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The Effect of the Addition of Gambir Catechins on Antioxidants and Antibacterial Hard Candy Products

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Abstract: The COVID-19 pandemic has changed consumer demands for food products due to the importance of eating functional foods capable of increasing the body's immune system, such as those containing natural bioactive compounds. Immune system enhancement through food intake is one of the top priorities for consumers globally. Hard candy products are ordinary foods consumed by people of all ages and social classes with numerous negative consumer perceptions due to their ability to cause dental caries. Therefore, it is imperative to develop hard candy as a functional food with antioxidant and antibacterial properties derived from natural ingredients. This research aims to innovate and design hard candy products that are acceptable to consumers with the addition of gambir catechins to its formulas. This research is considered novel for it provides innovation and design of hard candy products that are acceptable to consumers with the addition of gambir catechins. The result showed that all hard candy product formulas have *Staphylococcus aureus* with antimicrobial and antioxidant activities; hence, increased catechin concentration significantly affects these activities. Products in formulas 17, 8, 18, 4, and 7 with 4-5 ratings after organoleptic testing are standard products manufactured in accordance with SNI 3547.1:2018.

Keywords: catechins, antioxidants, antibacterial hard candy.

添加甘比尔儿茶素对抗氧化剂和抗菌硬糖产品的影响

摘要：由于食用能够增强人体免疫系统的功能性食品（例如含有天然生物活性化合物的食品）的重要性，新冠肺炎大流行改变了消费者对食品的需求。通过食物摄入增强免疫系统是全球消费者的首要任务之一。硬糖产品是所有年龄和社会阶层的人们食用的普通食品，由于它们能够引起龋齿，因此具有许多负面的消费者看法。因此，将硬糖开发为一种源自天然成分的具有抗氧化和抗菌特性的功能性食品势在必行。本研究旨在通过在配方中添加甘比尔儿茶素来创新和设计消费者可以接受的硬糖产品。这项研究被认为是新颖的，因为它通过添加甘比尔儿茶素提供了消费者可以接受的硬糖产品的创新和设计。结果表明，所有硬糖产品配方都含有具有抗菌和抗氧化活性的金黄色葡萄球菌；因此，增加儿茶素浓度会显着影响这些活动。配方17、8、18、4和7中的产品在感官测试后具有4-5级，是根据SNI3547.1:2018制造的标准产品。

关键词：儿茶素、抗氧化剂、抗菌硬糖。

1. Introduction

Consumer demand for food products is rapidly changing in accordance with their primary, secondary, and tertiary functions to boost the human immune system. Functional foods have a specific effect on health because they contain certain chemical compounds.

This food type is beneficial to consumers and provides opportunities for the food industry and the

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government to increase its economy. The advantages of consumers can be seen in the benefits of food that aid in preventing various diseases. Other indirect effects include boosting immunity, slowing aging, and improving physical appearance. For the food industry, high demand for functional foods means increasing profits by innovating product development and food formulations according to market demand. However, Indonesia's functional food industry is an opportunity to build national health.

The hard candy products are popular for people of all ages and social classes and considered to have common negative consumer perceptions because they cause caries/dental cavities in children. Conversely, it is the most favored confectionery product by all ages and social classes. The main ingredients are sucrose, glucose syrup, and water [1]. Several studies have concluded that refined sugar like sucrose is a significant cause of developing caries in children and adults [2].

This research aims to develop hard candy as a functional food with antioxidant and antibacterial properties derived from natural ingredients. Puji Lestari and Agustin [3] researched the production of hard candy with green tea extract. Therefore, based on this preliminary research, its development from gambier catechins was conducted through antioxidants and antibacterials activities. The various uses of catechins in gambier open up opportunities for this commodity to be used more widely.

Research on gambier is currently underway because the catechins contained are very high at 7-33%. It is used for pharmacological activity in developing pharmaceutical products and functional foods. Catechins also contain antioxidant and antibacterial agents [4] to enhance the body's immunity. Furthermore, it contains gambier [5], which has an immunomodulatory effect [6]. Immunomodulators are substances that play a role in strengthening the body's immune system. This study stated that taking lozenges for five days successfully boosted the human immune system. Gambir catechins are functional compounds of the polyphenol group, one of the antioxidant compounds that protect the body from free radical attack.

One of the possible innovation strategies to boost the immune system is to add gambier catechins to functional food products, such as hard candy. The results are expected to provide information to stakeholders on the manufacture of this product (*Uncaria gambir* Roxb.).

2. Methods

The ingredients used in this study were sucrose, glucose syrup, distilled water, mint flavor, strawberry flavor, citric acid, gambier catechins, and plastic candy wrappers. The hard candy reactors are printing and packaging equipment, measuring cups, and digital scales.

This study was conducted in five stages:

1. Preparing the raw materials: This stage starts by gathering the necessary raw materials and equipment. The type of flavor is determined based on a survey of consumer preferences, such as mint and strawberries;
2. Optimizing laboratory scale formulations using DesignExpert12.0 software with a d-optimal mix: The experimental design of the formulation can be seen in sub-chapter 3.4. This stage produces hard candy according to a standard experimental design set on a laboratory scale. The ingredients are cooked at high temperatures (140-150 ° C) until a solid, shiny, and clear texture is obtained;
3. Testing antioxidant and antibacterial activity;
4. Organoleptic testing of the product, including quality attributes of color, gloss, taste, and texture (mouthfeel), was carried out on 15 respondents using the scoring method. The hedonic evaluation scores used ranged from 1 to 5 with the following criteria: very dislike - 1, dislike - 2, enough - 3, like - 4, very like - 5;
5. Testing the hard candy according to SNI 3547.1:2008 [1] (Table 1).

Table 1 Hard candy quality requirements according to SNI 3547.1:2008 [1]

No	Criteria	Unit	Hard Candy
1	Condition Shape, Taste, Smell		Normal
2	Water content	% (b/b)	Max. 3,5
3	Ash content	% (b/b)	Max. 2,0
4	Reducing sugar (as invert sugar)	% (b/b)	Max. 24
5	Saccharose/Sucrose	% (b/b)	Min. 35
6	Additive		
6.1	Artificial sweeteners		Negative
6.2	Artificial Dyes		Negative

This formula was determined using a statistical data processing program such as Design Expert 12.0 software with the d-optimal mix method. The optimal formula consists of three stages:

1) Formula planning;
2) Drafting;
3) Analysis rating.

The first step in determining a variable is to combine its concentration. The variables in this design are gambier catechin (X_1), sucrose (X_2), glucose syrup (X_3), and (X_4) water. Based on preliminary studies, the lowest and highest values are indicated by minimum and maximum limits (Table 2).

Table 2 Minimum limits (low) and maximum limits (high) of each variable [7]

Variable	F_1 (Low)	F_2 (High)
Catechin (X_1)	0.5	1.0
Sucrose (X_2)	45	48,75
Glucose Syrup (X_3)	15	16,25
Water (X_4)	34	38

The model used is a d-optimum mixture comprising 20 formulations obtained using the d-optimum mixture design, as shown in Table 3.

Table 3 Formulation of hard candy with the addition of catechins from gambir (%)

Run	Catechin (X_1)	Sucrose (X_2)	Glucose Syrup (X_3)	Water (X_4)
1	0.75	45.00	14.75	35.50
2	0.74	45.76	14.74	34.76
3	0.50	45.85	15.21	34.44
4	0.74	45.76	14.74	34.76
5	0.72	46.50	14.00	34.78
6	0.50	45.84	14.23	35.44
7	0.74	45.76	14.74	34.76
8	0.75	45.00	14.75	35.50
9	1.00	45.68	14.00	35.32
10	0.50	45.15	15.50	34.85
11	1.00	45.94	15.00	34.06
12	1.00	46.09	14.31	34.61
13	0.50	46.38	15.00	34.13
14	0.50	46.39	14.53	34.58
15	0.74	45.76	14.74	34.76
16	1.00	45.33	14.62	35.06
17	0.82	45.68	15.50	34.00
18	0.50	45.84	14.23	35.44
19	1.00	46.34	14.53	34.13
20	1.00	45.06	15.24	34.70

This research consists of an antioxidant activity reaction using the DPPH spectrophotometer method [8] and disc diffusion. Additionally, an optimization analysis of the formula obtained using the Design Expert method was conducted after the reaction values were obtained. The descriptive text method was used to determine the organoleptic response of 15 panelists on the nature of color quality, luster, taste, and texture. The data collected were analyzed in accordance with SNI 3547.1:2008 to determine the water [1] and hardness content using a penetrometer [9].

3. Result and Discussion

Approximately 20 experimental runs were used to determine the antioxidant and antibacterial activities. The response values for antioxidant activity, antibacterial activity, catechin composition, sucrose, glucose syrup and water ranged between 38.26 - 64.50%, 118.5 - 182.5 mm, 0.5 - 1.0%, 45 - 48.75%, 15 - 16.25% and 34 - 38%.

The parameters analyzed by ANOVA showed significant and valid values of antimicrobial and antioxidant response models. The highest F-value in the antimicrobial reaction was 121.04, at a 5% significance level. This can be seen from the p-value < 0.05. R-squared values comprise 87.78% antioxidants and 95.91% antimicrobial activities with reasonable agreement. The criterion is that the difference between Adj and Pred R-squared is less than 0.2, while the value of Adeq Precision is above 4. The test results showed that the equations formed by the ANOVA in predicting the response indicate that the model is acceptable and can be used in the design space in the form of linear equations.

A surface reaction model was calculated with Design Specialist software to evaluate the contribution of the four components and determine the quantitative effects of different proportions on the antioxidant activity response (Y_1). The final equation of the model describing the antioxidant activity is as follows:

$Y_1 = 5182.78X_1 - 277.86X_2 + 381.32X_3 + 235.48X_4$

where Y_1 - antioxidant activity, X_3 - glucose syrup, X_1 – catechin, X_4 – water, X_2 – sucrose.

Based on the antioxidant activity equation results, it was found that the concentration of catechin, glucose, and water has a synergistic effect with the ability to increase the value of the antioxidant activity. Meanwhile, sucrose has an antagonistic value to the resulting antioxidant activity. Therefore, to illustrate the optimization of hard candy, the reaction analysis of antioxidant activity was plotted in a 3D graphical model, as shown in Fig. 1.

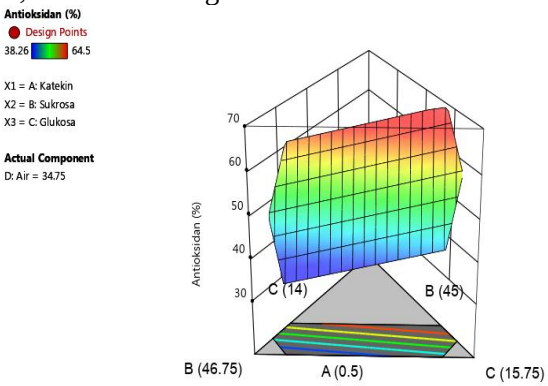


Fig. 1 3D graph illustrating the effect of hard candy composition on antioxidant activity

The final equation of the model describing the antibacterial activity is written as follows:

$Y_2 = 1552.34X_1 + 210.55X_2 + 952.26X_3 + 560.67X_4 -17.39X_1X_2 - 22.45X_1X_3 - 21.86X_1X_4 -11.66X_2X_3 - 13.99X_2X_4 - 24.66X_3X_4$

where Y_1 - antioxidant activity, X_3 - glucose syrup, X_1 – catechin, X_4 – water, X_2 – sucrose.

Hard candy optimization visualization and antibacterial activity response analysis were plotted in a 3D graphical model, as shown in Fig. 2.

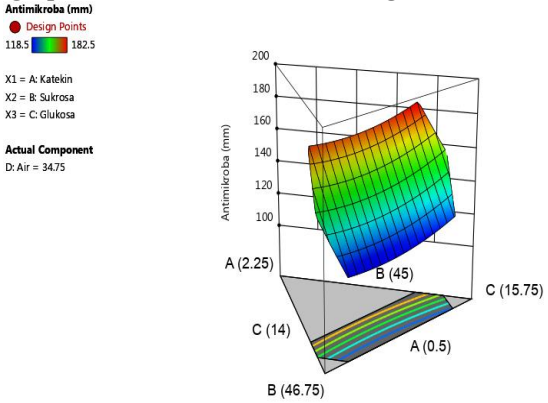


Fig. 2 3D graph illustrating the effect of hard candy composition on antibacterial activity

The antioxidant activity of hard candy is expressed in percent (%). The results indicate that the increase in catechin concentration in hard candy significantly affected the value of the antioxidant activity. Figure 3 shows that the antioxidant activity of hard candy with the addition of catechin is higher than hard candy control (0%).

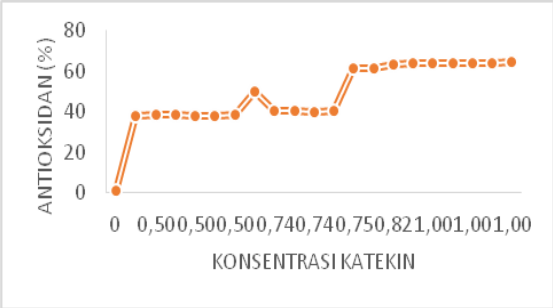


Fig. 3 Antioxidant activity at various catechin concentrations

The IC50 value of catechins in conventional preparations was 2.62 ± 1.75 ppm [10]. Antioxidant intensities are categorized as very strong IC50 values when smaller than 50 ppm [11]. This very powerful antioxidant activity of catechins protects the body cells from free radical damage and plays an important role in preventing degenerative diseases [12].

The value of the antioxidant activity is also affected by a high temperature of 150°C during the cooking process. This is because antioxidants are sensitive to high thermal processes, damaging their compound's chemical structure. Oligomers can also be hydrolyzed into simpler compounds during acid hydrolysis, contributing significantly to their antioxidant properties [13]. According to Andres et al. [14], the antioxidant content decreases with an increase in temperature due to the decomposition of phenolic compounds. Puspitasari [15] stated that the antioxidant content is very sensitive, unstable, and easily degraded. Flavonoid compounds, which act as antioxidants, are also reduced during the soursop leaf extraction in candy manufacturing at high temperatures. This is supported by Wirani's [16] research on hard candy manufacturing, stating that temperature treatment significantly affects antioxidant activity.

The hard candy inhibition zone was investigated by observing the diameter of the clear region around the disc as a potential determinant of the presence or absence of antibacterial compounds [17]. Therefore, the wider the clear zone, the greater the substance's ability to inhibit bacterial growth. Test results of antibacterial activity showed that the increase in catechin concentration in hard candy significantly affected the rise in diameter of the Streptococcus aureus inhibition zone. Fig. 4 shows that the antibacterial activity increased with a rise in the catechin concentration. This is consistent with preliminary studies stating that the effectiveness of antibacterial compounds is determined by the concentration and type of active compounds present in a substance. Therefore, the higher the concentration of the active ingredient, the greater its effectiveness [18].

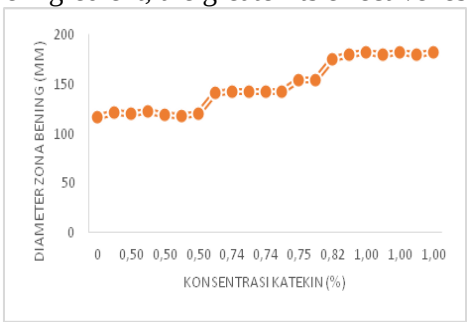
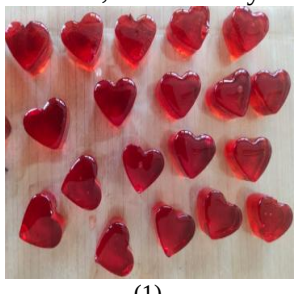


Fig. 4 Antibacterial activity at various catechin concentrations

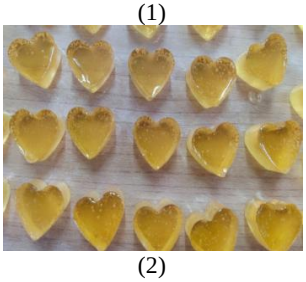
The antimicrobial activity of *Staphylococcus aureus* produced by hard candy ranged from 119.5-182.5 mm, where all formulas had a clear zone diameter larger than the control sample of 117 mm. This indicates that the formula has the antimicrobial activity of *Staphylococcus aureus*. It is also in accordance with preliminary research stating that 4% of commercial gambier product extract can kill the *Staphylococcus aureus* bacteria. Furthermore, the addition of 6% gambier product extract can kill 100% of Staphylococcus aureus within an hour [19].

Organoleptic testing of hard candy products was evaluated to determine the level of consumer acceptance. With 15 respondents with hedonic tests, this process was conducted subjectively based on observations with four senses. The parameters observed include color, luster, taste, and texture.

Technically, respondents evaluated the product by filling out the organoleptic test form following their priority levels. Therefore, based on the respondents' evaluation, it was found that the products with formulas 17, 8, 18, 4, and 7 are highly consumed and within the 4-5 range. Hard candy products have two flavor variants: strawberry and mint (Fig. 5). The hardest one commonly accepted by consumers with the highest average rating on each organoleptic test quality parameter is formula 13, a strawberry variant.



(1)



(2)

Fig. 5 Hard candy product for strawberry (1) and mint (2) variants

Product testing based on SNI hard candy 3547.1: 2008 was conducted on those with organoleptic test results criteria highly preferred by consumers (value 4-5) with an optimal formula of 17, 8, 18, 4, and 7. Table 4 shows the test results with water content between 0.42 and 2.78% at a maximum rate of 3.5%.

The low water content of a food item can impact its storage capacity because microbes are increasingly inhibited at this rate [20]. Buckle et al. [21] stated that sugar (sucrose) is added to foodstuffs at high concentrations of at least 40%. The ability to bind water is a property that causes sugar to reduce the water content of supplements. Apart from the composition aspect, another factor capable of influencing the moisture content of hard candy is the final manufacturing temperature. In this study, 150 ° C, which is 10°C below the melting point of sucrose, was used in its production. Arvianasari [22] stated that the product's water content varies due to the determination of the endpoint of crystal formation. This is the point where the flame is turned off and stirred continuously to avoid scaling. The product tends to have a high water concentration supposing the endpoint is faster than normal.

Table 4 Hard candy quality test results in accordance with SNI 3547.1:2008 [1]

No	Criteria	Unit	Standard	Result				
				17	**8**	**18**	**4**	**7**
1	Shape, Taste, Smell		Normal	Normal	Normal	Normal	Normal	Normal
2	Water	% (b/b)	Max. 3,5	0.73	0.80	0.47	0.42	2.78
3	Ash	% (b/b)	Max. 2,0	0.39	0.37	0.39	0.42	0.47
4	Reducing Sugar	% (b/b)	Max. 24	9.91	7.11	13.7	14.2	14.3
5	Sucrose	% (b/b)	Min. 35	61.2	68.4	61.2	58.2	56.4
6	Additives							
	Artificial sweeteners		Negative	Negative	Negative	Negative	Negative	Negative
	Artificial color		Negative	Negative	Negative	Negative	Negative	Negative

Table 4 shows that the test results of hard candy ash content between 0.37 to 0.47% met the product standard requirements of SNI 3547.1:2008 [1] at a maximum rate of 2.0%. The resulting ash content is related to the minerals contained in the sucrose, which greatly affects the candy's appearance. The lower the ash content, the better the candy's appearance. High levels of sugar purity and low ash content will produce sweets with good clarity, similar to water [23]. According to Winarno [24] and Wahyuni [25], the more calcium, phosphorus, and glucose concentrations added, the higher the mineral content. A maximum of 24% decrease in sugar is needed to obtain the standard range of 7.11-14.3% following SNI 3547.1:2008 requirement [1]. Formula 7 produces the product with the highest reducing sugar content with a value of 14.3%. This shows that the hard candy manufactured from this composition is easier to stick than other compositions due to its hygroscopic nature, making it easy to absorb water or air from the outside. Formula 8 produces hard candy with the lowest reducing sugar content, at a percentage of 7.11%.

According to Indahyanti [26], the more low-quality sugars formed, the more hygroscopic the hard candy products. Sugars with high glucose content or inverted sugars will be difficult to harden and have a short shelf life due to their high melting capability.

Indahyanti [26] reported that the quality of sugar is determined by the content of its sucrose, which is usually better when high. The overall composition produces a hard candy product with a sucrose content between 58.2-68.4%, according to SNI 3547.1:2008 [1], which is at least 35%.

According to the results of the organoleptic test, the selected formulation is included in the criteria preferred by consumers. The formula comprises 0.82%, 45.68%, 15.50%, and 34 % of the formula comprises catechin, sucrose, glucose, and air compositions. Based on the analysis results, the antioxidant and antibacterial activities of the selected formulation are 63.24% and 175.5 mm. Deviations that can occur in hard candy products are as follows:

1. *Crystallization (graining) during sugar product storage:* This is due to the recrystallization of sucrose in a very simple form, considered a transfer from the molecule to the nucleus. It is caused by one of the following factors:
   a. Lack of accuracy in the formulation;
   b. Unsuitable storage conditions due to water absorption on the product's surface and crystallization.
2. *Stickiness:* A possible mistake in manufacturing sugar products is that the product becomes sticky. These sugar products can absorb water from the air due to the storage space RH being 45% high.

4. Conclusion

In conclusion, the novelty of this research is the innovation and design of hard candy products that are acceptable to consumers with the addition of gambir catechins. This research has successfully formulated the hard candy products and received approval based on the organoleptic test. The final product corresponds to the standard requirements of SNI 3547.1:2008 [1] regarding hard candy as a functional food. Moreover, the consumers' negative perception about the product causing caries/cavities in the teeth is also resolved since it has high enough antioxidant and antibacterial

activity due to the addition of gambir catechins.

This research is limited to the antioxidant and antibacterial activities of the obtained product, which comprises 0.5-1% of catechins, increasing the number of consumers who dislike the product. Nevertheless, the hard candy product has the prospect of being developed as a functional food innovation on small, medium, and large industrial scales. Currently, people are more concerned about consuming food products that improve the immune system, such as functional food products containing natural bioactive compounds.

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