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Effect of carbon dioxide on colour stability and microbiological quality of bulk packaged shallot (*Allium ascalonium*) puree

[Kesan karbon dioksida terhadap kestabilan warna dan kualiti mikrobiologi puri bawang merah (*Allium ascalonium*) yang dibungkus secara pukal]

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Keywords: shallot puree, bulk packaging, carbon dioxide, microbiological, colour, storage

Abstract

Colour stability and microbiological quality of bulk packaged shallot puree were evaluated under modified atmosphere conditions (10% CO_2 + 90% N_2). Samples were packed in Ony/LLDPE bags of 0.07 mm thickness as primary packaging material and telescopic carton boxes as secondary packaging, then stored at 5 ± 1 °C (85–95% RH). Shallot puree packed under normal air was used as control and stored under the same conditions. Results showed that L* values and hue angle (H_{ab}) increased, while the chroma values (C*) decreased during the storage period. The Total Plate Count and *Lactobacillus* spp. count increased gradually during storage period in both samples. However, the populations of coliform, yeast and mould were undetected in all samples throughout storage. Modified atmosphere condition with 10% CO_2 + 90% N_2 was found to be a better storage condition for preserving the colour stability and microbiological quality of shallot puree up to 12 weeks at 5 ± 1 °C (85–95% RH).

Introduction

Shallot (*Allium ascalonium*), is one of the ingredients used in Malaysian dishes especially among the Malay and Indian communities. It is added to food not only to impart flavour but also to excite the taste buds to obtain a better appreciation of the dishes presented (Augusti 1996). Malaysia imported about 2.5 million tonnes of shallot from India, China, Thailand and Indonesia in 2003 valued at RM218 million (Department of Statistic Malaysia 2004). The high import value was due to the high per capita consumption of shallot which was reported to be about 2.45 kg per household (Lim 2000).

There are many shallot-based products such as shallot pulp, frozen shallots (or chopped shallots) and peeled shallots. Indeed, shallots are an authentic ingredient of many Asian cuisines from soups, red and green curries as well as fried rice dishes. It is also widely used in French cuisine such as beef bourguignon.

Shallot puree is a semi-processed product that offers convenience for the catering business, including restaurants, canteens (schools, hospitals, nursing homes, prisons, etc.) and also central kitchens as well as retail outlets such as supermarkets. This product can be utilized in preparing other food products, save labour cost and

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processing time without having to peel, wash, blend or pound. Purees can be bought earlier and kept in the refrigerator or chiller until they are required.

The semi-processed product is exposed to an increase in respiration rates, biochemical changes (because many cells are ruptured and intracellular products such as sugars are liberated) and microbial spoilage, which may result in degradation of colour, texture and flavour of the product (Cantwell 1998). According to Chen (2002), the wounded tissues release plant juices or cell contents that serve as nutrients for microorganisms. Thus, this product is ideal for containing a wide range of microorganisms including bacteria, yeasts, moulds, protozoa and viruses. Bacteria, yeasts and moulds have different respiratory and metabolic needs and can be grouped according to their oxygen needs. The anaerobic risks can be minimized by retaining a residual oxygen level of at least 2% within the packs (Hotchkiss 1987).

Modified atmosphere storage is one of the food preservation methods that maintain the natural quality of food products in addition to extend the storage life. The storage life of food products is considerably extended by modifying the atmosphere surrounding the food, which reduces the respiration rate of food products and activity of microorganisms in food (Jayas and Jeyamkondan 2002). Although carbon dioxide (CO_2) has a powerful inhibitory effect on bacteria growth, it does not retard the growth of all types of microorganisms. The growth of lactic acid bacteria, for example, is enhanced in the presence of carbon dioxide and low oxygen content (Parry 1993). High levels of carbon dioxide have generally been found to have an inhibitory effect on *Staphylococcus aureus*, *Salmonella* species, *Escherichia coli* and *Yersinia enterolitica* (Hintlian and Hotchkiss 1986).

In general, CO_2 increases the lag phase and generation time of microorganisms and this effect, as expected, is enhanced at lower temperatures. CO_2 inhibits the growth of bacterial at low temperatures for a restricted period and will restrict mould growth over a longer period. The inhibiting processes involve the dissolving of the gas in the product with the subsequent formation of carbonic acid which slightly reduces the pH of the product and so delays the growth of bacteria and moulds (Guise 1994). Depleted oxygen or enriched carbon dioxide levels can reduce respiration, delay ripening, decrease ethylene production and sensitivity, retard textural softening, reduce chlorophyll degradation and enzymic browning and preserve vitamins of fresh produce, thereby resulting in an extended quality shelf-life (Zagory and Kader 1988).

The food manufacturing and food service sectors remain the largest users of culinary products such as shallot puree. In 2008, the sale volume of culinary product increased by 3.7% valued at RM1,134.6 millions (80,000 tonnes) and estimated to increase up to RM 1,309.1 million (92,000 tonnes) in 2013 (Anon. 2008). Since there is a growing demand for culinary products (e.g. shallot puree), packaging in bulk must be implemented to meet market needs.

To ensure that shallot puree can be supplied to consumers with standard quality, stability of this product in bulk packaging form throughout storage should be studied. Therefore the objective of this study is to determine the influence of carbon dioxide treatment on colour stability and microbial safety as quality indicator of bulk packaged shallot puree during storage at 5 °C.

Materials and methods

Preparation of puree

Matured shallots of Indian variety were bought from a local wholesale wet market in Selangor. Preparation of the material was carried out at the Processing Laboratory, Food Technology Research Centre, MARDI, Serdang. The tail and top of shallots were removed manually with a sharp knife. This was followed by an abrasive peeling process where the product was held for 2 min in the

peeler, with a water jet provided to clean the shallots. After washing, the shallots were ground into a puree using a bowl chopper (Talleres Model AS-75, Palmerston, New Zealand) and then acidified by adding 0.3% (w/w) citric acid to obtain a pH in the range of 4.0–4.4. Finally, the acidified puree was heated to 60 °C in a steam-jacketed kettle (Hasimah 2003; Noor Azizah et al. 2005; Kaymak-Ertekin and Gedik 2005) to deactivate the enzymes.

Packaging and storage

The freshly processed shallot puree was packed immediately in 10 kg portions, into oriented nylon/linear low density polyethylene (Ony/LLDPE) pouches 45 x 35 cm with 0.07 mm thickness supplied by TIP Corporation Sdn. Bhd., Malaysia. The pouches were then placed in a blast chiller (TECNOMAC Model T14, Yorkshire, UK) to reduce the temperature of the product as rapidly as possible to 5 °C. After that, the air was removed from the cooled pouches by flushing with the desired gas atmosphere of 10% carbon dioxide and 90% Nitrogen (Noor Azizah et al. 2010) using a gas mixer (Gasetechnik Model WITT KM100-3MEM, Germany) and the pouches were then immediately heat sealed. The control sample was packed without gas flushing. CO_2 and N_2 gases were supplied by Syarikat Gas Pantai Timur, Selangor, Malaysia. Each pouch of shallot puree was then put into a telescopic carton box, flute B type of size 50 x 38 x 11.9 cm as a secondary package. All boxes were stored in the cold room at 5 ± 1 °C and 85–90 % relative humidity for 3 months. Samples were taken at two weeks interval for colour measurement and microbiological analyses which were done in duplicates.

Determination of colour

Surface colour of shallot puree was evaluated with a colorimeter (CR 300 Minolta, Osaka, Japan) by measuring the L*, a* and b* parameters on the flat sides of each quarter of the packages. The values of a* and b* were used to compute the hue angle ($\tan^{-1}(b^*/a^*)$) and chroma values ($\sqrt{a^{*2} + b^{*2}}$) (McGuire 1992). Results were expressed as hue angle, chroma and L* values for all samples during the storage period.

Microbiological analysis

Ten g portions of shallot puree were aseptically weighed and homogenized with 90 ml Ringer's solution (Oxoid, Hampshire, England) using a stomacher lab-blender (Seward Model 400, London, UK) at a normal speed for 1 min. Serial dilutions were prepared with the same diluent and duplicate counting plates were prepared using appropriate dilutions. For pour plating, 1 ml of the dilutions were mixed with molten (45 °C) media and poured into plates. Total mesophilic aerobic bacteria were counted by plating of sample on plate count agar (PCA; Difco, Detroit, USA). *Lactobacillus* spp. counts were carried out on the Man Rogosa Sharpe agar (MRS; Difco, Detroit, USA). Yeast and mould counts were cultivated on potatoes dextrose agar (PDA; Difco, Detroit, USA). Microbial colonies were counted after 72 h incubation at 31 ± 1 °C for PCA, MRS and PDA (ICMSF 1978). Total coliform counts were determined by the Most Probable Number (MPN) method in a three tube series using MacConkey Broth (Difco, Detroit, USA) incubated at 35 ± 1 °C for 48 h (ICMSF 1978). Microbiological counts were expressed as colony forming units (CFU) per g sample and MPN/g sample for total coliform.

Statistical analysis

Statistical analysis was performed using the SAS programme (SAS Inst. 1985). The values obtained were subjected to analysis of variance (ANOVA) and mean separation was done using the Duncan Multiple Range Test (DMRT) for differences among treatments and storage periods (Gomez and Gomez 1984).

Results and discussion

Changes in colour

Colour is the immediate criteria by which consumers judge the quality of shallot puree. The changes in colour measurements seem to be able to discriminate the shallot puree than the other quality characteristics considered. Normally, brown discolouration appeared together with a loss of the fresh look of the shallot puree during storage, which is undesirable to the consumer. The colour changes of the shallot puree was defined by the geometrical coordinates L* (lightness) and cylindrical coordinates (hue angle and chroma values), which represent intensity and saturation of colour. These were used to compare colour differences among the treated and control samples in this study.

The colour changes (L*, chroma and hue angle) of shallot puree treated with carbon dioxide and control are shown in *Figures 1–3* and *Table 1*. The results showed that the L* value tend to increase for both treated and control, indicating the product was turning lighter and paler compared to samples at the beginning of storage. The increase in lightness (L* values) indicated there was progressive loss of pigments during storage. The loss of colour might be due to the oxidation of anthocyanin pigment content in the shallots (Ferreres et al. 1996). *Figure 1* shows that the control sample had a significantly (p <0.05) larger increase in L* values compared to samples flushed with 10 % CO_2 during the storage period. A similar trend was reported by Leshuk and Saltveit (1990) on onions, where they found that controlled atmosphere storage could reduce colour change of intact onions during storage.

Colorimetry of shallot puree showed an intensification of colour change during storage, expressed as chroma (*Figure 2*) and hue angle values (*Figure 3*). Generally, there was a significant (p <0.05) difference between treatments for chroma values of shallot puree stored for 12 weeks at 5 ± 1 °C (*Figure 2*). At the end of the storage

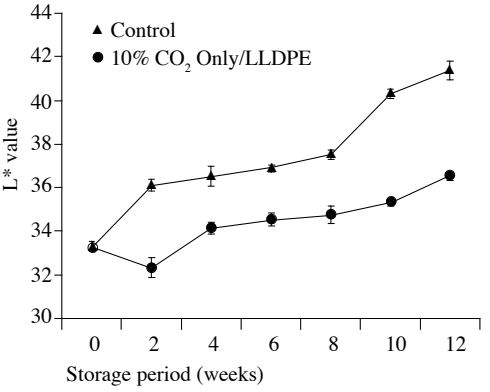

Figure 1. Changes in L values of bulk packaged shallot puree during 12 weeks storage at 5 ± 1 °C*

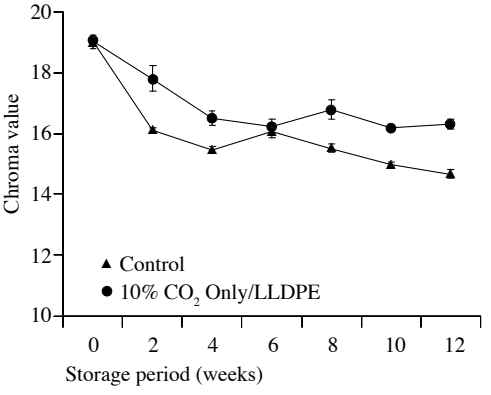

Figure 2. Changes in chroma values of bulk packaged shallot puree during 12 weeks storage at 5 ± 1 °C

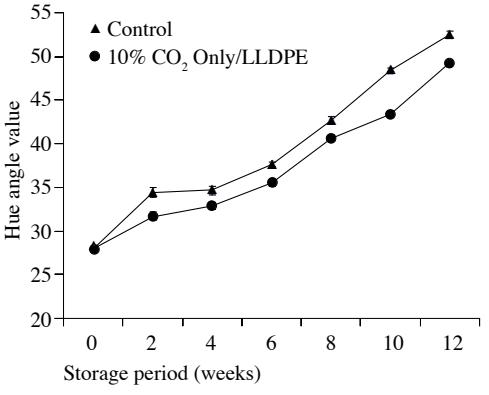

Figure 3. Changes in hue angle values of bulk packaged shallot puree during 12 weeks storage at 5 ± 1 °C

Table 1. Effect of modified atmosphere packaging (MAP) on colour (L* value, chroma value and hue angle) of bulk packaged shallot puree during 12 weeks storage at 5 ± 1 °C

Storage period (weeks)	L* value		Chroma value		Hue angle	
	Control	10% CO_2 Ony/LLDPE	Control	10% CO_2 Ony/LLDPE	Control	10% CO_2 Ony/LLDPE
0	33.29Da	33.29Da	19.03Aa	19.03Aa	27.96Fa	27.96Ga
2	36.12Da	32.33Eb	16.11Bb	17.81Ba	34.45Ea	31.67Fb
4	36.51Da	34.13CDb	15.46Cb	16.51Ca	34.69Ea	32.89Eb
6	36.90CDa	34.53BCb	16.08Ba	16.23Ca	37.63Da	35.48Db
8	37.49Ca	34.74BCb	15.52Cb	16.80Ca	42.66Ca	40.64Cb
10	40.29Ba	35.33Bb	14.99Db	16.18Ca	48.44Ba	43.43Bb
12	41.37Aa	36.53Ab	14.66Db	16.30Ca	52.52Aa	49.27Ab

Means with the same capital letter within a column and same small letter within a row are not significantly different at 5% level (p <0.05) using DMRT

period, there was a marked decrease in chroma value, i.e. the samples lost the typical red colour. Results showed that the chroma values of treated shallot puree decreased (p <0.05) rapidly from 19.03 (week 0) to 16.51 (week 4). However, there was no significant changes (p >0.05) of treated shallot puree at week 4 until the end of storage period. This indicated that samples treated with 10% CO_2 could retain the chroma values after 4 weeks storage preventing further loss in colour. For the control sample, chroma values decreased significantly (p <0.05) throughout 12 weeks of storage. The chroma values of the control sample also showed faster losses as compared to the treated sample.

A similar trend was reported by Rocculi et al. (2005) on minimally processed kiwifruit stored under MAP conditions at 4 °C. Modified atmosphere packaging has also been considered to be beneficial in maintaining high humidity, prevention of water loss and browning of litchi pericarp (Kader 1994; Pesis et al. 2002). The decreasing value of the chroma means that the colour of shallot puree was less saturated. In previous research, Nicolalde (2006) found that a greater chroma value represents a more pure and saturated colour. According to Jasim et al. (2004), decrease in colour values during storage was also likely due to oxidation of pigments.

The hue angle values increased significantly (p <0.05) during storage for both control and treated samples (*Figure 3*). However, higher values were detected in the control as compared to treated samples. Results showed that the changes in hue angle of shallot puree stored at 5 ± 1 °C for 12 weeks were statistically different between samples from week 2 until the end of storage (*Figure 3*). With regards to the hue angle, the rate of colour change was dependent on the CO_2 treatment for up to 12 weeks. Treatment with 10% CO_2 seemed to reduce colour change but was unable to prevent colour change completely at 5 ± 1 °C (*Figure 3*). According to Blanchard et al. (1996), the progress of colour change was slowed down slightly by lowering the oxygen content of the atmosphere, and more so by carbon dioxide enrichment. In this study, hue angle and L* values increased during storage, while chroma value tended to decrease progressively and statistical differences among samples were evident especially at week 2 of storage.

Changes in microbiological count

The total microbial count was associated with the spoilage of the shallot puree under atmospheric conditions. Lactic acid bacteria is the major bacterial group associated with the spoilage of refrigerated products packed under vacuum or modified atmosphere (Borch et al. 1996). Total plate count and

Lactobacillus spp. count were determined and expressed in colony forming units per gram sample (CFU/g) of shallot puree over the whole period of 12 weeks at 5 ± 1 °C (*Figures 4–5* and *Table 2*). Results indicated that growth was observed in both treated and control samples. The growth trend of microbial count in the shallot puree was generally similar to growth of *Lactobacillus* spp. which increased with storage time. However, the microbial count was significantly (p <0.05) higher in the control sample compared to 10% CO_2 treated sample. The number of bacteria in the control sample increased rapidly, whereby the population reached log 5 CFU/g within 12 weeks of storage.

According to Soliva-Fortuny et al. (2004), spoilage by aerobic microorganisms was delayed when bags of low permeability and a packaging atmosphere with low oxygen content were combined. Oriented nylons lamination or coextrusion with polyethylene offer better gas barrier (low permeability) and sealing properties (Gordon 1993; Kirwan and Strawbridge 2003). Therefore, this result was in line with the study on modified atmosphere packaging of shallot puree. Storage in reduced O_2 and elevated CO_2 was found to increase the edible shelf life of several products from 14 days to 21 days (Berrang et al. 1990).

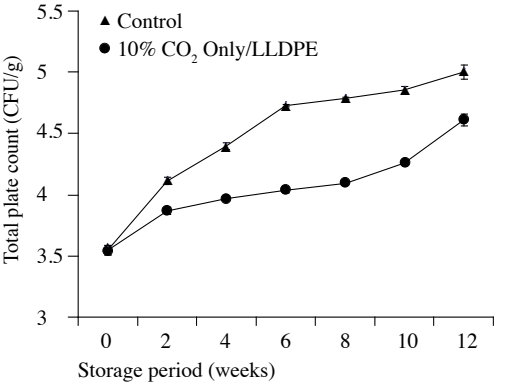

Figure 4. Changes in Total Plate Count of bulk packaged shallot puree during 12 weeks storage at 5 ± 1 °C

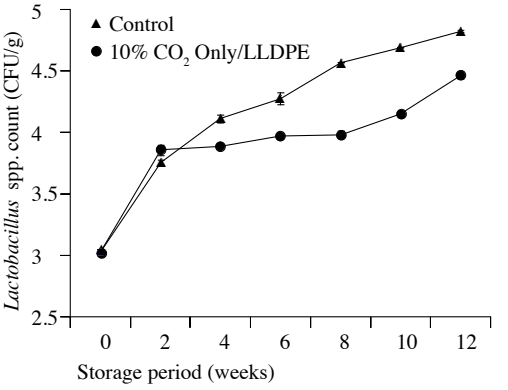

Figure 5. Changes in Lactobacillus spp. counts of bulk packaged shallot puree during 12 weeks storage at 5 ± 1 °C

Table 2. Effect of modified atmosphere packaging (MAP) on total plate count and *Lactobacillus* spp. counts of bulk packaged shallot puree during 12 weeks storage at 5 ± 1 °C

Storage period (weeks)	Total plate count (CFU/g)		*Lactobacillus* spp. counts (CFU/g)	
	Control	10% CO_2 Ony/LLDPE	Control	10% CO_2 Ony/LLDPE
0	3.54Da	3.54Fa	3.03Ga	3.03Ea
2	4.11Ca	3.87Eb	3.75Fa	3.85Da
4	4.40BCa	3.96Da	4.11Ea	3.89Db
6	4.73ABa	4.03CDb	4.27Da	3.97Cb
8	4.78Aa	4.09Cb	4.57Ca	3.98Cb
10	4.85Aa	4.26Bb	4.69Ba	4.15Bb
12	5.01Aa	4.62Ab	4.82Aa	4.46Ab

Means with the same capital letter within a column and same small letter within a row are not significantly different at 5% level (p <0.05) using DMRT

The changes in *Lactobacillus* spp. count was significantly (p <0.05) different between samples after 4 weeks of storage (*Figure 5*). Significant lower numbers of *Lactobacillus* spp. were observed in 10% CO_2 treated shallot puree when compared with the control sample from the fourth week of storage at 5 ± 1 °C. The counts started to increase rapidly from log 3.03 to log 4.46 CFU/g at week 0 and 12 respectively, for shallot puree treated with 10% CO_2, whilst the number of *Lactobacillus* spp. in the control sample increased to log 4.82 CFU/g at week 12 of storage. The growth was slower under 10% CO_2 and final population densities were reduced by less than one log unit as compared to growth under control conditions. The study also indicated that there was no growth of coliform, yeast and mould for both control and treated samples.

Conclusion

Modified atmosphere of 10 % CO_2 + 90 % N_2 can be used to maintain the quality of bulk packaged shallot puree for up to 12 weeks at 5 ± 1 °C (85–95% RH). This storage condition was able to delay colour degradation and slow down the microbial growth and *Lactobacillus* spp. A higher count was noted in the control sample during the 12 weeks storage.

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Abstrak

Kestabilan warna dan kualiti mikrobiologi puri bawang merah yang dibungkus secara pukal dalam atmosfera terubah suai 10% CO_2 + 90% N_2 telah dikaji. Sampel dibungkus menggunakan beg Ony/LLDPE sebagai bahan pembungkus primer dan kotak karton teleskopik sebagai pembungkus sekunder dan disimpan pada suhu 5 ± 1 ºC (85–95% RH). Puri bawang merah yang dibungkus dalam udara biasa digunakan sebagai sampel kawalan dan disimpan pada keadaan yang sama. Keputusan menunjukkan nilai L* dan *hue angle* (H_{ab}) meningkat, sementara nilai chroma (C*) menurun semasa tempoh penyimpanan. Kiraan jumlah plat dan *Lactobacillus* spp. meningkat secara perlahan semasa tempoh penyimpanan di dalam kedua-dua sampel. Walau bagaimanapun, populasi koliform, kiraan yis dan kulat tidak dikesan dalam semua sampel. Atmosfera terubah suai mengandungi 10% CO_2 + 90% N_2 didapati lebih baik untuk memelihara kestabilan warna dan kualiti mikrobiologi puri bawang merah sehingga 12 minggu penyimpanan pada suhu 5 ± 1 °C (85–95% RH).

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