PROCEEDINGS OF INTERNATIONAL SYMPOSIUM ON A NEW ERA IN FOOD SCIENCE AND TECHNOLOGY 2019

-PART 1-

INTERNATIONAL SYMPOSIUM ON A NEW ERA IN FOOD SCIENCE AND TECHNOLOGY 2019

ORGANIZER:

THE UNITED GRADUATE SCHOOL OF AGRICULTURAL SCIENCE, GIFU UNIVERSITY

-PART 2-

UGSAS-GU & BWEL JOINT POSTER SESSION ON AGRICULTURAL AND BASIN WATER ENVIRONMENTAL SCIENCES 2019

ORGANIZERS:

THE UNITED GRADUATE SCHOOL OF AGRICULTURAL SCIENCE, GIFU UNIVERSITY

GIFU UNIVERSITY REARING PROGRAM FOR BASIN WATER ENVIRONMENTAL LEADERS

DATE: OCTOBER 9-10, 2019

VENUE: GIFU UNIVERSITY, JAPAN

PROGRAM
–PART 1-

DAY ONE: Wednesday, October 9
Time: 9:00-16:45

Time Table

Venue: 6F in UGSAS Building, Gifu University

9:00-9:30 **Registration**

9:30-9:35 **Opening Remarks**
Prof. Masateru Senge (Dean of UGSAS-GU)

Kyenote Speeches

Venue: 6F in UGSAS Building, Gifu University
Session Chair: ***Prof. Tomio Yabe (Gifu University)***

9:35-10:05 **Keynote Speech 01**
Assoc. Prof. Nozomu Sakurai (National Institute of Genetics)

10:05-10:35 **Keynote Speech 02**
Dr. Shirley C. Agrupis (President of Mariano Marcos State University)

10:35-10:55 **Coffee Break**

10:55-11:25 **Keynote Speech 03**
Prof. Utpal Bora (Indian Institute of Technology Guwahati)

11:25-11:55 **Keynote Speech 04**
Assoc. Prof. Mohamad Yusof Maskat (Universiti Kebansaan Malaysia)

11:55-12:10 **Photo Shoot**

Venue: 6F in UGSAS Building, Gifu University

12:10-13:00 **Lunch Break**

Special Lecture

Venue: Room 100, Engineering Building, Gifu University

13:00-14:00 **Special Lecture**
Prof. Gary D. Christian (University of Washington)

14:00-14:30 **Break**

Scientific Sessions

Venue: 6F in UGSAS Building, Gifu University

Session 1 —Bioactives and Health / Prebiotics and Probiotics—
Session Chair: ***Prof. Tomoyuki Nakagawa (Gifu University)***

14:30-14:45 **01.** ***Prof. Das Shonkor Kumar (Bangladesh Agricultural University)***

14:45-15:00 **02.** ***Prof. Chen-jian Liu (Kunming University of Science and Technology)***

15:00-15:15 **03.** ***Prof. Xiaoxiong Zeng (Nanjing Agricultural University)***

15:15-15:45 **Coffee Break**

Session 2 —Physical Properties of Food—
Session Chair: ***Assis. Prof. Teppei Imaizumi (Gifu University)***

15:45-16:00 **01.** ***Assoc. Prof. Md. Sultan Mahomud***
(Hajee Mohammad Danesh Science and Technology University)

16:00-16:15 **02.** ***Assoc. Prof. Pongphen Jitareerat***
(King Mongkut's University of Technology Thonburi)

16:15-16:30 **03.** ***Dr. Maharani Pertiwi Koentjoro***
(Nahdlatul Ulama University of Surabaya)

16:30-16:45 **04.** ***Dr. Achmad Ridwan Ariyantoro*** **(*Sebelas Maret University*)**

Effect of Edible Laccase on Chinese Bread Texture

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SUMMARY

Laccase application in the food industry is an alternative and innovative solution for natural ingredients since it is able to improve the food quality especially for baking industry. Laccase is a natural catalyst which could improve the structure of gluten in dough by increasing phenol oxidation resulting a better bread texture, taste, volume, and freshness. The aim of this study was to evaluate *Trametes versicolor* laccase oxidation on physicochemical quality of Chinese bread. Laccase were pasteurized to obtain edible laccase with the final activity as 774.7 U/ml. Laccase treatment managed to reduce bread hardness until 9.55 N. The softening phenomenon was related to the laccase–mediated depolymerization of the cross–linked network. Chemical parameters of laccase treatment were 0.065 ppm lower than control. The functional group in the polymer pretreatment mixture of edible laccase observed by Fourier Transform Infrared Spectrometer (FTIR) showed the formation of disulfide group at a wavelength of 450–500 cm^{-1}. The Scanning Electron Microscope analysis revealed formation of gluten protein which has smaller and uniform pores compared to the control group. Based on the results obtained in the present study, laccase from *Trametes versicolor* can be considered a promising application to improve quality of Chinese bread at industrial level.

Keywords: laccase, *Trametes versicolor*, physicochemical quality, Chinese bread

Introduction

Laccase (EC 1.10.3.2) is a type of enzyme containing polyphenol oxidase, which can oxidize polyphenols, methoxy–substituted phenols, and diamine (Minnusi et al. 2002). Among plants, fungi, and bacteria, white–rot fungi are the major laccase producer. Laccase by *Trametes versicolor* has been produced using lignin containing materials (Shraddha et al. 2011; Imran et al. 2012; Offia–Olua, 2014).

Laccase in the food industry has been implemented in a dough as bread quality improver to increase volume or crumb structure and softness in the baked products (Minnusi et al. 2002; Imran et al. 2012; Osma et al. 2010). Laccase from *T. versicolor* have been evaluated to their ability to increase the strength of the gluten structure prior oxidizing lignocellulose in flour (Kaur et al. 2014; Minnusi et al. 2002). The elastic properties of low–protein flour used in making Chinese bread has a weak gluten index therefore it needs to be improved using laccase (Gulia and Khatkar, 2015).

Generally, the baking industry uses artificial food additives such as baking soda (sodium bicarbonate) to increase volume and delighting texture. Long–term of chemicals consumption could result negative impact of human health thereby reducing serum iron and calcium levels in the body which lead to specific doses toxicity (Fakhri et al. 2016). It is well known that the addition of certain enzymes to the dough have unwanted side effects. Since the enzyme are denaturated and inactivated by heat during cooking process (Gallagher et al. 2009). Therefore, the application of laccase as an enhancer of the texture of the bread is less health risks.

The objective of this study was to determine the effects of edible laccase from *T. versicolor* on the texture of Chinese bread by measuring phenol content, functional groups, and micro visual characters.

Materials and Methods

1. *Laccase production*

1.1 *Organism and cultivation conditions*

The *T. versicolor* fungi are a culture collection of Microbiology and Biotechnology Laboratory, Department of Biology, Institut Teknologi Sepuluh Nopember (ITS) Surabaya, Indonesia. The fungi cultures were maintained on Potato Dextrose Agar (PDA) medium and incubated at 28°C, for 6 days before using as seeding. The procedures of maintaining fungi culture is briefly explained as follows. Sporulated PDA medium were dislodged and washed with PBS sterile. As much as 10 mL of 10^8 CFU fungi spores' suspension were inoculated into 100 mL of Potato Dextrose Broth (PDB) (pH 6.0) and incubated for 6 days at room temperature (25°C) with a rotary shaker at 130 rpm (Jo et al. 2010; Qin et al. 2017).

1.2 *Partially edible laccase production*

All experiments were carried out in 300–mL Erlenmeyer flasks containing 100 mL culture medium. The medium contained 4.5 g/L rice husk, 1.5 g/L yeast extract, 1 g/L $C_6H_{12}O_6$, 0.5 g/L NH_4Cl and 100 mL of salt solution. The salt solution contained 2 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.1 g/L $CaCl_2.2H_2O$ and 0.5 g/L KCl and maintained pH 5.0, (Koentjoro et al. 2016). $CuSO_4$ was added by dissolving 100 mg in 1 L of dH_2O as laccase inducer. The medium was autoclaved at 121°C for 15 min. Each flask was inoculated using an agar piece 1 cm^2 cut from an actively growing fungal culture. The flasks were incubated at 30°C on a rotary shaker (130 rpm). The culture broth was filtered, clarified by centrifugation at 10,000 rpm for 15 min, frozen, defrosted and then filtered to remove the precipitated polysaccharides. The resulting clear filtrate was used for the further experiment. Partially edible laccase was sterilized by pasteurizing (or High Temperature Short Time) (HTST) is as follows, a total of 50 mL of laccase solution was placed in a water bath heated at 72°C for 25 seconds (Ranieri et al. 2009).

2. *Characterization of laccase*

2.1 *Laccase activity*

Laccase activity was determined as the increase in absorbance at 468 nm due to the oxidation of 2,2 –azino–bis (3 ethylbenzothiazoline–6–sulfonate) (ABTS) at 30°C (Koentjoro et al, 2016). The reaction mixture contained 200 µL 10 mM ABTS, 300 µL enzyme solution in 500 µL 100 mM citrate buffer, pH 4.5.

Oxidation of ABTS was observed spectrophotometrically at 436 nm for 5 minutes. The laccase activity is calculated based on the ABTS standard curve. One unit (U) of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol ABTS per minute and considered equivalent to 1 unit of enzyme activity ($\varepsilon 420 = 36000\ M^{-1}\ cm^{-1}$ and path length l = 1 cm) (Wolfenden and Wilson, 1982).

2.2 *Isoelectric point (pI)*

To investigate the isoelectric point of supernatant, as much as one mL of laccase was incubated in each tube containing a phosphate buffer with variable pH level (3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) at room temperature for overnight. The occurring of precipitation was monitored and justified as the isoelectric point of laccase in particular pH level.

2.3 *Total Protein determination in enzyme solution*

Total Protein concentration in supernatant was determined by Bradford method using Bovine Serum Albumin (BSA) as standard. Reaction was started by addition of 0.1 mL of supernatant and 5 mL of Bradford reagent (10 mg of Coomassie Brilliant Blue G–250 in 5 mL of 95% ethanol, 10 mL of 85% phosphoric acid, and 85 mL of dH_2O). The changes in absorbance due to laccase content in supernatant were recorded by spectrophotometer in absorbance at 595 nm.

3. *Preparation of laccase modified Chinese bread*

Dough was made manually by mixing flour with enzymes, yeast, water and sugar. The experimental formulations set are shown in Table 1. The average of duplicate data was used in analysis. The dough in each experimental formulation was divided into 6 similar weight. The dough was placed in a bowl covered with a

Table 1 Composition of bread with different levels of edible laccase

Sample	Sample 1	Sample 2	Sample 3	Sample 4
Treatment	Negative control	Non–Edible Laccase	Edible Laccase	Baking soda
Flour wheat low protein (gr)	100	100	100	100
Non–Edible Laccase	0	1.5	0	0
Edible Laccase	0	0	1.5	0
Baking soda (gr)	0	0	0	400
Yeast (gr)	2.2	2.2	2.2	2.2
Water (mL)	50	50	50	50
Sugar (gr)	20	20	20	20

plastic foil and allowed to stand at room temperature (28°C) for 45 minutes. The dough was then steamed for 7 minutes (Laohaprasit and Sricharoenpong, 2018).

4. *Laccase modified Chinese bread analysis*

4.1 *Total phenol content*

Total phenolic content was determined according to Folin–Ciocalteu method with slightly modified. Every extraction solution of bread (10 mL) was mixed with 10 mL of 96% ethanol and 5 mL of 0.1% HCl for 10 min. The reaction mixtures were allowed shaking in a rotary shaker for 30 minutes at room temperature. The mixtures were then centrifuged for 10 minutes at 4,000 rpm and the supernatant was collected. Every extraction of supernatant (1 mL) was mixed with 100 µL of Folin–Ciocalteu reagent and 1 mL of sodium carbonate 15% (w/v). After incubation for 1 hour at room temperature, the absorbance was measured at 710 nm using a spectrophotometer. Total phenol was calculated from the calibration curve of tannic acid and expressed as milligram of tannic acid equivalent per gram of dough (mg TAE/g).

4.2 *Texture profile analysis*

Evaluation of dough properties (hardness) was carried out by texture profile analysis. The test consists of compressing a bite–piece of bread two times in a reciprocating motion that imitates the action of the jaw and extracting from the resulting force–time curve a hardness parameters. The test was applied on bread by using following procedures. Each sample was placed on the Texture Analyzer (SMS) mod.TA.HDi 500 (Stable Micro Systems, Surrey, UK) preparation table followed by applying a pressure using a piston diameter of 75 mm (Ayala–Soto et al. 2017).

4.3 *Scanning Electron Microscope (SEM)*

The micro structural of starch granules in bread with different formulation were studied using Scanning Electron Microscope. Samples were prepared by knife cutting then placed on a carbon tip followed by gold and palladium coating. The SEM images of each samples were taken at magnification of 3,000 (Wojciechowicz–Budzisz et al. 2015).

4.4 *Fourier Transform Infrared Spectroscopy (FTIR) Analysis*

The FTIR analysis in this study has been carried out in order to identify functional groups shifting in every treatment at a wavelength between 800–3600 cm^{-1} (Amir et al. 2013).

5. *Statistical analysis*

The data obtained were then analyzed using of one–way variance (ANOVA) Duncan test using Minitab 14.0 (Minitab 14 Statistical software, 2014). Meanwhile, a qualitative descriptive design was conducted for texture and functional group analysis.

Results and Discussion

1. *Production and characterization of edible laccase*

The activity of laccase before and after pasteurization were calculated as 799.4 and 774.7 U/mL, respectively (Table 2). These results demonstrated the pasteurization processes has no effect on laccase activity.

Table 2 Activity and quantitative estimation of laccase production

Laccase	Activity (U/mL)	Protein Contain (mg/mL)
Before pasteurization	799.4	0.02
After pasteurization	774.7	0.11

The pI of the non–and edible laccase was determined on pH 3.0. This information is helpful to developing purification scheme as a crucial factor in enzyme purification, the precipitation occurs is avoided (Xia, 2007). This result is consistent with the study of Afreen et al (2017) which stated that generally, laccase has an isoelectric point between at pH 3.0–3.1.

The pasteurization did not change the total protein in laccase solution as shown in Table 2. It is indicated that laccase can be pasteurized using heating with a certain temperature and time that serves to kill pathogenic microorganisms while maintaining physical properties (Verhoeckx et al. 2015).

2. *Texture Analysis (Test Hardness)*

Bread treated with edible laccase was demonstrated in deformation in cavity characters (Fig. 1). The pretreatment–control had a greater cavity compared to non–and edible laccase pretreatment. Moreover, between non–and edible laccase Chinese bread has a small and uniform cavity. The result confirms the findings of Salinheimo et al (2007) and Tsegaye et al. 2018.that the bread treated with laccase has a small and uniform cavity. The small and uniform cavities, due to the ability of the laccase to form crosslinking in arabinoxilan through ferulic acid side groups allowing strengthen of gluten and more ability to retain gas (Meybodi et al. 2015). Therefore, the

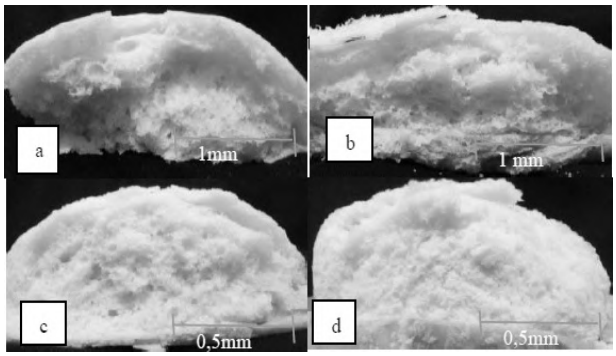

Fig. 1 Cross Section of Chinese Bread with a Variety of Pretreatment a) Control b) baking soda c) non–edible laccase d) edible laccase.

pasteurization does not affect the protein component of laccase.

3. *Total Phenol Analysis*

Analysis of total phenols in various treatments for dough are reported in Table 3. The non–pretreatment and edible laccase experimental group both of them have a lower total phenol concentration of 0.096 and 0.065 ppm respectively compared to a control of 0.168 ppm. This is because of ability of laccase to oxidize phenolic compounds such as ferulic acid that act as laccase mediator in a laccase–mediator system (LMS) reaction mode (Imran et al. 2012; Everette et al. 2014). The higher amount of oxidized phenol, it will increase the formation of disulfide groups (Linares–García et al. 2019). The ferulic acid is oxidized and formed radicals and continues to oxidize the sulfidryl (SH) groups. The oxidation can affect the natural disulfide formation (S–S) in gluten polymers and increase the rate of protein depolymerization lead to increase gluten networks (Linares–García et al. 2019).

Texture analysis on various treatments are shown in Table 4. Pretreatment of edible laccase and controls had a significantly different hardness value of 9.55 and 21.9 N respectively. These results indicated that the addition of laccase to the dough was able to reduce the hardness of the Chinese bread. This is consistent with the statement (Osma et al. 2010; Brijwani et al. 2010) that the laccase shows an oxidizing effect to improve the structure of gluten in the dough and can reduce stiffness, increase volume, improve crumb structure, and increase the softness of bread. Indeed, one good parameter of bread quality is a soft texture and low hardness (Borla et al. 2014). In addition, in Table 4 the non–and edible laccase pretreatment showed non–significantly different of hardness level which shows that pasteurization has no effect on the laccase protein component due to glycosylation. The edible laccase has a greater hardness value (9.55N) compared to non–edible one (9.15 N), this is most probably because of the different laccase activity shown in Table 2.

Table 4 A comparison of the physicochemical characteristics of bread

Pretreatment	Hardness (N)
Control	21.9 ± 0.269^{b}
Baking soda	17.97 ± 3.514^{b}
Non–Edible Laccase	9.15 ± 0.869^{a}
Edible Laccase	9.55 ± 0.262^{a}

Notes: Values with different superscript letters within a column are significantly different ($p \leq 0.05$. Data are expresses as means values of two samples ± standard deviation

4. *Scanning Electron Microscope (SEM) Analysis*

SEM analysis was taken to investigate the effect of laccase on microstructure of whole bread. This technique allowed a qualitative description of the structural cell and their distribution.

As shown in Fig. 2, that the microstructure of gluten bread was more uniform and more number of pores after adding of laccase due to higher volume of gas was released during dough fermentation.

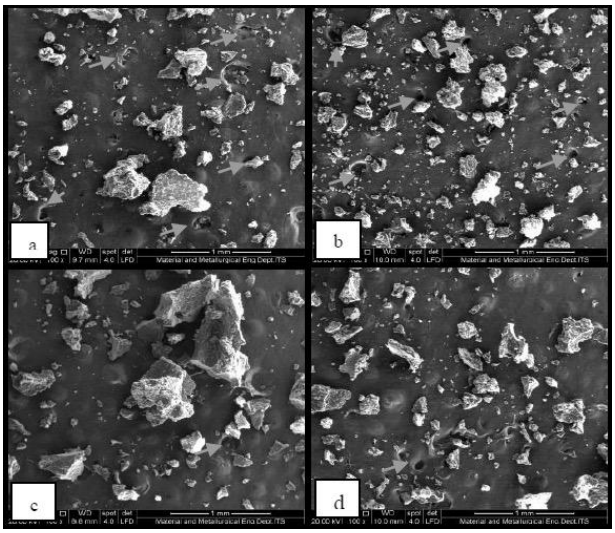

Fig. 2 SEM of a) control; b) baking soda; c) non–edible laccase; d) edible laccase at 1000X magnification Arrows show pores on gluten tissue.

The pore structure can be caused by the presence of an elastic gluten protein matrix which is able to withstand the development of gas while the cell walls was not easily broken (Ortolan and Steel, 2017).

5. *FTIR analysis*

FTIR spectroscopy of bread functional groups at various pretreatments was done in the range of 400–4000 cm^{-1} (Fig. 4). Characters of spectral bands were similar for control and laccase containing bread. Major peaks were indicated absorption of OH groups at a wavelength range of 3000–3,700 cm^{-1}.

In the range of 450–500cm^{-1} there is a change in the peak (peak) spectra at the pretreatment of non–edible laccase and edible laccase. At this wavelength range is the absorption of disulfide groups (S–S) (Amir et al. 2013). Disulfide groups are formed due to the oxidation process in sulfidryl groups which can affect the natural disulfide formation (S–S) (Linares–García et al. 2019).

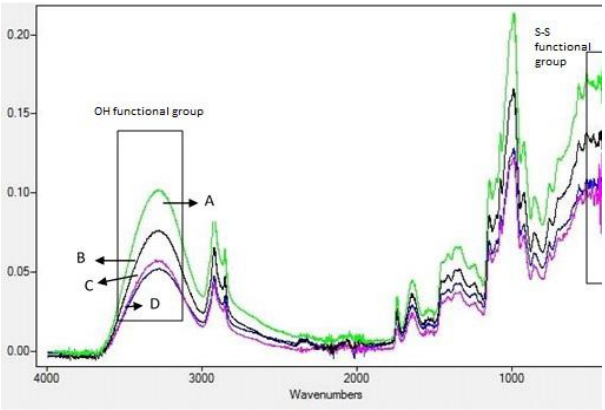

Fig. 3 FTIR spectra of bread in variety treatment

Conclusion

Laccase can be pasteurized excellently without decrease their activity. Pretreatment of edible laccase was able to reduce the value of bread hardness up to 9.55 N. The functional group in the polymer pretreatment mixture edible laccase formed a disulfide group formation at a wavelength of 450–500cm^{-1}. The structure of gluten was formed in uniform pores compared to controls. Therefore, applying laccase as natural food additives is able to improve bread texture quality.

Acknowledgements

The authors would like to thank the anonymous reviewers for their insightful suggestions.

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Physical, Chemical and Sensory Properties of the White Bread from Acetylated Jack Bean Flour

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SUMMARY

White bread is the preferred type of bread and it can be consumed by a variety of ages. Production of white bread in Indonesia is increasing every year. The main ingredients of white bread are wheat flour. Unfortunately, wheat could not grow very well in Indonesia. It will affect the increment of the number of imports in wheat flour. Therefore, it is necessary to substitute wheat flour with the others. The alternative is jack bean flour, which has been made from local commodities and it has high carbohydrate and protein content. Native jack bean flours still have weaknesses such as unstable viscosity on high temperature and shear stress, high retrogradation and syneresis. The modification can overcome these problems, one of the methods is acetylation process.

The aim of this research was to determine the selected formula of white bread, which has good sensory properties. Physical properties (baking expansion) and chemical properties (moisture, ash, fat, protein, carbohydrates and salt content) of white bread using acetylated jack bean flour (AJBF) also were investigated. The results show white bread with good sensory is F2. It has baking expansion (126.35 %), moisture (37.86 %wb), ash (1.93 %db), fat (2.70 %db), protein (5.68 %db), carbohydrate (52.26 %db) and salt content (1.88 %db). The results suggest that 10% of AJBF can be used in bread making and it has higher protein content than wheat bread (control).

Introduction

The production of white bread in Indonesia is increasing every year. According to Setyawan (2006), the production of white bread increased from 2000 to 2003 reached 25.106.495 kg. The main material of white bread is wheat flour. Unfortunately, wheat cannot be cultivated in Indonesia, so Indonesia have imported from abroad. Wheat flour imports in 2014 reached 756,241 tons. Therefore, it needs a substitute flour with other flour in making bread to reduce wheat imports One of flour which it is can be used as substitute is jack bean flour.

Jack bean has high potency because it has high nutrition such as protein, carbohydrates, amino acid non-essentials and mineral. The carbohydrates content on jack bean is very high (50-60%) (Olalekan, and Bosede, 2010). It has also contained protein content approximately 29-32% (Doss, 2011). Also, it has high starch content, so it can be changed to flour. The native jack bean flour has some weaknesses, such as low ability to swell and the unpleasant and taste. Therefore, it need to improve the functional properties with modification.

Starch modification is a method for improving the functional properties of native starch. There are many modification methods have been used to increase starch properties, which can be divided into physical, chemical, and enzymatic treatment (Zavarese and Dias, 2011). One of chemical modification method is acetylation. Starch acetate is widely used in the food industry because it increased viscosity, solubility compared with native starch. The objective of the research is to investigate the effect of acetylated jack bean flour on the properties of the white bread.

Materials and Methods

1. *Material*

The main material in this research is jack bean flour, which is obtained from the local market. The other material for the bread making and the chemical material for the

analysis.

2. *Acetylated Jack Bean Flour (AJBF)*

Jack bean seeds were soaked in 1% baking soda solution for 8 hours, and it was boiled for 5 minutes. It was removed the skin to obtain jack bean seed without skin. It was soaked again in 1% baking soda solution for 16 hours (with water changes every 8 hours). It was cut and it was dried in a cabinet dryer at 60ºC for 7 hours. Then, it was grounded and sieved to obtain jack bean flour.

Jack bean flour was soaked with ratio of 1: 3 with solution acetic acid (0.15% acetic acid in 450 ml of distilled water). It was heated in a water bath at a temperature of 45ºC for 90 minutes. The water was separated from the solution. Wet flour was dried using a cabinet dryer at 60ºC for 7 hours. It was grounded and sieved to obtain AJBF.

3. *Bread making*

All ingredients (wheat flour (WF), AJBF, milk powder, yeast, bread improver, water, sugar and salt) was mixed with mixer. The formula of breads are F1:100%WF;F2:90%WF:10%AJBF;F3:80%WF:20%AJBF;and F4:70%WF:30%AJBF). It was kneaded until smooth, fermented and baked at 180ºC for 20 minutes.

4. *Sensory test*

The sensory test was done using scoring method with panellist. Panellists give score on white bread parameter: colour, aroma, taste, texture and overall. They give score with range 1 to 5, which is 1 is disliked a lot and 5 is liked a lot.

5. Baking expansion test

The baking expansion of white bread was calculated by measuring the volume before and after baking process. The volume of white bread was performed by measuring the surface area of the sliced bread.

6. *Statistical analysis*

Data were analyzed statistically using the SPSS Software (Version 12, IBM, USA). Analysis of variance and DMRT tests with a significance threshold of $p < 0.05$ were used to compare the differences among samples.

Results and Discussion

1. *Sensory properties*

Sensory analysis was carried out in colour, texture, aroma, taste and overall. The sensory properties of white bread were shown in Table 1. The results show that the more AJBF lead to decrease in sensory properties (colour, aroma, taste, texture and overall). The AJBF has darker colour than wheat flour, so the use more AJBF cause the colour of white bread darker compare with white bread from wheat flour. The dark color of AJBF of white bread make the panellist give low score (2.93) compare with control (4.20).

The AJBF has unpleasant odour compare with wheat flour. The increment of more AJBF in bread making leads to decrease the aroma score by panellist. The aroma score of F1, F2, and F3 were 3.13, 2.63 and 2.50, respectively. The other sensory property in this research is taste. The AJBF has special taste with beany taste. White bread using more the AJBF could decrease panellist score.

Table 1 The effects of AJBF on sensory properties of white bread

Sample	Colour	Aroma	Taste	Texture	Overall
F0	4.20^{a}	3.93^{a}	4.23^{a}	3.90^{a}	4.23^{a}
F1	3.83^{ab}	3.13^{b}	3.20^{b}	3.70^{b}	3.37^{b}
F2	3.47^{b}	2.63^{c}	3.00^{b}	3.27^{bc}	3.20^{bc}
F3	2.93^{c}	2.50^{c}	2.77^{b}	3.00^{c}	2.90^{c}

Values represent the mean of triplicate measurements ± SD (Standard Deviation). Means within columns with different letters are significant.
Score : 1 = disliked a lot, 2 = disliked, 3 = neutral, 4 = liked, 5 = liked a lot.
Sample :
F0 : 100% wheat flour (WF)
F1 : 90% WF : 10 % AJBF
F2 : 80% WF : 20 % AJBF
F3 : 70% WF : 30 % AJBF

The texture of white bread from AJBF has harder than white bread. The AJBF has not gluten content compare with white bread. It cannot hold the gas from fermentation stage in the bread making. Therefore, the texture of the AJBF white bread has denser and harder compare with wheat flour. Affandi (2016) reported that brownies with modified jack bean flour lead to decrease the texture. The decrease in texture due to wheat flour has high water absorption capacity. In other hand, another flour has low water absorption capacity, so in the baking process resulted in more water evaporation lead to denser texture (Dahlia, 2014).

The panellist score in overall parameters shows the

same trend with the other parameter of sensory properties. The more AJBF lead to decrease overall score compare with white bread. But, the F2 has better sensory properties compare with F2 and F3 based on the results of sensory properties.

2. *Physical Properties*

Baking expansion test was used in this research for physical properties. The baking expansion of white bread was calculated by measuring the volume before and after baking process. The baking expansion of white bread was presented in Table 2.

The baking expansion is closely related to the ability of the dough to form and hold the gas produced during fermentation. The most important component of wheat flour is gluten, which affects the elasticity of the dough and support viscoelastic properties. The substitution of AJBF has significantly different effect on the baking expansion of white bread.

Table 2 The effects of AJBF on baking expansion of white bread

Sample	Baking expansion (%)
F0	157,78[a]
F1	126,35[b]
F2	112,41[b]
F3	91,50[c]

Values represent the mean of triplicate measurements ± SD (Standard Deviation). Means within columns with different letters are significant.

Sample :

F0 : 100% wheat flour (WF)

F1 : 90% WF : 10 % AJBF

F2 : 80% WF : 20 % AJBF

F3 : 70% WF : 30 % AJBF

It can be seen that the greater of AJBF, the baking expansion of white bread will be decreased from 157.78% to 126.35%. This is caused by a decrease in the gluten content in the white bread dough. If the gluten content in the dough was reduced, the development of the dough is also getting smaller. Gluten was only obtained from wheat flour because the AJBF does not have gluten. Gluten functions to keep the dough steady and can hold CO_2 gas during the fermentation process lead to the dough can expand. that the reaction of gliadin and glutenin with water (hydration) will form an elastic mass called gluten which is a gas-binding (Bennion and Hughes 1970). Gluten is a part of wheat protein which when hydrated cannot be dissolved in water. In addition, gluten is a wheat protein which is very important role in the final results of the dough.

3. *Chemical properties*

From the sensory and physical properties, F2 has chosen for chemical analysis. The result of chemical properties of white bread was presented in Table 3. The moisture, ash, fat, protein, carbohydrate and NaCl content were investigated. The results suggest that moisture, ash, fat and NaCl were not significantly differenced with F1.

Table 3 The effects of AJBF on chemical properties of white bread

Sample	Moist.	Ash	Fat	Protein	Carbo.	NaCl
F0	32.29a	2,32a	3,57a	4,55a	57,10a	2,03a
F1	37,86[a]	1,93a	2,70a	5,68b	52,26b	1,88a

Values represent the mean of triplicate measurements ± SD (Standard Deviation). Means within columns with different letters are significant.

Sample :

F0 : 100% wheat flour (WF)

F1 : 90% WF : 10 % AJBF

F2 : 80% WF : 20 % AJBF

F3 : 70% WF : 30 % AJBF

F2 has significantly different in protein content (5.68%) higher than F1 (4.55 because the AJBF has higher protein compared with wheat flour. The result in agreement with Ariyantoro (2014), the protein content of modified jack bean flour has higher than wheat flour. If the AJBF was used in the white bread-making, so white bread has higher protein content compare with wheat bread.

Conclusion

The making of white bread using AJBF were investigated. More concentration of AJBF in the bread-making lead to decrease sensory and baking expansion properties. 10% AJBF of white bread has not significantly different with wheat bread in moisture, ash, fat and NaCl. However, the use 10% AJBF in the white bread making has successfully increased protein content compare with wheat bread.

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