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Research Article

Analysis Contens of Natrium Benzoate and Sorbic AcidIn Beverage Snack Sold at SDN Karang Tengah Tangerang City

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ABSTRACT
The phenomenon of increase in food industry need a research about additional substances of food to get safety. This study aims at determining content of natrium benzoate and sorbic acid in snack which sold at in SDN Karang Tengah Tangerang City whether to standard of health minister number: 033 years 2012 or not. The research use HPLC method with KH2PO4 as a mobile phase. The result of this research showed the variation content from each beverage sample. Four of the five beverage sample showed content natrium benzoate with each level for sample A = 839,2145 mg/L, B=191,2495 mg/L, C=869,736 mg/L, E=226,9 mg/L. And one of the all-beverage sample showed the content of sorbic acid with the level D=522,013 mg/L.

Keywords: Beverage Packaging, Preservatives, Natrium Benzoate, Sorbic Acid

INTRODUCTION

Packaged drinks are one of the favorite drinks for all ages. Apart from being easily available on the market, this drink has a wide selection of flavors and is rich in nutritional content (Kregiel, 2015). The problem is, all types of drinks generally use additional substances such as preservatives, artificial sweeteners, essence (flavor enhancer) and coloring. The addition of preservative in foods can prolong their shelf-life, protect them against deterioration, and prevent the growth of pathogenic microorganisms (Xu et al., 2019). It is necessary to consider again the possible dangers of additives in drinks that have an adverse effect. Especially when consumed in large quantities and for a long time.

Natrium benzoate and sorbic acid are food additives which are used as food preservatives. Benzoic acid and sorbic acid are the most common food preservatives used to protect the longevity of food products (Akbari-Adergani et al., 2013; Xu et al., 2019).

The dangers of natrium benzoate and sorbic acid can cause Systemic Lupus Ertythematasus (SLE), this disease attacks the immune system. The role of experts is needed to provide scientific explanations and research, because food safety greatly affects the body's resistance. Some adverse of benzoic acid and sorbic acid reactions, including metabolic acidosis, convulsions, asthma, and allergic reactions (Tfouni & Toledo, 2002; Wen et al., 2007; Xu et al., 2019).

Natrium benzoate is the natrium salt from acids and exists in the form of salt. When dissolved in water. It can be produced by reacting natrium hydroxide with benzoic acid. This preservative is widely sold in the market and is used to preserve various foods and beverages such as fruit juices, soft drinks, tomato sauce, chili sauce, jam, jelly, sweets, soy sauce, and others (Wisnu, 2006). The chemical formula for natrium benzoate is C6H5COONa. Benzoic and sorbic acid and their respective sodium and potassium salts are com, is monly used preservatives for food protection (Akbari-Adergani et al., 2013).

Sorbic acid (C6H8O2) is generally used in the form of its potassium salt, has a wide spectrum of activity against many yeasts and molds, but is not selective against bacteria, many yeasts and fungi, but not as effective against bacteria, Lactobacilli, Staphyloccocci and Clostridia (including Clostridium botulinum) is not held back by Sorbat. Sorbic acid is more effective at higher pH than benzoic acid and is also allowed to be used in baked goods (bacterial products), (Tfouni & Toledo, 2002; Qi et al., 2009; Ayse et al., 2020) cheese and cheese products, high moisture prunes and some semi-wet foods, as an anti-starch. Because there is no taste, sorbate is also used in the wine industry to reduce the levels of sulfur dioxide used (Ayse et al., 2020).

Food additives are materials that are not normally used as food and are usually not a typical food ingredient, have or do not have nutritional value, which are intentionally added, processing, preparing, treating, packing, packaging, storing or transporting food to produce or are expected to produce. Food additives are substances which can be derived naturally or artificially and they are added deliberately to foods to prevent

degradation, to improve organoleptic properties and to retain their quality and colour (Etwaroo et al., 2019). The most used natural classes of additives are colourants, sweeteners, antimicrobials and antioxidants (Carocho et al., 2014) and they include beta carotenes, paprika, curcumin, lutein, anthocyanin, lycopene, capxanthin, chorophyll, annatto, beetroot red, polyphenols, tocopherols, natamycin and stevia (Etwaroo et al., 2019)

Directly or indirectly a component or affect the characteristics of these foods general, additives can be divided into two major parts, namely: Additives that are intentionally added, namely additives that are given intentionally with a specific purpose and purpose, for example to increase consistency, nutritional value, taste, control acidity or alkalinity, strengthen shape and appearance, and so forth. Additives are accidentally included in the manufacturing process, namely additives found in food in very small amounts as a result of processing (Winarno, 1997).

The use of preservatives is not only important to maintain food quality, but also to ensure the health and safety of consumers. Therefore, this study aims to determine content of natrium benzoate and sorbic acid in snack which sold at in SDN Karang Tengah Tangerang City whether to standard of health minister number: 033 years 2012 or not.

LITERATURE REVIEW

The Benefits of Using Preservatives in Food

The addition of preservatives to food in general aims to inhibit the growth of spoilage microbes in both pathogenic and non-pathogenic food, prolong the shelf life of food, does not reduce the nutritional quality, color, taste and smell of preserved food, does not hide the state of the food. which are of low quality, are not used to hide the use of materials that one does not meet the requirements, and are not used to hide food damage (Wisnu, 2006). Food preservation is defined as the pro- cesses or techniques undertaken in order to maintain internal and external factors which may cause food spoil- age (Amit et al., 2017). Food preservation is one of the oldest varieties of technology used by humans; different forms and means of preservation were found and perfected for this purpose (Mpountoukas et al., 2008; Bruna et al. 2018). Preservatives may be natural (salt and sugar) or chemical, and this is the most effective type in preservation for longer periods (Martyn et al., 2013; Pongsavee, 2015).

The Impact of Benzoic Acid and Natrium Benzoate Preservatives on Health: Excessive consumption of benzoic acid and natrium benzoate can cause stomach cramps, a numbness in the mouth for tired people. These preservatives exacerbate the situation as well as accumulative in nature which can cause cancer in the long term and there are also reports showing that these preservatives can damage the nervous system (Awang, 2003). Natrium benzoate is a preservative. As a food additive, natrium benzoate has the E number E211. It is bacteriostatic and fungistatic under acidic conditions (Pongsavee, 2015).

Benzoic acid metabolism in the body includes two stages of the reaction, the first catalyzed by the enzyme syntetase and the second reaction catalyzed by the enzyme acytransferase. The use of preservatives such as benzoate and sorbate has become more important to control microbial growth and extend the shelf life of foods (El-Ziney, 2009; Amirpour et al., 2015). The hippuric acid is tested in the liver, then excreted in the urine (Piper & Piper, 2017). So, in the body there is no accumulation of benzoic acid, the remaining benzoic acid, which is not excreted as hippuric acid, is removed from its toxicity in conjunction with glucoronic acid and excreted in urine. Patients with asthma and people suffering from urticaria are very sensitive to benzoic acid, if consumed in large quantities it will irritate the stomach (Gunawan & Gan, 2007)

High Pressure Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) developed during the 1960s as a direct offshoot of classic column liquid chromatography through improvements in the technology of columns and instrumental components (pumps, injection valves, and detectors) (Reuhs & Rounds, 1984). Chromatography is a general term used for various separation techniques based on the partition of the sample between a motion which can be a gas or a liquid and a stationary phase which can also be a liquid or a solid. According Amirpour et al., (2015) currently, the method most commonly used in the food industry is high performance liquid chromatography (HPLC). But while chromatographic methods are not considered specific enough for unambiguous identification, chromatographic retention time is often used as a second confirmatory identification test (Parente, 2020).

High Pressure Liquid Chromatography (HPLC) is a chemical and physicochemical method. HPLC is the newest analysis method, namely a chromatography technique with a liquid mobile phase and a liquid or solid stationary phase. HPLC can be applied to the analysis of any compound with solubility in a liquid that can be used as the

mobile phase (Reuhs & Rounds, 1984; Day & Underwood, 2002).

The Advantages of Using HPLC

Capable of separating molecules from a mixture, easy to implement, high speed of analysis and sensitivity, avoidance of decomposition or deterioration of the analyzed material, good resolution, can be used in various detectors, columns can be reused, and easy to recover sample (Day & Underwood, 2002). According Dong, (2013) amenable to diverse analyte or sample types, precise and highly reproducible quantitative analysis, flexible, customizable, automated operation and high separation power with sensitive detection.

Essential components of HPLC

The components of High Performance Liquid Chromatography (HPLC) can be seen in Figure 1, consists of:

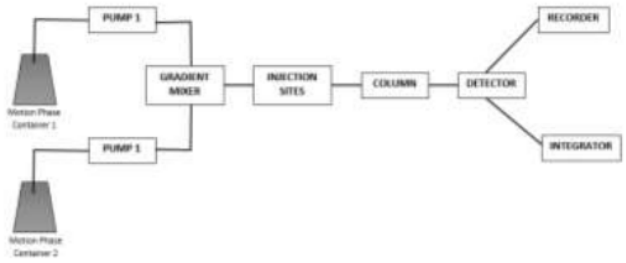

Fig.1: High Level Liquid Chromatogration Components

METHOD

Research Tools and Materials

Research Tools: HPLC consisting of an HPLC pump, column C18, detector, UV-VIS, recorder and integrator (Shimadzu), analytical balance (Mettle Toledo), ultrasonic mixer (Branson 2510), 0.45 μm filter filter (Whattman), pH meter (Metrohm), and glassware such as beaker glass, measuring flask, measuring pipette, measuring cup, and others.

Materials: The materials used in this study consists of: Sorbic acid, natrium benzoate ($C_7H_5O_2Na$), potassium hydrogen phosphate (KH_2PO_4), phosphoric acid (H_3PO_4), and 5 types of drinks sold at 11 SDN Karang Tengah Kota Tangerang.

Beverages Sampling: The sampling process for packaged drinks is carried out based on the brands circulating in the market in the Tangerang City area. Five packaged drinks were selected to be sampled in this study. The sample selection is based on the information on the ingredients that are added to the sample.

Mobile Phase Preparation: Phosphate buffer preparation pH 6.8 was carried out by weighing 2.5 grams of solid KH_2PO_4 in a weighing bottle. The two solids were then dissolved with aquabides in a 1 L flask to the right extent and shaken by an ultrasonic stirrer for about 20 minutes to remove the dissolved gases.

Standard Solution Preparation" Natrium Benzoic and Sorbic Acid Standard Solution Preparation

Weigh precisely 0.0300 g of natrium benzoate and 0.0300 g of sorbic acid. Put in a 100 ml volumetric flask, add 20 ml methanol then dilute with aquabides to 100 ml shake until homogeneous (Bui & Cooper, 1987; Javanmardi et al., 2014).

HPLC Instrument Preparation

First, the HPLC instrument was prepared by cleaning the HPLC column to remove any residual eluent that was still present in the column. After cleaning, it is followed by re-injection of the eluent or mobile phase for approximately 40 minutes until a flat baseline detector and baseline pump are obtained, indicating that the system is stable and the HPLC instrument is ready for analysis use.

Chromatograph Conditioning

The chromatographic conditions are adjusted in such a way by varying several parameters to get the optimum condition for the separation of the two preservatives natrium benzoate and sorbic acid.

Sample Testing

Qualitative Analysis

Qualitative analysis of the samples was carried out by comparing the results of the retention time analysis of each packaged drink sample to be tested with the retention of standard solutions of natrium benzoate and sorbic acid.

Quantitative Analysis

Each sample to be tested for the concentration level, is weighed as much as 1 gram and dissolved with 10 ml methanol solvent then stir well and homogeneously then put in a 50 ml volumetric flask, add distilled water to mark the line. Shake the solution for about 10 minutes, then each sample that has been dissolved is then filtered with Whatman filter paper no. 42 To remove other unwanted solids so as not to affect the chromatography column, the filter results are filtered again with a C-18 cartridge filter. The solution is ready for injection in HPLC.

After that, each sample was injected into the HPLC system and the peak area of natrium benzoate and sorbic acid was measured and then calculated using the regression line equation from the linearity curve to determine the levels of natrium benzoate and sorbic acid contained in each sample.

RESULT AND DISCUSSION

Each standard solution and test solution were injected into HPLC using the developed chromatography system. Then each chromatogram of standard solution and the test retention time was observed. The retention time of the standard solution chromatogram should be the same as the retention time of the test solution chromatogram. The value of the correlation coefficient (r) is an indicator of the quality of the linearity parameter which describes the proportionality of the analytic response (area) to the concentration being measured.

Based on the evaluation data of the standard series calibration of natrium benzoate at a concentration of 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 μg / ml, obtained a linear relationship with the correlation coefficient, r = 0.999293 and the linear regression equation y = 14734.3 * x + 1718.98. The coefficient value obtained shows good results because it is close to the value 1. This indicates that there is a proportional relationship between the analytical response and the measured concentration. From the linearity test results of the natrium benzoate calibration curve it can be seen in the Figure 2 below:

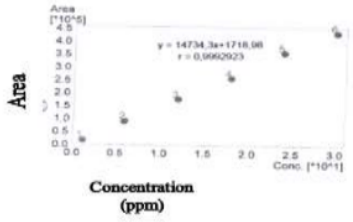

Fig.2: Natrium benzoate calibration curve

The sorbic acid calibration curve was determined based on the area at concentration level of 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 μg / ml, obtained a linear relationship with the correlation coefficient, r = 0.9990453 and the linear regression equation Y = 23907.9 * + 5308.94. The coefficient value obtained shows good results because it is close to the value 1. This indicates that there is a proportional relationship between the analytical response and the measured concentration. From the results of the linearity test, the sorbic acid calibration curve can be seen in the Figure 3 below:

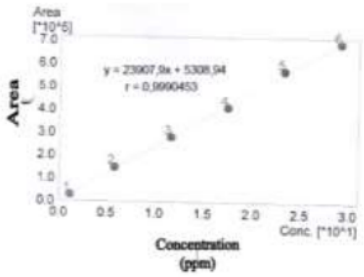

Fig.3: Sorbic acid calibration curve

Testing of additives in a product, especially food, is important in order to ensure the safety of product use. This analysis must yield correct data regarding the analyte content in the product. In addition, the method used in testing must be reliable so that it can guarantee the accuracy of the data obtained (Betz et al., 2011).

The results of the analysis of natrium benzoate and sorbic acid on labeled bottled beverage samples obtained different results for each brand. From the results of the analysis on the five samples tested showed that four samples contained natrium benzoate preservative whose results exceeded the level limit set by BPOM RI and two samples contained sorbic acid preservative but the results did not exceed the threshold set by BPOM RI.

The separation mechanism that occurs is based on the ability of the mobile phase and the sample to bind to the column (Coskun, 2016). The substance that comes out first, is the substance that is more polar than the substance which is the other, while the substance that is held out longer from the column is the more non-polar. The more polar the mobile phase, the slower the sample mooring time and the more non-polar the mobile phase, the faster the sample will come out (Merry, 2012; María José Ruiz-Ángel, 2019).

Column C18 with silica filling in which the silanol group is modified with an alkyl group, namely hydrocarbons, is widely used for the analysis of polar preservatives with a reverse phase mechanism. The interaction between the

preservative and the unprotected silanol group (stationary phase) allows the detention of the two preservatives in the column at different times according to the degree of polarity of each preservative. The mobile phase used in this study, a mixture of methanol with KH2PO4 phosphate buffer, is a polar solvent mixture . The interaction of the two preservatives can be removed from the C18 column. The role of phosphate buffer pH 6.8 as pH control for natrium benzoate and sorbic acid in the column also affects the separation conditions of the two preservatives.

Qualitative and quantitative analysis of natrium benzoate and sorbic acid using column C18 and the mobile phase in the form of a mixture of methanol: phosphate buffer KH2PO4 pH 6.8 with a ratio of 15:85 in methanol gave good separation results with a relatively short retention time of about 5 minutes. For each sample, the diplo and triplo analysis results were obtained, namely sample A 839,2145 mg / L, sample B 191,2495 mg / L, sample C 869,736 mg / L, sample D 522,013 mg / L, and sample E 226,900. mg / L chromatogram image of each sample is attached.

From this study, it was found that four samples of drinks containing natrium benzoate 0 - 869.736 mg / L from the four samples, there were two samples that exceeded the allowable threshold, namely 600 mg / L and one sample contained sorbic acid with a concentration of 0- 522.013 mg / L below the permissible threshold of 1000 mg / L. From the results of the concentration of each sample tested, it is described in the graphic image below.

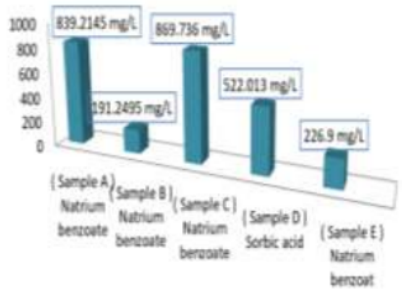

Fig.4: Graph of natrium benzoate and sorbic acid concentrations

CONCLUSION

The samples tested contained the preservative content of natrium benzoate with varying levels, namely sample A 839,2145 mg / L, sample B 191,2495 mg / L, sample C 869,736 mg / L, and sample E 226,900 mg / L and sorbic acid in sample D with a level of 522.013 mg / L. There are four packaged beverage products that use natrium benzoate as preservative and one sample uses sorbic acid. In the four samples tested, there were two samples whose levels exceeded the limits allowed by The Food and Drug Supervisory Agency (BPOM) based on Regulation of Indonesian Ministry of Health (PERMENKES RI), number 722/Menkes/Per/IX/1988.

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