EFFECTS OF PRODUCTION SYSTEMS AND OZONE TREATMENT ON NITRATE CONTENT AND *E. coli* O157:H7 CONTAMINATIONS OF BUTTERHEAD LETTUCE

By

SITI FAIRUZ BINTI YUSOFF

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Chairman : Professor Mahmud Tengku Muda Mohamed, Ph.D

Faculty : Agriculture

Butterhead lettuce, a salad crop pose to food safety risk issues because it is normally consume raw. The information regarding nitrate and *Escherichia coli* O157:H7 contaminations status on Butterhead lettuce in Malaysia is still scanty. Not much work of this nature is carried out locally. Recently, an outbreak of *E. coli* was happened in Germany, which one of the most stringent countries in term of food safety on fresh produce. Hence, this study seemed inevitable to be carried out. The first experiment, a market study, to determine the level of nitrate and *E. coli* O157:H7 contaminations of Butterhead lettuce. The result showed that nitrate levels exceeded the maximum limit but the bacteria contaminations were still under safe limit.

The second experiment was to determine the effect of harvesting stage on the nitrate content, quality and nitrate reductase activity (NRA) of Butterhead lettuce, grown by hydroponic and organic systems. After 35, 38, 41 and 44 days of transplanting (DAT), the lettuce harvested and the studied effects were determined. Nitrate content in

hydroponic lettuce was higher compared to organic lettuce. The accumulation varies with leaf parts, the highest being in midribs, followed by outer adult leaf blades and young leaves. For hydroponic lettuce, extended harvesting stage was found to reduce nitrate content. Fourty one DAT was the optimum stage to harvest with significantly higher reduction of nitrate content. At this stage, the fresh weight, firmness and color were still acceptable. However, harvesting stage had no effect on nitrate content in organic lettuce. NRA was found to be higher in young leaves compared with outer adult leaf blades and midribs.

The third experiment was to determine effect of different aqueous ozone concentrations on *E. coli* O157:H7, nitrate and nitrite contents, and postharvest quality of Butterhead lettuce. The lettuce was treated with aqueous ozone at concentrations of 0, 3 and 5 $mg.L^{-1}$ and stored at 10 °C for 12 days. The quality was assessed on day 0, 4, 8 and 12 of storage by comparing the changes. The number of *E. coli* in organic lettuce was found to be higher than hydroponic lettuce. The aqueous ozone at 5 $mg.L^{-1}$ treatments was effective in reducing *E. coli* colonies but with quality compromised and the effectiveness decreased as the storage period progressed. Ozone at 3 $mg.L^{-1}$ was a potential concentration on reducing *E. coli* without giving in to quality.

In conclusion, the consumers and producers should apply hygienic practices to ensure safe consumption. The optimum harvest stage of Butterhead lettuce is at 41 DAT. A potential concentration of aqueous ozone was 3 $mg.L^{-1}$ on reducing *E. coli* O157:H7 contamination without detrimental effects on lettuce quality. Lettuce can be stored in cool storage for up to eight days.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN-KESAN SISTEM PENGELUARAN DAN RAWATAN OZON KE ATAS KANDUNGAN NITRAT DAN PEMCEMARAN *E. coli* O157:H7 BAGI SALAD *BUTTERHEAD*

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SITI FAIRUZ BINTI YUSOFF

Ogos 2013

Pengerusi : Profesor Mahmud Tengku Muda Mohamed, PhD

Fakulti : Pertanian

Salad *Butterhead* adalah sejenis tanaman salad yang terdedah kepada isu risiko keselamatan makanan kerana ia biasanya dimakan segar. Maklumat tentang status pencemaran nitrat dan *Escherichia coli* O157:H7 ke atas salad *Butterhead* di Malaysia masih lagi kurang. Tidak banyak kajian tempatan dibuat dalam bidang ini. Baru-baru ini berlaku wabak *E. coli* di Jerman, yang merupakan salah sebuah negara yang ketat dari segi keselamatan makanan ke atas hasil segar. Oleh itu, kajian ini tidak dapat dielakkan. Eksperimen pertama, kajian pasaran untuk menentukan tahap pencemaran nitrat dan *E. coli* O157:H7 pada salad *Butterhead.* Keputusan menunjukkan tahap nitrat melebihi paras had maksimum tetapi pencemaran bakteria masih di bawah paras had yang selamat.

Eksperimen kedua adalah untuk menentukan kesan peringkat penuaian terhadap kandungan nitrat, kualiti dan aktiviti penurunan nitrat (NRA) salad *Butterhead* yang ditanam secara sistem hidroponik dan organik. Selepas 35, 38, 41 dan 44 hari selepas

pemindahan (DAT), salad dituai dan kesan-kesan kajian ditentukan. Kandungan nitrat dalam salad hidroponik adalah lebih tinggi berbanding dengan salad organik. Pengumpulannya berbeza mengikut bahagian daun, yang tertinggi adalah dalam midrib, diikuti oleh bilah daun luar dewasa dan daun muda. Bagi salad hidroponik, pelanjutan peringkat penuaian boleh mengurangkan kandungan nitrat. Empat puluh satu hari DAT adalah peringkat optimum untuk dituai kerana pengurangan nitrat yang ketara. Pada peringkat ini, berat segar, kerapuhan dan warna masih boleh diterima. Walaubagaimanapun, peringkat penuaian tidak mempengaruhi kandungan nitrat dalam salad organik. NRA ditemui lebih tinggi dalam daun muda berbanding bilah daun luar dewasa dan midrib.

Eksperimen ketiga menentukan kesan kepekatan akueus ozon yang berbeza ke atas *E. coli* O157:H7, kandungan nitrat dan nitrit, dan kualiti lepas tuai bagi salad *Butterhead*. Salad yang telah dirawat dengan akueus ozon pada kepekatan 0, 3 dan 5 $mg.L^{-1}$ disimpan pada suhu 10 °C selama 12 hari. Kualiti dinilai pada hari 0, 4, 8 dan 12 penyimpanan dengan membandingkan perubahan. Bilangan *E. coli* dalam salad organik ditemui lebih tinggi berbanding dengan salad hidroponik. Rawatan akueus ozon pada 5 $mg.L^{-1}$ berkesan bagi mengurangkan koloni *E. coli* tetapi dengan kualiti telah dikompromi dan keberkesanannya berkurangan semasa tempoh penyimpanan. Kepekatan ozon pada 3 $mg.L^{-1}$ berpotensi bagi mengurangkan *E. coli* tanpa menjejaskan kualiti.

Kesimpulannya, pengguna dan pengeluar perlu menerapkan amalan kebersihan bagi memastikan hasil yang selamat. Peringkat optimum penuaian bagi salad *Butterhead* adalah 41 DAT. Kepekatan ozon pada 3 $mg.L^{-1}$ berpotensi bagi mengurangkan pencemaran *E. coli* O157:H7 tanpa memberi kesan buruk pada kualiti salad. Salad boleh disimpan dalam penyimpanan sejuk sehingga lapan hari.

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Acetyl-CoA	: Acetyl-Coenzyme A
ADI	: Acceptable daily intake
ANOVA	: Analysis of variance
AOAC	: Association of Official Analytical Chemists
C*	: Chroma
BfR	: Federal Institute for Risk Assessment
BOD	: Biological oxygen demand
$Ca(OH)_2$	: Calcium hydroxide
CD	: Corona discharge
$CFUg^{-1}$	: Colony-forming units per gram
$CFUml^{-1}$	: Colony-forming units per milliliter
CH_3COOH	: Acetic Acid
cm	: Centimeter
COD	: Chemical oxygen demand
CQS	: Color quality scale
CRD	: Completely randomized designs
DAT	: Days after transplanting
DOA	: Department of Agriculture
DPPH	: 2,2-diphenyl-1-picrylhydrazyl
DW	: Dry weight
EC	: European commission
EFSA	: European Food Safety Authority
EU	: European Union
FAO	: Food Agriculture Organization
FCR	: Folin-Ciocalteu's phenol reagent
FW	: Fresh weight
g	: Gram
g/L	: Gram per liter

GAE	: Gallic acid equivalents
h	: Hour
h°	: Hue angle
H_2O	: Water
ha	: Hectar
HCl	: Hydrochloric acid
HPO_3	: Metaphosphoric acid
HUS	: Hemolytic-uremic syndrome
IFT	: Institute of Food Technologists
kg	: Kilogram
K_2HPO_4	: Dipotassium phosphate
KH_2PO_4	: Potassium dihydrogen phosphate
KNO_3	: Potassium nitrate
L*	: Lightness
L	: Liter
LSD	: Least significant difference
min	: minutes
mg/cm^2	: Milligram per centimeter square
$MgCO_3$	: Magnesium carbonate
$mg.kg^{-1}$	: Miligram per kilogram
$mg.L^{-1}$	: Miligram per liter
$mg.mL^{-1}$	: Miligram per mililiter
mL	: Mililiter
MNL	: Maximum nitrate limits
MRL	: Maximum recommended limits
MT	: Metric tons
N	: Newton
NAAS	: National Academy of Agricultural Sciences
$NaCO_3$	: Sodium carbonate
NADPH	: Nicotinamide adenine dinucleotiden phosphate
$NaNO_2$	: Sodium nitrite

NaOH	: Sodium hydroxide
NED	: N-(1-Naphthyl) ethylenediamine dihydrochloride
NH_4^+	: Ammonium
NiR	: Nitrite reductase
NIST	: National Institute of Standards and Technology
nm	: Nanometer
nmol	: Nanomoles
NO_2^-	: Nitrite
NO_3	: Nitrate
NO_3-N	: Nitrate-nitrogen
NR	: Nitrate reductase
NRA	: Nirate reductase activity
O_2	: Oxygen
O_3	: Ozone
OH	: Hydroxide
PAL	: Phenylalanine ammonia-lyase
ppm	: Part per million
PPO	: Polyphenoloxidase
PVC	: Polyvinyl chloride
QTLs	: Quantitative trait loci
R^2	: R-squared
RCBD	: Randomized complete block design
RMK-10	: Rancangan Malaysia ke-10
rpm	: Revolutions per minute
SA	: Sulfanilamide
SCF	: Scientific Committee on Food
SSC	: Soluble solids content
TA	: Titratable acidity
TCA	: Tricarboxylic acid cycle
TFTC	: Too few too count
TPC	: Total phenolic content

TPU	: Taman Pertanian Universiti
UPM	: Universiti Putra Malaysia
USA	: United States of America
UV	: Ultraviolet
v/v	: Volume per volume
WHO	: World Health Organization
w/v	: Weight per volume
^{o}C	: Degree celsius
%	: Percent
µL	: Microliter
$\mu L.L^{-1}$	: Microliters per liter
µmol	: Micromoles
$\mu mol\ mol^{-1}$	: Micromoles per moles

CHAPTER 1

GENERAL INTRODUCTION

Lettuce is a major leafy vegetable and commonly used as salad. There are five major types of lettuce; Butterhead, Crisphead (Iceberg), Romaine, Leaf, and Stem. In China and Egypt, stems rather than leaves of lettuce are consumed, mainly as a cooked vegetable. Humans have had a long history of domestication and cultivation of lettuce. The existence of many primitive forms of lettuce in the Middle East suggested that lettuce probably originated in the eastern Mediterranean basin. Lettuce-like plants were found in Egyptian tomb paintings dated from the Middle Kingdom, about 4, 500 years ago (Harlan, 1986). Human selection and later breeding efforts have led to changes in size, shape, color, texture, and taste of leaves and plants, resulting in modern-day lettuce. In Malaysia, lettuce is produce through hydroponic and organic systems. However, traditional planting on soil is also common. According to Nazaryuk et al. (2002), the production system has bearing on nitrate content in plant due to different types, amount, and frequency of fertilizer application.

The harvesting stage is one of the factors that affect lettuce quality. Lettuce maturity is reached when the heads are well formed and solid (Ryall et al., 1982). Maturity is also based on head compactness and firmness that is also related to its susceptibility to certain postharvest disorders (ZongQi, 2009). Delaying in harvest when the lettuce reaches its maximum yield decreases their quality as mentioned by Kader (2008) but in contrast, it reduced the nitrate content in lettuce (Santamaria et al., 2001).

In term of microbial food safety, the potential sources of preharvest contamination on fresh produce were recently reviewed and include the use of manure fertilizer, the presence of animals in fields and the use of poor quality water for irrigation (Beuchat and Ryu, 1997; Brackett, 1999; Beuchat, 2002; Steele and Odumeru, 2004; Brandl, 2006). The organic lettuce which contained ruminant manure and sewage were considered the main sources of *E. coli* O157:H7 (Olaimat and Holley, 2012) and they were able to survive in soils for months or years (Doyle and Erickson, 2008). Postharvest contamination might also occur in the packaging house due to cross-contamination with raw produce, during washing steps or poor sanitation. Thus, human pathogens might contaminate the fresh produce at any stage from farm-to-fork.

Ozone is one of the alternative sanitizing treatments tested for inactivation of microorganisms, removing toxic substances and extending the shelf life of fruits and vegetables. Ozone revealed promising results in solving problems of food industry like mycotoxin contamination and chemical or pesticide residues. In Oztekin et al. (2005) studies, a significant reduction in total bacteria, coliform and yeast counts on figs were observed after 3 hours treatment at 5 ppm. The decreased in total aerobic mesophyllic bacteria and yeast counts was 38 and 72%, respectively. All coliforms were inactivated. Nadas et al. (2003) stored strawberries for 3 days at 2 °C with or without 1.5 ppm ozone and then transferred to room temperature. Ozone treated fruits showed less weight loss than the non-treated fruits after cold storage. They stated that Ozone treatment reduced water loss through transpiration of the fruit, but this effect disappeared when the fruit returned to ambient air.

Ozonated water treatment resulted in no significant difference in total sugar content of celery (Zhang et al., 2005). Beltran et al. (2005) also reported that ozonated water maintained the initial visual appearance of fresh-cut lettuce and controlled browning during storage. However, some detrimental effects of ozone on certain products, such as bananas and leafy vegetables were also reported (Smilanick, 2003). Perez et al. (1999) stored strawberries for three days at 2 °C in an atmosphere containing 0.35 ppm ozone and found low contents of sugars at the third day of storage. They concluded, this could be due to an activation of sucrose degradation pathways in response to oxidative stress caused by ozone. Ozone is expected to cause the loss of antioxidant constituents, because of its strong oxidizing activity. However, ozone washing treatment was reported to have no effect on the final phenolic content of fresh-cut iceberg lettuce (Beltran et al., 2005). Vitamin C (ascorbic acid), present in fruits and vegetables gives an added value due to its important nutritional implications. It was reported that ozone decreases ascorbic acid in broccoli florets (Lewis et al., 1996). On the contrary, Zhang et al. (2005) reported that there was no significant difference between vitamin C contents of celery samples treated and non-treated with ozonated water.

Nitrate and *E. coli* O157:H7 are known to contaminate the fresh produce including lettuce. These contaminations could be harmful to human health if exceeded maximum limits. One of the major contamination sources is through production system. However, harvesting stage and storage duration also influenced the level of contamination. Ozone is an alternative sanitizer with no safety concerns with residual or by-products. Nevertheless, if not properly used, ozone can cause some deleterious effects on physiology and quality of produce. However, these kinds of studies are still scarce in Malaysia. Thus, this study carried out with the general objective of to evaluate the level of nitrate and *E. coli* O157:H7 contaminations in lettuce found in the market and interventions that can reduce the contaminations.

The specific objectives of this study were (i) to determine the nitrate and *E. coli* O157:H7 contaminations in Butterhead lettuce available in the market, (ii) to determine the effect of hydroponic and organic production systems and harvesting stage on nitrate accumulation, quality and nitrate reductase activity (NRA) of Butterhead lettuce, and (iii) to determine the effect of production system and different aqueous ozone concentrations on *E. coli* O157:H7, nitrate and nitrite contents and subsequently, postharvest quality of Butterhead lettuce.

CHAPTER 2

LITERATURE REVIEW

2.1 Lettuce

Lettuce (*Lactuca sativa* L.) is a leafy vegetable from Asteraceae family. It is mainly cultivated in temperate region countries such as England, France, the Netherlands and Western Europe (Vries, 1997) and also in some tropical regions. Lettuce is the only member of the *Lactuca* genus grown commercially (Koike et al., 2007). The Food and Agriculture Organization of the United Nations (FAO, 2010) reported that world production of lettuce and chicory for year 2010 was 23, 622, 366 metric tons. These are primarily from China (53%), the United States (17%) and India (4%). Although China is the top world producer of lettuce, majority of the crop is consumed domestically. Jore L. (2012) revised that Spain is the world's largest exporter of lettuce with US ranked second.

In Malaysia, it was reported that lettuce production in 2009 to 2010 increased from 19, 662 to 38, 597 metric tons, while the acreage also increased from 1, 182 to 2, 286 ha (DOA, 2010). In Tenth Malaysia Plan, (RMK-10), for years 2011 to 2015, the lettuce production is expected to increase from 10, 430 to 16, 362 metric tons and the planting area also increased from 883 ha to 1, 246 ha. Also, consumer's demand for lettuce is on the increase in Malaysia.

There are several types of lettuce, but most common are the Leaf, Head and Cos or Romaine lettuce (Katz and Weaver, 2003). Butterhead lettuce, a head type lettuce, is one of the most popular varieties in Western Europe, where it accounts for about 80% of lettuce consumption. In North America, this lettuce is gaining popularity and also is known as Boston or Bibb lettuce (Davey et al., 2007).

Butterhead lettuce, unlike other types of lettuce such as Iceberg, Romaine, or Leaf, forms open heads with softer leaves and has much smoother and delicate texture (Bradley et al., 2010). Head lettuce height was below 18 cm (Las Hojas, 2009). Elzebroek and Wind (2008) reported that nutrients of lettuce contain 96% water, 2% protein, 1% carbohydrate and 0.5% fiber. Lettuce is a good source of vitamin A and potassium, with higher concentrations of vitamin A found in darker green lettuces.

Vegetable production and marketing have received increasing attention with regard to quality and safety of produce (Midmore and Jansen, 2003; Kader, 2005; Hewett, 2006). In plastic bags, wounded lettuce leaves produce ethylene, which may compromise the product quality (Hodges et al., 2008). Although temperatures close to 0 °C are recommended, fresh vegetables may be prepared, shipped and stored at 5 °C and sometimes 10 °C (Watada et al., 1996). The high water content (94.9%) of lettuce creates a problem when attempting to preserve the plant because they are highly susceptible to water loss and mechanical damage during storage and transportation. Butterhead lettuce damaged much easily due to its exceptionally tender leaves. Crushing and bruising caused about 28% postharvest losses of fresh harvested Butterhead lettuce (Boonyakiat, 1999).