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Effect of Sterility Values and Retort Temperatures on the Antioxidant Activities, Soluble Protein, and PH of Canned Mushroom

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Abstract

This research aimed to study the effect of sterility values on antioxidant activity, soluble protein, and pH of canned *Agaricus bisporus* mushrooms processed at various retort temperatures (115, 121, and 130 °C) and processing times (2-97 minutes). The canning process was carried out by following commercial production procedures in one of the mushroom canning industries. Measurement of heat penetration into the product was carried out using a standard protocol. The sterility values (F_0-value) were calculated using the General Method. Our results indicate that antioxidant activity, soluble protein contents, and pH of canned mushrooms are not only affected by sterility value but also by the combination of temperature and time used to process the product. At the same F_0-value of 10 minutes, retorting of *A. bisporus mushroom* at a higher retort temperature (130 °C) resulted in higher antioxidant activity (RSA 73.73%) and soluble protein contents (24.1 mg/g), but resulted in lower pH-value as of (5.5±0.04 in drained liquid and 6.52±0.21 in drained solid) than other retort temperature of 115 and 121 °C.Since retort temperature is crucial parameters of chemical quality of product attributes in the canning industry, the selection of higher temperatures and shorter time of retorting will have a positive impact on quality parameter such as antioxidant activity and total soluble protein.

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Agaricus Bisporus; Antioxidant Activity; F_0-Value; PH-Value; Soluble Protein.

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Introduction

Interest in mushrooms has increased worldwide in recent years because of their nutritional and promoting health properties.[1] Generally, mushrooms are rich in dietary fiber, low in fat, and some essential amino acids, including bioactive polysaccharides (lentinan), ergosterol, vitamin B1 (thiamin), B2 (riboflavin), vitamin C, and minerals. Mushrooms have a high protein content[2] and highly efficient bio-conversion.[3] Beyond the nutritional characteristics, this is widely reported and extensively explored in the literature that mushrooms have also been attractive to be studied for their richness in bioactive compounds, such as antioxidant, anticancer, and antimicrobial properties, increased liver function, and cholesterol-lowering activity among other bio-activities.[4]

Agaricus bisporus mushroom is one of the more popular mushrooms,with almost 34.8 billion kg of total world production in 2013.[5] However, fresh *A. bisporusis* highly perishable by nature and has a short shelf-life in the ambient environment, owing to their high moisture contents, lack of physical protection to prevent water loss or microorganism attack[6,7] and extreme sensitivity to heating.[8] Therefore, mushrooms are usually used as processed products.[9] Among the various methods used in mushrooms preservation, canning is the most frequently used method on the commercial scale. However, it is well known that heat processing may cause quality changes and reduce the content or bioavailability of some bioactive compounds that are relatively unstable to heat.[10] Excessive heating during the sterilization process resulting in low quality.[11] These quality impacts of thermal processes have always been under constant primary attention and development for researchers and the food industry since product quality is always an essential parameter for consumer acceptance.

During the sterilization process, the effect of temperature and heating time on pathogenic microorganisms is commonly represented by the F_0-value. F_0-value is the time in minutes for the specified temperature that gives the same thermal lethality as at 121°C in one minute. Previous studies have explored the effects of thermal processing on antioxidant activities and soluble protein contents for various food products. Murcia, Martinez-Tome, Jimenez, Vera, Honrubia and Parras[12] also reported that canning reduced the antioxidant capacity of truffles. Phenolic compounds were decreased in grains[13] and curruquilla[14] after canning. The canning process produced a significant loss of total phenolic content in *A. bisporus*.[15] Choi, Lee, Chun, Lee and Lee[16] reported that antioxidant activities or DPPH radical scavenging activities (free and bound polyphenolic and flavonoid contents) of *Shiitake* mushroom decreased during 15 to 30 minutes at the heating temperature of 121°C. Previous research also showed that canning decreased the values of phenolics (38–56% and antioxidant activity (16–35%) of peach palm heart (*Bactris gasipaes*)[17] and also in grains.[18]

Furthermore, the effect of retorting on the soluble protein has been reported by previous researchers, such as Chiang, Yen and Mau,[19] who found that the total free amino acids contents are lost in canned mushrooms. Jaworska, Bernaś and Mickowska[20] also reported a significant decline of arginines, glycines, serines, histidines, methionine, and threonine in mushroom canned foods (1-31%). The process also resulted in a corresponding reduction of 80.1% in total free amino acids and 85% in essential amino acid contents in canned *A. bisporus*.[21] Researchers recently reported that high temperatures (130°C) reduce casein solubility that was associated to molecular interactions, and vary depending on the heating temperature.[22]

Interestingly, it is still arguable whether the change of food quality due to sterilization could also be solely described as a function of the F_0-value.[15,16,31] Limited data is available to systematically explore the impact of heating time-temperature on mushrooms. Therefore, this paper aims to study the effects of sterility values (F_0-value) and retort temperatures of various time-temperature combinations on antioxidant activities, total soluble protein, and pH-value of canned *Agaricus bisporus* mushrooms.

Materials and Methods

Materials

Fresh *A. bisporus* mushroom cultivated in Probolinggo, East Java, Indonesia, was kept fresh by storing at 4 °C.[23] The equipment used was a horizontal static retort with a diameter of 1.25 m and a length of 2.35 m (Chi Yinfa, Taiwan). Temperature measurement and recording were done using the OM-CP-Hitemp 140 data logger (Omega

Engineering, Norwalk, Connecticut, USA). OM-CP-Hitemp140 data logger can measure temperatures up to 140 °C (284 °F), with an accuracy of ±0.1 °C (0.18 °F). Eppendorf 5810R centrifuge (Hamburg, Germany), a UV-Visible Spectrophotometer (U-2900, Hitachi, Tokyo, Japan), a digital analytical balance (Fujitsu AR-210, Japan), a digital pH-meter (Milwaukee MW801, USA), and the eight-mesh stainless steel filter (Fisher Scientific Company, USA) were also used in this study. Methanol (Merck, Darmstadt, Germany), (DPPH) 2,2'-diphenyl-1-picrylhydrazyl (Sigma Aldrich, St. Louis, USA) were used in this study. All the chemicals were of high analytical grade. Bidistilled deionized water was obtained from IPB University.

Canning Procedure and Analysis

Preparation of *Agaricus bisporus* samples before canning followed the procedure used in one of the mushroom canning industries (PT. Suryajaya Abadiperkasa).[23] Mushrooms were stored one day at the temperature of 3-5 °C before the canning process. The canning steps were material preparation, blanching, filling into cans, filling medium (citric acid, ascorbic acid, and NaCl), exhausting, seaming, sterilizing, cooling, and storing (Figure 1). The retort consisted of three baskets loaded jumbly with the cans, and each basket was filled with 700 cans (full capacity conditions). Control mushroom samples were taken shortly after the seaming process or just before the retorting process. In contrast, the treated product samples were taken after the sterilization process according to the design of the experiment at different retort temperatures (115, 121, and 130 °C).

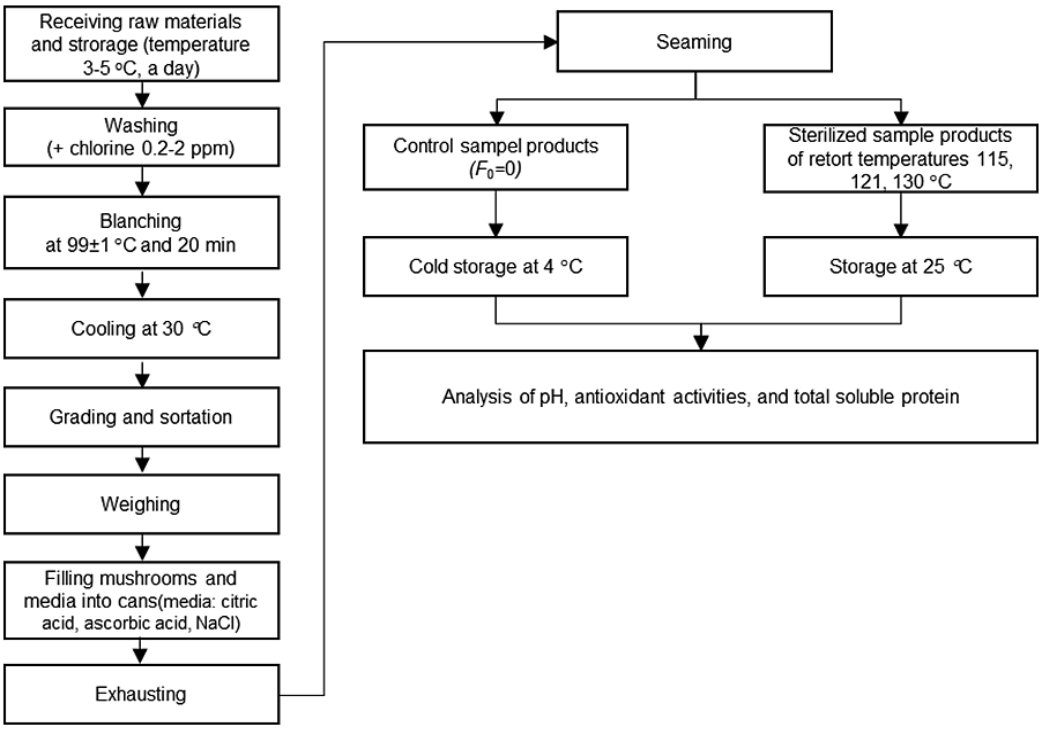

Fig. 1: The canning process of *Agaricus bisporus* packaged in 300x407, followed by product analysis[23]

F_0-Value Calculation

F0-value was calculated based on heat penetration data referred to the Institute for Thermal Processing Specialists (IFTPS) protocol.[26] Heat penetration tests were conducted by placing eleven data loggers (OM-CP-Hitemp 140, Omega Engineering, Norwalk, Connecticut, USA) in the slowest heating area. Data loggers were located at the center of the cans,

and their sensors were inserted into the mushroom. The sterility value was expressed as an F_0 calculated using General Method,[25] as shown in Equation 1.

$$F_0 = \int_0^t 10^{\frac{T-Tref}{z}} dt \quad ...(1)$$

where F_0 was the equivalent heating time (in minutes) at a constant temperature of 121.1 °C (250 °F) to inactivate C. botulinum spores, T was the product's temperature at any given time; T_{ref} was a reference processing temperature (121.1 °C or 250 °F), andz-value was 10 °C. Datalogger placement schemes and samples on a static horizontal retort is shown in Figure 2.[23]

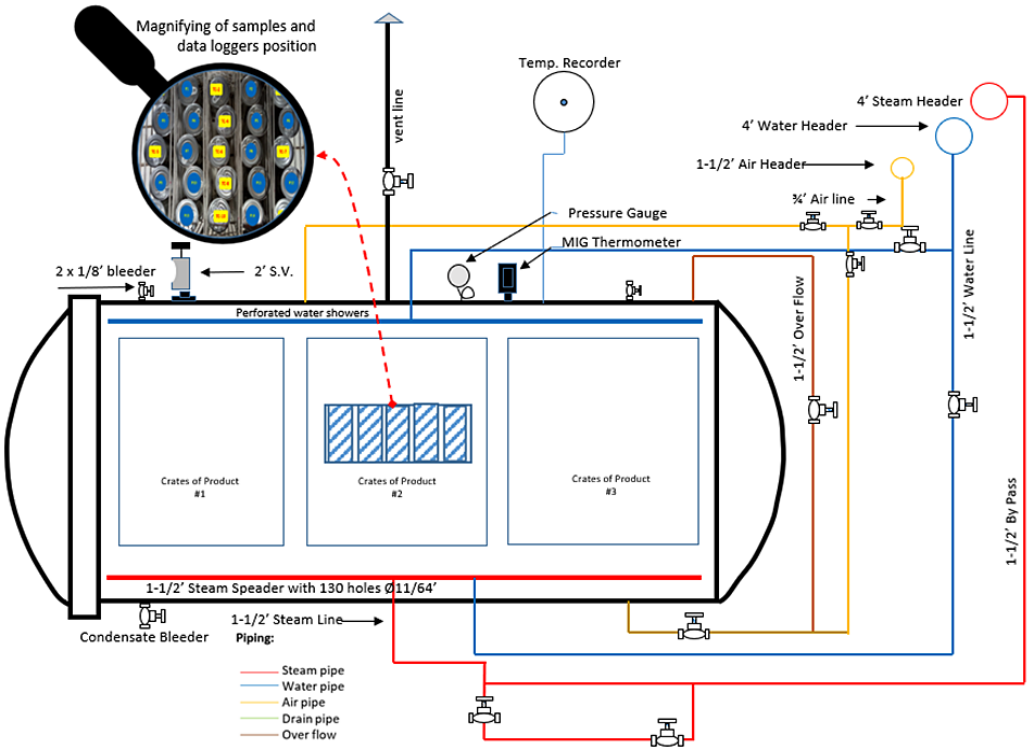

Fig. 2: Datalogger and samples schemes on the static horizontal retort[23]

Determination of Antioxidant Activity

Antioxidant activity was measured as free radical scavenging activity (RSA) and determined using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay method. DPPH solution was prepared by mixing 5 mg of DPPH with 96% methanol (Merck, Darmstadt, Germany) 250 mL, stirred, and stored at 4 °C in dark conditions for less than 8 hours. Ascorbic acid was used as a solution standard prepared in different concentrations (6.25, 12.5, 25, 50, 75, and 100 ppm). RSA was determined in two types of samples, drained solid mushroom and drained liquid. Both drained samples were obtained from drained weight measurement done by draining the whole content of canned mushroom on an eight-mesh stainless steel screen (Fisher Scientific Company, USA) positioned at an angle of 20° for two minutes at ambient temperature.

RSA in drained solid mushroom was determined first by blending 206.9 ± 1.19 gsample with 80% ethanol for 2 minutes. One g of composite sample was vortexed with 10 ml of ethanol for one minute and kept at room temperature for 30 minutes. The vortexed sample was centrifuged at 3,000 RPM to separate the supernatant from the solid. One ml of the supernatantand 0.1 ml of the drained liquid wasbothmixed with 5 ml of DPPH solution, vortexed for 1 minute before it was stored for 30 minutes in a dark room prior toanalysis. In the case of RSA

in the drained liquid of canned mushrooms, the same preparation as in drained solid mushrooms was applied. Furthermore, the absorbance of the sample was measured by a UV-Vis spectrophotometer at 517 nm (triplicates) and converted into a percentage of radical scavenging activity. The activity of arrest against radical DPPH is expressed as a percent inhibitory to radical DPPH. The DPPH radical scavenging activity was calculated by the following equation.[26]

$$\text{Radical Scavenging Activity (\%)} = (A_0-A_s)/A_0 \times 100 \quad ....(2)$$

A_0 is the absorbance of the control, and the A_s is the absorbance of samples.

The total RSA of canned mushrooms was the sum of both RSA in both drained solid mushrooms and drained liquid.

Determination of Soluble Protein

Soluble protein was determined according to Bradford Method (1976) in two types of samples, drained solid mushroom and drained liquid. Both drained samples were obtained from drained weight measurements done as described above. The sample was processed by using food blender for 2 minutes and filtered using a Whatman No.1 filter paper. One ml of the filtrate was taken and analyzed according to the Bradford Method (1976). Bovine Serum Albumin (BSA) was used as a solution standard ranging from a concentration of 0.1 to 1 mg/mL. The absorbance was measured with a spectrophotometer at 595 nm. The total soluble protein of canned mushrooms was the sum of both soluble proteins in both drained solid mushrooms and drained liquid.

Measurement of pH

As done in antioxidant activity and soluble protein determinations, the pH of canned mushrooms was measured in both drained solid and drained liquid. In both samples, the pH was measured after the drained solid mushrooms processed by using food blenderfor 30 seconds

Statistical Analysis

The result data were statistically analyzed for analysis of variance (ANOVA). $P<0.05$ was considered statistically significant. All statistical analyses were conducted using MS Office Excel (2016) and SPSS 20 (IBM Corp., Armonk, NY, USA).

Results and Discussion

Radical Scavenging Activity

Mushrooms contain various secondary metabolites, such as various phenolic compounds and ergothioneine, that have been shown to act as high antioxidants.[27] Phenolic acids are the major low molecular weight bioactive components usually found in mushroom species, responsible for their antioxidant properties.[28]

The total antioxidant activities of canned *A. bisporus* (drained mushroom and liquid), as determined by scavenging DPPH radical expressed in this article as RSA (Radical Scavenging Activity), are presented in Figure 3. Samples with F0=0 minutes are control samples taken before the retorting process. The RSA of mushroom samples decreases with increasing F_0-values at all sterilization temperatures. The result also shows that retort temperature significantly influences the change in the RSA of the product. The antioxidant activity after the commercial sterilization process compared with the antioxidant from the control decreased from 74.25% to 71.09%.

The decreasing rate at a retort temperature of 130 °C is lower than that of 121 and 115 °C due to shorter processing times. A previous study showed that the times needed to achieve an F_0-value of 10 minutes were 39.32, 11.22, and 1.30 minutes, respectively.[23] It can be seen, for example, at the same F_0-value of 10 minutes, the RSA at 115, 121, and 130 °C were 72.67%, 72.83%, and 73.73%, respectively. It shows that the sterilization process at a higher temperature at the same F_0-value results in slightly higher antioxidant activity (Figure 3). A similar result is shown at the same F_0-values. The RSA decreasing rate at a retort temperature of 130 °C is lower than other retort temperatures. At lower temperatures and a longer sterilization process, interactions between components in the food matrix occur, inhibiting the breakdown of antioxidant compounds (free polyphenol compounds) in drained liquid mushrooms. Due to the differences between thez-value of Clostridium botulinum (z = 10 °C) and the z-value of ascorbic acid (27 °C) 29, the activation energy of ascorbic acid is lower than the deactivation of microbial activity (C. botulinum). This value implies that the

rate of destruction of microorganisms will be much higher than the rate of antioxidant degradation at a higher temperature. Thus, the thermal processing of food products at higher temperatures can achieve commercial sterility with better retention of antioxidant quality.

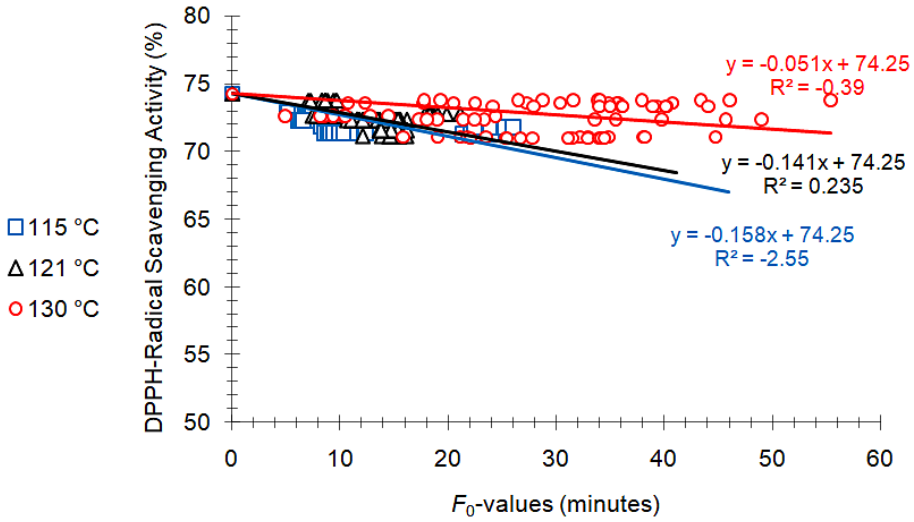

Fig. 3: The radical scavenging activity (RSA) of canned A*. bisporus* mushrooms, packed in 300x407 of cans as affected by F_0-value at three different retort temperatures

Although the total antioxidant activity of the canned *A. bisporus* mushrooms (drained solid mushroom and drained liquid) was decreasing, however, there was an increase in the antioxidant activity on drained solid mushrooms (Figure 4) and decreased antioxidant activity in drained liquid (Figure 5). Figure 4 shows that the (RSA) characteristic of antioxidant solubility from mushroom samples increases exponentially with increasing F_0-values at all sterilization temperatures. The result also shows that retort temperature significantly influences the change in the RSA of the product. The increasing rate at a retort temperature of 130 °C is lower than that of 121 and 115 °C. At the same F_0-value (10 minutes), the percentage of DPPH-radical scavenging activity at 115, 121, and 130 °C were 17.87, 17.62, and 15.94, respectively. Retorting the product at 115 °C and 121 °C caused the RSA to increase by 28.1– 30% from that of the control sample. Besides, retorting the product at 130 °C would increase RSA to 16.0%. The increase of antioxidant activity in drained solid mushrooms is probably due to the development of new compounds having antioxidant activity during heat or thermal treatment. Maillard's reaction products might be developed during prolonged heat treatment to increase antioxidant activity in this study. Peleg, Naim, Rouseff and Zehavi 30 stated that polyphenolic compounds in plant materials are mainly present as a covalently bound form with insoluble polymers. Choi, Lee, Chun, Lee and Lee[16] stated that thermal treatment disrupts the cell wall and releases antioxidants from the insoluble part of the mushroom, which increases the pool of antioxidant compounds. In previous studies,[31] canning resulted in the loss of water from mushroom products or decreased drained weight. This decreasing of drained weight was one of the reasons why the antioxidant characteristics of drained solid mushrooms increased during the retorting process. Moreover, Pogoń, Gabor, Jaworska and Bernaś[32] reported that adding vegetables and spices might have caused antioxidants to migrate from the added ingredients to the canned mushrooms in more significant quantities than may have been leached from the mushrooms to the brine. This phenomenon would impact overall antioxidant activity in mushrooms, whereas the (RSA) characteristic of antioxidant solubility from mushrooms increases along with increasing F_0-values.

Conversely, RSA decreases in the drained liquid of canned *A. bisporus* (Figure 5). The decrease rate of RSA is faster at a lower retort temperature of 115 °C due to longer processing times to achieve the same sterility value. It can be seen, for example, at the same F_0-value(10 minutes),the RSA detected at 115, 121, and 130 °C were 52.83, 54.40, and 55.16, respectively. Retorting the product at 115 °C and 121 °C decreased the RSA of 10.1-12.7% from that of the control sample, whereas at 130 °C would result in the RSA decrease of 8.8% due to shorter sterilization times.

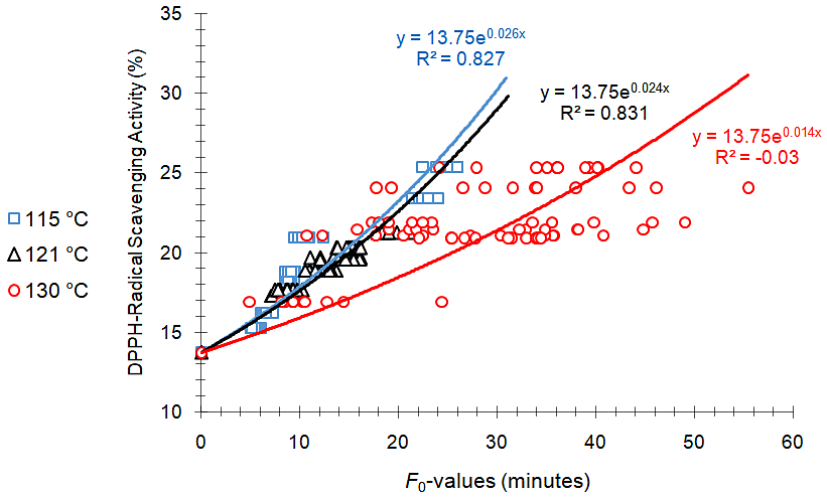

Fig. 4: The characteristic of DPPH-radical scavenging activity of drained solid *A. bisporus* mushrooms, packed in 300x407 of cans as affected by F_0-value at three different retort temperatures

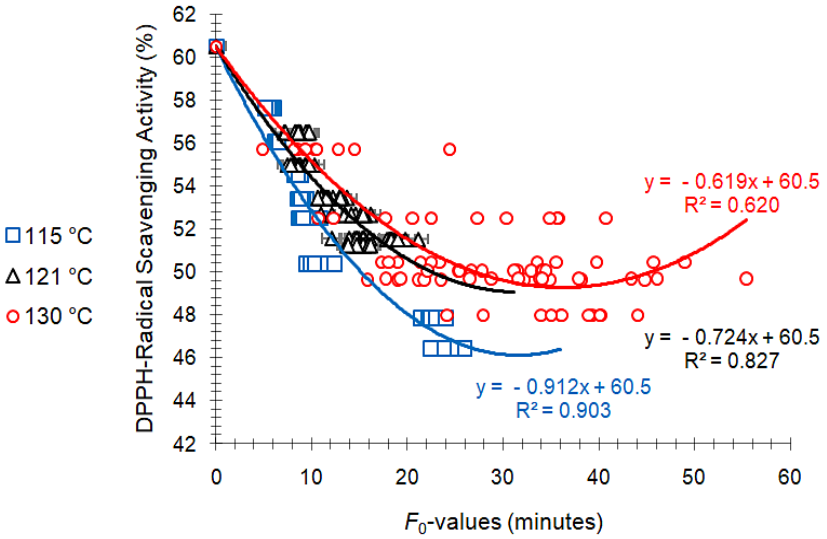

Fig. 5: The characteristic of DPPH-radical scavenging activity of drained liquid *A. bisporus* mushrooms, packed in 300x407 of cans as affected by F_0-value at three different retort temperatures

Soluble Protein Content

Nutritional contents and bioactive components of edible mushrooms depend on the processing technique used, which influences the chemical composition and commercial value of edible mushrooms.[8] The total soluble protein of samples (Figure 6) decreases with increasing F_0-values at all sterilization temperatures. The decreasing rate at a retort temperature of 130 °C is lower than that of the retort temperatures of 121 and 115 °C due to shorter processing times to achieve the same F_0-value. For example, at the same F_0-value of 10 minutes, it can be seen that the total of soluble protein at 115, 121, and 130 (C were 23.92, 23.97, 24.1 mg/g, respectively). Retorting the product at 115 °C and 121 °C decreased the soluble protein content by0.6-0.8% from that of the control sample.

Moreover, retorting the product at 130°C would decrease the soluble proteinby 0.1%. It shows that the sterilization process at a higher temperature at the same F_0-value will keep the soluble protein in drained solid mushrooms from denaturation or more stable. Decreasing soluble protein in drained liquid mushrooms probably due to thesterilizationprocessresultedinchanges in the chemical and physical structure of the mushroom tissues. Solubility often depends on increasing retort temperature, and increasing kinetic energy at higher temperatures allows solvent molecules to cause a change in theprimary, secondary,and tertiary structure of proteins. In addition, decreasing protein in drained solid mushrooms occurred at lower temperatures, probably due to a prolonged retorting process as compared to higher retort temperature (130 °C) to achieve the same F_0-value. Therefore, it causes protein denaturation and leachesin the drained liquid of canned *A. bisporus*. Jasinki, Stemberger, Walsh and Kilara[33] were observed using Transmission Electron Microscopy (TEM). They mentioned that high temperatures during canning caused coagulation of cytoplasmic material, and disruption of the intracellular membranes correlated mainly with the loss of cell membrane integrity. These findings of the current study are consistent with those of Zivanovic, Buescher and Kim,[34] who showed increasing soluble protein in the drained liquid of mushroom products as a function of temperature (85-121°C).

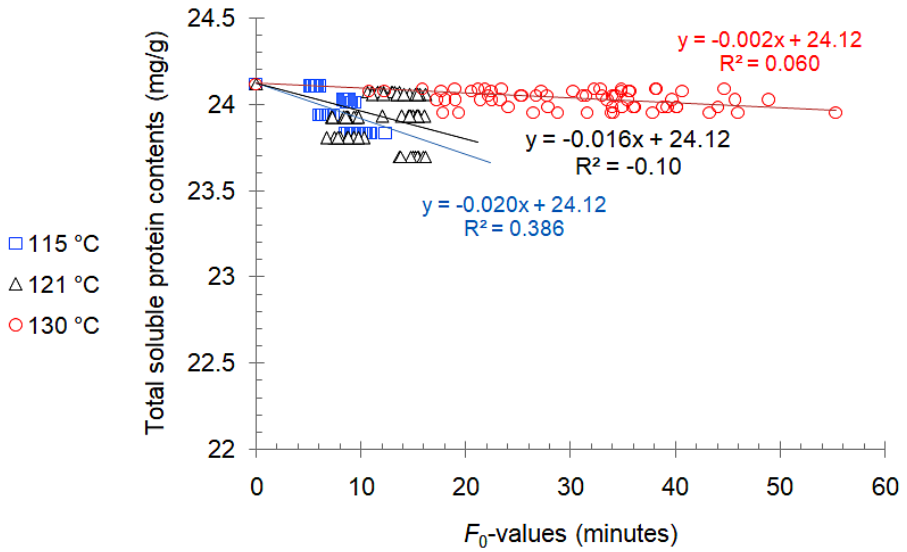

Fig. 6: Total soluble protein contents of canned *A. bisporus* mushrooms, packed in 300x407 of cans as affected by F_0-value at three different retort temperatures

Furthermore, Figures 7 and 8 show the characteristic of soluble proteins as an effect of retort temperatures (115, 121, and 130 °C) and F_0-values. Samples with a value of F_0=0 minutes are control samples taken before the retorting process. Figure 7 shows that the protein content decreased exponentially with

increasing F0-values at all sterilization temperatures. The result also indicates that retort temperature significantly influences the change in the soluble protein of the product (P<0.05). The decline rate at the retort temperature of 130 °C is slightly lower than that of 121 and 115 °C. At the same F_0-value, for example of 10 minutes, the percentage of soluble protein contents at 115, 121, and 130 °C were 10.50, 14.60, and 18.56, respectively. Retorting the product at 115°C and 121°C reduced the soluble protein contents by 33.6 – 52.3% from that of the control sample. On the other hand, retorting the product at 130 °C would reduce soluble protein contents by 15.6%.The soluble protein decreasing at 130°C of retort temperature is lower than other retort temperatures due to shorter processing times. In contrast, there is increasing soluble protein in the drained liquid of canned *A. bisporus* (Figure 8). The increased rate of soluble protein is faster at a lower retort temperature of 115 °C. At the same F_0-value achieved (10 minutes), the soluble protein content detected at 115, 121, and 130°C were 7.99, 4.09, and 3.20 (mg/g), respectively. Retorting the product at 115°C and 121°C significantly increased the soluble protein rate by 93.9-278.5% than that of control sample, whereas at 130°C would result in a soluble protein increase by 51.6%.A similar result is shown at the same F_0-value of 20 minutes.

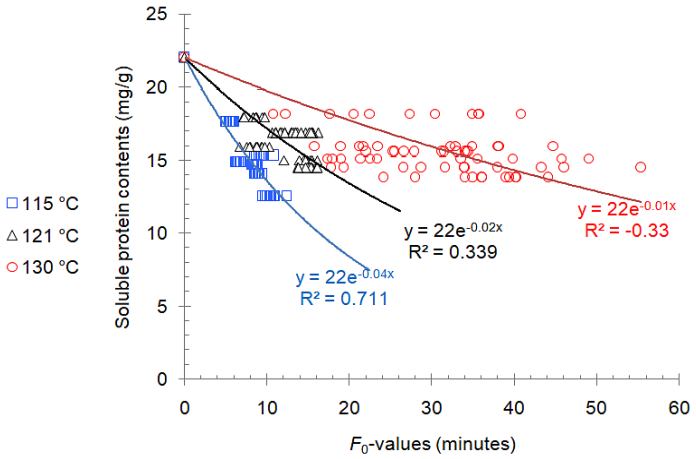

Fig. 7: The characteristic of soluble protein contents of drained solid *A. bisporus* mushrooms, packed in 300x407 of cans as affected by F_0-value at three different retort temperatures

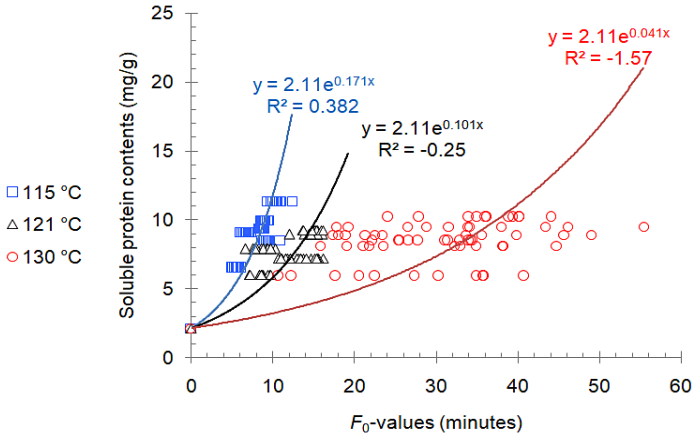

Fig. 8: The characteristic of soluble protein contents of drained liquid A*. bisporus* mushrooms, packed in 300x407 of cans as affected by F_0-value at three different retort temperatures

PH Value

Since pH significantlyinfluencesfood safety and quality attributes, it is essential to analyze pH change as an effect of the thermal process for the safety and quality of food products. One of the critical factors that affect the thermal resistance of bacterial spores, or their ability to germinate, from a sterilization point of view, is pH.[25] The pH or acidity of foods is generally used to determine processing requirements and applicability. In order to provide some safety margin, the USFDA has recommended a pH value of 4.6.[35]

Table 1: The pH-values of drained liquid and solid of the canned *A. bisporus* mushrooms, packed in 300x407 of cans as affected by F_0-value and retort temperature

Samples of Caned Mushrooms	Retort Temperatures (°C)	pH at different F_0-values		
		0 (Control samples)	10 minutes	20 minutes
Drained liquid	115	4.22±0.08[a]	5.83±0.15[b]	5.45±0.16[b]
	121		5.78±0.11[b]	5.42±0.10[b]
	130		5.50±0.04[c]	5.35±0.05[b]
Drained Solid	115	6.08±0.08[a]	6.58±0.13[b]	6.52±0.07[b]
	121		6.53±0.04[b]	6.50±0.10[b]
	130		6.52±0.21[b]	6.47±0.09[b]

Values are expressed as mean ± SD (n=3); different letters within columns indicate significant differences (P <0.05).

The pH change of canned *A. bisporus* (drained liquid and solid) as affected by F_0-value and retort temperature is presented in Table 1. Samples with a value of F_0=0 minutes are control samples taken before the retorting process. The pH value of mushroom samples slightly increases with increasing F_0-values at all sterilization temperatures than control samples (F_0=0 minute), P<0.05. The pH value of drained liquid and solid mushroom after the commercial sterilization process compared with the pH of the control samples increased from 4.22 to 5.83 and 6.08 to 6.58, respectively. The higher retort temperature and shorter processing time, the more it can maintain the pH value or be more stable.

While at lower sterilization temperatures and longer heating times result in more hydrolyzed mushroom proteins, especially basic amino acids, such as lysine, arginine, and histidine. As shown in Table 1, at F_0 = 10 minutes at retort temperatures 115, 121, and 130 °C, the pH values achieved are 5.83, 5.78, and 5.50, respectively. It shows that the process at a higher temperature at the same F_0-value will slightly protect the basic of amino acids in canned mushrooms from hydrolysis. A similar result is shown at F0=20 minutes for both drained liquid and solid mushrooms. This result supports other finding,[36] which showed that processing caused an increase in pH due to heating. Higher retort temperature causes more cell wall damage than lower retort temperature. At higher temperatures, the cell and tissue structure shrinks, releases water in the tissue, causes protein denaturation, and releases hydrogen ions.

Lund and Ray[37] reported that any solution's temperature increase would increase its ions' mobility in the solution. Increased temperature may also increase the number of ions in the solution due to molecular dissociation. Based on this fact, it can be said that the pH-value of the drained liquid of canned mushrooms is influenced by retort temperature and F_0-values.

Conclusion

This study demonstrates that the canning process of *A. bisporus* mushroom at various F_0-values and different sterilization temperatures of 115, 121, and 130 °C results in changes in chemical quality attributes. Our findings show that the canning process led to changes in antioxidant activities, soluble protein, and pH-value, which depend not only on the F_0-value but also on retort temperature. At the same F_0-value (e.g., 10 minutes), we found that retorting the product at a higher retort temperature of 130 °C would provide benefits such as alower decrease in the soluble protein and antioxidant activity of canned mushrooms. Moreover, selecting the higher retort temperature (130 °C) also results in a lower pH value than other retort temperatures of 115 and 121 °C. The use of a higher temperature and shorter time is more favorable than that of a lower temperature and longer time of retorting from antioxidant activity, soluble proteins content, and pH-value properties point of view.

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Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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