

(Journal of Food Technology and Nutrition)

ISSN: 1411-7096 eISSN: 2613-909x

KARAKTERISTIK FISIKOKIMIA DAN ORGANOLEPTIK SAMBAL TEMPE: PENGARUH KONSENTRASI ANGKAK DAN LAMA PENYIMPANAN

Physicochemical and Organoleptic Attributes of Sambal Tempe: Red Yeast Rice Concentration and Storage Duration Effects

Rachel Meiliawati Yosharia*, Megan Pakpahana, Susana Ristiarinia, Ira Nugerahania, Indah Kuswardania

^a Program Studi Teknologi Pangan, Universitas Katolik Widya Mandala Surabaya, Jl. Dinoyo 42, Surabaya, Indonesia

*Penulis korespondensi: rachelmy@ukwms.ac.id

Abstrak

Tempe merupakan produk fermentasi kedelai menggunakan kapang Rhizopus sp. Tempe memiliki komponen bioaktif yang berperan sebagai antioksidan, namun memiliki masa simpan yang pendek. Pengolahan tempe menjadi sambal tempe dapat memperpanjang masa simpan serta meningkatkan fungsi tempe. Bahan utama pembuatan sambal tempe pada penelitian ini meliputi tempe goreng, cabe, rempah-rempah (cabai, bawang putih, kencur), dan angkak. Angkak merupakan hasil fermentasi beras oleh kapang Monascus purpureus. Selama penyimpanan, komponen lemak pada sambal tempe dapat mengalami oksidasi. Penggunaan angkak dapat bertindak sebagai antioksidan, selain fungsinya sebagai pewarna makanan dan flavor enhancer. Penelitian ini bertujuan untuk mengkaji efek konsentrasi angkak dan lama penyimpanan terhadap karakteristik fisikokimia dan organoleptik sambal tempe. Rancangan penelitian yang digunakan adalah Rancangan Acak Kelompok desain faktorial, yaitu konsentrasi angkak (0%; 0,2%; 0,4%; 0,6%; dan 0,8%) dan lama penyimpanan (0, 7, dan 14 hari). Peningkatan konsentrasi angkak berpengaruh nyata terhadap parameter warna, dan penerimaan organoleptic terhadap warna, aroma, dan rasa sambal tempe. Interaksi konsentrasi angkak dan lama penyimpanan berpengaruh nyata terhadap angka peroksida (0-1,42 meg/kg sampel), % inhibisi 28,47-83,05%, dan total fenol 2,05-11,89 mg GAE/g sampel. Peningkatan konsentrasi angkak menurunkan kesukaan warna, rasa, dan aroma sambal tempe.

Kata Kunci: Sambal Tempe, Angkak, Lama Penyimpanan

Abstract

Tempe is a fermented soybean product using *Rhizopus* sp. It has bioactive components that act as antioxidants but has a short shelf life. Processing tempe into sambal tempe can extend the shelf life and improve the function of tempe. This study's main ingredients for making tempeh sauce include fried tempe, oil, chili, spices, and red yeast rice. Red yeast rice is the result of rice fermentation by *Monascus purpureus*. During storage, the fat component in sambal tempe can undergo oxidation. Apart the function as food colorant, red yeast rice can also act as antioxidant and flavor enhancer. This study aimed to examine the effects of red yeast rice concentration and storage time on the physicochemical and organoleptic characteristics of tempeh sauce. The research design used was a Randomized Block Design with a factorial design, namely red yeast rice concentration (0%; 0.2%; 0.4%; 0.6%; and 0.8%) and storage time (0, 7, and 14 days). Increasing the concentration of red yeast rice significantly affected the color parameters, and organoleptic acceptance of the color, aroma, and taste of tempeh sauce. The interaction between red yeast rice concentration and storage time significantly affected the peroxide value (0-1.42 meq/kg), % inhibition 28.47-83.05%, and total phenol 2.05-11.89 mg GAE/g. Red yeast rice concentration increasing led to reduction of color, taste, and aroma preferences of sambal tempe.

Keywords: Sambal Tempe, Red Yeast Rice, Storage Duration

Histori Artikel Submit: 16 April 2025 Revisi: 22 April 2025 Diterima: 23 April 2025 Dipublikasikan: 30 April 2025

(Journal of Food Technology and Nutrition)

ISSN: 1411-7096 eISSN: 2613-909x

INTRODUCTION

Tempe is a traditional food product originating from Indonesia. Indonesia's yearly average tempe consumption per capita in 2023 was 7.47 kg (Pusat Data dan Sistem Pertanian, 2024). Tempe is known as an affordable protein source and regarded as a superfood due to its bioactive compounds (Romulo & Surya, 2021). In addition, tempe is a source of antioxidants, which are produced during fermentation (Surya et al., 2024). However, the shelf life of tempe is less than 48 hours, so further processing is needed. The shelf life of tempe can be extended by processed into chips, tempe bacem, and sambal tempe.

One of the famous processed tempe products in Central Java is sambal tempe. The processing of sambal tempe uses spices such as garlic, shallots, curly red chili, cayenne pepper, galangal, salt, and sugar. Sambal tempe is processed through frying and sautéing, which used cooking oil. Frying and sautéing support in microbes inactivation to extend shelf life and improve flavor. However, during storage, the quality of sambal tempe will decrease because of lipid oxidation. Lipid oxidation of sambal tempe can be prevented by natural antioxidant, such as red yeast rice.

Red veast rice is produced by fermenting rice with Monascus purpureus. It is commonly used as a food preservative, flavor enhancer, and food-coloring agent (Zhu et al., 2019). Red yeast rice can also act as an antioxidant. Based on Peranginangin et al. (2018), IC₅₀ of red yeast rice ethanol extract is 2.6 ± 0.07 ppm, while IC₅₀ of vitamin C was 1.16 ± 0.00 ppm. According to Tisnadiaia (2006), the fermentation process of red yeast rice produced several secondary metabolite compounds in the form of other polyketides, such as monacolin K, which is identical to lovastatin or mevinolin and other monacolin compounds such as atorvastatin, mevastatin, and simvastatin. In addition, red yeast rice also contains a number of phenol compounds that will bind to hydroxyl, phenoxyl, and superoxide. The presence of these secondary metabolite compounds can act as antioxidants that can inhibit the rate of rancidity of sambal tempe, thus extending its shelf life. Red yeast rice can also be used as a natural food colorant, because red yeast rice has quite stable properties, can mix with other color pigments, and is non-toxic (Purwanto, 2011).

The pigments are also resistant to high temperature processes. Sambal tempe is generally yellowish in color, in contrast to chilibased sambal in general, which is red in color. Red yeast rice can be provided color that resembles sambal. According to Chairote et al. (2009), red yeast rice has alkaloid which may act as antioxidant. Most alkaloid is known to have bitter taste (Munoz et al., 2020). Thus, higher concentration of red yeast rice will cause a bitter taste in sambal tempe. An appropriate concentration of red yeast rice will support in evoking flavor due to the content of oligopeptides produced during fermentation (Trisnagati & Suprihartini, 2019).

Previous research that has been conducted showed that the addition of red yeast rice of more than 1% caused a darker color and bitter taste of sambal tempe. In this research, the addition of red yeast rice is limited to 0.8%. The addition of red yeast rice as a source of antioxidants is expected to inhibit the deterioration of the quality of sambal tempe. Therefore, the effect of the combined treatment of the addition of red yeast rice concentration and storage duration on physicochemical and organoleptic properties was studied.

MATERIAL AND METHOD

Material and Tool

The materials used in making sambal tempe with the addition of red yeast rice were tempe (Hienak, obtained from Pendekar Tempe Sakti Surabaya), cavenne pepper, curly red pepper, shallots, garlic, galangal, salt, sugar, mushroom flavoring. cooking oil, IR64 rice, and red yeast rice. All of the spices, herbs, cooking oil, and rice were obtained from local traditional market at Surabaya. Pure culture of Monascus purpureus JK2A (obatined from Biology and Food Industry Microbiologi Laboratory of Widya Mandala Surabaya Catholic University). The materials used for the analysis of sambal tempe were methanol (Merck 1.06009. 2500), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma D9132), gallic acid (Merck), sodium thiosulfate (Honevwell). sodium carbonate (HiMedia), hydrochloric acid (Honeywell), acetic acid (Fluka), chloroform (Honeywell), potassium iodide (Honeywell), potassium iodide (Emsure), amylum (Himedia), folin ciocalteu reagent (Merck), and distilled water.

The tools used to make sambal tempe were gas stove (Rinnai RI-602 BGX), non-stick pan (Maxim), grinder (Famoze Pro), kitchen scale (Ohaus), analytical scale (Denver Instrument), thermometer (TP101). aluminum induction seal, 150 mL plastic jar, and induction heater, Tools used for analysis were analytical scale (Metler Toledo), color reader (Konika Minolta CR-20 Chromameter), **UV-Vis** spectrophotometer (Shimadzu UV-1800), cuvette (Quartz), hot plate (Daihan), and centrifuge (Hettic EBA 20).

Sambal Tempe Production

Tempe was cut into 0.5 cm thick and then fried for 5 minutes, then drained. Separately, the spices (including red yeast rice) were fried for 2.5 minutes. 400 g of fried tempe then mixed with the cooked spices and grounded until soft. The concentration of red yeast rice used was 0, 0.2, 0.4, 0.6, and 0.8% (w/w) of fried tempe weight basis. The mixture was then sauteed for 45 minutes over medium heat. Sambal tempe then packed in a 150 mL plastic jar and sealed with aluminium foil then stored in room temperature for 0, 7, 14 days for further analysis. Analysis of tempe this sambal in study physicochemical properties (total phenolic content, antioxidant activity, and peroxide number) and organoleptic properties (preference of the color, aroma, and taste of the sambal tempe).

Color Properties of Sambal Tempe Analysis Sambal tempe color was analyzed with color reader CR-20 (Konica Minolta, 2024). Color

reader CR-20 (Konica Minoita, 2024). Color parameter that analyzed in this study were L* (lightness), a* (redness), b* (yellowness), c (chroma), and °H (hue).

Extraction Sambal Tempe for Total Phenolic Content and Antioxidant Activity

For total phenolic content (TPC) and antioxidant activity, sambal tempe were extracted with methanol. 25 g of sambal tempe were added by 50 mL of methanol then homogenized and centrifuged for 4000 rpm 15 min. Supernatant then filtered with Whatman number 42 filter paper then used for TPC and antioxidant activity analysis.

Total Phenolic Content of Sambal Tempe Analysis

TPC of sample were analyzed following Srianta et al. (2022). 0.25 mL sample extract were added by 1.25 mL Folin Ciocalteu reagent in 25

mL vacuum flask, homogenized, and added by 3.75 mL Na₂CO₃ 20% solution then added by distilled water. The mixture then homogenized and incubated for 30 min at room temperature. The absorbance of sample then measured at 750 nm with a UV–Vis spectrophotometer (Shimadzu, Japan). Gallic acid was used as a standard with distilled water as the solvent. The results were expressed in milligrams of Gallic acid equivalent per gram of sambal tempe (mg GAE/g).

Antioxidant Activity of Sambal Tempe Analysis

Antioxidant activity of sambal tempe were analyzed using DPPH free radical inhibiton method (Zou et al., 2011). 0.25 mL DPPH solution (400 ppm) was added to 4,75 mL extract sample or methanol (for control). The mixture then incubated for 30 min in dark room and the absorbance (A) was measured at 517 nm with a UV–Vis spectrophotometer (Shimadzu, Japan). Gallic acid was used as a standard with methanol as the solvent. Antioxidant activity of sample was expressed in % inhibition and calculated following to:

 $%Inhibition = {(A_{control} - A_{sample})/(A_{control})} \times 100\%$

Peroxide Number of Sambal Tempe Analysis

Peroxide number of sambal tempe were analyzed by iodometric titration method following Sudarmadji et al. (2010). 10 g of sample were extracted with 30 mL acetic acid-chloroform (3:2) then centrifuged (4000 rpm) for 15 min. The liquid phase obtained then added by 0.5 mL saturated KI and incubated in dark room for 1 min. The mixture then added by 30 mL free CO₂ water and titrated by 0.1 N sodium thiosulfate (standardized with 0.1 N KIO₃) and 1% amylum solution as indicator. For blank analysis, 30 mL of acetic acid-chloroform (3:2) was replacing the liquid phase of sample. Peroxide number of sample were presented as meq/kg sample and calculated following to:

Peroxide Number =
$$\frac{(S-B) \times N_{Na_2S_2O_3} \times 1000}{W}$$

 $S = Volume Na_2S_2O_3$ needed for sample titration (mL)

 $B = Volume Na_2S_2O_3$ needed for blank titration (mL)

N Na₂S₂O₃ = Concentration of Na₂S₂O₃ (N) W = sample weight (g)

(Journal of Food Technology and Nutrition) ISSN: 1411-7096

elSSN: 2613-909x

with 0,2% red yeast rice was the only sambal resembled to general sambal.

Organoleptic Properties of Sambal Tempe **Analysis**

Organoleptic properties of sambal tempe was conducted using a hedonic test (preference test) following Setvaningsih et al. (2010). Preference test in this study was conducted on the color, aroma, and taste of the sambal tempe. A hedonic 7-point scale was used, which 1 represent dislike very much and 7 represent like very much. 45 untrained panelist of Widya Mandala Surabaya Catholic University who have basic sensory knowledge, familiar and can consume spicy product. This analysis was conducted on fresh sambal tempe (day 0).

Statistical analysis

The data were analyzed using IBM SPSS Statistics (version 19.0). Red yeast rice concentration and storage duration of sambal tempe were the two factors that could affect the parameters tested. One way ANOVA was used and continued with post hoc analysis using Duncan Multiple Range Test (p < 0.05) when the effects were significant.

RESULT AND DISCUSSION

Effect of Red Yeast Rice Concentration on the Color of Tempeh Sambal

Color is a food product attribute that determines acceptance by consumers. Based on Ardila-Diaz et al. (2020), L* value indicates the difference in brightness with a range of values 0 (dark) to 100 (bright). A positive a* value indicates the color tends towards red and a negative a* indicates green. A positive b* value indicates the color tends towards yellow and a negative b* indicates blue. The chroma (C) value indicates color saturation. The higher the C value indicates the brighter/thicker color, while a low C value indicates the color is paler/faded and dull. Sambal tempe appearance during storage and L* (lightness), a* (redness), b* (yellowness), c (chroma), and oH (hue) of all sambal tempe sample are shown in Fig. 1. And Fig. 2.

The appearance of sambal tempe without addition of red veast (0%) rice was light brown. and increasing of red yeast rice made sambal tempe become redder and darker. Sambal tempe with 0.6% and 0.8% red yeast rice had too dark color and estimated will affected preference of consumers. Sambal tempe with 0,2% and 0.4% appearance were the closest to general sambal. After 14 day of storage, sambal tempe

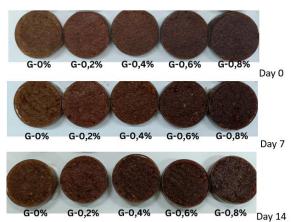


Figure 1. Sambal tempe appearance during storage

The concentration of red yeast rice significantly affected (p<0.05) the lightness (L), redness (a*), yellowness (b*), chroma (C), and ohue (H) of sambal tempe, while storage duration and the interaction of red yeast rice concentration and storage duration have no significant effect on those parameters. As the addition of red yeast rice concentration increased, the lightness of sambal tempe decreased. Red yeast rice was produced by fermentation of Monascus sp. Ristiarini et al. (2017) stated that during fermentation, Monascus creates at least six pigments including red, orange and yellow colors as its secondary metabolites. The more the red yeast rice is added, the more the pigment content will increase. Sambal in general tends to be red, because the main ingredient is chili. Berry et al. (2021) stated that carotenoids are the responsible for conferring piaments characteristic deep red colour to chilli.

The highest lightness and yellowness values were at 0% red yeast rice concentration. This phenomenon was due to the combination of fried tempe and spices producing yellower color of sambal tempe. Tempe is fermented soybean using Rhizopus sp. The most abundant carotenoids found in soybean flour are lutein and zeaxanthin. which both classified xanthophylls (Alamu et al., 2020). Xanthophylls are widely known as yellow-colored pigments. As the red yeast rice concentration increased, the lightness and yellowness of sambal tempe decreased.

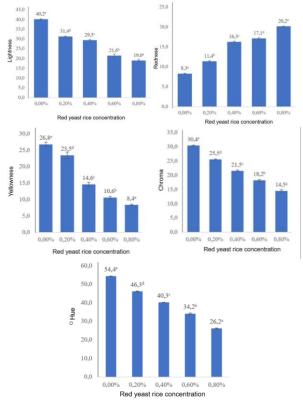


Figure 2. Effect of Red Yeast Rice Concentration on Lightness (L), Redness (a*), Yellowness (b*), Chroma (C), and °Hue (H) of Sambal Tempe. Mean values followed by different letters showed significant difference in the 5% DMRT test.

The higher concentration of red yeast rice led increasing of redness value of sambal tempe. As mention before, Monascus produces red, orange and yellow pigments. Rubropunctatin and monascorubrin contributes to orange color, while monascin and ankaflavin are yellow; and rubropunctamine and monascorubramine are two red pigments found in red yeast rice (Villaño et al., 2016). Despite of increasing yellow pigment as increasing of red yeast rice concentration, red and orange pigments also increasing.

The chroma value and ohue value of sambal tempe decreased as the concentration of red yeast rice increased. The chroma value and ohue value are influenced by the lightness, yellowness, and redness values caused by the red pigment contained in the red yeast rice. Therefore, the chroma value of sambal tempe as the concentration of red yeast rice increases caused the color of sambal tempe to be darken and reduce the sharpness of sambal tempe. While

the °hue value of sambal tempe is in the range of 0° - 90° which indicates a yellow - red color. As the storage duration was increased, all of the sambal tempe seen to be slightly darken but not significantly different. This finding showed us that pigment of red yeast rice remain stable during storage.

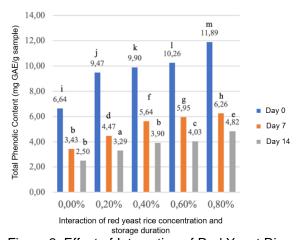


Figure 3. Effect of Interaction of Red Yeast Rice Concentration and Storage Duration on Total Phenolic Content of Sambal Tempe. Mean values followed by different letters showed significant difference in the 5% DMRT

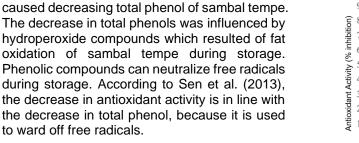
Effect of Red Yeast Rice Concentration and Storage Duration on Total Phenolic Content of Sambal Tempe

Total phenolic content analysis of sambal tempe was based on the folin ciocalteu method. The principle of this method is a reduction-oxidation (redox) reaction with electron transfer from the phenolic group to phosphomolybdate and phosphotungstic acid compounds in an alkaline medium to form a blue complex (Carmona-Hernandez et al., 2021). The concentration of red yeast rice, storage duration, and interaction of both significantly affected (p<0.05) TPC of sambal tempe. Effect of the interaction of the concentration of red yeast rice and storage duration on TPC of sambal tempe is presented at Figure 3.

Increasing of red yeast rice concentration affected the total phenolics of sambal tempe. According to Zhu et al. (2019), phenolic compound like daidzein, genistein, 5,5'-Dimethoxylariciresinol, lariciresinol, and scopoletin were found in red yeast rice. According to Hasim et al. (2018), red yeast rice extract had total phenolic content ranged from 5.17-66.23 mg GAE/g. Storage for up to 14 days

(Journal of Food Technology and Nutrition) ISSN: 1411-7096

ISSN: 1411-7096 eISSN: 2613-909x



Effect of Red Yeast Rice Concentration and Storage Duration on Antioxidant Activity of Sambal Tempe

Measurement of antioxidant activity of sambal tempe is based on the ability of antioxidant compounds to counteract free radicals. Concentration of red yeast rice, storage duration, and interaction of both significantly affected (p<0.05) antioxidant activity of sambal tempe. Effect of the interaction of the concentration of red yeast rice and storage duration on antioxidant activity of sambal tempe is presented at Figure 4.

The greater the concentration of red yeast rice led to increasing in the antioxidant activity of sambal tempe. Phenolic compounds can bind reactive oxygen specices (ROS), such as hydroxyl (HO•), phenoxyl, and superoxide (O2•-). Phenol is a bioactive compound that has the ability as an antioxidant by preventing oxidation reactions that can cause damage to packaged products. Phenolic compounds can also act as antimicrobials by damaging the cell walls and membranes of bacterial cells so that bacterial cells become lysed leading to cell death (Bouarab-Chibane et al., 2019), Ingredient of sambal tempe, such tempe, chilli, and garlic might also affect antioxidant activity of sambal tempe. According to Setiawati (2025), isoflavone was found in tempe. Chili contains capsaicin and flavonoid compounds which are antioxidant compounds (Kusnadi et al., 2019).

According to Minamizuka et al. (2021), red yeast rice had monacolin K that lower LDL cholesterol and blood pressure in Japanese with mild dyslipidemia. As stated before, hydroperoxide compounds from fat oxidation could be formed during storage of sambal tempe. Antioxidant on red yeast rice can interact with hydroperoxide or other ROS, then resulted lower antioxidant activity of sambal tempe during storage.

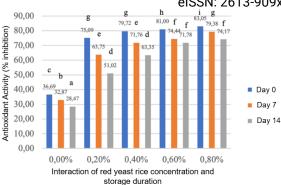


Figure 4. Effect of Interaction of Red Yeast Rice Concentration and Storage Duration on Antioxidant Activity of Sambal Tempe. Mean values followed by different letters showed significant difference in the 5% DMRT

Effect of Red Yeast Rice Concentration and Storage Duration on Peroxide Number of Sambal Tempe

Peroxide number testing is basically measuring the levels of peroxides and hydroperoxides formed in the early stages of fat oxidation reactions (Aminah, 2010). Factors that affect the rate of oxidation are the amount of oxygen, the degree of unsaturation of fatty acids in the oil, and the presence of antioxidants. The results caused by fat oxidation include peroxides, fatty acids, aldehydes and ketones (Mulyani & Sujarwanta, 2018). Rancidity is mainly caused by aldehydes and ketones. The higher the peroxide number, the higher the rancidity level of an oil caused by short carbon chains such as aldehydes and ketones.

The concentration of red yeast rice, storage duration, and the interaction of both had significant effect on the peroxide number of sambal tempe. Effect of the interaction of the concentration of red yeast rice and storage duration on peroxide number of sambal tempe is presented at Figure 5. Higher concentration of red yeast rice reduced peroxide number of sambal tempe. Jamaluddin et al. (2016) stated that solid state fermentation of Monascus purpureus enhanced antioxidant activity of rice. Zhu et al. (2019) stated that red veast rice had flavonoids, lignans, coumarin, and terpenoids. These compounds are widely known for its function as antioxidant that reduce effect of rancidity.

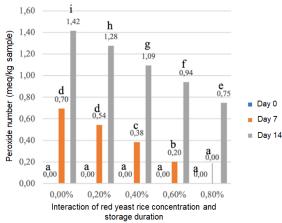


Figure 5. Effect of Interaction of Red Yeast Rice Concentration and Storage Duration on Peroxide Number of Sambal Tempe. Mean values followed by different letters showed significant difference in the 5% DMRT

Peroxide number of sambal tempe increased during storage. Fat compound on sambal tempe could undergone oxidation and formed peroxide. The result suggested adding 0.8% red yeast rice in sambal tempe could inhibit lipid oxidation until 7 days of storage, but prolonged storage until 14 days might lead further and faster oxidation. The presence of antioxidants obtained from the addition of red yeast rice helps neutralize free radicals by donating electrons and formation of free radicals are supressed.

Effect of Red Yeast Rice Concentration on the Sensory Properties of Sambal Tempe

Analysis of sambal tempe used fresh sambal tempe (0 days of storage) to see the effect of increasing the concentration of red yeast rice on the liking of color, aroma and taste. Effect of red yeast rice concentration on hedonic score of color, aroma, and taste of sambal tempe can be seen in Figure 5. Increasing concentration of red yeast rice led to significantly reduction of liking on color, aroma, and taste of sambal tempe (p<0.05). The red color produced got darker as the addition of red yeast rice concentration increases. The red color produced with the addition of red yeast rice concentration 0.6% - 0.8% caused a drastic decrease in liking.

The higher concentration of red yeast rice also caused a decrease in the liking of aroma. According to Indrawati et al. (2010), red yeast rice has a caramel-like aroma. The caramel-like aroma could cover the natural aroma formed by garlic, shallots, and galangal. Sauteing for a long

time could also caused the volatile components resembling caramel to become stronger, thus covering the volatile aroma sourced from spices.

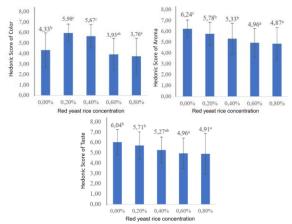


Figure 6. Effect Red Yeast Rice Concentration on Hedonic Score of Sambal Tempe.

Mean values followed by different letters showed significant difference in the 5% DMRT

The taste preference of sambal tempe decreased as the concentration of red yeast rice increased. Increasing the concentration of red yeast rice during heating causes the degradation of fat by heat to be inhibited, so that the formation of flavors that shape the taste of sambal tempe decreases. Red yeast rice contains a number of antioxidant compounds that inhibit fat degradation during heating. During cooking, cyclized compounds are formed that react with intermediate compounds and products of the Mailard reaction (non-enzymatic browning) and Strecker degradation. These reaction formed heterocyclic components resulting flavor components (Hustiany, 2016). In general, the addition of 0.2% red yeast rice produced the most preferred sambal compared to other treatments. In terms of taste preference, sambal tempe with a concentration of 0.2% had taste preference value that is not significantly different from the control treatment (no added red yeast rice), had the highest color preference compared to other treatments, and the aroma was quite preferred by panelists.

CONCLUSION

Red yeast rice concentration significantly affected color parameter of sambal tempe including lightness (L), redness (a*), yellowness (b*), chroma (C), and ohue (H). The concentration of red yeast rice, storage duration, and the interaction between red yeast rice

(Journal of Food Technology and Nutrition)

ISSN: 1411-7096 eISSN: 2613-909x

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concentration and storage duration affect the peroxide number, antioxidant activity, and total phenols of sambal tempe. Different concentrations of red yeast rice also significantly affected the sensory acceptance (color, aroma, and taste) of sambal tempe. Increasing concentration of red yeast rice reduced color, aroma, and taste panelist's preference. Based on physicochemical and organoleptic parameter, the addition of 0.2% red yeast rice significantly increased phenolic content, antioxidant activity, while decreased peroxide number compared to control, and also had higher sensory liking than other treatments.

ACKNOWLEDGMENT

The authors would like to thank Widya Mandala Surabaya Catholic University for the research funding provided through the Food Industry Microbiology Laboratory, Faculty of Agricultural Technology (ST No. 2934/WM01/N/2021).

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(Journal of Food Technology and Nutrition) ISSN: 1411-7096

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