SCREENING ON ANTIOXIDANT ACTIVITIES OF VEGETABLE AND FRUIT BY-PRODUCTS FROM THE MEKONG DELTA AND USING MANGOSTEEN PEEL EXTRACT FOR WHITE SHRIMP COLD STORAGE

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TÓM TẮT

SÀNG LỌC HOẠT TÍNH KHÁNG OXY HÓA CỦA CÁC MẪU PHỤ PHẨM RAU CỦ VÀ TRÁI CÂY VÙNG ĐỒNG BẰNG SÔNG CỬU LONG VÀ SỬ DỤNG DỊCH CHIẾT VỎ MĂNG CỤT TRONG BẢO QUẢN LẠNH TÔM THẺ CHÂN TRẮNG

*20 mẫu cao chiết ethanol từ các mẫu phụ phẩm rau củ và trái cây vùng đồng bằng sông Cửu Long được nghiên cứu hoạt tính kháng oxy hóa bằng hai phương pháp: ức chế gốc tự do DPPH và xác định tổng hàm lượng flavonoid (TFC). Trong số đó, mẫu cao trích từ vỏ măng cụt (**TNB-10**) và hạt xoài (**TNB-11**) thể hiện hoạt tính ức chế DPPH mạnh với giá trị IC_{50} lần lượt là 8,38 µg/mL và 1,84 µg/mL và mẫu **TNB-10** chứa tổng hàm lượng flavonoid cao nhất (422,97 mg QE/100g). Mẫu cao trích này được sử dụng để bảo quản tôm thẻ chân trắng (Litopenaeus vannamei) tại 2^oC, trong 7 ngày. Mẫu tôm ngâm bằng dịch chiết từ vỏ măng cụt có điểm cảm quan biến đen và giá trị TBARs thấp hơn mẫu đối chứng (ngâm trong nước), cho thấy mẫu **TNB-10** có khả năng làm chậm sự hình thành melanosis và quá trình oxy hóa chất béo ở tôm thẻ chân trắng trong quá trình bảo quản lạnh. Bằng kỹ thuật HPLC-EIS-MS, chín hợp chất kháng oxy hóa trong **TNB-10** được định danh gồm có: α-mangostin, β-mangostin, γ-mangostin, 8-dexoxygartanin, garcinone B, garcinone C, garcinone D, 9-hydroxycalabaxanthonevà garcinmangosone C.*

1. INTRODUCTION

In recent years, shrimp and shrimp products have occupied a large portion in the exported seafood products of Vietnam [1]. However, they are among the world's most

perishable commodities, and their spoilage begins soon after the death. Even when they are kept in cold storage, discoloration and oxidation in shrimp are serious problems affecting organoleptic, nutritional and economic value of shrimp. Many efforts resolved these problems including chilling, freezing, and preservatives. The addition of antioxidants is one of the most widely studied methods. However, many synthetic antioxidant compounds have shown toxic and/or mutagenic effects, which have stimulated the interest of many investigators to search natural antioxidant [2].

The Mekong Delta is one of the most fertile region in Vietnam. Every year, this region produces many kinds of food products for the domestic and international market. However, the content of by-products from vegetables and fruits are created very large, a part has used as a fertilizer, and the rest has discharged to the environment causing pollution. In our researh, we screened antioxidant activities of 20 by-product samples and preserved white shrimps (*Litopenaeus vannamei*) in the cold condition by the samples that showed strong activities.

2. EXPERIMENTAL

2.1. Chemicals

2, 2 – Diphenyl – 1 – picrylhydrazyl (DPPH), malonaldehyde (MAD) were purchased from Merck (Darmstadt, Germany). Trichloroacetic acid (TCA), thiobarbituric acid (TBA) and gallic acid, quercetin were purchased from Sigma Chem. Co. Ethanol solvent, aluminium tricloride ($AlCl_3$), sodium nitrite ($NaNO_2$) and sodium hydroxit (NaOH) were purchased from China.

2.2. Preparation of samples

Table 1. The list of 20 by-products and their antioxidant activities using DPPH assay and TFC

Sign	Local name	Scientific name [3,4]	Family [3,4]	Part Used	IC_{50} (µg/mL)	TFC (mgQE/100g)
TNB- 1	Pine-apple	*Ananas comosus*	Bromeliaceae	Stem	>100	243.23±3.54
TNB- 2	Water hyssop	*Bacopa monnieri*	Scrofulariacea	Stem	43.25	265.60±4.63
TNB- 3	Star apple	*Chrysophyllum cainino*	Sapotaceae	Peel	>100	-
TNB- 4	Star apple	*Chrysophyllum cainino*	Sapotaceae	Seed	>100	-
TNB- 5	Pomelo	*Citrus maxima*	Rutaceae	Peel	>100	90.49±3.54
TNB- 6	Pomelo	*Citrus maxima*	Rutaceae	Seed	>100	-
TNB- 7	Mandarin	*Citrus reticulata*	Rutaceae	Peel	87.32	321.92±4.82
TNB- 8	Mandarin	*Citrus reticulata*	Rutaceae	Seed	>100	-
TNB- 9	Longan	*Dimocarpus longan*	Sapindaceae	Seed	79.31	122.11±2.31

TNB- 10	Mango-steen	*Garcinia mangostana*	Clusiaceae	Peel	8.38	422.97±4.63
TNB- 11	Mango	*Mangifera indica* L.	Anacardiaceae	Seed	1.84	192.31±4.82
TNB- 12	Sapodilla	*Manilkara zapota*	Sapotaceae	Peel	>100	112.09±3.54
TNB- 13	Sapodilla	*Manilkara zapota*	Sapotaceae	Seed	>100	-
TNB- 14	Gac fruit	*Momordica cochinchinensis*	Cucurbitaceae	Peel	44.38	90.49±3.54
TNB- 15	Gac fruit	*Momordica cochinchinensis*	Cucurbitaceae	Seed	>100	86.63±4.82
TNB- 16	Banana	*Musa acuminata*	Musacae	Peel	>100	139.86±1.34
TNB- 17	Banana	*Musa acuminata*	Musacae	Seed	13.50	203.11±2.31
TNB- 18	Water mimosa	*Neptunia oleracea*	Fabaceae	Stem	>100	196.17±2.31
TNB- 19	Potato	*Solanum andigenum*	Convolvulaceae	Peel	27.18	325.00±4.82
TNB- 20	Ambarella	*Spondias dulcis*	Anacardiaceae	Peel	70.83	182.29±4.63

Fruit and vegetable by-products (50-100 g) were cleaned with water, air-dried, cut into small pieces and extracted with ethanol solvent (200 – 350 mL, reflux, 3 h, x3, 60-65ºC). The ethanol solutions were evaporated under reduced low pressure in order to give ethanolic extract. Samples were preserved and stored in biochemistry lab (Table 1).

White shrimps (*Litopenaeus vannamei*) with the size of 30-40 shrimps/kg were purchased from Thu Duc market, Thu Duc district, Hochiminh city. The shrimps were kept alive and transported to laboratory.

2.3. Screening on antioxidant activities

2.3.1. DPPH free radical scavenging assay

The stable free radical (DPPH) was used for determination of free radical scavenging activity of the extracts [5]. Briefly, a 0.1 mM solution of DPPH in 90% ethanol was prepared and then 1.5 mL of this solution was mixed with 1.5 mL of each sample (crude extract) at concentrations of 100, 50, 25, 10μg/mL in 90% ethanol. After 30 min incubation in the dark, the decrease in the solution absorbance was measured at 517 nm by Shimadzu UV-1800 spectrophotometer (Japan). DPPH inhibitory activity was expressed as the percentage inhibition (I%) of DPPH in the above assay system, calculated as (1−B/A) x100, where A and B are the activities of the DPPH without and with test material. IC_{50} (inhibitory concentration, 50%) values were calculated from the mean values of data from three determinations. Vitamin C at various concentrations (1.0, 2.5, 5.0, 10.0 μM) was used as a positive control.

2.3.2. Determination of flavonoid content

The total flavonoid content of ethanol was determined using the aluminium cloride assay through colorimetry [6].

Aliquots of extract solution (1 mg) were taken in 10 ml glass tube and made up to the volume 5 mL with ethanol. Later 150 μL $AlCl_3$ (10 %), 150 µL $NaNO_2$ (5 %), 1000 µL NaOH (4 %) and 1200 µL distilled water were added sequentially. After 30 min of incubation the mixture turns to pink whose absorbance was measured at 550 nm using the spectrophotometer. The contents of flavonoids in the samples were calculated from the calibration plot and expressed as mg quercetin equivalent per 100 gram of extract (mgQE/100g). All the determinations were carried out three times.

2.4. Applying for shrimp cold storage

2.4.1. Treatment of shrimp

The shrimps were immersed in selected extract solutions that were prepared in a weight ratio of 1:15 (extract/water) at room temperature for 10 minutes and in water (control sample), similarly. Shrimps were fished out and preserved in plastic box at 2^{o}C. Three shrimps from each treatment were taken every 0 days up to 7 days for evaluation of melanosis development and lipid peroxidation inhibition.

2.4.2. Sensory evaluation

Fiffteen candidates (19–22 years old) for panelists were selected from students of the Chemical and Food Technology Faculty. Candidates were carefully screened for ability to recognize and describe common aroma. Control sample (treated by water) and shrimp samples (treated by ethanol extracts) were evaluated during storage and classified according to the degree of black spot formation. The gray value in shrimp was evaluated directly using modified Montero's sensory evaluation [7]. Fiffteen candidates (n=15) evaluated gray values in shrimp by levels 1 to 5 scale as follow: point 0 = no point; point 1= light (about 20% of the surface area affected shrimp); point 2 = the average (accounting for 20-40% surface area affected shrimp); point 3= significant (accounting for 40 - 60% surface area affected shrimp); point 4= very severe (60-80 % occupied surface area affected shrimp); point 5= very terrible (80-100 % occupied surface area affected shrimp).

2.4.3. Lipid peroxidation inhibition assay

MDA is considered to be the final product of the oxidation process of lipid peroxidation. TBA reacts with MDA to form a di-adduct, a red chromogen, which can be detected spectrophotometrically at 532 nm [8]. Shrimps were grinded by machine, then was mixed with 10 mL TCA 7.5% solution. The mixture was filtered about 15 min, the filtrate was mixed with TBA 0.02 M solution equal volume rate, then the mixture was heated at 100^{o}C for 15 min.

Absorbance was measured at 532 nm by the spectrophotometer. MDA contents were calculated from standard curve built at concentrations from 0.01 to 0.05 μM and reported as mgMAD/kg shrimp. MDA content values were calculated from the mean values of data from three determinations.

2.5 HPLC-EIS-MS analysis of mangosteen peel extract

RP-HPLC was performed to determination antioxidants present in the ethanolic mangosteen peel powder. The separation module consisted of Agilent 1200 series HPLC (USA) equipped with ESI-MS system (micrOTOF-QII Bruker Daltonic, Germany). The samples was eluted on a column ACE3- C_{18} (4.6 ×150 mm, 3.5 μm, Merck, Germany) with a gradient system consisting of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol) used as the mobile phase, with a flow rate of 0.5 mL/min. The temperature of the column was maintained at 40 ^{o}C and the injection volume 20 μL. For ESI-MS, full scan mass spectra were measured between m/z 150 and 2000. High purity nitrogen was used as nebulizer gas at 1.2 bar, 200 °C and at a flow rate of 0.8 mL/min.

3. RESULTS AND DISCUSSION

3.1. Screening on antioxidant activities

The 20 ethanol extracts which were prepared from the 14 by-product vegetables and fruits were screened for their antioxidant activities by DPPH assay (Table 1). In total, nine ethanol extracts showed IC_{50} values below 100 μg/mL, six extracts with IC_{50} values less than 50 μg/mL, three extracts exhibited IC_{50} values below 25 μg/mL, and two extracts with IC_{50} values below 10 μg/mL. Two extract showed strong antioxidant activities were mango seed (**TNB-11,** 1.84 μg/mL) > mangosteen peel (**TNB-10**, 8.38 μg/mL). IC_{50} value of gallic acid was 4.66 μM (0.84 μg/mL).

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. On analyzing the results obtained in DPPH assay, it was noticed that extracts act as good hydrogen donating agent, there by bleaches the DPPH absorbance.

The total flavonoid contents of 20 ethanol extracts were determined in accordance with the equation $y = 231.43x + 3.6485$ ($r^2 = 0.9914$) and TFC values were shown in table 1. TFC values (mgQE/100g) of three samples having strong activities were arranged on decreasing order: mangosteen peel (**TNB-10**, 422.97) > potato peel (**TNB-19**, 325.00) > mandarin peel (**TNB-7**, 321.92).

In total, the mangosteen peel extract (**TNB-10**) showed strongest antioxidant with the highest content of flavonoid and the lowest IC_{50} value (DPPH assay). Therefore, **TNB-10** was selected and used as a natural

antioxidant food preservative for shrimp cold storage at 2 ºC and tested by sensory evaluation of melanosis development in shrimps and lipid peroxidation inhibition assay.

3.2. Applying for shrimp cold storage

3.2.1. Sensory evaluation

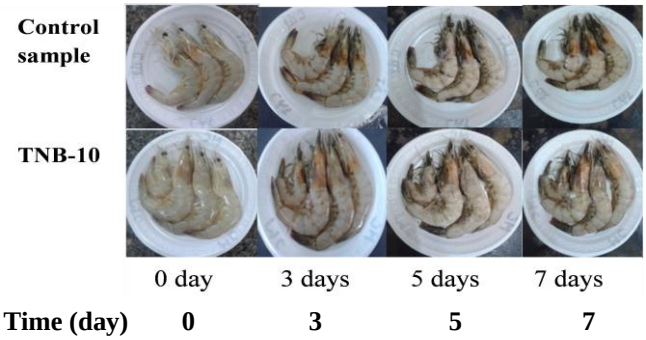

Fig 1: Development of melanosis in shrimps during the cold storage

Treated shrimp samples by **TNB-10** have gray values lower than the control sample (treated by water) in cold storage (Fig. 1,2). Gray values of the control sample after 3, 5, and 7 days were 3.5, 4.2, and 4.8, respectively. Meanwhile, gray values of treated shrimp samples by **TNB-10** were 2.8, 3.7, and 4.2, respectively. In general, gray values occurred significantly after 3 days of preservation. These results suggested that **TNB-10** is able to prevent melanosis development in shrimp during the cold processing.

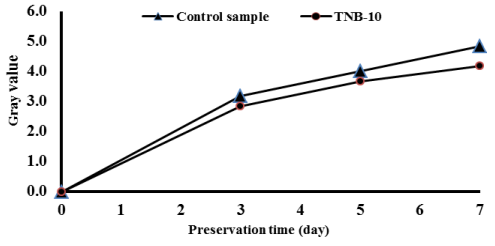

Fig 2: Changes in the mean gray value in shrimps before and after cold storage. Results are presented in terms of mean ± confidence value (n=15, p=95%)

3.2.2. Lipid peroxidation inhibition assay

In general, the TBARs values of the extract were found to be increased from the first day to the fifth day and to decreased significantly during 5-7 days of storage at 2 ^{o}C (Fig.3). Treated shrimp samples by **TNB-10** have TBARs values lower ($p<0.05$) than the control sample (treated by water). TBARs values of control sample after 3, 5, 7 days are 2.02, 2.96, 2.74 mgMAD/kg, respectively. Meanwhile, TBARs values of treated shrimp samples by **TNB-10** were 1.44, 1.86, and 1.61 mgMAD/kg.

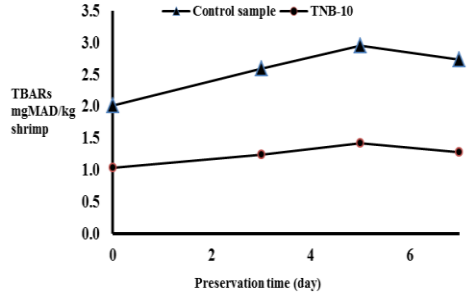

Fig 3: The TBARs value change of shrimp during preservation at 2^{o}C

The increasing TBARs values (0-5 days) is as fat oxidation powerful place in the first stage, the product of the fat oxidation such as hydroperoxit that formatted and oxidized rapid into secondary products like aldehyde. The secondary oxidation products continue to be converted to all other products under the effect of enzymes and microorganisms, leading to diminished TBARs value (5-7 days) [2]. Treated shrimp samples have lower TBARs value than the control sample. These results showed that **TNB-10** able to slow the process oxidation of fat in shrimp during the cold processing.

3.3 Indentification of antioxidants mangosteen peel extract

*Table 2: Identification of 9 compounds in **TNB-10** by HPLC-ESI-MS*

No.	Compounds	$[M-H]^-$ (m/z)	Predicted formula
1	α-mangostin	409.16565	$C_{24}H_{26}O_6$
2	β-mangostin	423.180215	$C_{25}H_{28}O_6$
3	γ-mangostin	395.148915	$C_{23}H_{24}O_6$
4	8-dexoxygartanin	379.154000	$C_{23}H_{24}O_5$
5	Garcinone B	393.133265	$C_{23}H_{22}O_6$
6	Garcinone C	413.159480	$C_{23}H_{26}O_7$
7	Garcinone D	427.175130	$C_{24}H_{28}O_7$
8	9-hydroxycalabaxanthone	407.148915	$C_{24}H_{24}O_6$
9	Garcinmangosone C	411.143830	$C_{23}H_{24}O_7$

Nine xanthones compounds namely, α-mangostin, β-mangostin, γ-mangostin, 8-dexoxygartanin, garcinone B, garcinone C, garcinone D, 9-hydroxycalabaxanthone, and garcinmangosone C were found **(**Table 2). According to Jung *et al.*, α-mangostin and γ-mangostin are xanthones having strong antioxidant using the authentic $ONOO^-$ and SIN-1-derived $ONOO^-$ methods [9]. Additions, these compounds were present at high content in the dried mangosteen peel [10]. Thus, the presence of xanthones may play an essential role in its antioxidant activity.

4. CONCLUSIONS

In conclusion, this study indicates that the extracts obtained from mangosteen peel (**TNB-10**), potato peel (**TNB-19**), mandarin peel (**TNB-7**), and mango seed **(TNB-11)** had significant free radical scavenging activity on stable DPPH and high flavonoid contents. Moreover, the data suggest that mangosteen peel (**TNB-10)** was able to slow down the oxidation of fat and melanosis development in shrimp during the cold processing using lipid peroxidation inhibition assay and sensory evaluation of melanosis

development in shrimps during the cold storage.

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