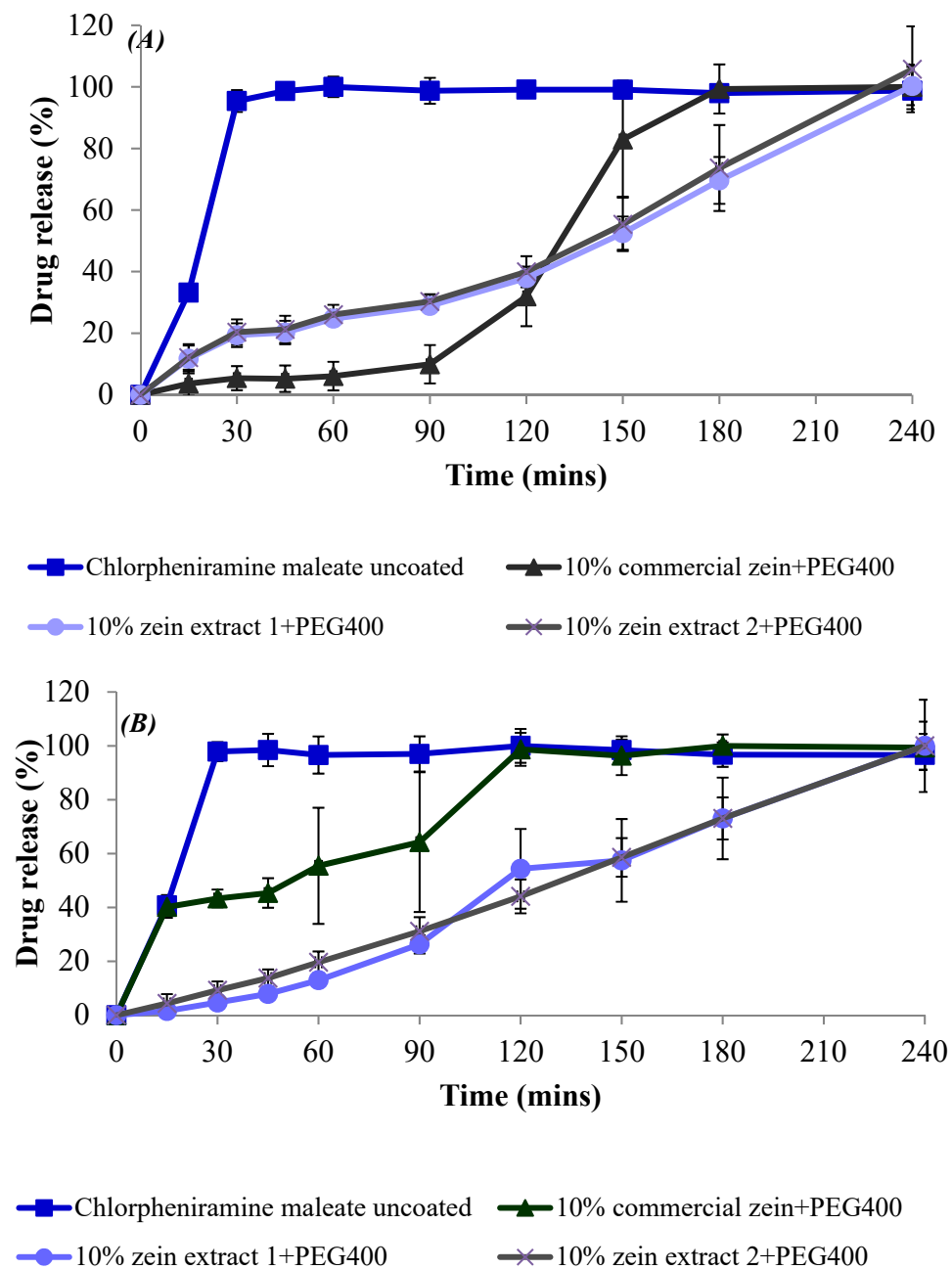


Figure 4. Dissolution profiles of tablets containing CPM; uncoated tablets, 10% commercial zein coating with PEG 400, 10% extracted zein 1 with PEG 400 and 10% extracted zein 2 with PEG 400; (A) 0.1 N HCl and (B) phosphate buffer pH 6.

4. Conclusions

Based on the results of this study, the most suitable solvent for zein extraction was 70% isopropanol, which had the highest % yield and protein content. However, we found that the extracted zein from acetic acid consisted of β -zein and α -zein compared with the extracted zein from 70% isopropanol having only α -zein. Thus, acetic acid could extract more of the smaller molecular weight β -zein than the alcohol solvents due to their hydrolytic property. The structures of both extracted zeins showed similar patterns. In this study, the two extracted zeins were applied in a tablet coating. PEG 400 was more effective as a plasticizer for improving the elongation properties than TEC, due to its high molecular weight and polarity, and thus the addition of PEG 400 in both types of extracted zein film could improve their elongation more than in the commercial zein film, especially for the zein extracted using the acetic acid solvent. The drug release of coated tablets from extracted zein showed a zero-order release profile with a linear relationship between time and drug release for both drug models.

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Effect of Additional Laser-Sensitive Pigment on Marking Brightness and Physical Property of Polypropylene Plastic

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Abstract

In this study, laser marking was performed by radiative laser beam having wavelength of 1064 nm onto the surface of polypropylene (PP) specimens with laser-sensitive pigment addition. The different amount of laser-sensitive pigment were added onto the PP plastic in the range from 0 to 4.5 wt%. The effect of laser marking parameters such as laser power, scanning speed and pulse frequency on the contrast between marking pattern and the specimens were investigated. In addition, the physical properties such as tensile strength, elongation at break and heat-resistant of the specimens with laser-sensitive pigment addition were determined. The results showed that the brightness of marking pattern was increased as the amount of pigment was increased due to the high absorption of laser-sensitive pigment in the specimens. The pigment content of 0.6 wt% was achieved for the highest contrast laser marking of PP. For the physical characteristics, PP plastic with laser pigment addition showed the decrease of tensile strength as the laser pigment was increased. However, the laser pigment addition can increase heat-resistance of PP plastic. The optimum laser parameter for maximization of brightness of marking pattern and maintaining the physical property were also found out in this study.

Keywords: laser marking, laser-sensitive pigment, PP plastic, tensile strength

1. Introduction

Indelible marking has been used in various manufacturing such as automotive, electronics, medical and food industries. The manufacturers use direct part marking to identify their components which are able to date codes, product name, bar codes or part numbers and company logos, etc. [1-4]. It is an important role to enable traceability, product unique identification and obstacle product counterfeiting. In general, laser can directly mark on glass, ceramics [3, 5-7], plastics [1-3, 8-15], metals [16-18], stainless steel [4, 19], and organic materials [4, 20] that provide many advantages over traditional methods such as heat pumps, ink-printing, ink-refilling, stamp, handwriting and dot peen marking [1-3, 5]. Because of these methods above mentioned, it is non-permanent marking on a high-density material and a glossy surface, so plastic material cannot absorb ink and hold it in place [2, 3].

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On the other hand, laser marking does not affect the environment and cost-effective management due to it does not use any consumables. It is the most flexible way of marking on plastics that provide a yield legible, high-contrast and indelible, with no direct parts contact with the material other than through the incident laser beam. This method is suitable for using on a complex curved or a rough surface and a small specimen. The automated systems would be designed in accordance with operation and can be moved easily [4, 21]. In addition, it is highly repeatability, high speed marking and reliability. However, the poor conditions of laser speed, laser power and frequency are affected to the low contrast or marks disappear on the material. And the dust control is required in the area where use to operate laser marking machine. If the computer control unit and lens are dirty, it will cause mark distortion. TEA-CO₂ laser, Nd: YAG laser and Excimer laser are currently used for marking on plastic material [1, 3, 22-30]. But, three lasers above mentioned can mark on PP plastic but it doesn't appear of brightly colored. Thus, the pigment and additive systems were considered to enable the contrasting colors and degrees of shading [1, 31]. Once of the pigment is (Mica + Fe₃O₄) that uses in additive on PP plastic. In addition, the advantage of this pigment is absorbing laser wavelength and cause the bright color marking [31]. PP plastic is widely used in automotive parts, pipes, tanks, batteries, bottles, packaging, medical equipment, sacks of rice and chemical bottle [32, 33]. It is a thermoplastic polymer that changes properties when heated and cooled. The melting point is approximately 165 ° to 177 °C and a density of 0.90 g/cm³. In addition, it has an excellent chemical resistance property, endurance, shock-resistant structure, lightweight, well formed, medium flexion, does not absorb moisture, high hardness and toughness. However, low temperatures can be more harmful to PP plastic than high temperatures, resulting in brittle and crack. The sun rays do not suitable for PP plastic because it affects to a pale color. Carbon black was also incorporated in a way to improve the property of PP plastic. Antioxidants help prevent thermal oxidation reactions when plastics are exposed to UV light. In this work, we were investigated the effects of (Mica+Fe₃O₄) pigment on the contrast of marking pattern and physical properties of PP plastic such as tensile strength, elongation at break and heat-resistant. The different amount of laser-sensitive pigment was added to the PP plastic in the range from 0 to 4.5 wt% with 0.2 wt% carbon black. The effect of laser marking parameters such as laser power, scanning speed and pulse frequency on the contrast of PP specimens was investigated.

2. Materials and Methods

2.1 Sample preparation

In the study, PP plastic mixing with laser-sensitive pigment (Mica CAS No. 12001-26-2 and Fe₃O₄ CAS No. 1317-61-9, MERCK) were used as laser marking specimens. The amount of laser pigment was varied between 0 and 4.5 wt% and fixed 0.2 wt% carbon black. The specimens were casting by injection molding process for a dimension of 3 in x 3 in x 3 mm (width x length x thickness). Figure 1 shows raw materials containing PP, laser pigment and carbon black. The specimens were laser-marked under the different laser conditions. In order to obtain highly brightness and keeping physical properties such as tensile strength, elongation at break and heat-resistant, the optimum content of laser-sensitive pigment with PP was investigated.



Figure 1. Raw materials (a) PP plastic, (b) laser pigment and (c) carbon black for casting process

2.2 Laser condition setting

In this experiment, Q-switched laser wavelength 1064 nm was used for laser marking. Laser has a maximum power of 20 W and the highest frequency range of 1000 kHz. The characteristics width pulse were 3-500 ns, focal plane diameter less than 20 μ m, pulse frequency up to 75 kHz, pulse energy at 0.7 mJ and the laser focus at 160 mm. Laser condition setting was important for marking brightness, marking depth, and plastic surface melting on the specimens. In this experiment, the variables such as power, frequency and marking speed were setting as shown in Table 1.

Table 1. Laser condition setting (marking speed, laser power, and frequency) for the experiments

Laser Condition Setting			
Experimental	Speed (mm/s)	Power (%)	Frequency (kHz)
1	500 - 2500	100	40
2	1500	75 - 95	40
3	1500	100	20 - 70

2.3 Sample Characterization

2.3.1 Color

The paint software was used to analyze the color of laser marking specimens by using Color Picker mode. Color measurement with paint software was processed through RGB system. In this study, the color that arises from laser marking is white color characteristic. The gray scale image system is available for color level from dark until white colors. The maximum white color level of 255 pixels and dark color level of 0 are defined. The value is the intensity of light/brightness in each of the positions in the gray scale [34-37]. In the order to measure color brightness with the paint software, we developed the setup to take the picture with a digital camera as shown in Figure 2. The lighting conditions were controlled at angle of 90° and it also controlled the condition of the camera to take a picture on the specimens.

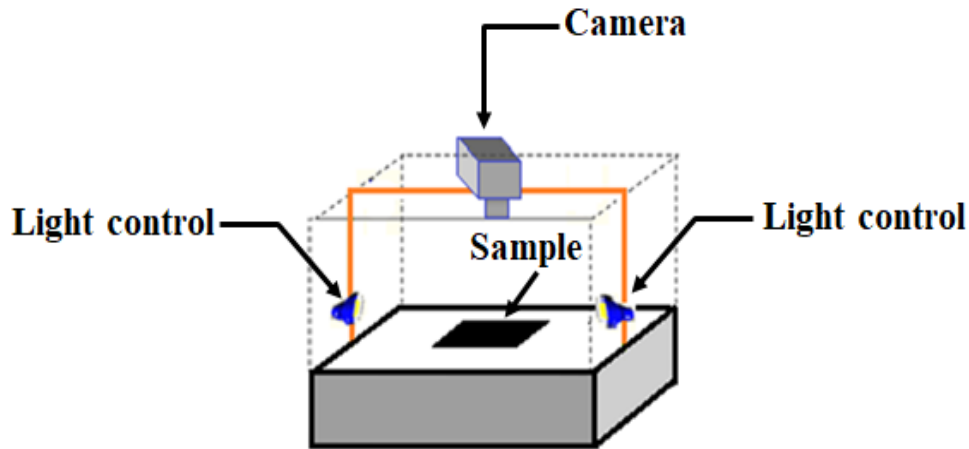


Figure 2. Schematic diagram of camera setup and imaging for laser marking samples

2.3.2 Tensile strength

Tensile strength analysis such tensile strength, Young's modulus and yield strength was used to determine the mechanical properties of laser pigment added in the specimens. The analysis was determined under the ASTM D638 standard. The different content of laser pigment in PP plastic at 0.2 wt%, 1.5 wt%, 3.0 wt% and 4.5 wt% were measured. For sample preparation, we prepared specimens in dimension of 19 mm x 165 mm x 3.2 mm, following the measurement standard [38, 39], as shown in Figure 3(a). After that the specimen brought to fix on both ends in tensile test machine as shown in Figure 3(b). The testing parameters consisted of pulling speed 50 mm/min, room temperature 23 ± 2 °C, and recorded the peak load until the maximum stress. Tensile tests showed the relationship between stress and strain.

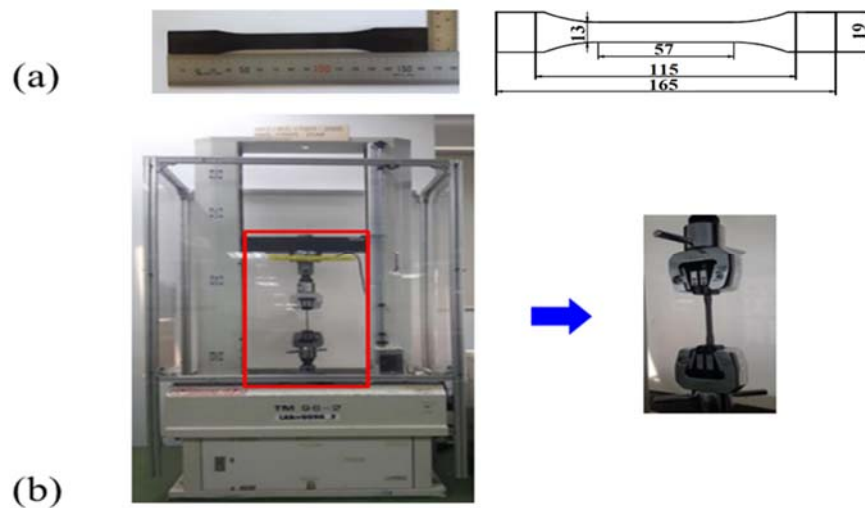


Figure 3. (a) Specimen used for tensile strength testing and (b) Testing equipment

2.3.3 Heat resistance analysis

Thermogravimetric analysis (TGA) is used to analyze the thermal stability of the laser pigment added in the specimens at different contents. Thermogravimetry measures the mass or weight loss of specimens related to temperature or time [40]. The specimens change the weight more or less during the temperature, depending on the characteristic of materials. The lost weight is caused by melting, evaporation or chemical reactions. The test conditions were shown as following; temperature at test start 50°C, maximum test temperature 600°C, temperature rising at 10°C/min.

3. Results and Discussion

3.1 Effect of laser pigment content on color brightness

The marking pattern was defined by the shape to the square 1cm x 1cm on PP plastic specimens with dimension of 3 in x 3 in x 3 mm as shown in Figure 4.

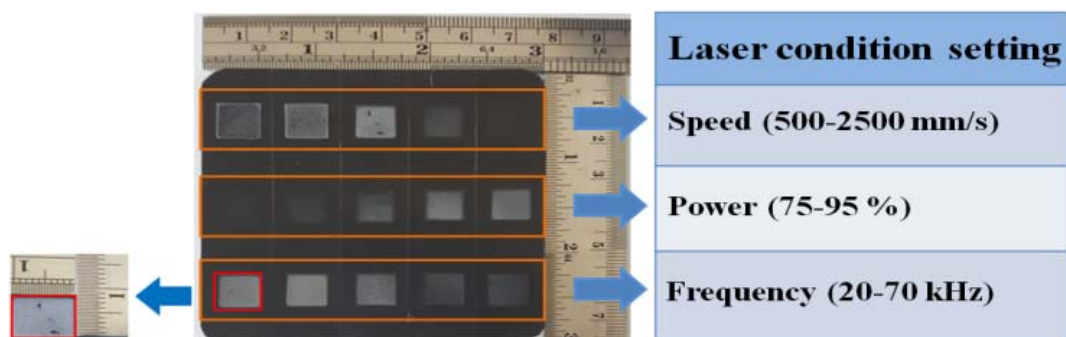


Figure 4. PP plastic specimen with dimension of 3 in x 3 in x 3 mm and color brightness analysis area of 1 cm x 1 cm by different laser condition setting

Figure 5 shows the color brightness of marking pattern with different laser-sensitive pigment content. It was obtained by paint software as shown in Table 2. The results presented in red, green and blue (RGB), following by $Y = 0.3R + 0.59G + 0.11B$ [36, 37]. It was found that the color brightness of the specimen without laser pigment was 56. The color brightness was obviously increased to 168 as the laser-sensitive pigment was increased up to 0.6 wt%. However, the laser-sensitive pigment beyond 1.0 wt% became the lower brightness levels. The color brightness was decreased from 168 to 46 as increase the laser-sensitive pigment to 4.5 wt%. It may be caused by the opaque of laser-sensitive pigment that affected the heat-chemical reaction. The laser-sensitive pigment permeated into the PP plastic and could not melt at the processing conditions. A high opaque was found at the excess laser-sensitive pigment or the poor condition that induced a low laser absorption and lower color brightness [1]. Laser-sensitive pigment of 0.6 wt% was optimized because the laser interaction with the material was complete. Indirect parameters such as, the surface texture of the specimen, the particle size distribution of pigments and the image brightness for shooting are an important role to control the quality of marking.

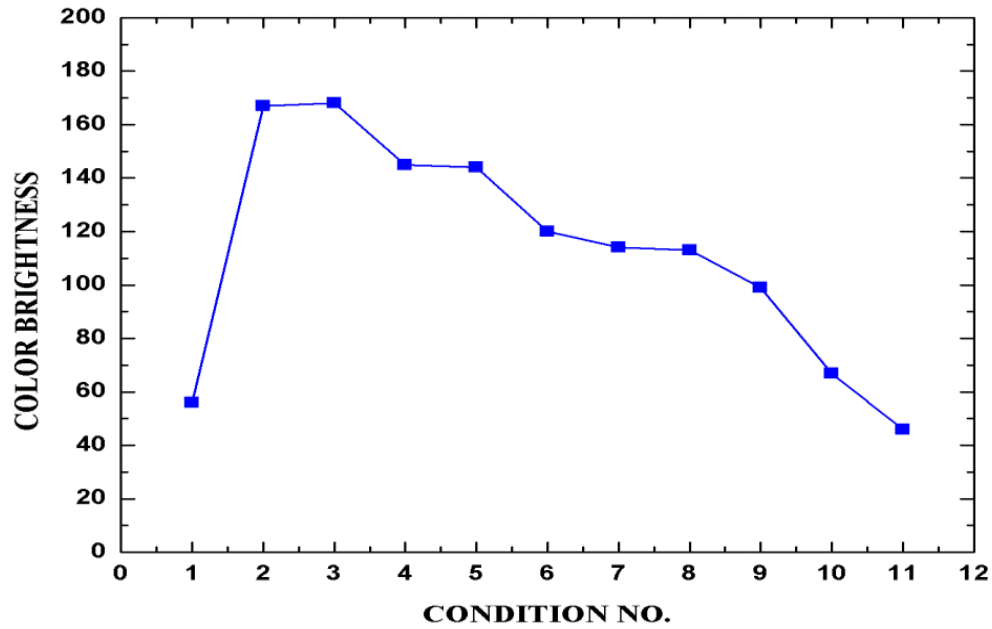


Figure 5. Color brightness of marking pattern on different amount of laser-sensitive pigment specimens

Table 2. Color brightness of marking pattern on different amount of laser-sensitive pigment specimens

Condition No.	Laser-Sensitive Pigment Content (wt%)	Color Brightness (Y)
1	0.0	56
2	0.2	167
3	0.6	168
4	1.0	145
5	1.5	144
6	2.0	120
7	2.5	114
8	3.0	113
9	3.5	99
10	4.0	67
11	4.5	46

3.2 Effect of laser condition setting on marking brightness

From the analysis results, 0.6 wt% of laser pigment was suitable for laser marking on PP plastic. Therefore, this content was chosen to study the laser marking parameters such as laser speed, power and frequency. Figure 6 shows the measurement results of color brightness in different setting speed at 500 mm/s, 1,000 mm/s, 1,500 mm/s, 2,000 mm/s and 2,500 mm/s with power at 100% and frequency at 40 kHz. The results shown that the speed at 1,500 mm/s provided the maximum marking brightness. For the speed setting lower than 1,500 mm/s, the color brightness was decreased due to melting of specimen surface. The surface of the specimen absorbed longer period of the laser energy, heated up, and melted. For the speed setting more than 1500 mm/s, the brightness of the marking pattern was gradually reduced as shown in Figure 6. It was due to the incomplete of decomposition reaction of laser pigment and laser energy. This result indicated that the laser scan speed was another important parameter for laser marking.

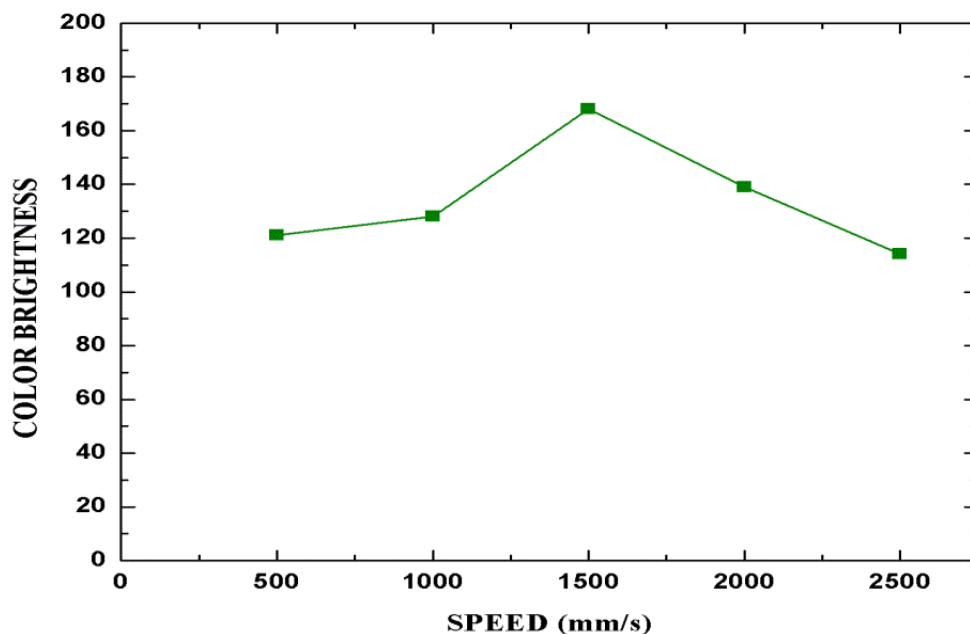


Figure 6. Color brightness of marking pattern as a function of laser speed on laser-sensitive pigment of 0.6 wt% specimen

Figure 7 shows the results of color brightness with the different laser power setting at 75%, 80%, 85%, 90% and 95%. The speed and frequency of laser was fixed at 1500 mm/s and 40 kHz, respectively. The maximum color brightness was obtained at the power of 95%. It was due to the high energy induced- melting of laser pigment that affect to the color reaction [3]. In addition, the suitable of laser speed and frequency were significant to the marking brightness.

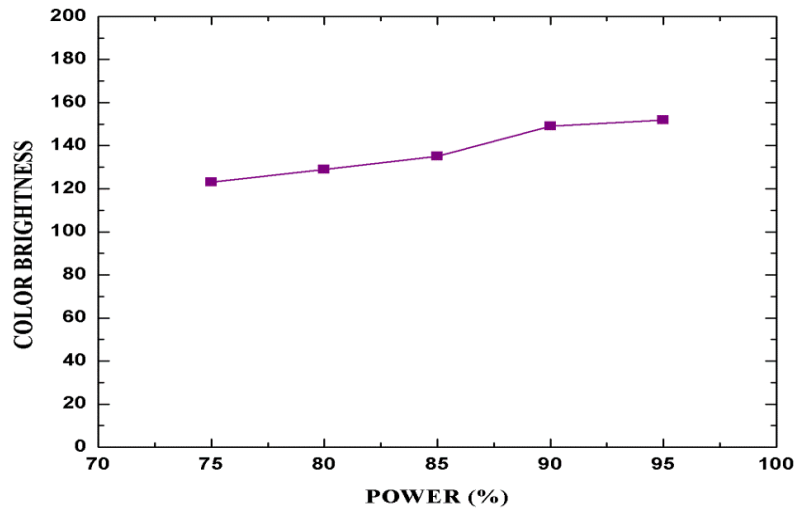


Figure 7. Color brightness of marking pattern as a function of laser power on laser-sensitive pigment of 0.6 wt% specimen

The difference of laser frequency was studied at 20 kHz, 35 kHz, 50 kHz, 65 kHz and 70 kHz under maintaining scan speed and power of 1,500 mm/s and 100%, respectively. Figure 8 shows the color brightness of the marking pattern on the specimen with 0.6 wt% pigment as a function of laser frequency. The color brightness was similar for the laser frequency of 20 to 35 kHz. However, the color brightness decreased as the increase of laser frequency from 35 to 70 kHz. It causes by the excessive repeatability per one pulse that affected to overlapping densities of the marking lines [4].

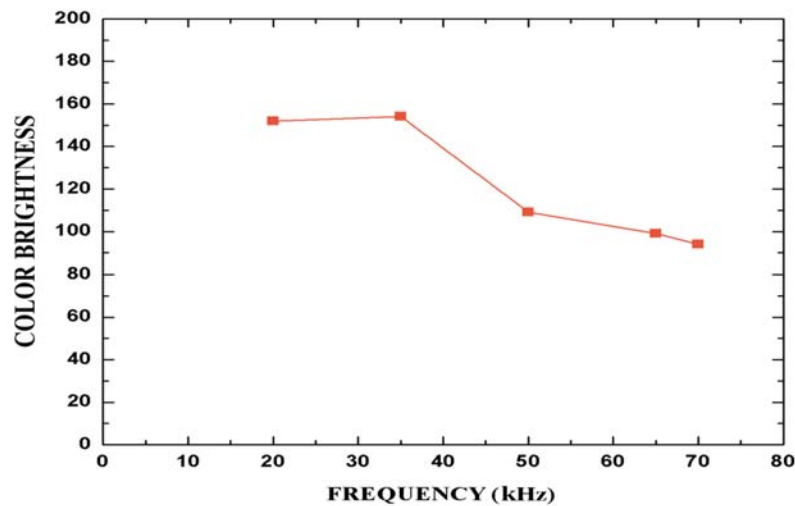


Figure 8. Color brightness of marking pattern as a function of laser frequency on laser-sensitive pigment of 0.6 wt% specimen

3.3 Effect of laser-sensitive pigment on physical property

Load versus elongation curve of specimens with different amount of laser pigment is shown in Figure 9. The calculation parameters such as tensile strength, Young's modulus, and yield strength were obtained in Table 3. The results shows that tensile strength, Young's modulus, and yield strength were slightly decreased as the laser pigment was increased from 0.2 wt% to 4.5 wt%. This result indicated that a small addition of laser pigment slightly affected the physical property of PP plastic. It caused by the laser pigment permeates into the PP plastic as foreign matter that affect physical properties on PP plastic.

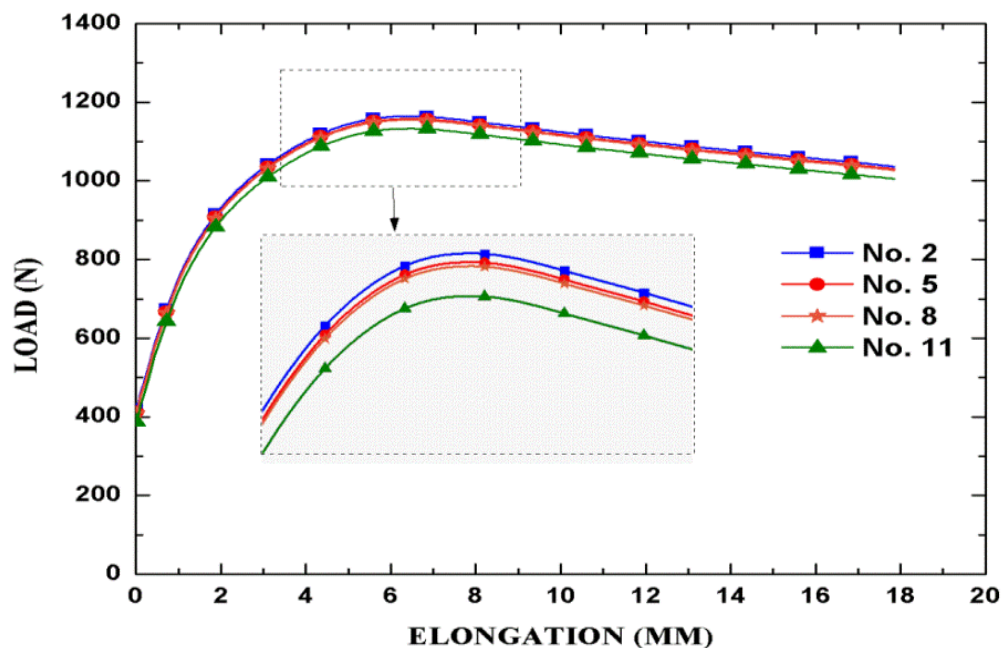


Figure 9. Tensile strength of PP plastic with the different amount of laser-sensitive pigment

Table 3. Tensile results of PP plastic with the different amount of laser-sensitive pigment

Condition No.	Test Results76				
	Laser-Sensitive Pigment (wt%)	Tensile Strength (N)	Tensile stress (MPa)	Young Modulus (MPa)	Yield Strength (MPa)
2	0.2	1164.614	27.996	426.520	21.870
5	1.5	1158.113	27.840	423.413	21.576
8	3.0	1155.169	27.766	415.406	21.296
11	4.5	1133.544	27.250	405.853	20.810

3.4 Effect of laser pigment on heat resistance

The heat resistance of the specimens was analyzed by thermogravimetric (TGA) in the thermogram pattern as shown in Figure10. The value of decomposition temperature was determined by a point that the specimen was lost the weight. The heat resistance was directly proportional to the decomposition temperature. It was found that the decomposition temperature of specimens depended on the content of laser pigment addition. The decomposition temperature increased from 314.38°C to 338.35°C as the laser pigment was increased from 0.2 wt% to 1.5 wt%. However, the temperature decreased after the laser pigment was increased from 1.5 wt% to 4.5 wt%, as shown in Table 4. The excessive laser pigment was not beneficial because it became a foreign matter on PP plastic. Thus, 1.5 wt% of laser pigment addition was suitable to enhance the heat resistance of PP plastic.

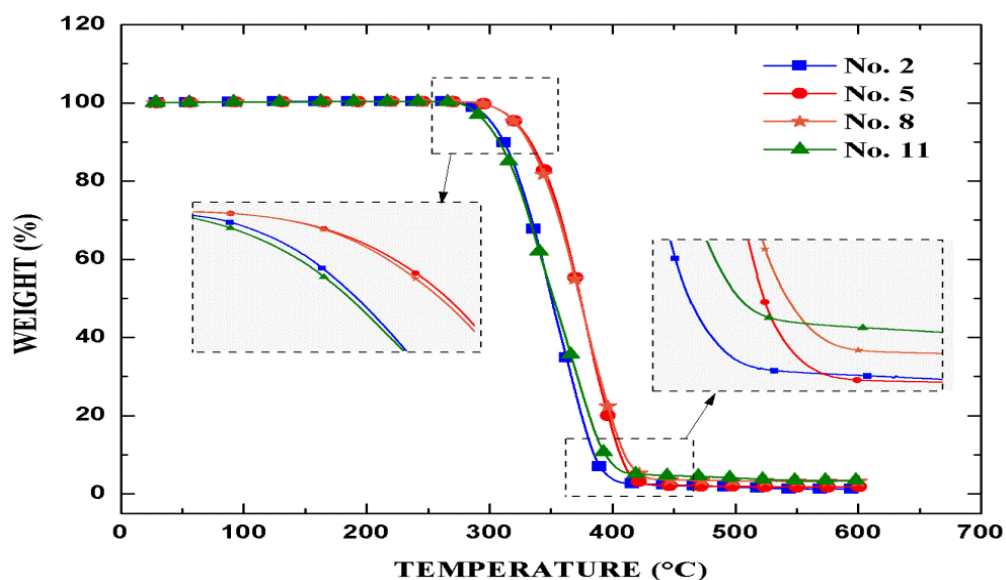


Figure 10. Heat resistance by Thermogravimetric Analysis (TGA)

Table 4. TGA measurement of specimens with different amount of laser-sensitive pigment

Condition No.	Laser-Sensitive Pigment Content (wt%)	Test Result	
		Temp (°C)	Weight (%)
2	0.2	314.38	98.865
5	1.5	338.35	98.298
8	3.0	335.35	96.826
11	4.5	305.14	96.609

4. Conclusions

The effect of laser-sensitive pigment addition on color brightness and physical property of PP plastic was investigated by using Q-switches laser making. The color brightness and physical property corresponded with the laser pigment content. The optimum laser pigment of 0.6 wt% was obtained that achieves both maximum color brightness and maintaining the physical property of PP plastic. In addition, laser parameters consisting of power, and speed were important factors to control the color brightness. Laser setting of speed at 1,500 mm/s, power at 100% and frequency at 40 kHz were optimized for the maximum color brightness on the laser-sensitive pigment of 0.6 wt% specimen. In the addition, the results of the physical properties of PP plastics revealed that small amount of laser-sensitive pigment slightly affect to tensile strength, Young's modulus, yield strength while it helped to increase heat-resistant of the specimens. Therefore, the appropriate laser pigment must be taken into consideration.

5. Acknowledgements

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A New Method To Detect IDH1 Synonymous SNP 105 C >T rs11554137

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Abstract

Alteration of cellular metabolism is one of the oncogenetic mechanisms. Furthermore, the defect of isocitrate dehydrogenase (*IDH*) enzymes lead to abnormal cellular metabolism. Mutation in the *IDH1* gene is common in glioblastoma multiforme (GMB), glioma, and acute myeloid leukemia (AML). The *IDH1* single nucleotide polymorphism (SNP) in codon 105 C>T rs11554137 is a prognostic factor in patients with glioma and AML. Currently, the most common technique used for *IDH1* SNP 105 C>T detection is the sequencing technique that expensive and time-consuming. We designed a new method through the Restriction Fragment Length Polymorphism (RFLP). Ninty Eight unrelated healthy volunteers comprising 49 males, and 49 females, which were randomly selected. Their ages ranged from 1 to 64 years with a median age of 33 years. Polymerase chain reaction (PCR) and *RsaI* fragmented *IDH1* SNP105 C >T were identified in 16.3% of our cases. So we concluded that we may use the PCR-RFLP assay instead.

Keywords: *IDH*, SNP, RFLP, AML, Glioma

1. Introduction

Isocitrate dehydrogenase (*IDH*) enzyme encoded from *IDH* gene is one of the β -decarboxylating dehydrogenase family of enzymes which can convert the isocitrate to α -ketoglutarate (α -KG) in the tricarboxylic acid (TCA) cycle. This conversion is a vital biochemical pathway for the production of amino acids, nucleotides, and lipids [1–3]. *IDH* enzymes have three isoforms with different subcellular localization in mammalian cells: the cytosolic *IDH1* enzyme and mitochondrial *IDH2* and *IDH3* enzymes [1, 3–7]. The location of *IDH1* gene is on chromosome band 2q33.3 [3] and its SNP located in codon 105 in exon 4 [8].

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IDH mutations are usually heterozygous with one wild-type allele and one mutant allele [8]. Furthermore, they are common mutations in patients with low-grade gliomas and secondary glioblastoma. It is also found in de novo AML, secondary AML (sAML) [9], cholangiocarcinomas, in both benign and malignant central cartilaginous tumors [10], angioimmunoblastic T cell lymphomas, thyroid cancer, colorectal cancer, and prostatic cancer [11]. *IDH1* synonymous SNP, representing a GGC to GGT conversion at the glycine residue 105 [12] is associated with bad prognosis in patients with CN-AML [8, 13], AML with NPM1/CEPB α mutations [14] and in Glioblastoma multiforme GMB [15].

Polymerase Chain Reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP) is a widespread technique for genetic investigations. It has been used for the detection of interspecies and intraspecies variations [16]. PCR-RFLP technique has many advantages including inexpensiveness and does not require advanced instruments or an extensive training of laboratory staff. The technique is also applicable for the analysis of single nucleotide polymorphisms as well as the availability for all molecular biology laboratories and it is easy to design [16].

2. Materials and Methods

2.1 Subject recruitment

Ninety-eight unrelated healthy volunteers comprising 49 males and 49 females were selected from blood donors. Their ages were ranged from 1 to 64 years with a median age of 33 years. All volunteers or their guardians for this study have signed a consent. PCR-RFLP was used for the assessment.

2.2 Analysis of *IDH1* SNP105 C>T rs11554137 by PCR-RFLP

DNA was extracted from EDTA anti-coagulated blood samples from normal volunteers by using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Genomic DNA was amplified specifically for exon 4 of *IDH1* gene, using forward primer: 5'-CCAAGGATGCTGCAGAAGC-3' and reverse primer: 5'-CCCCATAAGCATGACGACC-3'. PCR was performed in 20 μ l reaction mixture containing 10 μ l ready to use 2X master mixed solution (i-Taq) (Intron Biotechnology, Inc, Korea) containing dNTPs, 25 mM MgCl₂, PCR-buffer, i-Taq DNA polymerase and DNA loading dye, 100 ng/dl of genomic DNA, 0.4 μ l of 10 pmol/ μ l forward primer and 0.4 μ l of 10 pmol/ μ l reversed primer (Invitrogen - Thermo Fisher Scientific, Inc.) and the total volume was completed with nuclease-free distilled water. PCR condition was as follows: initial denaturation at 95°C for 150 seconds, followed by 35 amplification cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds followed by final extension step at 72°C for 10 minutes. The amplified products were separated on 2% agarose gel electrophoresis, stained with ethidium bromide. Nebcutter V.2-0 application (<http://www.labtools.us/nebcutter-v2-0>) was used to define suitable restriction enzyme for cutting variant type at the correct site of the SNP. PCR products were digested with *RsaI* enzyme (Fermentas - Thermo Fisher Scientific, Inc.), the reaction was done by addition of 10 μ l PCR product, 20 μ l reaction master mix containing (17 μ l nuclease-free distilled water, 1 μ l *RsaI* FastDigest enzyme and 2 μ l FastDigest 10X buffer) and incubated at 37°C for 60 min instead of 5 min to be sure the digestion is completely done.

2.3 Statistical analysis

Data were analyzed using the SPSS statistical package for social science version 16 (SPSS, Inc., Chicago, IL, USA). Median and range as appropriate were determined to represent numerical data. Frequency and percentage were used to express qualitative data. T-test was used for comparison of quantitative parametric data and Chi-square test for categorical variables. P value was set at 0.05 level.

3. Results and Discussion

3.1 Detection method of *IDH1* SNP 105 C>T rs11554137

The amplified product of *IDH1* gene produced a band at 240 bp (Figure 1). Restriction Fragment Length Polymorphism (RFLP) is a new method used to identify the *IDH1* SNP 105 C>T, where the digestion of PCR product is by enzyme *RsaI*, that recognizes the sequence GTAC and digests the sequence that possesses the variant type *IDH1* SNP into 146 bp and 94 bp fragments (Figure 2). The reactions run in simple thermocyclers, which are available in every laboratory working in molecular biology, and there is no need for a dedicated software to assess the results. This is the first analysis of the *IDH1* SNP 105 C>T by the PCR-RFLP technique.

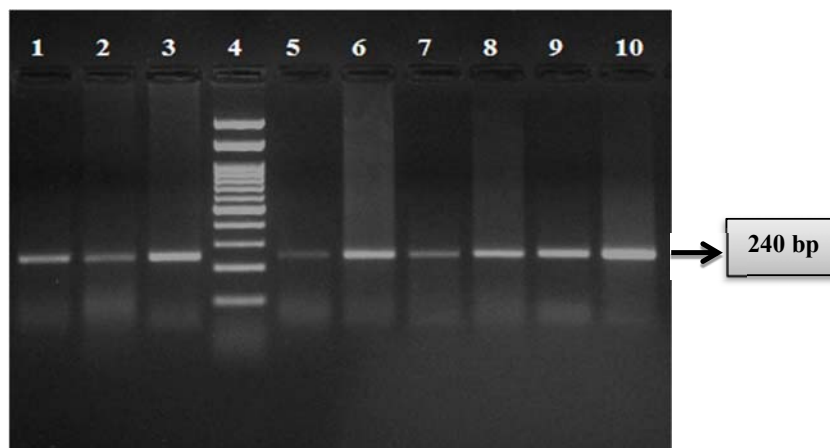
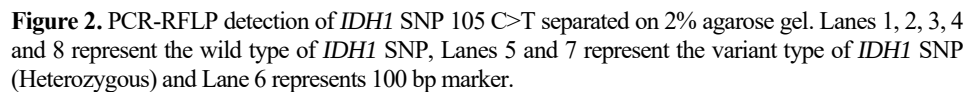


Figure 1. PCR product of *IDH1* SNP 105 C>T separated on 2% agarose gel. Lane 4 represents 100 bp marker and other lanes represent 240 bp amplified products.

Sequencing technique is the most common method which used to detect *IDH1* SNP 105 C>T rs11554137, but it is expensive and needs to specific machines that did not available in most laboratories. Many studies used sequencing technique in *IDH1* SNP 105 (C>T rs11554137 detection) [8, 12–15, 17–20] while some studies used other techniques and followed by sequencing as Single Strand Conformation Polymorphism (SSCP) [21] and PCR-Denaturing High-Performance Liquid Chromatography (PCR-DHPLC) [9, 22]. In addition, some studies use other methods like a light cyclers based melting curve assay [12] and real time PCR [23] (as shown in Table 1).



IDH1 SNP 105 C>T was found in 16 out of 98 healthy volunteers (16.3%), which all of them were heterozygous. Besides, there were no significant correlations between sex and age with *IDH1* SNP 105 C>T ($P=0.274$ and $P=0.136$ respectively) (Table 2).

IDH1 synonymous SNP 105 C>T rs11554137 found in many types of malignancies associated with poor prognosis in patients with glioma [15], CN-AML [13], and AML with negative NPM1 and CEPB α mutations [14]. *IDH1* SNP 105 C>T had a great variability in its incidence in normal volunteers according to the population number and ethnical variations.

Table 1. Detection methods of *IDH1* SNP in many diseases and ethnic populations

Method	Disease	Country	Reference
Direct sequencing	AML	Egypt	[14]
Direct sequencing	AML	China	[18]
	Control		
Direct sequencing	AML	Asia, Africa and America	[17]
Direct sequencing	AML	America	[3]
Direct sequencing	AML	Sweden	[8]
Direct sequencing	Control	Germany	[12]
Direct sequencing	CN-AML	Germany	[13]
	Control		
Direct sequencing	Control	Brazil	[19]
Direct sequencing	Malignant glioma	France and Germany	[15]
SSCP Followed by sequencing	Hematological disorders	China	[21]
	Control		
PCR-DHPLC followed by Direct sequencing	Control	Thailand	[9]
PCR-DHPLC followed by Direct sequencing	AML	Thailand	[22]
	Control		
NGS	Brain Tumors	Italy	[20]
	Control		
Real time PCR	Chronic Myeloproliferative Neoplasms	Turkey	[23]
Light cyler-based melting curve assay	AML	Germany	[12]

Abbreviations: AML; Acute Myeloid Leukemia, SSCP; Single Strand Conformation Polymorphism, DHPLC; Denaturing High Performance Liquid Chromatography, NGS; Next Generation Sequencing.

Table 2. Association between sex and age with *IDH1* SNP 105 C>T

	All cases n=98	Wild type n=82	Variant type n=16	P
Sex				0.274
Male, n%	49 (50%)	39 (47.6%)	10 (62.5%)	
Female, n%	49 (50%)	43 (52.4%)	6 (37.5%)	
Age, Median & Range	33 (1-64)	36 (1-64)	28 (10-48)	0.136

4. Conclusions

Sequencing technique with RFLP in our study can be used because of an accurate, simple, sensitive, inexpensive, available and speedy method for *IDH1* SNP105 C >T rs11554137 instead of the complicate method.

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Towards Sustainable Production of Potato under Climate Change Conditions

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Abstract

Nile delta of Egypt is one of the most vulnerable areas subjected to climate change impacts. A large portion of our agricultural cultivation is mainly accumulated in the Nile delta. As a result the agricultural cultivation is facing several challenges due to climate change impacts especially extreme climatic events. This leads to a significant drop in potato production consequently negative socio-economic impacts all over the country. The aim of this paper was to investigate the climate change impacts on one of Egypt's top strategic crops, potato. It was found that assessing climate change impacts on potato cultivation at local level is required. Increasing losses in crop production and quality have been taking place due to climate change in Egypt. It is very useful to identify proper adaptation practices for mitigating climate change impacts in potato production. Decision makers in developing adaptation strategies for potato production in Egypt should be assisted.

Keywords: Adaptation, Climate change, Economic analysis, Egypt, Potatoes

1. Introduction

Potato is one of the most important export vegetable crops in Egypt and the second most important vegetable crop after tomato in economic value. Egypt is one of the top 20 producers of potato worldwide and the top largest producer in Africa [1]. It reached a total production of 4.8 million tons/ year, including 637,434 ton for exportation with a market value of 250 million USD. About 19% of total area devoted for vegetable production is cultivated with potato [2]. Nowadays, potato cultivation is facing several challenges to maintain and improve production, from the point of view of both quality and quantity. In fact, there are many factors that can negatively affect this cultivation such as environmental factors, mainly linked to climate change. This demands a better comprehension of the relationship between climate (specially the extreme climatic events) and critical stages of the potato cultivation. Moreover, socio-economic factors have affected potato production as well [3]. Where, the last decade was increasingly characterized by a strong

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competition for land between urbanization and extensive crop systems, such as potato cultivation. All these factors can bring a significant drop in potato production and have negative socio-economic and environmental impacts all over the country. Few studies investigated the potential impacts of climate change on potato production in USA, some areas in Europe [4-6] and South Africa [7, 8]. As for Egypt, although studies agreed that the Nile Delta is one of the vulnerability areas subjected to climate change impacts and expanding urbanization; to date no similar studies were conducted addressing this issue in Egypt [9].

As a result, there is an increasing necessity to study the vulnerability of potato cultivation to climate change impacts and to identify the main driving factors that can influence the cultivation processes and finally to promote conservation and adaptation strategies specific for different targeted areas. The aim to study is to highlight the importance of investigating climate change impacts on potato production in Egypt to provide sustainable productivity.

2. Materials and Methods

Descriptive analysis was applied to characterize the problem in addition to the quantitative analysis method using some of the various measurements such as simple linear regression. Related data was based on annual data during the period (2000- 2016) obtained from the Central Agency for Public Mobilization and Statistics (CAPMAS), Ministry of Agricultural and Land Reclamation (MALR), the Egyptian Central Department of Agricultural Economics & statistics, the United Nations food and Agriculture Organization statistical database (FAOSTAT), etc.

3. Results and Discussion

3.1 Global potato production

The cultivation of potato is one of the main agricultural activities worldwide for both economic and social reasons. Potatoes were mainly produced and consumed in Europe, North America and the former Soviet Union till early 1990s. After that, potato production and demands increased in developing countries such as Asia, Africa and Latin America [10]. In 2014, the total world potato production was estimated at 381.7 million tons. China is the biggest potato producer with 96 million tons, followed by India with 45.3 million tons. The third of worldwide potatoes is harvested in China and India [11].

As for Africa, since the beginning of 20th century, potato production has been exponentially expanding in the continent reaching 30 million tons in 2013. The adaptation of potato production under wide range of climatic conditions was assisted in its expansion. Nowadays, potatoes are grown by irrigated commercial farms in Egypt and South Africa, and intensively cultivated in a tropical highland such as small farms in Eastern and Central Africa [12].

3.2 Production indicators of Egyptian potatoes

At the national level in Egypt, potato is the most important vegetable crop after tomato in terms of cash and production. It is the most important exportation vegetable crop. More than 105,000 ha are devoted for potato production [13]. Commercial production of potatoes in Egypt is focused in the Nile Delta (72% of total production) and Middle Egypt (16% of total production). El- Behera, Dakahlia, Gharbia and Menoufia governorates are the leading producers of potatoes with 27.4%, 9.17%, 8.3% and 8.29% of total production in the Nile Delta while production in Middle Egypt is

concentrated in Minia and Giza governorates (7.5% and 4.3% of total production in the Middle Egypt) [2].

Most of the exported potato production in Egypt is centered in the governorates of El-Behera, Menoufia and Gharbia, where yields range between 20 and 36 tons/ha [2]. El-Behera Governorate came in the first place in terms of total production with 1.1 million tons, while cultivated area in El-Behera Governorate was 105.4 thousand tons [2].

3.2.1 Total cultivated area of potato in Egypt

Table 1 showed that the total cultivated area of potatoes during the period of 2000-2016 reached 305.9 thousand feddan, while the cultivated area of potatoes was 178.7 thousand feddan in 2000, compared to about 437.4 thousand feddan in 2015. The equation 1 in Table 2 shows that the potatoes total area in Egypt had increased at annual statistically significant rate reached 16.6 thousand feddan during the period of 2000-2016.

3.2.2 Potato total production in Egypt

The results in Table 1 showed that the production of potatoes during the period of 2000-2016 reached 3,317.4 thousand tons. the total production was 1,769.9 thousand tons in 2000, reached the maximum production to 4,955.4 thousand tons in 2015 because of the increasing in cultivated area.

Table 1. Evaluation of total cultivation area, total production and yield of potatoes in Egypt during the period of 2000-2016

Years	Total area (Thousand feddan)	Potatoes Production (Thousand ton)	Potatoes yield (Ton/feddan)
2000	178.7	1769.9	9.9
2001	189.9	1903.1	10.0
2002	196.7	1985.3	10.1
2003	197.3	2039.4	10.3
2004	248.2	2546.6	10.3
2005	300.8	3167.4	10.5
2006	220.3	2312.8	10.5
2007	257.1	2760.5	10.7
2008	327.6	3567.1	10.9
2009	329.9	3659.3	11.1
2010	334.8	3643.2	10.9
2011	391.0	4338.4	11.1
2012	422.1	4758.0	11.3
2013	382.0	4265.0	11.2
2014	410.0	4611.0	11.3
2015	437.4	4955.4	11.3
2016	376.6	4113.4	11.4
Average	305.9	3317.4	10.8
Min	178.7	1769.9	9.9
Max	437.4	4955.4	11.3

Source: Compiled and calculated from: MALR and FAOSTAT

The equation 2 in Table 2 showed that the total production of potatoes in Egypt had increased annually at significant rate and reached 203.2 thousand tons during the period of 2000-2016, which means that the annual production increased basically attributed to increasing in cultivated area.

Table 2. General trend equations of total area and total production of potatoes in Egypt during the period of 2000- 2016

	Items	Equation	R ²	F-test	t-test	no
1.	Total area of potato cultivation (thousand feddan)	$\hat{Y}_i = 156.19 + 16.6X_i$	0.88	112	10.6	1
2.	Total production of potato (thousand tons)	$\hat{Y}_i = 1488.4 + 203.2X_i$	0.88	116	10.8	2

Where:

$\hat{Y}_i$ = the estimated value for the dependent variable in the year i.

X_i = reflect time variable in the year i.

i = 1, 2, 3.....17

Source: Calculated using the data from Table 1.

3.3 Economic analysis of potato cultivation in Egypt

3.3.1. Domestic prices for potatoes in Egypt

The results in Table 3 showed that the growth of domestic prices of potatoes was about 426 LE/ ton in 2000, reaching 1332 LE/ ton in 2013 with annual average of 750 LE/ ton during 2000-2013. The equation 1 in Table 4 showed that the annual domestic prices of potatoes in Egypt had increased at significant rate and reached 72.3 LE/ ton during the period of 2000-2013.

3.3.2 International prices for potatoes in Egypt

Table 3 showed that the growth of international prices of potatoes was about 175 USD/ ton in 2000, reached 479 USD / ton in 2013 with annual average of 311 USD / ton during 2000-2013. The international prices of potatoes were minimum in 2003 with 148 USD/ ton, and maximum in 2009 with 676 USD/ ton. The equation 2 in Table 4 showed that the annual international prices of potatoes in Egypt had increased at significant rate and reached 31 US\$ / ton during the period of 2000-2013.

Table 3. Evaluation of domestic prices, international prices and potatoes exports of Egypt's potatoes during the period 2000 -2013

Years	Domestic prices (LE/ Ton)	International prices (USD/ Ton)	Quantity of Potatoes Exports (Thousand ton)	% of Potatoes Exports
2000	426	175	156.6	8.8
2001	428	160	185.5	9.7
2002	443	186	229.4	11.6
2003	505	148	296.3	14.5
2004	499	176	381.5	15.0
2005	483	197	392.2	12.4
2006	635	178	367.1	15.9
2007	706	277	389.7	14.1
2008	828	443	397.9	11.2
2009	913	676	215.1	5.9
2010	979	434	298.6	8.2
2011	1107	393	637.4	14.7
2012	1216	431	699.2	14.7
2013	1332	479	705.4	16.5
Average	750	311	382.3	12.2
Min	426	148	156.6	5.9
Max	1332	676	705.4	15.9

Source: Compiled and calculated from: MALR and FAOSTAT

Table 4. General trend equations of domestic prices, international prices and exports quantity of Egyptian potatoes during the period of 2000 -2013

	Items	Equation	R ²	F-test	t-test	no
1.	Domestic prices (LE/ ton)	$\hat{Y}_i = 207.4 + 72.3X_i$	0.93	158	12.5	1
2.	International prices (US/ ton)	$\hat{Y}_i = 77.9 + 31X_i$	0.63	20.4	4.5	2
3.	Export quantity (thousand ton)	$\hat{Y}_i = 123.8 + 34.5X_i$	0.63	20.8	4.5	3

Where:

$\hat{Y}_i$ = the estimated value for the dependent variable in the year i.

X_i = reflect time variable in the year i.

i = 1, 2, 3.....14

Source: Calculated using the data from Table 3.

3.3.3 Quantity of Egyptian potato exports

The results in Table 3 showed that the average of potato exports quantity during the period of 2000-2013 was 382.3 thousand tons. The quantity of potato exports was 156.6 thousand tons in 2000 and reached the maximum of 705.4 thousand tons in 2013. The equation 3 in Table 4 showed that the quantity of potato exports in Egypt had increased annually at significant rate and reached 34.5 thousand tons during the period of 2000- 2013.

3.4 The effect of climate change on potato production and potato quality

Several studies have addressed the influence of climate change and extreme climate events on potato culture and agro-technical improvements. For instance, Tubiello *et al.* [4] investigated the role of simple adaptation techniques in minimizing negative climate impacts on US crop production and Hijmans [5] studied the same on potato production in Peru. Haverkort *et al.* [7] reviewed the repercussions of climate change on potato supply chain on North European and Mediterranean climates. These studies agreed that potato yields were highly sensitive to the projected climate changes. Tubiello *et al.* [4] and Hijmans [5] declared that early planting fully counterbalanced negative impacts of climate change, yield reductions ranges were 18% to 32% in case of no adaptation practices and 9% to 18% in case of adaptation practices. Mohamed *et al.* [14] found that climatic factors played an important role in the determination of the yield of potatoes, directly or indirectly, where they affected growth, production quality and quantity. The increasing of temperature by one degree Celsius during March at the Northern Delta caused the yield decreased by 0.67 ton per acre in summer potatoes crop.

As for potato quality, even though the potato plant has a wide range of adaptability, it requires sufficient cumulative temperature during the cultivation period. It was noted that during the cultivation time: a) a linear correlation exists between seeding days to emergence, flowering to harvest and mean temperature, b) insufficient cumulative temperature causes decrease in accumulation of dry matter, weight of tuber fresh, and final yield [15]. According to Haverkort *et al.* [7] potato quality in South Africa may be affected by climate change in form of larger tubers with higher dry matter concentration. At the same time, water efficiency is very important due to the reduction in rainfall worldwide, food shortages in Africa is expected [9]. A recent study by Haverkort *et al.* [7] found that climate change had an impact on potato production in South Africa

Regarding the impact of climate change on potato diseases and pests, few studies were conducted in this matter. It was reported that at higher temperatures and longer growing season, pests and diseases were expected to increase. Moreover, milder winters will lead to an increase in tubers survival, thereby acting as a source of inoculum and multiplication [16]. Van der Waals *et al.* [8] calculated past and future trends of pest and disease pressure in potato cropping. Their results helped in prioritizing research and breeding management strategies for diseases and pests reduction.

3.5 Research deficits and demands of future studies

Some general adaptation techniques for potato production under climate change conditions such as expanding rainwater harvesting, water conservation techniques, improve irrigation efficiency, adjust planting dates and relocate crops are suggested [9]. However, more in-depth analysis must be taken into consideration and especially on the study area and practices by local producers. In spite of potato production importance in Egypt and increasing demand for ensuring its production sustainability, no up-to-date investigations have been conducted assessing climate change impact on potato production in Egypt.

There is an increasing need to assess the potential impacts of climate change on potato production in Egypt and identify the proper practices for sustainable conservation and production. These practices can be propagated to stakeholders and decision makers to assist in developing adaptation strategies for potato production in Egypt.

4. Conclusions

Potato cultivation is one of the most important agricultural cultivations in Egypt. Assessing climate change impacts on potato cultivation at local level is necessary in order to avoid related losses in crop production and quality. Identifying the best practices for mitigating climate change impacts in potato production can be very useful for local farmers. Moreover, these results will also help decision makers in developing adaptation strategies for potato production in Egypt as well as in similar climatic areas.

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