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Texture Improvement of Filamentous Fungi Burger Derived from Biomass of *Rhizopus oligosporus*: Impact of Binding Agent on Physical, Sensory, and Microstructure

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Abstract

Despite its high nutritional value and low environmental impact, the nature of filamentous fungal hyphae might resemble the appearance of meat fibers. These properties make filamentous fungi as a promising protein alternative in the future. However, the texture of the mycelium is too weak to resemble meat fibers. This study was aimed to obtain non-animal binding agent to improve the texture of burger patty produced by fungal biomass of *Rhizopus oligosporus*. Different binding agents including sodium tripolyphosphate, transglutaminase, pectin, and modified tapioca were added to the fungi burger patty. The texture was analyzed by texture profile analyzer and sensory test. The texture profile analysis (TPA) showed that the addition of 10 % modified tapioca starch improved the patty structure, decreasing cooking loss by 46.05 %, while increasing hardness by 105.15 %, and chewiness by 83.55 %. Microstructure images confirm this finding by showing a compact and dense structure with discernible reduce in air pockets. This finding is in line to sensory evaluation result, in which the panels confirmed the improvement of fungi burger hardness. The Fourier Transform Infrared Spectroscopy (FTIR) result showed that the improvement might be due to the increase in hydrophobic interactions, hydrogen bonds, and disulfide bonds. These bonds were responsible for creating and stabilizing the structure of meat analogue. Overall, modified tapioca is a low-cost binding agent for texture improvement of fungi burger made from *R. oligosporus* biomass.

Keywords: Filamentous fungi, Burger patty, *Rhizopus oligosporus*, Modified tapioca starch

Introduction

The world's population is predicted to increase from 7.7 billion in the present to 9.7 billion by 2050 [1]. The rapid growth of the world's population poses a significant challenge and threat to food security. According to Godfray *et al*. [2], 1 in 7 people worldwide does not get enough calories and protein from their regular diet. At the same time, our existing animal-based food system is highly inefficient and environmentally damaging. The industry is only able to meet 37 % of the world's protein needs, while using more than 83 % of agricultural land and contributing to 56 - 58 % of global carbon emissions [3]. The high demand for land consequently leads to the conversion of forests, wetlands and grasslands into agricultural land, which has negative impacts on biodiversity. This leads to the search for environmentally friendly alternative protein sources. Plants such as soy and peas as protein alternatives often require extensive processing to achieve the desired meat-like texture, resulting in nutritional losses [4]. In contrast, filamentous fungi are interwoven fungal mycelia that can be easily molded into a meat-like fibrous structure.

The whole biomass of filamentous fungi which is produced by submerged liquid fermentation contains a high protein and safe for human consumption [5]. It contains a wide variety of amino acids, which makes it an excellent source of protein for the development of muscles [6]. Dunlop *et al*. [7] reported that the bioavailability of the amino acids in filamentous fungal biomass is comparable to that of milk. From an environmental perspective, this type of protein requires only one tenth of the water used by the meat industry [8]. The 1st filamentous fungus used for commercial production of fungi burger is *Fusarium venenatum*. This fungus was isolated from soil and was not previously consumed by humans. It has therefore taken many years to ensure that the fungus is safe and can be used in mass production. On the other hand, there are about 3,000 species of edible fungi, of which 200 species have been consumed by humans, one of which is *R. oligosporus* [9].

R. oligosporus has been employed for millennia in the production of tempeh, a fermented soybean product originating from Indonesia. The fungus rapid growth, neutral taste and GRAS (Generally Recognized as Safe) status make it a potentially useful addition to the food industry. Additionally, this fungus has the capacity to utilize food industry by-products as a sole carbon source, thereby creating potential for the implementation of circular economy systems [10]. Additionally, the elongated hyphae of *R. oligosporus* facilitate the formation of a fibrous structure. However, the intrinsic texture of filamentous fungal biomass is naturally soft, rendering it dissimilar to the texture of authentic meat. Several key factors affecting the texture quality of filamentous fungi patties as meat analogue include hardness, cohesiveness, and chewiness [11]. To enhance the cohesiveness and overall texture of a meat analogue, the molecular bridges between mycelium should be established, necessitating the incorporation of binding agents.

The most prevalent binding agent utilized in the fungal burger industry currently is albumen derived from egg white [11], which makes the product unappealing to the vegan market. Consequently, this study aimed to investigate the potential of alternative non-animal binding agents, including sodium tripolyphosphate, transglutaminase, pectin, and modified tapioca. In addition, the morphology of *R. oligosporus* is different to *Fusarium venenatum* which might requires different type of binding agent. The product selected for development in this research is the burger patty, which is a particularly popular imitation meat product [13]. Sodium tripolyphosphate has the capacity to form a cross-link with chitosan, which constitutes the primary component of the cell wall of *R. oligosporus* [14]. Transglutaminase has the capacity to bind lysine and glutamine residues of disparate polypeptide chains, thereby enhancing the textural integrity of textured plant protein products [4]. Pectin and modified tapioca are both carbohydrate polymers that are commonly added to plant-based meat alternatives for the purposes of binding water, improving texture, and reducing syneresis [4]. To characterize the *R. oligosporus* patty added with different types of binding agents, a series of analytical techniques were employed, including cooking loss, TPA, scanning electron microscopy (SEM), FTIR, and a sensory scoring test. The current study provides insight on the interaction between fungal mycelium and binding agents which affect the texture of burger patty made from whole biomass of *R. oligosporus*.

Materials and methods

Materials

Oat flour for *R. oligosporus* cultivation was purchased from a local market in Sweden. Glucose and rapeseed oil were also utilized during the fermentation process. Potato dextrose agar (PDA) was used to prepare the *R. oligosporus* inoculum. Ingredients for the patty formulation such as shallot, barbeque sauce, nutmeg powder, paprika powder, garlic powder, coriander, salt, mushroom bouillon, olive oil, and onion were all acquired from local market (Mirota Kampus, Yogyakarta).

R. oligosporus biomass fermentation setup

For submerged fermentation in a 1,200 L capacity bubble column reactor, scale-up was first carried out from a shake flask to a 26 L reactor and then to a 1,200 L reactor (modified from [15,16]).

Pre-inoculum was prepared prior to seeding. Commercial strain of *R. oligosporus* was cultivated on PDA at 30 °C for 3 days. The spore suspension was made by diluting the spore with 20 mL sterile distilled water. Subsequently, the 2 mL spore suspension was inoculated into ten 250 mL flasks each with 100 mL media containing 5 g/L glucose and 5 g/L yeast extract. The flasks were incubated for 24 h to obtain 1 L pre-inoculum for 26 L reactor [16].

The 26 L bubble column bioreactor (2 m high×15 cm diameter, Bioengineering AG, Wald, Switzerland) was sterilized by steam injection at 130 °C for 20 min [16]. The media containing 10 g/L glucose and 10 g/L yeast extract was wet sterilized using an autoclave at 121 °C for 20 min. Afterwards, the pre-inoculum was fed into the reactor alongside the media with the addition of 100 mL oil. Total volume of the mixture was 20 L, leaving 6 L of the headspace inside the reactor. Cultivation was performed at 35 °C for 24 h without pH modulation and aeration rate was 0.5 vvm (volume of air per volume of liquid per minute). Biomass from the 26 L reactor was used to inoculate the 1,200 L bioreactor [16].

Sterilization of 1,200 L airlift bioreactor (4 m high×0.65 m diameter, Process- & Industriteknik AB, Kristianstad, Sweden) was performed in 2 cycles. At the 1st cycle, empty reactor was sterilized with injected steam at 121 °C for 20 min [15]. While at the 2nd cycle, the reactor with substrate consisted of 30 g/L oat flour and 1.5 L cooking oil were sterilized with the same method as the 1st cycle [15]. Biomass suspension in the 26 L reactor then transferred into the 1200 reactor as a seeding. Fermentation was performed at 35 °C for 48 h without pH modulation and aeration rate was 0.45 vvm. After the fermentation, biomass was harvested using a stainless-steel kitchen sieve with 1 mm^2 pore area and then weighed. The suspension from the bioreactor was sieved with a vibration screen to recover the solid. The wet biomass was then pressed using a 12 L juice press to reduce the water content and stored afterwards in a freezer.

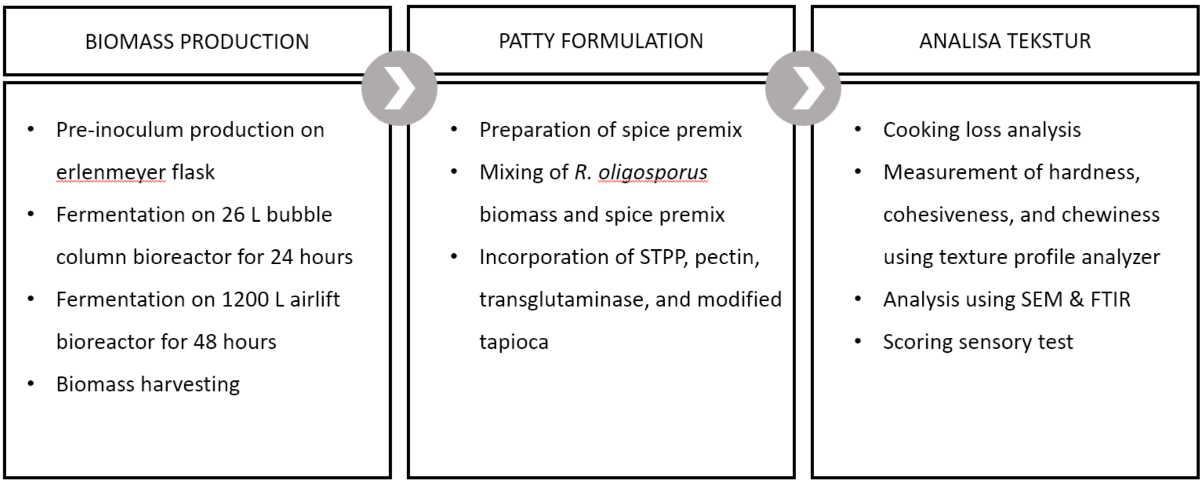

Figure 1 Overview flowchart of research method from biomass production, patty formulation, and analysis performed.

***R. oligosporus* patty and beef patty formulation**

R. oligosporus frozen biomass was thawed prior to the burger patty production. Afterwards, the biomass was manually pressed by hand to further lower the water content. Spice premix were prepared separately with the biomass. The formula of spice premix was 4.5 - 4.6 % shallot, 7.4 - 7.5 % barbeque sauce, 0.2 - 0.3 % nutmeg powder, 2.5 - 2.6 % paprika powder, 4.3 - 4.4 % garlic powder, 0.6 - 0.7 % coriander, 1.1 - 1.2 % salt, 0.8 - 0.9 % mushroom bouillon, 0.3 - 0.4 % olive oil, and 4.5 - 4.6 % onion. A food processor was used to prepare the spice premix. The fungi biomass was mixed with the spice premix by hand until a homogenous patty was obtained. Sodium tripolyphosphate (2.2 g/kg), pectin (1.5, 3, and 4.5 %) with calcium chloride flakes (added at 25 % of the pectin amount), transglutaminase (0.5, 1.5, and 2.5 %), and modified tapioca starch (5, 10, and 15 %) were then added into the patty mixture, and the samples were labeled accordingly. The patty samples were mixed evenly with the binders and molded into block shapes, each measuring 30×30 ×20 mm^3. A beef patty was also prepared using the same spice premix with identical dimensions to serve as a reference. All samples were stored in the freezer until analysis.

Cooking loss of *R. oligosporus*, beef, and commercial vegan patty

R. oligosporus, beef, and commercial vegan patties were thawed at 4 °C for 24 h and weighed before cooking. The cooking process was performed using laboratory hotplate with temperature set at 200 °C. Each sample was cooked without an oil for 10 min and flipped every 1 min to ensure the samples were cooked evenly. Cooked samples were weighed. The percentage of weight lost after cooking was defined as cooking loss. The experiment was conducted in duplicate.

TPA of *R. oligosporus*, beef, and commercial vegan patty

Cooked samples were subjected for the TPA using LLOYD TA1 Texture Analyzer (Ametek, Inc., Florida, USA) to measure hardness, cohesiveness, and chewiness. The dimensions of all samples were kept as homogeneous as possible. Each sample underwent a double compression cycle with test conditions were including 50 % deformation rate, compression speed of 0.5 mm/s, and 0.5 s wait time between 2 compression steps. Experiment was conducted in duplicate.

FTIR of *R. oligosporus* patty

FTIR spectroscopy was performed to investigate the bonds formed by the addition of binding agents that ameliorated the *R. oligosporus* patty texture. Samples were placed inside the FTIR spectrophotometer (Thermo Scientific Nicolet iS10, USA) sample compartment and scanning were performed in the wavelength of 4,000 - 650 cm^{-1} with KBr as the beam splitter. Scanning without samples was also conducted to obtain the background spectrum.

SEM of *R. oligosporus*, beef, and commercial vegan patty

Cooked *R. oligosporus* patty samples were observed under the SEM (JEOL JSM-6510LA, JEOL Ltd., Tokyo, Japan) to gain insights of changes in microstructure by addition of binding agents. Samples were mounted on top of the carbon tape inside the specimen holder and then dried using vacuum machine with pressure of –50 kPa for 1 h. Dried samples were subjected to coating process using an auto coater (120 s, 20 mA). The cross-section of each sample was finally observed with SEM. Microstructure images of the samples were captured at magnifications 100× with operation energy of 10 kV.

Sensory test of *R. oligosporus* and commercial vegan patty

A sensory test was performed to figure out does the results from an instrument are in consonance with consumers' perception of real-life consumption. Quantitative sensory test was performed with 60 nontrained consumer panels at the Sensory Evaluation Laboratory. Participants were informed about the tested products, test protocols, and the possibility of mushroom allergen prior to the tastings. A tray contained 6 different samples labelled with random 3-digit codes, plain crackers, and drinking water then served to each of the participants. The tested samples were a commercial vegan patty, *R. oligosporus* patty, and *R. oligosporus* patties enhanced with 4 different kinds of binding agents (2.2 g/kg sodium tripolyphosphate, 0.5 % transglutaminase, 3 % pectin, and 10 % modified tapioca). Participants were asked to give a score to 3 different texture attributes of each sample using a 7-point scale. An online form was provided to aid the participants with recording the sensory test results. A comment section on each sample was also provided to accommodate additional opinions of panel members regarding the tested samples.

Statistical analysis of the data

Statistical analysis was performed with IBM SPSS software version 25 (IBM Corporation, New York, NY, USA, 2017). Comparisons of means were made using one-way analysis of variance (ANOVA). A post hoc comparison between commercial vegan patty, *R. oligosporus* patties, and beef patty was carried out with Duncan's test. Observed differences were considered as significant at $p < 0.05$.

Results and discussion

Cooking loss of *R. oligosporus*, beef, and commercial vegan patty

Cooking loss describes the amount of moisture that is released by a given product during the cooking process. Cooking loss is a significant parameter for cooked products, as it is associated with yield and juiciness [17]. A low cooking loss indicates a high yield at the end of the cooking process, which also translates to a high level of juiciness. Additionally, a low cooking loss indicates that the food matrix can retain water-soluble nutrients, thereby enhancing the overall quality of the product [18]. This study assesses the impact of diverse binding agents on cooking loss, with the findings illustrated in **Figure 1**. The results demonstrated that the addition of binding agents resulted in a reduction in the cooking loss of *R. oligosporus* burgers. The lowest cooking loss was obtained with addition of 15 % modified tapioca and pectin. This reduction of cooking loss is associated to the water-binding ability of the modified tapioca, which positively impacted the water-holding capacity of the fungi patty. This result is consistent with that of a previous study, which reported that the addition of octenyl succinic anhydride-modified starch significantly reduced cooking loss of patty [19]. Besides modified tapioca, pectin also significantly reduced cooking loss of fungi patty. Pectin has been demonstrated to enhance water-binding properties and reduce the cooking loss of meat products [20].

A further finding of this study demonstrated that the *R. oligosporus* patty and all of its variants exhibited a lower cooking loss in comparison to beef and commercial vegan patties. This indicates that the *R. oligosporus* patty exhibited a higher degree of juiciness. The lower cooking loss observed for the *R. oligosporus* patty in comparison to the commercial vegan patty can be attributed to the porous and fibrous structure of the fungal mycelium, which is capable of retaining water within its pores [21]. In addition, the higher cooking loss of beef patty could be due to conformational changes in the beef protein by heating, thereby reducing the water retention capacity of the meat [18]. The incorporation of modified tapioca starch effectively reduced the cooking loss of the *R. oligosporus* patty. This finding aligns with the results reported by [19], where the addition of 5 and 15 % octenyl succinic anhydride-modified starch significantly lowered cooking loss in patties.

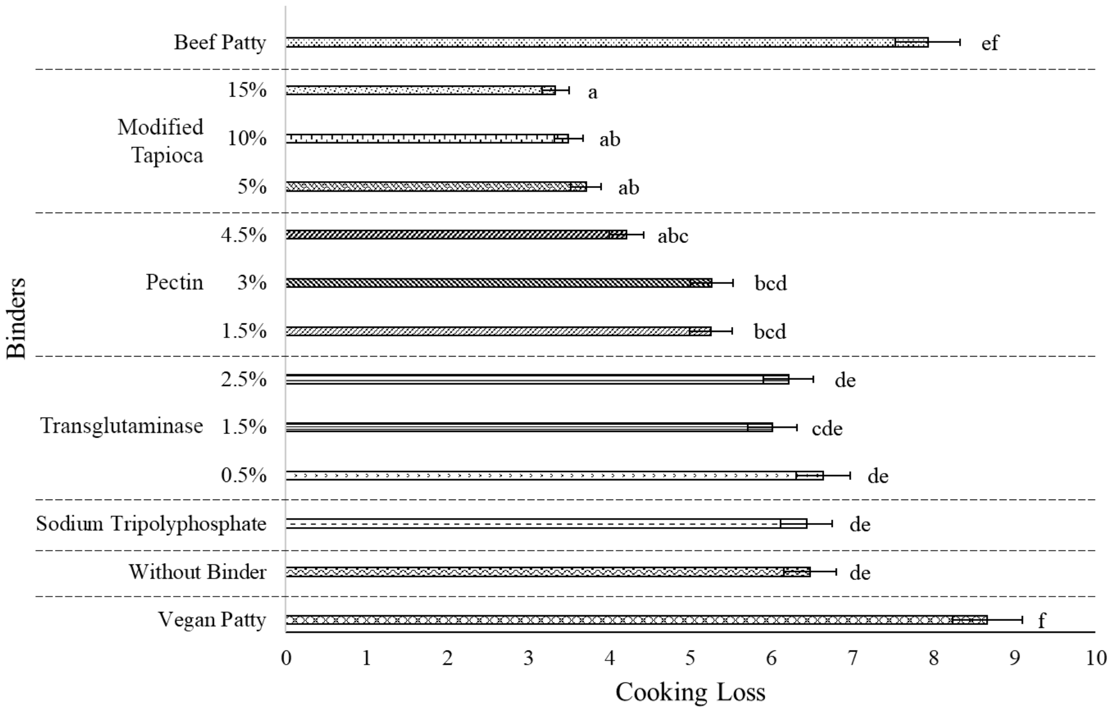

Figure 2 Effects of various binding agents on cooking loss (%) of burger patty.

TPA of *R. oligosporus*, beef, and commercial vegan patty

The textural properties of burger patties are essential parameters to evaluate as they are associated with eating quality and represent excellence in food processing. Incorporation of binding agents can improve the texture of *R. oligosporus* patty since the texture is inextricably linked to food structure and is highly influenced by the interactions of food biopolymers such as polysaccharides, proteins, and lipids [22]. In the current study, the main textural parameters in meat analogue including hardness, cohesiveness, and chewiness were evaluated [11]. These textural parameter values of *R. oligosporus* patties were extracted from the recorded force-deformation curves and are further elucidated below. Beef and commercial vegan patties were also included in this test as references.

Patty hardness can be referred to the force required to achieve a certain value of deformation [23]. The hardness of beef patty, vegan patty and *R. oligosporus* patty with addition of different binding agents are presented in **Figure 2**. The result showed that addition of binding agents increased the hardness value of *R. oligosporus* patty in general. A significant improvement of hardness was obtained from addition of modified tapioca starch which increased the hardness by 41.44 - 136.98 %. The highest improvement of hardness was obtained with the addition of 15 % modified tapioca starch, which was significantly higher than beef patty. However, the similar hardness to beef patty as reference was obtained by addition of 3 and 4.5 % of pectin as well as 5 and 10 % of modified tapioca starch. This finding could be a reference for further development of meat analogue with similar base ingredients [11]. Incorporation of modified starch increased the hardness of the *R. oligosporus* patty due to the swelling starch, which had a firming effect on the gel matrix. During the cooking process of *R. oligosporus* patty, the modified starch absorbs water in the food system and expands, which would build up pressure on the network, ultimately increasing the hardness [18]. Similar result has been reported by Pietrasik and Soladoye [24], who stated that starch improved the hardness of low-fat bologna. In addition, modified tapioca in *R. oligosporus* patty played a dual role, acting as both a binder and a filler [25]. The addition of pectin also improved the hardness of the *R. oligosporus* patty. Pectin was added together with calcium flakes as the source of calcium ion. The presence of calcium ion as divalent ion greatly assisted the formation of pectin gel in relatively neutral pH [26]. This gel-forming property of pectin may increase the hardness of *R. oligosporus* patty with similar mechanisms as modified starch. A stable protein-starch matrix could be formed during the cooking process of *R. oligosporus* patties, where hydrogen bonding, covalent bonding, and charge-charge interactions occurred [24]. In comparison with commercial vegan patty, all of *R. oligosporus* variants had higher value of hardness.

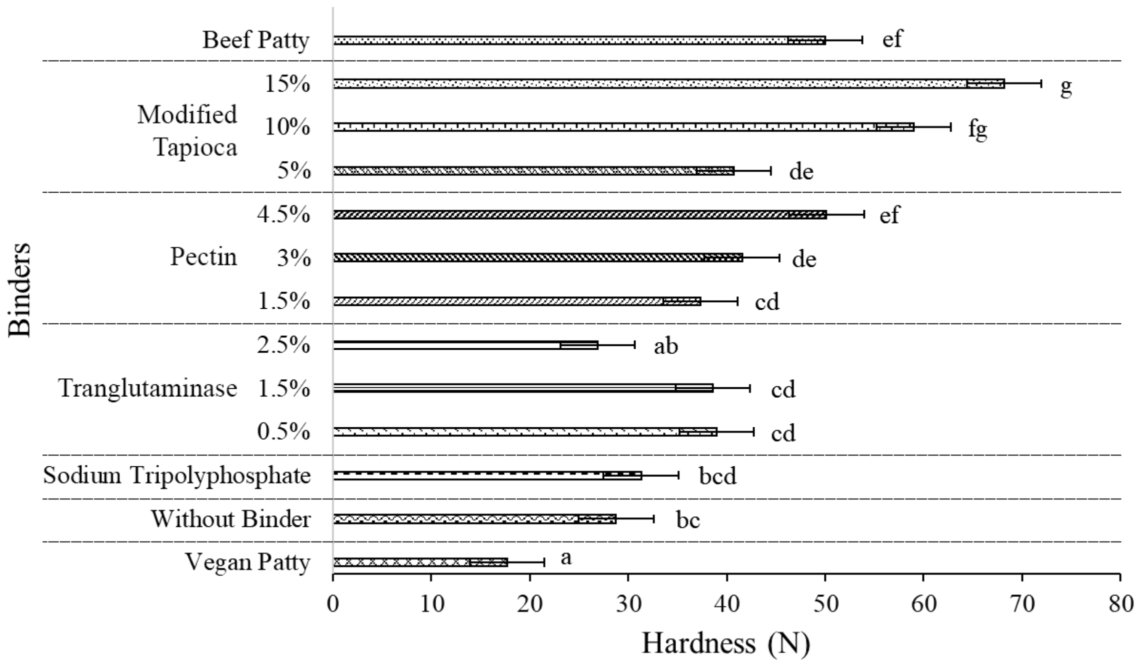

Figure 3 Effects of various binding agents on hardness (N) measured by instrument.

The cohesiveness of the patty is the extent to which the patty can withstand deformation until it fractures and suggests the internal bonds strength that composes the patty [11]. The value of cohesiveness can be determined from texture profile analyzer by calculating the ratio of the positive area during the 2nd compression to that of the 1st compression [27]. Generally, consumers prefer a firm meat analogue that can retain its shape which requires an adequate cohesiveness. **Figure 3** shows that the cohesiveness of all *R. oligosporus* patties was still lower than beef patty. High cohesiveness of beef patty was attributed to myofibrillar protein gel formed at the junction of meat chunks and particles which resulted in excellent adhesion ability [4]. This property was not present in *R. oligosporus* patty. Addition of binding agents did not change the cohesiveness of *R. oligosporus* patty in general. Addition of the highest concentration of modified tapioca starch and pectin even slightly reduce the cohesiveness. The decrease could be due to the rearrangement of the protein layer, which caused the dispersion of the air cell structure, resulting in a decrease in the internal structural strength of the sample, which directly affected the cohesiveness [28]. Air cells or air pockets were known to act as structure breakers in *R. oligosporus* patty [29]. This result is in line with previous findings that cassava starch reduced the cohesiveness of bologna compared to the control [24]. Additionally, starch added into various chicken analogue pieces decreased the TPA values for cohesiveness [29]. Although the cohesiveness values of *R. oligosporus* patties were lower than the beef patty, however the number is higher compared to commercial vegan patty.

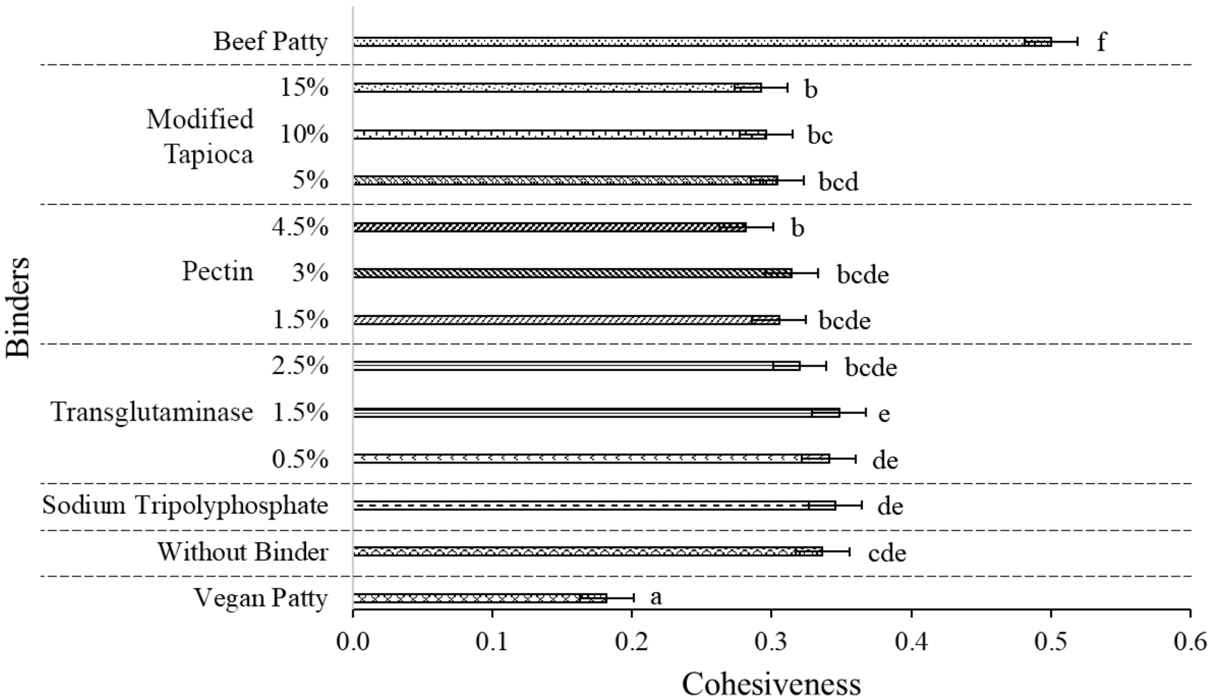

Figure 4 Effects of various binding agents on cohesiveness measured by instrument.

Chewiness quantifies the amount of energy necessary to masticate solid food until state ready for swallowing [27]. Tender and easy to chew meat analogue is more desirable to consumers [11]. This means that the ideal chewiness value of the developed *R. oligosporus* patty is similar to or slightly lower than that of the beef patty. Overall, the result showed that the addition of binding agents had no significant effect on the chewiness of the *R. oligosporus* patty (**Figure 4**). Only 10 and 15 % modified tapioca gave significant improvement in chewiness when incorporated into the *R. oligosporus* patty. The increase in chewiness by the addition of modified tapioca can be attributed to the high hardness value of the *R. oligosporus* patty, since chewiness is mainly influenced by hardness, cohesiveness, and springiness of the sample [30]. The chewiness of the *R. oligosporus* patty with addition of the best binding agents were higher than that of commercial vegan patty but lower than that of beef patty.

Three primary factors influencing the texture of *R. oligosporus* patties are hardness, cohesiveness, and chewiness [11]. These attributes are interrelated, with chewiness being a function of hardness, cohesiveness, and springiness. Consequently, variations in hardness and cohesiveness directly affect the chewiness of the patty [30]. This correlation is evident in the present study, where the incorporation of 10 % modified tapioca starch increased hardness, and, subsequently, the value of chewiness. This result suggested that the *R. oligosporus* patty had a softer and less chewy texture than the beef patty, which may still be acceptable to consumers.

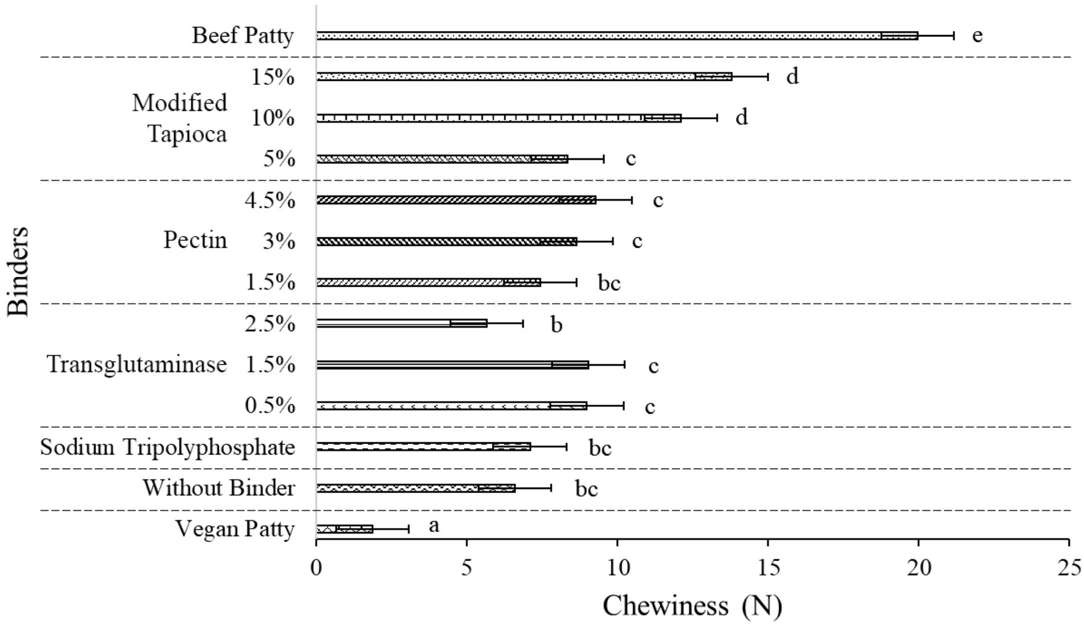

Figure 5 Effects of various binding agents on chewiness (N) measured by instrument.

Microstructure of *R. oligosporus* and commercial vegan patty

Images of the cross-sectional microstructure of patties were analyzed to observe a possible relationship between microstructure and texture properties (**Figure 5**). The result showed that *R. oligosporus* patty without binders, *R. oligosporus* patty with addition of sodium tripolyphosphate and transglutaminase, as well as commercial vegan patty had a rough and irregularly shaped aggregated form with large air pores microstructure. Correlating with the TPA result, the samples with large air pores had relatively lower hardness compared to other samples. Large air pores may act as structure breakers in the dough, resulting in lower hardness in the samples with observable large air pores [29]. In contrast, the *R. oligosporus* patty with addition of 3 % pectin and 10 % of modified tapioca had a smooth surface with small uniform air pores, which might be the reason for the higher hardness of the samples. The addition of 5 % pectin and 10 % modified tapioca to the *R. oligosporus* patty patched up the previously large air pores and helped to improve the microstructure, ultimately increasing the hardness of the patty. The microstructure images showed that the patty with starch-type binding agents had a substantial reduction in air pore size. Starch was able to swell and close the air pores in the microstructure during the cooking process of *R. oligosporus* patties [19]. The gradual expansion of the starch granules filled all the large air pores within the *R. oligosporus* patty matrices, resulting in expandable particles becoming interconnected. This filling behaviour of starch on the *R. oligosporus* patty gel network was related to pasting temperature and starch particle size. In this study, *R. oligosporus* patties were cooked at a surface temperature of 200 °C, which is higher than the temperature required for modified tapioca granules to undergo complete granulation, which was 80 °C. Therefore, no granulated modified tapioca starch was observed and the filling effect of modified tapioca starch on the *R. oligosporus* patty gel network reached an optimal result [18]. A similar effect of strengthening was also reported by Osman *et al*. [19], where the incorporation of 15 % octenyl succinic anhydride-modified starch showed a more compact and dense gel network with small air pores. Another possible explanation was that the binding between different proteins formed an aggregated structure leading to a reduction in the large air pores in *R. oligosporus* patty [28].

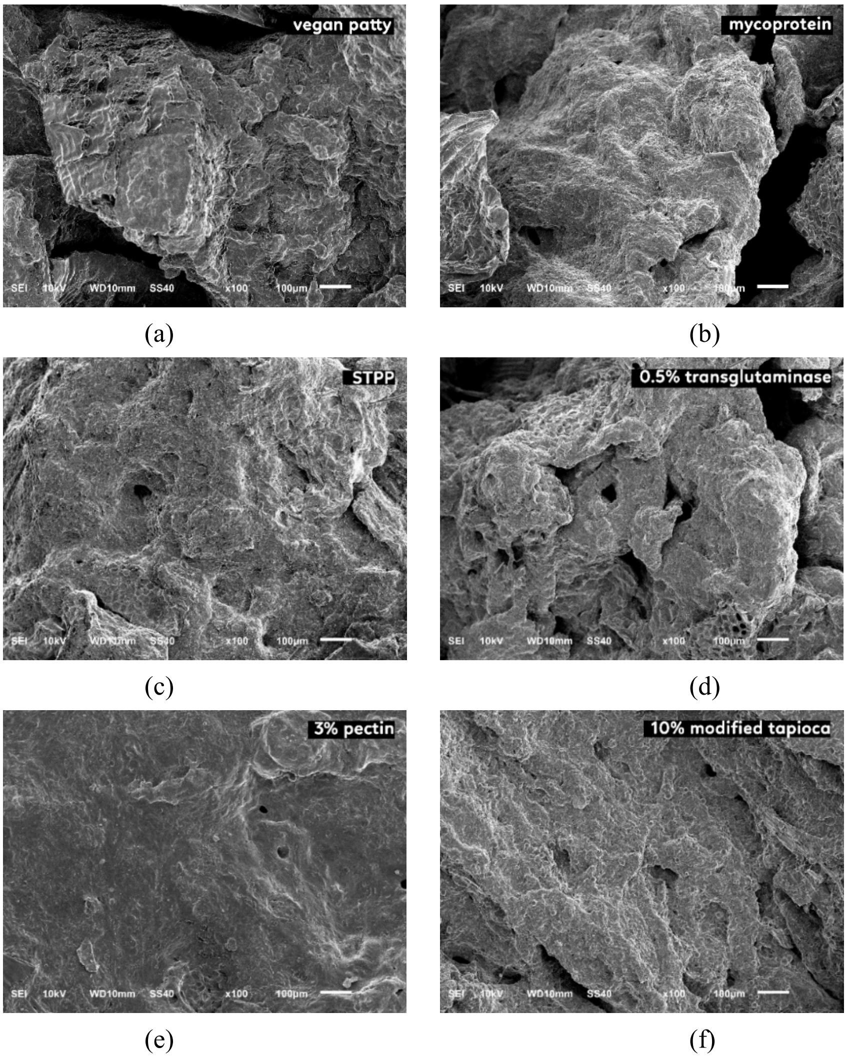

Figure 6 Scanning electron micrographs of (a) fungi burger patty, (b) vegan patty, fungi burger patty enhanced with (c) 2.2 g/kg STPP, (d) 0.5 % transglutaminase, (e) 3 % pectin, and (f) 10 % modified tapioca.

FTIR spectra of *R. oligosporus* and beef patty

In order to gain insights on chemical interactions behinds the texture improvement, *R. oligosporus* patty with addition of 10 % modified tapioca was subjected to FTIR since this patty showed the best texture improvement according to the texture pofile analyzer and SEM. The spectra was then compared to *R. oligosporus* patty without binding and beef patty. **Figure 6** showed that addition of 10 % modified tapioca changed the intensity of the peak and creation of a new absorbance peak compared with FTIR spectra of *R. oligosporus* patty without any binding agents. Intensity increase found on peak 1547 cm^{-1} from 78.48 % on pure *R. oligosporus* patty to 82.53 % on *R. oligosporus* patty with 10 % modified tapioca. Increase from 62.99 to 66.43 % was also found on absorbance peak number 1625 cm^{-1}. In the FTIR spectra, absorption band at 1500 - 1600 cm^{-1} was ascribed to amide II while absorption band band at 1620 - 1640 cm^{-1} was ascribed to β-sheets [31].

Amide II and β-sheet bands intensity increased was due to the rise of hydrogen and hydrphobic bonding in proteins caused by high temperature and pressure during cooking process of *R. oligosporus* patty [32]. Increase in hydrogen bonding can be explained by the nature of 10 % modified tapioca as a carbohydrate polymer that can bind water in *R. oligosporus* patty through hydrogen bonding via hydrophilic group [4]. Furthermore, a research conducted by Zhang *et al*. [33], reported that an increase on β-sheet band caused by heat resulted in a more rigid protein structure.

As a summary for FTIR results, incorporation of 10 % modified tapioca increased hydrogen and hydrophobic bonding in *R. oligosporus* patty. The structures inside *R. oligosporus* patty was created, retented, and stabilized by the presence of hydrogen bonds and hydrophobic interactions [28]. Therefore, hardness and chewiness improvement by the addition of 10 % modified tapioca on *R. oligosporus* patty can be correlated with the increase of hydrophobic interactions, disulphide, and hydrogen bonds which were confirmed by the the FTIR spectra result.

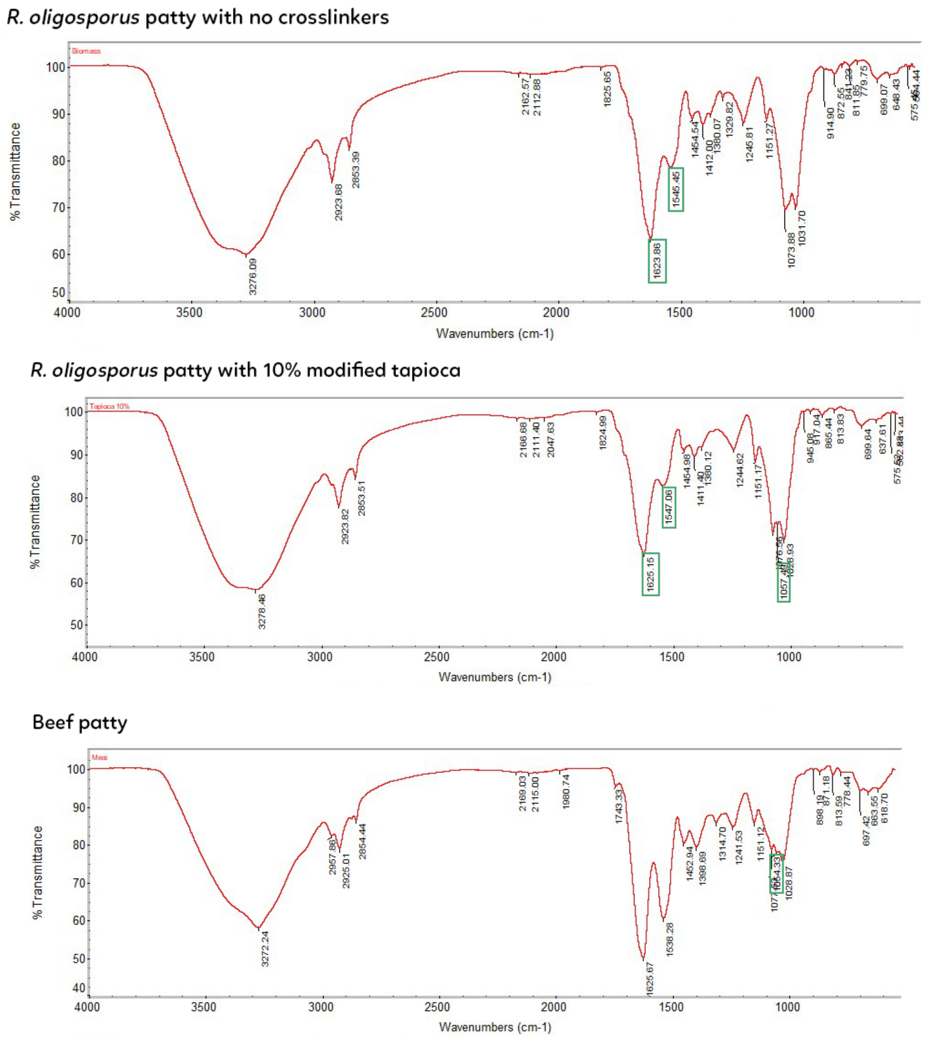

Figure 7 FTIR spectra of *R. oligosporus* patty without binding agents, with 10 % modified tapioca, and beef patty. The marked absorbance bands were the notable bands that responsible of texture amelioration of fungi burger patty

Sensory evaluation of *R. oligosporus* and commercial vegan patty

The sensory evaluation by scoring method was conducted to observe whether the positive effects suggested by the instruments can be well perceived by consumers or not. Although the instrumental evaluation of texture is highly valid and predictive, it is still important for actual consumers to evaluate the textural properties as it is more representative of the real-life situation. The same textural attributes assessed by the texture profile analyzer were also used for the sensory test. These attributes included hardness, cohesiveness and chewiness. A commercial vegan patty was also included in the sensory test as a reference.

Sensory definition of hardness is the amount of force required to compress a solid food between the molar teeth. Higher hardness means more force is required to compress the patty and the tougher the patty will be. This study shows that consumer panels can recognize the improvement of hardness by the addition of 10 % modified tapioca on *R. oligosporus* patty **(Figure 7)**. The increase in hydrophobic interactions, disulfide, and hydrophobic bonds by the incorporation of 10 % modified tapioca resulted in firmer and tougher patty that can be detected by both instrument and consumer palates. However, the other binding agents did not achieve a significantly higher score compared to the *R. oligosporus* patty without any binding agents enhancement. When compared to the commercial vegan patty, all *R. oligosporus* patty variants achieved higher hardness scores. Therefore, the incorporation of 10 % modified tapioca starch as a binder resulted in an increase in the hardness of the *R. oligosporus* patty, as confirmed by both TPA and sensory testing.

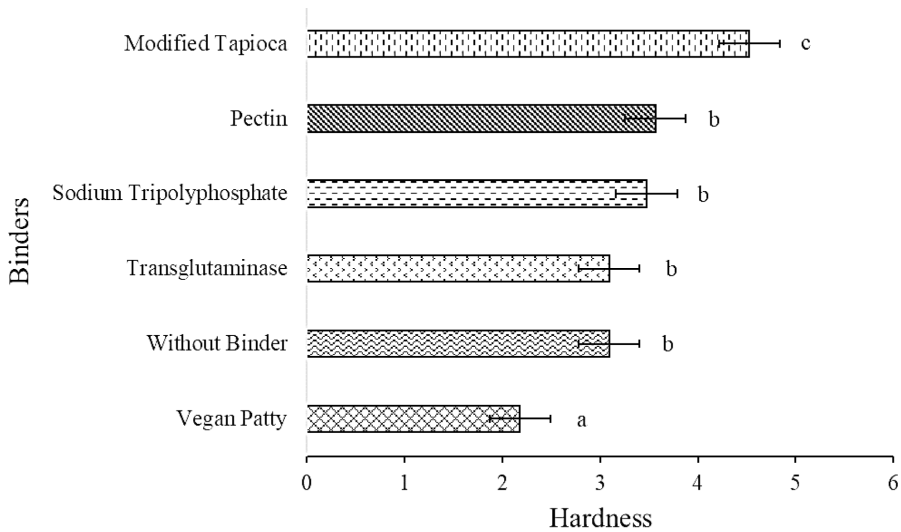

Figure 8 Effects of various binding agents on hardness according to sensory test.

Cohesiveness in sensory point of view is the degree to which a product can be compressed between the teeth before it ruptures or can also be defined as the ease of fragmentation. **Figure 8** showed that consumer panels were unable to distinguish the difference in cohesiveness with the addition of binding agents to the *R. oligosporus* patty. The texture test using a texture profile analyzer also showed that there was no improvement in cohesiveness with the addition of binding agents. Therefore, as suggested by a previous study, the TPA score for cohesiveness and hardness was predictive for the sensory test of the *R. oligosporus* analogue patty [29]. All the *R. oligosporus* patty variants had higher cohesiveness scores compared to the commercial vegan patty, indicating the superior ability of *R. oligosporus* patties to stick together. According to the results of the TPA and sensory testing, the incorporation of 10 % modified tapioca starch had no significant effect on the cohesiveness of *R. oligosporus* patty.

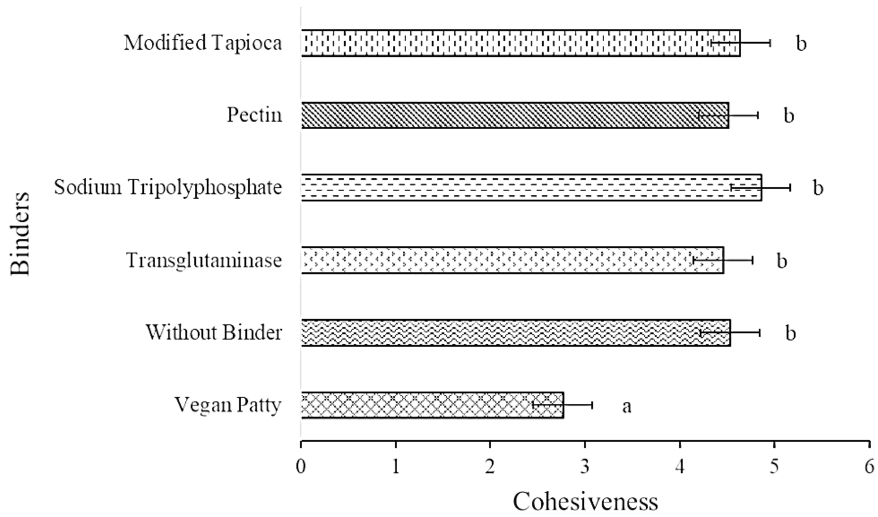

Figure 9 Effects of various binding agents on cohesiveness according to sensory test.

In sensory testing, chewiness is defined as the duration or time required to masticate or chew the product at a constant rate of force application to reduce it to a consistency suitable for swallowing. **Figure 9** showed that the addition of binding agents did not improve or change the chewiness of the *R. oligosporus* patties according to the panels. In contrast, texture profile analyzer showed that 10 and 15 % modified tapioca improved the chewiness of *R. oligosporus* patty product. The different results between these methods were due to the heterogenous panel thresholds. The improvement on the texture detected by texture profile analyzer was not enough to change the panelists' perception. Compared with commercial vegan patty, *R. oligosporus* patty of all variants had a significantly higher chewiness according to the panels. Therefore, while the TPA indicated an improvement in hardness with the incorporation of 10 % modified tapioca starch in *R. oligosporus* patties, this enhancement was not detectable by the sensory panel, as shown in the sensory test results.

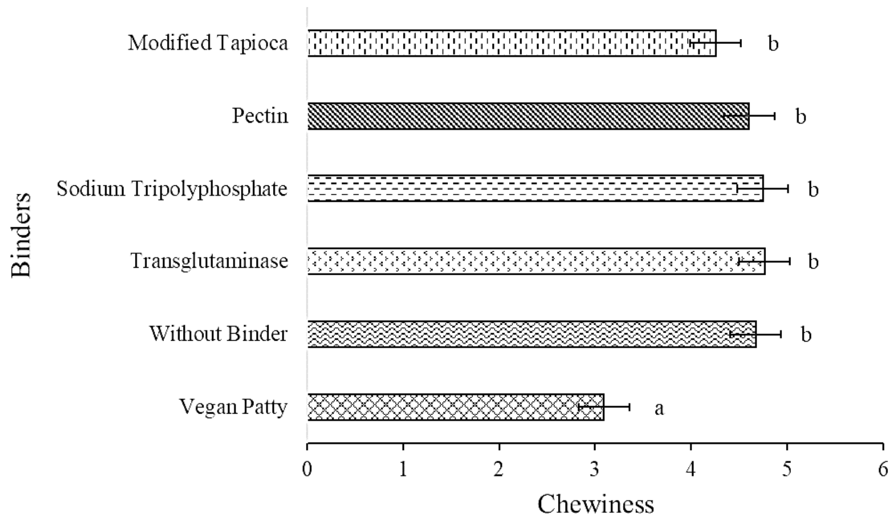

Figure 10 Effects of various binding agents on chewiness according to sensory test.

Additional comments from consumer panels were compiled to gain a better understanding of panel's collective opinion on the newly developed *R. oligosporus* patty. Based on the comments, it could be concluded that panels liked the *R. oligosporus* patty. The panels also stated that the fibrous and firm texture of *R. oligosporus* patty was the most appealing feature. The panelist were dissatisfied with the texture of the commercial vegan patty, which they found to be fragile and too crumbly. The incorporation of binding agents resulted in *R. oligosporus* patty with a firm and fibrous texture, which was appreciated by the panel. The finding of this study suggests that addition of binder, particularly 10 % modified tapioca starch could improve the texture of *R. oligosporus* patty. A future research into the development of *R. oligosporus* patties could focus on formulation to achieve a palatable taste of the patty.

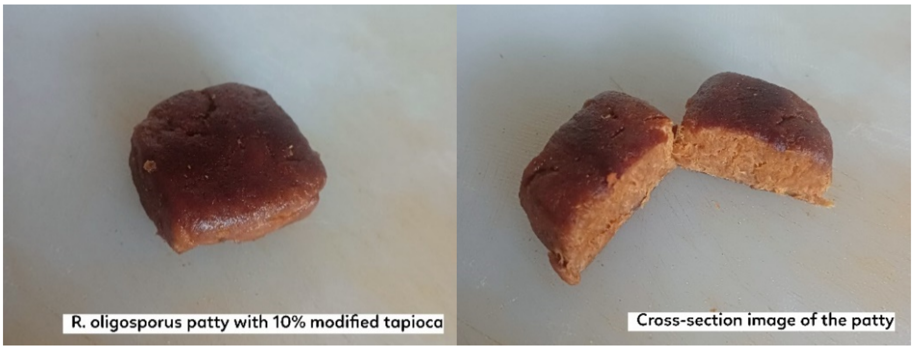

Figure 11 Surface and cross section image of *R. oligosporus* patty with 10 % modified tapioca.

Conclusions

This study investigates the impact of various binding agents on the texture of *R. oligosporus* patties. The findings of this study reveal that the addition of 10 % modified tapioca starch significantly improved the texture, particularly in terms of hardness. These textural enhancements are attributed to increased hydrophobic interactions, disulfide bonds, hydrogen bonds, and protein-starch interactions. Future research should focus on refining the patty formulation and conducting hedonic sensory tests to gain deeper understanding of consumer preferences.

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Compliance with ethical standards

All experimental protocols were approved by the Ethical Commission of Medical and Health Research Ethics Committee with protocol number of KE/1620/10/2023 with permits issued on October 31st 2023.

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