Formulation of Mahkota Dewa Leaf (*Phalria macrocarpa*) Extract Jelly Candy with Variation of Gelatin Concentration as Immunomodulator

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ABSTRACT

Jelly candy is one type of functional food that has great potential as a medium for delivering active herbal ingredients. Crownota dewa (*Phaleria macrocarpa*) leaf extract is known to contain bioactive compounds such as flavonoids and polyphenols that have immunomodulatory activity. For this reason, it needs to be formulated in an attractive dosage form such as jelly candy. Optimal formulation is required to maintain the stability and efficacy of active compounds during the production process. This study aims to develop a jelly candy formulation based on mahkota dewa leaf extract with variations in gelatin concentration as an immunomodulatory candidate. Variations in gelatin concentration (15%, 20%, 25%) were applied to evaluate its effect on the physical, chemical, and microbiological properties of the product. The results showed that the formula with 25% gelatin concentration (F3) had the best evaluation results, with gel strength reaching 1785 grams, 7% moisture content that meets SNI standards, and an acidic pH value (4.5-4.6) that supports product stability. Ash content in all formulas ranged from 0.43% to 0.45%, reflecting stable mineral content, while the number of mold colonies met the microbiological standards according to SNI. In addition, this product succeeded in maintaining the bioactive content of mahkota dewa extract, which has the potential to provide immunomodulatory benefits. This study concluded that formula F3 is an excellent candidate for further development as an herbal-based functional food.

Keywords: Crown of god, gelatin, jelly candy, immunomodulator, functional food

INTRODUCTION

Jelly candy is a functional food product that has great potential to be developed as a delivery medium for herbal active ingredients. The popularity of this product is not only based on its delicacy and textural appeal, but also its ability to accommodate various active ingredients, such as herbal plant extracts, while maintaining physical and sensory stability. One active ingredient that has great potential to be developed in jelly candy formulations is crownota dewa (*Phaleria macrocarpa*) leaf extract, which is widely recognized for its rich content of bioactive compounds and diverse pharmacological activities.

Crown of god leaves contain various phytochemicals, including flavonoids, alkaloids, saponins, and polyphenols, which contribute to their therapeutic properties (Altaf Asmawi M. Dewa A. Sadikun A. & Umar M., 2013; Sundari Soetikno V. Louisa M. Wardhani B. & Tjandrawinata R., 2018). These compounds are known to have anti-inflammatory, antioxidant, anticancer, and immunomodulatory effects (Rahmadi Dewi S. Nawawi A. Adnyana I. & Wachjudi R., 2018). Further research shows that ethanol extract of mahkota dewa leaves is able to suppress inflammatory pathways such as NF-κB and COX-2, as well as increase antioxidant activity which is important for maintaining cellular homeostasis (Putra Kusmardi K. Tedjo A. Estuningtyas A. & Rakasiwi M., 2020; Kusmardi Situmorang N. Zuraidah E. Estuningtyas A. & Tedjo A., 2021). This immunomodulatory activity shows the potential of mahkota dewa leaves as a prime candidate to support the body's immune system function.

However, a major challenge in the development of herbal-based products is maintaining the stability and efficacy of the active ingredients during the production and storage process. In jelly candy formulations, the stability of the active ingredients is greatly influenced by the interaction between the active ingredients and the main components such as gelatin. Gelatin, as a gelling agent, has an important role in determining the physical characteristics and stability of jelly candy. Varying gelatin concentration can affect the texture, hardness and shelf life of the product (Poçan E., 2019; Spinei and Oroian, 2023). Therefore, formulation optimization involving variations in gelatin concentration is crucial in the development of crown of god leaf extract-based jelly candy products.

The use of gelatin in jelly candy formulations offers advantages in terms of the ability to form stable gels that provide a distinctive and chewy texture. In addition, gelatin also functions as a stabilizer and emulsifier that improves the even distribution of flavor and color in the final product (Miharti and Kristopo, 2024). Variations in gelatin concentration not only affect the physical properties of jelly candy, but can also affect consumers' sensory perception of the product (Putri, Rosida and Rosida, 2024). Therefore, testing gelatin concentration variations is an important step to produce an optimal jelly candy formulation with immunomodulatory activity that remains effective.

Previous studies have shown that the formulation of herbal-active jelly candy products faces the challenge of maintaining the stability of the active compounds during the production process. This includes controlling water activity and processing conditions to minimize degradation of active compounds due to heat exposure or interaction with other ingredients (Cedeño-Pinos Marcucci M. & Bañón S., 2021). In this context, the selection of additives such as gelatin, as well as the optimization of its concentration, may provide a solution to these challenges.

The main objective of this research is to develop a jelly candy formulation based on mahkota dewa leaf extract with optimal gelatin concentration variation as an immunomodulator candidate from the resulting product. In addition, this research can contribute to the development of herbal-based functional food products, which can be a natural alternative to support immune system health.

METHODOLOGY

Sample Collection and Processing

Samples of mahkota dewa leaves were taken in Bone Regency, South Sulawesi Province. Collection of mahkota dewa (*Phaleria macrocarpa*) leaves was carried out then removed from the stalk and dirt then washed with running water. After the sample is washed, the sample is cut or sharpened. And then the sample is dried using an oven. Then the weight of the dry simplisia of the crown of god leaves that have been produced (Rahmadi Dewi S. Nawawi A. Adnyana I. & Wachjudi R., 2018)

Extraction of *Phalaria macrocarpa*

800 grams of crown of dawa leaves were macerated with 70% ethanol in a ratio of 1:10 for 3x 24 hours with occasional stirring, then filtered. The residue was re-macerated with the same liquid using the same ratio for 3x 24 hours. The filtrate obtained is then filtered, collected and evaporated until a thick extract is obtained and the extract is freezerdried to produce a dry extract (Mudalip Kathiman M. & Gimbun J., 2022).

Formula Design

Every 2 grams contains :

Material	Formula (%)		
	F1	F2	F3
Mahkota Dewa Leaf Extract	5	5	5
Gelatin	15	20	25
Sukrosa	50	50	50
High sirup fuktosa (HFS)	12	12	12
Sitrat Acid	0,8	0,8	0,8
Natrium benzoat	0,15	0,15	0,15
Essence strawberry	qs	qs	qs
Aquadest	ad 100	ad 100	ad 100

Prepare the tools and materials to be used and weigh the materials according to the calculations. Sucrose and HFS are heated to 80-900C. Gelatin is added, stirred until thickened. The temperature was lowered to 400C then added citric acid, sodium benzoate, strawberry essence, and mahkota dewa leaf extract stirred until homogeneous. The mixture was poured into a 2 gram mold container, dried at room temperature for 1 hour. Then stored in the freezer for 24 hours (Delgado S., 2017).

Evaluation of Jelly Candy Preparations

Organoleptic

Jelly candy is evaluated by direct observation which includes color, odor, taste and texture of the preparation (Baiti *et al.*, 2024).

Jelly Strength Test

Tensile strength aims to determine the force required when the jelly candy is pulled until the jelly candy tears for the first time. Measured using a texture analyzer (Vojvodić Cebin *et al.*,

2024).
Water Content Test
Determination of water content in jelly candy is done by weighing the weight of an empty porcelain cup, then weighing 2 grams of jelly candy and putting it in a porcelain cup, then putting it in an oven at a temperature of 105˚C. every 10 minutes the weight is weighed, but first cooled in a desiccator for ±5 minutes. The sample is ovened, cooled, and weighed repeatedly until a constant weight is obtained (Yuliana Agung B. Marwati M. & Candra K., 2020). The maximum limit of water content in jelly candy according to SNI is 20%. Determination of water content using the formula:

$$\text{Water Content} = \frac{W1 - W2}{W1 - W0} \times 100\%$$

Description:
W0 = weight of empty cup (g)
W1 = weight of cup and sample before drying (g)
W2 = weight of cup and sample after drying (g)

Ash Content Test
Jelly candy is weighed 2 grams and put in a porcelain cup, then heated using an electric bath in a fume hood until the smoke on the sample disappears and the color of the sample turns black. The sample is then ashed by being put into a furnace at a temperature of 550˚C until it becomes ash, then removed and left to cool and then weighed (Spinei and Oroian, 2023). The quality requirements for ash content in soft jelly confectionery products are a maximum of 3.0%

$$\text{Ash Content} = \frac{W1 - W2}{W1 - W0} \times 100\%$$

Description:
W0 = weight of empty cup (g)
W1 = weight of cup and sample before drying (g)
W2 = weight of cup and sample after drying (g)

pH Test
The pH evaluation test aims to determine the acidity level of the candy preparation. The pH acidity level of jelly candy should be adjusted to the National food standard. The pH test is carried out when the jelly candy is still in solution form. By dipping the pH meter into the solution (Song Chiou B. Xia Y. Chen M. Liu F. & Zhong F., 2021).

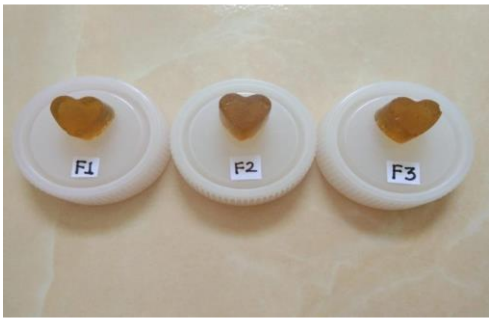
Microbiological Evaluation
For a 1:10 dilution, weigh 1 gram of jelly candy sample then melt and dissolve with 10 ml of distilled water. Pipette 1 ml of each dilution into a sterile petri dish in duplicate, pour 15-20 ml of PDA into each petri dish. Shake the petri dish carefully (rotate it by shaking it forward, backward, right and left) so that it is evenly mixed, let the mixture in the petri dish freeze, put

all the petri dishes in an incubator without turning upside down and incubate at 25˚C for 5 days. This is done by counting the colonies of mold growth on jelly candy by counting the colonies of mold growth on jelly candy by counting the colonies of mold growth on jelly candy (Baiti *et al.*, 2024).

RESULTS AND DISCUSSION

The results of this study indicate the effect of variations in gelatin concentration on the physical, chemical, and microbiological properties of jelly candy based on *Phaleria macrocarpa* leaf extract. This effect not only has implications for the texture quality and stability of the product, but also for the potential of the product as a functional food with immunomodulatory benefits.

The organoleptic test results were obtained as follows:



Formula	Organoleptic			
	Color	Odor	Texture	Taste
F1	Light brown	Strawberry aroma	Springy	Sweet
F2	Light brown	Strawberry aroma	Springy	Sweet
F3	Light brown	Strawberry aroma	Springy	Sweet

Description:
F1 = Jelly candy with 15% gelatin concentration
F2 = Jelly candy with 20% gelatin concentration
F3 = Jelly candy with 25% gelatin concentration

From the results of organoptic observations of jelly candy, the higher the concentration of gelatin used, the denser the jelly candy. The color of the jelly candy is brownish because it is influenced by the color of the extract. The resulting taste is sweet.

Jelly Strength Test Results

Gelatin functions to form a gel texture in making jelly candy mahkota dewa leaves. Gel formation is a combination of cross-linking of polymer chains to form a three-dimensional network. The higher the concentration of gelatin, the higher the gel strength. This is because gelatin is able to produce a good gel. The higher the concentration of gelatin used, the harder it is to tear.

Formula	Jelly Strength (gram)
F1	1314
F2	1359
F3	1785

Description:
F1 = jelly candy with 15% gelatin concentration
F2 = jelly candy with 20% gelatin concentration
F3 = jelly candy with 25% gelatin concentration

Water Content Test Results

Formula	Water Content
F1	26%
F2	10 %
F3	7 %

Description:
F1 = jelly candy with 15% gelatin concentration
F2 = jelly candy with 20% gelatin concentration
F3 = jelly candy with 25% gelatin concentration

From the observation results, it can be seen that the water content of jelly candy in this study ranged from 7-26%. Formula 1 and formula 2 have met the quality standards of jelly candy (SNI3547-2-2008) which is a maximum of 20%. Formula 1 does not meet the standard, this is because the more gelatin added to the jelly candy can bind the water contained in the jelly candy and can increase the viscosity of the material and reduce the water content of the material itself. The shelf life of various foods depends on their water content. The higher the water content in food, the faster the food will spoil and can cause bacteria, fungi and other types of microbes to grow and reproduce. Where water content is an important component in the processing of food ingredients, especially products in the form of candy. The water content in a food product will affect the level of acceptance, freshness, and shelf life.

Ash Content Test Results

The purpose of this ash content test is to determine the amount of mineral content in the sample.

Formula	Ash Content
F1	0,45%
F2	0,43 %
F3	0,45%

Description:
F1 = jelly candy with 15% gelatin concentration
F2 = jelly candy with 20% gelatin concentration
F3 = jelly candy with 25% gelatin concentration

From the observation results, it can be seen that the ash content of jelly candy in this study ranged from 0.43-0.45% and has met the quality standards of jelly candy (SNI 3547-2-2008) which is a maximum of 3%. The ash content in food ingredients is related to the mineral content of a material. According to (Amperawati *et al.*, 2019) gelatin contains 2-4% minerals. Ash content is a residual substance from the combustion of organic materials. Ash content is related to the minerals of a material. Ash content is one of the parameters determining the quality of jelly candy, where this ash content affects the candy will be better. Sugar with a high level of purity and low ash content will produce candy with good clarity.

pH Test Results

The pH test was conducted to determine the acidity level of jelly candy.

Formula	pH Observation
F1	4.5
F2	4.6
F3	4.6

Description:
F1 = jelly candy with 15% gelatin concentration
F2 = jelly candy with 20% gelatin concentration
F3 = jelly candy with 25% gelatin concentration

From the observation results, it can be seen that the pH of jelly candy in this study ranged from 4.5 to 4.6. The pH value produced by all treatments is classified as acidic because the pH value is below 7 (normal). This acidic condition is caused by the addition of citric acid. According to Koswara (2009), the success of making jelly candy depends on the degree of acidity to obtain the required pH. The pH value can be lowered by adding a little citric acid. This is also reinforced by Muawanah et al. (2012), the addition of citric acid in addition to adding flavor, will also lower the pH. The pH value of jelly candy is pH 4.5 to pH 6. All formulas 1, 2, and 3 meet the pH test requirements.

Formula	Number of colonies
F1	17
F2	13
F3	16

Description:
F1 = jelly candy with 15% gelatin concentration
F2 = jelly candy with 20% gelatin concentration
F3 = jelly candy with 25% gelatin concentration

Mold testing is done to determine the shelf life of the product. The shelf life of this jelly candy is influenced by several things such as the physical / chemical product, packaging and environment. Mold testing is done by counting the colonies of mold growth in jelly candy. Based on the results of the study, 0-day storage of the three jelly candy treatments did not show mold growth, while mold had grown on the jelly candy after 5 days of storage. However, the limit for mold growth according to the National Standardization Agency is a maximum of 1 x 102 colonies / gram and the jelly candy from the study met the requirements as determined.

This study proves that the gel strength of jelly candy increases with increasing gelatin concentration. Formula F3, with the highest gelatin concentration (25%), showed a gel strength of 1785 grams, much higher than F1 (1314 grams). This is in accordance with the mechanism of gelatin as a three-dimensional network former through polymer cross-linking, which provides structural strength to the product (Poçan E., 2019). The addition of gelatin not only increases the hardness but also the viscosity of the solution, which produces a chewy and sturdy texture. Consumer preference for this texture indicates that formula F3 has better market potential. Research (Mirarti & Kristopo, 2024) also describes the importance of texture in determining the level of acceptance of gelatin-based food products.
Chemical stability is an important aspect in ensuring product shelf life and quality. The results showed the highest water content in F1 (26%), which did not meet the SNI standard (maximum 20%). However, formulas F2 and F3 showed lower water content (10% and 7%, respectively), meeting the established standards. The ability of gelatin to bind water effectively at higher concentrations explains the decrease in water content in these formulas (Cedeño-Pinos Martínez-Tomé M. Murcia M. Jordán M. & Bañón S., 2020). Lower water content not only extends shelf life but also prevents the growth of microorganisms. Meanwhile, the stable ash content in all formulas (0.43-0.45%) indicates that the addition of gelatin does not affect the mineral content, which remains in accordance with quality standards. This low ash content is relevant to maintain the clarity and organoleptic quality of the product (Kurt Bursa K. & Toker Ö., 2021).

The acidity of the product, as seen from the pH value, plays an important role in the stability and shelf life of jelly candies. A pH value ranging from 4.5 to 4.6 indicates that the product is in an acidic condition suitable for preventing the growth of pathogenic microorganisms. The addition of citric acid not only provides a fresh taste but also helps lower the pH, as reported by (Souiy Amri Z. Sharif H. Souiy A. Cheraief I. Hamden K. … & Hammami

M., 2023). This pH stability also ensures that jelly candies remain stable during the storage process. A study (Silva Annetta F. Alves A. Queiroz M. Fadini A. Silva M. … & Efraim P., 2016) confirmed that the optimal acidity level contributes to sensory acceptance and overall product quality.

The extract of mahkota dewa leaves contains various bioactive compounds such as flavonoids, saponins, and polyphenols which are known to have immunomodulatory activity (Sundari Soetikno V. Louisa M. Wardhani B. & Tjandrawinata R., 2018). This jelly candy formulation shows stability for these active compounds without losing their efficacy during the production process. The immunomodulatory activity of this product can be linked to the ability of bioactive compounds to increase cytokine secretion, such as IL-2 and TNF-α, as reported by (Kusmardi Situmorang N. Zuraidah E. Estuningtyas A. & Tedjo A., 2021). Thus, this product not only offers functional value but also has the potential to be a natural alternative in supporting the body's immune system. So that the results of this study provide a scientific basis for the development of functional foods based on herbal ingredients.

The results of this study can provide implications for the development of the functional food industry. Integration of herbal ingredients into gelatin gelling agents, not only increases stability but also provides functional added value to the product. In formula F3 with a gelatin concentration of 25% has the best stability value based on physical, chemical, and microbiological characteristics.

However, this study has some limitations. Immunomodulation testing was only performed on initial parameters, so further studies are needed to test the product's efficacy in vivo or preclinical/clinical. In addition, the stability of the product during long-term storage also needs to be evaluated in more detail. For further research, it is recommended to integrate toxicity tests and clinical studies to evaluate product safety.

CONCLUSION

The results of the study showed that variations in gelatin concentration affected the physical, chemical, and microbiological stability of jelly candy based on Phaleria macrocarpa leaf extract. The formula with a gelatin concentration of 25% (F3) showed the best test results in various parameters. The gel strength in the F3 formula reached 1785 grams, indicating a strong and chewy texture, while its low water content of 7% ensured good stability and shelf life according to SNI quality standards. The pH value of this formula is in the acidic range (4.5–4.6), which contributes to product stability and prevents the growth of microorganisms. In addition, the ash content in all formulas is in the range of 0.43–0.45%, indicating the stability of the mineral content. From the microbiological test, it shows that the number of mold colonies meets the Indonesian national standard.

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