Methylation Formation to Improve Red Radish Anthocyanins Solubility in Cold Water

Jie Zhang, Xiaohua Zhou, Dan Wang, Xing Zhou, and Shiyu Tan

***Abstract*—This paper introduced an approach by methylation formation to enhance the solubility of red radish anthocyanins in cold water. FTIR spectrums indicated the formation of methylated red radish anthocyanins. The methylation yeild was 81.4 % under optimized conditions. The solubility of methylated red radish anthocyanins could reach 10.7 mg/mL in cold water (4 ℃). They were water soluble, which facilitated them incorporation into aqueous food systems. And the evolution of CIELab has been studied. Therefore, the information collected from this study is valuable for improving the quality of red radish anthocyanins with good solubility in cold water.**

***Index Terms*—Methylation, solubility, colour, red radish anthocyanins.**

I. Introduction

Color plays a very important role in the acceptability of foods. Consumers first judge the quality of a food product by its color, and the food industry has used colorants for centuries to enhance or restore original appearance of foods or to ensure uniformity, as indicator of food quality [1]-[5]. Nowadays, interest in natural colorants has significantly increased as a consequence of both legislative action and consumer awareness to the use of synthetic additives in their foods.

Red radish anthocyanins are potential economic sources for the natural colorants since they are rich in red radish and also grown all over the China [6]. Besides the color attributes, interest in anthocyanins has intensified because of their possible health benefits. Seventeen anthocyanins in red radish anthocyanins has been tentatively identified by mass spectroscopy as pelargonidin-3-sophoroside-5-glucoside derivatives with multiple acylation of hydroxycinnamic acids [7]-[9]. However, the limitation that has restricted the use of red radish anthocyanins in food systems is their relatively low stability in cold water.

The aim of this paper is to enhance the solubility of red radish anthocyanins in cold water by methylation. Dimethyl sulfate (DMS) was chosen as a methylating reagent The factors affecting reaction efficiency were investigated thoroughly. Moreover, the solubility and colour properties of methylated red radish anthocyanins were measured. This study could provide useful information towards developing making red radish anthocyanins attractive alternatives to synthetic dyes.

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II. Experimental

A. Materials

Red radish anthocyanins was donated by the Haiju Agriculture Development Co. Ltd., Chongqing, China. AB-8 resin was supplied by Tianjin Nankai Hecheng Technology Co. Ltd., Tianjin, China. Dimethyl sulfate (purity 99%) was obtained from Sigma-Aldrich, USA. Nylon membrane and syringe filter all with the aperture of 0.45 μm were purchased from Pall Co., USA. All the other reagents used were of analytical grade.

Deionized water was purified by a Milli-Q Water Purification system (Millipore, USA). The temperature controller in the circulator bath which had a precision of ±0.1 ℃ came from Zhengzhou Greatwall Scientific Industrial and Trading Co. Ltd (Zhengzhou, China). The weight measurements were done by using a precise balance (AUW220D, Shimadzu, Japan) with a resolution of ±0.01 mg. BSZ-100 automatic partial collector and automatic liquid chromatography system include glass chromatography column (40 cm×1 cm) were from Huxi Analysis Instrument Factory Co. Ltd. (Shanghai, China). Fourier transform infrared (FTIR) spectra of the samples were recorded using a MAGMA-IR550 spectrometer (Nicolet Co., USA). Colour was evaluated with a Hunter colour Quest XE colorimeter (Hunter Lab, Reston, USA).

B. Purification of Red Radish Anthocyanins

Red radish anthocyanins (0.5 g) was suspended in 100 mL deionized water. The solution was filtered through a nylon membrane with the aperture of 0.45 μm to remove water insoluble substance. The filter cake obtained was discarded. While the filtrate obtained was further purified by AB-8 column chromatography. The filtrate flowed through the glass column at the flow rate of 1.5 mL/min. After adsorption, the column was washed first with deionized water, and then eluted by 60 % ethanol solution. The desorbed solution was vacuum-dried at 60 ℃ after removing solvent, and the

purified red radish anthocyanins was obtained.

C. Methylation of Red Radish Anthocyanins

The 0.5 g purified red radish anthocyanins was dispersed in 100 mL deionized water. The pH 9.5 carbonate buffer solution (1.0mL) was added to the above solution. It was stirred vigorously for 30 min to form a suspension at different temperatures (10~70 ℃). 0.5mol/L aqueous solution of NaOH (3mL) and dimethyl sulfate (0.05~0.25 mol) were added to the suspension in an alternate mode. After 1 h, the solution flowed through the glass column, it was washed first with deionized water, and then eluted by 30 % ethanol solution. The desorbed solution was vacuum-dried at 60 ◦C after removing solvent, and the methylated red radish anthocyanins was obtained. Then the column was eluted by 60 % ethanol solution. The desorbed solution was vacuum-dried at 60 ℃ after removing solvent, and unreacted purified red radish anthocyanins was obtained.

D. FTIR Spectroscopy

FTIR analysis was carried out on a Nicolet, 550II spectrometer scanning from 4000 cm^{-1} to 500 cm^{-1} at room temperature. The purified red radish anthocyanins and methylated red radish anthocyanins were, respectively, mixed with KBr and pressed to plates for measurements.

E. Solubility Measurement

The gravimetric method was used in this study for solubility measurement. These experiments were carried out in a 250 mL double jacketed glass vessel equipped with a stirrer. A supersaturated solution of the original red radish anthocyanins in cold water (4.0 ℃) was prepared. The solution temperature was controlled using circulator bath. Solution was agitated with a magnetic stirrer at the temperature of interest for 30 min to ensure equilibrium was reached. Several samples were taken by a syringe and filtered with 0.45 μm syringe filter and poured in a pre-weighed 30 mL glass vials. Then, the glass vial was weighed again and placed inside a vacuum oven overnight till no change in the final mass of the vial was observed. The net mass of the original red radish anthocyanins divided by the sample's volume shows the solubility of red radish pigment at the temperature of interest. The same procedure was performed for purified and methylated red radish anthocyanins.

F. Colour Analysis

The color characteristics (CIELab parameters, L*, a*, b*, Hue and Chroma) of purified and methylated red radish anthocyanins were measured by a colorimeter, using illuminant D_{65} and 10 ° observer angle. The sample was placed in 1 cm path length cuvettes and values were measured in the total transmission mode.

III. Results and Discussion

A. Methylating Process

DMS is an attractive methylating reagent with superior reaction rates and higher yields than competitive products [10]. Typically, one methyl group is transferred more quickly than the second. Methyl transfer is typically assumed to occur via an SN2 reaction.

During the reaction, DMS content and reaction temperature are the main factors to methylation yield. Fig. 1 demonstrates the change of methlation yield with DMS content. The methlation yield increases with an increase in DMS content until reaching a maximum (81.4 %). The optimum content of DMS was found to be around 0.2 mol when the weight of purified red radish anthocyanins (0.5 g) was certain.

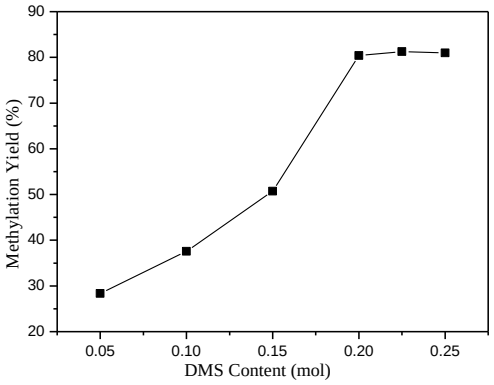

Fig. 1. Effect of DMS content on methylation yield.

Fig. 2 shows the experimental methlation yield at different temperatures. The methlation yield first increases with an increase temperature when it is below 30 ℃. However, after the methlation yield reaches a maximum when temperature is around 30 ℃, it ceases to increase and begin to decrease with a further increase temperature. The reason for this decrease is believed to be DMS is obviously hydrolyzed at high temperature.

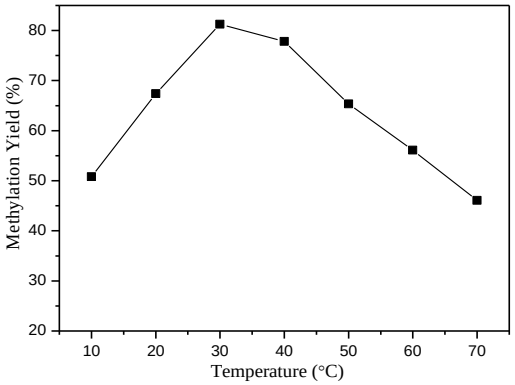

Fig. 2. Effect of reaction temperature on methylation.

B. FTIR Analysis

FTIR spectrums for the purified red radish anthocyanins as well as for the methylated products are presented in Fig. 3. In the both spectrum, the peaks at 1639 cm^{-1}, 1601 cm^{-1}, 1512 cm^{-1}, and 1445 cm^{-1} corresponding to the aromatic ring of benzopyran and heterocyclic in red radish anthocyanins. The peaks at 1268 cm^{-1}, 1178 cm^{-1} and 1071 cm^{-1} are attributed to $\nu_{C\text{-}O\text{-}C}$ in saccharide ring. The appearance of the new peak at 2848 cm^{-1} dues to methoxy group, which indicates the formation of methylated red radish anthocyanins.

C. Solubility

The concentration of three kinds of dissolved red radish anthocyanins in cold water (4.0 ℃) is depicted in Table I. after methylating the purified red radish anthocyanins with

DMS caused more solubility than the untreated one. The solubility is 10.7 mg/mL in cold water.

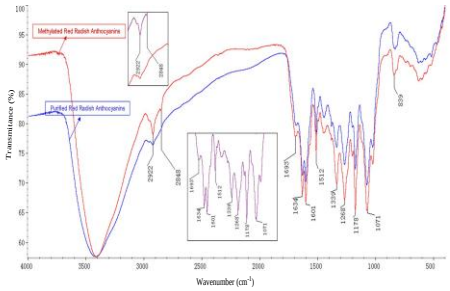

Fig. 3. Comparison of FTIR spectrum of the purified red radish anthocyanins with methylated red radish anthocyanins.

Methylation formation is the most common and effective method of increasing solubility. Methylation of the anthocyanins molecule improve their stability through intramolecular and/or intermolecular copigmentation, and self-association reactions [11], [12]. Therefore, sources of methylated red radish anthocyanins may provide the desirable stability for food applications.

TABLE I: THE SOLUBILITY OF ANTHOCYANINS

Components	Original red radish anthocyanins	Purified red radish anthocyanins	Methylated red radish anthocyanins
Solubility (mg/mL)	3.1	5.4	10.7

TABLE II: THE COLOUR PROPERTIES OF ANTHOCYANINS

Components	purified red radish anthocyanins	methylated red radish anthocyanins
*L**	19.26	22.47
*a**	34.73	36.21
*b**	9.54	14.48
Hue	17.55	17.39
Chroma	36.53	51.68

D. Colour Properties

Several models have been developed for the analysis of color, but the CIELab system is the one that nowadays presents a high acceptance since the color perception is uniform which means that the Euclidean distance between two colors corresponds approximately to the color difference perceived by the human eye [13]. The CIELab is an international standard for color measurement since 1976. *L** is the luminance component (from 0 to 100), and *a** (from green to red) and *b** (from blue to yellow) are the two chromatic components [14].

The CIELab parameters of purified and methylated red radish anthocyanins are presented in Table II. *L** increased from 19.26 to 22.47 means that methylated red radish anthocyanins get a bright colour. It also can be observed from the table that the hue stayed pretty constant. The chroma value increased from 36.25 to 51.68 indicating an increase in the stability of anthocyanin after methylated.

IV. CONCLUSIONS

The findings from the present study could serve as a foundation for developing red radish antyocyanins for purposes of health benefits or food systems. FTIR spectrums indicated the formation of methylated red radish anthocyanins. The methylation formation leads to a pronounced solubility enhancement of the compound in cold water. The evaluation of the final product with respect to CIELab, hue and chroma indicated an increase in the stability of anthocyanin after methtlated. They were evaluated as potential sources for anthocyanin-type colourants or value-added products. And they could be applied for commercially preparing the compounds of interests.

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