

**LAPORAN AKHIR**  
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**Hidrolisat protein hati dan paru-paru sapi  
sebagai antioksidan dan antiproliferasi sel kanker kolon  
(CaCo-2) *in vitro***

**Tahun ke 1 dari rencana 2 tahun**

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## ABSTRAK

Tujuan penelitian ini adalah mempelajari sifat fungsional protein dari konsentrat protein hati dan paru-paru sapi, mengetahui pengaruh hidrolisis enzimatis dengan pepsin terhadap kemampuan hidrolisat protein hati dan paru-paru sapi sebagai antioksidan dan mengetahui karakteristik hidrolisat protein antioksidatif. Hidrolisat protein hati dan paru-paru sapi diperoleh dengan melakukan serangkaian tahapan penelitian yaitu: pembuatan konsentrat protein hati dan paru-paru sapi dengan ekstraksi basa dan presipitasi asam, evaluasi sifat fisikokimia dan fungsional protein hati dan paru-paru sapi, filtrasi dan karakterisasi hidrolisat protein antioksidatif tertinggi (HPLC, FTIR, SDS-PAGE), dan evaluasi aktivitas antioksidan fraksi hidrolisat protein secara kimia *in vitro* (DPPH, FTC, dan TBA). Hasil penelitian menunjukkan bahwa hati dan paru-paru sapi mempunyai kadar protein 20,29% dan 19,07%, dengan rasio protein/DM yaitu 68,69% dan 85,13% untuk hati dan paru-paru sapi. Konsentrat protein yang diperoleh memiliki rendemen sebesar 40% untuk hati dan 30% untuk paru-paru. Protein hati dan paru-paru sapi memiliki sifat fungsional yang baik dilihat dari daya buih, stabilitas buih, sifat emulsi, kemampuan mengikat air dan kemampuan mengikat minyak. Kadar protein terlarut berkisar antara 1,28 – 7,02 mg/ml dan kadar peptida sebesar 1,904 – 3,052 mg/ml. Derajat hidrolisis yang diperoleh sebesar 3,06 – 37,03%. Hidrolisat kedua protein mempunyai kemampuan scavenging radikal bebas DPPH berkisar antara 8,65 – 40,58%, dengan uji TBA sebesar 22,44 – 64,92  $\mu\text{mol/kg}$ . Hidrolisat kedua protein memiliki aktivitas antioksidan berkisar 2,46 – 50,28% dengan metode tiosianat. Disimpulkan bahwa konsentrat protein hati dan paru-paru sapi yang dihasilkan dari ekstraksi alkali dan presipitasi asam dengan pengeringan menggunakan pengering microwave mempunyai sifat fungsional yang cukup baik. Hidrolisis dengan enzim pepsin menghasilkan hidrolisat protein hati dan paru-paru sapi yang memiliki kemampuan antioksidan.

**Kata kunci:** hati sapi, paru-paru sapi, konsentrat protein, hidrolisat protein, antioksidan

## ABSTRACT

The purpose of this research was to study the functional properties of proteins from beef liver and lung protein concentrates, to know the effect of enzymatic hydrolysis with pepsin to the ability of the beef liver and lung protein hydrolyzate as antioxidants and to know the characteristics of antioxidative protein hydrolysates. Beef liver and lung protein hydrolyzate were obtained by conducting a series of stages of the research: the manufacture of beef liver and lung protein concentrates with alkaline extraction and acid precipitation, the evaluation of physicochemical and functional properties of beef liver and lung proteins, filtration and characterization of the highest antioxidative protein hydrolysates (HPLC, FTIR, SDS - PAGE), and the evaluation of the antioxidative activity of protein hydrolyzate fractions chemically *in vitro* (DPPH, FTC, and TBA). The results showed that the beef liver and lung have a protein content of 20.29% and 19.07%, with the ratio of protein/DM is 68.69% and 85.13% respectively. Protein concentrate obtained has a yield of 40% for the liver and 30% for lungs. Beef liver and lungs protein have good functional properties viewed from foam capacity, foam stability, emulsion properties, water holding capacity and oil holding capacity. Soluble protein content ranged from 1.28 to 7.02 mg/ml and peptide contents of 1.904 to 3.052 mg/ml. The degree of hydrolysis was 3.06 to 37.03%. Both proteins hydrolyzate have the ability to scavenge free radical DPPH ranged from 8.65 to 40.58%, with TBA test of 22.44 to 64.92  $\mu\text{mol/kg}$ . Both proteins hydrolyzate have antioxidant activity ranged from 2.46 to 50.28% using thiocyanate method. It was concluded that beef liver and lungs protein concentrate resulting from alkali extraction and acid precipitation and dried using microwave dryers have a good functional properties. Hydrolysis by pepsin produced a beef liver and lungs protein hydrolyzate that have an antioxidant activity.

**Key words:** beef liver, beef lungs, protein concentrate, protein hydrolysate, antioxidant

## RINGKASAN

Pemanfaatan jeroan (hati dan paru-paru) saat ini hanya untuk manusia, hewan piaraan dan pakan ternak serta pupuk atau langsung dibuang saja. Di sisi lain, hati dan paru-paru sapi memperlihatkan kadar protein yang tinggi, antara 15-20% (w/w), sehingga dapat diupayakan sebagai alternatif sumber protein dan hidrolisat protein melalui hidrolisis enzimatik. Konsentrat protein memiliki sifat fungsional yang beragam. Hidrolisat protein yang diperoleh juga dapat memiliki fungsi fisiologi dan biologi yang tertentu, dalam hal ini sebagai antioksidan.

Tujuan jangka panjang dari penelitian ini adalah pengembangan calon ingredien untuk pangan fungsional berbasis hidrolisat protein dari protein hati dan paru-paru sapi yang memiliki sifat antioksidan dan antikanker kolon. Tujuan penelitian tahun pertama ini adalah : 1) mempelajari sifat fungsional protein dari konsentrat protein hati dan paru-paru sapi yang dihasilkan dengan metode ekstraksi alkali dan presipitasi asam dengan pengering microwave, 2) mengetahui pengaruh hidrolisis enzimatik dengan pepsin terhadap hidrolisat protein yang dihasilkan, 3) mengetahui kemampuan hidrolisat protein sebagai antioksidan, dan 4) mengetahui karakteristik hidrolisat protein antioksidatif. Manfaat penelitian ini adalah memberikan informasi mengenai metode yang dapat digunakan dalam mempersiapkan konsentrat protein dan hidrolisat protein hati dan paru-paru sapi yang memiliki aktivitas antioksidan tinggi sehingga dapat digunakan dalam pengembangan pangan fungsional baru.

Materi yang digunakan dalam penelitian ini adalah hati, paru-paru sapi dan pepsin. Bahan kimia yang digunakan untuk analisis kimia memiliki grade pro analisis. Tahapan penelitian pada tahun I yaitu: 1) pembuatan konsentrat protein hati dan paru-paru sapi dengan ekstraksi basa. Konsentrat protein hati dan paru-paru sapi dilihat jumlah yang diperoleh (rendemen) dan diuji kadar protein dengan metode Kjeldahl, kadar protein terlarut dengan metode Lowry, kadar peptida dengan metode OPA dan sifat fungsional protein seperti daya buih dan stabilitas buih, sifat emulsi (daya emulsi dan stabilitas emulsi), kapasitas mengikat air dan kapasitas mengikat minyak. 2) Pembuatan hidrolisat protein hati dan paru-paru sapi dilakukan dengan hidrolisis enzimatik menggunakan enzim pepsin. Penelitian dilakukan dengan memanaskan substrat konsentrat protein pada suhu 90°C selama 5 menit atau tanpa pemanasan awal. Kemudian dilanjutkan dengan hidrolisis menggunakan enzim pepsin selama 0,5; 1 dan 6 jam. Hidrolisat protein hati dan paru-paru sapi yang diperoleh dievaluasi tentang: a) kadar protein terlarut, b) kadar peptida, c) derajat hidrolisis, d) berat molekul menggunakan SDS-PAGE dan e) aktivitas antioksidan. Uji aktivitas antioksidan hidrolisat protein hati dan paru-paru sapi: a) uji aktivitas antioksidan hidrolisat protein terhadap radikal bebas DPPH, b) uji aktivitas antioksidan hidrolisat protein dengan metode tiosianat, dan c) uji aktivitas antioksidan hidrolisat protein dengan metode TBA. 3) Filtrasi dan karakterisasi hidrolisat protein antioksidatif tertinggi (HPLC, FTIR, SDS-PAGE), dan evaluasi aktivitas antioksidan fraksi hidrolisat protein secara kimia *in vitro* (DPPH, FTC, dan TBA).

Hasil yang diperoleh menunjukkan bahwa hati sapi yang digunakan memiliki kadar air 70,44%, protein 20,29%, lemak 3,95%, abu 1,68% dan karbohidrat 3,64%, dengan rasio protein/DM yaitu 68,69%. Paru-paru sapi yang digunakan mempunyai kadar air 77,6%, protein 19,07%, lemak 2,15%, abu 1,02% dan karbohidrat 0,15% dengan rasio protein/DM sebesar 85,13%. Konsentrat protein yang diperoleh memiliki



## SUMMARY

Utilization of offal (beef liver and lungs) was currently only for humans, pets and livestock feed as well as fertilizer or disposed of it. On the other hand, beef liver and lungs showed a high levels of protein, between 15-20% (w/w), so it could be suggested as an alternative source of protein and protein hydrolysates by enzymatic hydrolysis. Concentrated proteins have diverse functional properties. Protein hydrolyzate obtained by enzymatic hydrolysis could also have specific physiological and biological functions, in this case as an antioxidant.

The long term goal of this research was the development of ingredient candidate for functional food based on protein hydrolyzate from beef liver and lungs protein that have antioxidant and colon anticancer properties. The purpose of this first year study was: 1) to study the functional properties of proteins from beef liver and lungs protein concentrates produced by alkaline extraction and acid precipitation with microwave dryer, 2) to determine the effect of enzymatic hydrolysis with pepsin to protein hydrolysates produced, 3) to determine the ability of the protein hydrolyzate as an antioxidant, and 4) to determine the characteristics of antioxidative protein hydrolyzate. The benefits of this study was to provide information on methods that could be used in preparing protein concentrates and protein hydrolyzate from beef liver and lungs that have a high antioxidant activity and could be used in the development of new functional food.

The material used in this study was beef liver, beef lungs, and pepsin. Chemicals used for chemical analysis have pro analysis grade. Stages of research in year I were: 1) the manufacture of beef liver and lungs protein concentrates with alkaline extraction. Beef liver and lungs protein concentrate measured the protein yield and protein content tested by Kjeldahl method, soluble protein content tested by Lowry method, peptide content measured by OPA method and functional properties of proteins such as foam capacity and foam stability, emulsion properties (emulsion activity and emulsion stability), water holding capacity and oil holding capacity. 2) Preparation of beef liver and lungs protein hydrolyzate was conducted by enzymatic hydrolysis using pepsin. The study was conducted by heating the substrate protein concentrate at a temperature of 90°C for 5 minutes or no preheating. Then followed by hydrolysis using pepsin for 0.5, 1 and 6 hours. Beef liver and lungs protein hydrolyzate obtained were evaluated on: a) the content of soluble protein, b) peptide content, c) the degree of hydrolysis, d) molecular weight using SDS - PAGE and e) antioxidant activity. The antioxidant activity tests of beef liver and lungs protein hydrolysates were: a) the antioxidant activity test of protein hydrolysates on DPPH free radicals, b) the antioxidant activity test of protein hydrolysates using thiocyanate method, and c) the antioxidant activity test of protein hydrolysates with TBA method. 3) Filtration and characterization of highest antioxidative protein hydrolysates (HPLC, FTIR, SDS - PAGE), and the evaluation of the antioxidative activity of protein hydrolyzate fractions chemically in vitro (DPPH, FTC, and TBA).

The results obtained indicated that beef liver used has a water content of 70.44%, 20.29% protein, 3.95% fat, 1.68% ash and 3.64% carbohydrates, with the ratio of protein/DM was 68.69%. The beef lungs used has a water content 77.6%, 19.07% protein, 2.15% fat, 1.02% ash and 0.15% carbohydrate with the ratio protein/DM of

85.13%. Protein concentrate obtained has a yield of 40% for beef liver and 30 % for beef lungs. Beef liver and lungs protein have a good functional properties in terms of the foam capacity (54.6% and 49.4%), foam stability (46 and 55 minutes), emulsion activity (0.52 and 0.54), emulsion stability (17 and 15 minutes), water holding capacity (1.89 ml/g and 2.05 ml/g) and oil holding capacity (6.28 ml/g and 5.86 ml/g). Soluble protein content in beef liver protein concentrate and hydrolyzate ranged from 1.28 to 6.95 mg/ml and peptide content of 1.904 to 3.052 mg/ml, and the degree of hydrolysis obtained for 3.06 to 37.03%. Beef liver protein hydrolyzate has the ability to scavenge free radical DPPH ranged from 8.65 to 40.58%, with TBA test of 22.44 to 64.92 umol/kg. The antioxidant activity ranged from 2.46 to 50.28% with thiocyanate method. Soluble protein content in beef lungs protein concentrate and hydrolyzate ranged from 1.42 to 7.02 mg/ml and peptide content of 1.962 to 2.968 mg/ml. The degree of hydrolysis obtained for 4.11 to 33.09%. Beef lung protein hydrolyzate has the ability to scavenge free radical DPPH ranged from 9.52 to 39.48%, with TBA test of 24.18 to 64.23 umol/kg and has antioxidant activity ranged from 3.64 to 48.91% using thiocyanate method.

From these results, it could be concluded that the beef liver and lungs protein concentrate produced by alkali extraction and acid precipitation and dried using microwave dryers have a good functional properties. Hydrolysis by pepsin produced a beef liver and lung protein hydrolyzate that have an antioxidant activity.

## DAFTAR PUSTAKA

- Al Awwaly, K.U., Natsir, M.H., Manab, A. dan Widati, A.S., 2009. Produksi Hidrolisat Protein Pollard dan Evaluasi Aktivitas Antioksidannya serta Aplikasinya dalam Pengolahan Bakso. Laporan Penelitian Hibah Penelitian Strategis Nasional Tahun 2009. Universitas Brawijaya, Malang.
- Anonim, 2008. Serat Pangan (Dietary Fiber). <http://duniapangankita.wordpress.com/>. Diakses tanggal 12 Februari 2010.
- Anonim, 2011. Statistik Peternakan dan Kesehatan Hewan 2011. Ditjen Peternakan dan Kesehatan Hewan. Jakarta.
- Anonim, 2012. Statistik Peternakan dan Kesehatan Hewan 2012. Ditjen Peternakan dan Kesehatan Hewan. Jakarta.
- Arihara, K., 2006. Strategies for designing novel functional meat products. *Meat Sci.* 74: 219-229.
- Arihara, K. and Ohata, M., 2008. Bioactive Compounds in Meat. In F. Toldra (Ed) *Meat Biotechnology*. Springer Science + Business Media, LLC. London. 231-249.
- Chan, W.K.M., E.A. Decker, J.B. Lee and D.A. Butterfield, 1994. EPR spin-trapping studies of the hydroxyl radical scavenging activity of carnosine and related dipeptides. *J. Agric. Food Chem.* 42: 1407-1410.
- Chen, H.M., K. Muramoto and F. Yamauchi, 1995. Structural analysis of antioxidative peptides from soybean  $\beta$ -conglycinin. *J. Agric. Food Chem.* 43: 574-578.
- Chen, H.M., K. Muramoto and F. Yamauchi, and K. Nokihara, 1996. Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. *J. Agric. Food Chem.* 44: 2619-2623.
- Decker, E.A., A.D. Crum and J.T. Calvert, 1992. Differences in the antioxidant mechanism of carnosine in the presence of copper and iron. *J. Agric. Food Chem.* 40: 756-759.
- Dinata, A., 2008. Hidrolisat protein ikan dan bahan fortifikasi makanan. <http://foodreview.biz/preview.php?view&id=78>. Diakses 1 Agustus 2008.
- Fadda, S., G. Oliver and G. Vignolo, 2002. Protein degradation by *Lactobacillus plantarum* and *Lactobacillus casei* in a sausage model system. *J. Food Sci.* 67 (3): 1179-1183.
- Hattori, M., K. Yamaji-Tsukamoto, H. Kumagai, Y. Feng and K. Takahashi, 1998. Antioxidative activity of soluble elastin peptides. *J. Agric. Food Chem.* 46: 2167-2170.
- Iwaniak, A. and B. Dziuba, 2009. Motif with potential physiological activity in food proteins-biopep database. *Acta Sci. Pol. Technol. Aliment.* 8 (3): 59-85.
- Jang, A., and M. Lee, 2005. Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. *Meat Science.* 69:653-661.
- Kim, H.S., H.S. Chae, S.G. Jeong, J.S. Han, S.K. Im, C.N. Ahn, and J.M. Lee, 2005. Antioxidant activity of some yogurt starter cultures. *Asian-Aust J. Anim Sci.* 18 (2):255-258.
- Kukizaki, H. and N. Nakatani, 1993. Antioxidant effect of some ginger constituents. *J. Food Sci.* 58 (6):1407-1410.
- Lawal, O.S., 2005. Functionality of native and succinylated Lablab bean (*Lablab purpureus*) protein concentrate. *Food Hydrocolloids.* 19:63-72.



- Sukanto, 2008. Eksplorasi Fraksi Globulin 7S dan 11S Komak (*Dolichos lablab*) dan Interaksinya dengan Gum Xanthan. Disertasi Program Pascasarjana Fakultas Pertanian Universitas Brawijaya, Malang.
- Sun, W., Zhao, H., Zhao, Q., Zhao, M., Tang, B., Wu, N. and Qian, Y., 2009. Structural characteristics of peptides extracted from Cantonese sausage during drying and their antioxidant activities. Abstract. *Innovative Food Sci. & Emer Tech.* 10 (4): 558-563.
- Susilo, A. dan K.U. Al Awwaly, 2005. Tinjauan kualitas fisik bakso dari pemanfaatan edible meat (hati sapi) sebagai substitusi daging dan sodium tripolyphosphate (STPP) sebagai bahan pengental. *Jurnal Ilmu-ilmu Hayati (Life Science)* 17 (2): 107-116.
- Vass, N., Czegledi, L. and Javor, A., 2008. Significance of functional foods of animal origin in human health. *Lucran stiinfice zootechnie si biotehnologie* 41 (2): 263-270.
- Vastag, Z., Popovic, L., Popovic, S., Petrovic, L. and Pericin, D., 2010. Antioxidant and angiotensin-I converting enzyme inhibitory activity in the water-soluble protein extract from Petrovac Sausage (Petrovska Kolbasa). Abstract. *Short com. Food Control* 21 (9): 1298-1302.
- Winarno, F.G., 1993. *Pangan, Gizi dan Konsumen*. PT. Gramedia. Jakarta.
- Yamashoji, S. and G. Kajimoto, 1980. Antioxidant effect of Gly-Gly-His on Cu (II)-catalyzed autoxidation and photosensitized oxidation of lipids. *Agric. Biol. Chem.* 44: 2735-2736.