Proteomic Examination of Cheese Slices With Smart Active Coating and Enhanced From Pumpkin *(Cucurbita moschata)*

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Abstract. Degenerative diseases are the number one disease in the world, especially in Indonesia, which causes almost 17 million people to die. One of the preventive efforts is by consuming foods that contain antioxidants, such as beta-carotene. This study aims to determine the activity of beta carotene and proteomic studies on cheese slices with the addition of beta carotene with treatment P0 (control), P1 (5% beta carotene), P2 (10% beta carotene), P3 (15% beta carotene). The variables of this research include proteomic analysis, fat content, water content, IC50 antioxidant activity, pH, WVP, antimicrobial, transparency, tensile strength, solubility, thickness, shelf life, and color. The research method used RAL with four treatments and three replications as a laboratory experiment. The research showed that the chromatogram of cheese slices with microbial rennet and the addition of beta carotene identified using LC-MS/MS showed that the peptides are β-casein and phenylalanine. The antioxidant test results showed that the greater the percentage of beta carotene addition, the better the IC50 value. The addition of beta carotene from pumpkin extract had a significant effect ($P<0.01$) on the antimicrobial properties of the coating, thickness, and fat content in cheese slices. Adding beta carotene from pumpkin extract significantly affects ($P<0.05$) pH and WVP values. However, the addition of beta carotene did not have a significant effect ($P>0.05$) on the water content, antioxidant activity of cheese slices, solubility, transparency, and tensile strength of the coating.

1 Introduction

People's unhealthy lifestyles cause various diseases in the body. One is a degenerative disease, ranked in the top 10 in Indonesia. Degenerative diseases are non-communicable diseases that last chronically with a decline in cell and organ function.

According to WHO, degenerative diseases have caused the deaths of nearly 17 million people worldwide, making degenerative diseases the biggest killer in the world. Therefore, efforts are needed to prevent degenerative diseases from occurring. One way is to consume foods that contain antioxidants. Antioxidants are compounds that can inhibit or prevent the

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oxidation process of other compounds, which produce free radicals that can damage cells. Natural antioxidants can also inhibit lipids' oxidation, which can damage food and become rancid. This natural antioxidant is obtained by extracting plants that contain antioxidants; one of the compounds contained in antioxidants is beta-carotene. Beta carotene is an antioxidant that inhibits free radicals, which can cause disease, especially degenerative diseases [9]. Beta carotene is also the primary precursor for vitamin A in humans. Beta carotene also positively affects degenerative diseases [5]. Beta carotene is naturally obtained from vegetables and fruit. One source of beta-carotene that is good for human consumption is pumpkin [6]. The high value of beta carotene in pumpkin is very good for food additives, which can inhibit lipid oxidation, which causes undesirable taste, loss of color, and food nutrition. This event was also explained by Rakcejeva et al. [13], who explained that the content of pumpkin is high in beta carotene and moderate in carbohydrates, vitamin B6, and K thiamine.

However, because beta carotene chemically has low solubility in water and is sensitive to heat, light, and oxygen, it isn't easy to use directly in food [2]. Therefore, efforts are needed to utilize the beta carotene compound in pumpkin to inhibit the rate of oxidation, which can be used indirectly in food. One way is using beta carotene with other compounds used in food packaging that are easily oxidized, such as cheese slices. Cheese slices are cheese in sheets, usually added to food or eaten directly. Because of its thin shape, cheese slices are easily oxidized and damaged. Additional control methods are needed to quickly inactivate pathogens on the surface of cheese slices after the packaging stage, such as packaging or coating with antimicrobial materials, which are a more effective solution for controlling microbial contaminants on the cheese surface, compared to adding preservatives to food. Antimicrobial activity is based on reducing the pH of food, which can suppress microbial growth. A natural polymer that can suppress microbial growth and oxidation in cheese slices is needed. Adding pumpkin beta carotene to the cheese slice coating is expected to suppress microbial growth and oxidation on the surface of the cheese slice and increase its shelf life. The coloration of beta carotene pigment could change under oxidizing agents (from orange to light yellow), indicating that color change is a factor in determining expiration time [1]. Based on the description above, this research aims to analyze the activity of pumpkin beta carotene as an intelligent coating on cheese slices and a proteomic analysis of functional cheese slices based on smart coating.

2 Materials and Methods

2.1 Materials

The materials used in this research were pumpkin fruit, skim milk powder, gelatin, LAB starter, microbial rennet, table salt, sp, cheddar cheese, maltodextrin, lactic acid, potassium sorbate, 70% alcohol, methanol, distilled water, glycerol, palm oil o, DPPH, H2SO4, ascorbic acid, kappa-carrageenan, vaseline, paraffin, beta carotene (Sigma), MCA (Mac Conkey Agar).

2.2 Methods

The method used in this research used a laboratory experimental method with a completely randomized design with four treatments, namely P0 (control), P1 (5% beta carotene), P2 (10% beta carotene), P3 (15% beta carotene) and three replications. The stages include extracting beta carotene, making functional cheese slices, making smart active coating, and testing. The results obtained were analyzed using ANOVA, and if differences were found, then further analysis was carried out using Duncan's Multiple Range Test.

3 Results and discussion

3.1 Identification of Beta Carotene in Pumpkin Extract

Beta carotene was identified using visible spectrophotometry with a wavelength of 450 nm and a maximum absorbance of 0.554. The results obtained from the value of beta carotene content from pumpkin extract were 10.27%. Previous research stated that the beta carotene value in yellow pumpkin was 14.59% [7].

3.2 LC-MS/MS Proteomics Assay

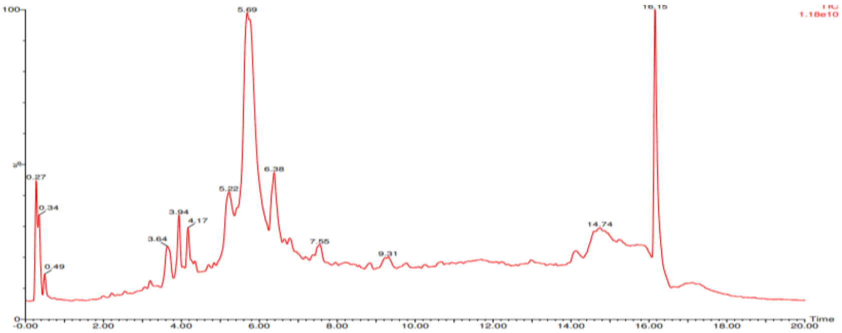

Fig. 1. LC-MS/MS TCI Chromatogram (Total Ion Chromatogram) of cheese slice samples with the addition of beta carotene by LC-MS/MS.

Figure 1 is a chromatogram of cheese slices with microbial rennet and the addition of beta carotene, which was identified using LC-MS/MS as being able to identify and quantify the proteome of complex proteins in the cheese slice matrix for characterization of casein micelles that are hydrolyzed in making cheese slices. The chromatogram identified the highest peaks at retention of 5.69 minutes and 16.15 minutes, with molecular mass ion values of 83.26 and 143.36 m/z, respectively. Interpretation of the LC-MS/MS chromatogram shows the presence of β-Casein and phenylalanine peptides, which are indicated as the result of microbial hydrolysis of rennet on casein micelles and several organic acids which are indicated as the result of enzyme activity during the cheese fermentation and coagulation process as well as in the process of making cheese slices.

3.3 Test Smart Active Coating

Based on the research that has been carried out, results were obtained regarding the effect of adding beta carotene from pumpkin extract on the antimicrobial value and water vapor permeability (WVP) of bright, active coating. The average antimicrobial and WVP values of smart active coating can be seen in Table 1.

Based on Table 1, adding beta-carotene pumpkin extract has a very significant effect ($P<0.01$) on the antimicrobial properties of the smart active coating. The resulting inhibitory response is vital, so it can be stated that the sample is suitable for food packaging material because it can make food last longer and protect against unwanted bacteria such as E.coli. The highest clear zone value in treatment P3 was 20.467, with the lowest value in P0 being 6.175. The addition of beta carotene from pumpkin extract significantly affects ($P<0.05$) the WVP value. Jafarzadeh et al. [3] explained that the barrier properties of packaging have an essential role in maintaining shelf life by minimizing the transmission of moisture from the surrounding environment, so it is essential to know the water vapor permeability. The WVP value for all treatments meets the Japanese Industrial Standards (1975), which states that the maximum WVP value is 7 G/M2/day. Other factors that influence the WVP value are also explained by Tran et al. (2020), who explain that adding filler and glycerol will slow down the rate of water vapor passing through the coating. Packaging materials can cause an increase in the WVP value during storage, which can be influenced by the higher permeability of the packaging material to water vapor, the hygroscopic properties of the packaged material, and the level of environmental air humidity for the food (Lobo et al., 2013).

Table 1. Analysis values of antimicrobial smart active coating and WVP tests

Treatment	Average Antimicrobial	Average WVP
P0	6,175±0,843^{a}	0,23±0,036^{a}
P1	11,053±0,926^{b}	0,15±0,081^{ab}
P2	14,737±0,515^{c}	0,09±0,125^{b}
P3	20,467±0,712^{d}	0,13±0,048^{b}

Based on the research that has been carried out, results were obtained regarding the effect of adding beta carotene from pumpkin extract on the values of tensile strength, solubility, transparency, and thickness in smart active coatings. The average tensile strength, solubility, transparency, and thickness values for smart active coatings are presented in Table 2.

Table 2. Analysis values of innovative active coating tests for tensile strength, solubility, transparency, and thickness'

Treatment	Average Tensile Strength	Average Solubility	Average Transparency	Average Thickness
P0	0,875±0,419	2,167±0,357	0,059±0,084	0,153±0,008^{b}
P1	0,727±0,252	2,534±0,967	0,150±0,026	0,141±0,005^{b}
P2	0,720±0,204	2,675±0,714	0,126±0,008	0,134±0,004^{ab}
P3	0,649±0,195	3,327±0,268	0,262±0,092	0,116±0,013^{a}

The addition of yellow pumpkin beta carotene extract with different concentrations had a significant effect ($P<0.05$) on the thickness of the smart active coating. Based on the analytical and quantitative results in Table 2, the highest thickness value was 0.154 mm in the P0 treatment without the addition of beta carotene extract, and the lowest value was 0.117 mm in the P3 treatment with the addition of 15% beta carotene extract. This is comparable to the statement Putri et al [12] that the thickness of the coating decreases as the concentration of active ingredient extract increases. The maximum coating thickness value of Japanese Industrial Standards [4] is 0.25 mm. The thickness values of the smart active coating from all treatments have met the standards. The addition of yellow pumpkin beta carotene extract with different concentrations did not have a significant effect ($P<0.05$) on the solubility of the smart active coating. The highest solubility value was 3.328% with the addition of 15% beta carotene extract in treatment P3, while the lowest average value was 2.168% in treatment P0 without the addition of beta carotene extract. Coatings with high solubility are suitable for ready-to-eat food products because, when consumed, they will dissolve quickly. Based on Japanese Industrial Standards [4], the coating category for food packaging has a maximum solubility in water of 14%. The best treatment is P3 treatment with 15% beta carotene extract. The addition of yellow pumpkin beta carotene extract with different concentrations had no significant effect on the transparency of the intelligent, active coating. The highest transparency value was 0.787 in treatment P3, while the lowest average value was 0.179 in treatment P0, presented in Table 2. Warkoyo et al. [15] stated that transparency tends to increase as the concentration of active ingredients increases. The tensile strength value of smart active coating with the addition of beta carotene extract is 0.649 Mpa to 0.875 Mpa; all treatments have met coating standards; according to Japanese Industrial Standards [4], the minimum tensile strength value is 0.39 Mpa. The data obtained shows that the tensile strength of the coating decreases as the concentration of the added extract increases; this is because the beta-carotene extract interferes with the formation of the smart active coating matrix.

Based on the research that has been carried out, results were obtained regarding the effect of adding beta carotene from pumpkin extract on the pH value of smart active coating. The average pH value of smart active coating is presented in Table 3.

Table 3. Analysis values of innovative active coating tests for tensile strength, solubility, transparency, and thickness'

Treatment	Average pH
P0	18,45±0,18^{d}
P1	16,79±0,10^{c}
P2	14,61±0,20^{b}
P3	12,88±0,20^{a}

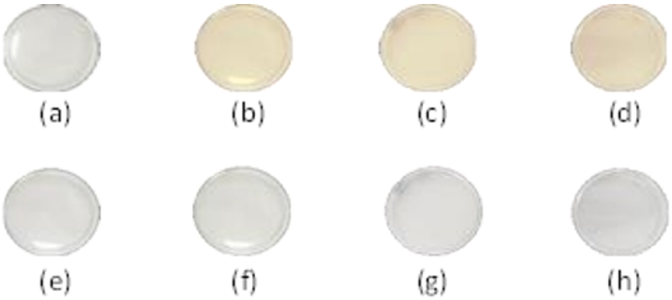

Fig. 2. Color Test (a) P0 (0 days), (b) P1 (0 days), (c) P2 (0 days), (d) P3 (0 days), (e) P0 (8 days), (f) P1 (8 Days), (g) P2 (8 Days), (h) P3 (8 Days).

Based on Table 3, pH testing, the addition of beta carotene from pumpkin extract had a significant effect ($P<0.05$) on the pH value of the smart active coating. The pH value of the smart active coating has a lower average as the addition of beta carotene increases. The addition of 15% beta carotene extract to functional cheese slices based on smart active coating showed that shelf life decreased after eight days (Figure 2). Meanwhile, for functional cheese slices without additional coating, shelf life decreased after 24 hours. β-Carotene is based on proper physico-chemical bonds; apart from that, it aims to preserve and extend the shelf life of food [2].

3.4 Functional Cheese Slice Test

Based on the research that has been carried out, results were obtained regarding the effect of adding beta carotene from pumpkin extract on the values of water content, fat content, and antioxidant activity in functional cheese slices. The average values of water content, fat content, and antioxidant activity in functional cheese slices are presented in Table 4.

Table 4. Analysis of functional cheese slice testing

Treatment	Average Water Content	Average Fat Level	Average Antioxidant Activity (IC50)
P0	45,26±0,295	31,84±0,917^{ab}	1,353±0,603
P1	45,30±0,781	31,75±0,808^{ab}	1,259±0,081
P2	44,87±0,277	34,11±0,793^{b}	1,174±0,177
P3	45,37±0,509	29,86±0,861^{a}	1,084±0,554

Based on Table 4, the water content test shows that the addition of beta carotene had no significant effect ($P>0.05$), with water content results between 44.87%-45.37%. The lowest water content was in treatment P2 with the addition of 10% beta carotene, which shows that treatment P2 had a low whey content. This value follows the standard for processed cheese: a maximum water content of 45% [11]. The fat content test showed that adding beta carotene had a significant effect ($P<0.01$), with fat content results between 29.86%-34.11%. The P3 treatment with 15% beta carotene added the highest fat content, with the minimum processed cheese fat content standard being 25% [11]. This research shows that fat content results are inversely proportional to water content. The lower the fat content of the functional cheese slice, the higher the water content, and vice versa [17].

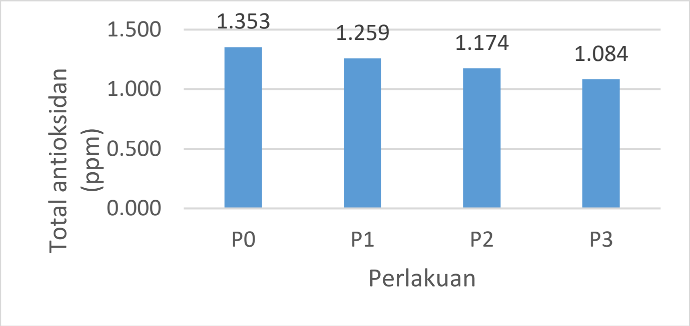

Fig. 3. Antioxidant activity IC50 with the addition of different beta carotene (0%, 5%, 10%,15%).

In the antioxidant activity test (IC50), Figure 3 shows results between 1,084-1,353 ppm. The lower the IC50 value, the better the antioxidant activity [10]. Beta carotene has high antioxidant activity. The highest value in the P3 treatment with the addition of 15% beta carotene had the most potent antioxidant value of 1.054 ppm at the IC50 value. If a compound has an IC50 value of less than 50 ppm, it has potent antioxidant activity and is said to have potential. Still, the antioxidant activity could be better if it has an IC50 value of more than 200 ppm [16].

4 Conclusion

The addition of beta carotene from pumpkin extract showed that the best treatment in the antimicrobial test had a significant effect ($P<0.01$) on the antimicrobial properties of the coating and the fat content value of the functional cheese slice. The addition of beta carotene from pumpkin extract significantly affected ($P<0.05$) pH, WVP, and thickness values. However, the addition of beta carotene did not have a significant effect ($P>0.05$) on the water content and antioxidant activity of the IC50 cheese slice's functional and solubility, transparency, and tensile strength of the coating. The results of the functional cheese slice chromatogram with microbial rennet and the addition of beta carotene identified using LC-MS/MS were able to identify and measure the complex protein proteome in the functional cheese slice matrix for characterization of hydrolyzed casein micelles in making functional cheese slices and the antioxidant test results showed that the greater the percentage of beta carotene added means the IC50 value gets better.

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