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The effect of gelatin and konjac glucomannan concentrations on moisture content, water activity, texture, density, and protein content in synbiotic marshmallows

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Abstract

Along with current lifestyle changes, consumers seem to want food that satisfies their appetites and provides health benefits to the body. Synbiotic products are believed to benefit the body's health, particularly the gastrointestinal tract. Apart from being healthy, consumers also like practical snacks. Hence, synbiotic marshmallows were developed as a novel form of synbiotic or marshmallow products. This research aimed to determine the effect of various gelatin and konjac glucomannan concentrations on synbiotic marshmallows' physicochemical characteristics and texture profile and to find the optimum formulation. Synbiotic marshmallows were prepared by mixing sugar solution with gelatin and konjac glucomannan gel in the following formulas: P1 (9%:0%), P2 (8.5%; 0.5%), P3 (8%;1%), P4 (7.5:1.5%), and P5 (7%:2%) (w/v). The dough was mixed with 15 g of skim milk probiotic before being moulded and cooled at room temperature. Once solid, marshmallows are sowed with corn flour. Moisture content was analyzed gravimetrically, water activity was determined using an a_w meter, texture profile analysis was evaluated using a texture analyzer, density was determined by measuring mass and volume, and protein content was analyzed according to the Kjeldahl method. The results showed that the formulation of 8.5% gelatin and 0.5% KGM produced the best synbiotic marshmallows regarding water content, water activity, texture (hardness, gumminess, chewiness), density, and protein content. Based on the results, it can be concluded that synbiotic marshmallows can be developed for future innovation.

Introduction

Increased public awareness of the importance of living a healthy lifestyle has led to a desire for food that satisfies appetite and gives health benefits. The demand for foods that contribute to human health, such as functional foods, has increased interest in recent years. Functional food is a type of dietary item that provides basic essential nutrients to the body, improving general and physical conditions and lowering the risk of various disease disorders (Miranda *et al*., 2015). One of the considered functional foods is synbiotic products. Due to the use of a combination of prebiotics and probiotics, synbiotics are believed to be optimal for increasing probiotic bacteria while decreasing pathogenic bacteria (Pandey *et al*., 2015).

Probiotics are live microorganisms that could benefit their hosts by providing nutritional benefits and protection against pathogenic bacteria (Pérez-Sánchez *et al*., 2014). The majority of probiotic strains are lactic acid bacteria from the genera Leuconostoc, Lactobacillus, Lactococcus, Pediococcus, and Streptococcus. *Lactobacillus plantarum* Dad-13 is one of the lactic acid bacteria strains currently being developed. *L. plantarum* Dad-13 is an Indigenous probiotic isolated from *dadih* produced from the spontaneous fermentation of buffalo milk (Kamil *et al*., 2021). This strain has been identified for its probiotic potential because it has antimicrobial activity and can survive in the digestive tract (Kamil *et al*., 2020). Previous research on adding *L. plantarum* Dad-13 to purple sweet potato extract yoghurt increased its health effects on lowering the risk of diarrhoea and reducing free radicals (Tari *et al*., 2020). Therefore, it could underlie *L. plantarum* Dad-13 has the potential to be added as a probiotic on food products.

Prebiotics are selectively utilized substrates by host microorganisms to provide a health benefit (Núñez-Sánchez *et al*., 2021). Prebiotics work by selectively

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stimulating the growth or activity of beneficial bacteria in the gastrointestinal tract (Bandyopadhyay *et al*., 2014). One of the potential prebiotic sources is glucomannan which can be derived from *Amorphophallus konjac*. Glucomannan comprises monomers D-glucose and D-mannose with β-1,4 glycosidic linkages (Zhang, 2014). Glucomannan has been shown to be effective in stimulating the growth of beneficial microflora in the gastrointestinal tract, particularly *Lactobacillus* sp. (Tester and Al-Ghazzewi, 2013), and modulate gut-associated lymphoid tissue immunity in mice (Changchien *et al*., 2020). Glucomannan also helps to lower triglycerides and cholesterol levels, stabilizing blood pressure and blood sugar levels and facilitating weight loss (Devaraj *et al*., 2019).

The most common synbiotic or probiotic products available on the market are synbiotic drinks or fermented foods like yoghurt, kefir, and *tape* (fermented cassava). Products that do not require fermentation are needed to increase the diversification of probiotic and synbiotic products. Synbiotic product innovation is possible based on previous research on gummy *L. plantarum* Dad-13, which was successfully developed and can serve as a carrier for the treatment of malnourished children (Kamil *et al*., 2021; Kamil *et al*., 2022). In line with this, consumers also prefer snack products that are practical, quick, and convenient. Marshmallow candy is an example of a popular snack item. Marshmallow is a food confectionary product with soft, foamy, and chewy textures and comes in various shapes, flavours, aromas, and colours. Gelatin contributes to the chewy texture of the whipped confections by lowering the surface tension of the syrup, stabilizing the foam through increased viscosity, setting the foam via gelation, and preventing sugar crystallization (Du Toit *et al*., 2016). Aside from being a prebiotic, konjac glucomannan also has hydrocolloid characteristics and could be used as a gelling agent, thickener, emulsifier, stabilizer, and improve food texture (Kaya *et al*., 2015).

Various concentrations of gelatin and konjac glucomannan in the production of synbiotic marshmallows can affect their physical and chemical properties. Adding konjac glucomannan to gelatin with or without sucrose can reduce the hardness and sensory stickiness, making the gel easier to chew and swallow (Tomczyńska-Mleko *et al*., 2014). The gelatin and konjac glucomannan concentration in marshmallows can also affect their moisture content. The moisture content of a product will influence the water activity (a_w) or the amount of free water that microorganisms can use to grow. A good marshmallow has a water content of 12-20% and a water activity value of 0.60-0.75. (Hartel *et al*., 2018). The moisture content and water activity will interfere with the shelf life or durability of the marshmallow and its texture. Substitution of Gelatin with konjac glucomannan will also affect the resulting protein content in marshmallows. Therefore, this study aimed to determine the effect of various gelatin and konjac glucomannan concentrations on moisture content, water activity (a_w), texture, density, and protein content in *L. plantarum* Dad-13 synbiotic marshmallows.

2. Materials and methods

2.1 Materials

Gelatin (Hakiki), konjac glucomannan (El Masta), sucrose (Gulaku), glucose syrup, corn flour (Maizenaku), and skim milk powder containing *L. plantarum* Dad-13 supplied from the Centre for Food and Nutrition Studies of Universitas Gadjah Mada with the production method by Kamil *et al*. (2020).

2.2 Synbiotic marshmallow preparation

Production of the synbiotic marshmallow follows the method described by Santoso *et al*. (2019) with modifications. Gelatin and konjac glucomannan with the concentration as follows: P1 = 9:0; P2 = 8.5:0.5; P3 = 8:1; P4 = 7.5:1.5; P5 = 7:2 %w/v were soaked in 20 mL warm water for 15 mins. The sugar solution was made by boiling 150 g of sucrose, 45 g of glucose syrup, and 45 mL of water at 105-115°C for 15 mins until completely dissolved. The gelatin and konjac glucomannan solution was mixed into the sugar solution with high speed whipped until it was foamy. A total of 15 g of probiotic skimmed milk containing *L. plantarum* Dad-13 was added to the mixture at 40°C, followed by slow whipping until the mixture was evenly whipped. The mixture was then poured into a mould and left to cool for 1 hr at room temperature before being stored in the chiller for 24 hrs. Once solid, marshmallows are sowed with corn flour to decrease the stickiness and then cut and stored in an aluminium-sealed pouch for further analysis.

2.3 Physicochemical analysis

2.3.1 Moisture content

Moisture content was evaluated following SNI number 3547-2-2008, using the gravimetric method (Badan Standardisasi Nasional, 2008). The porcelain cup was heated in an oven at 100°C for 1 hr, cooled in a desiccator for 30 mins, and weighed on an analytical balance (W_0). Then 5 g of sample was weighed in the cup (W_1), heated at 100°C for 3 hrs, cooled in a desiccator for 30 mins, and weighed. This process is carried out until the weight of the sample is constant (W_2). The moisture content was calculated using the

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following formula:

$$\text{Moisture content} = \frac{W_1 - W_2}{W_1 - W_0} \times 100\%$$

Where W_0 = Mass of the cup (g), W_1 = Mass of the sample and cup before heating (g) and W_2 = Mass of the sample and cup after heating (g)

2.3.2 Water activity (a_w)

The samples' water activity (a_w) was measured using the Lab-Master a_w meter (Novasina, Swiss). The sample was put into the a_w meter after the calibration process. The a_w value will be displayed on the screen after the automatic analysis is completed.

2.3.3 Marshmallow density

Marshmallow density was determined according to the method used by Mardani et al. (2019). The sample was cut into 3 × 3 × 1.5 cm pieces. The sample was then weighed and measured for volume. The density was calculated using the following formula:

$$\rho = \frac{m}{v}$$

Where ρ = density (g/cm^3), m = mass (g) and v = volume (cm^3)

2.3.4 Crude protein content

Crude protein content (N×6.25) was measured using the Kjeldahl method (Legowo *et al*., 2005) by weighing approximately 0.5 g of the macerated sample and putting it into the Kjeldahl flask. Then, add 0.3 g of selenium and 10 mL of concentrated H_2SO_4. The Kjeldahl flask is then reconstructed in a smoke cupboard until the color is clear green. After cooling, carefully add 100 mL of distilled water, followed by 40 mL of 40% NaOH. The distillation process is then completed by trapping 5 mL of 4% H_3BO_3 mixed with MR and MB indicators. The distillation process is repeated until 40 mL of distillate is produced. The distillate is then titrated with 0.1 N HCl until a light purple color appears. The process of destruction, distillation, and titration is also performed on blank solutions containing only aqueous and NaOH solutions. Calculation of protein content by the formula:

2.3 Texture profile analysis measurement

$$\%\ \text{Protein} = \frac{(\text{mL HCl sample} - \text{mL HCl blank}) \times \text{N HCl} \times 14.008}{\text{mass sample} \times 1000} \times 6.25 \times 100$$

The texture profile of the marshmallow was evaluated following the method described by Mutlu *et al.* (2018) with some modifications. A texture analyzer with a cylindrical probe (diameter of 38.1 mm) was used in this measurement. The analysis was carried out at room temperature with the setting of pre-test speed: 2 mm/s; test speed: 1 mm/s; post-test speed: 1 mm/s, the distance between probe and sample is 10 mm, and trigger force is 2 g. The measurement was conducted with two compressions with a delay between the first and second compressions was 5 s. Parameters observed included hardness, chewiness, and gumminess obtained from the software provided along with the instrument.

2.4 Data analysis

Statistical analysis was processed by using IBM Statistics SPSS 26.0 for Windows. All the data were analyzed using analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) with a significance level of α = 5%. In addition, Pearson correlation analysis was also conducted to determine the correlations between each variable tested. Statistically different data was considered if the p-value was lower than 0.05.

3. Results and discussion

3.1 Moisture content

The results of the moisture content of all synbiotic marshmallow formulations can be seen in Table 1. According to the result of statistical analysis, adding various concentrations of gelatin and konjac glucomannan had a significant effect ($p<0.05$) on the moisture content. The moisture content of synbiotic marshmallows tends to decrease as gelatin concentration decreases and konjac glucomannan concentration increases.

The concentration of gelatin used influenced the moisture content of marshmallows. A higher concentration of gelatin will increase moisture content (Wang and Hartel, 2022). Gelatin has a polypeptide chain with an amino group at the left and a carboxyl group at the right end, allowing gelatin to absorb water (Laksanawati *et al*., 2022). However, the addition of konjac glucomannan resulted in a decrease in the moisture content of the marshmallows. The formation of a synergistic complex between gelatin and konjac glucomannan, both of which have the ability to bind water, might have been responsible for the decrease in the moisture content of marshmallows. It has been known that konjac glucomannan can bind water through its hydroxyl groups. Liu *et al*. (2019) reported that adding konjac glucomannan at higher concentrations causes a decrease in the moisture content in edible films from gelatin caused by synergistic interactions that may contribute to a decrease in water binding capacity. Hydrogen bonds are formed due to the interaction of the two hydrocolloids (Prateepchanachai *et al*., 2019). Hydrogen bonding between gelatin and konjac glucomannan results in a reduced availability of free

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hydroxyl groups that can bind water molecules, decreasing water content. According to a previous report by Hazirah *et al*. (2016), the hydrocolloid xanthan gum can interact with gelatin via hydrogen bonds, preventing the hydroxyl groups from associating with water and thus decreasing the water content in the gelatin film. This mechanism is also present when gelatin hydrocolloid and konjac glucomannan are combined; hydrogen bonds are formed between the amino groups of gelatins and the hydroxyl groups of konjac glucomannan.

Table 1 shows the moisture content values ranging from 9.74-13.8%. Overall, this study's moisture content of marshmallow samples complies with the Indonesian quality standards regarding soft confectionery items (SNI 3547-2-2008). The maximum moisture content is 20% (National Standardization Agency, 2008). The moisture content of food products will determine the final quality produced. The low water content will also help to extend the shelf life of food longer during storage (Sohaimy *et al*., 2015).

Table 1. The moisture content of synbiotic marshmallow.

Formulation	Moisture content (%)
P1	13.80±1.26^a
P2	11.20±0.72^b
P3	10.36±0.93^b
P4	9.90±0.86^b
P5	9.74±0.99^b

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

3.2 Water activity

Table 2 summarizes the water activity (a_w) of synbiotic marshmallows. Statistical analysis revealed that the addition of gelatin and konjac glucomannan with various concentrations had a significant effect ($p<0.05$) on the water activity of marshmallows. The water activity of marshmallow control (P1) with 9% gelatin and 0% konjac glucomannan had the highest value.

Water activity expresses available water in the system. It is an essential factor for evaluating microbiological and physical characteristics (Kamil *et al*., 2021) and the shelf-life of food products (Mardani *et al*., 2019). The water activity of the synbiotic marshmallow in this study was lower than the marshmallow from previous research by Mardani *et al*. (2019). Synbiotic marshmallows with water activity ranging from 0.607 to 0.619 are considered safe from bacteria, yeast, and mold, which grow at $a_w > 0.90$, $a_w > 80$, and $a_w > 0.70$, respectively. According to Hartel *et al*. (2018), as a confectionery product, marshmallow has water activity in the range of 0.60-0.70. The results (Table 2) showed that the water activity of synbiotic marshmallows decreases as the concentration of gelatin decreases and konjac glucomannan increases. This was due to the reduced availability of water in the synbiotic marshmallow due to hydrogen bonds formed between the amino groups of gelatins and the hydroxyl groups of konjac glucomannan, causing a decrease in water binding capacity. The reduced availability of hydroxyl groups in polysaccharides will limit their interactions with water molecules, hence reducing the product's moisture content (Balasubramanian *et al*., 2018). The decrease in water activity corresponds to the decrease in moisture content. Generally, the lower the moisture content, the lower the water activity value (Beuchat *et al*., 2013).

Table 2. The water activity of synbiotic marshmallow.

Formulation	Water activity (a_w)
P1	0.619±0.008^a
P2	0.609±0.002^b
P3	0.609±0.008^b
P4	0.609±0.001^b
P5	0.607±0.001^b

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

3.3 Density

Table 3 lists the result of the density analysis for synbiotic marshmallows. Statistical test results showed that the use of gelatin and konjac glucomannan at various concentrations had a discernible influence ($p<0.05$) on the density of synbiotic marshmallows. Control synbiotic marshmallow (P1) had the highest density, while the lowest density value was found in the formula of 7% gelatin and 2% konjac glucomannan (P5). In this study, the density of synbiotic marshmallows ranged from 0.77 to 1.13 g/cm^3. These values were slightly higher than the density of regular marshmallows. According to Hartel *et al*. (2018), the density of marshmallow products with high aeration generally decreases to 0.25 g/cm^3 and can reach more than 0.70 g/mL for denser marshmallow products. Marshmallows with lower density values are more desirable and indicate a better quality compared to marshmallows with high density due to a softer and lighter texture when eaten, as well as having a higher volume (Yuddhistira *et al*., 2021). The density value of marshmallows is affected by the concentration of gelatin used in the production. The greater concentration of gelatin resulted in marshmallows with low density because the more air trapped within them, making them lighter. The more gelatin molecules involved, increase the thickness and stability of the air-water interface film, resulting in an increase in foaming properties (Feng *et al*., 2021).

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Table 3. The density of synbiotic marshmallow.

Formulation	Density value (g/cm^3)
P1	0.77 ± 0.18^{a}
P2	0.91 ± 025^{b}
P3	1.06 ± 0.36^{c}
P4	1.08 ± 0.01^{c}
P5	1.13 ± 0.01^{d}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

The addition of konjac glucomannan and the decreasing concentration of gelatin caused the density of marshmallows to increase. Konjac glucomannan is a non-gelatin polysaccharide that cannot form foam; moreover, adding konjac glucomannan in powder can increase the number of solids. This was supported by the observation made by Puvanenthiran *et al*. (2014), who reported that increasing solids in food products would result in higher density. Previous research indicates that adding inulin extract from white potatoes causes an increase in the density of marshmallows. The increment is because white sweet potato inulin extract belongs to a type of fibre containing pectin. This polysaccharide could work as a gelling agent, thus affecting the quality and texture of marshmallows (Yudhistira *et al*., 2021). According to the results, marshmallows with lower density have more pores than marshmallows with higher density (Figure 1). This was due to the trapped air forming pores in the food matrix. The more air is entered, the more pores will be formed (Charcosset, 2021).

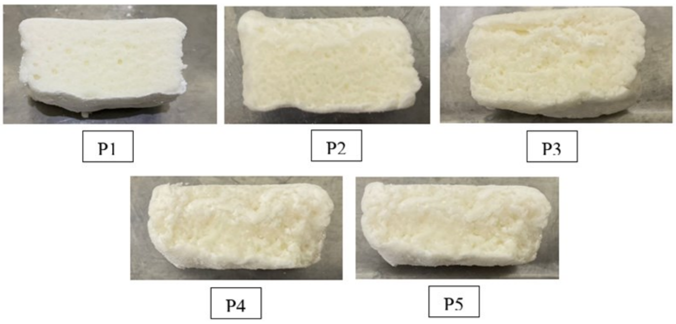

Figure 1. Cross-section of synbiotic marshmallows.

3.4 Texture profile analysis

The most common parameter used to evaluate the acceptability of a food product is texture (Sohini *et al*., 2015). The results for texture parameters can be seen in Table 4. According to statistical analysis, the addition of gelatin and konjac glucomannan at various concentrations had a significant effect ($p<0.05$) on the hardness, gumminess, and chewiness of synbiotic marshmallows.

Control synbiotic marshmallow (P1) produced the highest hardness value of 1535.7 g, while 8% gelatin formulation and 1% konjac glucomannan (P3) produced the lowest hardness. The amount of gelatin used influences the hardness of marshmallows. The addition of gelatin concentration can significantly increase the hardness of the marshmallow because the formed gel is stiffer and more rigid, requiring more force to deform the product (Wang and Hartel, 2022). The gelatin gel network contributes to the hardness of the marshmallow; the gel is formed by the formation of junction zones in a three-dimensional network, which affects the gel's strength. Elsewhere, Santoso *et al*. (2019) reported that the increase in gelatin concentration resulted in a more hardened marshmallow due to gelatin gel network formation contributing toward the hardening of marshmallow texture. As seen in Table 4, the addition of konjac glucomannan decreases marshmallow hardness due to its elasticity. According to Tomczyńska-Mleko *et al*. (2014), a mixture of gelatin and konjac glucomannan produced lower hardness than a gel made solely from gelatin. In a previous report by Kaya *et al*. (2015), the addition of konjac glucomannan produced a gel with higher elasticity due to its ability to reduce the surface tension in the kappa carrageenan and glucomannan gel mixture. The hardness value initially decreased and then increased again when 1.5% konjac glucomannan was added. The increased hardness was likely due to the lower gelatin concentration, which inhibits foam formation. Similarly, He *et al*. (2020) discovered that adding konjac glucomannan initially reduced the hardness of steamed buns, however, it increased again due to the higher concentration of konjac glucomannan, which was caused by the interaction of konjac glucomannan with gliadin protein forming hydrogen bonds, thus reducing gliadin's ability to bind water and decreasing foam volume, resulting in the firmer texture of steamed buns.

Table 4. Texture profile analysis of synbiotic marshmallow.

Formulation	Hardness (g)	Gumminess (g)	Chewiness (g)
P1	1535.7 ± 460.7^{a}	588.7 ± 109.6^{a}	3559.8 ± 71^{a}
P2	$1082.\ 5\pm215.5^{ab}$	316.1 ± 42.2^{b}	1077.2 ± 140^{b}
P3	731.3 ± 68.3^{b}	215.7 ± 13.6^{b}	776.8 ± 167.4^{c}
P4	1224.5 ± 177.3^{a}	315.7 ± 24.3^{b}	996.5 ± 44^{b}
P5	1296.4 ± 412.6^{a}	317.4 ± 81.6^{b}	984.9 ± 186.4^{b}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

Gumminess is a secondary parameter of texture profile analysis. The gumminess value of synbiotic marshmallows is presented in Table 4. Gumminess was measured by multiplying hardness and cohesiveness. Hence gumminess is directly related to hardness (Chandra and Shamsudar, 2015). The Gumminess value

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of the marshmallow was high in P1 with 9% gelatin and 0% konjac glucomannan. In line with hardness, the gelatin concentration also affects the gumminess value. By forming a gel network through the junction zone of a three-dimensional network, gelatin can increase the gumminess value of marshmallows. Once the gelatin is dissolved in warm water, a dispersion forms, resulting in a cross-linking of its helix-shaped chains; this linkage in the gelatin protein network is referred to as 'junction zones' (Du Toit *et al*., 2016). According to Jiamjariyatam (2018), gelatin is required to form a gel network through a junction zone in the production of gummy jelly. Adding konjac glucomannan may contribute to the decreased amino groups of gelatins and the gumminess of marshmallows. The low content of amino acid hydroxyproline is the main reason for weakening the gel strength of gelatin (Sarbon *et al*., 2013). Marshmallows with slightly low gumminess are the most desirable. A high gumminess value causes the gel to stick to the teeth and the hard palate, making it more difficult to chew and swallow (Tomczyńska-Mleko *et al*., 2014).

In addition to gumminess, chewiness is also a secondary parameter of texture profile analysis. The chewiness results from multiplying hardness with gumminess (Li *et al*., 2020). Table 4 shows the chewiness value for all synbiotic marshmallow formulations. The same result as hardness and gumminess was found in the chewiness value. The highest chewiness value was obtained in the 9% gelatin and 0% konjac glucomannan formulation (P1). The chewiness was affected by the gel structure of gelatin (Guo *et al*., 2021). Therefore, the higher the concentration of gelatin, the marshmallow chewiness increased. Gelatin has the ability to form a tangled three-dimensional network of polymer chains, thus allowing it to generate chewy marshmallows (Santoso *et al*., 2019). Since konjac glucomannan does not have the ability to produce a stronger gel network structure in comparison with gelatin, its addition decreased the chewiness of the marshmallow. According to Xie *et al*. (2022), konjac glucomannan was a kind of non-gelatin polysaccharide and could not form a tightly connected gel network structure. A high chewiness value means more energy is required during mastication in order for food to be ready to swallow.

3.5 Protein content

The protein content of synbiotic marshmallows is presented in Table 5. The statistical analysis showed that the use of gelatin and konjac glucomannan at various concentrations had a discernible influence ($p<0.05$) on the protein content of synbiotic marshmallows. The protein content of synbiotic marshmallows ranges from 2.14-3.63%.

The highest protein content was found on marshmallow control (P1), while P5, with 7% gelatin and 2% konjac glucomannan, had the lowest protein content. Gelatin has a significant impact on the protein content of marshmallows. Gelatin is an amino acid-based hydrocolloid derived from the hydrolysis of collagen in the skin, bone, or animal tissue (Godoi *et al*., 2021). The amino acid content increases as gelatin concentration increases. The type of gelatin also influences the protein content. Previous report by Rawdkuen *et al*. (2013), showed that the protein content in beef skin gelatin was 88.3%, which was higher than gelatin from catfish (85.3%) and tilapia (84.3%) but lower than food-grade halal bovine gelatin (90.2%). The protein content in gelatin determines its amino acid content (Pradarameswari *et al*., 2018). The protein content decreased due to the addition of konjac glucomannan. Gelatin and konjac glucomannan molecules could interact by forming hydrogen bonds (Prateepchanachai *et al*. 2019), thereby reducing the availability of amino acid groups and decreasing the protein content.

Table 5. The protein content of synbiotic marshmallow.

Formulation	Protein content (%)
P1	3.63 ± 0.17^{a}
P2	2.84 ± 0.09^{b}
P3	2.41 ± 0.17^{c}
P4	2.32 ± 0.09^{cd}
P5	2.14 ± 0.17^{d}

Values are presented as mean±SD. Values with different superscripts within the same column are statistically significantly different ($p<0.05$).

3.6 Correlation analysis

Pearson correlation analysis was conducted to evaluate the relationship between variables. Table 6 summarizes the results of Pearson correlation analysis between the variables tested. The results of Pearson correlation analysis (Table 6) showed there was a significant correlation ($p<0.001$) between moisture content and water activity (a_w). A correlation value of 0.681 was obtained; based on this analysis, it can be known that the water activity value was 68% influenced by moisture content with strong interpretation. The varying concentrations of gelatin and konjac glucomannan decreased both moisture content and water activity. The data analysis showed a positive correlation (positive r-value), implying that the two variables tested are directly proportional. According to Beuchat *et al*. (2013), foods and food ingredients with low water activity generally have low moisture content.

As seen in Table 6, moisture content and density have a significant correlation ($p<0.001$). A correlation value of 0.782 indicated that moisture content strongly influenced the density of synbiotic marshmallows by 78%. The addition of various concentrations of gelatin and konjac glucomannan decreased water content in synbiotic marshmallows but increased density. The results of data analysis also showed a negative correlation (negative r-value), so it can be interpreted that the two variables tested are inversely proportional, i.e., the lower the water content, the greater the density. Reducing the concentration of gelatin and adding konjac glucomannan can reduce the ability of gelatin to bind water resulting in a soft gel that cannot trap air optimally, thus inhibiting foam formation. Gelling is the process of forming hydrogen bonds through the interaction between semi-solid gelatin molecules and water component; these molecules form multiple crystals to form a three-dimensional network that binds the fluid and forms a strong cross-link, allowing the formation of the gel (Santoso *et al*., 2019).

The results revealed no significant correlation between moisture content and hardness (Table 6). With a low interpretation, the correlation value obtained was 0.205, indicating that the hardness of the synbiotic marshmallow is only 20% affected by moisture content. The decrease in moisture content did not occur in large amounts in each formulation. Thus, it had less of an impact on the hardness of marshmallows. The results also showed that the moisture content and gumminess had a significant correlation ($p<0.001$), as well as the moisture content and chewiness, which had a discernible correlation ($p<0.001$). A correlation value of 0.601 indicates that moisture content affected the amount of gumminess by 60%, with a strong interpretation.

In contrast, a correlation value of 0.789 indicates that moisture content affected the amount of chewiness by 79%, with a strong interpretation. Moisture content, gumminess, and chewiness of synbiotic marshmallows decreased as gelatin concentration decreased and konjac glucomannan concentration increased. This relationship can also be seen by a positive correlation (positive r-value), which can be interpreted as a unidirectional relationship between moisture content, gumminess, and chewiness. The decrease in water content is due to the reduced ability of gelatin to form a sturdy gel structure as a result of the decreased gelatin concentration and the addition of konjac glucomannan, therefore lowering the gumminess and chewiness values. The chewiness value is affected by the gel network's structure (Guo *et al*., 2021). According to a previous study by Hazirah *et al*. (2016), hydrocolloids such as xanthan gum can interact with gelatin and inhibit its association with water, causing a decrease in the moisture content of the gelatin film.

Table 6 shows a significant correlation ($p<0.001$) between hardness and gumminess. The two variables are highly correlated, as evidenced by the correlation value of 0.832, indicating that the hardness value influenced the gumminess of the synbiotic marshmallow by 83%. According to the Pearson correlation analysis results, there was also a significant correlation between chewiness and hardness ($p<0.05$). The correlation value was 0.547, so it can be interpreted that the chewiness value is influenced 55% by hardness with a moderate interpretation. It can also be seen from the results of the Pearson correlation analysis that chewiness and gumminess also have a significant correlation ($p < 0.001$). The correlation value of both variables is 0.908, which means that the gumminess value strongly influences the chewiness value by 91%. The data analysis also revealed a positive correlation (positive r-value), indicating that the three variables tested were in line. The lower the hardness, the lower the gumminess and chewiness value. According to Chandra and Shamsudar (2015), gumminess is produced by multiplying hardness and cohesiveness, this is also supported by the opinion of Li *et al*. (2020) that chewiness is a product of hardness multiplied by gumminess, so the variation trend of the three parameters is similar.

Table 6 shows a discernible correlation ($p<0.001$) between synbiotic marshmallows' moisture content and

Table 6. Pearson correlation analysis results.

	Pearson correlations					
	MC	a_w	Density	Hardness	Gumminess	Chewiness
a_w	0.681**					
Density	-0.782**	-0.723**				
Hardness	0.205	0.416	-0.312			
Gumminess	0,601**	0.689**	-0.729**	0.832**		
Chewiness	0.789**	0.779**	-0.846**	0.547*	0.908**	
PC	0.895**	0.745**	-0.946**	0.313	0.725**	0.872**

** Correlation significant at the 0.001 level (2-tailed)
* Correlation significant at the level 0.05 (2-tailed)
MC: Moisture content, PC: Protein content

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protein content. The correlation value of 0.895 indicates that the amount of moisture content is influenced 89% by protein content, with a powerful interpretation. The data analysis results also showed a positive correlation, so it can be interpreted that the two variables tested are directly proportional. The moisture content decreases due to the reduced amino acid content, thereby inhibiting gelatin from binding to water in gel formation. According to Rafieian *et al*. (2015), hydrogen bonds between water molecules and the hydroxyl groups of gelatin amino acids can affect the strength of the gel formed.

The results showed that water activity significantly correlated with protein content ($p < 0.001$). Both of these variables are correlated with a strong interpretation seen from the correlation value of 0.745, indicating the amount of protein content is affected 74% by moisture content. Using gelatin and konjac glucomannan formulations with various concentrations reduced protein content and water activity. The reduction of amino acids impacts moisture content because gelatin molecules cannot sufficiently associate with water. In contrast, a decrease in moisture content affects water activity's value.

The results in Table 6 showed that density and water activity are significantly correlated ($p < 0.001$). A correlation value of 0.723 was obtained, revealing that moisture content affects protein by 72%, with a strong interpretation. Both variables have an inversely proportional relationship seen from the negative correlation value. The decrease in water activity value correlates with a decrease in water content (Beuchat *et al*., 2013). The lower water content affects the formation of a strong gelatin gel structure, resulting in poor air capture and causing an increase in density.

According to the results presented in Table 6, there was no significant correlation between water activity and hardness. Water activity influences hardness by 42%, seen from the correlation value of 0.416, indicating water activity's low impact on hardness. Due to the low amount of water activity decrease in each formulation may have caused no significant effect on hardness. However, there was a significant correlation between water activity, gumminess, and chewiness ($p < 0.001$). The correlation value between water activity and gumminess is 0.689, indicating that water activity affects gumminess by 69%, with a strong interpretation. The correlation value of water activity with chewiness is 0.779, indicating that water activity strongly influences chewiness by 78%. The data also revealed a positive correlation which can be interpreted between water activity, gumminess, and chewiness inline relationship. The decrease in gumminess and chewiness is due to the structure of the gelatin gel, which is softened due to inhibition of the hydrogen bonds formed between gelatin and water molecules, resulting in the decrease of moisture content and water activity. Production of jelly with a gummy texture requires gelatin to form a gel network through the junction zone (Jiamjariyatam, 2018).

Table 6 shows a significant correlation between density and protein content ($p<0.05$). A correlation value of 0.946 was obtained, indicating that protein content strongly influences the density value by 95%. The decrease in protein content affects the formation of foam. Hence the marshmallows produced are denser and have greater density. The analysis also revealed a negative correlation between the two variables. Jansens *et al*. (2019) reported that the ability of the protein to adsorb quickly at the air-water interface influences foam formation.

The correlation analysis between density and hardness revealed no significant correlation (Table 6). The value of hardness is only affected 31% by density with low interpretation. The increase in density value may not have been major enough to give a significant impact on hardness. The results showed a significant correlation between variable density with gumminess and chewiness ($p < 0.001$). Density affects the gumminess value by 73% with a strong interpretation, while density influences the chewiness value by 85% with a robust interpretation. Reduced gelatin concentration and increased konjac glucomannan concentration resulted in a higher density due to the gel's soft structure, weakening its ability to trap air; the marshmallows formed heavier, denser, and the texture of the chewiness and gumminess of the marshmallows decreased. The data also revealed a negative correlation. According to Eddy and Editya (2020), the higher the gelatin concentration, the decreased density value and the higher the chewiness texture.

The results in Table 6 show that variable hardness and protein content showed no significant correlation. The results showed that protein content had a 31% effect on hardness, with a low interpretation. This is likely due to a decreased protein content that was not significant enough to affect hardness. However, there was a significant correlation between gumminess, chewiness, and protein content ($p<0.001$). The correlation value showed that protein content influences gumminess by 73% with a strong interpretation, while protein content influences chewiness by 87% with a very strong interpretation. The protein content contributed to the texture of the finished marshmallows. The increase in gelatin concentration causes protein molecules to cross-link more tightly to form a linkage or network, increasing gumminess and chewiness, thus requiring more energy to chew and break the gummy before swallowing it (Kumkong *et al*., 2020).

4. Conclusion

In this study**,** a synbiotic product in the form of a marshmallow was developed by conjoining probiotic *L. plantarum* Dad-13 and konjac glucomannan as a prebiotic. The formulation of gelatin and konjac glucomannan with various concentrations significantly affected moisture content, water activity, density, texture (hardness, gumminess, chewiness), and protein content on synbiotic marshmallows. A combination of 8.5% gelatin and 0.5% konjac glucomannan was the most preferred formula to produce synbiotic marshmallows with the best characteristics in terms of low moisture content and water activity, decent density and protein content, and slightly low hardness, gumminess, and chewiness. According to the results, a synbiotic marshmallow with *L. plantarum* Dad-13 as a probiotic and konjac glucomannan as a prebiotic can be developed as a future innovation.

Conflict of interest

All authors declare there is no conflict of interest.

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