JKKI 2016;7(5):219-226

Jurnal Kedokteran dan Kesehatan Indonesia

Indonesian Journal of Medicine and Health

Journal homepage : www.journal.uii.ac.id/index.php/jkki

Comparison of reduction sugar analysis method in cilembu sweet potato (Ipomoea batatas l.) using luff schoorl and anthrone method

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Original Article

ARTICLE INFO

Keyword:
Sweet Potato Cilembu,
Reducer Sugars,
Luff Schoorl,
Anthrone

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DOI : 10.20885/JKKI.Vol7.Iss5.art8

ABSTRACT

Background: Cilembu Village - Sumedang, West Java is the producer of sweet potato that tastes sweet. The sweet taste was obtained from potato that has been through the process of incubation for 3 weeks, with a result of the change of starch by amylase enzyme into reducer sugars in the sweet potato Cilembu.

Objective: The aim of the study is to verify the levels of reducer sugar of sweet potato Cilembu the analysis of reducer sugar itself is required and the comparison of the methods used.

Methods: Among many calorimetry methods that used to analyze reducer sugar, the most widely used is Anthrone. The analysis of total carbohydrates, especially reducer sugar is quite simple and sensitive. but the analysis method for reducer sugar is quite simple SNI 3457.1:2008 using Luff Schoorl method that based on titrimetric, it is consuming time, difficult to do for untrained analyser and the reduction reaction is not stoikiometric. the candidate method that using Anthrone method is recommended in order to replace Luff Schoorl method of reducing sugar in SNI 3457.1:2008.

Results: The average levels of reducer sugar in sweet potato Cilembu that used Luff Schoorl as a method was 14.88 ± 0.44 (%) whereas Anthrone method was 21,60 1,31 (%). The average levels of reducer sugars in sweet potato Cilembu from Luff Schoorl method completed the Indonesia Pharmacopoeia conditions, that is has a recovery value a 92,49 % % and but has 3,25% CV value. The average levels of reducer sugar in sweet potato Cilembu with Anthrone method did not complete the Pharmacopoeia Indonesia conditions, which has a recovery value a 67,58 % and has CV 6,56 % value.

Conclusion: Anthrone method not recommended for analyze the reducer sugars in sweet potato Cilembu.

Latar belakang: *Desa Cilembu – Sumedang, Jawa Barat adalah penghasil ubi jalar yang rasanya manis. Rasa manis tersebut didapatkan dari ubi yang telah mengalami proses pengeraman selama 3 minggu, sehingga terjadi perubahan pati oleh enzim amilase menjadi gula pereduksi pada ubi Cilembu tersebut.*

Tujuan : *Untuk membuktikan kadar gula pereduksi ubi Cilembu maka diperlukan analisis kadar gula pereduksinya dan membandingkan metode yang digunakan.*

Metode: *Diantara banyak metode kolorimetri yang ada untuk menganalisis gula pereduksi, yang paling*

banyak digunakan adalah Anthrone. Analisis total karbohidrat terutama gula pereduksi cukup sederhana dan sensitif. Tetapi metode analisis untuk gula pereduksi SNI 3547.1:2008 menggunakan metode Luff Schoorl yang berprinsip pada titrimetri, banyak memakan waktu, sulit dikerjakan bagi analis yang tidak terlatih dan reaksi reduksinya tidak stoikiometri. Metode kandidat yang menggunakan metode Anthrone dianjurkan untuk dapat menggantikan metode gula pereduksi Luff Schoorl dalam SNI 3547.1:2008.
Hasil: *Kadar rata-rata gula pereduksi pada ubi Cilembu dengan metode Luff Schoorl adalah (14,88 ±0,44)% sedangkan metode Anthrone adalah (21,60 1,31)%. Kadar rata-rata gula pereduksi pada ubi Cilembu secara metode Luff Schoorl memenuhi persyaratan Farmakope Indonesia yaitu memiliki nilai recovery sebesar 92,49 % dan akan tetapi memiliki nilai CV 3,25%. Kadar rata-rata gula pereduksi pada ubi Cilembu secara metode Anthrone tidak memenuhi persyaratan Farmakope Indonesia yaitu memiliki nilai recovery sebesar 67,58 % dan memiliki nilai CV 6,56 %.*
Kesimpulan: *Metode Anthrone tidak dapat direkomendasikan untuk analisis gula pereduksi pada ubi Cilembu.*

INTRODUCTION

Cilembu sweet potato (*Ipomoea batatas* L.), which generally known as ubi Cilembu in Indonesia, has fourth highest carbohydrate content after rice, corn, and cassava.[1] Cilembu sweet potato plant has good adaptation ability towards the environment, thus it could grow in tropical and subtropical area in 0 until 3000 m above sea level area in various soil conditions.[2] Currently the trend of sweet potato utilization has shifted from staple food into processed food, industrial raw material and mainly for comestibles.[3]

Methods used for direct sugar reduction analysis that are determined by BSN (Badan Standarisasi Nasional/ National Standardization Bodies) through SNI 3547.1:2008, about sugar reduction method analysis (counted as inversion sugar) is Luff Schoorl methods. Other instances of direct sugar analysis are Dinitrosalysilate, Samogyi Nelson, and Hagedorf Jansen methods. Anthrone Method, is generally used for total sugar analysis using Visible Light Spectrophotometry.[4,5]

Until now, the comparison between Luff Schoorl and Anthrone method in analyzing reducing sugar in Cilembu sweet potato had never been done. Even though Luff Schoorl is an approved method[6] both Luff Schoorl and Anthrone methods are not standardized method, thus they need validation. This study aims to compare the level of reducing sugar between both methods to determine the better ones in order to utilize in routine analysis.

METHODS

Tools and ingredients

Ingredients used are rancing genus Cilembu sweet potato, H_2SO_4, Barfoed, P., Benedict, Molisch P., H_2O free O_2, Luff Schoorl, p., citrate acid, KOH, KI 20% HCl, NaOH 0,1 solution, starch indicator 0,5%, standard glucose, $Na_2S_2O_3$0,1 N, Anthrone 0,1 %.

Tools used are Oven Slicer, 80 mesh strainer, Stirrer Hot Plate Stirrer, vacuum evaporator, centrifuge, Halogen Moisture Analyzer, Spectrophotometer UV-Vis (Pharmaspec 1700, SHIMADZU).

Powder Production

Cilembu sweet potato was stored for 3 weeks then sliced and dried up in 60°C temperature for 10 hours, and in 60°C temperature for 5 hours when using cabinet dryer. Lastly, it was strained into powder (80-100 mesh).[1]

Cilembu Sweet Potato Powder Standardization

The measurement of drying weight shrinkage uses Hb 3 Halogen Moisture Analyzer of Mettler Toledo with the formula :

$$\% \text{ water} = \frac{\text{(water weight)}}{\text{(fresh ingredient weight)}} \times 100\%$$

Determination of Total Ash Content approximately 2-3 g Cilembu sweet potato powder, inserted into crucible porcelain and added with some samples, heated and measured

up to a constant weight.[7]

The ash gained from Total Ash Conrent measurement is boiled in 25 mL weak hydrochloric acids for 5 minutes, acid-insoluble part is then gathered. The concentration of acid-insoluble ash content is then compared with material that has dried up in the air and then counted.[7]

Water Content Determination is done by distillation using toluene distillation.[7]

Extraction

As much as 5 gram of Cilembu sweet potato powder is mixed with 40 mL boiling water while being stirred, and pH is maintained between 6.5-8 by adding 0.05 N KOH or 0.05 N HCl. Extract, which pH has been regulated, is then moved into 100 mL volumetric flasks and calibrated until 100 mL. Temperature of the extract is maintained between 85°C ± 2°C with continuous stirring for 10 minutes.[8]

Analysis

Qualitative analysis is done using tube test including a) Molisch test, b) Benedict test, c) Barfoeddan test, and d)Thin Layered Chromatography (TLC).[9] Quantitative analysis is done by Luff Schoorl and Anthrone methods.

Luff Schoorl Method (Titrimetry)

1. Prior to quantitative analysis using Luff Schoorl method, standardization of $Na_2S_2O_3$ is done using KIO_3.
2. The determination of Cilembu sweet potato sample concentration

The result of Cilembu sweet potato extraction in the concentration of 50 mg/mL, is added with H_2O free O_2. Extract solution is inserted into iodine flask 250,0 mL, added with 25,0 mL Luff Schoorl reactant and some boiling chips, heated for 4-5 minutes while closing the iodine flask; After cooled, 10 KI 20 % solution and 25 mL H_2SO_4 25% solution are added, then titrated with sodium thiosulphate 0,1N solution using starch 0,5 % solution as indicator.[10]

Anthrone Method (Visible Spectrophotometry)

1. The making of Anthrone 0,1% reactant in thick sulfuric acid.
2. Determination of operating time and maximal wavelength absorption.
3. The making of standard curve
4. Determination of sugar content in samples

The measurement using this method is by determining the sugar concentration of the examples using the standardized curve in linear regression formula Y = Bx+A.

Validation and Verification

The comparison between the methods is done to evaluate how far both methods would result in the same measurement.[11] Validation and verification are done using the Cilembu sweet potato extract that has been stored for 3 weeks. The determination of validity includes precision with RSD cost,[12] accuracies using recovery test, determination of sensitivity using Limit of Detection (LOD) value and Limit of Quantitative (LOQ) value. Specificity is measured by the ability to determine expected analyte specifically and appropriately with the presence of other components in the samples, like impurities and degradation products.[12] Efficiency is measured by the use of resources in a process. An efficient process is characterized by process improvement, making it faster and more cost effective.

RESULTS

Powder of Cilembu Sweet Potato

Cilembu sweet potato powder was made directly from Cilembu sweet potato that was sliced and dried. Then, it was inserted into the oven in 50° C temperature for 24 hours and then blended.

The result of drying weight shrinkage was (6,63±1,24)%. The general requirements for drying weight shrinkage is should be no more than 10%, in which enzymatic reaction would still be present.

The result of simplicia ash content was (4,10 ±0,13)%. The requirement of ash content, as regulated in Materia Medika Indonesia, should

not exceed 4,9%. Samples simplicia in this study has met this requirement.[7]

The determination of acid-insoluble and acid-soluble ash content was done to recognize the HCl-insoluble metal like Hg, Pb, and Ag. The result of acid-insoluble ash content determination was (0,86 ±0,14)%. The acid-insoluble ash content was found less than 1,7% indicating that the samples or powder had met the requirements as regulated by Materia Medika Indonesia.[13]

The determination of water content in Cilembu sweet potato powder is done using toluene distillation. The tolerable water content in a simplicia, according to Materia Medika Indonesia[13], should be less than 10% in order to prevent mold growth and enzymatic activities. During the drying process of this study, the water content had not yet been determined less than 10%. The result of this study showed that the water content was (6,72 ±0,23)%.

Extraction of Cilembu Sweet Potato Powder

Extraction of Cilembu sweet potato powder was done using boiling aquadest solvent, the purpose is to determine the concentration of its reduction sugar. The addition of HCl 0,01 N and NaOH 0,01 N was to maintain a neutral pH of the samples. If the pH was too high or too low, the analysis process would be altered. The pH of extract in this research was pH 7. To prevent the extract from damage or microbe contamination, it was stored in cool temperature or sometimes it was frozen in 0°C inside the freezer.

Qualitative analysis

Qualitative analysis of Cilembu sweet potato extract was done using 4 means, which were Molish tube test, Benedict tube test, Barfoed tube test, and TLC.[14] The results of the tube tests can be seen in Table 1. The result of TLC could be seen in Figure 1.

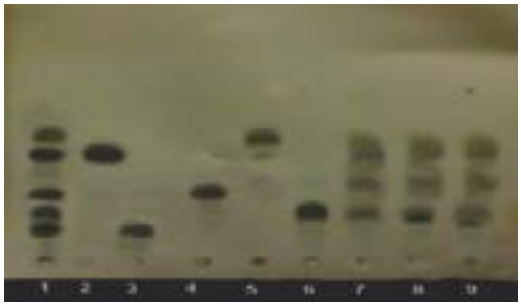

Figure 1. TLC profile Cilembu sweet potato extract and the standards. Figure explanation : (1) glucose, lactose, sucrose, fructose, galactose; (2) glucose; (3) lactose; (4) galactose; (5) fructose; (6) sucrose (7),(8) and (9) Cilembu potato extract 1; (8) Cilembu potato extract 2 and (9) Cilembu potato extract 3.

Table 1. Qualitative testing of Cilembu sweet potato extract

Tube Test	Figure	Characteristics	Results
Molish		Violet ring formation	+
Benedict		Brownish orange	+
Barfoed		Sedimentations form in less than 2 minutes	+

The results of elution using 5 types of standard monosaccharides showed that Cilembu sweet potato contained glucose (Rf 0,50), fructose (Rf 0,56), galactose (Rf 0,37), Sucrose (Rf 0,31).

Luff Schoorl Method

Mean concentration seen in this study was (14,88±0,44)%. 5 gram Cilembu sweet potato contained 14,88% reducing sugar or 744 mg. It was assumed that the remaining 4.256 mg sugar, contained others non-reducing sugar, such as sucrose, protein, and water-soluble glucosides.

Table 2. Result of Reduction Sugar Measurement

Replication	Titrant Volume (mL)	Concentration (%)	$\bar{X}$ ±LE (%)	CV (%)
1	21,87	15,16	14,88 ± 0,44	3,25
2	21,90	15,02		
3	21,85	15,26		
4	21,82	15,40		
5	21,95	14,79		
6	22,10	14,00		
7	22,00	14,55		

Anthrone Method (Visible Light Spectrophotometry)

Prior to the measurement of reducing sugar concentration, operating time was measured. The result of the measurement showed that sample could be stable in minute 21st. While maximum wavelength absorption was 623,80 nm. The reaction could be seen in Figure 2.

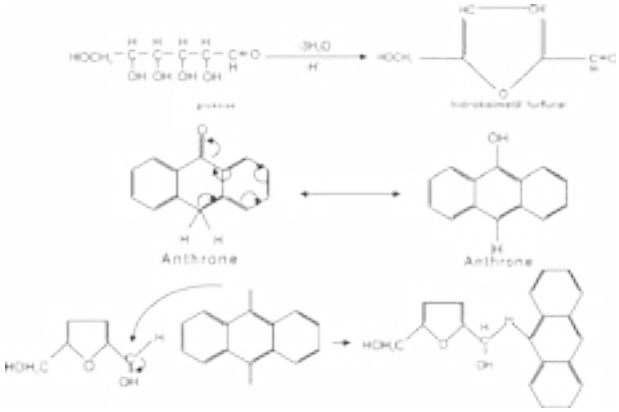

Figure 2. Anthrone Reaction[15]

Generating Standard Curve

As seen on Figure 3, the result of OT determination and maximum wavelength 623,80 nm, generated linear regression formula as such y=0,5661x + 0.1535 r count = 0,972, while the value of r table for N = 6 with 95% Confidence Interval was 0,811. Because the value of r count (0,972) was bigger than r table (0,811) in 95% Confidence Interval or error bars 5%, it could be concluded that there is positive and significant correlation between standard glucose absorbance value and standard glucose concentration.

The Measurement of Glucose Concentration in Cilembu Sweetpotato Extract

Samples concentration were obtained by inserting the absorbance of samples (Y) from the Table into standard curve formula Y = 0,5661 X + 0,1535, so that sugar reduction (glucose) concentration of the samples could be obtained. The results of absorbency and analysis of sample concentration of Cilembu sweet potato using Anthrone method could be seen in Table 3.

Validation and Verification Method

The validation of Luff Schoorl and Anthrone method results were tested.[16] The validity could be seen in Table 4.

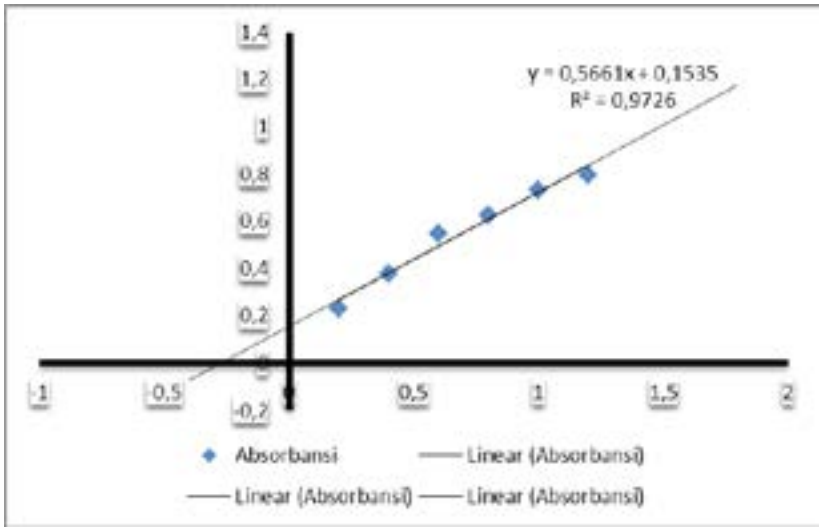

Figure 3. Glucose Standard Curve

Table 3. The results of Cilembu sweetpotato sample concentration using Anthrone method

Replication	Absorbance	Concentration (%)	$\overline{X}$ ±LE (%)	CV (%)
1	0,272	20,93	21,60 ± 1,31	6,56
2	0,283	22,87		
3	0,268	20,22		
4	0,289	23,93		
5	0,278	21,99		
6	0,267	20,04		
7	0,274	21,28		

Table 4 The results of Validation and Verification of Luff Schoorl and Anthrone Method

Validation and Verification Parameter	Absorbance	Concentration (%)	Notes
	Luff Schoorl	Anthrone	
Accuracy	X Recovery = 92,42% (qualify)	X Recovery = 67,58% (not qualify)	Range 90,0 -110,0% *
Precision	CV= 3,25% (not qualify)	CV= 6,56% (qualify)	CV < 2%**
LOD and LOQ	-	LOD= ,019mg/10mL LOQ= 0,06 mg/10mL	<2mg/10mL
Specificity	Specific	Unpecific	
Efficiency	Complicated and long process	Simple and easy	

*Farmakope Indonesia IV edition

**Other requirement[13]

DISCUSSION

The comparison of reduction sugar analysis method using two different methods, which were Luff Schoorl which uses titrimetry, and candidate method which uses Anthrone by visible light spectrophotometry, in Cilembu sweet potato samples showed different accurate values. Accuracy value obtained from recovery of Luff Schoorl method was found better because it met the range required by Farmakope Indonesia, while Anthrone method did not.[17] The coefficient variance value (CV) of Luff Schoorl method was also found better than Anthrone method in term of accuracy. In addition to that, the result of T test and F test showed that both methods were statistically significantly different in 95% Confidence Interval. Based on its efficiency, Anthrone method was more simple and modern compared to Luff Schoorl method.

CONCLUSION

Based on Cilembu sweet potato powder characteristics, it was found that drying weight shrinkage: 6,62 ± 1,24 (%); Total ash-content: 4,10± 0,13 (%); Acid-insoluble ash content: 0,86 0,14(%); and Water content: 6,72±0,23 (% v/b).

The mean concentration of Cilembu sweet potato reducing sugar using Luff Schoorl method was (14,88±0,44) %. The mean concentration of Cilembu sweetpotato reducing sugar using Anthrone method was (21,60±1,31)%.

Luff Schoorl method and Anthrone method was found significantly different, thus Anthrone method is not recommended to analyze Cilembu sweet potato reducing sugar and could not substitute Luff Schoorl method.

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