Quantitative Determination of Vanillin in Coated Paper Using Gas Chromatography

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Abstract. One of the strategies to improve the food shelf – life is to utilize functional packaging. The functional packaging could contain antimicrobial agents to inhibit pathogenic microorganisms or antioxidants to prevent oxidative degradation. Vanillin, known as one of the most common food flavoring ingredients, could serve as a potent antioxidant. This paper focuses on the development of a methodology to determine vanillin content in the coated paper using gas chromatography. The vanillin was extracted from vanillin-coated paper using water as the aqueous phase and dichloromethane (DCM) as the organic phase. The effect of filtration on vanillin extraction and water to DCM ratio used for extraction were investigated. It was concluded that the extraction without filtration with water to DCM ratio of 1:2 was the best setting for the vanillin extraction from vanillin-coated paper.

1. Introduction

Paper is commonly used for packaging material of food products [1]. To prevent microbial growth on the product surface and food spoilage and increase the shelf life of food products, many antimicrobial substances are coated either directly on food surfaces or into the food packaging materials [17]. However, sometimes these substances immobilized on the surface of the packaging material could migrate into the food products, thus leading to unacceptable product quality [2-4]. Therefore, the antimicrobial compounds used for food packaging or even coming into human contact should be safe and correspond to the regulatory concern. As a result, the industry is searching for a natural compound that has antimicrobial with abilities. There are some studies in the literature showing that polyphenols have antimicrobial activity. Vanillin is a familiar flavour and has shown to be a potential antimicrobial agent due to the effect of the phenolic compound that prevents bacteria, yeasts, and molds to grow [1, 5]. The antimicrobial property of vanillin is the effect of the phenolic group in chemical structure. One of the most promising possibilities of stabilizing aromatics such as vanillin is their inclusion complex formation (molecular encapsulation) with β-cyclodextrin as a powder-like crystalline, which made its industrial utilization possible. However, the main applications of the microencapsulated vanillin powder are as food additives [6].

One other potential application is as a coating agent for the production of aroma paper [7, 8]. Despite vanillin’s advantages as an antimicrobial agent in the packaging material for the enhancement of food’s shelf-life, no study has reported the use of vanillin in the paper packaging or materials [1, 9].
Furthermore, methods for extraction and identification of vanillin from the coated paper is absent. Consequently, there are still industrial challenges with standardized methods for the extraction and determination of vanillin from coated paper [1, 10]. Thus, this paper focuses on the methodology developments for quantifying vanillin content in vanillin coated paper using Gas Chromatography (GC).

2. Experimental Section

2.1. Liquid – liquid extraction
For liquid-liquid extraction, 1 g of commercial vanillin (obtained from PT Mane Indonesia) and 0.05 g of ethyl vanillin (obtained from PT Mane Indonesia) were completely dissolved in 15 mL of deionized water at 100 °C and 300 rpm during 10 minutes. In the single-step extraction, the solution were cooled down, and mixed with 10 mL of dichloromethane (DCM). and the mixture was stirred for 10 minutes at 300 rpm. The aqueous phase (water) and the organic phase (DCM) were separated using the decantation method. The organic phase was then centrifuged at 4000 rpm for 5 minutes and filtered before injection to GC.

In the multi-step extraction, the solution was cooled down, then 5 mL of DCM was added to the solution, and the mixture was stirred for 10 minutes. The aqueous phase (water) and the organic phase (DCM) were separated using the decantation method, while the organic phase was centrifuged at 4000 rpm for 5 minutes. The supernatant was filtered to remove solid impurities and 0.5 mL of the aliquot was then mixed with ethyl vanillin solution. In the next step of extraction, another 5 mL of DCM was added to the aqueous phase and the extraction, recovery, and sample preparation for analysis as described previously were repeated. The extraction steps were repeated five times.

2.2. Vanillin coated paper sample preparation
An A4 size paper was soaked in 3.5 % commercial vanillin solution for one minute. Excess water was removed by using blotting paper. The paper was then dried at 60°C for two minutes.

2.3. Effect of filtration on vanillin extraction method from coated paper
Approximately 1 g of coated paper and 0.05 g of ethyl vanillin were added into 15 mL of deionized water. The sample was heated at 100 °C and stirred at 300 rpm for 10 minutes. The sample was then left to cool down. After cooling, both vanillin and ethyl vanillin were extracted from the sample using 10 mL of DCM by manually stirring the mixture for 2 minutes. The organic phase was then recovered according to the method described in section 2.1.

In another experiment, a different approach was taken, in which after the sample was cooled down, the aqueous phase was recovered by filtration first before adding 10 mL of DCM to the aqueous phase. The mixture was then stirred for 2 min and the DCM was recovered according to the method described in section 2.1.

2.4 Effect of water to DCM ratio on vanillin extraction method from coated paper
The extraction was carried out using the protocol described in section 2.3 without filtration. To obtain water to DCM ratio of 1:2, the amount of DCM added was 30 mL instead of 10 mL.

2.5 Analysis of vanillin by GC – FID
Before injection, all samples were filtered using a Minisart membrane filter with 0.2 μm pore size. The analysis was performed using gas chromatography (GC) (Thermo Scientific™ TRACE™ 1300) with an FID detector. The capillary column TG-5MS column (Thermo Scientific) with the dimension of 30 m x 0.25 mm and 0.25 μm film thickness was used. The oven temperature started at 150°C kept for 1 minute, and then increased to 210 °C at the rate of 15°C/min for 2 min. The injector temperature were maintained at 290 °C and the detector temperature at 325 °C. 0.8 μL of the sample was injected in a split mode with the split ratio of 75. Nitrogen gas was maintained at 40 mL/min, while air and hydrogen were
maintained at 350 mL/min and 35 mL/min, respectively. Helium gas was used as carrier gas at a flow rate of 1 mL/min.

Pure vanillin (analytical grade, Merck) was used for calibration. The pure vanillin was dissolved in DCM (analytical grade) at the concentration ranging from 0.05% - 0.25% w/v. In addition, the sample solution contained 0.25% w/v ethyl vanillin (analytical grade) as an internal standard.

2.6. Statistical analysis
In this study, all experiments were carried out in replicates. The data were analysed using the statistical tool of Excel 2016, MS Office. The graphs were plotted using mean values and the standard deviation is shown as error bars.

3. Result and Discussion

3.1. Extraction of vanillin from commercial vanillin powder
As one of the most common flavouring agent in the food industry, vanillin is commonly used in food packaging in a small amount. Vanillin is usually supplied to the market in the form of powder, containing carriers such as maltodextrin. The carrier is used to improve the property of the powder such as flowability, and to increase the yield during spray drying. Although maltodextrin is highly soluble in water, it has minimal solubility in DCM. Therefore, for quantification of vanillin using GC, it was necessary to release the entrapped vanillin from the powder by dissolving the powder in water. Afterward, the released vanillin was extracted from the aqueous phase using DCM. Preliminary experiments were conducted to determine the optimum time for extraction and the total content of the vanillin in the powder. It was concluded that 10 minutes of mixing was enough for vanillin to diffuse to DCM.

Figure 1 shows the vanillin content detected using single-step extraction and multiple-step extraction. In the single-step extraction, 8.23±0.06% vanillin in commercial powder was reported. Meanwhile, a higher vanillin content of 8.57±1.11% was reported in multi-step extraction. The result of a one-way analysis of variance indicated that both methods gave a similar result with a 95% confidence level. Although multi-step extraction could extract more vanillin, the standard deviation was relatively larger due to the volatility of the solvent and difference in solvent recovery in each stage of extraction.

![Figure 1. Comparison of single- step and 5- steps vanillin extraction from commercial powder](image-url)
3.2. Extraction of vanillin from coated paper
As demonstrated in section 3.1. using commercial vanillin for the vanillin extraction, the single-step vanillin extraction gave a smaller variability. Hence, the single-step extraction was adapted for the extraction of vanillin from coated paper. The result is presented in Figure 2. The vanillin content detected was 0.17±0.01%. A challenge occurred during the dispersion of paper in water, in which the paper disintegrated into fibers. The dispersion of fibers in water modified the consistency of the suspension such that the suspension had a slurry-like consistency. The consequence of this was an increase in energy required for mixing to ensure that the vanillin was completely extracted. In addition, the difficulty of organic phase recovery at a later stage was higher. To solve this problem, the fibers were separated from the slurry before DCM was added. This gave rise to a lower vanillin recovery, with vanillin content of 0.14 ± 0.00% (Figure 2). Furthermore, two other methods were also investigated. First, increasing the extraction step (data not shown), and second by increasing the water to DCM ratio, while maintaining the volume of the water constant. The result showed that changing the water to DCM ratio from 3:2 to 1:2 while maintaining the water volume constant gave the best result in terms of the vanillin extracted and the lowest variability, with the vanillin content from 1:2 ratio was 0.30 ± 0.00% (Figure 3).

![Figure 2. Effect of adding filtration step in single-step vanillin extraction. Method A: the paper was dispersed in the water before DCM addition. Method B: the recovered filtrate after dispersing the paper in the water mixed with DCM.](image2)

![Figure 3. Effect of water to DCM ratio.](image3)
A higher amount of organic solvent is needed in the extraction from coated paper compared to that of commercial vanillin powder. It was speculated that the fibers hindered the diffusivity of vanillin to both water and DCM. The presence of hydroxyl groups in cellulose fiber renders the paper hydrophilic. As such, the paper is easily wetted and the fiber will disperse in water. However, the same functional group is responsible for inter and intra-chain bonds in the fiber. As such, the paper will only dissolve in certain solvents, of which water and DCM do not belong to [11]. The implication to the vanillin extraction process from the coated paper was a three-phase extraction process. Solute recovery using liquid-liquid extraction is an already established method with the effect of most factors known. However, when the solute is dispersed in a heterogeneous matrix-like fiber in water, the complexity for modeling and prediction rises exponentially, as both the structure of the solid phase and the interaction between the solid phase and the solute affect the diffusivity of the solute to the solvent [12-14].

4. Conclusion
The goal of the experiment was to establish the protocol for extraction and quantification of vanillin from the coated paper. A modification was needed in terms of water to DCM ratio in which the ratio of 1:2 was concluded to be the best for the vanillin extraction process from paper. The protocol could be useful to consistently recover and quantify vanillin from coated paper. Thus, the method would be appropriate for sampling and quality control. Future work includes modification of protocol for faster analysis.

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