The Physical, Chemical and Microbiological Quality of Tempe Treated with High Pressure of Carbon dioxide

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Abstract The objective of this research was to analyze the physical, chemical, and microbiological quality of tempe treated with CO_2 under 900 psi and 1100 psi. The treatment was designed as Randomized Completely Block Design with two factors as treatments and each treatment was repeated three times. The first factor was the pressure under 1100 psi and 900 psi, and the second factor was holding time for 5, 10, 15, and 20 minutes. The physical quality (texture), chemical quality (water content and protein content) and reduction number of microbial loads were determined in control and treated tempe. The result showed that at the different pressure, holding time and combination of both of the treatments were significantly affected the texture, protein and water content of tempe ($P<0.05$). Tempe treated with CO_2 under 1100 psi for 5 minutes had the highest hardness (314,73 gf) and water content (39,9%), but the lowest protein content (16,74%). The reduction number of microbial load was 1 log cycle and less than 1 log cycle in tempe treated with CO_2 undert 1100 psi and 900psi for 20 minutes, respectively.

Keywords: High pressure CO_2, tempe, physical, chemical, microbiological.

1. Introduction

Tempe, a fermented food originated from Indonesia, are made from cooked soybeans and fermented by *Rhizopus oligosporus*. Tempe is of remarkable interest due to its freshness quality and has superior nutrition. Tempe is not consumed in raw but consumer acceptability on tempe is greatly determined on the freshness of tempe. The fresh tempe describes as having white moldy color, compact and sliseable texture of a cake like, mushroomy aroma, and beany flavor. Beside its superiority in nutrition, tempe is perishable food. Fresh tempe is only stand for 48 hours at room temperature [1]. Methods for extending the storage time of fresh tempe have been sought by tempe manufacturers. Application of heat on tempe produced the cooked tempe with changing the freshness of tempe. In addition, loss of fresh taste and texture occurred at refrigerated as well as freezed storage of tempe [2]. At the same time there is increasing demand for minimally process and fresh-tasting foods. Therefore, an advanced in technology that cause least deterioration in product quality is required. Study on the processing of tempe with high pressure carbon dioxide (CO_2) has not yet been done. One of the interesting properties of CO_2 is that its critical point can be reached at relatively low temperature and pressure values (31.1°C and 75 bar/ 7.4 MPa/1073 psi) which can be applied for mild heat food processing [3]. At the supercritical phase CO_2 exhibits both gas-like transport characteristics and liquid-like solvent properti, which makes them useful for reactions and separations [4]. The properties of supercritical CO_2 can be controlled by manipulation of the temperature and pressure. Near the critical point, small changes in temperature or pressure lead to significant changes in the density and density-dependent solvent properti such as solubility parameter, the partition coefficient and the dielectric constant [5]. Beside these interesting physical properties, specific interest in CO_2 is magnified by its perceived green properties as it is relatively non-toxic, relatively inert, and non-residual left. The objective of this research was to analyze the changes in texture, chemical contents, and microbial reduction of tempe treated with high pressure CO_2 at 1100 psi and 900 psi.

2. Materials and Methods

Tempe used in this experiment was obtained from tempe producer in Palembang. The materials used were gas CO_2, chemicals for analysis, Plate Count agar (PCA) for bacterial growth, and mold extract agar (MEA, Difco) for mold growth medium.

2.1. High pressure carbondioxide (HPCD) treatment.

The design of the CO_2 apparatus is shown in Figure 1. The system is equipped with a stainless steel chamber, CO_2 gas tank, and container with hot water to control the pressure. The CO_2 gas reservoir tank is regulated with a temperature-controlled heater to achieve the pressure required e.i, the supercritical phase ($scCO_2$) was at the condition of 7,6 Mpa (1100 psi) and 45°C, and the liquid phase ($lqCO_2$) was at the condition of 6,2 Mpa (900 psi) and 25°C. Tempe, a 29-30 g, cylindrical long shape 10x2x2 cm3, were placed into a stainless steel chamber. Tempe was then pressurized with CO_2 at 900 psi and 1100psi by adjusting the temperature of the CO_2 in the system. Approximately 2 min was needed to achieve a pressure of 1100 psi. When the required pressures were reached, the process was hold for 5, 10, 15, and 20 min. Following was the decompression time which was approximately 2 min to reach the pressure at an ambient temperature.

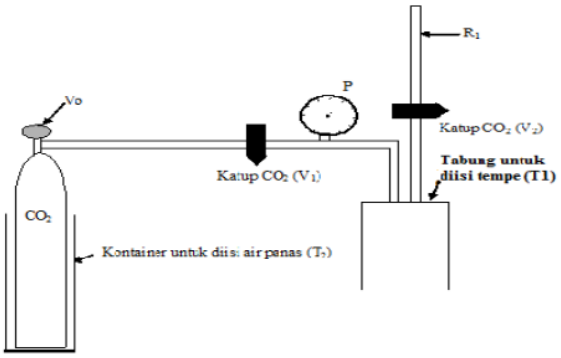

Figure 1. The diagram of CO_2 instrument used in the study.

2.2. Analysis

2.2.1. Microbial analysis

Tempe (5g) was homogenized using Stomacher Bag and the macerate was serially diluted with peptone water. Total viable counts were determined as duplicate spread plates of 1 mL ofdiluted sample in Plate Count agar (Difco). Plates were incubated for 24 h at 32o. The counts were reported as log CFU/g of tempe. Reduction number was calculated as the difference between the number of viable cell before the treatment and after the treatment in log.

2.2.2. Physical analysis

Texture measurements of the hardness of tempe was analysed using LFRA textur analyzer Brook Field. Scanning electron microscopy (SEM JEOL JSM 5310 LV) was used to study the surface properties and the mycelium of the tempe. Examination of the sampel was done under 1000, 2000

and 10.000 magnifications, with an acceleration potential of 20 kV.

2.2.3. Chemical analysis

The proximate analysis of the treated and non-treated tempe were done including the protein content and moisture content followed the method of AOAC [6].

2.3. Experimental design and data analysis

The experiment was designed as Randomized Completely Block Design with two factors of high pressure as treatment and each treatment was repeated three times. The first factor was high pressure CO2 under 1100 psi (7,6 MPa) and 900 psi (6,2 MPa), and the second factor was holding time at 5, 10, 15, and 20 minutes. One way ANOVA was applied to all the data and the significant different results were analyzed further using Duncan Multiple Range Test (DMRT).

3. Result and discussion

3.1. Texture analysis

Texture is commonly related to physical properties such as firmness, hardness and viscosity measurements. Based on ANOVA and the Duncan (DMRT) Test, treatment with high pressure of CO_2, the duration of treatment in minutes, and the combination of both of treatment and duration of the treatment significantly changed the texture of tempe ($P \geq 0,05$) (Table 2). Treatment under 1100 psi for 20 minutes decreased the hardness of texture tempe (272,88 gf ± 32,54). Another study reported the textural changes of carrot dried with CO_2 under 100-550 MPa for 20 minutes [7]. The result of our study suggested that the high pressure might rupture the bean's cell wall which then decreased the cellular turgor and cell wall integrity. In addition, the microstructure analysis (Figure 2) showed that the bean's cell wall was shrinkage that could be as a result of pressure released, but it retained the circularity of the cell. At the pressure of 900 psi CO_2 was thought to diffuse into the bean's cells and the water present in the cell was expected to be dissolved into. Upon depressurization, the CO_2 was expelled from the bean's cell and maintain the cell volume, since the CO_2 was able to occupy volume that was originally occupied by water. This explained the ability of bean's cells to retain their circularity well. Yet, depressurization caused the shrinkage of the bean's cell wall resulting in the decrease in hardness. Study done by Son and Lee [8] reported that pasteurization of kimchi with high pressure CO_2 caused the texture became firmer. This was not in agreement with our study because high pressure when applied to the vegetables produced the cell wall firmer than the raw one [9].

Tabel 1. Texture of tempe treated with CO_2 under 1100 psi and 900 psi for different holding time.

Pressure/holding time	Texture (gf)
Control	371,73± 0,22a
900 psi/5 min	300,18±42,03b
900 psi/10 min	292,02± 15,20c
900 psi/15 min	295,08 ±20,79d

900 psi/ 20 min	293,83 ±29,99e
1100 psi/ 5 min	314,73± 56,12f
1100 psi/ 10 min	302,00 ±46,03g
1100 psi/ 15 min	291,52 ±27,50h
1100 psi/ 20 min	272,88± 32,54i

Means followed by the same letter within a column are not significantly different at $P < .05$.

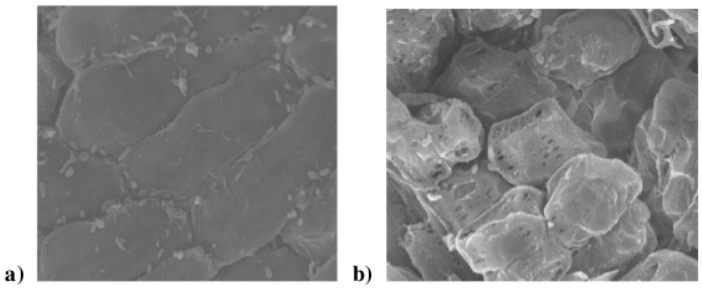

Figure 2. Scanning electron microscope of the surface tempe**:** a) surface tempe without treatment, b) surface tempe treated with CO_2 under 900 psi (6,2 MPa) for 5 min.

3.2. Chemical analysis

3.2.1. Protein content

Based on ANOVA and the Duncan (DMRT) Test, the treatment of high pressure of CO_2, the duration of the treatment, and the combination of both of them significantly changed the protein content (*P≥0,05*). The treatment of CO_2 under 1100psi for 5 minutes provided the lowest protein in tempe, but the protein content was high on tempe treated for 20 minutes at 900 psi or 1100 psi. Another study reported that supercritical CO_2 can stabilize the secondary structure as a result of solvent effect [10]. VanHekken *et al* [11] reported that application of milk with high pressure CO_2 under 5,52 MPa resulted in precipitation of casein. The result of our study suggested that during the application of high pressure under 1100 psi (7,6 Mpa), CO_2 which posses high solubility power may diffuse into the protein and the tertiary structure which is held by hydrophobic interaction may be disrupted and dissolved into CO_2. In addition, because the dielectric constant of supercritical CO_2 is weak, it is immiscible with organic solvent such protein molecules, resulting in the precipitation. When the pressure was released, the CO_2 reformed and left precipitated protein. The decrease of protein in our study may as a result of the denaturation and the precipitation. Moreover, the high water content in tempe may contribute to the denaturation and the protein precipitation, because CO_2 dissolved in the aqueous phase of the tempe resulting in a decrease in the pH which remained in the tempe when CO_2 was dissipated out during depressurize. The low pH causes precipitation of protein tempe. Therefore, it could be suggested that high pressure treatment applied in our study may cause denaturation and a reversible changes of protein, as the secondary structure of protein is disrupted at pressure of ≥ 300 MPa [12], [13].

3.2.2. Water content

Based on ANOVA and the Duncan (DMRT) Test, the treatment of high pressure of CO_2, the duration of the treatment, and the combination of both of them significantly changed the water content of tempe ($P \geq 0,05$). The water contents in tempe treated with CO2 under 1100 psi were higher than that of treated under 900 psi CO_2 (Table 2). Another study reported that water was more soluble in supercritical CO_2 than in liquid CO_2 [14]. The high pressure of CO_2 processing is actually involved an exothermic reaction [4]. The result of our study suggested that when 1100 psi of CO_2 was applied, the high compressibility is used to absorb excess heat evolved in the reaction and the vapor pressure surrounding the system was very dense. Upon the pressure released the CO_2 reformed and the vapor state was changed to liquid state, resulting in an increased of RH. When RH in the atmosphere is high, tempe would absorb more water, and as a result in high water content. Water content in tempe treated with CO_2 under 1100 psi was higher than that of under 900 psi, because the temperature, pressure and subsequently vapor pressure were higher.

Tabel 2. Protein and water content of tempe treated with CO_2 under 1100 psi and 900 psi for different holding time.

Pressure/holding time	Crude Protein (% dry base)	Water content (% dry base)
Control	24,57±0,05i	35,72±0,19
900 psi/5 min	22,51±0,17a	34,79±0,69a
900 psi/10 min	24,43±0,21b	34,63±0,95c
900 psi/15 min	23,87±0,26c	35,66±0,63d
900 psi/ 20 min	25,63±0,24d	38,10±0,21b
1100 psi/ 5 min	16,74±0,29e	39,9±0,52a
1100 psi/ 10 min	19,92±0,50f	40,43±0,27a
1100 psi/ 15 min	23,03±0,20g	39,97±0,60a
1100 psi/ 20 min	27,46±0,31h	38,45±0,23a

Means followed by the same letter within a column are not significantly different at $P < .05$.

3.2.3. Microbial analysis

Figure 3 showed that the reduction number of treated tempe with CO_2 under 1100 psi for 20 minutes was 1 log, while the reduction number was less than 1 log on treated tempe under 900 psi. Another study reported that CO_2 under 75-110 bar (1087-1595 psi) reduced the microbial load of tomato paste less than 1 log [15]. Werner and Hotchkiss [16] reported that CO_2 under 20,7 MPa (3002 psi) reduced the microbial load of milk by 5,36 log. Those differences in the reduction number of microbial loads may be due to the properties of the food constituents which influenced the rate transport of CO_2 [18] . The result of our study suggested that the destruction of microorganisms may be as a result of acidification of the tempe being treated. The acidification occurs as a result of CO_2 interaction with water in the tempe, this interaction ultimately results in the generation of H_2CO_3. H_2CO_3 exists in equilibrium with HCO^-_3, this equilibrium is responsible for the release of protons in the bacterial environment, thus decreasing pH [17]. Low pH then can induce structural changes in the bacterial membrane cell which will increase the permeability of the membrane to CO_2. CO_2 then easily diffuse and accumulate in the cell, interfering the normal metabolism of the living cell

resulting in the death of the bacterial cell.

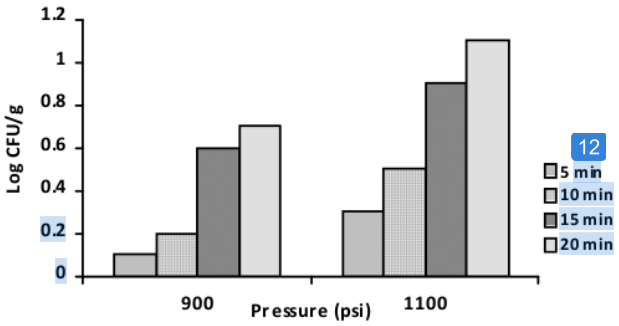

Figure 3. Reduction number (log CFU/g) of microbial loads in tempe treated with CO_2 under 900 psi and 1100 psi at different holding time.

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