Color Test For Screening Chemical Components

by Putut Riyadi

Submission date: 08-Nov-2020 03:20PM (UTC+0700)
Submission ID: 1439414258
File name: Color_Test_For_Screening_Chemical_Components_IJSTR.pdf (307.72K)
Word count: 2544
Character count: 14544

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

Putut Har Riyadi, Yudhomenggolo Sastro Darmanto, Apri Dwi Anggo, Sumardianto, Laras Rianingsih

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 (Sephia 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.

- *Department of Fisheries Product Technology, Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Central Java, Indonesia*
- *Corresponding author: P. H. Riyadi, putut.riyadi@live.undip.ac.id*
- *ORCID iD : 0000-0003-2940-4108*

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 Aldrigh) 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).

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 FeCl3 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 × 105 μg of methanol extract was put into 2 x 103 l of 96 % ethanol in a test tube. The mixture was added five × 103 μL of distilled water and five × 102 μL of Follin-Ciocalteau reagent (50 % v / v), then the mixture was left for 5 minutes. Then it was added 1 × 103 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	Alkaloids		
		Meyer	Dragendrof	Bouchardat
extracts of crab waste protein (Portunus pelagicus) non-shell waste				

TABLE 2

COLOR TEST RESULTS OF THE NON-SHELL CRAB WASTE PROTEIN HYDROLYZATE EXTRACT FOR TANINS, TERPENOIDS, POLIFENOL, SAPONIN

Sample	Compounds			
	Tannins	Terpenoids	Polifenols	Saponins
extracts of crab waste protein (*Portunus pelagicus*) 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 Dagendorf	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 penyphenols. The extract has the potential to be developed in the field of health as a nutraceutical or pharmaceutical.

5 ACKNOWLEDGMENT

The author would like to thank the Faculty of Fishery and Marine Sains, Diponegoro University for Scheme B Outcome-Based Research Grant.

REFERENCES

[1]. N. H. Ishak, & N. M. Sarbon, Optimization of the enzymatic hydrolysis conditions of waste from shortfin scad (Decapterus Macrosoma) for the production of angiotensin I-converting enzyme (ACE) inhibitory peptide using response surface methodology, International Food Research Journal, Vol. 24, no. 4, pp. 1735–1743, 2017.

[2]. P. A. Harnedy, & R. J. FitzGerald, 2012. Bioactive peptides from marine processing waste and shellfish: A review. Journal of Functional Foods, Vol. 4, no. 1, pp. 6–24, 2012.

[3]. R. Balti, A. Bougatef, N. E. H. Ali, D. Zekri, A. Barkia, & M. Nasri, Influence of degree of hydrolysis on functional properties and angiotensin I-converting enzyme-inhibitory activity of protein hydrolysates from cuttlefish (Sepia officinalis) by-products, Journal of the Science of Food and Agriculture, Vol. 90 no. 12, pp. 2006–2014, 2014.

[4]. O. Abdelhadi, M. Jridi, I. Jemil, L. Mora, F. Toldra, M-C. Aristoy, & R. Nasri, Combine biocatalytic conversion of smooth hound viscera : Protein hydrolysates elaboration and assessment of their antioxidant, anti-ACE and antibacterial activities, Food Research International, Vol. 86, pp. 9–23, 2016.

[5]. A. Bougatef, N. Nedjar-Arroume, R. Ravallec-Plé, Y. Leroy, D. Guillochon, A. Barkia, & M. Nasri, Angiotensin I-converting enzyme (ACE) inhibitory activities of sardinelle (Sardinella aurita) by-products protein hydrolysates obtained by treatment with microbial and visceral fish serine proteases, Food Chemistry, Vol. 111, no. 2, pp. 350–356, 2008.

[6]. J. Roslan, K. Faezah, N. Abdullah, & S. Mazlina, Characterization of Fish Protein Hydrolysate from Tilapia (Oreochromis niloticus) by-Product, Agriculture and Agricultural Science Procedia, Vol. 2, pp. 312–319, 2014.

[7]. P. H. Riyadi, E. Suprayitno, A. Aulanni'am, & T. D. Sulistiyati, Chemical Characteristics and Amino Acids Profile of Protein Hydrolysates of Nile Tilapia (Oreochromis niloticus) Viscera, World Vet. J., Vol. 9, No. 4, pp. 324-328, 2019.

[8]. P. H. Riyadi, Bioactive Peptide for Lowering Pressure Blood from Fisheris by-Product, J. Peng & Biotek Hasil Pi, Vol. 7, No.1, pp. 1–6, 2018.

[9]. Y. Mine, E. Li-Chan, & B. Jiang, Bioactive Proteins and Peptides as Functional Foods and Nutraceuticals. Wiley-Blackwell, USA, 2010, ISBN: 9780813813110, (Book style).

[10]. H. Nasri, A. Baradaran, Shirzad, & M. Rafieian-Kopaei, New concepts in nutraceuticals as alternative for pharmaceuticals. International Journal of Preventive Medicine, Vol. 5, No. 12, pp. 1487–1499, 2014

[11]. G. Hardy, Nutraceuticals and functional foods: introduction and meaning. Nutrition, Vol. 16, No. 7/8, pp. 688–689, 2000.

[12]. E. Susanto, & A. S. Fahmi, Senyawa Fungsional Dari Ikan : Aplikasinya Dalam Pangan. Jurnal Aplikasi Teknologi Pangan, Vol. 1, No. 4, pp. 95–102, 2012.

[13]. N. T. Hoyle, & J. H. Merrit, Quality of Fish Protein Hydrolysates from Herring (Clupea harengus). Journal of Food Science, Vol. 59 No. 1, pp. 76–79, 1994.

[14]. M. A. Amiza, Y. L. Kong, & A. L. Faazaz, Effects of degree of hydrolysis on physicochemical properties of cobia (Rachycentron canadum) frame hydrolysate. International Food Research Journal, 19(1), 199–206, 2012.

[15]. J. B. Harborne, Phytochemical methods, 2nd edition. Chapman And Hall Publications, Vol. 288, 1984.

[16]. S. Skrovankova, D. Sumczynski, J. Mlcek, T. Jurikova, J. Sochor, Bioactive Compounds and Antioxidant Activity in Different Types of Berries, Int J Mol Sci, Vol. 16, pp. 24673–24706, 2015.

[17]. A. P. Gollucke, O. Jr. Aguiar, L. F. Barbisan, D. A. Ribeiro, Use of grape polyphenols against carcinogenesis: putative molecular mechanisms of action using in vitro and in vivo test systems. J Med Food, Vol. 16, pp. 199–205, 2013.

[18]. L. Marín, E. M. Miguélez, C. J. Villar, F. Lombó, 2015, Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties, Biomed Res Int, 905215, 2015 (Book style).

[19]. N. Gonzalez-Abuin, M. Pinent, A. Casanova-Marti, L. Arola, M. Blay, A. Ardevol, Procyanidins and their healthy protective effects against type 2 diabetes, Curr Med Chem, Vol. 22, pp. 39–50, 2015.

[20]. R. Firm, Nature's Chemicals, Oxford: Biology, 2010, (Book style).

[21]. A. Salminen, J. Ojala, J. Huuskonen, A. Kauppinen, T. Suuronen, K. Kaarniranta, Interaction of aging-associated signaling cascades: inhibition of NF-kappaB signaling by longevity factors FoxOs and SIRT1, Cell

Mol Life Sci, Vol. 65, No. 7-8, pp. 1049-1058, 2008.
[22]. L. Melanie, Pentacyclic Triterpenes of the Lupane, Oleanane and Ursane Group as Tools in Cancer Therapy. Planta Medica, Vol. 75, No. 15, pp. 1549-1560, 2009.
[23]. Battineni, J. Kumar, P. K. Koneti, V. Bakshi, N. Boggula. Triterpenoids: A review. International Journal of Research in Pharmacy and Pharmaceutical Sciences, Vol. 3, No. 2, pp. 91-96, 2018.
[24]. S. Lecour, K. T. Lamont, Natural polyphenols and cardioprotection. Mini Rev Med Chem, Vol. 11, pp. 1191–1199, 2011.
[25]. J. Pérez-Jiménez, V. Neveu, F. Vos, A. Scalber, Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol Explorer database. Eur J Clin Nutr, Vol. 64, pp. S112–S120, 2010.
[26]. A. Singh, S. Holvoet, A. Mercenier, Dietary polyphenols in the prevention and treatment of allergic diseases, Clin Exp Allergy, Vol. 41, pp. 1346–1359, 2011.

Color Test For Screening Chemical Components

ORIGINALITY REPORT

19%	%	19%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Eko Sri Sulasmi, Murni Saptasari, Kuni Mawaddah, Firda Ama Zulfia. "Tannin Identification of 4 Species Pterydophyta from Baluran National Park", Journal of Physics: Conference Series, 2019 Publication	5%
2	Hannah Cory, Simone Passarelli, John Szeto, Martha Tamez, Josiemer Mattei. "The Role of Polyphenols in Human Health and Food Systems: A Mini-Review", Frontiers in Nutrition, 2018 Publication	4%
3	N. Bhaskar, T. Benila, C. Radha, R.G. Lalitha. "Optimization of enzymatic hydrolysis of visceral waste proteins of Catla (Catla catla) for preparing protein hydrolysate using a commercial protease", Bioresource Technology, 2008 Publication	2%
4	Sridhar Reddy Vulapula, Srinivas Malladi. "Attribute-based Encryption for Fine-Grained	2%

Access Control on Secure Hybrid Clouds", International Journal of Advanced Computer Science and Applications, 2020
Publication

---

5 "Proceedings of the Andalas International Public Health Conference 2017", BMC Public Health, 2017 — 1%
Publication

---

6 Nura Ruhaya Abdul Halim, Azrina Azlan, Hayati Mohd Yusof, Norizah Mhd Sarbon. "Antioxidant and anticancer activities of enzymatic eel (monopterus sp) protein hydrolysate as influenced by different molecular weight", Biocatalysis and Agricultural Biotechnology, 2018 — 1%
Publication

---

7 I Wijayanti, E S Susilo. " Proximate content of wild and cultured eel ( ) in different part of body ", IOP Conference Series: Earth and Environmental Science, 2018 — 1%
Publication

---

8 Veymar G. Tacias-Pascacio, Roberto Morellon-Sterling, El-Hocine Siar, Olga Tavano et al. "Use of Alcalase in the production of bioactive peptides: A review", International Journal of Biological Macromolecules, 2020 — <1%
Publication

9	Ali Topçu, Ilbilge Saldamli. "Proteolytical, Chemical, Textural and Sensorial Changes During the Ripening of Turkish White Cheese Made of Pasteurized Cows' Milk", International Journal of Food Properties, 2006 Publication	<1%
10	Ola Abdelhedi, Leticia Mora, Ines Jemil, Mourad Jridi, Fidel Toldrá, Moncef Nasri, Rim Nasri. "Effect of ultrasound pretreatment and Maillard reaction on structure and antioxidant properties of ultrafiltrated smooth-hound viscera proteins-sucrose conjugates", Food Chemistry, 2017 Publication	<1%
11	Seafood Processing By-Products, 2014. Publication	<1%
12	Asadatun Abdullah, Hartmut Rehbein. "Authentication of closely related scombrid, catfish and tilapia species by PCR-based analysis and isoelectric focusing of parvalbumin", European Food Research and Technology, 2015 Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

Color Test For Screening Chemical Components

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4