Effect of Soaking Formalin Solution on the Quality in Bean Sprout

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Abstract: Formaldehyde is believed to preserve several agriculture produces not only seafood but also fruits and vegetables. There are news spreading on the illegal adding this compound in bean sprout and other vegetables but no scientific document has been investigated the effect of formaldehyde on it. Therefore, this study was focused on the effect of formaldehyde on quality changes of bean sprout during chilled storage. Bean sprout were soaked in three levels (10, 100 and 500 ppm) of formaldehyde solution for 15 min, rinsed and stored at 8ºC for 18 days, the samples were taken every 3 days to evaluate free and total formaldehyde content, firmness, browning index (BI) and weight loss. It was found that the higher concentration of soaking, the higher residues were significantly remained in free and total formaldehyde content ($p \leq 0.05$). However, both residuals rapidly decreased and mostly was statistically indifference within 9 days ($p > 0.05$). After 9 days storage, the BI and weight loss were higher as increasing soaking concentration especially soaking with 500 ppm formaldehyde solution ($p \leq 0.05$). For firmness, there were insignificantly among untreated and treated samples ($p > 0.05$). In conclusion, soaking with formaldehyde solution could not help for preserving bean sprout but speedily deteriorate of this produces.

1 INTRODUCTION

Thailand cultivates vast variety of fruits and vegetables. These nutrition produces contain high amount in fiber, vitamins and minerals which are benefits for health especially lower the risk of intestinal cancer and other disease (Thai Health, 2017). In Southeast Asia, bean sprout is commonly well-known in many varieties of soup, salad, stirred fried vegetables and side dishes (Liu, 2008). It is made either from soybean or mung bean by germinating in the dark. It is rich in vitamins, minerals, and phytochemicals (Guo *et al*., 2012). Unfortunately, this sprout has a short period of shelf-life which is about two weeks in a refrigerator (Fresher Pantry, 2017). This produce is easily infected by mold and bacteria and rapidly become rotten because it is cultivated under conditions of wetness and darkness (Hur & Koh, 2002). Therefore, the agriculturists may use some chemicals for extending shelf-life.

The rumor of using illegal chemicals in foods is widely spread in the country. One of them is formaldehyde (or methanol) or generally known as formalin. This chemical is a precursor to produce the resin for particle bonding or coating in several industrial applications for example, textile and furniture. Formaldehyde is a hazardous chemical for human. At low volume of formaldehyde in food, it is metabolized to formate and CO_2 and then excreted from body but the metabolite may be toxic to liver, kidneys, heart and nervous system. However, at high volume of formaldehyde, it is collected in formic acid form which can be toxic with cells, tissues, digestive system and may result in death (Silpakorn University, 2017; Changsap, 2015). The minimum risk level (MRL) for oral exposure to formaldehyde is 0.3 mg/kg/day which is derived for intermediate-duration exposure and an MRL of 0.2 mg/kg/day is derived for chronic-duration exposure (ATSDR, 1999). The United States Environmental Protection Agency (US EPA) advises the recommended daily intakes (RDI) for formaldehyde is 0.2 mg/kg/day (Yeh *et al*., 2013). In addition, this chemical is also recognized as biocide to fix animal organs and bodies. Therefore, the agriculturists who lack of knowledge may misuse to preserve meats, fruits and vegetables. This chemical does not be legally allowed to add into any agricultural produces and foods. However, formaldehyde is naturally presented in fruits and vegetables when plant cell wall undergoes to de-methyl-esterified process. It produces

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methanol which convert to formaldehyde by the catalase-H_2O_2 system, cytochrome *P*450 (CYP2E1)-mediated oxidation and the alcohol dehydrogenase 1 (ADH1) class of enzymes (Dorokhov *et al*., 2015).

The previous studies revealed various amounts of formaldehyde in a variety of fruits and vegetables. Formaldehyde content in cauliflower was reported at 5.94 ppm using HPLC procedure (Wahed *et al*., 2016) and 26.9 ppm using colorimetric method (CFS, 2009). Some fruits and vegetables were also reported high formaldehyde content for example, apple (17.3 ppm) (Tsuchiya *et al*., 1975). However, the high amount of formaldehyde may contain in the fresh produces and cause adverse effect for human (Yuyong, 2016). In addition, there is controversial on the effect of formaldehyde in fruits and vegetables. The previous study revealed the formaldehyde could extend shelf-life of mushrooms while it presented negative effect on fruits and vegetables (Antora *et al*., 2018). The objective of this study was to investigate the effect of soaking formaldehyde solution on the quality in bean sprout during chilled storage.

2 MATERIALS AND METHODS

2.1 Chemicals

Formaldehyde solution (36-38%) was purchased from GPO (Thailand). Trichloroacetic acid (TCA) was bought from Fisher Scientific (England). Phosphoric acid was obtained from MERCK (Germany). Ammonium acetate was purchased from LOBA Chemie (India). Acetic acid and Potassium hydroxide (KOH) was bought from SIGMA-ALDRICH (United States). Acetylacetone was purchased from CARLO ERBA (New Zealand).

2.2 Sample Preparation

First, the Bean sprout was purchased from the Amornphan village market, Bangkok, Thailand. Next, four different concentrations of formaldehyde solution (0, 10, 100 and 500 ppm) were prepared by dilution with distilled water. Each sample about 200 g was soaked in 1L formaldehyde solution for 15 min. After that, the sample was rinsed with tap water, drained and put in to HDPE bag. All samples were kept in refrigerator at 8 °C and were sampling every 3 days for analysis.

2.3 Methods

2.3.1 Determination of Weight Loss

Every sample was weighed before and after storage by 2 digits analytical balance. The weight loss percentage was calculated by using the following formula:

$$\%\ Weight\ loss = \frac{W_0 - W_1}{W_0} \times 100 \quad (1)$$

2.3.2 Determination of Browning Index

The sample of each treatment was tightly arranged in area of 4 x 6 cm. After that, it was determined L^*, a^* and b^* by Hunter Lab (Color Quest XE, USA) which, the reflectance specular excluded (RSEX) mode, D65 of illuminant and 10° of observer was set for this operation (Palou *et al*., 1999).

The browning index was calculated by using the following formula:

$$Browning\ index = \frac{[100 \times (X - 0.31)]}{0.172} \quad (2)$$

$$X = \frac{[a^* + (1.75 \times L^*)]}{[(5.645 \times L^*) + [a^* - (3.012 \times b^*)} \quad (3)$$

2.3.3 Determination of Firmness

The samples were investigated with a Texture Analyzer (TA-XT Plus, UK) couple with needle probe which, the compression mode, 10 g of contact force and 2 mm/sec of test speed was set for this operation (Paciulli *et al*., 2015).

2.3.4 Determination of Free and Total Formaldehyde Contents

For free formaldehyde extraction, 5 g of minced sample was mixed with 30 ml of 5% trichloroacetic acid and homogenized at a speed of 11,000 rpm for 2 min. Next, the homogenate was filtered with the Whatman No. 4 filter paper. Then, the filtrate was adjusted to pH of 6.0-6.5 using 1 N KOH and was made up to a final volume of 50 ml with distilled water. This made-up volume filtrate was used for the determination.

For total formaldehyde extraction, 20 g of minced sample was mixed with 10 ml of 10% phosphoric acid and 200 ml of distilled water. Next, the mixture was homogenized with a homogenizer at a speed of 11,000 rpm for 2 min. Then, the homogenate was transferred to distillation flask and the distillation was conducted for approximately 1

hour or until the distillate of around 15 ml was obtained. The distillate was used for the determination.

For formaldehyde determination, 3 ml of filtrate or distillate was mixed with 3 ml of Nash reagent (0.2% Acetylacetone; 0.1 M Acetic acid; 3.89 M Ammonium acetate). After that, the mixture was reacted at 60 °C in the water bath for 15 min and cooled with running water. Finally, the sample was measured by Spectrophotometer (Evolution 201, USA) at 412 nm of absorbance and the formaldehyde content was calculated from the standard curve prepared using standard formaldehyde solution ranging 0-10 ppm (Sochaya & Soottawat, 2013).

3 RESULTS AND DISCUSSIONS

The weight loss of treated sample during chilled storage is shown in Figure 1. During first 15 days of storage, the weight loss in samples continuously increased and were insignificantly among untreated and treated samples ($p>0.05$). After that, during 15 to 18 days, the weight loss in sample soaked with 100 and 500 ppm formaldehyde solution was rapidly increased and much higher than other samples especially control. Weight loss occur when plant transpire for heat transferring from respiration (Siripanich, 2006). This result indicated that soaking with formaldehyde could not extend the shelf-life but it accelerated the deterioration of bean sprout ($p\leq0.05$).

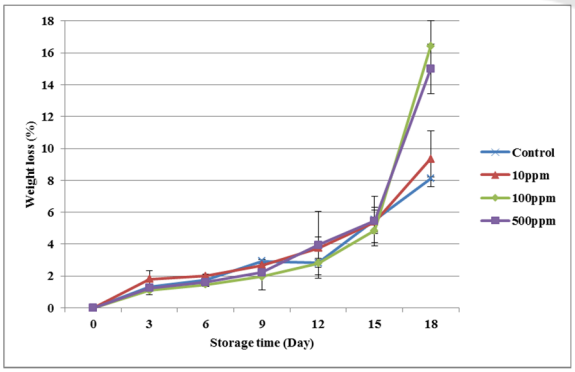

Figure 1: Change of weight loss percentage for formaldehyde treated bean sprout graph.

The result of change in BI is presented in Figure 2. For the first 9 days of storage, BI slightly increased after that, rapidly increased. The treated sample at higher concentration were significantly higher BI compared with control ($p\leq0.05$). During storage, plant slightly produce ethylene which is speedily respiration rate and browning reaction (Siripanich, 2006). Soaking with formaldehyde solution, the formaldehyde residues may be corroded and oxidized to formic acid (Val Tech, 2014) causing cell lysis. The enzyme such as polyphenol oxidase will react with the substrate resulting in formation of browning compounds and, subsequently, increasing BI value. The result also confirmed that soaking with formaldehyde is speeding the deterioration of bean sprout. The final sentence of a caption must end with a period.

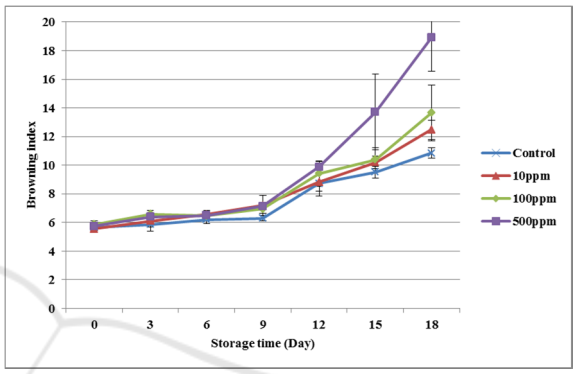

Figure 2: Change of browning index for formaldehyde treated bean sprout graph.

The firmness of all treated samples and control were insignificantly different ($p>0.05$). However, all samples were slowly decreased when the storage time increased ($p\leq0.05$). This phenomena is a natural deterioration of plant caused by the change of enzymatic pathways activation (Swieca & Dziki, 2015) and mechanism of cell wall will destroy pectin that is structure of cell wall when plant cell develop and grow (Dorokhov *et al*., 2015).

The free formaldehyde content in sample as shown in Table 1. The soaked with water (Control) was 0.58 ppm. After soaking in the formaldehyde solution, the treated samples contained higher free formaldehyde contents compared with control (0 ppm). As soaking the samples in higher concentrations, the formaldehyde contents in the samples were significantly higher ($p\leq0.05$). This result indicated that the compound can diffuse in the plant tissue. It was noted that the intense of formaldehyde smell was so strong after treating the bean sprout at the concentration of 100 and 500 ppm and could be noticed at the first sight. During the chilled storage, the free formaldehyde content in control slightly increased at the first 3 days of storage from 0.58 to 0.79 ppm and after that, it remained constant until the end of storage. For

Table 1: Change of free formaldehyde content in treated bean sprout.

Soaking formaldehyde concentration (ppm)	Storage time (day)						
	0	3	6	9	12	15	18
0	$0.58\pm0.19^{c,a}$	$0.79\pm0.21^{a,a}$	$0.73\pm0.08^{b,a}$	$0.72\pm0.15^{a,a}$	$0.71\pm0.08^{a,a}$	$0.70\pm0.15^{a,a}$	$0.79\pm0.12^{a,a}$
10	$0.73\pm0.03^{bc,a}$	$0.79\pm0.21^{a,a}$	$0.72\pm0.12^{b,a}$	$0.72\pm0.17^{a,a}$	$0.74\pm0.13^{a,a}$	$0.69\pm0.16^{a,a}$	$0.84\pm0.19^{a,a}$
100	$0.77\pm0.01^{b,a}$	$0.73\pm0.23^{a,a}$	$0.75\pm0.12^{b,a}$	$0.72\pm0.20^{a,a}$	$0.71\pm0.16^{a,a}$	$0.67\pm0.22^{a,a}$	$0.67\pm0.13^{a,a}$
500	$2.69\pm0.05^{a,a}$	$1.01\pm0.27^{a,b}$	$0.96\pm0.18^{a,b}$	$0.91\pm0.20^{a,b}$	$0.82\pm0.16^{a,b}$	$0.86\pm0.29^{a,b}$	$0.91\pm0.30^{a,b}$

Free formaldehyde content is expressed in ppm.

For the first letter, means with different letters within the same column are significantly different ($p\leq0.05$).

For the second letter, means with different letters within the same row are significantly different ($p\leq0.05$).

Table 2: Change of total formaldehyde content in treated bean sprout.

Soaking formaldehyde concentration (ppm)	Storage time (day)						
	0	3	6	9	12	15	18
0	$0.10\pm0.03^{c,a}$	$0.10\pm0.00^{b,a}$	$0.08\pm0.01^{c,b}$	$0.07\pm0.01^{b,b}$	$0.08\pm0.01^{c,b}$	$0.07\pm0.01^{c,b}$	$0.09\pm0.01^{c,ab}$
10	$0.10\pm0.01^{c,ab}$	$0.10\pm0.02^{b,ab}$	$0.09\pm0.02^{c,ab}$	$0.07\pm0.01^{b,c}$	$0.09\pm0.01^{c,bc}$	$0.08\pm0.01^{c,bc}$	$0.11\pm0.02^{bc,a}$
100	$0.31\pm0.01^{b,a}$	$0.18\pm0.05^{b,b}$	$0.17\pm0.02^{b,b}$	$0.14\pm0.01^{b,b}$	$0.16\pm0.03^{b,b}$	$0.15\pm0.02^{b,b}$	$0.14\pm0.02^{b,b}$
500	$4.44\pm0.06^{a,a}$	$1.23\pm0.15^{a,b}$	$1.07\pm0.04^{a,c}$	$0.92\pm0.19^{a,d}$	$0.88\pm0.06^{a,de}$	$0.75\pm0.05^{a,e}$	$0.77\pm0.03^{a,de}$

Total formaldehyde content is expressed in ppm.

For the first letter, means with different letters within the same column are significantly different ($p\leq0.05$).

For the second letter, means with different letters within the same row are significantly different ($p\leq0.05$).

sample soaked with 10 and 100 ppm, the free formaldehyde contents tended to be constant along with the storage period. However, for the sample soaked with 500 ppm, the free formaldehyde content significantly decreased from 2.69 to 1.01 ppm at the first 3 days storage which indicated that the formaldehyde could be volatized at the chilled temperature. However, the free formaldehyde content seemed to remain constant (0.81-0.95 ppm) for the rest of storage period same as other samples. The final sentence of a caption must end with a period.

For total formaldehyde content as shown in Table 2. In control, we found that formaldehyde contents were insignificantly changed during storage ranged from 0.07 - 0.10 ppm. Surprisingly, the amounts of total formaldehyde contents were lower than those of free formaldehyde content. It indicated that the condition of steam distillation may cause loss of formaldehyde during extraction. After soaking with formaldehyde solution, all samples except soaked at 10 ppm had significantly higher amount of total formaldehyde contents ($p\leq0.05$). These results agreed with the result obtained from those of free formaldehyde content.

4 CONCLUSION

In conclusion, bean sprout soaked with formaldehyde solution prior to storage could not help for preservation. Moreover, it was speedily the deterioration especially soaking at high concentration. Therefore, this result was scientific proof that the belief in its preservation benefit in bean sprout was wrong.

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