

Color Test For Screening Chemical Components Of Protein Hydrolyzed Extract From Non-Shell Small Crab (*Portunus Pelagicus*) Waste

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Abstract: Fisheries industry wastes such as crab (*Portunus pelagicus*) have the shell and non-shell waste. The effort to overcome and reduce waste is to use it optimally. One of them is by hydrolysis technology which can produce extracts that have bioactivity. The purpose of this study was to determine and obtain a description of the chemical components in the protein extracts of crab waste (*Portunus pelagicus*) non-shell crab. The study was conducted by color testing, namely flavonoids, alkaloids (Meyer, Dragendorf, Bouchardat), tannins, terpenoids (steroids, triterpenoids), polyphenols, and saponins. Screening and identification results show the presence of chemical components tannins, triterpenoids, and polyphenols from protein crab waste hydrolysates (*Portunus pelagicus*) non-shell, whereas for flavonoids, alkaloids, steroids, and saponins are not was found. These chemical components have activities as antioxidants, anti-cancer, antimicrobial, cardioprotective, antidiabetic, anti-obesity, hepatoprotective, anti-bacterial, anti-anxiolytic, analgesic, anti-nociceptive. The extract has the potential to be developed in the health field as a pharmaceutical or nutraceutical.

Index Terms: color test, chemical components, hydrolizate, small scrab, waste.

1 INTRODUCTION

Fisheries industry wastes such as crab (*Portunus pelagicus*) have the shell and non-shell waste. Non-shell waste has high protein and unsaturated fat content. The non-shell waste can be used as a source of raw material for making protein hydrolyzate [1] and can minimize environmental and health problems and can reduce economic impacts [2]. A number of studies utilizing fisheries industry waste that have the potential to become bioactive peptides include viscera and squid skin (*Sepia officianalis*) [3], viscera of smooth hound fish (*Mustelus mustelus*) in Tunisia [4], head and viscera of sardine fish (*Sardinella aurita*) [5], as well as the utilization of the head, fins and tail of tilapia (*Oreochromis niloticus*) [6], fish viscera tilapia (*Oreochromis niloticus*) [7] and various other fishery waste raw materials [8]. One of the most prospective is to develop food products from bioactive peptides produced from the fisheries industry wastes into nutraceutical products [9]. Nutraceutical is a substance that has physiological benefits or provides protection against chronic diseases [10]. Even in 2018, there is an estimated increase in trade in nutraceutical products reaching US \$ 250 billion [11] and will continue to grow in the future [12]. In this research, the use of non-shell crab waste will become hydrolyzate with enzymes. The purpose of this study was to determine and obtain a description of the chemical components of flavonoids, alkaloids, tannins, terpenoids (steroids, triterpenoids), polyphenols and saponins in extracts of crab waste protein hydrolysates (*Portunus pelagicus*) non-shell. The results of this study are expected to provide useful information about the chemical components of the extract to be developed in the field of health as a pharmaceutical or nutraceutical.

2 MATERIAL AND METHOD

2.1 Material

The material used in this study is small crab waste from the small canned meat canning industry of PT Windika Utama, Semarang City. On the way to the laboratory, non-shelled crab (*Portunus pelagicus*) waste is put in an ice-cooled container to keep the temperature cool. After arriving at the laboratory, non-shell crab waste (*Portunus pelagicus*) is cleaned, then non-shell crab waste (*Portunus pelagicus*) is weighed, and a hydrolysis process is carried out. While the protease enzyme used is the alcalase enzyme (Sigma Aldrich) with activity ≥ 0.75 Anson units/ mL. The tools used include: test tubes, test tube clamps, stainless steel spatulas, drop pipettes, measuring cups, bunsen, glass funnels, micropipettes, beaker glass, incubators, 1000 ml Erlenmeyer, vacuum filters, rotary evaporators.

2.2 Making of Hydrolyzate

Raw material in the form of small crab waste (*Portunus pelagicus*) and aquades (1 : 1) is mashed using a blender until homogeneous, then heated at 85 °C or 20 minutes to inactivate endogenous enzymes. The sample was then centrifuged at 10 °C for 20 minutes at a speed of 5,800 rpm to separate fat and protein, the fat was removed, and the result was a protein-rich residue. The solid-rich protein was extracted three times with distilled water at 1 : 1 (w/ v) to collect protein extracts. Furthermore, the protein extract was centrifuged at 10 °C for 20 minutes at a speed of 5,800 rpm to separate the fat and protein, the fat was removed, and the result was a protein-rich residue. The protein extract (EP) was hydrolyzed to the desired level with 1 N sodium hydroxide using a digital pH meter (Cyberscan 1001, Eutech, Singapore). The solution is inactivated at a temperature (80-85) °C for 20 minutes with the aim of stopping the hydrolysis process. After that, the samples were allowed to stand at 4 °C for 24 hours and centrifuged cold for 20 minutes and dried using a freeze dryer. The degree of hydrolysis calculated by the SN-TCA method [13] is referred to in [14]. A total of 20 mg of protein hydrolyzate was added with a 10 % TCA (w / v) of 20 mL. The mixture is then allowed to stand for 30 minutes for sedimentation, then centrifugation (7,800 rpm, for 15 minutes).

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The supernatant obtained is a non-shell crab waste protein hydrolyzate extract (*Portunus pelagicus*) for further testing.

2.3 Color Test

Screening analysis and identification of compounds from non-shell crab protein hydrolyzate extract (*Portunus pelagicus*) based on color test refer to the Harbone method [15], which is characterized by changes in the color of the extract after the addition of certain reagents. The parameters include flavonoids, alkaloids, tannins, terpenoids (steroids, triterpenoids), polyphenols, and saponins. The data obtained are presented in tabular form and analyzed descriptively, and a conclusion is drawn.

Identification of Flavonoids

2 ml of sample extract was added 8 ml of water which had been heated for ± 10 minutes. The resulting filtrate was filtered and put in a test tube. Then a few drops of concentrated HCl are added. Next, a little Mg powder is added. Positive results show when dark red/pink colored filtrate is produced.

Identification of Alkaloids

2 ml of sample extract was added 8 ml of water which had been heated for ± 10 minutes. The resulting filtrate is filtered and put in a test tube. Then six drops of Meyer reagent were added to the first test tube, six drops of Dragendorf reagent to the second test tube, six drops of Bouchardat reagent to the third test tube. Positive results show when white sediment is produced in Meyer's reagents, orange sediment deposits in Dragendorf reagents, and brown sediment deposits in Bouchardat reagents.

Identification of Tannins

2 ml of sample extract was added 8 ml of water which had been heated for ± 10 minutes. The resulting filtrate is filtered and put in a test tube. Three drops of FeCl_3 1 % were added to the filtrate. Positive results show when either blackish brown, blackish-blue, or blackish green colored filtered is produced.

Identification of Terpenoids (steroids, triterpenoids).

2 ml of sample extract was added 8 ml of water which had been heated for ± 10 minutes. The resulting filtrate is filtered and put in a test tube. Three drops of Bouchardat were added to the filtrate. Positive results contain steroids when bluish-green colored filtrate is formed, and the results contain pyrenoids when a brownish-orange colored filtrate is formed.

Identification of Polyphenols

A total of 1×10^5 μg of methanol extract was put into 2×10^3 l of 96 % ethanol in a test tube. The mixture was added five $\times 10^3$ μL of distilled water and five $\times 10^2$ μL of Follin-Ciocalteau reagent (50 % v / v), then the mixture was left for 5 minutes. Then it was added 1×10^3 μL sodium carbonate solution (7.5 % w / v), homogenized and incubated at room temperature for 1 (one) hour under no-light conditions (dark). Positive results show when either blackish green, blackish blue, or blackish brown colored filtered is formed.

Identification of Saponins

2 ml of sample extract was added 8 ml of water which had been heated for ± 10 minutes. The resulting filtrate is filtered and put in a test tube. Two ml of hot water was added to the

filtrate. The test tube containing the filtrate was then shaken firmly. Positive results show when the permanent foam is not lost.

3 RESULTS AND DISCUSSION

The results of the screening of the chemical components of the non-shell crab waste (*Portunus pelagicus*) protein hydrolyzate extract by the color test as in Table 1, 2, and 3. Protein hydrolyzate extract of small crab waste (*Portunus pelagicus*) containing tannins compound. Tannins include organic glycoside polymers. Tannins have a -OH (hydroxyl) group on the aromatic ring. This component is effective in capturing free radicals and donating electrons and hydrogen atoms. Tannins have antioxidant activities [16], Anti-cancer [17], Antimicrobial [18], Cardioprotective, Anti-diabetic and anti-obesity [19]. Protein hydrolyzate extract of small crab waste (*Portunus pelagicus*) containing triterpenoid compounds. About 60 % of known natural products are terpenoids [20]. Triterpenoids have a relatively complex cyclic structure, consisting of alcohol, aldehydes or carboxylic acids. Triterpenoids are crystalline in shape and have a high melting point. Triterpenoids have anti-inflammatory and anti-cancer potential [21], antioxidants [22], anti-colon cancer, Hepatoprotective, anti-bacterial, anti-anxiolytic, Analgesic and Anti-Nociceptive [23]. Protein hydrolyzate extract of small crab waste (*Portunus pelagicus*) containing polyphenol compounds. A growing body of research indicates that polyphenol consumption may play a vital role in health through the regulation of metabolism, weight, chronic disease, and cell proliferation. Over 8,000 polyphenols have thus far been identified, even though their short- and long-term health effects have not been fully characterized [24]. Animal, human and epidemiologic studies show that various polyphenols have antioxidant and anti-inflammatory properties that could have preventive and therapeutic effects for cardiovascular disease, neurodegenerative disorders, cancer, and obesity [25]; [26].

TABLE 1
COLOR TEST RESULTS OF THE NON-SHELL CRAB WASTE PROTEIN HYDROLYZATE EXTRACT FOR FLAVONOIDS AND ALKALOIDS

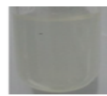


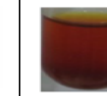
Sample	Compounds			
	Flavonoids	Meyer	Dragendorf	Bouchardat
extracts of crab waste protein (<i>Portunus pelagicus</i>) non-shell waste				

TABLE 2
COLOR TEST RESULTS OF THE NON-SHELL CRAB WASTE PROTEIN HYDROLYZATE EXTRACT FOR TANNINS, TERPENOID, POLIPHENOL, SAPONIN





Sample	Compounds			
	Tannins	Terpenoids	Polifenols	Saponins
extracts of crab waste protein (<i>Portunus pelagicus</i>) non-shell waste				

TABLE 3
SCREENING RESULTS AND IDENTIFICATION OF CHEMICAL COMPONENTS OF
PROTEIN EXTRACTS OF CRAB WASTE (NON-SHELL WASTE HYDROLYZATE)

No	Compounds	Standard	Conclusion
1.	Flavonoids	The color orange, pink, or orange	Negative
2.	Alkaloid		
	Metode Meyer	White deposits	Negative
	Metode Dagensdorf	Orange deposits	Negative
	Metode Bouchardar	Chocolate deposits	Positive
3	Tannins	Blackish green, blackish blue, blackish brown	Positive
4	Steroids	Bluish green	Negative
5	Triterpenoids	orange, brownish orange	Positive
6	Polifenols	blackish green, blackish blue, blackish brown	Positive
7	Saponins	stable foam for 15 minutes	Negative

4 CONCLUSION

Extracts of crab waste protein (*Portunus pelagicus*) non-shell waste containing chemical components such as tannins, triterpenoids, and phenols. The extract has the potential to be developed in the field of health as a nutraceutical or pharmaceutical.

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