

# Processing of Vanilla Pods Grown in Thailand and Its Application

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## Abstract

*This research aimed to determine the method for processing of vanilla pods grown in Thailand. The three methods (different in killing step) used were KW, BB and MB. KW was the method of the Royal Project, Khun Wang Center, which was based on soaking of green vanilla pods in hot water (80 °C) for 1 h, whereas BB and MB were based on soaking in 63-65 °C water for 4 min and soaking in 80 °C water for 10 sec 3 times with 30 sec interval, respectively. Sweating was done by sun-drying from 11.00 am to 2.00 pm for 7 days. The pods were then slowly dried in shed for 30 days. The last step was the conditioning step for which the pods were kept in an air tight container for 3 months. Vanillin contents of these pods were significantly different ( $p < 0.05$ ). The vanillin contents of BB and MB were approximately 2.5%, while that of KW was 2.3%. The application of Thai vanilla pods in butter cake was then studied in comparison with synthetic vanilla. Butter cake using BB pods had the highest score in all attributes. The use of natural vanilla pod decreased the butter flavor intensity, resulting in mellowing of the butter cake flavor. The product also had higher volume and higher consumer acceptance (59%) than that of synthetic vanilla. In addition, the antimicrobial properties of Thai vanilla pod were investigated. Crude vanilla solutions were extracted using different concentrations of ethanol, with a 1:1 ratio of vanilla pod to solvent; and then tested for antimicrobial properties against *E. coli*, *B. cereus*, *S. aureus*, *L. monocytogenes* and *Sal. typhimurium* using disc diffusion method. Standard vanillin solutions were also investigated. *E. coli* was the most resistant microorganism to all crude vanilla extracts, whereas, *L. monocytogenes* and *Sal. typhimurium* were the most sensitive. In addition, antimicrobial properties of standard vanillin (120 mM) were not found in this research. The crude extracts from BB and MB had better antimicrobial effect than that of KW when they were extracted by using 75% ethanol.*

**Keywords:** Royal Project, vanillin, antimicrobial properties, butter cake, flavor.

## 1. Introduction

Vanilla (*Vanilla planifolia* L.) is an economic plant with a high market demand. It was originated in the tropical area in Mexico and Central America and also cultivated in the forest around South America (Moorthy and Moorthy 2002; ACD 2000). In Thailand, the natural vanilla is only imported despite the high prices due to a lack of vanilla plantations. The Royal Project has foreseen the economical importance of this plant; therefore, an

experimental plantation of vanilla has been set up at Khun Wang, Chiang Mai, where the climate is suitable for its growth. The Royal Project also encourages the local farmers to grow this plant. There are many processes of vanilla pods after harvesting which affect the quality of vanilla pods (Anon. 2005). These processes, such as Mexico, Indian Ocean and Modified Indian Ocean methods, are mainly different in a killing step, the process that uses heat treatment to activate enzymes in the vanilla pods, which affects the development of vanillin during the curing process.

Apart from using natural vanilla as the flavoring agent in food products, such as butter cake and ice cream, natural vanilla pods contain natural antimicrobial substances, such as vanillin, p-hydroxybenzaldehyde, p-hydroxybenzyl methyl ether and other trace constituents, which are developed during fermentation of vanilla pods (Kazer 2003). Rupasinghe *et al.* (2006) reported that the growth of bacteria, yeast and mold, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Salmonella enterica* subsp. *enterica* serovar, *Candida albicans*, *Lactobacillus casei*, *Penicillium expansum* and *Saccharomyces cerevisiae*, were inhibited with the minimal inhibitory concentration (MIC) of 6-18 mM. They also demonstrated that with a concentration of 12 mM, the total microorganism count was reduced by 37-66% in a fresh-cut apple. Moreover, Fitzgerald *et al.* (2002) reported that vanillin in vanilla pod could inhibit the growth of yeasts, *S. cerevisiae*, *Zygosaccharomyces bailii* and *Z. rouxii* at the MIC of 21, 20 and 30 mM, respectively. Vanillin concentrations of 15, 35 and 75 mM also reduced the growth of *E. coli*, *L. plantarum* and *Listeria innocua*, respectively (Fitzgerald *et al.* 2004), which was similar to the result obtained by Corte *et al.* (2004) and Delaquis *et al.* (2004). Moreover, Fitzgerald *et al.* (2005) demonstrated the inhibitory effect of vanillin against the spoilage mold and yeast at the MIC of 5.71 mM and they found that the aldehyde structure of vanillin played the important role. Furthermore, the growth of *L. monocytogenes* and *E. coli* O157:H7 at pH 3.5-4.5 in model apple juice (MAJ) was also inhibited (Moon *et al.* 2006).

Figueiredo *et al.* (2008) reported that phenolic aldehydes, sinap-aldehyde, coniferaldehyde, p-hydroxybenz-aldehyde, 3,4-dihydroxybenzaldehyde and 3,4,5-trihydroxybenzaldehyde found in vanilla pods inhibited the growth of *Oenococcus oeni*.

Therefore, this research aimed to study the suitable processing method of vanilla pods, including the application of vanilla pods in butter cake, in comparison with synthetic vanilla used in the food industry. Simultaneously, the antimicrobial properties of

natural vanilla pods produced in Thailand were also investigated.

## 2. Materials and Methods

### 2.1 Processing of Vanilla Pods

Fully mature green vanilla pods, obtained from the Royal Project Khun Wang Center, Chiang Mai, were graded based on the length of the pods as grade A (>15 cm), B (10-15 cm) and C (<10 cm); and then cleaned with tamarind juice. The pods were divided into 3 parts for three different processing methods (different killing step) as KW, BB and MB. KW was the method of the Royal Project, Khun Wang Center, which was based on soaking of green vanilla pods in hot water (80°C) for 1 h, whereas BB and MB were based on soaking in 63-65°C water for 4 min and 80°C water for 10 sec three times with 30 sec interval, respectively. The pods were then wrapped with woolen fabric and stored in a wooden box lined with woolen fabric for 24 h. The following day the pods were sun dried from 11.00 am to 2.00 pm, wrapped with the woolen fabric and placed under the sun for 1 h before to be stored back in the wooden box. This process was repeated for 7 days. The pods were then dried under the shade for 35 days in a well ventilated room at 35°C and 75% relative humidity. In the end, the pods were made tight in a 250 g bundle, packed in the wooden box for 3 months at 35°C and then stored in an airtight container for further study.

### 2.2 Analysis of Vanillin

The vanilla pods produced by using KW, BB and MB were analyzed for vanillin contents using AOAC 966.12 (2000).

### 2.3 Application of Vanilla Pods in Butter Cake in Comparison with Synthetic Vanillin

**2.3.1 Production of butter cake:** Butter cakes were produced by using natural vanilla pods (KW, BB and MB) and synthetic vanilla (liquid and powder). The formula used was 120 g butter, 200 g ground sugar, 3 g salt, 13 g

skim milk powder, 2 eggs, 120 g water, 200 g cake flour, 8 g baking powder and 2 g vanilla. The vanilla pods were ground into powder before to be used.

**2.3.2 Sensory evaluation:** Butter cakes produced as described in Section 2.3.1 were screened by using 9-point hedonic scale and 30 panelists for either natural vanilla or synthetic vanilla. The chosen vanillas of each type were then compared using descriptive analysis and 8 trained panelists. The consensus terms of flavor were also defined (Vara-Ubol *et al.* 2004; Matta *et al.* 2005; Chambers *et al.* 2006). The panelists were trained until the standard deviation for scores of each attribute was less than 1.5 before testing the sample.

**2.3.3 Consumer acceptance:** The consumer acceptance was conducted in eight public places, four places in Bangkok and four places in suburbs of Bangkok. Based on the method of Cochran (1963), 400 consumers who like butter cake were voluntarily selected without compensation. They were asked to answer a questionnaire and scored the products based on their preferences.

## 2.4 Antimicrobial Properties of Crude Vanilla Extract

**2.4.1 Preparation of crude vanilla extract:** Ground natural vanilla pods were extracted by using 0, 25, 50, 75 and 100% ethanol, with a 1:1 ratio of vanilla pod to alcohol. The mixtures were then placed on an orbital shaker at 150 rpm for 24 h, and centrifuged at 4,000 rpm. The ethanol was evaporated from the supernatants at 45°C. The remaining solutions were filter sterilized by using a cellulose acetate filter with a pore size of 0.22  $\mu\text{m}$  before antimicrobial testing.

**2.4.2 Preparation of culture:** There were five microorganisms used, *Sal. typhimurium*, *E. coli*, *B. cereus*, *S. aureus* and *L. monocytogenes* V7. All cultures were propagated in nutrient broth except for *L. monocytogenes*, which was enumerated in trypticase soy agar containing 0.6% yeast

extract (TSAYE). The cultures were incubated at 37°C for 18 h and then diluted to obtain the same concentration (0.5 A) used before.

**2.4.3 Antimicrobial testing:** The antimicrobial testing was conducted by using disc diffusion method. The 0.6 mm sterile discs were immersed in the crude vanilla extracts and then placed on agar plates that had previously been inoculated with the testing cultures. The sterile standard vanillin (99.9%) (Aldrich, Germany), at the concentration of 5, 10, 15, 20, 40, 60, 80, 100 and 120 mM, was also tested. The sterile distilled water was used as a control. The inhibition zones were measured after incubation at 37°C for 24 h.

## 2.5 Statistical Analysis

A randomized block design with three replications was used. The mean differences were analyzed by using Duncan's Multiple Range Test.

# 3. Results and Discussion

## 3.1 Processing of Vanilla Pods

There were three processing methods used, KW, BB and MB. After the conditioning step, the vanilla pods were analyzed for vanillin contents. It was recognized that the processing methods had significantly ( $p < 0.05$ ) affected the vanillin contents of the products (see Table 1). The vanillin content of KW was 2.3%, which was lower than those of BB and MB (2.5 and 2.6%, respectively); indicating that the heat treatment of green vanilla pods in the killing step played the important role. The high heat intensity used in KW (80°C for 1 h) might possibly destroy enzymes necessary for vanillin development of the further process, whereas the low heat intensity used in BB and MB did not only inhibit the physical changes of vanilla pods, but also enhanced the enzymatic reactions responsible for the vanillin development.

Table 1. Vanillin contents of vanilla pods produced by using different methods.

Method	Vanillin content (%)
KW	2.3 <sup>b*</sup>
BB	2.6 <sup>a</sup>
MB	2.5 <sup>a</sup>

\*Values followed by the same letters are not significantly different ( $p > 0.05$ ).

### 3.2 Application of Vanilla Pods in Butter Cake in Comparison with Synthetic Vanilla

**3.2.1 Sensory quality of butter cake:** The sensory quality of butter cakes produced from natural and synthetic vanillas was evaluated using 30 panelists and 9-point hedonic scale. It was noticed that between synthetic vanilla given as liquid or powder, butter cake produced by using vanilla powder had significantly ( $p < 0.05$ ) higher overall liking score than that of liquid vanilla in all attributes, except for texture (see Fig. 1). The scores of color, flavor, vanilla flavor and overall liking of butter cake produced by using vanilla powder were 7.5, 7.1, 7.4 and 7.3, respectively. This might be possible due to the dark color of liquid vanilla, resulting in the dark color of the product. Moreover, the butter cake produced by using vanilla powder had a stronger vanilla flavor than that of the liquid one, which might be caused by the presence of a solvent in the liquid vanilla, leading to a low concentration of vanillin content.

For natural vanilla, it was recognized that although the sensory quality of butter cakes produced by using three types of natural vanilla pods (see Fig. 2) was not significantly different ( $p > 0.05$ ), the butter cake that was made from BB had slightly higher scores than the others. This might be possible due to the observation that the panelists were unfamiliar with the butter cake produced by using natural vanilla. The natural vanilla pods sold in Thailand were mainly imported despite the high prices, therefore, only some of the consumers were familiar with the butter cake produced by using this vanilla. In addition, most of the butter cakes in Thailand were produced by using synthetic vanilla, which

was a lot cheaper than the natural one. Consequently, most of the panelists could not differentiate the differences among vanilla pods produced by using different methods. However, the BB vanilla pod was chosen for the further experiments due to the highest score of preference.

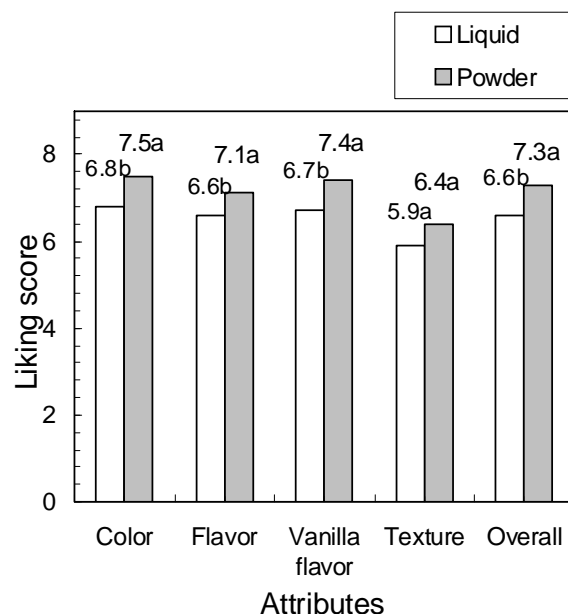


Fig. 1. Sensory quality of butter cakes produced by using synthetic vanilla.

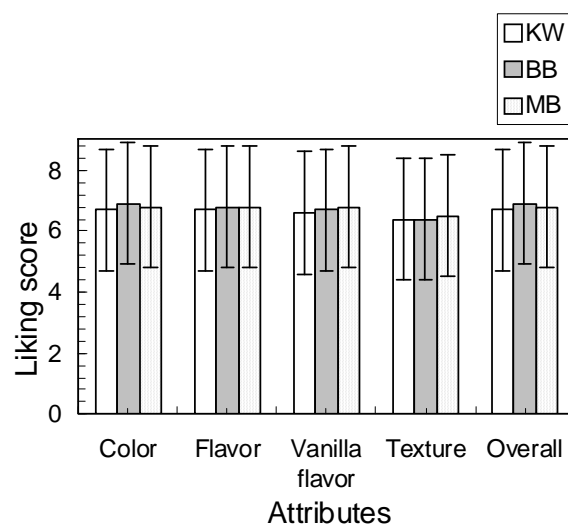


Fig. 2. Sensory quality of butter cakes produced by using three types of natural vanilla pods.

**3.2.2 Flavor profile:** Descriptive analysis with 8 trained panelists was used for the construction of a flavor profile (see Fig. 3). Several consensus terms of butter cake flavor were defined, such as butter flavor, egg flavor,

milky, sweetness and saltiness. The use of natural vanilla pods in the butter cake significantly ( $p < 0.05$ ) reduced the butter flavor intensity, resulting in mellowing of the butter cake flavor. The butter flavor intensity of vanilla pods was 9.6, whereas that of synthetic vanilla was 7.4.

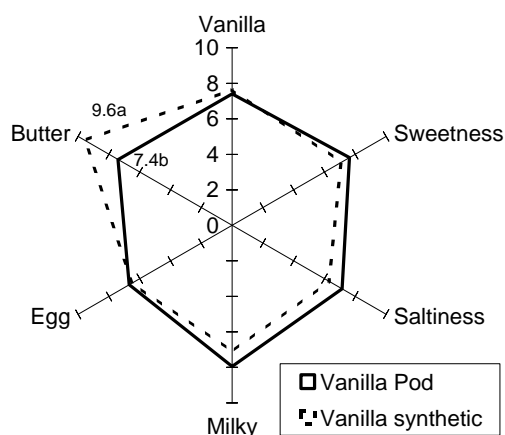


Fig. 3. Flavor profile of butter cake flavor produced by using natural and synthetic vanilla.

**3.2.3 Consumer acceptance:** The consumer acceptance of butter cakes produced by using natural and synthetic vanilla was conducted using four hundreds consumers (54% male and 46% female) in Bangkok and suburbs of Bangkok. Most of the consumers were in the age of 21-25 years (52%) with a bachelor degree of education (67%), from which 67% were students. It was noticed that the butter cake made from natural vanilla had significantly ( $p < 0.05$ ) higher liking scores than that of synthetic vanilla in all attributes, except for texture, which was similar in both cases (see Fig. 4). This might be caused by other compounds in vanilla pods, resulting in balancing of product flavor, whereas, only vanillin was found in synthetic vanilla powder. Moreover, 59% of the consumers preferred the butter cake made from natural vanilla pods, simultaneously, 41% preferred the butter cake made from synthetic vanilla, which was possibly due to the observation that the consumers were unfamiliar with the natural

vanilla pods in the butter cake (see Fig. 5). The price of the natural vanilla is also high, leading to its limited use in premium products only.

In addition, the volume of the butter cake produced by using natural vanilla was significantly ( $p < 0.05$ ) higher (4.0 cm) than that of synthetic vanilla (3.4 cm) (see Fig. 6). The pieces of natural vanilla pods might interrupt the structure of the butter cake, resulting in discontinuous and larger air cells in the cake structure than that of synthetic vanilla.

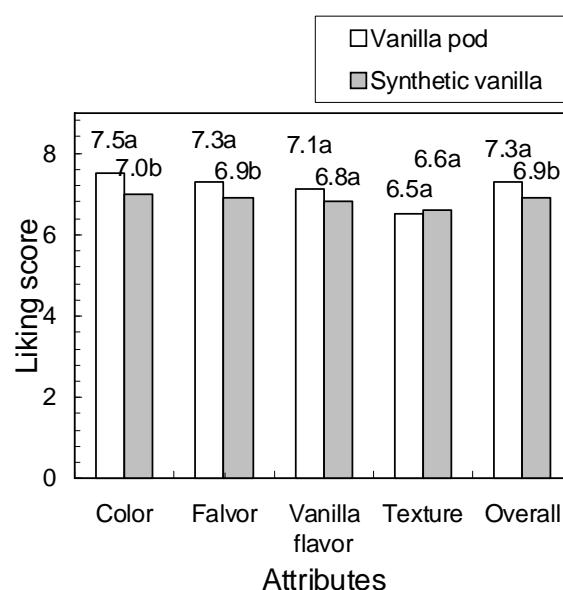


Fig. 4. Sensory quality of butter cakes made from natural and synthetic vanilla by 400 consumers.

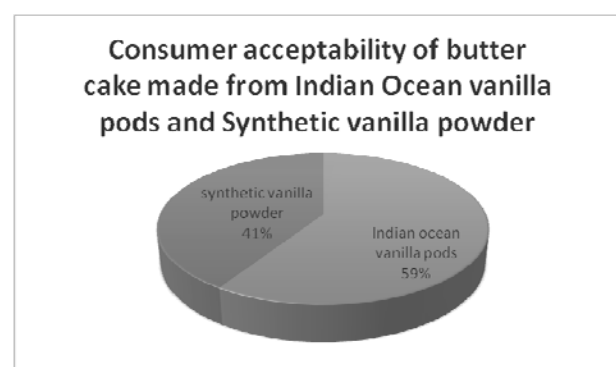


Fig. 5. Consumer acceptance on butter cakes made from natural and synthetic vanilla by 400 consumers.



Fig. 6. Volume of butter cakes made from natural and synthetic vanilla.

### 3.3 Antimicrobial Properties of Crude Vanilla Extracts

Crude vanilla extracts were produced by using ethanol extraction at 0, 25, 50, 75 and 100% and tested for antimicrobial properties against *Sal. typhimurium*, *E. coli*, *B. cereus*, *S. aureus* and *L. monocytogenes* (see Table 2). It was found that crude vanilla extracts from KW, BB and MB could inhibit the growth of *Sal. typhimurium* and *L. monocytogenes*, which are food poisoning bacteria. The solution extracted by using 75% ethanol provided the highest inhibitory effect, followed by the cases with the use of 50% and 100% ethanol. On the other hand, the crude vanilla extracts could not inhibit *E. coli*. All crude vanilla extracts had significantly ( $p < 0.05$ ) different inhibition against *B. cereus* and *S. aureus*. The KW extract could not inhibit these microorganisms; conversely, the highest inhibitory effect occurred in the MB extract. The inhibition zone of the MB extract at 75% ethanol against *Sal. typhimurium*, *B. cereus*, *S. aureus* and *L. monocytogenes* was 0.93, 1.06, 0.76 and 0.95 mm, respectively. This implied that the difference of inhibitory properties of crude vanilla extracts was influenced by the different processes used during the production of vanilla pods, leading

to differences in types and amount of antimicrobial compounds developed in natural vanilla. Moreover, crude vanilla extract produced by using water extraction (control) could not inhibit these microorganisms, demonstrating that the inhibitory compounds in vanilla pods were not water soluble; therefore, only solvent extraction could be used.

In addition, standard vanillin (99.9%) at 5, 10, 15, 20, 40, 60, 80, 100 and 120 mM was also tested for antimicrobial properties. It was recognized that no inhibitory effect was found in all concentrations used. This was an indication that vanillin was not the major component responsible for the inhibitory effect, alternatively to the studies of other authors (Cerrutti and Alzamora, 1996; Fitzgerald *et al.* 2002, 2004, 2005; Rupasinghe *et al.* 2006). They reported that vanillin could inhibit the growth of *E. coli*, *L. plantarum* and *L. innocua*. Moreover, Moon *et al.* (2006) reported that vanillin at 40 mM inhibited the growth of *L. monocytogenes* and *E. coli* O157:H7 at pH 3.5-4.5 in apple juice media. Recently, Char *et al.* (2009) also reported that combination of vanillin and heat treatment at 55°C inhibited the growth of *L. innocua* in orange juice.

Table 2. Antimicrobial properties of crude vanilla extracts produced by using different types of vanilla pods and ethanol concentrations.

Microorganisms	Type of vanilla pods	Inhibition zone (mm) at different concentration of ethanol				
		0%	25%	50%	75%	100%
<i>E. coli</i>	KW	N*	N	N	N	N
	BB	N	N	N	N	N
	MB	N	N	N	N	N
<i>B. cereus</i>	KW	N	N	N	N	N
	BB	N	0.18 <sup>e</sup>	0.69 <sup>b</sup>	0.75 <sup>b</sup>	0.40 <sup>d</sup>
	MB	N	N	0.42 <sup>c</sup>	1.06 <sup>a</sup>	0.69 <sup>b</sup>
<i>S. aureus</i>	KW	N	N	N	N	N
	BB	N	N	N	N	N
	MB	N	N	0.31 <sup>b</sup>	0.76 <sup>a</sup>	0.71 <sup>a</sup>
<i>L. monocytogenes</i>	KW	N	N	0.36 <sup>f</sup>	0.60 <sup>c</sup>	0.48 <sup>d</sup>
	BB	N	N	N	0.69 <sup>b</sup>	0.40 <sup>ef</sup>
	MB	N	N	0.40 <sup>e</sup>	0.95 <sup>a</sup>	0.39 <sup>e</sup>
<i>Sal. typhimurium</i>	KW	N	N	0.63 <sup>d</sup>	0.72 <sup>b</sup>	0.63 <sup>d</sup>
	BB	N	N	0.73 <sup>b</sup>	0.75 <sup>b</sup>	0.68 <sup>c</sup>
	MB	N	0.41 <sup>e</sup>	0.71 <sup>bc</sup>	0.93 <sup>a</sup>	0.74 <sup>b</sup>

\* N = no inhibitory effect

\*\* The same letters mean significant difference at 95%, the statistical analysis was done separately for each bacterium.

#### 4. Conclusion

During the processing of vanilla pods after harvesting, the killing step played an important role in vanillin development. The use of too high temperatures for a long time, like with the KW method, provided lower vanillin content than the use of low temperature (BB) or the use of high temperature/short time (MB). BB and MB methods produced vanilla pods with 2.5% vanillin content. The application of natural vanilla pod in the butter cake reduced the high intensity of butter flavor, resulting in the mellowing of the butter cake flavor with consumer acceptance as high as 59%. Moreover, crude vanilla extract produced by using 75% ethanol had the highest inhibitory effect against *L. monocytogenes* and *Sal. typhimurium*, leading to its further application in food products, such as fresh-cut fruits and vegetables, in order to replace the use of chemical preservatives.

#### 5. Acknowledgements

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