Original Article

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Preparation and characterization of rice gels containing tooth bleaching agent

Adchareeya Kaewpinta[1], Sakornrat Khongkhunthian[2,3], Pisaisit Chaijareenont[2,4], Siriporn Okonogi[2,5,*]

[1] *Interdisciplinary Program in Nanoscience and Nanotechnology, Chiang Mai University, Chiang Mai, Thailand;*
[2] *Research Center of Pharmaceutical Nanotechnology, Chiang Mai University, Chiang Mai, Thailand;*
[3] *Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand;*
[4] *Department of Prosthodontics, Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand;*
[5] *Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, Thailand.*

Summary **In the present study, the modified white rice of Jasmine (JM) and Saohai (SH) were used to prepare the rice gels. Carbamide peroxide (CP) containing rice gels (CP rice gels) of JM (CP-JM) and SH (CP-SH) were prepared. The rice gels and CP rice gels show homogenous texture. Rice variety influences the characteristics and properties of the rice gels. Amylose content of JM was lower than SH. Rheological behavior of JM and CP-JM was pseudoplastic without thixotropy whereas that of SH and CP-SH was pseudoplastic with thixotropy. CP-SH showed higher adhesive property and viscosity than CP-JM whereas CP-JM showed faster *in vitro* drug release than CP-SH. For *ex vivo* efficacy evaluation, 55 normal human teeth were subjected to the CP rice gels. Samples were applied on tooth surface according to the dental bleaching techniques. For at-home bleaching technique, the CP rice gels with 10% and 20% CP were used with bleaching time of 8h and 4h, respectively. For in-office bleaching technique, the CP rice gels with 35% CP was used with bleaching time of 1 h. The developed CP rice gels showed significantly higher efficacy than the positive and negative controls. For at-home bleaching technique, CP-SH was the most effective gels whereas for in-office bleaching technique, CP-JM was the most effective gels.**

Keywords: Jasmine rice, Saohai rice, modified rice, carbamide peroxide, tooth bleaching

1. Introduction

Tooth color is an important issue in aesthetic dentistry because the abnormal tooth color can affect the quality of life (*1*), physical appearance, beauty, and self-confidence (*2,3*). Abnormal tooth color is caused by aging, high levels of fluoride, and other drugs. It is also caused by chromogenic agents such as dental plaque, food, colored beverage, and smoking (*4*). Dissatisfaction with tooth color is widely reported (*5*). An oral health related quality of life questionnaire for use among young adults reported that tooth color was the most important concern (*6*). This concern has been shown to be associated with increased desire for treatments that improve color of the human teeth.

Tooth color can be improved by many methods including toothpastes (*7*), scaling and polishing (*8*), bleaching, enamel microabrasion (*9*), placement of crowns and veneers (*10*). Among those methods, bleaching is the most convenient treatment for tooth color improvement (*11*). Carbamide peroxide (CP), hydrogen peroxide, sodium percarbonate, and calcium peroxide have been widely used as bleaching agent for the teeth (*12*). Peroxide will diffuse into the teeth and bleach the color or darker shades called chromogens that are accumulated in the tooth to give brighter teeth (*13*). CP is more commonly used than others because of its effectiveness and safety for oral use with a controlled concentration (*14*).

The tooth bleaching techniques are divided into two categories; at-home bleaching and in-office bleaching. For at-home bleaching technique, CP at low

**Address correspondence to:*
Dr. Siriporn Okonogi, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand.
E-mail: okng2000@gmail.com

concentrations, usually between 10 to 20% is used. In general, 10% CP was used for 8 h per day, and 15 to 20% CP was used for 3 to 4 h per day. For in-office bleaching technique, CP at high concentration like 35% was used for short time of 30 min to 1 h per day (*9*). Tooth bleaching products generally help to improve the overall color. Gels are the formulations that enhanced ability of bleaching agents and have become widespread worldwide as an effective tooth bleaching treatment (*15*). Moreover, gels was a preferable formulation in terms of patient compliance, comfortable, and easy to apply on the tray that used for bleaching the teeth (*12*).

Most of gelling agents using nowadays are usually made from synthetic polymers which mostly produced from chemical polymerization and may cause serious problems to the environment. Therefore using natural polymer particularly from the edible raw material is increase because of environmental benefits (*16*).

Rice (*Oryza sativa* L.) is a plant in family Gramineae. The main element of rice is carbohydrates or starch. Fat and protein can be found in rice but very small amount. Starch is polysaccharides which are long chains of monosaccharide linked by glycosidic bonds. The main components of starch are amylose and amylopectin. Amylose is a linear polymer chain and amylopectin is branch polymer chain. Many researches have been reported that the structure of rice starch can be modified and change the physicochemical properties of starch. Structural modification of rice starch can be made by heat, chemicals, or enzymes. The modified rice is widely used in pharmaceutical field. It can be used as gelling agents, flocculants, thickeners, stabilizers, fillers, binders, and disintegrate (*16*). Many researches have studied about rice structural modification, and it is able to make a good natural gelling agents (*17*). We have reported that the gels developed from rice powder of some rice varieties showed suitable properties of buccal drug delivery (*18,19*). Recently, we reported that colored rice gels could be feasible to be the good gelling agents for CP (*20*) however, colored rice gels may decrease patient compliance due to their color which were red-brown or yellow-brown (*21*), whereas rice gels obtained from white rice are normally white or colorless. Furthermore, there is limited information available about using different rice varieties of white rice as gelling agent for CP. The present study emphasized on using white rice as a main component in gel formulation as drug delivery system.

2. Materials and Methods

2.1. *Materials*

Two rice varieties; Jasmine (JM) and Saohai (SH) were from a local supermarket in Chiang Mai province, Thailand. CP, triphenylphosphine (TPP), silver nitrate, glacial acetic acid, sodium hydroxide, monochloroacetic acid and amylose standards were from Sigma chemical Co. (St. Louis, MO, USA). Dichloromethane, methanol, and glacial acetic acid were obtained from RCI Labscan Co., Ltd. (Bangkok, Thailand). CP commercial gels with 10%, 20%, and 35% CP concentration were from manufacturer (Ultradent Product Inc, Salt Lake City, UT, USA). All other chemicals and solvents were analytical grade or the highest grade available.

2.2. *Preparation of modified rice powder*

2.2.1. *Preparation of rice powder*

Raw rice powder was prepared from JM and SH rice grains by wet milling method previously described by Okonogi *et al.* (*17*) with some modification. Briefly, the clean water-soaked rice grains were blended and filtered through 80 mesh sieve. The water of the filtrates was removed and the solid mass obtained was dried in the hot air oven. The dried solid mass was pulverized and kept in a desiccator for further study.

2.2.2. *Determination of amylose*

The analysis of amylose content in the rice was done according to the iodine colorimetric method described by Juliano (*22*) with some modification. Briefly, 0.1 g of rice powders was mixed with 1 mL of 95% ethanol and 9 mL of 1 N sodium hydroxide. The mixed solution was heated to 100°C for 10 min. Then, distilled water was added to make 100 mL solution. A 5 mL of the obtained solution was added to a volumetric flask, 1 mL of 1 M acetic acid and 2 mL iodine-potassium iodide were added into the flask and the volume was adjusted to 100 mL by using distilled water. The solution was protected from light and incubated for 20 min at room temperature. Spectrophotometer measurements were made at 620 nm by using UV-Spectrophotometer (UV 2450 Spectrophotometer, Shimadzu Corporation, Kyoto, Japan). Standard curve was generated using the amylose standards.

2.2.3. *Modification of rice*

The raw SH and JM powder was subjected to chemical modification according to the previous method described by Okonogi *et al*. (*17*) with some modification. Briefly, a solvent of methanol-water was mixed with sodium hydroxide solution. The raw rice powder was added to the mixture followed by monochloroacetic acid solution. The mixture was refluxed at 50 ± 1°C for 3 h. The mixture was adjusted to neutral pH. The solid mass was collected and washed by using methanol until the chloride of the filtrate was negative to silver nitrate testing. The dried solid mass was pulverized and filtered through the 80 mesh sieve.

The fine powder of the modified rice was kept in a desiccator for further study.

2.3. *Morphology of rice particles*

The morphology of rice particles was characterized using scanning electron microscope (SEM) (JEOL JSM-5910LV, JEOL Ltd., Tokyo, Japan). The samples were placed on the surface of the stub and coated with gold before examination. The excitation voltage of 10-20 kV under low vacuum mode (0.7 - 0.8 torr) and 10,000 magnifications were used.

2.4. *Preparation CP rice gels*

Rice gel base was prepared by hydration method previously described (*19*) with some modification. Briefly, exact weight of each modified rice powder was dispersed in distilled water to obtain 10% w/w, and then heated to 90°C and stirred for 1 h to obtain homogenous gels. CP powder was incorporated in the prepared gel base to obtain JM gels containing CP (CP-JM) with concentrations of 10% (10CP-JM), 20% (20CP-JM) and 35% (35CP-JM) and SH gels containing CP (CP-SH) with concentrations of 10% (10CP-SH), 20% (20CP-SH) and 35% (35CP-SH). The gels were kept in 4°C until further study.

2.5. *Outer appearance of CP rice gels*

In order to detect miscibility, rice gels containing CP were visually observed for drug precipitation and gel separation over the whole study period of 5 months.

2.6. *Rheological behavior and viscosity of the gels*

Rheological characterization and viscosity of the rice gels and rice gels containing CP were determined using Rheometer (Rheometer R/S-CPS, plate&plate, Brookfield engineering laboratories, Middleboro, MA, USA) with P25 DIN plate. Shear rates from 1 to 360 s^1 and back from 360 to 1 s^1 were used to determine the rheological properties of the gels under shear stress. The temperature was maintained at 25 ± 0.2°C.

2.7. *Adhesive property of the gels*

The adhesive property of the gels was investigated by an *in vitro* adhesive test previously described (*23*) with some modification. Briefly, the exact amount of gels was applied on the smooth surface plate with a width of 20 mm and a length of 100 mm. This surface plate was set next to the angle 30° inclined plate. A 15 mm diameter glass ball was released from the top of the inclined plate with a ball running length of 200 mm and further run on the surface plate until it stopped by gel adhesion. The length of the ball running from the beginning of the surface plate to the stop point was recorded.

2.8. *In vitro drug release*

In vitro drug release was studied by using an activated dialysis bags with a molecular weight cut-off at 12,000 daltons (Cellu Sep® T4 regenerated cellulose tubular membrane, Membrane filtration products Inc., Seguin, TX, USA) were used. The 50-mL artificial saliva was used as a release medium. The amount of 1 g of tested gels was filled in the dialysis bag. The bag was sealed and added to the medium with the controlled temperature at 37 ± 1°C and stirring speed of 100 rpm. After 5, 10, 15, 20, 30, 40, 50, and 60 min, the samples were collected and the fresh medium with the same volume was replaced. High-performance liquid chromatography (HPLC) (Hewlett Packard series 1,100, Agilent technologies, Santa Clara, CA, USA) was used for drug determination. Briefly, 1,000 µL of samples was mixed with 1,000 µL of 0.1M TPP and stirred for 2 h and protected from light. A reversed phase column 4.6 × 250 mm Hypersil ODS Agilent technologies with UV detection at 225 nm was used, the injection volume was 10 µL and the flow rate was adjusted to 1.0 mL/min. Determination was carried out at 25 ± 0.2°C with a mobile phase containing acetonitrile-water at a ratio of 50:50. At 6.5 min of running time, the composition of mobile phase was changed to a ratio of 100:0. After that, at 10 min, the composition of mobile phase was changed back to a ratio of 50:50 until the retention time of 25 min was reached. The calibration curve was prepared using solution of CP at 50-200 µg/mL and a linear response were obtained with correlation coefficient (r^2 = 0.9997).

2.9. *Ex vivo bleaching efficacy*

2.9.1. *Preparation of the teeth*

This investigation was approved by the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University (No. 58/2016). Fifty-five human teeth from normal volunteers of Chiang Mai University were collected by dentists. Cleaned teeth without caries or structural enamel defects were selected and kept in saturated 0.1% thymol solution at 4°C until further test.

2.9.2. *Tooth color measurement*

The teeth were randomly allocated into 11 experimental groups according to gel formulations; the treatment groups were JM gels and SH gels with 10%, 20%, and 35% CP. Commercial gels containing 10% CP (10CP-PC), 20% CP (20CP-PC) and 35% CP (35CP-PC) were used as positive controls. Rice gel bases; SH gels and JM gels were used as negative controls. The bleaching

protocol used in the present study was according to the dental bleaching techniques (*12,24*). At-home bleaching technique, 10% CP was applied for a long period (8 h per day) and 20% CP was applied for a medium period (4 h per day). In-office bleaching technique, 35% CP was applied for a short period (1 h per day). Duration of the bleaching was 14 days.

To demonstrate bleaching efficacy, tooth color values were measured using colorimeter (Fru WR10 portable precision colorimeter, Shenzhen wave optoelectronics technology Co.,Ltd, Shenzhen, China). The tooth color values were determined based on the Commission international de l'Eclairage (International commission on illumination: CIE) b* (yellow–blue) scales (*25*). After initial tooth color measurement, each day, the 0.1 mL samples were placed on tooth surface surrounded with 0.05 mL artificial saliva and kept in close container. The relative humidity was controlled at 100% and the temperature was maintained at 25 ± 1°C. After that, the gels were removed by using deionized water and the teeth were measured for color changing and stored in artificial saliva until the next bleaching session. The collected data was calculated to measure the bleaching efficacy.

2.10. *Statistical analysis*

Descriptive statistics for continuous variables were calculated and reported as a mean ± standard deviation (SD). Data were analyzed using a one-way analysis of variance (ANOVA) and followed by Duncan's multiple range test ($p < 0.05$) using SPSS statistic a software version 22.

3. Results

3.1. *Raw rice and modified rice*

Raw JM powder was white powder whereas raw SH powder was yellowish powder. After both raw rice powders were chemically modified by etherification, the obtained modified rice powders from both rice varieties were white. The outer appearances of raw rice powder and modified rice powder were shown in Figure 1.

3.2. *Amylose content*

Different amylose levels were found in the rice samples. Amylose content of JM was found to be 15.24 ± 0.07% whereas that of SH was 22.75 ± 0.06%. These values were used to classify the rice based on their amylose content. Generally, amylose content was categorized into five classes: waxy (0-2%), very low amylose (3-9%), low amylose (10-20%), intermediate amylose (20-25%) and high amylose (above 25%) (*26*). JM were classified as low amylose and SH were classified as intermediate amylose.

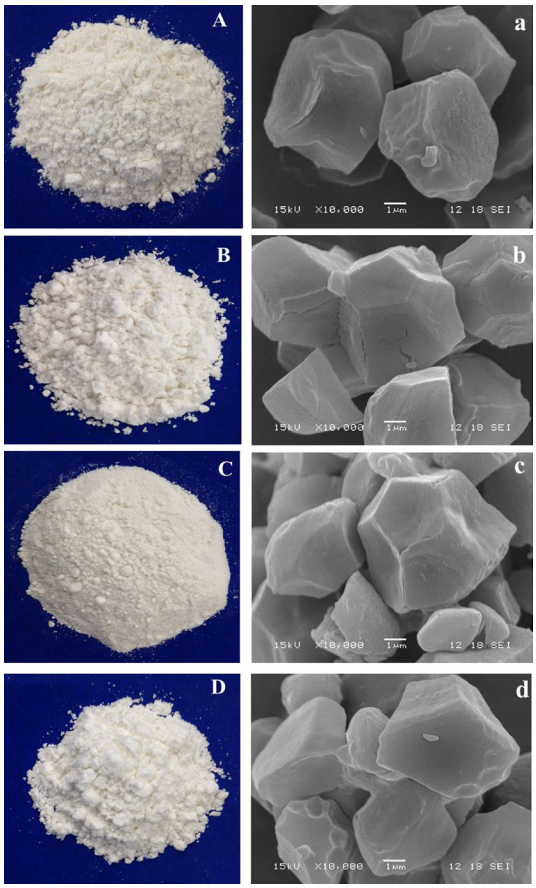

Figure 1. Outer appearance of raw JM (A), raw SH (B), modified JM (C), and modified SH (D) powders and SEM morphology of raw JM (a), raw SH (b), modified JM (c), and modified SH (d) powders.

3.3. *Morphology of rice particles*

The SEM results demonstrated different morphology between raw and modified rice of each rice varieties as presented in Figure 1. The raw rice showed polygonal shapes with heterogeneous sizes. Size of raw rice particles was approximately 5-7 µm. The modification of rice caused a change to the rice particles. The modified rice particles were swollen and merged together. Some small particles were attached to the surface of the large. Some of surface edges were slightly unsharpened.

3.4. *Outer appearance of CP rice gels*

The obtained rice gels and CP rice gels showed transparent semisolids and homogenous textures. After incorporating CP into the rice gel bases, both gels showed good compatibility to the drug. The CP rice gels showed no phase separation and drug precipitation after keeping at room temperature for 5 months.

3.5. *Rheological behavior*

The rheological behavior of the rice gel bases, CP rice gels, and CP-PC was shown in Figure 2. The stress-

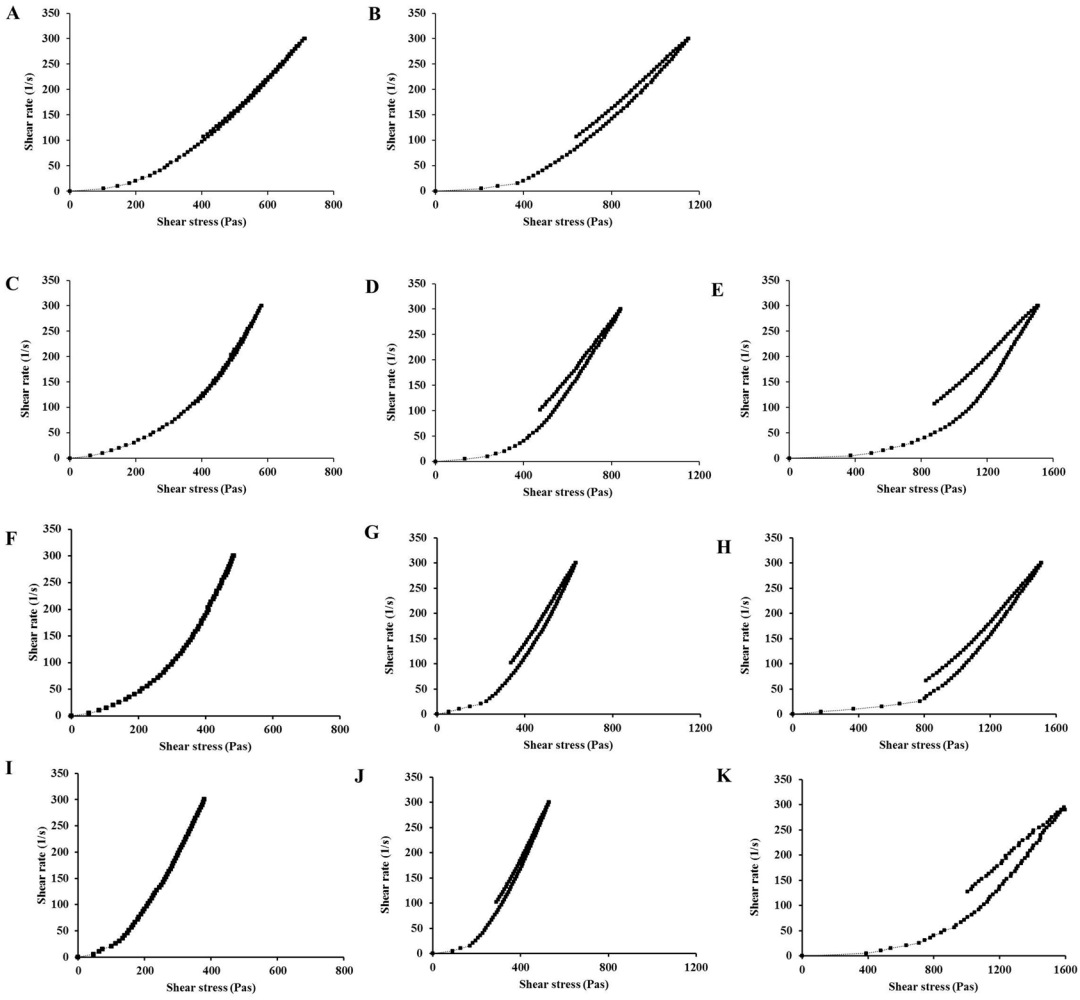

Figure 2. Rheograms of JM gels (A), SH gels (B), 10CP-JM (C), 10CP-SH (D), 10CP-PC (E), 20CP-JM (F), 20CP-SH (G), 20CP-PC(H), 35CP-JM (I), 35CP-SH (J), and 35CP-PC(K).

strain relationship was non-linear for all samples. The rheological behavior of the rice gel bases, CP rice gels and CP-PC was non-Newtonian flow. JM gel base and CP-JM gel showed pseudoplastic flow without thixotropy. SH gel and CP-SH gel showed pseudoplastic flow with thixotropy.

The viscosity of the rice gel bases, CP rice gels, and CP-PC was presented in Table 1. CP-PC showed the highest viscosity. It was found that the viscosity of SH gels was higher than that of JM gels. The viscosity of the CP rice gels was slightly lower than their corresponding gel bases.

Table 1. Viscosity of the gels and the distance of running ball

Formulations	Viscosity (Pas)	Distance (cm)
JM	4.17 ± 0.27	5.40 ± 0.11
SH	8.98 ± 0.18	4.78 ± 0.10
10CP-JM	3.52 ± 0.24	6.12 ± 0.12
10CP-SH	7.75 ± 0.17	5.23 ± 0.10
10CP-PC	13.20 ± 0.26	1.60 ± 0.18
20CP-JM	2.16 ± 0.35	8.50 ± 0.21
20CP-SH	6.84 ± 0.50	6.10 ± 0.14
20CP-PC	12.50 ± 0.31	1.88 ± 0.28
35CP-JM	1.75 ± 0.12	11.43 ± 0.10
35CP-SH	4.88 ± 0.20	8.72 ± 0.12
35CP-PC	14.01 ± 0.22	1.90 ± 0.15

3.6. *Adhesive property*

The adhesive property of the gels as shown in Table 1 was expressed as the distance of the running ball on the surface covered with the test gels. The shorter the distance indicated the higher the adhesive property of the gel samples. Among CP rice gel formulations, 10CP-SH showed the shortest distance. The results indicated that SH gels possessed higher adhesive property than JM gels.

3.7. *In vitro drug release property*

Drug release profile of CP rice gels and CP-PC was presented in Figure 3. The determination of CP was based on an oxidation of TPP into triphenylphosphine oxide (TPPO) (*27*). TPP and TPPO are the agents that can be detected by HPLC. TPP was oxidized by peroxide and formed TPPO. Determination of drug was

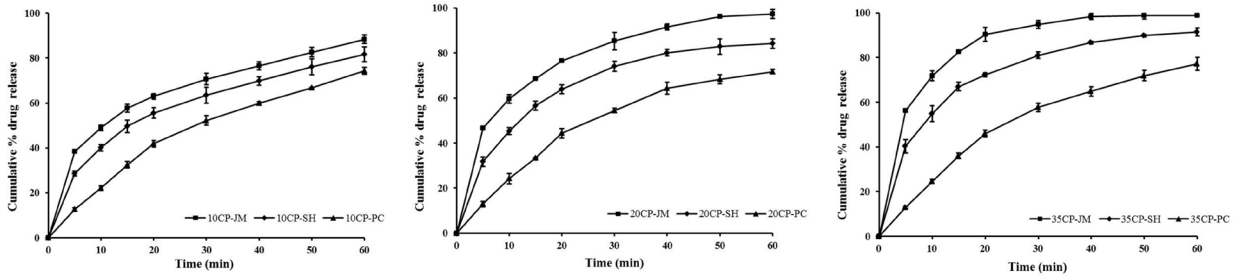

Figure 3. ***In vitro*** **cumulative drug release profiles from the gels.**

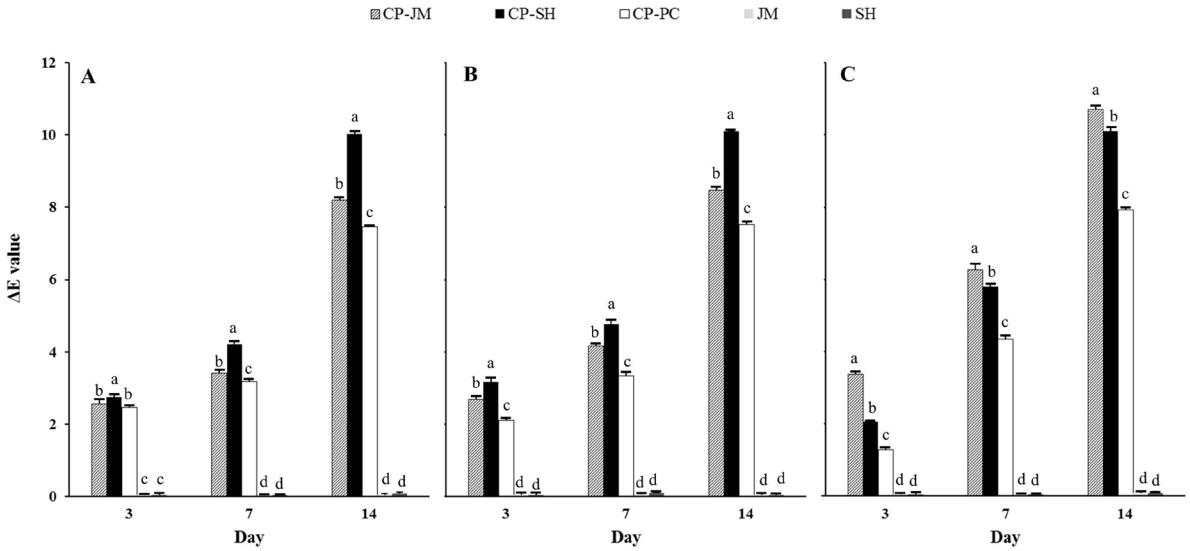

Figure 4. ***Ex vivo*** **tooth bleaching efficacy of CP-JM, CP-SH, and CP-PC, at 3 different CP concentrations, 10% CP (A), 20% CP (B), and 35% CP (C).** Data are mean ± SD. Different lowercase letter in same day for each group implied the statistically different ($p < 0.05$).

made by external quantification using TPPO peak area. All CP rice gels demonstrated faster drug release than CP-PC. Among the CP rice gels, 35CP-JM showed the highest amount of drug release.

3.8. *Ex vivo bleaching efficacy*

Visual observation revealed that tooth color of CP rice gels groups and positive groups after treatment was highly whiter than the initial day. The values of color changing were collected by colorimeter. The result was expressed by the mean value of measurements of the tridimensional coordinates of the CIELab system. In this system, the color was determined by the relationship between three axes. The L* axis represents the lightness with values ranging from 0 (black) to 100 (white). The a* axis represents the amount of red (positive a* value) or green (negative a* value). The b* axis represents the amount of yellow (positive b* value) or blue (negative b* value). The values of L*a*b* were calculated for color changing (ΔE) by using an equation following (*25*); $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. After calculations, the results indicated that the groups of CP rice gels and CP-PC were obviously effective. The mean ΔE values of these groups were significantly higher than the negative control group. Moreover, the mean ΔE values of day 14 were the highest when compared to those of day 3 and day 7. The results of 10% CP gel groups were presented in Figure 4A. At day 14, 10CP-SH showed the significant highest mean ΔE value of 10.04 ± 0.07 ($p < 0.05$). Even ΔE value of 10CP-JM was lower than 10CP-JM, but it was significantly higher than 10CP-PC. The mean ΔE values of 10CP-JM and 10CP-PC were 8.20 ± 0.08 and 7.46 ± 0.04, respectively. The results of 20% CP gel groups were presented in Figure 4B. At day 14, it was also found that ΔE value of 20CP-SH was the significantly highest of 10.10 ± 0.03. Similar to the 10% CP gels, the ΔE value of 20CP-JM (8.47 ± 0.09) was significantly higher than that of 20CP-PC. However, the results of 35%CP gel groups were different from 10% and 20% CP gel groups as presented in Figure 4C. At day 14, 35CP-JM showed significant tooth bleaching effective with the highest ΔE value of 10.71 ± 0.11 followed by 35CP-SH and 35CP-PC with mean ΔE values of 10.11 ± 0.12 and 7.92 ± 0.07, respectively. These values indicated that 35CP-SH had significantly higher ΔE values than 35CP-PC ($p < 0.05$).

4. Discussion

Chemical modification of rice base on carboxymethylated etherification can improve the properties of rice (*16*). This modification method can enhance water soluble property of the rice starch. The particle morphology of the obtained modified rice is obviously changed from the raw rice. The modified rice of both JM and SH shows good property for forming hydrogels by using simple hydration method. As CP is a water soluble active compound. Therefore, this drug can miscible well with the rice hydrogels after incorporation to the gel bases leading to obtain the good texture CP rice gels.

The suitable topical gel formulations should have suitable rheological behavior. This property can provide the flow behavior information and microstructural environment of the gels which are responsible for drug diffusion and also possible drug compatibility (*28*). The rheological property of the developed JM and SH gels is non-Newtonian behavior. Both gels can immediately flow after stress application, and their viscosity changes during the process of shearing. Rice variety plays important role on rheological behavior of the rice gels. JM gels possess pseudoplastic flow without thixotropy whereas SH gels possess pseudoplastic flow with thixotropy. Thixotropy indicates the reformation of gel structure after shear stress is applied. Higher thixotropy indicates the lower gel structure reformation. It has been reported that a synthetic polymer, carboxypolymethylene, is commonly used as gelling agent in many tooth bleaching commercial gels (*24*) and gives non-Newtonian behavior, pseudoplastic flow with thixotropy (*29*). The obtained CP-SH rice gels developed in our present study possess pseudoplastic flow with thixotropy, the same rheological behavior as the commercial gels used as positive controls.

High adhesive property helps the topical gel formulations to retain at the application area for the desired duration of action whereas the formulations with low adhesive property are easily removed. For tooth bleaching gels, high adhesive property is important for efficacy and safety (*30,31*). Considering rice gel formulations, the viscosity and adhesive property of SH gels are higher than JM gels. Recent research has reported that the amylose content effects to rice swelling after modification and properties of gels (*32*). The results from our present study indicate that JM can be classified as a low amylose content group whereas and SH can be classified as an intermediate amylose content group. The different properties of rice gels are considered to be due to the amylose content in different rice variety. Moreover, CP also affects to the rice gels, increasing CP concentration decreases viscosity and adhesive property of the gels.

The release study demonstrates that 35CP-JM has the highest drug release property. It is considered that the release property of the gel is influenced by the viscosity of the gel base. Drug molecules can diffuse easily through the low viscosity gel and pass the definite MW cut-off dialysis membrane to the medium. High viscosity of gels may prolong releasing of the drug. This effect causes 35CP-JM with low viscosity possesses the fastest drug release. The *ex vivo* study in human teeth was done in order to compare the bleaching efficacy. The treatment sessions of 10%, 20%, and 35% CP used in this study are according to the common bleaching technique. All CP gel formulations possess high effective for tooth bleaching. CP rice gels and CP-PC have the similar tooth bleaching efficacy but in different level, which the developed CP rice gels are higher efficacy than the CP-PC and the negative controls.

Comparing ΔE values of CP rice gels and CP-PC, it is indicated that for at-home bleaching technique, the efficacy of 10CP-SH and 20CP-SH is higher than the others. For in-official bleaching technique, the efficacy of 35CP-JM is the highest which is considered to be due to the fastest drug release of this formulation. Considering other factors that can affect bleaching efficacy, it is noted that not only the release property of the gels but also the adhesive property. SH gels had the suitable adhesive property and good ability of drug release. Therefore, at the medium and long period, 10CP-SH and 20CP-SH showed the highest tooth bleaching efficacy.

In conclusion, the developed rice gels containing tooth bleaching agent, CP-JM and CP-SH are homogeneous texture with tooth bleaching effectiveness. Rice variety plays a role on the characteristics and properties of the derived gels CP rice gels showed higher efficacy than the positive controls. Moreover, CP-SH is the most effective gels for at-home bleaching technique whereas CP-JM is the most effective gels for in-official bleaching technique.

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