

Effect of the proportion of stevia leaf extract (*Stevia rebaudiana B*) on the chemical characteristic properties of functional pudding

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Abstract

The proportion of stevia leaf extract (*Stevia rebaudiana B*) will influence the physicochemical properties of food. Functional pudding, a popular dessert, has attracted attention in recent years due to its potential as a vehicle for delivering health-promoting ingredients. This study aim to investigates the impact of different proportions of stevia leaf extract (*Stevia rebaudiana B*) on the physicochemical properties of functional pudding. Stevia leaf extract, known for its natural sweetness and negligible impact on blood sugar levels, holds promise as a low-calorie alternative to traditional sweeteners. Through systematic experimentation, various concentrations of stevia leaf extract are incorporated into pudding formulations, and their effects on water content, ash content, fat content, protein content, and carbohydrate content are evaluated. The results indicate significant alterations in the physicochemical characteristics of the pudding samples, with variations observed in water content, ash content, fat content, protein content, and carbohydrate content. Specifically, sample F6, containing the highest proportion of stevia leaf extract, exhibits notable changes, including a high-water content of 88.31%, low ash content of 0.49%, moderate fat content of 0.81%, elevated protein content of 5.35%, and reduced carbohydrate content of 5.02%. These findings underscore the potential of stevia leaf extract as a functional ingredient in pudding formulations and provide insights for the development of healthier dessert options.

KEYWORDS

Gelatin, Natural sweetener, Low calorie, Stevia leaf, Pudding

1. INTRODUCTION

Puddings, a popular dessert enjoyed worldwide, have undergone significant transformations to meet the growing demand for healthier food alternatives (Abdulan et al., 2023). In recent years, the exploration of the physicochemical characteristic properties of functional pudding,

particularly with the incorporation of stevia leaf extract (*Stevia rebaudiana B*), has gained prominence (Schiatti-Sisó et al., 2023). This study addresses the need to understand and enhance the nutritional profile, sensory attributes, and overall quality of functional pudding (Li et al., 2022). Moreover, the increasing prevalence of health-

related concerns, such as diabetes and obesity, has intensified the search for low-calorie sweeteners and alternatives to traditional sugar (Saraiva et al., 2020). Stevia leaf extract, derived from the *Stevia rebaudiana* B plant, emerges as a promising natural sweetener due to its negligible impact on blood sugar levels (Chughtai et al., 2020). The potential of stevia to impart sweetness without the caloric burden of sugar has prompted its exploration in functional pudding formulations, aligning with the broader trend of healthier food choices.

Considerable strides have been made in the study of physicochemical characteristic properties of functional pudding (Dhar et al., 2023). Researchers have delved into the effects of various additives, alternative sweeteners, and formulation changes, aiming to create a balance between indulgence and health consciousness (Almeida et al., 2024). Investigations have explored alterations in texture, flavor, and nutritional content, reflecting the dynamic nature of consumer preferences and the continuous evolution of food science.

However, despite the advancements, certain gaps and challenges persist in the existing body of research on the physicochemical characteristic properties of functional pudding (Altemimi et al., 2024). The need for a comprehensive understanding of the impact of stevia leaf extract proportions remains a notable gap (Raspe et al., 2022; Wang et al., 2020). Previous studies have often focused on singular aspects or neglected the optimization of stevia leaf extract levels, leaving room for a more nuanced exploration of its effects on the overall quality of functional pudding. Addressing these gaps is crucial for advancing the development of healthier pudding options.

This research aims to build upon existing knowledge by systematically investigating the influence of different proportions of stevia leaf extract on the physicochemical properties of functional pudding. By systematically exploring different concentrations, we aim to identify the optimal balance that enhances water, ash, fat, protein, and carbohydrate content. This study holds

significant implications for both the food industry and consumers, providing valuable insights into the development of functional puddings that align with contemporary health-conscious preferences. The outcomes of this study will contribute to the enhancement of pudding formulations, fostering the creation of delectable yet health-promoting dessert options in the market.

2. MATERIALS AND METHODS

The study was conducted at the Laboratory of Food Science and Technology, Faculty of Agriculture and Animal Husbandry, University of Muhammadiyah Malang. This study used a simple randomized group design (RAK), namely the proportion of stevia leaf extract which was divided into 6 formulas, namely F1 (0 g stevia leaf extract + 170 g sugar), F2 (0.17 g stevia leaf extract + 136 g sugar), F3 (0.34 g stevia leaf extract + 102 g sugar), F4 (0 g stevia leaf extract + 0.51 g stevia + 68 g sugar), F5 (0.68 g stevia leaf extract + 34 g sugar), F6 (0.85 g stevia leaf extract + 0 g sugar). Tools that are used scale, stainless steel basin, measuring cup, spoon, plate, juicer, pan, ladle (centong), gas stove and pudding cup. The ingredients used in making the pudding are jelly powder (swallow), stevia leaf extract (stevia), sugar (gulaku), salt (anak pintar), and water (aqua). The materials used in the analysis are H₂SO₄, boric acid, and HCl 0.02 N was purchased from Kimia Farma company (Malang city, East Java province, Indonesia). Making pudding by cooking jelly powder, water, sugar, stevia leaf extract and salt to boil. The next activity, pudding molded and cooled until it has a chewy texture and dense (Mouritsen & Styrbæk, 2017). The quality of the chemical content of stevia leaf extract as the main raw material was analyzed, including water content, ash content, protein content, fat content, and carbohydrate content. Similarly, the pudding obtained was also analyzed for its chemical content, which includes water content, ash content, protein content, fat content, and carbohydrate content. The organoleptic properties

of the pudding were also studied as a reference for the level of acceptance by the public.

2.1 Water content analysis

A higher water content value will shorten the storage time, as free water in a product or food ingredient becomes a breeding ground for bacteria, leading to a shorter shelf life and quicker spoilage (Mayasari, 2016). In determining the water content, an empty porcelain crucible was dried in an oven for 24 hours at a temperature of 100-105 °C. The porcelain crucible was cooled in a desiccator for 15 minutes and weighed as the weight of the crucible. The sample was crushed using a mortar. Two grams of the sample were weighed and placed into the dried porcelain crucible. The sample was dried in the oven at a temperature of 100-105 °C for 4 hours. The sample was cooled in a desiccator for 15 minutes and re-weighed as the final sample weight. The water content of the sample was calculated using the [formula 1](#) (Myers et al., 2016) :

2.2 Ash content analysis

Ash content analysis was conducted with the aim of determining the mineral content present in a product or food ingredient (Lisa et al., 2015). Samples obtained from the drying process in the oven during the water content analysis were further processed for ash content testing. The samples were placed in a muffle furnace at a temperature of 600 °C for 5 hours. Afterward, the samples are taken out and placed in a desiccator for 15 minutes. The samples are then weighed as the final mass. The ash content is calculated using the [formula 2](#) (Picó, 2012).

2.3 Protein content analysis

Protein was a crucial component of human food with essential functions in various biological processes such as catalysis, transportation, involvement in molecules like oxygen, immune system support, and nerve impulse transmission. Food processing significantly affects the damage that occurs to proteins (Antonio et al., 2014). In the

protein analysis, a sample (0.1 g), 1 catalyst spatula (Na₂SO₄: HgO), and 2 ml H₂SO₄ were added to a Kjeldahl flask. The sample was then destructed until the solution becomes clear. After the sample cools, 10 ml of 50% NaOH and 15 ml of distilled water are added. The sample was distilled and collected in 15 ml of 4% H₃BO₃ with 1% methyl red - bromothymol blue indicator. The sample was distilled until it turns green. The titrate was then titrated with 0.02 N HCl until it turns pink. The calculation formula for the Kjeldahl method was [3 and 4](#):

2.4 Fat content analysis

The fat content in a food material plays a crucial role in human health. The fat content will impact the shelf life of flour as a higher fat content in food materials tends to result in rancidity more easily (Micha et al., 2017). The analytical process involves drying the fat flask in an oven at a temperature of 100-110 °C. The fat flask was then cooled in a desiccator for 15 minutes. The empty fat flask is weighed. Two grams of the crushed sample were weighed and added to the flask. 30 ml of petroleum benzene solution are added. It was raised in a water bath for 4 hours and then placed in the oven until the sample thickens. The final weight of the flask was measured. The fat content was calculated using the [formula 5](#) (Gordon et al., 2021).

2.5 Carbohydrate content analysis

Carbohydrates were the primary source of calories for almost all living organisms (Slavin, 2013). In addition to being an energy source, carbohydrates also play a crucial role in determining the characteristics of food materials, such as taste, colour, texture, and others (Mann & Truswell, 2017). Calculating the percentage carbohydrate in food materials can be done using the following [formula 6](#) (Sizer & Whitney, 2010).

2.6 Statistical analysis

For each treatment, the mean value and standard deviation of water, ash, fat, protein, and

carbohydrate content were computed and displayed. The data underwent statistical analysis using IBM SPSS Statistics 24. The results were presented in the form of mean standard deviation (SD). Utilizing

one-way analysis of variance (ANOVA) and Duncan's test ($P < 0.05$), statistical differences between the treatments were examined.

$$\text{Water content (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight}} \times 100\% \quad (1)$$

$$\text{Ash content (\%)} = \frac{\text{mass of ash (g)}}{\text{initial mass of sample (g)}} \times 100\% \quad (2)$$

$$N = \frac{(\text{HCL sample} - \text{HCL blank}) \times \text{HCL} \times 14.007}{\text{mg sample}} \times 100\% \quad (3)$$

$$\text{Protein content} = N (\%) \times \text{correction factor (Chang \& Zhang, 2017)} \quad (4)$$

$$\text{Fat content (\%)} = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Weight of the material (g)}} \times 100\% \quad (5)$$

$$\text{Carbohydrate content (\%)} = 100\% - (\% \text{protein} + \% \text{fat} + \% \text{water} + \% \text{ash}) \quad (6)$$

3. RESULTS AND DISCUSSION

3.1 Raw material analysis

The raw material under investigation exhibits diverse physicochemical characteristics, with notable values across different parameters. The raw material in this study was stevia leaf extract. Extract of stevia leaves in the analysis of its quality in the form of water content, ash content, protein content, fat content, and carbohydrate content. The results of quality analysis on stevia leaf extract were shown in [Table 1](#).

Table 1. Chemical content of stevia leaf extract

Parameter (%)	Sample	Reference (Abou-Arab & Abu-Salem, 2010)
Water content	7.33	7.00
Ash content	3.19	7.41
Fat content	0.13	3.73
Protein content	5.13	11.4
Carbohydrate content	84.2	61.9

Based on the analysis in Table 1. It was obtained the chemical composition of raw materials stevia leaf extract sample. The water content of the sample stands at 7.33%, indicating a relatively low moisture level. But, the water content of stevia leaf extract has a higher yield compared to Abou-Arab and Abu-Salem (2010). This is likely due to higher carbohydrate. Carbohydrates that contain many -OH groups make it possible to form a larger hydrogen bond. This leads to binding more water. This finding was crucial as water content significantly influences the texture, shelf life, and overall quality of food products.

In terms of ash content, the sample demonstrates a value of 3.19%, suggesting the presence of inorganic mineral elements. Ash content was often indicative of the overall mineral composition in food and can provide insights into its nutritional profile. Ash content in this study was lower than the literature Abou-Arab and Abu-Salem (2010). Differences ash content can be caused by mineral contributions from the soil where stevia leaves grow, which usually consists of zinc, potassium, magnesium, calcium, iron, and sodium (Lemus-Mondaca et al., 2012). The fat content in the sample was minimal, registering at 0.13%. This low-fat content aligns with the current trend toward

healthier food alternatives, catering to consumers who prioritize reduced fat intake without compromising on flavor and texture. The value of fat content of stevia leaf extract in this study was lower than the literature (Abou-Arab & Abu-Salem, 2010).

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A noteworthy protein content of 5.13% was observed in the sample. Proteins were essential building blocks for various physiological functions, and their presence in the sample contributes to its nutritional value. The value of protein content in this study was lower than the literature (Abou-Arab & Abu-Salem, 2010) of 11.4%. Understanding the protein content was crucial for assessing the sample's potential as a protein source in a broader dietary context. Carbohydrate levels in this study was quite high than the previous study of 84.2%. This difference was possible due to the variety of varieties of stevia leaves. Carbohydrates serve as a primary energy source, and their abundance in the sample indicates its potential to provide a quick and sustainable energy boost (Zhu & Xu, 2020). In summary, the sample exhibits a balanced composition with low fat, moderate protein, and high carbohydrate content. These findings provide a foundational understanding of the sample's nutritional composition.

3.2 Chemical analysis of pudding

Based on the results of the variance analysis showed that the addition of stevia leaf extract

treatment has a value of $\alpha = 0.000$ which means that the effect was very real ($P < 0.05$) on water content, ash content, protein content, fat content, and carbohydrate content (Table 2).

3.3 Water content

Based on Table 2, the data depicts the water content in percentage for various treatments labeled from F1 to F6. Each treatment corresponds to a different level of water content. Treatment F1 exhibits a water content of 76.19%, marked with the label "a". Meanwhile, treatment F2 shows a water content of 78.77%, labeled "b". There was a noticeable increase in water content for treatment F3, reaching 81.69%, labeled "c". Subsequently, treatment F4 demonstrates a more significant increase in water content, reaching 84.77%, labeled "d". Treatment F5 also shows a high-water content, recording 86.06%, with the same label "d" as F4. Finally, treatment F6 exhibits the highest water content, reaching 88.31%, labeled "e". The results of statistical analysis of water content (Table 2) showed a very distinct difference in the six samples. This data provides insight into the impact of treatments on the water content of the sample, with a noticeable increase corresponding to higher treatment numbers. It indicates that the treatments exert a significant influence on the water content of the sample.

Water content in this study was higher than the water content of pudding in the previous study amounted to 75,62% in pudding strawberry powder (Srimati et al., 2023) and 74.13% in pudding moringa (Windari et al., 2021). Water content greatly affects the quality of pudding. High water content results in easy bacteria and fungi and other microbes to multiply (Gupta et al., 2017). One parameter that was quite important in food content is water content because in addition to affecting the shelf life of food, water contained in food can also affect the appearance, texture, and taste (Ergun et al., 2010). The higher the percentage of water content in food stuffs, the shorter the shelf life of these food stuffs and can lead to a decrease in product quality.

Table 2 Chemical analysis results pudding

Treatment	Water (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)
F1	76.19 ^a	0.88 ^d	3.34 ^a	0.45 ^a	19.12 ^f
F2	78.77 ^b	0.82 ^d	3.65 ^{ab}	0.52 ^a	16.21 ^e
F3	81.69 ^c	0.71 ^c	4.08 ^b	0.60 ^b	12.89 ^d
F4	84.77 ^d	0.66 ^c	4.81 ^c	0.63 ^b	9.11 ^c
F5	86.06 ^d	0.57 ^{ab}	5.05 ^{cd}	0.72 ^c	7.57 ^b
F6	88.31 ^e	0.49 ^a	5.35 ^d	0.81 ^d	5.02 ^a

Description: *) the numbers followed by different letters show a very real difference (P=5%) according to Duncan's test

3.4 Ash content

The data represents the ash content in percentage for different treatments labeled from F1 to F6. Each treatment corresponds to a specific level of ash content. Treatment F1 shows an ash content of 0.88%, labeled as "d". Treatment F2 exhibits a slightly lower ash content of 0.82%, also labeled "d". A notable decrease in ash content is observed for treatment F3, which records 0.71%, labeled as "c". Subsequently, treatment F4 demonstrates a further decrease in ash content, measuring 0.66%, also labeled "c". Treatment F5 presents a relatively lower ash content of 0.57%, with a combined label "ab". Finally, treatment F6 exhibits the lowest ash content of 0.49%, labeled "a". This data provides insights into the influence of treatments on the ash content of the sample, with varying levels observed across different treatments. It suggests that the treatments exert a significant impact on the ash content of the sample, influencing its overall composition.

Ash content was an element that makes up the extract and can affect the composition of organic matter (Ani & Abel, 2018). Ash content was also a measure that is used to determine the total amount of minerals that contained in food material (Al-Farga et al., 2016). In food stuffs determination of ash content was very important to determine good or not the product results and shows the total

minerals contained in the material, where the higher the ash content, the quality of the product decreases. Ash content analysis was also often done as an indicator to determine the quality of other foods (Harris & Marshall, 2017). The ash content in the pudding that has been determined by SNI jelly was <6.99%, thus the ash content of pudding in this study was in accordance with SNI jelly (Kusumawati et al., 2020).

3.5 Fat content

The data provided represents the fat content in percentage for various treatments labeled from F1 to F6. Each treatment indicates a specific level of fat content. Treatment F1 shows a fat content of 0.45%, labeled as "a". Following this, treatment F2 exhibits a slightly higher fat content of 0.52%, also labeled "a". An increase in fat content becomes apparent with treatment F3, registering 0.60% and labeled as "b". This trend continues with treatment F4, which records a further increase in fat content at 0.63%, also labeled "b". Treatment F5 demonstrates a relatively higher fat content of 0.72%, receiving the label "c". Finally, treatment F6 presents the highest fat content at 0.81%, labeled as "d". These data points highlight the impact of different treatments on the fat content of the samples, indicating a progressive increase from F1 to F6.

Fat content plays a crucial role in pudding for several reasons. Firstly, fat contributes to the smooth

and creamy texture of pudding, enhancing its mouthfeel and overall sensory experience (Zhou et al., 2022). Without adequate fat content, pudding may appear grainy or lacking in richness. Secondly, fat helps to bind and stabilize the ingredients in pudding, ensuring a cohesive and uniform structure (Udo et al., 2023). This is particularly important in recipes that require baking or heating, as fat helps to prevent the pudding from separating or curdling. Additionally, fat adds flavor to pudding, contributing to its richness and depth of taste. It also helps to carry and enhance the flavor of other ingredients, such as vanilla, chocolate, or fruit extracts. Moreover, fat plays a role in providing satiety, making pudding a more satisfying and filling dessert option (Alam et al., 2024). This can be beneficial for individuals seeking to manage their appetite or maintain a balanced diet. Overall, while fat content should be moderated for health reasons, it remains an essential component in pudding recipes to achieve the desired texture, stability, flavor, and satiety.

3.6 Protein content

The data represents the protein content in percentage for different treatments labeled from F1 to F6. Each treatment corresponds to a specific level of protein content. Treatment F1 exhibits a protein content of 3.34%, labeled as "a". Treatment F2 shows a slightly higher protein content of 3.65%, labeled "ab". A notable increase in protein content is observed for treatment F3, recording 4.08%, labeled as "b". Subsequently, treatment F4 demonstrates a further increase in protein content, measuring 4.81%, labeled "c". Treatment F5 presents a relatively higher protein content of 5.05%, with a combined label "cd". Finally, treatment F6 exhibits the highest protein content of 5.35%, labeled "d". This data provides insights into the influence of treatments on the protein content of the sample, with varying levels observed across different treatments. It suggests that the treatments have a significant impact on the protein content of the sample,

potentially altering its nutritional composition and quality.

Protein was an essential component of the human diet that was needed for tissue replacement, energy intake, and versatile macromolecules in living systems (Kumar et al., 2022). Proteins also have important functions in all biological processes such as catalysts, transport, various other molecules such as oxygen, as immunity, and conducting impulse nerve impulses (Wu, 2016). Low protein content in pudding can be caused by the length of boiling pudding resulting in protein contained in it damaged by heat (Naseer et al., 2022). This is thought to be due to the use of relatively high temperatures in the boiling process which causes in greater protein damage. Food processing greatly affects the damage that occurs to proteins. The higher the temperature and the longer the processing time the higher the protein damage that occurs in these foods (Bhat et al., 2021).

3.7 Carbohydrate content

The data provided represents the carbohydrate content in percentage for various treatments labeled from F1 to F6. Each treatment indicates a specific level of carbohydrate content. Treatment F1 shows a carbohydrate content of 19.12%, labeled as "f". Following this, treatment F2 exhibits a lower carbohydrate content of 16.21%, labeled "e". A notable decrease in carbohydrate content is observed with treatment F3, registering 12.89% and labeled as "d". This trend continues with treatment F4, which records a further decrease in carbohydrate content at 9.11%, labeled "c". Treatment F5 demonstrates a relatively lower carbohydrate content of 7.57%, receiving the label "b". Finally, treatment F6 presents the lowest carbohydrate content at 5.02%, labeled as "a". These data points highlight the impact of different treatments on the carbohydrate content of the samples, indicating a progressive decrease from F1 to F6. Such variations are crucial for understanding how different factors influence the carbohydrate composition of the product and its potential implications for nutritional value and dietary considerations.

Carbohydrates were the main source of calories for almost all living things. Carbohydrate also plays an important role in determining the characteristics of food ingredients such as taste, color, texture, and others (Kokkinidou et al., 2018). In the test of carbohydrate table obtained different results with the highest carbohydrate content of 19.12% this is due to the addition of sugar in the process of making the F1 sample while the lowest carbohydrate content of 5.02% in the F6 sample, this is because in the F6 sample there is no addition of sugar so that the resulting carbohydrate levels are low.

4. CONCLUSION

This study aims to build upon existing knowledge by systematically investigating the influence of different proportions of stevia leaf extract on the chemical properties of functional pudding. Stevia leaf extract has nutritional components that support its use as a raw material for functional foods and was considered safe when mixed with food ingredients such as pudding. The test results show a significant effect on water content, ash content, fat content, protein content, and organoleptic properties. This research indicates that the test results for sample F6 have a high-water content of 88.31 %, ash content of 0.49 %, fat content of 0.81 %, protein content of 5.35 %, and carbohydrate content of 5.02 %. Further study could focus on Investigate the effect of incorporating stevia leaf extract on the shelf-life stability of functional pudding. Assess changes in texture, flavor, and microbial growth over time to determine the suitability of stevia-based formulations for commercial production and consumer acceptance.

Stevia leaf extract has nutritional components that support its use as a raw material for functional foods and was considered safe when mixed with food ingredients such as pudding. The test results show a significant effect on water

content, ash content, fat content, protein content, and organoleptic properties.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Damat: Conducted the research trial. Rahayu Relawati: Supervised the trial. Mujiyanto and Lili Zalizar: Cosupervised the trial. Iswahyudi and Sustiyana: Technical assistance for lab analysis. Burhanuddin Harahap: Statistical analysis and Proof reading and final editing.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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