International Journal of Scientific Engineering and Research (IJSER)
ISSN (Online): 2347-3878
Index Copernicus Value (2015): 62.86 | Impact Factor (2015): 3.791

Analysis of Amylase Inhibitors Content and Characterizations of Cassava Adira - 1 Variety: *(Manihot esculenta Crantz)* as a Functional Food

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Abstract: ***The results of activity test analysis of amylase inhibitors early stages obtained 16 samples amylase inhibitors extracted from cassava varieties of Adira-1 (Manihot esculenta crantz) by using the α-enzyme amylase of marine animals Brevibacterium sp. Based on the analysis, that the sample number 2, 3, 4, 6, 10, 11, 13, 14 and 15 had a positive value, it means predictable that to 9 samples amylase inhibitors that have the ability to inhibit α-amylase. Based on the protein content test in amylase inhibitors using the method of Lowry, that the protein value of 16 samples ranged from 0.325 mg / mL to 0.588 mg / mL. After purification the result that the molecular weight amylase inhibitors ranged from 20 kD to 113 kD. In this study, acquired 100% inhibition of the activity of amylase inhibitors against the action of the α-amylase enzyme in the samples of cassava were experiencing treatment dredged mucus layer cassava and experience of immersion in water for 12 hours (P1L2) occurs at a volume of amylase inhibitor 400 ng, a temperature of 30 ° C, the concentration of 1% starch, α-amylase enzyme volume 0.4 mg / mL and pH 6.7. Based on these results, amylase inhibitors of cassava (Manihot esculenta Crantz) had a potential inhibitory action of the α-amylase enzyme, so that it could be made as a functional food which could be used for prevention, treatment of obesity and diabetes mellitus due to the isolation, purification and characterizations of specific amylase inhibitors in casava varieties of Adira-1 (Manihot esculenta Crantz) had made it known. For further research on the type of protein in cassava of amylase inhibitors with Immunobloting method or Western Bloting.***

Keywords: Amylase inhibitors, inhibition, cassava, α-amylase enzyme and Functional Food

1. Introduction

A. Background.

Cassava is a traditional food crop widely grown in subtropical and tropical Southeast Asia, South Asia, Latin America and South Africa. Bulbs can be consumed as a source of starch or carbohydrates are developed locally are usually used as a staple food. Nutritional evaluation of cassava shows the storage of biologically active substances that have anti-nutritional properties, namely, reducing the availability of nutrients for animals and humans, among these substances are inhibitors of amylase (McEwan, 2008).

Amylase inhibitors is proteins include widespread in cassava tubers. Inhibiting the amylase enzyme is believed to be deadly to insects, thus contributing several advantages for plants (Sasikiran et al., 2002). Amylase inhibitors are known as "starch blockers" or starch blockers, because it can prevent the starch food from being digested and absorbed by the body. This could be useful for treating obesity and diabetes mellitus and metabolic disorders characterized by chronic hyperglycemia due to damage to the insulin secretion (Ali et al., 2006). Cassava plays an important role in the treatment of diabetes, especially in developing countries where most people have limited resources and do not have access to modern medicine.

Amongst the medical world amylase inhibitors on cassava, very important presence and development in plant physiology of animals and human nutrition, extensive research has been conducted in the laboratory and have already acquired biological effects (Garcia-Olmedo et al, 1987; Silano, 1987).

In most cases the mechanism of inhibition by amylase inhibitors occurs through direct blockage of the active center of the enzyme in several sub sites (Payan, 2004). Sharma and Pattabiraman, (1980, 1982); (Ida et al., 1994) stated that many of amylase inhibitors present in the tuber active against mammalian amylase, but showed no amylase activity in the plant. Sharma and Pattabiraman (1982), have reported similar results for Dioscorea alata. Bifunctional nature has been shown by a number of inhibitors and has received special attention as an attractive candidate for experimental animals (Maskos et al., 1996). Amylase inhibitors are also found in wheat (Franco et al., 2000), barley (Richardson, 1991) and finger millet India (Campos and Richardson, 1983). Amylase inhibitor has been shown to efficiently inhibit the action of the enzyme amylase in different insects.

Based on these results, of the 16 samples studied only 9 amylase inhibitors that have a positive value, it means to the amylase inhibitor 9 has the ability to inhibit the activity of amylase or having inhibitors. Amylase inhibitors that have inhibitors activity was a factor that greatly affect not only the thickness of dredging factors but the duration of immersion time (12-48 hours) has a critical determinant of inhibitors activity. For samples that undergo soaking for 48 hours already do not have the power inhibitors activity, although it contains amylase inhibitor.

According to Boivin et al; 1988, amylase inhibitors can inhibit the breakdown of starch, then people do not get some of its energy from carbohydrate-containing amylase

inhibitors. Things like this can be used as a regulator in the management of diet in patients with diabetes mellitus, because of the effect of work amylase inhibitors with a certain amount in the body can lower blood glucose levels, by not stimulating the release of insulin by the pancreas and increase its effectiveness, because it works by delaying absorption glucose in the gut, so that it can control blood glucose levels, it is in line with research from Boivin et al; 1988; on "Gastrointestinal and metabolic effects of amylase inhibition in Diabetics" as well as the operation and Murugesan; 2012; about "Invitro α-amylase and α-glucosidase inhibition activity of crude ethanol extract of Cissus arnottiana".

Duncan's Multiple Range Test (DMRT) based on a confidence level of 5%, that the interaction dredging thickness and length of time soaking up the very real significant effect on the working mechanism of inhibition of the amylase inhibitors. In this research, the inhibition of 100% of the activity of amylase inhibitors against the action of the amylase enzyme in the samples of cassava are being treated not dredged mucus layer cassava and experienced immersion in water for 12 hours (P1L2) occurs in the volume of amylase inhibitors of 400 ng, temperature of 30 ° C, starch concentration of 1%, the volume of amylase enzyme 0.4 mg / mL and a pH of 6.7. Based on the test Additional research is needed to further investigate this possibility.

2. Research Methodology

Materials and Tools

Raw materials used in this study is Cassava Varieties Adira-1 obtained from the city of Palembang South Sumatra. Cassava is milled and sieved to 80 mesh size and then made into cassava flour. Chemicals used in this study is sulfuric acid, sodium sulfate, HgO, sodium hydroxide, saturated boric acid, hydrochloric acid, amylase enzyme of Brevibacterium sp, 20 mM phosphate buffer pH 6.6 and pH 6.7, 0.5% Starch; 1%, 2%, 3% (Merck), Ammonium sulfate (Merck), DNS (Sigma), Materials for SDS-PAGE consisting of: acrylamide-bis acrylamide 30% (Biobasic), TEMED (Sigma), APS (Sigma) , 10% SDS, water millipure, Buffer 0.5 M Tris-HCl pH 6.8; 1.5 M Tris HCl buffer pH 8.8, Mercaptoethanol (Merck), running a sample buffer for SDS-PAGE consist of: Bromophenol blue, 10% SDS, Glycin, running buffer for SDS-PAGE consist of: Glycin (Merck), SDS (Nacalai), Tris-HCl (Promega), Fixing solution consisting of 50% methanol (Merck), 10% glacial acetic acid (Merck) and milipure, Staining solution consisting of 0.1% Comassie brilliant blue R-250, 50 % methanol, 10% glacial acetic acid and milipure, destaining solution consisting of 40% methanol, 10% glacial acetic acid and milipure, test materials Lowry consists of reagent analysis, solution Folin-Ciocalteu, standard BSA (Sigma), and Marker for protein (Biorad).

Research Equipment

Research tools that are used for the manufacture of cassava flour and inspection levels proximate and cyanide is cup porcelain, clamp plate, desiccator, muffle furnace (furnace), an analytical balance, desiccator which already contains silica gel, a set of tools Kjeldhal consisting of pumpkin micro Kjeldhal to destruction process, heating pumpkin Kjeldahl, distillation equipment, and tools titration, set Soxhlet apparatus along with condenser and pumpkin (round bottom flask), mortar, oven blower, goblet, micrometer screw, knife stainlees steel, sieve with a size of 80 mesh and steamed , plastic washbasins, flask, incubator shaker, burette, beaker glass. These tools are in the Chemical Laboratory Results Agriculture, Department of Agricultural Technology, Sriwijaya University and the Laboratory of Animal Nutrition and Feed the Faculty of Agriculture, University of Sriwijaya, Indralaya.

The tools used for analysis of amylase inhibitors in cassava flour, among others: the spectrophotometer, waterbath, centrifuges, PCR machine, electrophoresis for protein / SDS PAGE for Protein, shaker incubator, and autoclave. These tools are in the Laboratory of Bioprocess Sector biocatalyst and Fermentation Biotechnology Research Center LIPI Cibinong, Bogor. The study design used was completely randomized factorial design (Ralf), replicated three times for each treatment combination. The combination treatment significantly done by using Duncan's Multiple Range Test (DMRT) at the level of 1%.

Making Cassava Flour

Selection of a good quality bulbs (old optimum, aged 7-10 months). Starting with washing fresh cassava, to clean up the soil and dirt. Cassava which has been cleaned and then will be stripping and disposal of mucus layer by dredging 0 mm, 0.5 mm, 1.0 mm and 1.5 mm without soaking and stripping without mucus discharge by immersion for 0 hours, 12 hours, 24 hours and 48 hours. Followed by washing three times to re soaked. After flushing both cassava soaked or not, cut into small pieces. Cassava is cut into small pieces drained to remove the excess water to cassava through a process of soaking before being dried. The drying process is done by using a blower oven temperature of 50 ºC for 72 hours to produce a moisture content of cassava with a predetermined minimum is 12.5%, according to the formula:

Pieces of dried cassava is then ground and sieved (80 mesh), thus producing cassava flour with a mesh or a desired level of refinement.

Experimental Procedure

Extraction Amylase Enzyme Inhibitor. A total of 0.1 grams of cassava flour dissolved in 1 mL of 20 mM posphate buffer pH 6.7 and incubated for 1 h at 4 ° C. Furthermore, the solution was centrifuged at 10,000 rpm for 10 min at 4 ° C. Preparation of enzyme of Brevibacterium sp. Amylase enzyme used in the activity test glukoprotein / amylase inhibitor derived from marine bacteria Brevibacterium sp.

Amylase enzyme activity test. Amylase enzyme activity measurements performed by the reaction of 250 mL to 250 mL of enzyme substrate (starch 0.5%) at room temperature for 30 minutes. Then added 750 mL of DNS solution and

incubated at a temperature of 100 ° C for 15 minutes. The solution was cooled with ice for 10 minutes and its absorbance was measured at a wavelength of 540 nm.

Protein Concentration Test Method Using Lowry. 2 ml reagent is added to the analysis of standard solution of 200 mL / sample at room temperature for 10 minutes. The solution mixture was homogenized using a vortex. Furthermore, as many as 200 mL Folin-Ciocalteu reagent was added and incubated at room temperature for 30 minutes. Absorbance values mixture solution was measured at a wavelength of 660 nm with the spectrophotometer instrument.

3. Results and Discussion

Chemical Composition of Cassava Flour

Analysis of the chemical composition of cassava flour with different treatment of dredging thickness (0-1.5 mm), length of immersion (0-48jam) and the drying temperature (50 ° C), long drying time (72 hours) include; amylase inhibitor (Method Bernfeld (1955), Kumari et al (2012) with modifications), the levels of proximate (Method AOAC, 1995) starch content, moisture, ash, fat, cyanide, protein, yield, carbohydrate (by difference), amylose (Method AOAC 2005), as well as fiber content (Van Soest method) NDF, ADF, hemicellulose, cellulose, lignin and pectin as presented in Table 1 and Table 2.

Table 1: Comparison of Composition and Fiber Content of Amylase Inhibitors of Cassava Flour with a Variety of Treatments

Code	NDF (%)	ADF (%)	Hemicellulose (%)	cellulose (%)	Lignin (%)	Pectin (%)	Amylase Inhibitors (%)	Cyanide (ppm)
P1L1	1,54	1,263	0,277	0,383	0,41	0,225	0,084	44,41
P1L2	2,432	1,275	1,158	0,163	0,31	0,194	0,092	39,31
P1L3	1,829	1,08[D]	0,749	0,272	0,29	0,143	0,050	38,29
P1L4	1,679	0,759	0,759	0,576	0,25	0,105	0,027	37,25
P2L1	1,436	1,126	0,31	0,897	0,03	0,32	0,036	44,03
P2L2	1,547	1,363	0,183	0,382	0,12	0,378	0,074	31,12
P2L3	2,046	0,908	1,137	0,31	0,15	0,273	0,031	30,35
P2L4	1,39	1,127	0,263	0,401	0,06	0,12	0,014	30,06
P3L1	1,378	0,705	0,673	1,704	0,51	0,303	0,010	42,51
P3L2	1,332	1,16	0, 172	0,715	0,01	0,368	0,070	29,81
P3L3	1,929	0,8	1,126	0,302	0,09	0,105	0,062	29,49
P3L4	1,251	1,06	0,192	0,451	0,08	0,264	0,079	29,08
P4L1	1,104	0,93	0,173	1,814	0,32	0,119	0,008	40,32
P4L2	1,357	1,082	0,275	0,783	0,12	0,287	0,016	27,52
P4L3	1,81	0,715	1,095	0,138	0,04	0,115	0,002	27,34
P4L4	1,148	1,059	0,088	0,939	0,05	0,239	0,041	27,05

Table 2: Comparison of Chemical Composition of Cassava Flour with Different Treatment

Code	Carbohyd-rate (%)	Water (%)	Ash (%)	Fat (%)	Yield (%)	Protein (%)	Glucose (mg)	Starch (%)	Amylose (%)
P1L1	84,23	9,36	1,64	1,56	34	3,44	13,75	80,29	31,57
P1L2	87,66	7,90	1,72	1,14	42	2,78	13,80	80,62	23,60
P1L3	87,98	7,89	1,77	1,06	39	2,25	13,88	81,06	23,38
P1L4	87,93	9,19	1,32	0,77	42	1,71	13,82	80,69	19,98
P2L1	85,66	8,79	1,52	1,47	40	1,74	13,77	80,42	31,70
P2L2	86,12	8,30	1,49	1,43	41	2,92	13,71	80,08	20,98
P2L3	87,99	6,58	1,57	1,24	39	2,82	13,79	80,51	28,92
P2L4	87,51	8,43	1,59	0,88	38	1,73	13,88	81,09	23,57
P3L1	86,80	8,26	1,58	1,28	38	1,41	13,78	80,47	30,65
P3L2	87,36	8,28	1,38	0,92	43	2,25	13,81	80,66	20,54
P3L3	88,09	7,30	1,65	0,88	41	2,25	13,80	80,60	28,56
P3L4	88,94	7,86	1,64	0,49	43	1,17	13,91	81,23	23,26
P4L1	87,03	9,09	1,62	0,71	40	0,87	13,78	80,50	26,87
P4L2	87,59	8,65	1,58	0,61	50	1,72	13,83	80,79	18,44
P4L3	89,60	6,98	1,68	0,66	43	1,19	13,86	80,93	28,19
P4L4	88,72	8,47	1,65	0,36	45	0,87	13,91	81,24	19,61

The chemical composition of cassava flour has different levels for different treatments, as shown in Table 1 and Table 2. The carbohydrate content is highest at treatment P4L3 (89.60%), namely cassava the treated dredged 1.5 mm and soaked 24 hours in the process of making cassava flour processing, while the carbohydrate content was lowest for the treatment P1L1 (84.23%) that cassava is not dredged and not soaked. Based on Table 1 and Table 2, that dredging and length of time of immersion in the process of making cassava flour greatly affect the chemical composition, for example in the treatment of dredging of 0.5-1.5 mm and 12-24 hours of immersion can increase the levels of carbohydrates, amylose, starch, and the yield of cassava flour compared to cassava that are not dredged and not experience immersion. For cassava is not dredged and not experience immersion precisely moisture content, protein content and higher fat content means that the

mucus lining the outside of turns cassava contains a lot of water, protein and fat.

Based on Table 1 shows that the fiber content of NDF (2.432%), ADF (1.275%) and hemicellulose (1.158%) is highest in the treatment P1L2 ie with no treatment dredged and soaked for 12 hours, while the cellulose content highs (1.184%) on P4L1 treatment that is dredged 1.5 mm and not soaked, lignin (0.51%) at treatment P3L1 namely dredged 1 mm is not soaked, pectin (0.378%) in the treatment of dredged P2L2 ie 0.5 mm and soaked 12 hours and the content of inhibitors the highest amylase present in the treatment P1L2 (0.092%) is not dredged and soaked for 12 hours and P1L1 (0.084%) is not dredged and not soaked.

The enzyme production Amylase Derived from Brevibacterium sp.

Amylase enzyme used to test the activity of amylase inhibitors derived from marine microbial Brevibacterium sp with activity of 2.2 U / mL and the protein concentration of 1.32 mg / mL.

Amylase Inhibitors Activity Test of Cassava Flour

The first phase extraction is conducted to obtain a sample supenatant amylase inhibitors containing as many as 1 mL of each of the samples of cassava flour. Based on each of these samples were tested for inhibitors activity by using Method Bernfeld (1955), Kumari et al (2012) with modifications. Amylase inhibitors activity test was carried out by using the enzyme amylase by 20 mL (1:32 mg). Data amylase inhibitor activity assay results of the three trials can be seen in Table 3.

Table 3: Analysis of Amylase Inhibitors Activity Test of Cassava Flour (amylase 20 mL / 1.32mg)

No	Sample Code	Control : Amylase (A)	Sample : Amylase + Inhibitor (A+I)	(A+I) - A	*Inhibitor activity*
1	P1L1	1,665	1,749	-0,084	-
2	P1L2	1,731	1,715	0,092	+
3	P1L3	1,621	1,571	0,050	+
4	P1L4	1,734	1,707	0,027	+
5	P2L1	1,625	1,661	-0,036	-
6	P2L2	1,602	1,528	0,074	+
7	P2L3	1,579	1,610	-0,031	-
8	P2L4	1,579	1,593	-0,014	-
9	P3L1	1,573	1,583	-0,010	-
10	P3L2	1,723	1,653	0,070	+
11	P3L3	1,740	1,661	0,079	+
12	P3L4	1,661	1,723	-0,062	-
13	P4L1	1,731	1,723	0,008	+
14	P4L2	1,665	1,573	0,016	+
15	P4L3	1,625	1,623	0,002	+
16	P4L4	1,668	1,709	-0,041	-

Based on data analysis of the early stages of the 16 samples amylase inhibitors extracted from the cassava flour, amylase inhibitors activity assay using amylase enzyme derived from Brevibacterium sp. According to Table 3, that the sample number 2, 3, 4, 6, 10, 11, 13, 14 and 15 has a positive value means that it is expected that all nine samples of the amylase inhibitor has the ability to inhibit amylase inhibitors activity or have any power. Assay Test Method Using Protein Lowry.

BSA standard absorbance data used to calculate the protein content can be seen in Table 2 and Figure 1. The protein volume value of 16 samples between 0,3- 0.5 mg / mL (Table 5).

Table 4: Values BSA Absorbance at a Wavelength of 660 nm (Lowry's Method)

Volume BSA (ppm)	abs (660 nm)
50	0,002
100	0,036
150	0,067
200	0,090
250	0,136
300	0,164
350	0,195
400	0,219

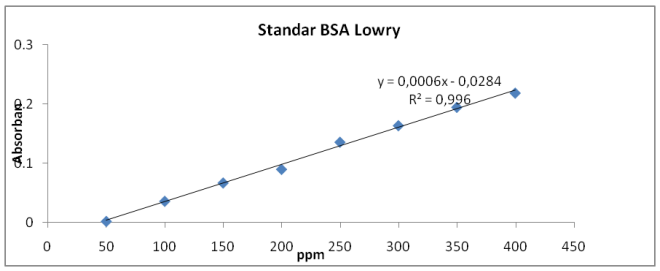


Figure 1: Graph of Regression Equation BSA Standard Curve

Table 5: Data Analysis of Protein Content Using Lowry's Method (mg / mL)

No.	Sampels Code	Absorbance Samples (660 nm)	mg/mL
1	P1L1	0,242	0,543
2	P1L2	0,206	0,483
3	P1L3	0,181	0,442
4	P1L4	0,193	0,462
5	P2L1	0,269	0,588
6	P2L2	0,149	0,388
7	P2L3	0,184	0,447
8	P2L4	0,228	0,520
9	P3L1	0,198	0,470
10	P3L2	0,135	0,365
11	P3L3	0,181	0,442
12	P3L4	0,263	0,578
13	P4L1	0,178	0,437
14	P4L2	0,111	0,325
15	P4L3	0,137	0,368
16	P4L4	0,166	0,417

To determine protein levels of a protein in this case amylase inhibitors used method of Lowry. Data obtained in the form of absorbance value at a wavelength of 660 nm. To convert into mg / mL (units of protein content), this value was added to the regression equation of the standard BSA used in Lowry methods as follows: The regression equation can be seen in the graph (Figure 1), namely: Y = 0.0006X - 0.0284. The value of X is sought (mg / mL), while the value of Y is the absorbance at 660 nm wavelength. Conversion value from ppm to mg / mL was divided in 1000.

The Trial SDS-PAGE

SDS-PAGE trial was conducted on 16 samples to see amylase inhibitors protein in amylase inhibitors that has been isolated / extraction from cassava flour. The results of SDS-PAGE sample numbers 1-8 can be seen in Figure 2, while sample numbers 9-16 can be seen in Figure 3. The size of the protein band varied and still in a state of crude / rude.

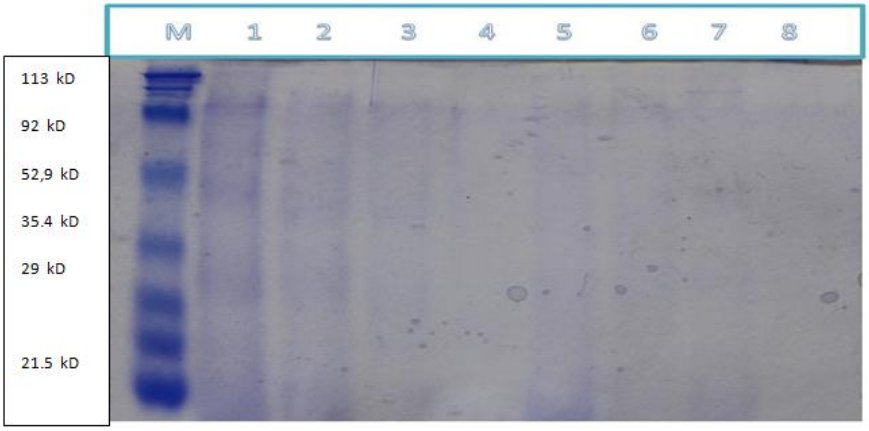
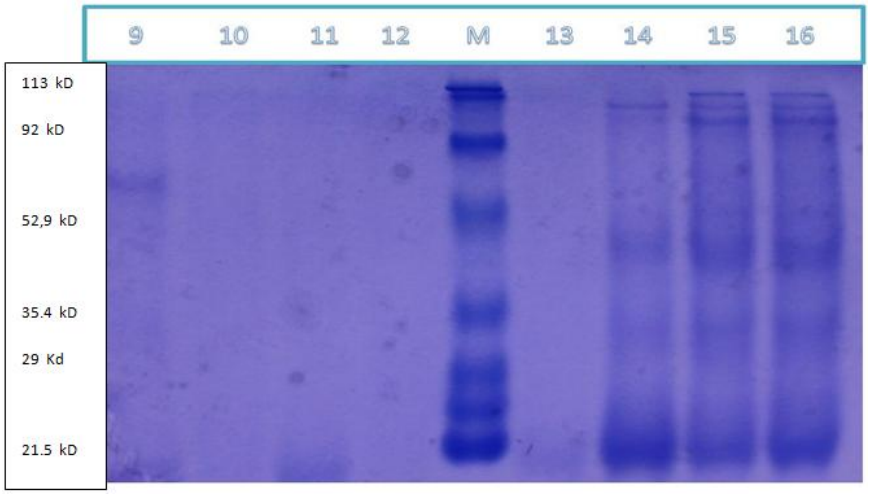


Figure 2: The Results of SDS-PAGE Sample No. 1-8 Amylase Inhibitors. Running at 40 mM 3 hours



The molecular weight of each sample is already readable though still rough protein bands (crude). Based on the protein bands in the image above amylase inhibitors molecular weight ranging from 20 kD to 113 kD. Thus obtained several bands with different size. To get a definitive molecular weight samples must be purified. The results showed that the molecular weight of the protein amylase inhibitor compound cassava flour has a protein band 16, respectively, are: 21.87; 25.34; 29.95; 33.29; 38.79; 41.76; 47.56; 58.85; 67.75; 69.73; 75.13; 81.15; 102.49; 106.57; 109.34; 113.75 kD (kilodalton).

Chemical Characteristics of the Flour of Cassava Adira-1 Varieties

Diversity analysis showed that the treatment of dredging and long soaking time cassava varieties Adira-1 as well as the interaction between treatments significantly to highly significant levels of proximate, fiber and amylase inhibitors. The test results Duncan's Multiple Range Test (DMRT) at the level of 1%. Factors Influence Treatment of measurement parameters can be seen in Table 6 to Table 9.

Table 6: Test Duncan Multiple influences the thickness of the dredging, without dredging, long soaking time and without soaking the moisture content, ash content, fat content, yield, carbohydrate, protein, starch, amylose content, and fiber-like; NDF, ADF, hemicellulose, cellulose, lignin, pectin

Treatment	Water content (%)	Ash content (%)	Fat content (%)	yield (%)	carbohydrate(%)	Protein (%)	Amylose content (%)	Starch content (%)
P1	$8,585^{D}$	$1,6125^{B}$	$1,2375^{C}$	$39,25^{A}$	$86,95^{B}$	$2,545^{D}$	$24,632^{B}$	$80,665^{A}$
P2	$8,025^{B}$	$1,5425^{A}$	$1,365^{D}$	$39,5^{A}$	$86,82^{A}$	$2,3025^{C}$	$26,292^{D}$	$83,025^{D}$
P3	$7,925^{A}$	$1,5625^{A}$	$0,9725^{B}$	$41,25^{A}$	$87,7975^{C}$	$1,77^{B}$	$25,752^{C}$	$80,74^{B}$
P4	$8,297^{C}$	$1,6325^{C}$	$0,675^{A}$	$44,5^{B}$	$88,235^{D}$	$1,1625^{A}$	$23,277^{A}$	$80,865^{C}$
L1	$8,875^{D}$	$1,59^{B}$	$1,3775^{D}$	38^{A}	$85,93^{A}$	$1,865^{B}$	$30,197^{D}$	$80,42^{A}$
L2	$8,2825^{B}$	$1,5425^{A}$	$1,1175^{C}$	44^{C}	$87,1825^{B}$	$2,4175^{D}$	$20,89^{A}$	$83,037^{D}$
L3	$7,1875^{A}$	$1,6675^{C}$	$1,035^{B}$	$40,5^{B}$	$88,415^{D}$	$2,1275^{C}$	$27,262^{C}$	$80,775^{B}$
L4	$8,4875^{C}$	$1,55^{A}$	$0,6825^{A}$	42^{BC}	$88,275^{C}$	$1,37^{A}$	$21,605^{B}$	$81,062^{C}$

Description: The Numbers followed by the same letters in the same column show the differences are not real. Capital letters: Test DMRT 1%. Lowercase: DMRT 5%

Tabel 7: DMRT Influence dredging thickness, without dredging, long soaking time and without soaking the fiber content of NDF, ADF, hemicellulose, cellulose, lignin, pectin, and amylase inhibitors

Treatment	NDF (%)	ADF (%)	Hemicellulosa (%)	cellulosa (%)	Lignin (%)	Pectin(%)	Amylase Inhibitor (%)	cyanide (ppm)
P1	$1,87^{D}$	$1,0942^{B}$	$0,7357^{D}$	$0,3485^{A}$	$0,315^{D}$	$0,1667^{A}$	$2,6527^{C}$	$44,41^{BC}$
P2	$1,6048^{C}$	$1,131^{B}$	$0,4732^{B}$	$0,4975^{B}$	$0,09^{A}$	$0,2727^{B}$	$2,421^{B}$	$44,03^{DE}$
P3	$1,4718^{B}$	$0,9312^{A}$	$0,5407^{C}$	$0,793^{C}$	$0,1725^{C}$	$0,26b^{B}$	$3,243^{D}$	$42,51^{AB}$
P4	$1,3547^{A}$	$0,9465^{A}$	$0,4077^{A}$	$0,9185^{D}$	$0,1325^{B}$	$0,19^{A}$	$2,1422^{A}$	$40,32^{FG}$
L1	$1,3645^{A}$	$1,006^{B}$	$0,3582^{A}$	$1,1995^{D}$	$0,3175^{B}$	$0,2417^{B}$	$2,0895^{A}$	$38,29^{B}$
L2	$1,667^{B}$	$1,22^{C}$	$0,447^{B}$	$0,5107^{B}$	$0,14^{A}$	$0,3067^{C}$	$3,783^{D}$	$30,35^{J}$
L3	$1,9028^{C}$	$0,8757^{A}$	$1,0267^{C}$	$0,2555^{A}$	$0,1425^{A}$	$0,159^{A}$	$2,226^{B}$	$29,08^{I}$
L4	$1,367^{A}$	$1,0012^{B}$	$0,3255^{A}$	$0,5917^{C}$	$0,11^{A}$	$0,182^{A}$	$2,3605^{C}$	$27,52^{L}$

Description: The numbers followed by the same letters in the same column show the differences are not real. Capital letters: Test DMRT 1%. Lowercase: DMRT 5%

Table 8: Test Duncan Multiple combinations dredging thickness, without dredging, long soaking time and without soaking the moisture content, ash content, fat content, yield, carbohydrate, protein, amylose content and starch content

Treatment	Water content (%)	Ash content (%)	Fat content (%)	yield (%)	carbohydrate (%)	Protein (%)	Amylose content (%)	Starch content (%)
P1L1	9,36^{K}	1,64^{FG}	1,72^{N}	34^{A}	84,23^{A}	3,44^{H}	31,57^{N}	80,29A
P1L2	7,9^{D}	1,72^{H}	1,23^{J}	42^{BCD}	87,66^{I}	2,78^{F}	23,6^{H}	80,62^{DE}
P1L3	7,89^{D}	1,77^{I}	1,15^{I}	39^{BC}	87,98^{JK}	2,25^{E}	23,38^{G}	81,06^{I}
P1L4	9,19^{J}	1,32^{A}	0,85^{F}	42^{BCD}	87,93^{J}	1,71^{D}	19,98^{C}	80,69^{F}
P2L1	8,79^{H}	1,52^{C}	1,61^{M}	40^{BC}	85,66^{B}	1,74^{D}	31,7^{O}	80,42^{B}
P2L2	8,3^{E}	1,49^{C}	1,56^{M}	41^{BCD}	86,12^{C}	2,92^{G}	20,98^{E}	90,08^{K}
P2L3	6,58^{A}	1,57^{D}	1,33^{K}	39^{BC}	87,99^{K}	2,82^{F}	28,92^{L}	80,51^{C}
P2L4	8,43^{F}	1,59^{DE}	0,96^{GH}	38^{AB}	87,51^{G}	1,73^{D}	23,57^{H}	81,09^{I}
P3L1	8,26^{E}	1,58^{DE}	1,4^{L}	38^{AB}	86,8^{D}	1,41^{C}	30,65^{M}	80,47^{C}
P3L2	8,28^{E}	1,38^{B}	1,01^{H}	43^{CD}	87,36^{F}	2,25^{E}	20,54^{D}	80,66^{EF}
P3L3	7,3^{C}	1,65^{FG}	0,95^{G}	41^{BCD}	88,09^{L}	2,25^{E}	28,56^{K}	80,6^{D}
P3L4	7,86^{D}	1,64^{FG}	0,53^{B}	43^{CD}	88,94^{N}	1,17^{B}	23,26^{F}	81,23^{J}
P4L1	9,09^{L}	1,62^{EF}	0,78^{E}	40^{BC}	87,03^{E}	0,87^{A}	26,87^{I}	80,5^{C}
P4L2	8,65^{G}	1,58^{DE}	0,67^{C}	50^{E}	87,59^{H}	1,72^{D}	18,44^{A}	80,79^{G}
P4L3	6,98^{B}	1,68^{GH}	0,71^{D}	43^{CD}	89,6^{O}	1,19^{B}	28,19^{J}	80,93^{H}
P4L4	8,47^{F}	1,65^{FG}	0,39^{A}	45^{D}	88,72^{M}	0,87^{A}	19,61^{B}	81,24^{J}

Description: The numbers followed by the same letters in the same column show the differences are not real. Capital letters: Test DMRT 1%. Lowercase: DMRT 5%.

Table 9: Test the thickness Duncan Multiple combination of dredging, without dredging, long soaking time and without soaking the fiber content of NDF, ADF, hemicellulose, cellulose, lignin, pectin, and amylase inhibitors.

Treatment	NDF (%)	ADF (%)	Hemicellulosa (%)	cellulosa (%)	Lignin (%)	Petin (%)	Amylase Inhibitor (%)	cyanide (ppm)
P1L1	1,54^{E}	1,263^{F}	0,277^{DE}	0,383^{CDE}	0,41^{E}	0,225^{CDE}	5,045^{O}	44,41^{BC}
P1L2	2,432^{J}	1,275^{FG}	1,158^{G}	0,163^{A}	0,31^{D}	0,194^{BCD}	0,887^{D}	39,31^{A}
P1L3	1,829^{GH}	1,08^{DE}	0,749^{F}	0,272^{B}	0,29^{D}	0,143^{BC}	4,540^{M}	38,29^{B}
P1L4	1,679^{F}	0,759^{AB}	0,759^{F}	0,576^{F}	0,25^{D}	0,105^{A}	5,526^{P}	37,25^{F}
P2L1	1,436^{D}	1,126^{DE}	0,31^{E}	0,897^{H}	0,03^{A}	0,32^{EFG}	1,557^{F}	44,03^{DE}
P2L2	1,547^{E}	1,363^{G}	0,183^{BC}	0,382^{CDE}	0,12^{BC}	0,378^{G}	2,458^{I}	31,12^{G}
P2L3	2,046^{I}	0,908^{C}	1,137^{G}	0,31^{BCD}	0,15^{C}	0,273^{DEF}	1,963^{G}	30,35^{J}
P2L4	1,39^{CD}	1,127^{DE}	0,263^{CD}	0,401^{DE}	0,06^{AB}	0,12^{AB}	4,619^{N}	30,06^{O}
P3L1	1,378^{CD}	0,705^{A}	0,673^{F}	1,704^{I}	0,51^{F}	0,303^{EFG}	4,063^{L}	42,51^{AB}
P3L2	1,332^{BC}	1,16^{E}	0,172^{B}	0,715^{G}	0,01^{A}	0,368^{FG}	0,462^{B}	29,81^{M}
P3L3	1,929^{H}	0,8^{B}	1,126^{G}	0,302^{BC}	0,09^{ABC}	0,105^{A}	3,733^{K}	29,49^{P}
P3L4	1,251^{B}	1,06^{D}	0,192^{BC}	0,451^{E}	0,08^{ABC}	0,264^{DE}	0,924^{E}	29,08^{I}
P4L1	1,104^{A}	0,93^{C}	0,173^{B}	1,814^{J}	0,32^{DE}	0,119^{AB}	0,636^{C}	40,32^{FG}
P4L2	1,357^{CD}	1,082^{DE}	0,275^{DE}	0,783^{G}	0,12^{BC}	0,287^{DEF}	2,215^{H}	27,52^{L}
P4L3	1,81^{G}	0,715^{AB}	1,095^{G}	0,138^{A}	0,04^{AB}	0,115^{AB}	0,123^{A}	27,34^{H}
P4L4	1,148^{A}	1,059^{D}	0,088^{A}	0,939^{H}	0,05^{AB}	0,239^{DE}	3,085^{J}	27,05^{E}

Description: The numbers followed by the same letters in the same column show the differences are not real. Capital letters: Test DMRT 1%. Lowercase: DMRT 5%

Amylase inhibitors in cassava are those in which the branch of carbohydrates that bind to proteins (backbone) in the form of glucose. Glucose in the amylase inhibitors can stand alone as a type of glucose hexose or glucosamine. Some types of inhibitors of amylase in the form of the receipt of other chemicals have been isolated from various fruit and tuber crops, such as amylase inhibitors in the skin of berries (Pentadiplandra brazzeana), curculin (Curculigo latifolia) located in West Africa and potatoes, beans / whitebean (Phaseoluss vulgaris) in Taiwan, and fruit Cissus arnottiana in india and amadumbe (Colocasia esculenta) in southern Africa and Leaves (Vaccinium arctostaphylos) in Iran (Gutierrez et al; 1993; Ishimoto et al; 1996; Khalil et al; 2012; Wanga et al ; 2011; Nickavara and amen; 2011).

Amylase inhibitors protein is bound to the carbohydrate group, which work by blocking enzymes amylase in the human digestive tract. Amylase enzyme is required, especially by men who consume lots of carbohydrates derived from plants, such as cassava that contain lots of starch. Starch must be hydrolyzed into simpler carbohydrates molecules into disaccharides and monosaccharides, for use in the human body's metabolic system (Gutierrez et al; 1993; Murray et al; 1998). Based on Table 1 and Table 2, that of 16 samples obtained by the content of inhibitors of amylase highest in treatment P1L2 (0.092%), which is in the process of making cassava flour mucus layer is not dredged / not removed and undergo soaking for 12 hours, while the content of inhibitors of amylase lowest for the treatment P4L3 (0.002%), which is in the process of making cassava flour mucus layer dredged / dumped as much as 1.5 mm and experience soaking for 24 hours. This indicates that the dredging peroses thickness and length of time of immersion significant, even very significant to the content of amylase inhibitor and also the fiber content, the content of cyanide and proximate (Table 6 to Table 9).

Based on these results, as shown in Table 3 that, of the 16 samples studied only 9 amylase inhibitors that have a positive value, it means to 9 amylase inhibitors that have

the ability to inhibit / inhibit the activity of amylase or having inhibitors. Amylase inhibitors that have inhibitors activity was a factor that greatly affect not only the thickness of dredging factors but the duration of immersion time (12-48 hours) has a critical determinant of inhibitor activity. For samples that undergo soaking for 48 hours already do not have the power inhibitors activity, although it contains amylase inhibitors. According to Boivin et al; 1988, amylase inhibitors can inhibit the breakdown of starch, then people did not get some of its energy from carbohydrate-containing amylase inhibitors. Things like this could be used as a regulator in the management of diet in patients with diabetes mellitus, because of the effect of work amylase inhibitors with a certain amount in the body can lower blood glucose levels, by not stimulating the release of insulin by the pancreas and increase its effectiveness, because it works by delaying absorption glucose in the gut, so that it could control blood glucose levels, it is in line with research from Boivin et al; 1988; on "Gastrointestinal and metabolic effects of amylase inhibitions in Diabetics" as well as the operation and Murugesan; 2012; about "Invitro α-amylase and α-glucosidase inhibition activity of crude ethanol extract of Cissus arnottiana".

Table 4 of the protein assay using the method of Lowry, that the absorbance value Bovin Serum Albumin (BSA) at a wavelength of 660 nm is between 0,002- 0.219 nm. The absorbance values largely determine the protein content to 16 amylase inhibitors calculated into the regression equation of the standard BSA used is Y = 0,0006X - 0.0284, so that the protein content values obtained ranged from 0.3-0.5 mg / mL (Table 5).

Then all 10 amylase inhibitors either crude or after the enzyme was concentrated with ammonium sulfate in SDS-PAGE (Figure 4). Molecular weight to 16 amylase inhibitors of SDS-PAGE results are respectively 21.87; 25.34; 29.95; 33.29; 38.79; 41.76; 47.56; 58.85; 67.75; 69.73; 75.13; 81.15; 102.49; 106.57; 109.34; 113.75 kD (kilodalton).

Based on the results DMRT the confidence level of 5% (Table 6 to Table 9) data showed that differences in the thickness of dredging the mucus layer of cassava and the length of time of immersion causes a real difference to a very significant effect on the levels of amylase inhibitors, moisture content, ash content, fat content, yield, carbohydrate, protein, amylose content, starch, fiber (NDF, ADF, hemicellulose, cellulose, lignin, and pectin) cassava flour. The thicker the layer of slime dredging cassava and the longer the soaking time, the smaller the content and the lower the power of amylase inhibitors activity.

McEwan, 2008 amylase inhibitors extracted from plants, doing research with Amadumbe and showed specificity in the inhibition of the amylase enzyme that is at the center of the target in this study. This study was the same as that of Weselake et al, 1983; Franco et al, 2000, in most cases the mechanism of inhibition occurs through direct blockage of the active center of the enzyme in several sub sites (Payan, 2004); (Sharma and Pattabiraman, 1980, 1982; Ida et al., 1994) showed that many contained in the amylase inhibitors active against amylase bulbs and mammals, but showed no amylase activity in the plant.

Amylase inhibitors found in Amadumbe also have amylase inhibitors active against mammalian amylase but no effect on Aspergillus amylase. Amadumbe grow in damp, humid conditions are fertile place to grow the fungus that causes Amadumbe many who become infected, so it is possible that the fungus has become resistant to the action of the amylase inhibitors. Sharma and Pattabiraman (1982), have reported similar results for Dioscorea alata.

Bifunctional nature has been shown by a number of amylase inhibitors, and therefore, the amylase inhibitors received particular attention as an attractive candidate for pestcontrol (Maskos et al., 1996). -amylaseα inhibitors are also commonly found in wheat (Franco et al., 2000), barley (Richardson, 1991) and in the Indian finger millet (Campos and -amylase fromαRichardson, 1983) has been shown to efficiently inhibit different sources insects. There is a possibility that two amylase inhibitors found in Amadumbe can complement each other, with defense against a broader spectrum.

Table 8 and Table 9 shows the interaction of the thickness of the dredging, the length of time of immersion of the levels of amylase inhibitors, proximate and fiber cassava flour. Results DMRT shows a tendency, the thicker dredging mucus layer of cassava and the longer the soaking time lead to decreased levels of inhibitors of amylase levels proximate, cyanide and the levels of soluble fiber such as pectin, so that if we treat foods derived from cassava should have to consider from In terms of varieties for consumption, and the processing must be true.

Herbs and natural products to function slows the absorption of glucose and inhibits carbohydrate hydrolysis by the amylase enzyme and glucosidase. Some synthetic amylase inhibitors include acarbose, voglibose and miglitol clinically used for the treatment; but the price is still high and clinical side effects can occur (Scott et al; 2000), therefore the study challenged glucosidase inhibitor and amylase inhibitors from plant sources is increasing.

According to the researchers that the cassava plant can offer an attractive strategy for the purpose of prevention, promotion and treatment in patients hyperglikemik or diabetes mellitus. This is in line with research from Nickavara and Amin (2011), about the "Enzyme Assay Guided Isolation of an α-amylase inhibitors of flavonoids from Vaccinium arctostaphylos Leaves". In his research on plant leaf extract "Vaccinium arctostaphylos" which contains amylase inhibitors and useful for the treatment of diabetes mellitus in multiple countries. In his research for anti-diabetic compounds from natural sources, the author found that the methanol extract of leaves of "Vaccinium arctostaphylos" found that a strong inhibitory activity on pancreatic α-amylase activity (IC50 = 0.53 (0.53 to 0.54) mg / mL). Compounds found from the leaf extract "Vaccinium arctostaphylos" after isolated and purified compound is obtained in the form of quercetin as α-amylase inhibitor active. Quercetin showed an inhibitory

effect with IC50 values of 0.17 (0.16 to 0.17) mM to work amylase enzyme derived from the pancreas of mammals.
Jung et al; 2006, has developed various inhibitors (amylase and glucosidase) from a natural source. In his research, in vitro effects in different concentrations of ethanol extract of the fruit Cissus arnottiana evaluated. The result obtained at a concentration of 20 mg / mL of the plant extract, shows inhibitory activity of the amylase enzyme and glucosidase enzymes. Statistical analysis showed a significant relationship, that extracts of the fruit Cissus arnottiana can be useful in the management of postprandial hyperglycemia or blood glucose 2 hours after a meal. In in vitro tests do not necessarily relate inhibitory activity in vivo tests straight to the appropriate activity. Therefore a proof of concept for the anti-diabetic needs shown in preclinical animal studies (Subramanian et al; 2008). As a suggestion for the safety and efficacy of the research to be established, it is important to be confirmed by in vivo studies.

4. Conclusions and Recommendations

A. Conclusions

Based on the research results and the description above, it can be concluded as follows:

1. Based on the results of treatment effect factor DMRT dredging mucus layer thickness and the length of time of immersion in the processing of cassava flour until very real significant effect on levels of amylase inhibitor cassava flour at a level of 5%.
2. Based on the results of treatment effect factor DMRT dredging mucus layer thickness and the length of time of immersion in the processing of cassava flour until very real significant effect on levels of proximate, cyanide levels and dietary fiber content of cassava flour.
3. Based on the effect of combined treatment DMRT factors dredging mucus layer thickness and the length of time of immersion in the processing of cassava flour until very real significant effect on levels of glukoprotein / amylase inhibitor cassava flour at a level of 5%.
4. Based on the results of the effect of combined treatment DMRT factors dredging mucus layer thickness and the length of time of immersion in the processing of cassava flour until very real significant effect on levels of proximate, cyanide levels and dietary fiber content of cassava flour.
5. Based on the analysis of 16 samples studied obtained the highest levels of amylase inhibitors (1.090 mg / ml), and has a 100% inhibition properties of the amylase is the amylase inhibitors number 2 (P1L2) that are treated are not dredged mucus layer cassava and soaked for 12 hours.
6. The results of the isolation and purification of amylase inhibitors (SDS-PAGE) of cassava flour obtained Molecular Weight (BM) amylase inhibitor are respectively 21.87; 25.34; 29.95; 33.29; 38.79; 41.76; 47.56; 58.85; 67.75; 69.73; 75.13; 81.15; 102.49; 106.57; 109.34; and 113.75 kD (kilodalton).

B. Recommendations

Based on the results of the study suggest that:

1. To obtain cassava flour which contains high levels of amylase inhibitors best and have the nature of inhibition of amylase, the necessary treatment in the mucus layer of cassava may not be removed and must be soaked for 12 hours (P1L2).
2. Recommended that do further research to find out the amylase inhibitors compound of any kind contained in extracts of cassava flour.
3. For further research on the type of protein in cassava flour amylase inhibitors with Immunobloting Method or Western Bloting (WB).

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