



EFFICACY AND STABILITY OF ANTHOCYANIN; *ORYZA SATIVA* ON EMULSION FORM

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Article Received on 09/11/2021

Article Revised on 30/11/2021

Article Accepted on 21/12/2021

ABSTRACT

Extraction from rice berry are important substances; Anthocyanin (AT) has many properties such as Anti-oxidants, Inhibitor cancer cell, Anti-allergic, Anti- Cholesterolemia and Anti-atherosclerosis. However, AT deteriorate quickly. This research aims to develop AT in emulsion form and study its stability, the color change of the extracts under different solvent conditions. The study found that the extraction extracted with ethanol and acids developed as emulsions form have colorlessness and antioxidant efficiency of $28.76 \pm 0.13 \mu\text{g/ml}$ and non-significant $p > 0.01$ compared with BHT and smallest particle size $29.21 \pm 0.87 \text{ nm}$. However, the substance was suitable for cosmetic and supplementary in the future study.

KEYWORD: Rice berry, Anti-oxidant, Emulsion, Cosmetic, Anthocyanin.

INTRODUCTION

Oryza sativa (rice berry) was considered the staple food in Thailand.^[1] which has been abundantly grows in the northeastern regions of Thailand. The aforementioned region near and contains minerals that were suitable for growing rice thus finding a place where rice is grown in that region. However, black rice classified as rice that contains Anthocyanin high because the appearance was purple to black.^[2] which such color is the source of a very common substance named cyanidin. When considering the chemical structure, it is in the group of flavonoids.^[3] Prevalent study was showing the properties of this anti-oxidant, against cancer cells in vitro^[4], reduce hairs loss and gray hairs.^[5] However, anthocyanin don't stability on pH environment,^[6] charge of structure could change dependent on solvent that not suitable efficacy for used.

The extraction process validity to employ the active substances from the plants. Each extraction has a different specificity in order to obtain chemical in the plants for their intended use in the extraction of rice berry there are also researches that use different extractions.^[7] This may be due to the purpose of other researchers to continue to use the substance, toothpaste, lotion and beverage which may be because rice berry extract does not maintain its condition; different pH.^[8] In this study, the researcher was interested to use rice berry extract to develop into an emulsion form to study the effect of stability in order to be used in the development of cosmetic and dietary supplement product formulations in the future to come.

Emulsion was the proposition in pharmaceutical composition of oil surfactant and water. After constituted on the water the emulsifier of component shown oil in water, the droplet size of oil was dispersion inner emulsion (hydrophobic) and constituent that soluble in water was outer (hydrophilic). However, the preparation of emulsion was achieved to enhancer of extracted for increased efficacy, deep for skin and stability. Anthocyanin form rice berry have polars for soluble in water^[9] but non stability in environment form structure.^[10] The purpose of this study was to investigation anthocyanin stability. Previous studies, it was found that under pH different conditions had an effect on the color change of the substance. which from the problem caused by the dissociation of the charge in the solvent. In order to maintain the decomposition state, the experiment was conducted to study the packaging of extracts obtained from rice into the form emulsion for further stability studies.

The aims of this research were to study the stability of anthocyanins that naturally tend to change color when environmental pH, making unsuitable for use. On the other hand, anthocyanins have many properties, they are suitable for use as medicines, supplements or cosmetics. In this study, the extracts were developed in emulsion form and stored under the accelerated condition at 40 °C and tested for discoloration and DPPH method.

MATERIALS AND METHODS

Reagents

Rice berry were collected from the Ban Yang, Mueang Buriram, Buriram, Thailand Ethanol, Deionized Water, Hydrogenated Castor oil 40 and Polysorbate 80 were purchased from Krungtepchemi Corporation (Thailand)., White oil was purchased from Cosme-plus Corporation (Thailand)., 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich Corporation (United States), Gallic acid was purchased from Sigma-Aldrich Corporation (United States), Citric Acid Anhydrous was purchased from Sigma-Aldrich Corporation (United States).

Plant extraction

45 g of rice berry was extracted with 900 ml of water, 900 ml of Ethanol 96% and 900 ml of 1 M Citric acid in ethanol 96%. The extracts were sonicated at 50°C for 50 min. The solutions were filtered through an Whatman No.1 filter paper and vacuum-dried in a rotary evaporator at 40 °C. The crudes of extract were stored in a brown bottle at 2-8 °C until further analysis.

Preparation emulsion of rice berry

1 g of the extracts extracted by water, alcohol and acid was used to create emulsions consisting of 0.5 g of white oil and polysorbate-80, homogeneous texture. Briefly, each emulsion was stored for 40 °C for 1 week and measure anti-oxidant by DPPH in the future and stability in another pH on solvent, dispersion emulsifying 500 µl on solvent 10 ml. Color on each emulsion solvent was study.

100 µl of emulsion (citric acid solvent) was dispersion on 100 mL of DI water. The texture was homogenous by vertex 800 rpm to 5 min. The particle size was studied.

Radical scavenging activity using DPPH method

The radical scavenging activity (RSA) which is modified from Chumchuen S.^[11] Briefly, 300 µl each of extracts and emulsions were transferred into a 96-well microplate. Then 100 µl of 9 µM DPPH (in absolute ethanol) were added into each well. Incubation at the dark room temperature for 30 min. The blank was prepared as above without the extract and ethanol was used for the baseline correction. Adjust in the absorbance of the extract samples were measured at 516 nm using the UV-visible spectrophotometer. The RSA were calculated in the equation here.

$$\% \text{ of inhibition RSA} = [(A_{516 \text{ blank}} - A_{516 \text{ sample}}) / A_{516 \text{ blank}}] \times 100$$

Stability of color in solvents, acid neutral and alkaline

0.5 g of the extracts and emulsions were dispersion on different solvent (pH 1.2, 7.0 and 9.2) and then homogenous mixture 30 s.

Statistical analysis

Statistical analysis was performed ANOVA method, confidence level 99 % of the comparison was compared by individual pair Tukey's test.

RESULTS AND DISCUSSION

Percent yield topic

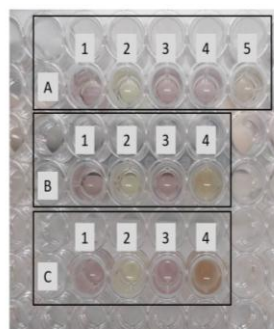
Rice berry was extracted in different solvents, so the content of the extracts was different. The extracts extracted from cetric acid were the highest. This may be because the solids in the solution of acid were evaporated and crystallized, which may be the amount of such acid crystals. Which did not find salt born accordingly, may be deduced that such a document may be the texture of the extract.

Free radical scavenging activity by DPPH method

For anti-oxidant activities, DPPH scavenging activity assay have IC₅₀ of the extraction employee citric acid was 10.34 ± 0.74 µg/ml, the highest all of extraction. white positive control, BHT presented higher activity with IC₅₀ 9.08 ± 0.74 µg/ml. However, the extractions studied in 40 °C for 1 week can't affected for anti-oxidant that show in table 2. On the other hand, the extraction storage in 40 °C that show in emulsion form in citric acid has IC₅₀ 28.76 ± 0.13 µg/ml, the highest all of 40 °C storage for 1 weeks. Therefore, a statistical test of the anti-oxidant efficiency by DPPH methods in the extraction of citric acid solvent compared to positive control showed insignificant differences p > 0.01.

Stability and Anti-oxidation in emulsion form

The extract of anthocyanin was developed to emulsion 40 °C for 1 week, after which it was tested to determine the disadvantages, they tend to change color in environments with different pH, the presence of charge in their structure. When the extract is developed into an emulsion form. It was found that they did not change, possible the formulation water (anthocyanin) in oil affected non-reaction of environments, thus the control relief.

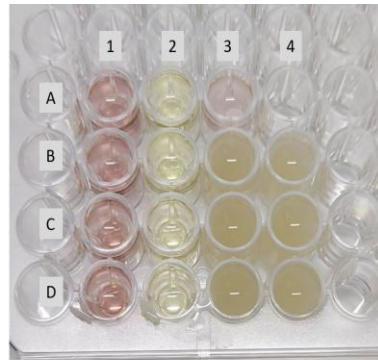


A1 = DPPH, A2 = DPPH + Gallic acid, A3 = DPPH + Alcohol, A4 = DPPH + Acid, A5 = DPPH + Extract

B1 = DPPH, B2 = DPPH + Gallic acid, B3 = DPPH + Alcohol, B4 = DPPH + Extract

C1 = DPPH, C2 = DPPH + Gallic acid, C3 = DPPH + Water, C4 = DPPH + Extract

Figure 1: DPPH method of the extraction after incubated at the dark room for 30 min.



A1 = DPPH, A2 = DPPH + Gallic acid, A3 = DPPH + Base Emulsion(White oil + water + Surfactant)
 B1 = DPPH, B2 = DPPH + Gallic acid, B3 = DPPH + Emulsion Initial 25°C, B4 = DPPH + Emulsion Initial 40°C
 C1 = DPPH, C2 = DPPH + Gallic acid, C3 = DPPH + Emulsion Overnight 25°C, C4 = DPPH + Emulsion Overnight 40°C
 D1 = DPPH, D2 = DPPH + Gallic acid, D3 = DPPH + Emulsion 1 Week 25°C, D4 = DPPH + Emulsion 1 Week 40°C

Figure 2: DPPH method of the extraction that emulsions form storages at 45 °C for 1 week after incubated at the dark room for 30 min.

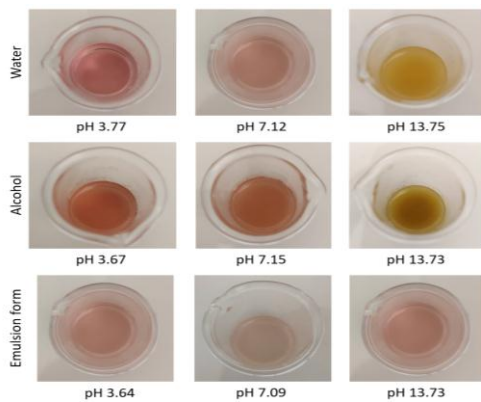


Figure 3: Different form of extraction varied in environments.

Table 1: Percent yield of the various solvent of *Oryza sativa* were extracted by ultrasonic-assisted extraction.

The solvent extracted of <i>Oryza sativa</i>	Percent yield
Di water	9.25 ± 0.56
Ethanol	4.81 ± 0.87
Citric acid 0.1 M	42.08 ± 0.91

Table 2: Antioxidant activities of *Oryza sativa*.

Organs of plant	DPPH IC ₅₀ (µg/ml)
The extraction by di water in 25 °C	98.78 ± 0.19 ^b
The extraction by ethanol 96 % in 25 °C	38.09 ± 0.12 ^c
The extraction by Citric acid in 25 °C	10.34 ± 0.74 ^a
The extraction by di water in 40 °C	ND
The extraction by ethanol 96 % in 40 °C	ND
The extraction by Citric acid in 40 °C	ND
Emulsion forms the extraction by citric acid in 40 °C	28.76 ± 0.13 ^d
BHT (positive control)	9.08 ± 0.74 ^a
Ethanol 96 % (negative control)	ND
Di water (negative control)	ND
Citric acid 1 M (negative control)	ND

ND = Not detected

*The different letters in the same column showed the significant difference $p < 0.01$

Table 3: The characteristic of formulation in emulsion.

Periods	Temperature (°C)	Color	Particle size	PDI
Initial	25	purple	27.67 ± 0.76 nm	0.567
	40	purple	29.21 ± 0.87 nm	0.365
Overnight	25	purple	29.65 ± 0.76 nm	0.678
	40	purple	28.12 ± 0.56 nm	0.789
1 week	25	purple	28.74 ± 0.36 nm	0.125
	40	purple	28.32 ± 0.65 nm	0.675

CONCLUSION

The extraction of rice berry was found to contain anthocyanin as an extract but this substance was not stable and changed color according to different pH conditions. More color change and stability when developed into emulsion form. when stored under accelerated conditions of 40 °C are also effective in antioxidants by DPPH method.

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