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The effect of vacuum-packaging on nutrient and *Salmonella* growth of sautéed vegetables

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Abstract

Low consumption of vegetables was associated with a lack of cooking and preparation skills. In addition, in modern times, people tend to choose foods that can be served quickly. Vacuum packaging is a simple method of preserving food that can be done at home. Therefore, sautéed vegetables in vacuum packaging were developed and evaluated for their nutrients and microbiological properties. The vegetables, including spinach, carrots, and water spinach, were sautéed before being vacuum packaged. The related parameters, including carbohydrate, protein, ash, moisture, vitamin C, pH, and *Salmonella* sp., were evaluated. Some parameters were measured at 0, 7, and 14 days. The results showed that the samples contained carbohydrates ranging from 8.26-17.08%, 0.145-1.90% protein, 2.23 -2.49% fiber, 1.46-3.10% fat, 0.13-0.10% ash and 82.44-86.08% moisture. The Vitamin C content was similar during storage time, at day 0, 7 and 14. The pH of sautéed vegetables was relatively constant on days 0, 7 and 14. A high *Salmonella* sp. content was found during storage time on days 7 and 14. The vacuum-packaging of sautéed vegetables potentially maintained the nutrients during storage time until day 7. However, the results of this study indicated that there is still a possibility that vacuum packaging has not been able to reduce the growth of micro-bacteria in the package. Overall, this study implied that vacuum packaging with other treatments is needed for food safety reasons.

1. Introduction

Noncommunicable diseases (NCDs) are the leading risk factor in 71% of all deaths globally (World Health Organization (WHO), 2021). It aligns with the increase in NCDs prevalence in developing countries like Indonesia (Health Ministry of Indonesia, 2018a). Previous studies reported that people with NCDs tended to have low consumption of vegetables (Jakkaew *et al.*, 2019; Yazew and Daba, 2020; Kang *et al.*, 2021). Multiple studies revealed a positive correlation between vegetable consumption and the reduction of cardiovascular disease risk (Borgi *et al.*, 2016; Li *et al.*, 2016; Zhan *et al.*, 2017). The meta-analysis research shows that vegetables can increase the body's immunity and reduce inflammatory biomarkers (Health Ministry of Indonesia, 2014a; Craddock *et al.*, 2019).

Unfortunately, the mean consumption of vegetables in 136 countries is 186 g/day, below the existing recommendations from WHO (≥420 g/day) (Kalmpourtzidou, 2020). The low consumption of vegetables was associated with food availability in the household. Individual barriers like lack of cooking and preparation skills were inversely related to low vegetable consumption (Larson *et al.*, 2012; Graham *et al.*, 2013). A qualitative study showed that people consume fewer vegetables because they are labor-intensive and have longer cooking times (Merchant *et al.*, 2022). The preparation of vegetables needs time to go shopping, cleaning, picking, and processing until the food is ready to be served. On the other hand, the vegetables have a relatively short shelf life, mainly green leaves. Several factors that affected the shelf life were pre- and post-harvest environments (Pollard *et al.*, 2002; Arah *et al.*, 2015). This issue generates an opportunity to develop processed vegetables to provide consumers with healthy and quick-prepared products (Zhang *et al.*, 2018; Otto *et al.*, 2021).

The development of effective food preservation methods to increase the shelf life of food continues to be implemented to meet daily food needs and reduce food waste (Alice *et al.*, 2020; Sridhar *et al.,* 2020). Food packaging keeps food safe and protects food quality against negative ambient influences (Otto *et al.*, 2021). Vacuum packaging is a popular way of extending the

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shelf life of food. Vacuum packaging reduces atmospheric oxygen, limits the growth of aerobic bacteria or fungi, and prevents the evaporation of volatile components (Pennacchia *et al*., 2011). The vacuum packages have been used for preserving foods, including meat and lamb (Lorenzo and Gomez, 2012; Fuentes *et al*., 2014; Duran and Kahve, 2020). The studies that identify vacuum packaging for cooked vegetables, particularly sautéed vegetables, are limited. Hence, the study aimed to evaluate the organoleptic properties, the nutrient content, and the contamination content (*Salmonella* sp.) of sautéed vacuum-packaged vegetables.

2. Materials and methods

2.1 Materials and sample preparation

Vegetable samples used include spinach, carrot and water spinach. These vegetables are the most consumed in Indonesia (Health Ministry of Indonesia, 2014b). The standard seasoning of sautéed used consisted of shallot (12.0%), garlic (11.6%), chili (4.0%), oyster sauce (4.0%), salt (0.4%) and sugar (2.0%). All the seasonings were sliced, mixed, and used to saute the samples over medium-low heat. The vegetables and seasonings were obtained from PT. Pangan Sari Padjajaran, Karawang, Indonesia.

2.2 Sauteing process and vacuum

The vegetables were cleaned and chopped into small pieces using chiffonade cut for spinach, water spinach, and julienne cut for carrots. According to Rani and Fernando (2015), the sautéing process is recommended to obtain the high antioxidant activity of vegetables. All seasonings were fried with a small amount of vegetable oil. The vegetables were then added and stirred regularly. The sautéing process took 5-7 mins. The sautéed vegetables were kept at room temperature. Subsequently, the vegetables were packaged using a plastic vacuum bag and kept at 4°C until use. The vegetables were evaluated on days 0, 7, and 14 for the organoleptic test (color, aroma, flavor, texture and aftertaste) and nutrient analysis.

2.3 Organoleptic test

The organoleptic tests of the samples were carried out on days 0, 7 and 14. Prior to the organoleptic test, the panelists (thirty people) were trained to evaluate the attributes of the samples. The organoleptic test is carried out through three types of tests; the hedonic test, the hedonic quality test, and the ranking test. They were conducted once for each day of the test. The samples were rated on a 7-point scale in the hedonic test (1, 2, 3 = dislike; 4 = somewhat like; 5 = like; 6, 7 = really like) and a 5-point scale in hedonic quality based on parameters: color, aroma, flavor, and texture. Meanwhile, the panelists chose the three scores (1, 2, and 3) for the ranking test for each sample.

2.4 Nutrient and microbiological evaluation

Nutrient and microbiological analyses were carried out in the Laboratory of Vegetable Crops Research Institute, Lembang, Bandung, Indonesia 40391, a laboratory that has been certified by the National Accreditation Committee of Indonesia (KAN). The nutrient evaluations comprised proximate analysis (carbohydrate, protein, fat, moisture, ash content and vitamin C). Proximate analysis was performed only on day 0. In contrast, vitamin C and microbiological evaluations were performed on days 0, 7 and 14.

2.4.1 Moisture

The moisture content was measured using the Association of Official Analytical Chemists (AOAC) method (AOAC, 2005). The sample was dried in an oven to a constant weight. The percentage of moisture content was calculated by subtracting the dry weight from the wet weight. The result was divided by the wet weight and then multiplied by 100.

2.4.2 Protein

Protein content was determined using the Kjeldhal method, following the procedure of AOAC (2005). A Kjeltec™ 2200 Auto Distillation Unit was used to measure nitrogen content. The protein content was obtained by multiplying the content of nitrogen by 6.25.

2.4.3 Fat

Fat was determined using the Soxhlet method (AOAC, 2005). The Soxtec™ 2050 automated analyzer was used with petroleum ether as a solvent. Fat content was obtained by dividing the extraction residue's weight by the sample's weight and multiplying it by 100.

2.4.4 Ash

A furnace was used to determine the ash following the procedure of AOAC (2005). The sample was incinerated at a temperature of 550°C. The remaining inorganic material was cooled and weighed to obtain the percentage of ash.

2.4.5 Carbohydrate

Carbohydrate content was calculated by subtracting the total percentage of protein, fat, ash and ash from the weight of the wet sample.

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2.4.5 Fibre

Fibre content was analyzed using the enzymatic-gravimetric method, following the procedure of AOAC (2005). Dietary fibre was isolated using a heat-stable alpha-amylase (termamyl) (pH 6, 100°C), protease (pH 7.5, 60°C), and amyloglucosidase (pH 4.5, 60°C) for 30 mins, respectively.

2.4.6 Vitamin C

Vitamin C was measured using the titration method. Metaphosphoric acid was used to extract the vitamin C, which was then titrated with 2,6-dichlorophenol indophenol. The titration was terminated when a blue color became a colorless form (AOAC 2005).

2.4.7 Microbiological evaluation

The microbiological evaluation was conducted for *Salmonella* sp. using the MPN (Most Probable Number) method (Wardani and Tanikolan, 2021). The sample was suspended in buffered peptone water (BPW). Pre-enrichment was performed by incubation for two days and was then transferred to a 24-well plate containing 2.5 mL Modified Semi-solid Rapoirt-Vassiliadis (MSRV). XLD agar was used to subculture *Salmonella* at 37°C for 24 hrs (De Man, 1983).

2.5 Data analysis

The data were descriptively analyzed. The differences in the organoleptic test between the groups and between the times were performed using One-Way ANOVA with Tukey Post-hoc Test. The significant differences were determined at p-value < 0.05.

3. Results and discussion

3.1 Organoleptic properties

This study aimed to evaluate the organoleptic properties of sautéed vacuum-packaged vegetables. Generally, based on the three types of organoleptic tests, all of the products can still be accepted up to the 7^{th} day (Table 1). According to the hedonic test, the preference level for water spinach and spinach started declining on day 7. Meanwhile, the preference level for the carrot sample persisted until the 14^{th} day. Additionally, the sautéed vacuum-packaged carrot sample performed better on the hedonic quality and ranking test on days 0, 7 and 14 than other samples. The other two samples, connected to shelf life, darkened in color as they were held for extended periods. Overall, sautéed vacuum-packaged carrots outperformed other samples until day 14 regarding color, flavor and texture.

3.2 Nutrient composition

The proximate composition of the three sautéed vegetables is shown in Table 2. This study found that the vegetables had a similar proximate composition, where the main content was water, followed by carbohydrates, fat, fiber, protein and ash. The moisture content ranged from 82.44 to 86.08%. The moisture content was decreased by sauteing as compared to the fresh vegetables (fresh spinach 94.5%, fresh carrot 89.9% and water spinach 91.0%) due to water loss during cooking (Health Ministry of Indonesia, 2018b, Ishfaq *et al.,* 2021; Sabu and Kalpana, 2021). In contrast, the sauteing process increased the fat content of these vegetables. When comparing the fat content to the fresh vegetables (spinach 0.4%, carrot 0.6%, and water spinach 0.7%) (Ministry of Health, 2018b), the content increased 2-4 times. Sauteing is a method of cooking that uses a small amount of oil, which can increase the fat content of food (Fabri and Crosby 2016).

Carbohydrate is the second primary component of sautéed vegetables, with a content of 8.26-17.08%. The composition is in agreement with the study results by Patricia *et al.* (2014). The carbohydrates in vegetables are mainly starch and fiber (Slavin 2013). The fiber content of the sautéed vegetables ranged from 2.23 to 2.49%. The content seemed not to be different between the sautéed vegetables and fresh vegetables (spinach 2.2%, carrot 2.8% and water spinach 2.1%) (USDA 2021). The findings are also supported by the previous study that the method of processing did not affect the content of fiber (Bember and Sadana, 2013). The ash content in the present study, which indicates mineral content, was comparable to the previous reports that showed the content ranged from 0.25 to 1.91% (Hanif *et al.,* 2006; Afify *et al.,* 2017). As vegetables are not a source of protein, the protein content of the sautéed vegetables was low, between 0.15 and 1.90%, comparable to a previous report (Hanif *et al.,* 2006).

The vitamin C content of all vegetables was relatively constant from day 0 to day 14 (p-value > 0.05). This indicated that vacuum packaging could potentially preserve the vitamin C content of sautéed vegetables. However, the content of vitamin C in the sautéed vegetables was relatively low (<1 mg/100 g) (Table 3). The vitamin C was lower as compared to the fresh spinach (17 mg/100 g), carrot (41 mg/100 g), and water spinach (18 mg/100 g). The low content of vitamin C might be affected by leaching during preparation and thermal breakdown during the sauteing process (Bembem and Sadana, 2014). The previous study revealed that heating affects the vitamin C content, as the heating time increases the vitamin C content decreases

Table 1. Organoleptic properties of each sample.

Organoleptic properties	Length of vacuum	Water spinach	Spinach	Carrots
Hedonic				
Colour	Day-0	4.83 c,x	6.33 b,x	7.74 a,x
	Day-7	4.52 b,x	4.74 b,y	7.12 a,y
	Day-14	4.88 b,x	4.23 b,y	6.80 a,y
Aroma	Day-0	6.00 a,x	6.33 a,x	6.53 a,x
	Day-7	5.30 a,x	5.00 a,y	5,07 a,xy
	Day-14	4.15 b,y	3.81 b,z	5.54 a,y
Flavor	Day-0	5.73 b,x	4.83 b,x	6.70 a,x
	Day-7	5.44 a,x	4.48 ab,x	4.22 b,y
	Day-14	4.34 a,y	3.61 a,y	4.96 a,y
Texture	Day-0	6.24 b,x	5.63 b,x	6.93 a,x
	Day-7	5.33 a,xy	6.48 a,x	5.67 y
	Day-14	4.77 ab,y	4.11 b,y	5.38 a,y
Aftertaste	Day-0	5.67ab,x	5.03 b,x	6.50 a,x
	Day-7	5.12 a,x	4.67 a,x	4.67 a,y
	Day-14	3.88 a,y	3.62 a,y	4.46 a,y
Overall	Day-0	6.38 ab,x	5.90 b,x	7.00 a,x
	Day-7	5.11 a,y	4.67 a,x	4.81 a,y
	Day-14	4.08 ab,z	3.69 b,y	4.81 a,y
Hedonic quality				
Colour	Day-0	5.60 a,x	3.80 b,y	1.53 c,y
	Day-7	5.59 a,x	4.63 b,y	2.33 c,x
	Day-14	5.62 a,x	5.50 a,x	2.42 b,x
Aroma	Day-0	4.30 a,y	4.27 a,y	3.40 a,y
	Day-7	4.96 a,xy	5.69 a,x	4.70 a,x
	Day-14	5.50 a,x	6.08 a,x	4.23 b,xy
Flavor	Day-0	4.40 b,y	5.27 a,y	3.17 c,y
	Day-7	5.04 a,xy	6.07 a,xy	5.26 a,x
	Day-14	5.81 ab,x	6.77 a,x	5.23 b,x
Texture	Day-0	4.77 a, x	4.83 a, x	4.83 a, x
	Day-7	4.81 a, x	4.52 a, x	5.00 a, x
	Day-14	5.15 a, b, x	4.77 a, x	4.69 a, x
Aftertaste	Day-0	4.80 b, x	4.40 b, x	6.53 a, x
	Day-7	5.15 a, b, x	4.56 b, x	5.70 a, x, y
	Day-14	5.08 a, x	4.92 a, x	5.45 a, y
Ranking test				
Colour	Day-0	2.6 a, x	2.0 b, x	1.22 c, x
	Day-7	2.62 a, x	2.27 a, x	1.19 c, x
	Day-14	2.52 a, x	1.92 b, y	1.60 b, x
Aroma	Day-0	2.07 a, x	2.13 a, x	1.77 a, x
	Day-7	2.00 a, x	2.19 a, x	1.92 a, x
	Day-14	2.00 a,x	2.08 a, x	2.04 a, x
Flavor	Day-0	1.97 a, b, x	2.33 a, x	1.63 b, x
	Day-7	1.69 b, x	2.27 a, x, y	2.15 a, b, x
	Day-14	2.08 a, x	2.08 a, y	1.76 a, x
Texture	Day-0	2.17 a, x	2.10 a, y	1.60 b, x
	Day-7	2.00 b, x	2.50 a, x	1.62 b, x
	Day-14	2.00 a, x	2.24 a, x	1.80 a, x
Aftertaste	Day-0	1.97 a, b, x	2,40 a, x	1.57 b, x
	Day-7	1.77 a, x	2.04 a, x	2.27 a, y
	Day-14	2.16 a, x	1.96 a, x	2.00 a, x, y
Overall	Day-0	2.17 a, x	2.17 a, y	1.67 b, x
	Day-7	1.88 a, x	2.35 a, x	1.96 a, x
	Day-14	2.12 a, x	2.16 a, x, y	1.76 a, x

a,b,c = difference between formulas in one point in time
x,y,z = organoleptic differences between the times of each formula

Table 2. Proximate composition of the sautéed vegetables at day 0.

Vegetables	Carbohydrate (%)	Protein (%)	Fiber (%)	Fat (%)	Ash (%)	Moisture (%)
Spinach	8.26	0.145	2.26	3.10	2.64	84.06
Carrot	11.50	1.90	2.49	1.46	0.13	82.44
Water spinach	17.08	1.36	2.23	3.10	0.18	86.08

Table 3. Vitamin C (mg/100 g) content of the sautéed vegetables at day 0, 7 and 14.

Vegetables	Day-0	Day-7	Day-14
Spinach	0.43	0.33	0.32
Carrot	0.23	0.18	0.21
Water spinach	0.11	0.15	0.19

(Gahler *et al.,* 2003). In addition, as a water-soluble micronutrient, vitamin C is easily leached into the water (Igwemmar *et al*., 2013). However, these results indicated that vacuum packaging could maintain nutrient content, particularly vitamin C during storage.

Table 4 shows the pH of sautéed vegetables. The pH of these samples was relatively constant, ranging from 5.00 to 6.15 (p>0.05). Microorganisms mostly cannot survive at low pH as their maximum pH grows between 6.0 and 7.5 (Srivastava *et al*., 2021). The pH decrease protects food from bacterial spoilage (Brenesselová *et al.,* 2015). This result was consistent with Lee *et al*. (2016), who demonstrated that the vacuuming of kimchi products did not change pH quality. They also found that vacuum treatment assists in preserving the aqueous product (Lee *et al*., 2016). In addition, the pH of sautéed spinach continuously increased until day 14. Seleshe *et al*. (2021) reported that an increase in pH during the storage of vacuum packaging may be related to the accumulation of microorganism metabolites, including ammonia and glucose (Kim *et al*., 2018).

Table 4. pH of the sautéed vegetables at day 0, 7 and 14.

Vegetables	Day-0	Day-7	Day-14
Spinach	5.90	5.85	6.15
Carrot	5.00	5.55	5.20
Water spinach	5.15	5.20	5.15

3.3 Microbiological evaluation

The microbiological analysis revealed *Salmonella* sp. was detected (<30×10^2) on day 7 and between 300 and 600 (MPN/ g) on day 14 (Table 5). This result is consistent with other studies that found increasing microbiological counts started on day 7 of storage (Brenesselová *et al*., 2015). The shelf-life of food is influenced by the number of present microorganisms and is related to the pH. The pH on day 14 is higher than on day 7; thus, the number of *Salmonella* sp. increased.

Salmonella spreads by the fecal-oral route and can be transmitted by food and water. It is one of the most common foodborne infections, with a 12-72 hrs or longer incubation period (Centers for Disease Control (CDC), 2016). Wen *et al.* (2012) reported that vacuum packaging under chilled conditions (4°C and 10°C for 28 days) did not result in a significant reduction in the mean numbers of *Salmonella* in moisture-enhanced pork (Wen and Dickson, 2012). Given that *Salmonella* sp. may thrive in both aerobic and anaerobic environments, the samples' high water content after sauteing suggests that it flourished under vacuum packaging (CDC, 2016).

Table 5. *Salmonella* sp. (MPN/g) in the sautéed vegetables at day 7 and 14.

Vegetables	Day 7	Day 14
Spinach	<30×10^2	300
Carrot	<30×10^2	1600
Water spinach	<30×10^2	1600

*MPN: the most probable number

The current study is the first one to discuss the vacuum packaging of sautéed vegetables, especially in Indonesia. Vacuum packaging with low-temperature storage has proven to be very effective in extending the shelf-life of various perishable foods (Pennachia *et al*., 2011). Interestingly, findings in our study revealed that the nutrients of sautéed vegetables, especially vitamin C, could be maintained during storage in vacuum packaging. Despite maintaining nutrient content, this study reported the occurrence of contamination bacteria (*Salmonella* sp.) in vacuum packaging. Therefore, the findings of this study suggest the consideration of safety practices in the cooking process and vacuum packaging for sautéed vegetables due to bacterial growth.

4. Conclusion

This study revealed that the effect of vacuum packaging on sautéed vegetables helped preserve nutrients and protected them from microbiological growth. The findings in the present study proved that vacuum packaging was able to maintain the quality of sautéed vegetables for 7 days.

Conflict of interest

The authors declare no conflict of interest.

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