Protein Profile Based on SDS-PAGE Sago Caterpil (*Rhynchophorus ferruginesus*) Processed with Variation of Grilling Type with Carcoal, Oven, and Microwave and Grilling Time

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Abstract. Sago is the staple food of the people of Eastern Indonesia, especially in the Papua and Maluku regions. Sago trees that have been cut down and decay will be seized by beetles and larvae, making them sago worms (*Rhynchophorus ferruginesus*). Based on the results of the proximate analysis, sago caterpillars contain 13.80% protein, 18.09% fat and 64.21% water. Sago caterpillars are usually processed in various ways such as fried and baked using charcoal, roasting in an oven or microwave. The purpose of this study was to determine changes in the characteristics of protein band patterns in sago caterpillar samples using the SDS-PAGE method based on variations in type and time of roasting. The results of research on roasting time of 1, 2, and 3 minutes on sago worms with charcoal, oven, and microwave resulted that the longer roasting time showed that the longer the roasting time the lower total protein content and the more protein bands and smaller molecular weight sizes in sago caterpillar samples, so that the higher the level of protein denaturation and produce a protein profile that is decreasing / worse, so that the effective time of roasting that produces a protein profile of sago worms is still good is 1 minute. Roasting sago caterpillars with a variety of roasting types for 1 minute produced the highest total protein and the best protein profile was roasting sago worms using charcoal.

Key words: protein profile, sago caterpillar, roasting time, roasting type

1. Introduction

Sago is the staple food of the people of Eastern Indonesia, especially in the Papua and Maluku regions. Sago trees that have been cut down and decay will be seized by beetles and larvae so that they become sago caterpillars (Rhynchophorus ferruginesus) (Hastuty, 2016, Purnamasari). Based on the proximate analysis, sago caterpillars contain 13.80% protein, 18.09% fat and 64.21% water (Wikanta, 2005; Widiastuti).

Sago caterpillars are usually processed in various ways such as fried and baked by heating from wood (Lidaya, et al., 2018), oven roasting, and microwave roasting (Elvira et al., 2018). An oven is a grill that uses gas, wood or electricity as a source of heat. Microwave is a grill that utilizes electromagnetic wave technology, whose position is between radio waves and infrared radiation in the electromagnetic spectrum. The process of roasting both charcoal, oven and microwave on sago caterpillars can cause

changes in appearance, texture and nutritional value of sago worms. Changes in physical and chemical properties that occur during the heating process (temperatures above 65 ° C) one of which is protein denaturation (Saputra, 2014).

According to Palupi (2015), sago caterpillar protein can be reduced due to processing by heating. Heating causes protein solubility, thus affecting the amount and type of protein that can be extracted in the process of protein isolation and causing coagulation, namely clumping / viscosity and loss of solubility. Coagulation is caused by protein molecules undergoing aggregation and the formation of bonds between molecules is hydrophobic, hydrogen bonds and disulfide bonds.

Characteristics of proteins and DNA in living things can be studied by molecular analysis methods based on electrophoresis principles (Darmawati et al., 2012; Ethica et al., 2013; Feri et al., 2017). The protein profile of sago caterpillars can be known by using electrophoresis, one of them is by using the SDS-PAGE method. Sodium Dodecyl Sulfate Method Polyacrilamide Gell Electrophoresis (SDS-PAGE) is a method used to separate protein subunits based on molecular weight through a polyacrylamide matrix which is electrified and migrates from the negative pole to the positive pole (Darmawati et al., 2012 dan Saputra, 2014). The purpose of this study was to determine the total protein and protein profile of sago caterpillar based on variations in type and time of roasting.

2. Method

The type of this study was an experiment with the independent variable of sago caterpillar type, namely baking with charcoal, oven, and microwave and roasting time of 1, 2 and 3 minutes. The dependent variable in this study is the total protein content and protein profile of sago caterpillar that has been roasted with charcoal, oven, and microwave with 1, 2, and 3 minutes roasting time.

2.1 The object of the research

The object of the research was 9 sago caterpillar samples which were purchased at the Sore market in Merauke city. Furthermore, each sago caterpillar was cut into 4 parts and each part was treated with no processing, charcoal roasting, oven roasting, and microwave roasting with 1, 2 and 3 minutes roasting time each repeated 3 times.

2.2 The Research

This research was conducted at the Biotechnology Laboratory of Gadjah Mada University, Yogyakarta.

2.3 The Tools and Reagent

The tools used in this study are oven, microwave, electrophoresis chamber, combs, glassplate, spaser, micropipette, cone tube, eppendorf tube, microtube, power supply, vortex device, gloves, mask, biological liquid flue, centrifuge, waterbath, yellowtip, bluetip, whitetip, erlenmeyer, rotator, grinding,

spectrophotometer, beaker glass, spatula, wire mesh, and furnace. The ingredients needed are 9 sago caterpillars, sterile aquabidest, 30% polyacrylamid, 1.5 M tris (pH 6.8 and 8.8), 10% SDS, 10% APS, TEMED, bromophenol blue, glycerin, coomassie briliant blue R-250, methanol, glacial acetic acid, and charcoal.

2.1 Research procedure

Nine Sago caterpillars were washed with water, then drained in a plastic basket container. One sago caterpillar is cut into 4 parts, part 1 without treatment, parts 2, 3, and 4 are grilled with charcoal, oven, and microwave respectively with 1 minute roasting time. The procedure is repeated for 2 and 3 minutes roasting time. The next step is isolation of the protein by means of sago caterpillar after receiving the treatment smoothed on a borer, PBS 1x is added and then centrifuged and the supernatant is taken and then added with biorad. The absorbance of the sample is read by using a spectrophotometer to obtain the sample protein concentration value. The sample protein is then recycled using the SDS-PAGE method.

3. Research Result

The total protein of sago worms with variations in roasting and roasting time is shown in Table 1.

Table 1. Total levels of sago caterpillar protein (µg/µl) with variations in roasting and roasting time

No.	Variation of roasting	Grilling Time Variations (minute)			
		0	1	2	3
1	Without treatment	4.93	-	-	-
2	Charcoal	4.93	3.03	2.95	2.67
3	Oven	4.93	2.46	2.30	1.63
4	*Microwave*	4.93	2.19	1.97	1.94

Table 1 shows that the total level of protein control is higher than roasting with charcoal, oven and microwave, and the total protein yield will be a calculation of the number of protein samples to be included in the gel. Protein profile analysis using the SDS-PAGE method for sago caterpillars baked with variations in the type of roasting and roasting time are shown in Figure 1 and Figure 2.

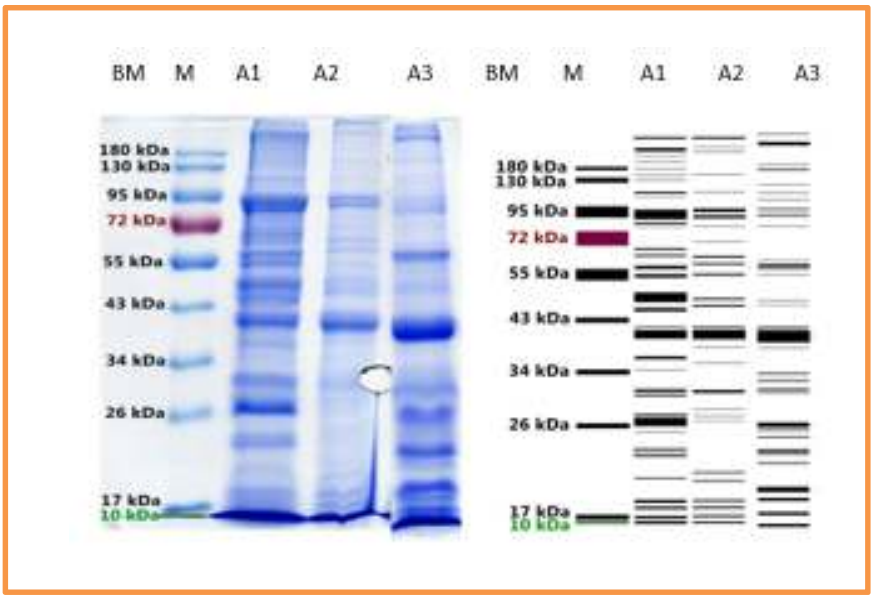

Figure 1. SDS-PAGE sago caterpillar electrophoretic results (left) and conversion results of SDS-PAGE gel visualization with Autocad program (right) Gel 1.

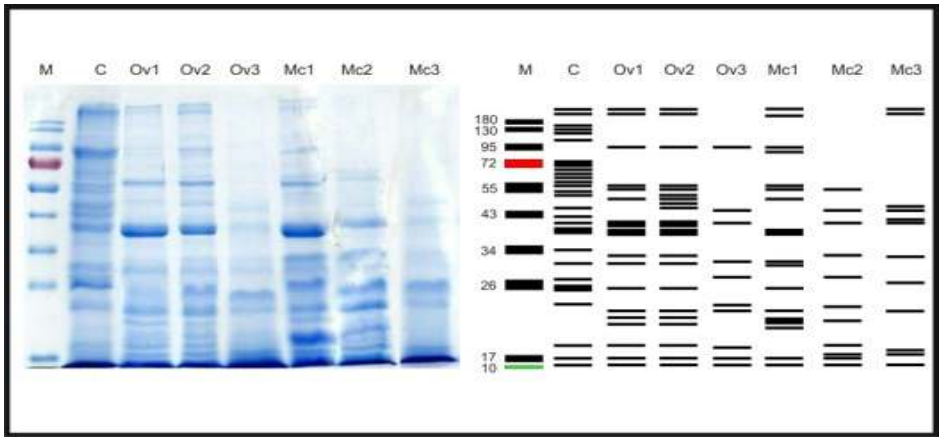

Figure 2. SDS-PAGE sago caterpillar electrophoretic results (left) and Conversion results of SDS-PAGE gel visualization with Autocad program (right) Gel 2.

Information:

M : Protein markers
C : Samples without treatment (control)
A1 : Charcoal with 1 minute roasting
A2 : Grilling with Charcoal 2 minutes
A3 : Grilling with Arabg 3 minutes
Ov1: Grill with 1 minute oven
Ov2: Grill with a 2 minute oven
Ov3: Grill with a 3-minute oven
Mc1: Grill with 1 minute microwave
Mc2: Microwave with 2 minutes roasting
Mc3: Grill with 3 minutes microwave

According Gunanti (2010), protein molecular weight determination is performed by calculating RF (Retardation Factor) of each band (band) protein with the following formula:

$$Rf = \frac{\text{Jarak pergerakan pita protein dari tempat awal}}{Jarak\ pergerakan\ warna\ dari\ tempat\ awal}$$

Distance, Rf, and molecular weight marker and samples are shown in Table 2 and Table 3.

Table 2. Rf and BM Marker Gel 2

Distance Marker	Rf Marker	Molecular Weight Marker (kDa)
0.6	0.10	180
0.8	0.14	130
1.2	0.20	95
1.6	0.27	72
2.2	0.37	55
2.8	0.47	43
3.6	0.61	34
4.3	0.73	26
5.7	0.97	17
5.8	0.98	10

Table 3. Rf and BM Marker Gel 1

Distance Marker	Rf Marker	Molecular Weight Marker (kDa)
0.3	0.05	180
0.5	0.09	130
0.9	0.16	95
1.1	0.20	72
1.7	0.30	55
2.3	0.41	43
3.0	0.54	34
3.8	0.68	26
5.3	0.95	17
5.6	1.00	10

To determine the protein molecular weight of the sample in the gel, it was calculated using Rf and plotted on the logarithmic graph of the Rf protein marker whose molecular weight was known (Darmawati et al., 2012). BM sago worms with variations in roasting with charcoal, oven and microwave and variations in roasting time are listed in Table 4.

Tabel 4. Analysis Result and sample Molecular Weight at gel 1 and gel 2

Gel	**Sample Type**	**Sample Protein**	**Molecular Weight (kDa)**
2	K	3 major ribbons	72, 38 dan 26 kDa
		23 minor ribbons	156, 130, 121, 106, 70, 68, 64, 60, 57, 53, 51, 47, 42, 40, 34, 30, 27, 24, 18, 17 dan 13 kDa
1	A1	4 major ribbons	95, 50, 38, dan 26 kDa
		21 minor ribbons	180, 165, 121, 93, 87, 81, 72, 68, 62, 57, 46, 42, 36, 33, 29, 28, 24, 23, 21, 19, dan 10 kDa
	A2	4 major ribbons	93, 55, 42, dan 34 kDa
		23 minor ribbons	180, 121, 115, 87, 81, 78, 72, 68, 62, 52, 49, 44, 41, 39, 31, 28, 26, 25, 24, 23, 19, 17, dan 10 kDa
	A3	1 major ribbons	40 kDa
		30 minor ribbons	180, 130, 121, 115, 106, 95, 93, 81, 68, 66, 62, 57, 55, 52, 50, 49, 42, 39, 36, 34, 31, 30, 28, 27, 25, 23, 20, 19, 18, dan 10 kDa
2	O1	2 major ribbons	40 dan 38 kDa
		18 minor ribbons	95, 57, 55, 51, 49, 47, 45, 32, 30, 26, 22, 21, 20, 18, 17 dan 13 kDa
	O2	2 major ribbons	40 dan 38 kDa
		15 minor ribbons	95, 57, 55, 49, 32, 30, 26, 22, 21, 20, 18, 17 dan 13 kDa

Gel	Sample Type	Sample Protein	Molecular Weight (kDa)
2	O3	10 minor ribbons	95, 45, 40, 31, 28, 25, 22, 17 dan 13 kDa
	M1	2 major ribbons	38 dan 19 kDa
		14 minor ribbons	180, 95, 72, 57, 55, 49, 31, 30, 26, 22, 21, 19, 17 dan 13 kDa
	M2	12 minor ribbons	47, 45, 41, 40, 32, 26, 23, 18, 17 dan 13 kDa
	M3	11 minor ribbons	55, 45, 40, 33, 28, 25, 21, 19, 17 dan 13 kDa

Figure 1, Figure 2 and Table 4 show that the visualization of the representation of sago caterpillar protein bands based on variations in the type of roasting with charcoal, oven, and microwave respectively resulted in a decrease in the number of major ribbon proteins and a smaller band increasing. Increasing the roasting time of both sago caterpillar baked with charcoal, oven, and microwave also produces smaller total protein levels and the greater number of major protein ribbons decreases and minor bands increase. The type and time of roasting are effective in producing total protein and the protein profile of sago caterpillars is best for roasting using charcoal for 1 minute.

This is consistent with Palupi's study (2015), sago caterpillar protein can be reduced due to processing by heating. Heating causes protein solubility, thus affecting the amount and type of protein that can be extracted in the process of protein isolation. The process of processing materials by heating will also cause coagulation, namely changes in the structure of the protein of sago caterpillars, changes in shape from liquid (sol) to solid or semi-solid (gel) and protein molecules undergoing aggregation and the formation of bonds between molecules that are hydrophobic bonds hydrogen bonds and disulfide bonds resulting in increased viscosity and loss of solubility.

4. Conclussion

Based on the results of research on protein profiles in sago caterpillars with SDS-PAGE based on variations in the type and time of roasting, it was concluded that the longer the roasting time of sago worms, both with charcoal, oven and microwave, the lower total protein levels and the reduced protein profile. The type of roasting in sago caterpillar with 1 minute roasting time which produces the best protein profile is roasting using charcoal.

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Oral Presentation Acceptance

November 13th, 2018

Dear **Ana Hidayati Mukaromah,**

Thank you for submitting a paper to present at **"1st International Conference On Food Science & Technology 2018"**. On behalf of the Organising Committee, I am delighted to inform you that your abstract **Protein Profile Based on SDS-PAGE Sago Caterpil *(Rhynchophorus ferruginesus)* Processed with Variation of Grilling Type with Carcoal, Oven, and Microwave and Grilling Time,** has been accepted for an **oral presentation** at the conference.

Details of your Paper are as fellows :

Title :	**Protein Profile Based on SDS-PAGE Sago Caterpil *(Rhynchophorus ferruginesus)* Processed with Variation of Grilling Type with Carcoal, Oven, and Microwave and Grilling Time**
Authors :	Ana Hidayati Mukaromah, S N Ethica, S Darmawati, N Lidaya, and S. Elvira
Presenting Author :	**Ana Hidayati Mukaromah***

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SURAT TUGAS

Nomor : 968/UNIMUS.G/ST/2018

1	Pejabat yang berwenang memberi perintah	Dekan Fakultas Ilmu Keperawatan dan Kesehatan Universitas Muhammadiyah Semarang
2	Nama yang diperintah	**Dr. Ana Hidayati Mukaromah, M.Si**
3	a. Jabatan b. Beban Anggaran	Dosen Fakultas Ilmu Keperawatan dan Kesehatan (FIKKES – UNIMUS) ----------
4	Maksud Tugas	1st International Conference on Food Science and Technology 2018 (1th ICHESTECH)
5	Alat Angkutan / Transportasi	Dinas / Umum / Pribadi
6	a. Tempat berangkat b. Kota / Tempat Tujuan c. Lama Tugas d. Hari / Tanggal Berangkat e. Hari / Tanggal Kegiatan f. Hari / Tanggal Kembali	FIKKES – UNIMUS Universitas Muhammadiyah Semarang 2 hari Rabu, 28 November 2018 Rabu-Kamis, 28-29 November 2018 Kamis, 29 November 2018
7	Kepada yang bersangkutan diharapkan : a. Dapat melaksanakan tugas dengan sebaik-baiknya. b. Mengembalikan surat tugas kepada administrasi bag. Keuangan FIKKES c. Memberikan laporan kepada Pimpinan Fakultas tentang hasil yang diperoleh dalam kegiatan diatas	
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