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Fast and simple method for semiquantitative determination of calcium propionate in bread samples



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ABSTRACT

Calcium propionate has been widely used as a preservative in bakery and in bread. It is sometimes not carefully used, or a high concentration is added to preserve products. High consumption of calcium propionate can lead to several health problems. This study aims to develop a fast and simple semiquantitative method based on color complex formation for the determination of calcium propionate in a bread sample. A red–brown complex was obtained from the reaction of ferric ammonium sulfate and propionate anion. The product was rapidly formed and easily observed with the concentration of propionate anion >0.4 mg/mL. A high-performance liquid chromatography (HPLC) method was also developed and validated for comparison. Twenty-two bread samples from three markets near Bangkok were randomly selected and assayed for calcium propionate using the above two developed methods. The results showed that 19/22 samples contained calcium propionate >2000 mg/kg. The results of the complex formation method agreed with the HPLC method.

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1. Introduction

In the countryside areas of Thailand, some health problems come from dietary foods. Local food manufacturers do not usually know about good manufacturing practices, as well as country food laws. Calcium propionate has been widely used as a preservative in bakery and in bread. It has a

potency for preventive bacteria and fungi. Calcium propionate is listed as E number 282 in the Codex Alimentarius and is allocated an acceptable daily intake as “not specified” or “not limited” [1,2]. However, the fact that acceptable daily intake is not specified does not mean that unlimited intake is acceptable. It could, in principle, be allowed for use in foods in general with no limitation other than being in accordance with good manufacturing practices. The United

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States Food and Drug Administration recommended that the daily intake of calcium propionate for adults is 1 mg/kg/d [3]. If 1 kg of food was taken per day for a man of 50 kg body weight, the amount of calcium propionate in the food should not exceed 2000 mg/kg.

In Thailand, there were notifications of the ministry of Public Health of Thailand announced in 1984 and 1989. The allowed concentration of calcium propionate in food according to those announcements was 0.2%. Although oral calcium propionate might not cause severe toxicity, long term health effects have been reported. Propionate preservatives can contribute to or cause hyperactivity [4], visual hallucinations [5], irritability, restlessness, inattention, and sleep disturbance in some children [6–8]. Some researchers found that brief infusions of propionic acid in rats produced short bouts of behavioral, i.e., hyperactivity, object fixation, social impairments and other effects such as seizures, similar to those seen in autistic spectrum disorders [9,10].

Food safety is a key factor of good health. Unfortunately, some preservatives are illegally added or exceed regulation limits in foodstuffs. Several analytical methods have been developed for determination of these illegally used preservatives including food contaminants [11–13]. For propionic acid, the reported quantitative determination methods were gas chromatography [14] and gas chromatography/mass spectrometry [15,16]. Although these methods have been accepted for their accurate and precise results, but they still have some drawbacks such as requiring expensive instruments and well-trained analysts. Moreover, toxic organic solvent waste was also produced. In this study, a simple and cheap method of calcium propionate investigation in bread products based on the colorimetric method was developed. The developed colorimetric method was successfully applied to determine calcium propionate in bread products and the results were in agreement with high-performance liquid chromatography (HPLC). This indicated the accuracy of the colorimetric method.

2. Materials and methods

2.1. Preparation of standard solution of calcium propionate

About 200 mg of calcium propionate was accurately weighted into a 100-mL volumetric flask, then dissolved and diluted with deionized water. Further dilution of this stock solution was employed with deionized water to obtain a standard solution of 0.6 mg/mL and 0.4 mg/mL.

2.2. Preparation of ferric ammonium sulfate test solution

About 8.0 g of ferric ammonium sulfate was dissolved and made up to 100 mL with deionized water.

2.3. Bread sample preparation

A sample was cut into small pieces and 10 g weighed accurately into a 250-mL beaker. Some 100 mL of deionized water

was added, and the mixture was left standing for 2 hours before filtering. The filtrate was used to test for calcium propionate in the colorimetric method. For the HPLC assay, the filtrate was further diluted 10 times with a mobile phase before injection.

2.4. Testing for calcium propionate

Some 1.0 mL of 0.6 mg/mL calcium propionate standard solution was added to a 1.0-mL bread sample preparation. The color of the reaction was monitored after a few drops of ferric ammonium sulfate test solution (TS) was added. The color of the reaction tube was compared with the standard preparation tube and blank tube. Standard preparation was performed by adding a few drops of ferric ammonium sulfate TS to 2.0 mL of 0.4 mg/mL of calcium propionate. The blank tube was performed by adding a few drops of ferric ammonium sulfate TS to 2.0 mL of deionized water. A red–brown color complex was formed indicating the presence of calcium propionate. For the sample containing calcium propionate < 2000 mg/kg, the color was weaker than that of the standard tube, but stronger than that of the blank tube.

2.5. HPLC condition

Quantitative determination of calcium propionate in bread samples was employed using Shimadzu model LC-10 AD (Shimadzu Corporation, Kyoto, Japan) equipped with an SPD-10A UV detector. A symmetry C18 HPLC column (4.6 mm × 150 mm, 5 μm) was utilized. A mixture of 0.05% phosphoric acid and methanol with the ratio of 90 and 10 by volume was the successively mobile phase. The flow rate was 1.0 mL/min and the presence of calcium propionate was monitored at 210 nm.

2.6. HPLC method validation

Method validation parameters, i.e., linearity, accuracy precision, and specificity were evaluated. Linearity was performed by using a series concentration of standard solutions in the range of 25.0–250.0 μg/mL. The concentrations (X-axis) were then plotted with their corresponding peak areas (Y-axis). Linear least square regression was used to calculate the correlation coefficient (*r*).

Accuracy of the HPLC method was studied with standard addition approach. Three concentrations, low, middle, and high, of standard calcium propionate covering the linearity range were spiked into equal amounts of bread samples. Three replicates were performed for each concentration level. Accuracy of the method was expressed as recovery percent of standard found from the sample.

Precision was evaluated with three concentrations of the desired compound covering linearity range and three replicate samples for each concentration. Precision was expressed as relative standard deviation percent (% RSD) of replicate samples of each concentration.

Specificity of the HPLC method was obtained by comparing the chromatograms of the sample with the standard and standard spiked-sample. Retention times of calcium propionate in the sample and standard spiked-sample might equal that of the calcium propionate standard.

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