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IAI CONFERENCE: Hypoglycemic activity test on smooth pigweed (*Ammaranthus Hybridus* L) leaf water extract on male Wistar rats

Asti Yunia Rindarwati (Author)

p. 56-60



IAI CONFERENCE: Mother's knowledge and practices towards self-medication of fever among children under five years in Muncar Banyuwangi, Indonesia

Sinta Rachmawati, Khusnul Khotimah, Ika Norcahyanti (Author)

p. 264-268



IAI CONFERENCE: Correlation between the antioxidant capacity of plasma and blood glucose level

Eva Nurinda, Emelda, Nurul Kusumawardani (Author)

p. 108-115



IAI CONFERENCE: Enhancing solubility and antibacterial activity using multi-component crystals of trimethoprim and malic acid

p. 296-304



IAI CONFERENCE: Acute toxicity study of the ethanolic extract of *Eleutherine bulbosa* Urb in Wistar rats

Helmina Wati, Rahmi Muthia, Kartini, Finna Setiawan (Author)

p. 143-147



IAI CONFERENCE: The effect of biofilm formation on the outcome therapy of diabetic foot infections (DFIs) patients in the outpatient clinic and inpatient ward of Dr Sardjito General Hospital Yogyakarta

Ika Puspitasari, Titik Nuryastuti, Rizka Humardewayanti Asdie, Hemi Sinorita, Nusaibah Umaroh4, Wahyu Tri Hapsari (Author)

p. 172-177



IAI CONFERENCE: Adverse drug reactions associated with successful treatment of multidrug-resistant tuberculosis patients in Cempaka Putih Islamic Hospital Central Jakarta

Adin Hakim Kurniawan, Harpolia Cartika, Siti Aisyah (Author)

p. 15-21



IAI CONFERENCE: Acute toxicity test of 96% ethanol extract of *Syzygium myrtifolium* leaves in white mice (*Mus musculus*)

Lusi Indriani, E. Mulyati Effendi, Kevin Christofer Fadillah (Author)

p. 201-204



IAI CONFERENCE: Exploring pharmacist experience and acceptance for debunking health misinformation in the social media

p. 42-47



IAI CONFERENCE: Comparing the quality of life of neuropathic patients treated with gabapentin and pregabalin at the neuropathic poly of the NTB provincial hospital in 2019

Nurul Qiyaam, Baiq Leny Nopitasari, Haerul Muhajiji (Author)

p. 225-229



IAI CONFERENCE: A pharmacoeconomic study: cost-utility analysis of modern wound dressings vs conventional wound dressings in patients with diabetic foot ulcer

Cyntiya Rahmawati, Baiq Leny Nopitasari (Author)

p. 71-76



IAI CONFERENCE: Medicine management in districts and primary health care centres (PHC) in the national health insurance (JKN) programme

Raharni, Rini Sasanti, Yuyun Yuniar (Author)

p. 251-257



IAI CONFERENCE: The evaluation of compounding prescription and its availability of a licensed product for children at a private hospital in Yogyakarta, Indonesia

p. 93-97



IAI CONFERENCE: Proximate analysis on animal feed granules composed of raw material from fish innards wastes

Wahyuning Setyani, Christine Patramurti, Agatha Budi Susiana Lestari, Raysha Mcseer, Day Stella Maris Gewab, Maria Felix Zita Ina Bulu, Maria Regina Lusiana Kya (Author)

p. 281-286



IAI CONFERENCE: The potential effect of the green coffee extract on reducing atherogenic index in hyperlipidemic rats

Fransiska Maria Christianty, Fifteen Aprila Fajrin, Andrean Roni (Author)

p. 126-131



IAI CONFERENCE: Antibiotic use on paediatric inpatients in a public hospital in Bangil, Indonesia

Ika Norcahyanti, Malikatur Rosyidah, Abdul Kadir Jaelani, Antonius N.W Pratama (Author)

p. 163-167



IAI CONFERENCE: Analysis of pharmacists' knowledge and attitude in the pharmaceutical industry of halal certification and their readiness to produce halal medicine

Abdul Rahem, Mustofa Helmi Effendi, Hayyun Durrotul Faridah (Author)

p. 1-7



IAI CONFERENCE: Microencapsulation of Jeringau Rhizome essential oils (*Acorus calamus* L.) using β -Cyclodextrin

Ledianasari, Deby Tristiyanti, Elva Mauldyha Tanjung, Lovelyta Barani (Author)

PDF	p. 189-194
IAI CONFERENCE: Identification of herbal products used by families in the campus of Darussalam Gontor University	
Amal Fadholah, Solikah Ana Istikomah, Cania Sofyan Islamanda, Evi Rohana Ma'rufi Jannah (Author)	p. 31-35
PDF	
IAI CONFERENCE: Effectiveness of combination of Moringa Leaf Extract (<i>Moringa oleifera</i> Lamk.) and Papaya Seed Extract (<i>Carica papaya</i> L.) in reducing blood sugar levels of diabetic rats	
Novi Ayuwardani, Yetti Hariningsih (Author)	p. 215-219
PDF	
IAI CONFERENCE: Correlation between the level of knowledge of drug managers and drug management in several primary health centres in Malang regency	
Ayuk Lawuningtyas Hariadini, Nur Ishmah, Hananditia Rachma Pramestutie (Author)	p. 61-66
PDF	
IAI CONFERENCE: Effectiveness of public service advertisements on the use of antibiotics in Pangkalpinang	
	p. 241-245
PDF	
IAI CONFERENCE: In silico screening of mint leaves compound (<i>Mentha piperita</i> L.) as a potential inhibitor of SARS-CoV-2	
	p. 81-86
PDF	
IAI CONFERENCE: The comparison of the actual cost to case-mix of type 2 diabetes mellitus inpatient in Pandan Arang Boyolali hospital	
Sri Bintang Sahara Mahaputra Kusuma Negara, Devi Ristian Octavia, Primanitha Ria Utami (Author)	p. 269-274
PDF	
IAI CONFERENCE: Fluconazole-tartaric acid co-crystal formation and its mechanical properties	
Fikri Alatas, Nia Suwartiningsih, Hestiary Ratih, Titta Hartyana Sutarna (Author)	p. 116-122
PDF	
IAI CONFERENCE: Sambiloto (<i>Andrographis paniculata</i> Nees.) leaf extract activity as an α-Amylase enzyme inhibitor	
Yustina Sri Hartini, Dewi Setyaningsih, Maria Josephine Vivian Chang, Maria Cyrilla Iglesia Adi Nugrahanti (Author)	p. 305-308
PDF	
IAI CONFERENCE: Evaluation of antidiarrheal effect of combination of Salam Leaves (<i>Syzygium polyanthum</i>) and Jackfruit Leaves (<i>Artocarpus heterophyllus</i> Lam.) infusum in rats induced by castor oil	
Husnul Khuluq, Evi Marlina (Author)	p. 148-151
PDF	
IAI CONFERENCE: The effect of loss-of-function allele (CYP2C19*3) with Clopidogrel efficacy in coronary heart disease patients	
Ike Dhiah Rochmawati, Nur Hidayat, David Pomantow (Author)	p. 178-183



IAI CONFERENCE: The effect of ethanol extract from *Portulaca oleracea* on inhibiting total cholesterol on animal subjects

Afifah Bambang Sutjiatmo, Fahmy Ahsanul Haq, Sulaeman Al Jati, Suci Nar Vikasari (Author) p. 22-26



IAI CONFERENCE: The development of *Origanum vulgare* L. into nanoparticles in dosage forms

Lutfi Chabib, Arman Suryani, Muhammad Iqbal Pangestu, Adnan Muhammad Uno J Hidayat, A.M. p. 205-209
Bagas Trianloka (Author)



IAI CONFERENCE: The evident gap between actual and perceived facilities supporting value-added pharmacy services

Andi Hermansyah, Catur Dian Setiawan, Firqin Fuad Riansyah (Author) p. 48-51



IAI CONFERENCE: Analysis of white pepper essential oil components using gas chromatography-mass spectroscopy

Purwaniati, Gilang Eka Permana, Indro Pamudjo (Author) p. 230-234



IAI CONFERENCE: Dissolution profile of Curcumin from solid dispersion prepared at a high drug load of Curcumin using Poloxamer 407 as the carrier

Dewi Setyaningsih, Yustina Sri Hartini, Christine Patramurti, Sastira Putri, Yosi Bayu Murti (Author) p. 77-80



IAI CONFERENCE: Antiseptic gel formulated from ethanol extract of *Citronella grass* (*Cymbopogon nardus*) using CMC-Na, arabic gums, and gelatin as gelling agents

Reynelda Juliani Sagala, Lorensia Yolanda, Michael (Author) p. 258-263



IAI CONFERENCE: The potential of banana fruit Ranggap (*Musa paradisiaca* var. *Troglodytarum*) as an excipient alternative to oral tablet dosage form

Dolih Gozalil, Iyan Sopyan, Resmi Mustarichi, Wahyu Priyo Legowo (Author) p. 98-107



IAI CONFERENCE: The therapeutic outcomes and adverse drug reactions study of Clozapine on Schizophrenia inpatients in the Grhasia psychiatric hospital Yogyakarta, Indonesia

p. 287-295



IAI CONFERENCE: Pandemic 2020: Economic pressure and evaluation of a primary health care innovation programme for type 2 diabetes mellitus treatment

Fransiskus Samuel Renaldi, Rani Sauriasari, Woro Riyadina (Author) p. 132-142



IAI CONFERENCE: The influence of extracts and fractions from matoa leaves (*Pometia pinnata*) on angiotensin I levels

Ika Purwidyaningrum, Jason Merari Peranginangin, Iyem Sahira (Author) p. 168-171



IAI CONFERENCE: The impact of pharmacist shortage on the inventory management of medicines at primary healthcare centres in East Java, Indonesia

Abdul Rahem, Umi Athiyah, Catur Dian Setiawan, Andi Hermansyah (Author)

p. 8-14



IAI CONFERENCE: IR spectroscopy coupled with chemometrics used as a simple and rapid method to determine the caffeine content of tea products

Lestyo Wulandari, Diana Hanifiyah Sutipno, Dwi Koko Pratoko (Author)

p. 195-200



IAI CONFERENCE: The remuneration of the community pharmacist in the developing world: the case in Indonesia

Andi Hermansyah, Anila Impian Sukorini, Abdul Rahem (Author)

p. 36-41



IAI CONFERENCE: Activity test of fruit and pomegranate seeds (*Punica granatum* L) as a hepatoprotector against white male Wistar rats

p. 220-224



IAI CONFERENCE: The effect of stress level on the therapeutic outcomes of type 2 diabetes mellitus at the regional public hospital of West Nusa Tenggara province

Baiq Leny Nopitasari, Baiq Nurbaety, Made Krisna Adi Jaya (Author)

p. 67-70



IAI CONFERENCE: Satisfaction of drug information services implementation in Air Itam, Selindung, Girimaya, Gerunggang and Melintang health centre Pangkalpinang City

Rachmawati Felani Djuria (Author)

p. 246-250



IAI CONFERENCE: Formulation and physical evaluation of facial cream preparations from Ceremai fruit juice (*Phyllanthus acidus* (L.) Skeels)

Danang Indriatmoko, Nani Suryani, Tarso Rudiana, Mila Kurniah (Author)

p. 87-92



IAI CONFERENCE: Role of pharmacist in providing drug information and education for patients with chronic diseases during Transition of Care

Umi Athiyah, Abdul Rahem, Catur Dian Setiawan, Andi Hermansyah (Author)

p. 275-280



IAI CONFERENCE: Formulation and evaluation of Kirinyuh Leaf effervescent granules (*Chromolaena Odorata*. L) as an antioxidant

Firman Gustaman, Keni Idacahyati, Winda Trisna Wulandari (Author)

p. 123-125



IAI CONFERENCE: Drug interactions in patients with hypertension at Persahabatan hospital in 2015

Zainul Islam, Atika Vitasari, Muhammad Arif Ridwan (Author)

p. 309-314



IAI CONFERENCE: Self-medication and self-treatment with short-term antibiotics in Asian countries: A literature review



p. 152-162

IAI CONFERENCE: In vivo activity of *Phaseolus vulgaris* as an anti-hypercholesterolemic

Keni Idacahyati, Yedy Purwandi Sukmawan, Nopi Yanti, Winda Trisna Wulandari, Firman Gustaman, Indra (Author)

p. 184-188



IAI CONFERENCE: In vitro antimalarial activity assay of *Ashitaba* Leaf ethanolic extract (*Angelica keiskei*)

Alvi Kusuma Wardani, Abdul Rahman Wahid, Miftahul Jannah (Author)

p. 27-30



IAI CONFERENCE: Therapeutic potential of *Cymbopogon schoenanthus* (L.) developed into nanoparticle technology

Lutfi Chabib, Adnan Muhammad Uno J Hidayat, A.M. Bagas Trianloka, Muhammad Iqbal Pangestu, Arman Suryani, Yulianto (Author)

p. 210-214



IAI CONFERENCE: Description of medication adherence in hypertensive respondents at Mandalika Mataram elderly social centre

Anna Pradiningsih, Dzun Haryadi Ittiqo, Neti Puput Arianti (Author)

p. 52-55



IAI CONFERENCE: Formulation and physical properties of lotion *Kalakai* root ethanol extract (*Stenochlaena palustris* Bedd)

Rabiatul Adawiyah, Riska Apriliyanti, Agustinawati Umaternate (Author)

p. 235-240



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RESEARCH ARTICLE

Proximate analysis on animal feed granules composed of raw material from fish innards wastes

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Keywords

Animal feed granules
Animal feed proximate analysis
Fish innards
Fish innards wastes
Fishery wastes

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Abstract

Introduction: Fish innards contain 14.01% protein, 20% lipid, 4.75% ash, and 60.62% water. Fish innards are formulated into granules for practicality in their application as animal feed. **Aim:** This research on the proximate analysis of animal feed granules composed of raw material from fish innards wastes used a descriptive quantitative method. **Results:** The result indicated that the water content measured using the thermogravimetric method was 6.62%, the ash content observed using the dry ashing method was 10.25%, the protein content checked using the biuret method was 37.03%, fat content using the soxhlet method was 6.13%, and carbohydrate content measured using phenol sulfate method was 26.14%. **Conclusion:** These findings show that nutrient contents in the composition of animal feed granules of raw material from fish innards wastes fulfill the regulation of animal feed content based on SNI-8509-2018.

Introduction

Fish is one of the food sources with many nutritional benefits. It is rich in essential amino acids, unsaturated fats, vitamins, and minerals and is easily digested (Wibowo & Darmanto, 2014). Besides producing useful products to fulfil food, industry, and daily needs, fish production also generates wastes, reaching up to 500,000 tons each year (Harianti, 2012). Wastes resulting from the fish industry consist of fish that are no longer good to consume or process, fish innards, and other non-commercial parts. These wastes pile up every day due to the lack of skills of the people handling them (Komariyanti & Surachman, 2018). These unutilized fish wastes can cause environmental pollution (Hildawianti, Vanny & Abram, 2017) as they become ideal media for microbes to grow, causing unpleasant odours (Jayanti, Herpandi & Lestari, 2018).

Fish innards are wastes resulting from the fish industry that, if not used, will cause harm to the environment, health, and economy (La Apu, 2017). In Indonesia,

several types of research were conducted on the use of fish innards, one of which is to transform fish processing wastes into animal feed (Komariyanti & Surachman, 2018). Other research explored the utilization of fish waste as animal feed raw material (Sihite, 2013).

The formulation of artificial animal feed is based on the producer's considerations. Animal feed production should acknowledge animal nutritional needs, sources, raw material quality, and economic value (Niode & Nasriani & Irdja, 2017). Based on SNI-8509-2018, content requirements of quality animal feed are as follows: 12.00% water (max), 14.00% ash (max), 16.00% crude protein (min), and 14.00% carbohydrates (min) (Standar Nasional Indonesia, 2018). Based on several studies in Indonesia, which found protein and fat contents in fish innards and used them as animal feed, this research aims to do a proximate analysis on animal feed granules composed of raw material from fish innards wastes.

Method

The material used was fish innards, thick H₂SO₄, H₂SO₄ 52%, aqua dest, alcohol 95%, petroleum ether, glucose, phenol, biuret reactor, and BSA (Bovine Serum Albumin). Formula modification of other researchers entitled: "Optimization of Tapioca Flour and Molasses Flour in Cat Food and Dog Food Pellets From Fish Innards Wastes Raw Material with the Factorial Design Method".

Ash content determination

The working cup was firstly dried for 30 minutes in the oven at 100-105°C or until the fixed weight was obtained. It was then cooled in a desiccator for 30 minutes and then weighed (B1). Five grams of sample were put into the formerly weighed cup and burned on a Bunsen burner, a smokeless stove. It was then put into an ashing furnace to be burned at 400°C until greyish ash was obtained or the sample had fixed weight. Then the furnace temperature was increased to 550°C for 12-24 hours. The sample was then cooled in a desiccator for 30 minutes and weighed (B2) (Hafiludin, 2011). Ash content can be calculated as follows:

$$\text{Ash content (\%)} = \frac{B2-B1}{\text{sample weigh}} \times 100\%$$

Water content determination

The working cup was firstly dried for 30 minutes in the oven at 100-105°C or until the fixed weight was obtained. It was then cooled in a desiccator for 30 minutes and then weighed. Five grams of sample (B1) were weighed on the cup and then dried in an oven at 100-105°C until the fixed weight was obtained (8-12 hours). The sample was then cooled in a desiccator for 30 minutes and then weighed (B2) (Hafiludin, 2011). Water content can be calculated as follows:

$$\text{Water content (\%)} = \frac{B1-B2}{\text{Sample weight}} \times 100\%$$

Protein content determination

Standard solution preparation

One gram of Bovine Serum Albumin (BSA) was dissolved in distilled water in a volumetric flask 10 mL up to the designated mark to obtain a standard solution of 10% W/V.

1. Optimum wavelength determination

Five per cent BSA Standard solution was put in a test tube by sampling 2.5mL of BSA d with 0,8 mL biuret reactor, and distilled water was added to

make a total of a 5 mL solution. The solution was allowed to sit and react for \pm 10 minutes; the absorption was then measured at a wavelength of 450-600 nm. The maximum absorption of the wavelength was recorded.

2. Standard curve making

Six test tubes were prepared. The first one was filled with a blank solution (solvent), while the five others were filled with BSA standard solution at concentrations of 1, 2, 3, 4, and 5%, and completed with 0.8mL aqua dest to a total volume of 5 mL. The solution was allowed to sit for 10 minutes, and then each absorption was measured using a UV-VIS spectrophotometer at the maximum wavelength.

3. Measurement of the protein content of the sample

Each sample weighing 25 grams was put into a beaker added with 250 mL of distilled water, smoothly ground, and filtered with a filter paper.

Protein content measurement was carried out as follows: 2.5 mL of protein sample was added to 0.8 mL of biuret reactor and completed with distilled water to a total of 5 mL solution. The solution was then vortexed let sit for 30 minutes to make a perfect purple. The maximum absorption of the wavelength was measured and recorded (Keppy & Allen, 2016).

Protein content calculation was obtained by:

$$\text{Protein content} = \frac{\text{Protein Weight}}{\text{Sample Weight}} \times 100\%$$

Protein weight: Sample volume x Protein concentration of a sample

Fat Content Determination

A round bottom flask was made fat-free using alcohol 95%, and 3 grams were wrapped with a filter paper and put into a Soxhlet tool. Then 200 mL of petroleum ether was put into the round bottom flask, and the Soxhlet toolset was connected to continue with sample filtering for 8 hours until the sample became clear. The solvent in the round bottom flask was evaporated until almost dry. Then it was put into an oven at 100°C for 30 minutes, then cooled in a desiccator for 30 minutes. The fat was then weighed (Suriani, 2015).

The fat content calculation was obtained by:

$$\text{Fat content (\%)} = \frac{(B-A)}{\text{Sample weight}} \times 100$$

Carbohydrate content determination

Sample preparation

One gram of animal feed pellet sample was added to 10 mL of aqua dest while stirring, then 13 mL H₂SO₄ 52% were added while stirring for 20 minutes using a magnetic stirrer and put into a test tube. Aluminium foil was used as a lid to cover the tube. One hundred mL of aqua dest were added and filtered into a 250 mL volumetric flask then aqua dest was added to the volumetric flask up to the designated mark.

Carbohydrate content measurement

Standard glucose solution was prepared at concentrations of 0, 100, 200, 300, 400, and 500 ppm. Each solution measuring 0.5 mL was put into separate flasks, then soaked in water, then 0.5 mL of phenol 5% and 2.5 mL of thick H₂SO₄ were added carefully and slowly along the wall of the flasks. Those solutions were allowed to sit for 10 minutes, then vortexed before being allowed to sit for 20 minutes. The absorption was then measured using a spectrophotometer at the wavelength of 490 nm. The linear equation was then made as a standard curve. Sample measuring was done by putting 0.5 mL of sample solution into a flask, then soaking it in the water, then adding 0.5 mL of phenol 5% and 2.5 mL H₂SO₄ slowly. The following process was the same as it was with a standard glucose solution, then the measurement value was plotted on the standard curve (Bintang, 2018). Carbohydrate content calculation was obtained by:

$$\text{Percent of glucose (\%)} = (G)/W \times 100\%$$

G = glucose concentration (g); W = sample weigh (g)

Results

In this research, the average contents were as follows: 10.25% ash, 6.62% water, 37.30% protein, 6.13% fat, and 26.14% carbohydrate (Table I). The samples of ash, water, protein, fat, and carbohydrate are shown in Figures 1-5.

Table 1: Proximate analysis result of animal feed granules composition

Content	Average (%)
Ash	10,25
Water	6,62
Protein	37,30
Fat	6,13
Carbohydrate	26,14



Figure 1: Sample of ash content



Figure 2: Sample of water content



Figure 3: Sample of protein



Figure 4: Sample of fat



Figure 5: Sample of carbohydrate

Based on the standard curve solution absorption, linear regression equation was $y = 0.148x + 0.088$ (Figure 6).

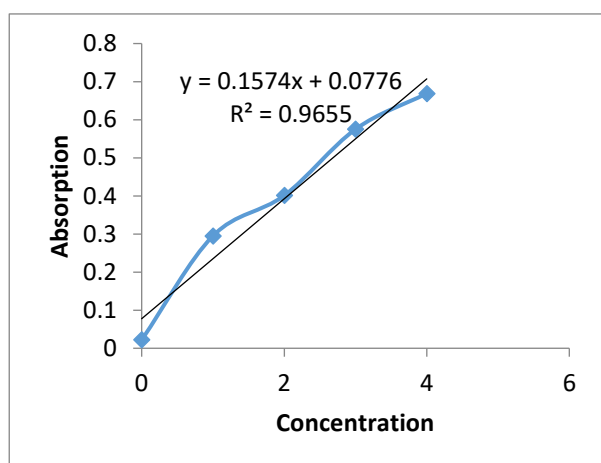


Figure 6: BSA Standard curve

Based on absorption measurement of glucose standard solution, the regression value obtained was $y = 0.006x + (-0.066)$ (Figure 7).

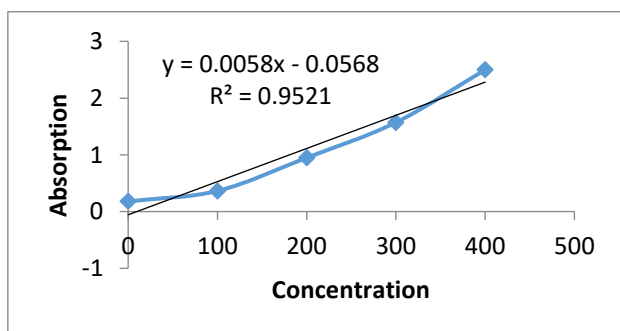


Figure 7: Glucose Standard curve

Discussion

Ash content determination of animal feed granules was done through the dry ashing method using a furnace to determine how good a process was in animal feed production (Widarta, 2015) and find the nutrient value of animal feed granules composition. In this research, the average amount of ash content with three replications was 10.25%. Referring to SNI-8509-2018, this amount should not exceed 14%. Hence, our results complied with SNI-8509-2018.

Water content determination of animal feed granules was done through the thermogravimetric method to find the nutrient content of the animal feed and check whether it meets the standards (Widarta, 2015). Water content determination is crucial because excess water can affect the appearance, texture, and taste of animal feed and support the growth of bacteria, mold, and yeast, resulting in the change of the animal feed. In this research, the average amount of water content with three replications was 6.62%. According to SNI-8509-2018, it should not be higher than 12%. Thus, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

The protein content of animal feed granules was determined through the biuret method, based on radiation absorption, using a spectrophotometer to measure protein content quantitatively, find the nutrient content in the sample, and verify whether it meets the standards Niode & Nasriani & Irdja, 2017). The biuret reactor forms complexes, which help identify the substances. The CuSO_4 produced by heating urea had a similar structure to that of the peptide bond of protein. The principle of the biuret reactor is based on the existence of a reaction between copper sulfate and alkalis with other compounds resulting in a distinct purple-blue (Machin, 2012) or red-violet or blue-violet (Purnama & Retnaningsih & Aprianti, 2019) solution. In this research, the average amount of protein content was 37.30%. Referring to SNI-8509-2018, this amount should not be any less than 16%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

The fat content of animal feed granules was determined using a Soxhlet, which extracted fat using petroleum ether solvent, to calculate calories in animal feed (Pargiyanti, 2019). In this research, the average amount of fat content with three replications was 6.13%. According to SNI-8509-2018, this amount should not be any less than 2%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

Carbohydrate content determination of animal feed granules used the phenol sulfate method. The principle of this method is that simple sugar and oligosaccharides

can react with phenol resulting in a stable yellowish-orange or orange colour. The standard carbohydrate used in this study was glucose. Based on absorption measurement, the regression value obtained was $y = 0.006x + (-0.066)$. What follows is the glucose identification reaction by adding phenol and sulfate acid, which form the colour complex. In this research, the colour complex formed was orange. Based on Figure 8, the reaction shows that glucose was hydrated by thick sulfate acid to form hydroxymethyl-furfural.

The colours obtained by hydroxymethyl furfural were various, ranging from orange-green to brown and purple, depending on the glucose concentration of the sample. In this research, the average amount of carbohydrate content with three replications was 26.14%. Referring to SNI-8509-2018, the amount should not be any less than 14%. Hence, our results complied with SNI-8509-2018 (Standar Nasional Indonesia, 2018).

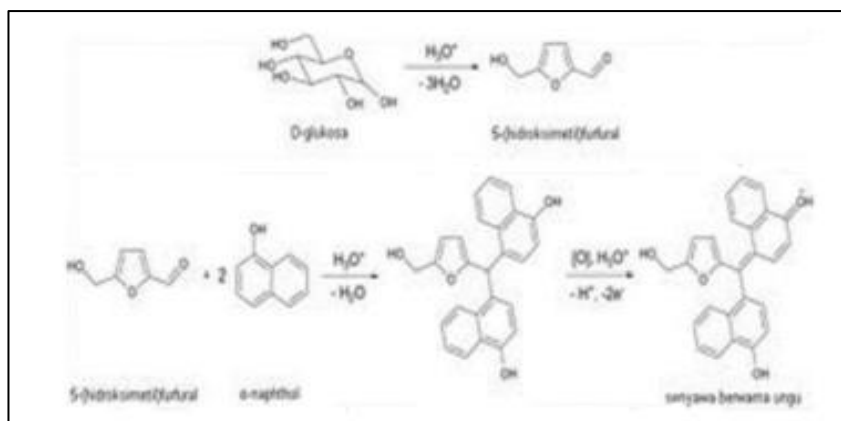


Figure 8: Glucose identification reaction with phenol-sulfuric acid (Wiyantoko, Rusitasari, Putri, dan Muhaimin, 2017)

Conclusion

The findings of this study show that nutrient contents in the composition of animal feed granules of raw material from fish innards wastes fulfil the regulation of animal feed content based on SNI-8509-2018.

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