Saccharin

by Yusnidar Yusuf

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REVITALIZATTION OF PROFESSIONAL ASSOCIATION AND SCIENTIFIC KNOWLEDGE FOR HRD OF HIGHER EDUCATION

(Workshops as an Organization Profession, International Conference, MoA/MoU Multy Kampus, OJS Training)

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PREFACE

Praise being said to Allah Almighty God for all the grace and guidance that has been given to us all, so the Proceedings of the ADRI 2016 International Multidisciplinary Conference and Call for Papers. Proceedings contains a number of articles and research papers from lecturers, teachers, students, researchers and / or observers of the development of science and technology.

This seminar is the series of the International Seminar organized by ADRI, the first was held in Lombok, Mataram; The second was held in Denpasar, Bali October 15 to 17, 2016; the third was held in Surabaya, East Java, on November 10, 2016 and the fourth was held in Pontianak, West Kalimantan, on 6 to 7 November 2016. The fourth International Seminar in Pontianak's speakers came from 5 countries; Indonesia, Taiwan, United Kingdom, Italy and Malaysia. Call papers Participated in an international conference in Pontianak as much as 103 paper came from 5 countries, with a number of writers were 156 persons, from Indonesia came from 15 provinces. Most writers of West Kalimantan: 67 person and East Java: 41 people.

The international conference has been made to be held as the realization of cooperation between ADRI, National University of Kaohsiung in Taiwan, Universiti Tun Hussein Onn Malaysia, STKIP Singkawang, and all the universities participating in the MoU / MoA multi campus.

On this occasion let us give awards and gratitude to:

Keynote speaker

1. Prof., dr. Ali Ghufron Mukti, M.Sc., Ph.D., Dirjen Sumber Daya Ilmu Pengetahuan, Teknologi dan Pendidikan Tinggi
2. Dr. Ir. Jumain Appe, M.Sc., Direktur Jenderal Penguatan Inovasi, Kemenristek Dikti.
3. Prof. Dr. Paulina Pannen, M.Ls., Staf Ahli Bidang Akademik, Menristek Dikti.
4. Prof. Dr. I-Hsien Ting (Associate Professor Department of Information Management, National University of Kaohsiung, Taiwan)
5. Prof. Dr. Wahid Bin Razzaly, Universiti Tun Hussein Onn Malaysia (UTHM)
6. International speakers; Tirthendu Bagchi (Nottingham University, UK) and Cristina Lanteri (Italy)

In special award and we thank to:

1. Drs. Cornelis, M.H., Gubernur Kalimantan Barat.
2. Dr. H. Achmad Fathoni Rodli, M.Pd., General Chairman DPP P-ADRI Board.
3. Dr. M. Zeet Hamdy, Sekretaris Daerah Propinsi Kalimantan Barat.
4. Board of DPP ADRI
5. The Board of Trustees and Governing ADRI DPD Kalbar
6. Rector and Leadership College participant MoU / MoA multi-campus
7. Board of Editor, executive Editors and the Executive Committee in ADRI International Multidisciplinary Conference and Call for Papers in Pontianak
8. The sending of paper and parallel scientific conference speaker

In addition to the international conference, at the same time as a multi-campus realization cooperation activities, as well as activities carried out:

1. Inauguration of ADRI DPD West Kalimantan.
2. Training Open Journal System, as we know that from 2017 Kemenristek Dikti already requires all scientific journals should be based online by implementing OJS and scientific work for the maintenance of mandatory functional academic journals published in the OJS.

Proceedings are published in book form only contains abstract, distributed to participants in the form of compact disks (full paper) and published online at:

www.p-adri.or.id/prosiding/prosiding4pontianak.

Hopefully, these proceedings may give benefit to us all, for the development of science, technology, arts, culture, and sports. In addition, it is also expected to be a reference for the nation and state-building efforts so that science and technology become a strong pillar in the face of the ASEAN Economic Community.

Lastly, we are sorry if there are things less pleasing.

Sincerely,

Pontianak, December 6, 2016.
Chief Executive,

Drs. Andi Mursidi, M.Si.
Chairman ADRI DPD Kalbar

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TABLE OF CONTENTS

ANALYSIS ON LEVEL OF SACCHARIN AND CYCLAMATE ADDITIVES INSIDE CUP-PACKAGING-DRINK ATSD KARANG TENGAH, TANGERANG CITY

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Abstract. The use of food additives today were extremely diverse, from preservatives to flavor concentrates and sweeteners, the circulation needs to attention. This study aims at analyzing the levels of saccharin and cyclamate at children elementary school's beverage at Karang tengah, Tangerang City. This study uses HPLC with a C18 column with mobile phase KH2PO4 and methanol (70:30), with UV-Vis detector for cyclamate at 200 nm wavelength and for saccharin at 227 nm wavelength. The results of this study showed that five samples containing saccharin (sample A = 73.059 mg/L, the sample B = 56.624 mg/L, the sample C = 0.73 mg/L, the sample D = 0.82 mg/L, the sample E = 61,52 mg/L), this figure was still below the recommended threshold BPOM RI. Whereas four ofthe sampleincluding, containingcyclamate (sample A = 1184.42 mg/L, the sample B = 1409.95 mg/L, the sample D = 1418.31 mg/L, the sample E = 444.38 mg/L), it's levels exceeding BPOM RI's threshold recommended and one sample containing cyclamate whose levels do not exceed the threshold (sample C = 117.095 mg/L).

Keywords: Saccharin and Cyclamate, Sweeteners, Beverage Packaging

I. INTRODUCTION

Food additives, such as preservative, additive, and food coloring,were easily found in various food and beverage. Factory-based-industries used those additives for their products as well as home-based-industries. The safety of school's snacks had to be paid attention because it related to students' physical-growth. Food that was often become source of intoxication were snacks. This was due to lack of quality control from the manufacturer (that was often come from home-based-industry) (Adriani M, Wirjatmadi B, 2012).

Additive that was often used was artificial sweetener. It was processed chemically, and its compound was not found naturally (Anonym, 2012). It was purposely added to increase flavor, aroma and physical appearance of food or drink. Sweetener was divided into 2 (two): synthetic sweetener (cyclamate, aspartame, saccharin) and natural sweetener (sucrose, fructose, glucose, sorbitol).

Saccharin was artificial sweetener in form of salt, consisted of calcium, potassium and sodium saccharin. In general, saccharin salt was crystal white, odorless, soluble in water and very sweet (Head of National Agency of Drug and Food Control, 2014). Saccharin was 200-700 times sweeter than sucrose. Unfortunately, it left bitter or metal after taste in middle until high concentration. This was due to low purity as result from synthetic process. To remove the after taste, saccharin could be mixed with cyclamate by comparison of 1:10 for cyclamate (Putri Intan Eriska, 2013).

In 1971, a research conducted by Wisconsin Alumni Research Foundation (WARF) proved that saccharin classified as carcinogenic substance. From 15 rats which were given saccharin, 5% (or 7 rats among them) had cancer on gall bladder after consuming saccharin for 2 years(Djojosoebagio, Soewando dan G, Wiranda, 1996). In 1977, Canada's Health Protection Branch reported that saccharin was responsible as the cause of urine bladder cancer. Afterwards, the use of saccharin was banned in Canada,except as sweetener (Winarno F.G, 2008).

Initially, cyclamate was only use in drug industry. The purpose was to cover bitter taste from active drug substance, like in antibiotic and pentobarbital. After it was proclaimed safe on 1958, cyclamate was more popular as low-calorie artificial sweetener. However, research in 1969 showed that it could cause urine bladder cancer on mice that was given cyclamate regularly (Anonym, 1998). As result, United States withdrew its distribution from food industrytotallyon 1969 and continued by United Kingdom on 1970.

Cyclamate ($C_6H_{12}NNaO_3S$) was often found in form of calcium salt, potassium, and sodium cyclamate. Cyclamate was quite cheap additive, 30 to 50 times sweeter than sucrose. Unlike saccharine, cyclamate seldom leave bitter after taste like saccharin (Anonym, 2009). Cyclamate had 0 kkl/gor equal to 0 kJ/g and ADI: 0 mg/kg - 11 mg/kg per body weight. Based on regulation from Head of National Agency of Drug and Food Control (NADFC) No. 4 year 2014 regarding maximum limit of sweetener additives allowed in flavored drink, including

sport drink or electrolyte and particle drink, was 350 mg/kg (Head of National Agency of Drug and Food Control, 2014).

High Performance Liquid Chromatography (HPLC) was a chromatography method which could separate macro molecule, ionic compound, unstable natural products, polymeric compound and poly-functional groups with high mass molecule. It worked by fractional filtering, absorption or ion substitution that used interactive active phase and active solid/liquid passive phase(Ibrahim Slamet S, 1995). Its instruments consisted of: active phase's container, pump, tool to insert sample (injection container), column, detector, container to collect emission from active phase and a computer or integrator or recorder. Advantages of using this method were fast, well-separation power, sensitive to unique detector, column could be reused, ideal for big molecule and ion, and easy to reclaimed.

II. RESEARCH METHOD

A. Material and Instrument

Materials used in this research were plastic-cup-drink with P.I.R.T label (Product of Home-Industry), saccharin, cyclamate, potassium dehydrogen phosphate (KH2PO4), methanol, aquades.

Instruments used in this research included: Shimadzu SPD-20A as HPLC with C 18 (150 mm x 4.6 mm) of nucleosil column, analytic scale, whatman filter paper no. 42, volumetric flask, beakerglass, pipet volume.

B. Analysis Method

Drink sampling

Sampling method used in this research was purposive sampling; sample was taken intentionally depended on sample requirements needed. Researcher decided himself which sample taken due to particular consideration; sample must have P.I.R.T label (label for home-made industry product) and potentially contained saccharin or cyclamate. Drink with cup-package was chosen and five samples were taken.

1. Making of standard solution (British International Standard, 1997).
   a. Making of saccharin standard solution 1000mg/l
      Measure 0.0103 gram of saccharin. After that, pour it into 100 ml volumetric flask. Then dissolved it with KH2PO4solution – methanoluntil the border and homogeneous. 10 ml was taken from the 100 mg/l source solution. It was then dissolved and diluted into 100 ml volumetric flask with KH2PO4solution – methanol. Series of standard solution was then made; 10, 50, 100, 300,and 500 mg/l by pipetting 0.1 ml, 0.5 ml, 1.0 ml, 3.0 ml, and 5.0 ml solution. These standard solution series was then poured into 10 ml volumetric flask. Afterwards, it was dissolved with KH2PO4solution – methanol.
   b. Making of cyclamate standard solution
      Measure 0.0508 gram of cyclamate. After that, pour it into 50 ml volumetric flask. Then dissolved it with KH2PO4solution – methanoluntil the border and homogeneous. Series of standard solution was then made; 10, 50, 100, 300, and 500 mg/l by pipetting 0.1 ml, 0.5 ml, 1.0 ml, 3.0 ml, and 5.0 ml solution. These standard solution series was then poured into 10 ml volumetric flask. Afterwards, it was dissolved with KH2PO4solution – methanol until the border and homogeneous.
2. Sample Preparation
   Measure 2 gram of sample. After that KH2PO4solution and methanol were addeduntil the border. It was then shaken until dissolved and homogeneous. After that, it was filtered by whatman filter paper no, 42. The solution was ready to be injected into HPLC (British International Standard, 1997).
3. Conditioning Instrument
   a. Conditioning Instrument for cyclamate
      HPLC's optimal condition for this experience was as follow : C-18 (150 mm x 4.6 mm) of nucleosil column, the flow speed was 1 ml/minute, 200 nm Ultraviolet detector, composition of active phase from potassium dehydrogen phosphate (KH2PO4) - methanol was (70:30) with 20 µl injection volume.
   b. Conditioning Instrument for saccharin
      HPLC's optimal condition for this experience was as follow : C-18 (150 mm x 4.6 mm) of nucleosil column, the flow speed was 1 ml/minute, 220 nm Ultraviolet detector, composition of active phase from potassium dehydrogen phosphate (KH2PO4) - methanol was (70:30) with 20 µl injection volume.
   c. Determining level ofsynthetic additives (saccharin and cyclamate)
      Sample, which had been diluted by KH2PO4solution – methanol, was taken for the amount of 5 µl. It was then injected into HPLC. The area obtained from the result was recorded, and calculated using calibration curve from each element. Concentration of saccharin and cyclamate synthetic additives was calculated using standard curve by linear regression equation as follow (Winarno F.G, 1997):

      y = a + bx

      whereas: y = area's width
      x = sample's concentration
      a = intercept
      b = slope

      While level of synthetic additives could be calculated using formula as follow (Winarno F.G, 1997) :

$$Synthetic\_Additives\_Level = \frac{C_{sample} \times Dillution\_factor}{Ws} = ppm$$

      Note: Csample = concentration of sample
      Ws = weight of sample

III. RESULT AND DISCUSSION

A. Conditioning HPLC Instrument

Conditioning instrument in this research was conducted with the objective to find optimal condition for HPLC

method. Cyclamate optimal conditioned obtained from this research was C-18 (150 mm x 4.6 mm) column, flow speed 1 ml/minute, 200 nm Ultraviolet detector, and 70:30 composition of active phase from KH2PO4-methanol.Saccharin optimal conditioned obtained from this research was C-18 (150 mm x 4.6 mm) column, flow speed 1 ml/minute, 220 nm Ultraviolet detector, and 70:30 composition of active phase from KH2PO4-methanol.

We could differentiate between saccharin and cyclamate with instrument's condition stated above. Separation between saccharin and cyclamate could be seen from its retention period. Retention period was time needed after sample was injected to bring out an analit peak by detector (IbrahimSlamet S, 1995).

Sample Preparation

Sample preparation was conducted by dissolving sample with the fit solvent and conducting filtration (Ibrahim Slamet S, 1995). The solvent used was KH2PO4 - methanol (70:30). Meanwhile the filtration process was using whatman filter paper no. 42 so that sample would still be in clear condition.

Determining Calibration Curve

Correlation coefficient value (r) was a quality indicator from linier parameter which described proportionality of analytic respond (area width) towards the measured concentration. Data of area width generated from saccharin standard series (with concentration of 0.101; 0.505; 1.009; 3.028; 5.047 µg/ml) showed that it had linier relation with correlation coefficient value (r = 0.9999987) by using linier regression equation; y = 69020.9 x – 112.852. The coefficient obtained showed good result (close to 1 score). This informed that there was proportional relation between area width and concentration measured. Result from linier test of saccharin calibration curve could be found in Figure 1:

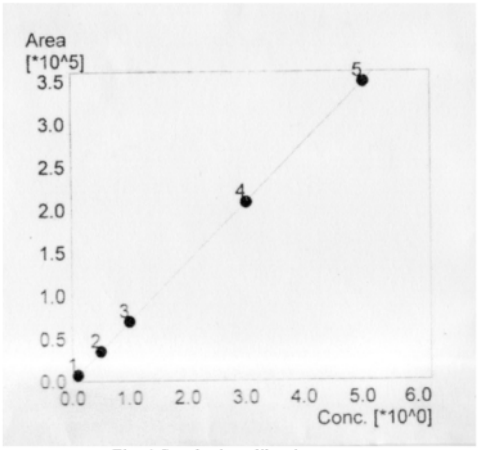

Fig. 1 Saccharin calibration curve

From series of saccharin concentration (9.957; 49.784; 99.568; 298.704; 497.840 µg/ml), correlation coefficient value (r) was obtained (r = 0.9999088) using linier regression equation; y = 929.899 x – 1479.25.The coefficient value obtained revealed good relation between concentration and area width (because the score was close to 1). The linier test result from cyclamate calibration curve was shown in Figure 2:

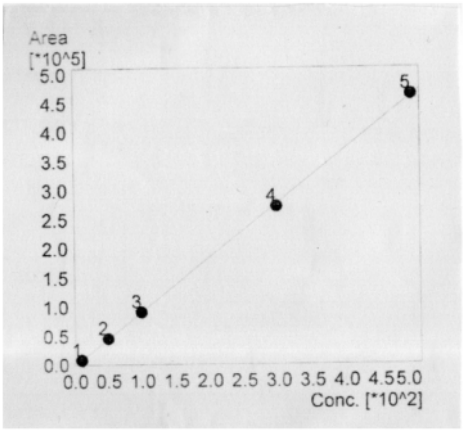

Fig. 2 Cyclamate calibration curve

B. Analysis Result of Saccharin and Cyclamate Content

Based on linier regression calibration curve obtained from saccharin and cyclamate on Picture 1 and 2 above, saccharin and cyclamate level content was found in various amounts within all five samples. All five samples tested positively contained cyclamate. One sample showed safe level of cyclamate as determined by NADFC, while the rest four samples had cross it.Those five samples also positively contained saccharin in safe level.

The analysis result was shown on table below:

Table 1 :Analysis result towards saccharin and cyclamate content in drink sample

Sample	Saccharin		Cyclamate	
	Curve's width (ppm)	Level (mg/L)	Curve's width (ppm)	Level (mg/L)
A	0.735	73.059	47.704	1184.422
B	0.575	56.624	57.447	1409.954
C	0.030	0.73	4.754	117.095
D	0.0083	0.82	59.289	1418.306
E	0.615	61.522	17.854	444.385

This research was conducted to test whether cup-package-drink with P.I.R.T (Home-Made Industry Product) label contained synthetic additives, such as saccharin and cyclamate. Based on result from the research, the five samples contained saccharin with concentration between 0 – 73.059 ppm, while cyclamate's concentration ranged between 0 – 1418.306 ppm. After calculating the content level, saccharin found in all five samples didn't exceed safe

level of 120 mg/L as determined by NAFDC's regulation No. 4 Year 2014. The highest found only contained 73.059 ppm saccharin. However, the cyclamate found in all five samples range between < 2.00 mg/L – 1418.306 mg/L, whereassafe level of cyclamate determined by NAFDC must not exceed 350 mg/L.

The sweet taste of every synthetic additive was always evaluated towards its relativity with the sweet taste from sucrose. Cyclamate was 30 – 50 time sweeter than sucrose, while saccharin was 200 – 700 times sweeter. Due to its strong sweet taste, the use of synthetic additive was thought to be more efficient that natural additive (Wisnu C, 2006).

The use of cyclamate had been banned in United States. However in Indonesia, it wasn't forbid and could be used with certain daily intake which had been determined for each food and drink substance. Considering the danger that might cause by this synthetic additive, supervision and regulation towards the use of synthetic additives for commercial food and drink needed to be stricter.

Cyclamate from the analysis result had exceeded safe level determined by NADFC. This could cause negative effect for children in the future. This issue needed to be anticipated. Government in general and NADFC in particular, as authorized institution needed to warn manufacturer which already P.I.R.T label but still used cyclamate.

CONCLUSION

Based on research towards saccharin and cyclamate inside commercial cup-drink with P.I.R.T (Home-made industry product) label using HPLC method, it can be concluded that :

1. All five samples positively contained saccharin and cyclamate
2. Saccharin levels in all five samples were still in safe level as determined by NADFC. However, cyclamate level in four samples had exceeded limit set by NADFC, while one sample was still in safe level.

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