THE EFFECT OF BLANCHING TREATMENT ON CHEMICAL AND SENSORY PROPERTIES OF BROCCOLI

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Abstract - The physicochemical and sensory properties of on blanched broccoli at 70˚C for 3 minutes and untreated broccoli were determined. The parameters of the blanching treatment were obtained by conducting response surface methodology with temperature and time as the factors. Results showed that the vitamin C in untreated sample (31.00 ± 1.41 mg/100g) was higher compared to the treated samples (15.03 ± 0.81 mg.100g). The same went for peroxidase it was determined that peroxidase activity was higher in untreated broccoli (0.13 ± 0.01 Δ485nm/min/gram and 0.90 ± 0.87 kg) whilst for treated broccoli was 0.04 ± 0.01 Δ485nm/min/gram and 0.77 ± 0.27 kg respectively. As for nonanal compound quantified in the analysis, untreated broccoli showed low value of 0.46% ± 0.00 while it accelerated in treated broccoli to 0.71% ± 0.00. Sensory properties were evaluated in term of firmness, greenness and overall acceptability where treated broccoli exhibited higher result with value of 5.77 ± 1.17, 6.30 ± 1.06 and 6.10 ± 1.16 respectively.

Keywords - Broccoli, Response Surface Methodology (RSM), Boiling, Sensory Properties,Vitamin C, Peroxidase Enzyme Activity

I. INTRODUCTION

There are consequential evidences for the role of diet in the prevention of cancer (Link and Potter, 2004), cardiovascular, and other degenerative diseases (Kaur and Kapoor, 2001). Vegetables such as broccoli are considered to be protective thanks to their content of phytochemical for examples glucosinolates, dithiolthiones, indoles and glucoraphanin.

These naturally occurring compounds in vegetables has contribute to their own antioxidant properties and their implications in variety of biological mechanism at the base of the degenerative process. Such compounds are identified to be the secondary plant metabolites which are responsible for plant food color, smell, flavor, bitterness and consist of a variety of different molecules. Due to the detection of many bioactive compounds in the food with possible antioxidant activities, relationship between antioxidants and disease risk has start gaining interest among researchers (Nilsson et al., 2004). Furthermore, Zhang and Hamauzu (2004) stated that the cooking method affected the antioxidant activity of broccoli for instance its ability to trap the free radicals inside broccoli, which inhibit degenerative diseases.

Most of the vegetables are commonly cooked before consumption either by boiling, microwave or even frying. These cooking processes would bring about numbers of changes in terms of physical characteristic and chemical composition of vegetables (Rehman et al., 2003 and Zhang and Hamauzu, 2004). In term of physical properties' effects, texture and color are considered to be very significant parameters in the cooking quality of broccoli. These two parameters may influence customers' choices as the appearance of the vegetables play important roles in determining their quality and helping the customer in making a choice.

In addition, cooked vegetables usually exhibit poor color quality in comparison with fresh ones (Turkeman et al., 2006). These data proves that cooking method will affects the vegetables either physically or chemically, and this includes broccoli. General studies show that people starts to be more aware on their health and practice on taking healthier food such as vegetables. And according to Nilson (2004), relationship between antioxidants and disease risk has start gaining interest among researchers as antioxidant is known in preventing disease. Broccoli is a green vegetable which is high in vitamin C, and can contribute to health benefit if the amount of its nutrient is maintained as much as possible. Applying heat treatments without proper condition such as proper cooking temperature and cooking time can alter the nutrients reside in broccoli, especially vitamin C which is the least stable nutrient among all (Franke et al., 2004; Lathrop and Leung, 1980). Improper cooking methods on broccoli can also affect the physical appearance of the vegetables as well as it flavor. Therefore, the present study was undertaken to investigate the effect of heat treatment on physicochemical properties in term of texture, color, vitamin C, peroxidase activity, volatiles compound and also sensory properties of broccoli.

II. RESEARCH CONTEXT

This study will be conducted to study the effect of blanching treatment on the physicochemical and sensory properties of broccoli. It will also provide information to the consumer of the proper cooking method to prepare the vegetable, specifically broccoli, in order to maintain as much nutrient as it can.The objectives of the study are to determine the effect of blanching treatment on texture, color, vitamin C, peroxidase activity, volatiles compound of broccoli and also to determine the effect of blanching treatment on sensory properties of broccoli.

2.1. Definition of Broccoli

Broccoli or sprouting broccoli, with a scientific name of Brassica oleracea var. Italia, is a family member of Brassicaceae. According to Podsedek (2007), Brassicaceae also commonly known as Crucifers, includes genera of cabbage, broccoli, cauliflower, Brussels sprouts, kale, etc. By 8000 year ago, broccoli along with the wild cabbage are traced to start growing along the coastal Europe and chalks along the cliff of England. After the Aryan invasion of Europe, the plants were taken to the Persian, from where they start to be introduced into the Mediterranean region. Because of the broccoli's origin, brassicas are best grown in cool climates with high humidity. A temperature between 10˚C and 25˚C are deemed to be the optimum temperature for the vegetables' growth. Whereas, temperature above 27˚C will slow down and arrest the growth. In Malaysia, brassica is best yet to be nurtured at Cameron Highland due to the natural low temperature and high humidity. Broccoli has the image of being the same with the cauliflower, but with different color. Most fresh brassicas are deep green in color. Its flower stalks are less clenched compare to the cauliflower, forming an irregular, open head.

The texture of the brassica, if it hasn't been exposed to any treatment, can be considered as hard especially around its stalks. Furthermore, members of Brassicaceae are shallow-rooted and perform well in well-drained, fertile soils rich in organic matter. The presence of the organic matter is essential not only because it's able to maintain high fertility, but also proper soil aeration and water holding capacity. Brassica do not tolerate waterlogged soil, drought or poor soil. Constant and continuous moisture is needed throughout the growing season; otherwise supplemental inundation may be necessary. Brassica vegetables are said to be a good source of antioxidant because of their high phenolic and glucosinate content (Moreno et al., 2006, Bruce and Pickett, 2007 and Jahangir et al., 2008). Other than that, broccoli is said to be the excellent source of vitamin C and dietary fibre and is claimed to also contain vitamin A and a good source of potassium. Broccoli can be mixed with a variety of foods and usually is used in soup, salad or even the main dish itself.

2.2. Response surface study (RSM)

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for the analysis of problems in which a response of interest is influenced by variables and the objective of solving the problem by using RSM is to optimize this response (Montgomery, 2005). For instance taking this research into an example, the peroxidase activity resides in the broccoli depends on the temperature and time of boiling. This response can give different result under any combination of temperature and time. When the variables are from a continuous range of values, then a response surface methodology is useful for developing, improving, and optimizing the response variable. In this case, peroxidase activity is the response variables, and it is the function of time and temperature. If the response can be defined by linear function of independent variables, then the approximating function is a first-order model. In cases where there is a curvature in the response surface, then a higher degree polynomial should be used. The approximating function with 2 variables is called a second-order model. Other than that, RSM has been applied widely across industries and the most common applications of RSM are in Industrial, Biological and Clinical Science, Social Science and Food Science. Since RSM has an extensive application in the real-world, it is important to know how and where response surface methodology started in history. According to Hunter et al., (2005), RSM method is introduced by G.E.P Box and K.B Wilson in 1951. These historical figures suggested to use a first-degree polynomial model to approximate the response variable.They acknowledged that this model is only an approximation, not accurate, but such model is easy to estimate and apply, even when little is known about the process.

2.3. Boiling

Boiling is one of the most used methods that have been practiced since centuries in any meal preparation. It is the method of cooking the food in boiling water, or any water-based liquids such as stocks, milk and even yoghurt. Boiling point of water is generally known to be 100˚C, but it can be altered by changing the composition of the liquid. Adding cooking substances such as sugar or salt can increase the boiling point and is termed as boiling-point elevation. And depending on the type of food to be used, the water may not be hot enough to cook that particular food. Commonly, type of foods that are suitable for boiling consist of vegetables, starchy food e.g. rice, noodles and potatoes, eggs, meat and soups. Despite the boiling-point elevation and the fact that it is simple cooking method as the advantages, Podsedek (2007) claimed that processed vegetables

will have lower antioxidant contents compare to the fresh, unprocessed samples.

2.4. Sensory properties

The demand for broccoli either fresh broccoli or ready to eat salad consumption has increased greatly as this indirectly reflects the natural taste of the broccoli to be desirable. Sensory properties is a scientific discipline that analyses and measures human response to the food's composition e.g. appearance, texture, flavor, odour, color, etc. These properties play essential role in food industry especially new food product to predict the sale outcome and the consumer's acceptance of the food once the food has been released to the public.The sensory quality in broccoli actually varies between different cultivars (Schonhof, Krumbein, & Brückner, 2004), but only few cultivars are usually used. Although the knowledge of climatic effect on the brassica's sensory is limited, some studies have linked the relationship between the contents of specific volatiles of secondary metabolites and the specific flavours of Brassica vegetables

2.5. Vitamin C

Broccoli has been labelled as one of the nutrient-rich vegetables and is a rich source of vitamin C. The content of vitamin C in the broccoli can be influenced by diverse factors such as growing season, cultural practices and genotypic differences (Lee and Kader, 2000; Abd El-Hamed and Elwan, 2010). Vitamin C consist of L-ascorbic acid (AA) and its oxidized form L-dehydroascorbic acid (DHA), is the major antioxidant in Brassica vegetables (Davey et al., 2000; Podsedek, 2007). Vitamin C contents in fruits and vegetables are observed to decrease gradually with increasing in storage's temperature and duration after harvest (Lee and Kader, 2000).

2.6. Peroxidase enzyme activity

Peroxidase (POD) is an enzyme that can be found in plant-based foods. This type of enzyme attract the food processor's attention due to its high thermal stability and its ability to get involve with the oxidation of organic compound, which will contribute to deterioration in terms of flavour, colour and also the nutritional quality. These changes have been found particularly in canned and frozen fruits and vegetables during storing. Because of its high thermal stability and the simplicity of POD's measurement, it has been used as the benchmark of the adequacy of fruits and vegetables blanching (Anthon & Barrett, 2002). These active enzymes are usually inactivated by using heat treatment, commonly blanching and boiling

III. RESEARCH METHODOLOGY

The broccoli obtained from the grower at Cameron Highland, selected of high quality in term of bright green color and strong texture. The vegetables were washed with tap water, followed by drying with a soft cloth. The broccoli was then cut into small pieces, and will be divided into portions where the portion contains of 800 ± 5 gram. One portion will be retained as untreated while the other one will be cooked using method specified.

3.1. Response surface methodology (RSM)

The first goal for response surface methodology was to find the optimum response. In this research, two variables have been selected which are temperature and time. Peroxidase activities (y_1) was the response of this method in which they require the optimum cooking temperature and time to determine the responses. The temperature ranges (x_1) for this method started from 60˚C up to 100˚C with range of time (x_2) between 1 min and 10 minutes. Statistical analysis of RSM is performed by using MINITAB 16 software. The experimental design conducted was central composite design of (2 factors, 5 levels).Peroxidase activity resided in the broccoli was determined by using spectrophotometer with wavelength of 465 nm and distilled water as the reference (Toivonen and Sweeney, 1998; Rojas Grau et al, 2008). The experimental value of the response was compared to the predicted value of response at optimum condition.

3.2. Boiling treatment

In the study, a big portion of broccoli equivalent to 800 ± 5 g was subjected to boiling at optimum temperature (70˚C) and time (3 min) decided by response surface methodology (RSM). The broccoli was soaked in cold water bath right after the heat treatment finished for 3 minutes to avoid overheating.

3.3. Sensory properties

Sensory evaluation of these broccolis was conducted to compare the difference between the treated and untreated samples. The purpose of this evaluation is to discern the preference and the difference perceived in attributes (firmness, green colour) by consumers. Consumers received small portion of broccoli of 2 to 3 branches that have been treated with boiling, including an untreated sample to describe the intensity of attributes in the vegetables. 30 consumers evaluated the firmness of the treated and untreated broccoli by using a 7-point hedonic scale (7 being "like extremely', 4 being 'like accepted' and 1 being 'dislike extremely').

3.4. Vitamin C

Using method by Zenebon et al., (2008),about 100 g of treated and untreated will be weighed and cut into uniform sizes as much as possible. The samples would be first treated with homogenization using 50 mL of 10% TCA solution for about 5 minutes. Then, the extracts were clarified by centrifugation at 5500 ppm for 5 minutes, followed by filtration using a

vacuum pump. The filtrates would be analysed directly using iodometric titration. 1 mL of 10 g/L starch solution and 1 mL of 100 g/L potassium iodide solution were added into prepared samples. The samples were titrated with 0.002 mol/L potassium iodide solution until the mixture became dark blue and persisted for more than 60 seconds. Each mL of 0.002 mol/L potassium iodide was equivalent to 0.8806 mg of L-AA. Vitamin C content in treated and untreated samples were determined by observing and calculating the amount of 0.002 mol/L potassium iodide used.

3.5. Peroxidase enzyme activity

Based on the combined procedure of Toivonen and Sweeney (1998) and Rojas-Grau et al. (2008), POD extraction and assay were conducted. The samples (5g) were homogenized with 10 mL phosphate buffer (pH 6.6, 50 mM containing 1M NaCl) by using a Waring blender for 30 s. At 18900 g and 4˚C, the mixture was centrifuged (Beckman J2-21M/E, USA) for 15 minutes. The supernatant was then filtered by using 0.45µm filter and will be kept in water bath prior to analysis. Based on the oxidation reaction of guaiacol by hydrogen peroxide, H2O2, peroxidase activity was determined by using spectrophotometer (Ultrospec 3300 Pro, GE Healthcare, New Zealand). The assay mixture contained of 2.7 mL phosphate buffer (50 mM, pH 6.5 containing 1M NaCl), 0.2 mL guaiacol (1% w/v in water), 0.1 mL H2O2 (1.5% w/v in water) and 20 µL enzyme extract. The formation of oxidized tetraguaiacol polymer was monitored at 20˚C for 3 minutes. Substrate solution was prepared on the same day prior to enzyme measurement to ensure the freshness of the solution. The changes in absorbance at 485 nm and 20˚C were followed by using kinetic software (Swift II, GE Healthcare, New Zealand). Enzyme activity was calculated based on the established slope of the linear portion of the curve and expressed as change in absorbance (ΔA485nm) per minute per gram broccoli tissue. The enzyme activity measurement was done in triplicate.

DISCUSSION AND CONCLUSION

The aim of this study is to determine the effect of blanching treatment on sensory analysis, vitamin C, peroxidase activity, volatiles compound of broccoli and also to determine the effect of blanching treatment on sensory properties of broccoli.Response surface methodology (RSM) was applied to the broccoli for optimization of blanching treatment to determine the peroxidase activity. A total of 13 runs with two factors were used to fit the central composite design for the optimization process.

The target value for peroxidase activity of 0.0712 was found at temperature 70˚C and 3 minutes.The optimal level of the variables and the effect of their interaction were further investigated by plotting the contour plot and response surface plot.

No. of run	Factors (x)		Response (y)	Fits
	Temp (˚C)	Time (min)	Peroxidase activity (abs)	Predicted (abs)
1	70.0	3.0	0.0733	0.07161
2	90.0	3.0	0.0004	0.00234
3	70.0	7.0	0.0329	0.03114
4	90.0	7.0	0.0002	0.00207
5	65.9	5.0	0.0651	0.06758
6	94.1	5.0	0.0007	-0.00196
7	80.0	2.2	0.0353	0.03516
8	80.0	7.8	0.0064	0.00636
9	80.0	5.0	0.0144	0.01016
10	80.0	5.0	0.0140	0.01016
11	80.0	5.0	0.0101	0.01016
12	80.0	5.0	0.0052	0.01016
13	80.0	5.0	0.0071	0.01016

Table 1: Independent variables and response in the central composite design

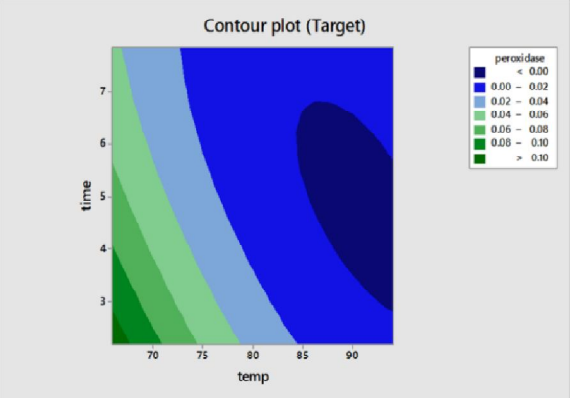


Figure 1: Countour plot for target value of 0.0712

An elliptical contour plot implied that interaction between the variables used for the model were significant (Muralidhar et al., 2001; Xu et al., 2008). In this study, the elliptical contour plot indicated that the mutual interaction between the variables, which were temperature and time, was significant. The color of the contour plot determined the peroxidase activity at certain temperature and time. At 3 min and 70℃, an optimcal condition, showed the enzyme activity was still present but it wasn't in the highest amount. For the 3D-plot, it showed that the peroxidase activity increased at lower temperature and time. While peroxidase activity decreased gradually with increased of time at lower temperature, it was reduced rapidly with increasing of time and temperature. These findings were in agreement with Thongsook and Barrett (2005) who found that peroxidase activity treated at 60˚C for 5 minutes increased rapidly after the treated enzyme was cooled and incubated at room temperature. Saedian and Ghasemifar (2013) and Raseetha et al. (2013) also revealed that the peroxidase activity for broccoli at 70˚C with 10 minutes heating time decreased greatly

to 10% and 0.09 ±0.00 of peroxidase activity respectively.

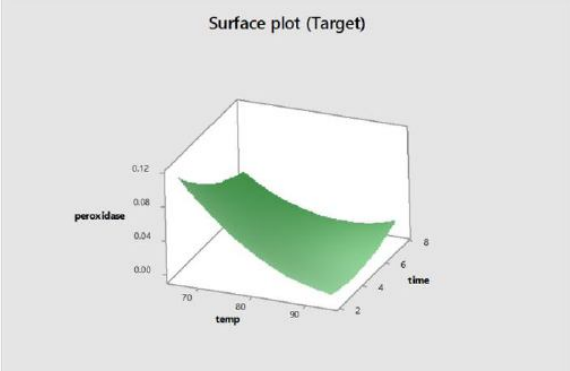


Figure 2: Surface plot for target

Based on the Figure 1 and 2, the optimal blanching condition to determine peroxidase activity in cooked broccoli was at temperature of 70˚ in 3 minutes. Under this optimal condition, the optimum predicted peroxidase activity was 0.0712 Δ/min/gram. The observed value obtained was 0.0713 Δ/min/gram which suited the target goal and no significant differences between the predicted and observed were found. These results proved that the model was valid and practical.

Sample	Verification*
Experimental	0.0713 ± 0.00^{a}
Predicted	0.0712 ± 0.00^{a}

^{a} means no significant difference

Table 2: Verification table for target condition

Using the optimum condition found by RSM method, the broccoli was then boiled at 70℃ for 3 min and was categorized as treated sample. Together with the control sample (fresh broccoli), they were subjected to sensory analysis, vitamin C and peroxidase enzyme's determination. The result of the sensory evaluation for the broccoli samples was tabulated as the table above. There were no significance differences ($p>0.05$) in term of firmness and overall acceptability between the control and treated samples. Based on the consumer acceptance, the broccoli sample cooked at 70˚C for 3 minutes managed to maintain its attribute (firmness) similar to the fresh broccoli. However, there was significance difference ($p<0.05$) between the samples regarding the greenness of the broccoli. Studies from Ansorena et al. (2014) and Garitta et al. (2013) also showed that appearance acceptability for the broccoli samples changed significantly. The appearance of the treated broccoli did change as compared to the fresh broccoli and these changes significantly caused difference in greenness and affected the sensory acceptability and thus, it could be concluded that the appearance of the broccoli was a critical attribute evaluated by the consumer. The greenness of the fresh broccoli scored 5.8 whereas the treated broccoli score 6.3 amidst it had been applied with heat treatment for 3 minutes. The use of short cooking times and low temperature might have led to this difference (Zhong et al., 2015). Chlorophyll plant pigment was the plant pigment that was responsible to the green color of the broccoli (Almela, Lopez & Roca, 2000). Since the chlorophyll pigment was easily destroyed by heat, the treated broccoli went through the chlorophyll degradation when heat was applied onto the sample. Erge, Karadeniz, Koca and Soyer (2008) implied that the optimum temperature for the cholorphyllase activity range from 60˚C to 82˚C with substantial formation of chlorophyllides and some pheophorbides only at low-temperature (60-70 °C). The treated broccoli undergo chlorophyll degradation from chlorphyll (green) to considerably large amount of chlorphyllides (bright green), which was preferred more by the consumer.

Sample	Firmness	Greenness	Overall acceptability
Control	5.57 ± 1.13^{a}	5.80 ± 0.76^{b}	5.80 ± 0.81^{a}
Treated	5.77 ± 1.17^{a}	6.30 ± 1.06^{a}	6.10 ± 1.16^{a}

Table 3: Result of sensory evaluation

The analysis for vitamin C was conducted via iodometric titration and the result revealed that there was a significant difference ($p<0.05$) between the control sample and treated sample. The control sample showed a higher amount of vitamin C with a value of 31.00 mg/100g fresh weight (FW) compared to the treated sample. The treated sample displayed small amount of vitamin C (15.03 mg/100g F.W) as the extent of vitamin C loss during processing was also affected by temperature (Davey et al., 2000; Lee & Kader, 2000). The fact that the result established the evidence of vitamin C in the control sample was higher than treated sample was supported by study from Zhong et al. (2015) who found that vitamin C content in uncooked broccoli (630 mg/100g dry mass (D.M.)) was significantly different with steamed broccoli (570 mg/100g D.M).

	Amount of Vitamin C (mg/100g F.W)
Control	31.00 ± 1.41^{a}
Treated	15.03 ± 0.81^{b}

Table 4: Result of Vitamin C

The peroxidase activity for control and treated samples were determined by observing the absorbance of the sample at ΔA485nm and the slope of the line was calculated. Peroxidase is used as the index of blanching adequacy, since the inactivation of this enzyme help in increasing the shelf life of

vegetables during the frozen storage (peroxidase temperature journal). This enzyme concern the manufacturers since it can lead to deteriorative changes in quality of the processed products (Saedian & Ghasemifar, 2013). Enzymatic browning was one of the most essential color reactions that affected the fruits and vegetables (Saedian & Ghasemifar, 2013). Browning in the broccoli was catalysed by the interaction between the peroxidase enzyme activity and polyphenoloxidases (Saedian & Ghasemifar, 2013).

The result stated that there was a significance difference ($p<0.05$) between the two samples where the control and treated sample gave values of 0.1280 and 0.0391 respectively. Control sample showed a higher amount of peroxidase activity compare to the treated sample. This result agreed with Raseetha et al. (2010), Saedian and Ghasemifar (2013), Raseetha et al. (2013), Blancas, Chandia and Zevallos (2002) and Rasheeta et al. (2011). All studies showed a significantly high peroxidase activity for the control sample and they decreased gradually over time. However, studies from Saedian and Ghasemifar (2013) and Raseetha et al. (2013) revealed that the peroxidase activity for treated broccoli at 70˚C with 10 minutes heating time decreased greatly to 10% and 0.09 ±0.00 of peroxidase activity respectively. The drop in peroxidase activity at temperature 70˚C indicated that the enzyme had slowly denatured and this was due to the unfolding of tertiary structure of enzyme to the secondary structure (Saedian & Ghasemifar, 2013). A high F-value from the table implied that the temperature had a greater effect on enzyme activity compared to the control treatment. Boiling the broccoli at 94.1˚C ±0.5 resulted in complete inactivation of POD with peroxidase activity of 0.0007. Although the absence of peroxidase activity helped in extending the shelf life of the broccoli, but changes in enzyme's activity affected the quality of the broccoli in term of its color, flavours and nutritional value.

	Peroxidaseactivity (Δ485nm/min/gram)
Control	0.13 ± 0.01^{a}
Treated	0.04 ± 0.01^{b}

Table 5: Result of peroxidase activity

This finding demonstrated that the treated sample managed to maintain the same attributes similar to the fresh broccoli especially in term sensory properties when it was cooked at 70˚C for 3 minutes. Fresh broccoli showed higher content of vitamin C (31.00 mg/100g F.W) and lower content of nonanal (0.46%) compound, which were better compared to the treated broccoli as higher vitamin C gave lots of benefit to the consumer and lower nonanal compound minimized the off-odour of broccoli. However, fresh broccoli gave a higher amount of peroxidase activity (0.13 Δ485nm/min/gram), which would soon cause faster browning reaction than treated broccoli. For the firmness (0.77 kg), color (41.35 L*, -9.61 a* and 7.69 b*) and sensory properties (6.10), treated broccoli exhibited better result and was favoured more by the consumer. And by studying and observing all the result from the analysis conducted, treated broccoli cooked at 70˚C for 3 minutes was better compare to the fresh broccoli. Further studies regarding optimization of different cooking methods on organoleptic properties (taste, odour, color, texture) of broccoli and volatile compound quantification using SPME-GCMS of broccoli were recommended.

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