

Factors Affecting Health-Beneficial Compounds in Lettuce

Shimin Fan

Department of Plant Science

McGill University, Montreal

Macdonald Campus of McGill University

21,111 Lakeshore Road, Sainte-Anne-de-Bellevue, Quebec, Canada

November 2013

A thesis submitted to McGill University in partial fulfillment of the requirements of the
degree of Master of Science

© Shimin Fan 2013

Table of Contents

Table of Contents	i
List of Tables	iv
List of Figures	v
Abstract	vii
Résumé	viii
Acknowledgements	x
List of Abbreviations	xii
Chapter I Introduction	1
1.1 General Introduction	1
1.2 Objectives and Hypotheses of the Study	2
1.2.1 Broad Objective	2
1.2.2 Specific Objectives	2
1.2.3 Hypotheses:	2
Chapter II Literature Review	4
2.1 General Presentation of Lettuce.....	4
2.2 Nutraceuticals and Functional Food: Definition.....	6
2.3 Benefits and Importance of Health-Beneficial Compounds in Lettuce	7
2.4 Factors Affecting Concentration of Health-Beneficial Compounds in Lettuce	9
2.4.1 Genetic Factors	9
2.4.1.1 Differences among Cultivars of Lettuce for Health-beneficial Compounds Concentrations	9
2.4.1.2 Importance of the GXE interaction in the determination of the concentrations of health-beneficial compounds in lettuce.....	10
2.4.2 Environmental and Agronomic Factors	11
2.4.2.1 Temperature	11
2.4.2.2 Soil Fertility and Salt Stress	12
2.4.2.3 Light Duration and Quality	13
2.4.3 Use of Elicitor Compounds	14
Chapter III Health-Beneficial Compounds in Different Lettuce Genotypes	18
3.1 Abstract.....	18
3.2 Introduction	19
3.3 Materials and Methods	20
3.3.1 Treatments and Growing Conditions	20
3.3.2 Laboratory Analyses.....	22
3.3.2.1 Extraction of Total Flavonoid, Total Phenolics and FRAP Assay	23

3.3.2.2 Determination of Total Flavonoid	23
3.3.2.3 Determination of Total Phenolics	24
3.3.2.4 FRAP (Ferric-Reducing Antioxidant Power) Assay	24
3.3.3 High-Performance Liquid Chromatography (HPLC) Analysis	25
3.3.4 Data Analyses	25
3.4 Results	26
3.4.1 Relationship among Tested Environments	26
3.4.1.1 Total Flavonoids	26
3.4.1.2 Total Phenolics	27
3.4.1.3 FRAP (Ferric-Reducing Antioxidant Power)	27
3.4.2 Ranking of the Genotypes	27
3.4.2.1 Total Flavonoids	27
3.4.2.2 Total Phenolics	29
3.4.2.3 FRAP (Ferric-Reducing Antioxidant Power)	29
3.4.2.4 Caffeic Acid	30
3.4.2.5 Chicoric Acid	31
3.5 Discussion and Conclusion	31
Chapter IV Heat Stress Effects on the Concentration of Health-Beneficial Compounds in Lettuce	57
4.1 Abstract	57
4.2 Introduction	58
4.3 Material and Methods	59
4.3.1 Plants Selection and Treatments and Grow Conditions Description	59
4.3.2 Laboratory Analyses	62
4.3.2.1 Extraction of Total Flavonoids, Total Phenolics and FRAP	62
4.3.2.2 Determination of Total Flavonoids	62
4.3.2.3 Determination of Total Phenolics	63
4.3.2.4 FRAP (Ferric-Reducing Antioxidant Power) assay	63
4.3.3 HPLC (High-Performance Liquid Chromatography)	64
4.3.4 Data analysis - Statistical Analyses	65
4.4 Results	65
4.4.1 Plant Growth	65
4.4.2 Health-beneficial Compounds at the Ten-leaf Stage	66
4.4.3 Health-beneficial Compounds at the 'Maturity' Stage	67
4.5 Discussion and Conclusion	71
Chapter V General Conclusions	82
Chapter VI Suggestions for Future Research	84
Chapter VII Contributions to Knowledge	85

References.....	86
Appendix	100

List of Tables

Table 3.1: List of 38 lettuce genotypes evaluated	35
Table 3.2: Total flavonoid concentration of 38 lettuce genotypes harvested at two stages ('ten-leaf stage' and 'maturity' stage) and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).....	36
Table 3.3: Total phenolics concentration of 38 lettuce genotypes harvested at two stages ('ten-leaf stage' and 'maturity' stage) and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).	38
Table 3.4: FRAP values of 38 lettuce genotypes harvested at two stages ('ten-leaf stage' and 'maturity' stage) and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).	40
Table 3.5: Caffeic acid concentration of 38 lettuce genotypes harvested at the 'maturity' stage and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).	42
Table 3.6: Chicoric acid concentration of 38 lettuce genotypes harvested at the 'maturity' stage and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).	44
Table 3.7: Correlation matrix for total flavonoid concentrations among 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).....	47
Table 3.8: Correlation matrix for total phenolics concentrations among 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).....	49
Table 3.9: Correlation matrix for antioxidant activity (FRAP value) among 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).....	51
Table 4.1: Harvest date of six lettuce cultivars grown at two different temperature regimes in 2012. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown 28/20°C day/night.....	73
Table 4.2: Analysis of Variance of Concentrations Table of Total Flavonoid, Total Phenolics, FRAP, Chlorogenic Acid, Caffeic Acid, Chicoric Acid and Luteolin-7-O-Glucoside at 'Ten-Leaf Stage'	74
Table 4.3: Analysis of Variance of Concentrations Table of Total Flavonoid, Total Phenolics, FRAP, Chlorogenic Acid, Caffeic Acid, Chicoric Acid and Luteolin-7-O-Glucoside at 'Maturity stage'	75

List of Figures

Figure 2.1: Different types and species of lettuce. a) crisphead, b) romaine, c) green leaf, d) red leaf, e) butterhead, f) Batavia, g) latin, h) stem, i) buds of wild type lettuce <i>L. saligna</i> , j) wild type lettuce include <i>L. serriola</i> , <i>L. saligna</i> and <i>L. virosa</i> , only <i>L. saligna</i> shown in this figure.	16
Figure 2.2: The structures of luteolin and quercetin; two key flavonoids in lettuce.	17
Figure 2.3: The structures of three key phenolic acids in lettuce: caffeic acid, chicoric acid and chlorogenic acid.	17
Figure 3.1: The environment-vector view of GGE biplot illustrating the relationship among tested environments for total flavonoids concentrations in 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).	46
Figure 3.2: The environment-vector view of GGE biplot illustrating the relationship among tested environments for total phenolics concentrations in 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).	48
Figure 3.3: The environment-vector view of GGE biplot illustrating the relationship among tested environments for antioxidant activity (FRAP value) in 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).	50
Figure 3.4: Mean-stability ranking for total flavonoid concentrations of 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).....	52
Figure 3.5: Mean-stability ranking for total phenolics concentrations of 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).	53
Figure 3.6: Mean-stability ranking for antioxidant activity (FRAP value) of 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).....	54
Figure 3.7: Mean-stability ranking for caffeic acid concentrations of 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).....	55
Figure 3.8: Mean-stability ranking for chicoric acid concentrations of 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).....	56

Figure 4.1: Number of leaves in six lettuce cultivars grown at two different temperature regimes. Control plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night.....	76
Figure 4.2: Appearances of six lettuce cultivars grown at two different temperature regimes. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown 28/20°C day/night. Heat tolerant indicates cultivars that have been previously reported to be tolerant to heat-stress. Photos were taken 25 days after transplantation.....	77
Figure 4.3: Effect of heat stress on total flavonoid concentration in lettuce sampled at the 'maturity' stage. Control plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night. Estival, QSJ-09 (Champlain) and New Red Fire have previously been classified as being heat tolerant cultivars, the others were not. New Red Fire, Lolla Rossa 'Sanguine' and Ruby Sky are red-leaf cultivars, while the other three cultivars are green-leaf cultivars. Treatment means followed by different letters are significantly different ($P<0.05$). Vertical bars indicate standard deviation.	78
Figure 4.4: Effect of heat stress on total phenolic concentration in lettuce sampled at the 'maturity' stage. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night. Estival, QSJ-09 (Champlain) and New Red Fire have previously been classified as being heat tolerant cultivars, the others are not. New Red Fire, Lolla Rossa 'Sanguine' and Ruby Sky are red-leaf cultivars, while the other three cultivars are green-leaf cultivars. Treatment means followed by different letters are significantly different ($P<0.05$). Vertical bars indicate standard deviation.	79
Figure 4.5: Effect of heat stress on antioxidant activity (FRAP value) in lettuce sampled at the 'maturity' stage. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night. Estival, QSJ-09 (Champlain) and New Red Fire have previously been classified as being heat tolerant cultivars, the others are not. New Red Fire, Lolla Rossa 'Sanguine' and Ruby Sky are red-leaf cultivars, while the other three cultivars are green-leaf cultivars. Treatment means followed by different letters are significantly different ($P<0.05$). Vertical bars indicate standard deviation.	80
Figure 4.6: Effect of heat stress on chlorogenic acid concentration in lettuce sampled at the 'maturity' stage. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night. Estival, QSJ-09 (Champlain) and New Red Fire have previously been classified as being heat tolerant cultivars, the others are not. New Red Fire, Lolla Rossa 'Sanguine' and Ruby Sky are red-leaf cultivars, while the other three cultivars are green-leaf cultivars. Treatment means followed by different letters are significantly different ($P<0.05$). Vertical bars indicate standard deviation.	81

Abstract

Lettuce contains several health-beneficial compounds. Increasing the concentration of key health-beneficial compounds in lettuce has thus become an objective of several breeding programs and producers. In a first experiment, 38 genotypes of lettuces including crisphead, butterhead, romain, leaf lettuce, stem, Latin and wild species, were grown in greenhouses at two sites and concentrations of major flavonoids and phenolic compounds were quantified. In a second experiment, 6 cultivars of lettuce were grown in growth chambers with high (20°C night/28°C day) or control (14°C night/18°C day) temperatures to study the effect of heat stress on the concentration of health-beneficial compounds in lettuce. The results indicated that concentrations of total flavonoids, total phenolics, Ferric-Reducing Antioxidant Power (FRAP) and chicoric differed significantly among lettuce genotypes. Results at the two sites were highly correlated, thus selection at one site may be sufficient. Among the most commonly cultivated types, red leaf lettuces had the highest total flavonoids concentration at the maturity stage, followed by butterhead, and green leaf, while crisphead and batavia had the lowest concentrations. Health-beneficial compounds could be affected by heat stress in lettuce, however, different lettuce cultivars responded differently to the heat stress.

Résumé

La laitue contient plusieurs composés bénéfiques pour la santé. L'augmentation de la concentration des principaux composés bénéfiques pour la santé dans la laitue est ainsi devenue un objectif de plusieurs programmes d'amélioration et de producteurs. Dans une première expérience, 38 génotypes de laitues dont pommée, butterhead, Romain, laitue en feuilles, les espèces latine et sauvages, ont été cultivés dans des serres à deux sites et les concentrations des principaux flavonoïdes et des composés phénoliques ont été quantifiés. Dans une seconde expérience, 6 cultivars de laitue ont été cultivés dans des chambres de croissance avec températures élevées (20°C nuit/28°C jour) ou un témoin (14°C nuit/18°C jour) pour étudier l'effet du stress thermique sur la concentration de composés bénéfiques pour la santé dans la laitue. Les résultats indiquent que les concentrations de flavonoïdes totaux, composés phénoliques, le pouvoir de réduction antioxydant de l'ion ferrique (FRAP), l'acide chlorogénique et l'acide chicorique diffèrent significativement entre les génotypes de laitue. Les résultats aux deux sites étaient fortement corrélés, et une sélection à un site pourrait suffire. Parmi les types, les plus couramment cultivées, les laitues à feuilles rouges avaient la plus forte concentration totale en flavonoïdes, suivie par les types semi-pommés (butterhead), et ceux à feuilles vertes, tandis que la laitue pommée et batavia avaient les concentrations les plus faibles. Les composés

bénéfiques pour la santé dans la laitue étaient affectés par un stress thermique cependant la réponse dépendait des cultivars.

Acknowledgements

I would like to express my sincere gratitude to my co-supervisors Dr. Philippe Seguin and Dr. Sylvie Jenni. Both of them provided me very helpful advices and suggestions for my project, especially for the project set up and data analysis. Dr. Seguin also helped me a lot with my paper editing, and gave me constant encouragements and support during my study in McGill. I would also like to thank Dr. Katrine Stewart for serving on my research advisory committee and for her advices during the course of my project.

I would like to thank Agriculture and Agri-Food Canada Horticulture Research and Development Centre (AAFC) for providing the lettuce seeds used for this project. I am most grateful to the technicians both at the Macdonald Campus of McGill University and Agriculture and Agri-Food Canada Horticulture Research and Development Centre for their great help during my project, especially Mr. Wucheng Liu for his advice with HPLC analyses and laboratory work; Mr. Luc Marchand, Mr. Daniel Rolland and Ms. Éléonore Tremblay who helped me to take good care of my plants at the Agriculture and Agri-Food Canada Horticulture Research and Development Centre, as well as their technical help with my plant sampling. Thank Dr. Marie-Thérèse Charles, post-harvest researcher at AAFC for her help with this project. Thanks to Guy Rimmer for providing me with some experimental advices and materials. I would like to thank Dr. Kebba Sabally, Dr. Arif Mustafa and Dr. Jacqueline Bede who let me use

the laboratory equipment from their lab, especially Dr. Kebba Sabally also gave me advice about my experiments. I would like to thank Centre SÈVE for travel funding for my conference.

I would thank my laboratory mates, Pratyusha Chennupati, Erik Delaquis and Marie-Pier Aubin who are very nice and I was very happy to become friends with them. I would like to express my greatest appreciation to my parents who supported me to study in Canada, and always encourage me. Thanks to my good friends, Kimi, Luan, Chen, Shuopeng, Selina etc. who make me feel warm like a family here.

List of Abbreviations

ABA	Absciscic Acid
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	Aluminium Chloride Hexahydrate
CH_3COOK	Potassium Acetate
COX	Cyclooxygenase
CV%	Conversion Percentage
DW	Dry weight
Fe^{2+}	Ferrous
Fe^{3+}	Ferric
FeSO_4	Ferrous Sulphate
FeCl_3	Ferric Chloride
FRAP	Ferric-Reducing Antioxidant Power
HPLC	High-Performance Liquid Chromatography
LPO	Lipoxygenase
MeJA	Methyl Jasmonate
NaCl	Sodium Chloride
TPTZ	Tripyridyl Triazine

Chapter I Introduction

1.1 General Introduction

Lettuce is one of the most widely consumed leafy vegetables in the world, which contains several health-beneficial compounds such as antioxidant, flavonoids and phenolic acids. The use of these health-beneficial compounds has been considered in the prevention and treatment of diseases such as cancers and heart diseases (Arai et al., 2000; Birt et al., 2001; Hu, 2003; Jang et al., 1997). For example, people with high carotenoids plasma levels have a lower risk of cancer (Paiva and Russell, 1999), the antioxidant activity of flavonoids can also prevent cancers and cardiovascular diseases (Gregory et al., 1990). Increase of key nutritional components in lettuce, especially those with health-beneficial properties thus has become a hot topic these days. However, there is limited published data reporting on the concentrations of many of these health-beneficial compounds including flavonoids and phenolic acids in different genotypes and species of lettuce, and on how the concentration of health-beneficial compounds is affected by environmental factors. In this project, 38 genotypes of lettuces were studied, including crisphead, butterhead, romaine, leaf lettuce, stem, Latin and wild species, for their concentration of major flavonoids and phenolic compounds. The project includes two parts: first, the concentration of health-beneficial compounds in different lettuce species and genotypes was determined; second, the effect of one environmental stress (i.e., temperature) on the concentration

of health-beneficial compounds in lettuce was studied. This research could contribute to new utilizations of lettuce and to the development of new markets for lettuce producers in the long term.

1.2 Objectives and Hypotheses of the Study

1.2.1 Broad Objective

The long-term objective of this study was to contribute to the development of strategies that can be used to improve the concentration of health-beneficial compounds in lettuce, with the goal of developing common lettuce into a functional food.

1.2.2 Specific Objectives

- a. To determine the concentration of health-beneficial compounds in different genotypes and species of lettuce.
- b. To determine how a given environmental stress (i.e., heat stress) affect health-beneficial compounds in lettuce and determine if there is a link between concentration of certain health-beneficial compounds and heat stress tolerance.

1.2.3 Hypotheses:

- a. Lettuce genotypes differ in their concentration of health-beneficial compounds.
- b. The ranking of different genotypes and species of lettuce in terms of health-beneficial compounds concentration is consistent across different sampling stages and environments.

- c. Heat stress increases the concentration of health-beneficial compounds in lettuce.
- d. There is an association between the concentration of health-beneficial compounds in lettuce and reported lettuce heat tolerance.

Chapter II Literature Review

2.1 General Presentation of Lettuce

Lettuce is a cool season annual crop. Both cultivated and wild lettuce species are native from Europe and Asia. Germination of lettuce is favoured by uniformly cool temperatures (optimum of 18 to 21 °C). Temperatures of 26 °C and above inhibit germination severely in some cultivars (Peirce, 1987). Lettuce does not grow optimally in highly acidic soils and lime must generally be applied if the soil pH is below 5.5. Lettuce is somewhat tolerant of alkaline soils, but it may still be beneficial to reduce the soil pH to avoid mineral deficiencies and toxicities (Decoteau, 2000). Lettuce is one of the most popular leafy vegetables in the world. The Food and Agriculture Organization of the United Nations (FAO) reports that from 2011, worldwide production of lettuce and chicory are around 24 million metric tons, a 3% increase when compared to the data from 2010 (FAO 2011). China is the largest lettuce producer with more than 50% of the world production; the United States is the second largest producer. While the Chinese production is mostly for the domestic market, production in the United States is both for the domestic market as well as for exportation; most of this lettuce is exported to Canada and Mexico. The value of the American exports of lettuce to Canada increased from 50 million US dollars in the year 1989 to 230 million US dollars in the year 2004 (FAO 2011).

There are several types of lettuce, which can be classified based on their appearance.

The following are the most common types of lettuce (Figure 2.1):

Crisphead: Crisphead lettuce, also referred to as Iceberg lettuce has tightly folded, large, heavy and brittle- textured leaves, which make it resistant enough for long-distance shipping. Leaves in different parts of the Crisphead lettuce vary in color, the outer ones are green but the interior of the head is more white or yellowish. There are many types of Crisphead lettuce including Imperial, Great Lakes, Empire, Vanguard, Salinas etc. (Peirce, 1987; Wittwer and Honma, 1979).

Butterhead: Butterhead lettuce has very soft and buttery textured leaves. Compared with Crisphead lettuce, the veins and midribs of Butterhead lettuce are less prominent, and not suitable for shipping. However, it is popular for local sales. There are two types of Butterhead lettuce, respectively called Bibb and Boston lettuce (Peirce, 1987; Wittwer and Honma, 1979).

Romaine lettuce: Romaine lettuce also called cos lettuce has elongate heads of long heavy textured leaves. Outer leaves are dark green and look tough, while inner leaves are fine textured and excellent in eating quality, also suitable for local sales (Decoteau, 2000; Peirce, 1987).

Leaf lettuce: Leaf lettuce is one of the most popular types of lettuce for market sales and home gardens. The leaves of leaf lettuce are variable in shape and color and form a compact rosette. The quality especially the nutritional quality of leaf lettuce is

superior to that other heading forms because most leaf-type cultivars withstand greater environmental variations (Decoteau, 2000; Peirce, 1987; Wittwer and Honma, 1979).

Stem lettuce: Stem lettuce is a popular crop in Asia. It was first selected to be grown commercially in China. During the growth of stem lettuce, the stems enlarge and the leafy portion and the outer skin of the plant will be peeled away before consumption. The remaining soft and translucent green core can be eaten either fresh or cooked (Decoteau, 2000; Peirce, 1987).

Latin lettuce: The leaves of Latin lettuce are elongated and more leathery than Romaine lettuce. This type is predominantly grown in the Mediterranean region and South America (Decoteau, 2000; Peirce, 1987).

2.2 Nutraceuticals and Functional Food: Definition

The term 'nutraceutical' is composed by 'nutrition' and 'pharmaceutical', which was coined by DeFelice in 1989 (Kalra, 2003), the definition being a food which provides medical or health benefits, including the prevention and treatment of diseases (Brower, 1998). Health Canada defines functional food as "similar in appearance to a conventional food, consumed as part of the usual diet, with demonstrated physiological benefits, and/or to reduce the risk of chronic disease beyond basic nutritional functions" (Bagchi, 2008). Market statistics demonstrates that the global functional food and nutraceutical market is growing much faster than the traditional

processed food market (Wildman, 2006). A poll conducted by the Council for Responsible Nutrition (CRN) indicated that, in 2007, the percentage of American people who identified themselves as regular users of dietary supplements was up to 52%, compared to 46% in 2006 (Dickinson et al., 2009). Only in the United States, the consumer spending of dietary supplements and functional foods reached a reported \$22.4 and \$31.4 billion in sales, respectively, these amounts being more than two times compared to those spent in 1994 (Bagchi, 2008). However, a research from The Institute of Food Technologists (IFT) reported in 2012 more people preferred getting their vitamins and minerals from food they eat and beverages they drink instead of taking supplements.

2.3 Benefits and Importance of Health-Beneficial Compounds in Lettuce

A diet which is mainly composed of fruits and vegetables has been considered as one of the best ways to decrease the risk of suffering from cardiovascular diseases and cancer (He et al., 2007). The action of health-beneficial components such as antioxidants, flavonoids and phenolic acids, has been implicated in providing some of those health-promoting effects (Halliwell et al., 2005; Kaur and Kapoor, 2001; Yao et al., 2004). Lettuce is one of the most widely consumed leafy vegetables (Van Duyn and Pivonka, 2000), thus increasing the concentration of key health-beneficial components in lettuce has become an objective of some breeding programs and producers.

There are several health-beneficial compounds in lettuce, including two main groups namely flavonoid and phenolic acids. Large variations exist in term of concentrations of specific compounds (Crozier et al., 1997; Ordidge et al., 2010). Flavonoids are a class of plant secondary metabolites (Ahuja and Ahuja, 2011; Harborne and Williams, 2000; Tanwar and Modgil, 2012), most commonly known for their antioxidant activity in vitro (Heim et al., 2002; Hertog et al., 1992; Pietta, 2000; Rice-Evans et al., 1996). Consumers and food manufacturers have become interested in flavonoids for their role in prevention of cancers and cardiovascular diseases (Chandan and Shah, 2006; Ransley, 2001). The main dietary sources of flavonoids are fruits, vegetables and beverages such as tea and red wine (Harborne and Williams, 2000; Hertog et al., 1993; Hertog et al., 1992; Yao et al., 2004). Luteolin and quercetin are two key flavonoids found in lettuce (Figure 2.2).

Phenolic acids are also one of the major groups of polyphenols (Bravo, 1998; Scalbert and Williamson, 2000). They are a type of aromatic secondary metabolites biosynthesized by plants, containing a phenolic ring and an organic carboxylic acid function (Robbins, 2003). Due to the antioxidant activity in phenolic acids decreasing the risk of cardiovascular diseases and cancers, they have attracted considerable attention in recent years (Hasler, 1998; Huang and Ferraro, 1992). There are nearly 8,000 naturally occurring phenolic compounds and developing a uniform extraction method for different phenolic acids from different matrices is thus difficult (Luthria and Mukhopadhyay, 2006;

Luthria et al., 2006). The structures of some major phenolic acids in lettuce include caffeic acid, chicoric acid and chlorogenic acid (Figure 2.3).

2.4 Factors Affecting Concentration of Health-Beneficial Compounds in Lettuce

2.4.1 Genetic Factors

2.4.1.1 Differences among Cultivars of Lettuce for Health-beneficial Compounds Concentrations

The content and profile of health-beneficial compounds such as flavonoids and phenolics vary considerably among cultivars, as well as the concentrations of these compounds among different lettuce types and genotypes. In the US, a study reported on the variation of phenolics in red and green lettuce. Red leaf lettuce had higher concentration of lipoxygenase (LPO) and cyclooxygenase -1 and -2 (COX-1 and -2) enzyme inhibitory activities than green leaf lettuce which lacks anthocyanins found in high concentration in red leaf types (Mulabagal et al., 2010). A similar research was done in France with six cultivars of lettuce to determine the variation of antioxidants. Results indicated that the red oak leaf cultivar had much higher concentrations of hydroxycinnamic acids, dicaffeoyl tartaric acid, chlorogenic acid and one form of quercetin than the green cultivars (Nicolle et al., 2004). Similar results was reported in Spain, with a large variation in antioxidant compounds, flavonols and polyphenols in varieties of lettuce. Anthocyanins could only be found red leaf cultivars, which also had a higher concentration of the main phenolics such as caffeic acid derivatives than

green leaf cultivars (Llorach et al., 2008). A study on the carotenoid concentration in 52 genotypes of lettuce including wild species, reported substantial variation in carotenoid concentration. Among cultivated lettuce cultivars, concentration of carotenoid followed the following order: green leaf or romaine > red leaf > butterhead > crisphead (Mou, 2005).

2.4.1.2 Importance of the GXE interaction in the determination of the concentrations of health-beneficial compounds in lettuce

Genetic, environmental and management factors affecting the concentration of health-beneficial compounds in lettuce. The genetics of plants has an important role in determining the concentration of health-beneficial compounds in plants but environmental factors as well as the interaction between these and genetic factors are important (Harborne, 1998). Significant genotype x environment interaction (G x E) was recently reported on antioxidant activity and chlorophyll content in lettuce (Hayashi et al., 2012). The G x E interaction has also been reported to play an important role in determining the concentration of health-beneficial compounds in other crops such as soybean and wheat. Flour, bran and grain composition of 27 cultivars of wheat grown at six locations significantly varied in mineral and protein concentrations (Peterson et al., 1986). Another project reported that the G x E interaction affected the most isoflavones concentration in soybean grown at different locations in Korea (Lee et al., 2003). Such results demonstrate that the performance

of cultivars in terms of concentration of health-beneficial compounds may vary depending on the growing conditions and environment. Consequently, environmental factors should also be considered as important factors affecting concentration of health-beneficial compounds in lettuce.

2.4.2 Environmental and Agronomic Factors

2.4.2.1 Temperature

It is often reported that temperature is one of the most important environmental factors affecting the concentration of nutritional compounds in lettuce, for example in terms of the accumulation of polyphenols (Tomás-Barberán and Espin, 2001). In Italy, a research project analyzed the concentrations of polyphenol compounds including caffeic acid derivatives, quercetin and kaempferol glycosides, in order to verify if the qualitative and quantitative phenol patterns can be affected by different temperatures. The result demonstrated that lettuce grown in open air with temperatures between 10°C and 20°C had higher concentration of flavonoids than lettuce grown in greenhouse with temperature between 15°C and 29°C (Romani et al., 2002).

Another study examined more closely the relationship between temperature and health-beneficial compounds in lettuce (Oh et al., 2009). They grew five-week-old lettuce plants in growth chambers with different temperatures. In one growth chamber plants were subjected to a 40°C heat shock for 10 minutes, the other one was subjected to 4°C chilling for 1 day. The result indicated that all stressed lettuce plants

had higher (up to two to three-fold) total phenolic content and antioxidant capacity compared to the plants grown in control. The concentrations of two major phenolic compounds in lettuce, chicoric acid and chlorogenic acid increased greatly after stress treatments. On the other hand, quercetin-3-O-glucoside and luteolin-7-O-glucoside were not significantly affected.

2.4.2.2 Soil Fertility and Salt Stress

While it is well documented that temperature may have an important effect of lettuce health-beneficial compounds concentration, the impact of soil fertility and fertilization has been less documented. In Argentina, a project investigated the impact of nine different soil fertilization treatments on lettuce health-beneficial compounds concentration (Shahidi and Naczki, 1995). Different types of composts were evaluated, including organic wastes compost, fruit and vegetable solid waste compost, cow manure compost, cattle slaughterhouse waste compost, and urban solid waste compost. Compared to growth in untreated soil, the yield of lettuce and the concentration of main pigments were increased by all treatments except the one with urban solid compost. It was concluded that treatments increased the nitrogen level in soil which in turn had positive effects on the yield and pigment contents of lettuce. However, concentration of antioxidants and phenolics was significantly decreased by all treatments compared to an untreated control (Coria-Cayupán et al., 2009).

Salt stress in irrigation water also may affect the concentration of nutritional compounds in lettuce. A study reported on lettuce response to salinity, by subjecting romaine lettuce to different concentrations of NaCl (Kim et al., 2008). After a long-term treatment with 5mM NaCl, the concentration of the two main carotenoids in romaine lettuce, lutein and β -carotene, increased by 37 and 80% respectively, when compared to an untreated control. In contrast the total phenolics concentration was not affected by a long term 5mM NaCl treatment, but was decreased by a short-term treatment. Similar research was also done with broccoli, and results demonstrated that glucosinolates and phenolic compounds were significantly increased under saline stress (López-Berenguer et al., 2009).

2.4.2.3 Light Duration and Quality

Sun irradiation, light intensity and quality also have marked effects on phenolics metabolism (Dussi et al., 1995). Application of UV-rays may affect the concentration of flavonoid and phenolic acid contents in red leaf lettuce (García-Macías et al., 2007). Increased level of UV radiation during cultivation increased the concentration of main flavonoids such as cyanidin, quercetin and luteolin, and phenolic acid such as caffeic acid. A similar study also determined the effect of light quality on the levels of health-related phytochemicals in lettuce, strawberry, raspberry, and blueberry fruits (Ordidge et al., 2010). The concentration of total phenolics, anthocyanin, luteolin and quercetin

were all increased by changing the UV blocking film from a low UV transparency to a high UV transparency one.

Another study reported that the concentration of some antioxidants varied in lettuce grown under different light conditions (Zhou et al., 2009). Lettuce plants were grown under low (LL), middle (ML) and high light (HL) treatments, and the relationship between photoacclimatory plasticity, light energy utilization, and antioxidant capacity were observed. Lettuce plants grown under high light treatment had higher concentration of carotenoids than the ones grown in middle light and low light conditions. These studies showed that light quality could affect concentration of health-beneficial compounds in lettuce.

2.4.3 Use of Elicitor Compounds

Elicitors compounds (i.e. abscisic acid, Methyl Jasmonate solutions) effect on lettuce health-beneficial compounds have also been investigated. A study looked at the effects of spraying 150 ppm and 300 ppm abscisic acid (ABA) to lettuce 30 and 39 days after sowing (Li et al., 2010). The yield of both green-leaf and red-leaf cultivars was reduced, but concentrations of total phenolic, total chlorophyll, and total carotenoid were increased compared to the water control. The response however differed qualitatively between lettuce types, as specific anthocyanins including cyaniding 3-glucoside, cyaniding 3-(3"-malonoyl)-glucoside and cyaniding 3-(6"-

malonoyl)-glucoside could not be observed in green-leaf lettuce after treatment with ABA, but were quantified in red-leaf lettuce.

The effect on lettuce of various Methyl Jasmonate (MeJA) solutions treatments was also investigated (Kim et al., 2007). The concentration of total phenolic acids like chlorogenic acid and caffeic acid, and antioxidant capacities in lettuce increased after being treated with MeJA .



Figure 2.1: Different types and species of lettuce. a) crisphead, b) romaine, c) green leaf, d) red leaf, e) butterhead, f) Batavia, g) latin, h) stem, i) buds of wild type lettuce *L. saligna*, j) wild type lettuce include *L. serriola*, *L. saligna* and *L. virosa*, only *L. saligna* shown in this figure.

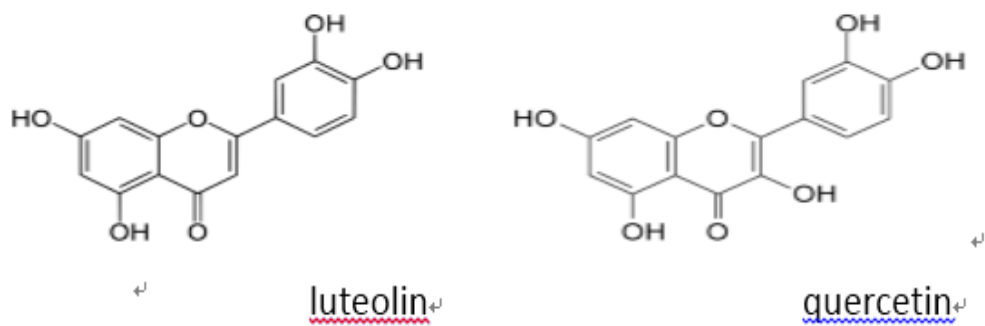


Figure 2.2: The structures of luteolin and quercetin; two key flavonoids in lettuce.

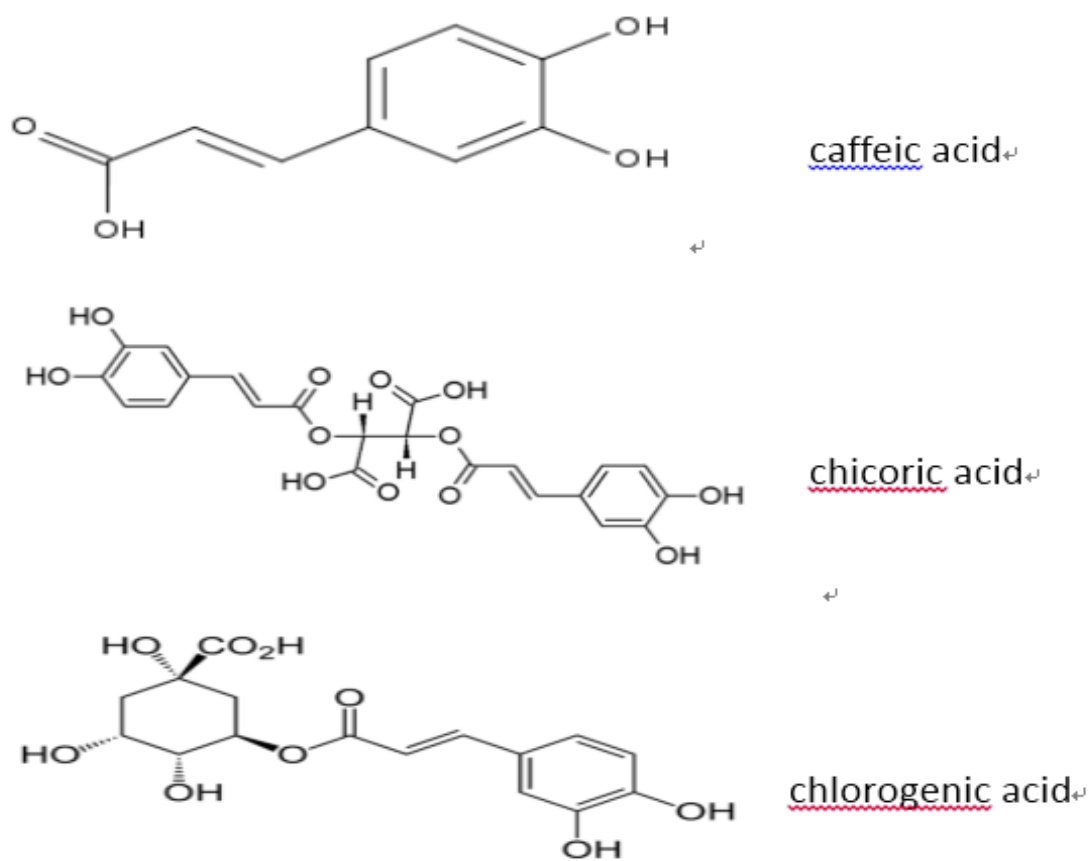


Figure 2.3: The structures of three key phenolic acids in lettuce: caffeic acid, chicoric acid and chlorogenic acid.

Chapter III Health-Beneficial Compounds in Different Lettuce Genotypes

3.1 Abstract

Lettuce contains several health-beneficial compounds including total flavonoids and total phenolics. Increasing their concentrations could lead to new utilizations of lettuce and to the development of new markets for lettuce producers in the long term. In this experiment, 38 genotypes of lettuces including crisphead, butterhead, romain, leaf lettuce, stem, Latin and wild species, were grown in greenhouses at two sites (Sainte-Anne-de-Bellevue and Saint-Jean-sur-Richelieu, QC). Concentrations of major flavonoids and phenolic compounds were determined at two growing stages as well as FRAP (Ferric-Reducing Antioxidant Power) values. Concentrations of all compounds studied significantly differed among lettuce genotypes, and the ranking of genotypes significantly varied when plants were harvested at the 10-leaf and at the maturity stages. Concentrations at the maturity sampling stage at the two sites were, however, highly correlated, thus selection at one site may be sufficient. Among the most commonly cultivated types, red leaf lettuces have the highest total flavonoids concentration at the maturity stage, followed by butterhead, and green leaf, while crisphead and batavia have the lowest concentrations. Concentration of total phenolics and FRAP values followed the trend as total flavonoids.

3.2 Introduction

Lettuce contains several health-beneficial compounds, including flavonoids and phenolic acids. The use of these health-beneficial compounds has been considered in the prevention and treatment of diseases such as cancers and cardiovascular diseases (Llorach et al., 2008). Increasing their concentrations could thus be desirable as it could lead to new utilizations of lettuce and to the development of new markets for lettuce producers in the long term.

There are several factors affecting the concentrations of total flavonoids, total phenolics, and antioxidant capacity in lettuce, including genetic and environmental factors. Previous studies have demonstrated that lettuce types and varieties vary significantly in their content of health-beneficial compounds. For example, the concentration of phenolic compounds has consistently been reported to be higher in red-leaf than green-leaf cultivars (Nicolle et al., 2004). In green-leaf types (iceberg, and romaine) the main phenolics are caffeic acid derivatives. Red-leaf types (red oak leaf and lollo rosso) have higher concentrations of caffeic acid derivatives, while anthocyanins can only be found in red-leaf types (Llorach et al., 2008). The red-leaf types had higher concentration of flavones and a higher antioxidant activity than green leaf cultivars (Llorach et al., 2008). Carotenoid concentrations was reported to vary significantly among in 52 genotypes of lettuce including wild species (Llorach et al., 2008). The concentrations of β -carotene and lutein (the two predominant types of

carotenoids) in wild species were higher than in cultivated lettuces. Among cultivated species, concentrations of carotenoid followed the following order: green leaf or romaine > red leaf > butterhead > crisphead. Carotenoid concentration was higher when lettuce was harvested in the summer than in the fall (Llorach et al., 2008).

Genotype (G) by environment (E) interactions (GxE) also could be a factor affecting concentrations of health-beneficial compounds in lettuce. A study reported on a significant genotype x environment interaction for antioxidant activity and chlorophyll content in lettuce (Hayashi et al., 2012). The importance of the GxE interaction had also been reported by previous researchers in other crops such as soybean and wheat (Lee et al., 2003; Peterson et al., 1986). Environmental factors such as temperature, soil fertility and salt stress, light duration and quality, and use of elicitors can also effect the concentrations of health-beneficial compounds in lettuce (Coria-Cayupán et al., 2009; Kim et al., 2008; Li et al., 2010; Oh et al., 2009; Zhou et al., 2009).

The objective of this study was to determine the concentrations of health-beneficial compounds in different genotypes and species of lettuce, and determine if these concentrations were affected by the environments and harvest stage.

3.3 Materials and Methods

3.3.1 Treatments and Growing Conditions

An experiment was replicated in two greenhouses, one on the Macdonald Campus of McGill University, Sainte-Anne-de-Bellevue, Quebec, Canada, the other at the

Agriculture and Agri-Food Canada Horticulture Research and Development Centre in Saint-Jean-sur-Richelieu, Quebec, Canada. A total of 38 different genotypes and species of lettuce were evaluated, including crisphead, green leaf, red leaf, romaine, butterhead, stem, Latin, Batavia and wild type (Table 3.1). Each genotype was replicated three times at each site.

All plants were initially seeded in a growth chamber; ten seeds of each genotype being sown in plastic transplanting trays (200 cells). The growth chamber was maintained at 18/16°C day/night temperatures with a 14h/10h day/night photoperiod. Plants were watered with tap water every day. Twenty-five days after seeding, when most plants were at the four to five leaves stage, nine plants (three plants for each replicate) of each lettuce genotype were transplanted into pots of two different sizes. Three plants for the ten-leaf sampling stage were transplanted into four-inch pots; the other three plants for the maturity sampling stage were transplanted in seven-inch pots, filled with commercial peat-based medium (Promix BX, Premier Horticulture, Rivière-du Loup, Quebec).

Plants were then grown in greenhouses at both sites with 18/16°C day/night temperatures and a 14h/10h day/night photoperiod until harvest. Plants were fertilized with a nutrient solution of 20-8-20 at a rate of 150 ppm of N three times a week and only with water on the other days.

Plants at each site were harvested at two sampling stages: the 'ten-leaf stage' and the 'maturity stage'. At the 'ten-leaf stage', all the cultivars including wild types and stem lettuce were harvested when the plants had ten leaves on average. At the 'maturity stage', cultivated types of lettuce were harvested when they had reached what was considered commercial size before starting to bolt, while wild species and the stem lettuce were harvested when the plants reached full size, before the first flower appeared. Two (out of three) better plants were harvested at each sampling stage. Base-leaves and wrapper-leaves were removed from crisphead and butterhead lettuce plants at harvest. At the 'ten-leaf stage', sampling dates of all the plants were the same, 18 days after transplantation. Sampling dates varied depending on the genotype for the 'maturity stage', ranging between 31 to 59 days after transplanting (Table 3.2). At the 'ten-leaf' sampling stage, the whole plant without the core but including leaves and ribs were sampled. At the 'maturity stage', six leaves from different parts of the plants were taken, two leaves of the outer part, two of the middle and two of the inside part, to take into consideration the possible variation in concentrations of variables quantified in different parts of the plant (Hohl et al., 2001). Leaves were grinded into small pieces in liquid nitrogen, freeze dried for forty-eight hours then grinded again into fine powder and stored at -80°C in the dark until use in laboratory analyses.

3.3.2 Laboratory Analyses

3.3.2.1 Extraction of Total Flavonoid, Total Phenolics and FRAP Assay

Total flavonoids, total phenolics and FRAP (Ferric-Reducing Antioxidant Power) assays were extracted and/or analyzed following the procedure of (Norris et al., 1995) with minor modifications. First, 0.1g of freeze-dried lettuce fine powdered sample was placed in 15 mL centrifugal tubes, then 2 mL of 95% methanol was added and the mixture was vortexed well. The samples were then sonicated in a cold room and in the dark for 30 min, centrifuged at 10,000g at 4°C for 10 min. The supernatant was collected in a new 15mL centrifugal tube. One mL of 95% methanol was added to the pellet and the extraction and centrifugation steps were repeated. The extract was stored at -20°C in the dark before analyses.

3.3.2.2 Determination of Total Flavonoid

The total flavonoids content was measured using a colorimetric method described by (Chang et al., 2002) with minor modifications. Plant extracts were diluted 10 times before analysis. Aliquots (0.5 mL) of diluted lettuce extract or standard solutions were pipette into 15 mL polypropylene conical tubes containing 2.8 mL of double-distilled water and mixed with 1.5 mL of 95% methanol, 0.1 mL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution and 0.1 mL of 1M CH_3COOK solution. The reaction solution was vortexed and kept at room temperature for 40 min. The absorbance was determined at 415 nm. The total flavonoids content was calculated using a standard curve (5-300 mg L^{-1} quercetin) and expressed as equivalent in μg of quercetin g^{-1} DW.

3.3.2.3 Determination of Total Phenolics

Total phenolics were determined using Folin-Ciocalteu assay (Kang and Saltveit, 2002). Two mL of distilled water and 200 μ L of 2N Folin-Ciocalteu-reagent were added to 100 μ L of extract; the mixture was then vortexed and incubated at room temperature for 30 minutes. One mL of 20% sodium bicarbonate solution was then added to the mixture, which was vortexed, and absorbance was measured at 765 nm after incubating the mixture at room temperature for 1 hour. The total phenolics content was calculated using a standard curve (5-1000 mg L⁻¹ gallic acid) and expressed as equivalent in μ g of gallic acid g⁻¹ DW.

3.3.2.4 FRAP (Ferric-Reducing Antioxidant Power) Assay

The FRAP assay was conducted followed the procedure of (Nilsson et al., 2005) with minor modifications. The principle of the FRAP assay is that the yellow ferric tripyridyl triazine (Fe^{3+} -TPTZ) complex is reduced to the blue Fe^{2+} TPTZ complex by electron donating substances under acidic conditions. The FRAP reagent was made from a mixture of 0.1 mol L⁻¹ acetate buffer (pH 3.6)/10 mM TPTZ solution/2 mM FeCl_3 solution/distilled water (50:5:5:6 v:v:v:v); the mixture was then incubated at 37°C for 10 min before use. An aliquot of 10 μ L of diluted lettuce extract was added to 30 μ L of distilled water and 200 μ L of FRAP reagent into a micro plate; mixed 10 s and incubated at room temperature for 8 min before the absorbance reading (593 nm).

Calibration was made against a standard curve using freshly prepared FeSO₄ (0.1-1 nM).

3.3.3 High-Performance Liquid Chromatography (HPLC) Analysis

Caffeic acid and chicoric acid were extracted and analyzed by HPLC, using the procedure of Ferreres et al. (1997) with minor modifications. First, 0.1g of freeze-dried lettuce fine powdered plant material was placed in 15mL centrifugal tubes. Three mL of mixture of methanol/water/formic acid (25:24:3 v:v:v) was added to the tube and vortexed well and centrifuged twice at 10,000 g.

Separation of caffeic acid and chicoric acid were carried out using a Varian system (Walnut Creek, CA, USA) equipped with a Prostar 210 solvent delivery system, a model 410 autosampler and a Prostar 330 photodiode array detector (PDA). One mL of the extract were used for analyses. The mobile phases were water with 5% formic acid (A) and methanol (B) with a solvent flow rate of 1 ml min⁻¹, in a gradient program starting with 5% B in A, reaching 40% B at 25 min, and then remaining isocratic for 5 min. The UV chromatograms were recorded at 520 nm. The identification of phenolic compounds was based on their UV spectra and retention times. Calibration curves were prepared using caffeic acid (Sigma, Saint Louis, MO, USA) and chicoric acid (Sigma, Saint Louis, MO, USA) as standards. Concentrations of all the compounds detected were expressed on a dry matter (DM) basis.

3.3.4 Data Analyses

Treatments (i.e., genotypes) were replicated three times and assigned to a randomized complete block design. All data were subjected to an analysis of variance (ANOVA) using the general linear model (GLM) procedure in SAS (version 9.2) to identify significant treatment effects. Comparisons between means were made using LSDs at a 0.05 probability level when ANOVA indicated model and treatment significances. Only significant effects ($P < 0.05$) were discussed.

In order to show the relationship between traits and dissect the total variation into G (genotype), E (environment: site-stage combinations), and GxE interaction, different biplots were generated by the GGE biplot software (Yan, 2001). A biplot of the Average Environment Coordination (AEC) was drawn by the GGE biplot software to show the mean versus stability of the genotypes in order to compare the mean performance and stability among genotypes.

3.4 Results

3.4.1 Relationship among Tested Environments

3.4.1.1 Total Flavonoids

The biplot explained 89.7% of the variation in total flavonoid concentrations. All the environments were correlated ($P < 0.05$) (Figure 3.1, Table 3.7), particularly A2 (Ste. Anne-de-Bellevue plants harvested at the maturity stage) and J2 (Ste. Jean-Sur-Richelieu plants harvested at the maturity stage) for which the r value was 0.877 indicating that results at the maturity stage were very stable across sites.

3.4.1.2 Total Phenolics

The biplot explained 87.5% of the variation in total phenolic concentrations. Results from the two sites for plants harvest at the ten-leaf stage (i.e., J1 and A1) were not significantly correlated, however, those for plants harvested at the maturity stage were highly correlated ($r=0.803$; $P<0.01$) (Figure 3.2, Table 3.8); demonstrating that results for plants harvested at that stage were very stable across sites.

3.4.1.3 FRAP (Ferric-Reducing Antioxidant Power)

The biplot explained 82.7% of the variation of FRAP values. All environments were correlated, particularly A1, A2 and J2 (Figure 3.3, Table 3.9). Again, as for total flavonoids and phenolics concentrations, FRAP results were highly correlated ($r=0.817$; $P<0.01$) for plants harvested at the maturity stage at both sites (i.e., A2 and J2) demonstrating the stability of results across sites.

3.4.2 Ranking of the Genotypes

3.4.2.1 Total Flavonoids

The total flavonoid concentration in lettuce genotypes harvested at the ten-leaf stage averaged $11057 \mu\text{g g}^{-1}$ DM over all genotypes and ranged from 6725 to $15173 \mu\text{g g}^{-1}$ DM at Saint-Jean-sur-Richelieu; averaged $12604 \mu\text{g g}^{-1}$ DM and ranged from 9139 to $17220 \mu\text{g g}^{-1}$ DM at Sainte-Anne-de-Bellevue (Table 3.2). At the maturity stage, concentrations averaged $10768 \mu\text{g g}^{-1}$ DM, ranged from 5811 to $20350 \mu\text{g g}^{-1}$ DM at Saint-Jean-sur-Richelieu; and averaged $8943 \mu\text{g g}^{-1}$ DM, ranged from 3282 to 21114

$\mu\text{g g}^{-1}$ DM at Sainte-Anne-de-Bellevue. There was significant genetic variation ($P < 0.001$) for total flavonoids concentration among the lettuce genotypes evaluation (Table 3.2).

Figure 3.4 illustrate the ranking of genotypes along the average environment coordination (red axis) for total flavonoid. The closer the projection of the genotype on the red axis, the more stable the genotype is across environments. For example if a genotype is toward a particular environment (e.g., A1, J1, A2, or J2), this indicated a better performance in that environment. For example, in Figure 3.4 the performance of W25 was superior in environments A2 and J2, compared to A1 and J1. The position on the red axis shows the average value of each genotype; genotypes with concentrations greater than the average are located to the right of the blue axis and those with values below the average to its left. Wild type *L. saligna* [e.g., PI 509525 (code: W25) and PI 490999 (code: W99)] had the highest concentrations of total flavonoids among all the genotypes of lettuce evaluated. Among of the cultivated lettuce types, red leaf lettuce - Ruby Sky (code: Res), green leaf lettuce - Royal Oak Leaf (code: Gro) and type latin/red sucrine lettuce - Kendo (code: Lrk) had higher concentration of total flavonoid content than other types, followed by Boston type genotypes which had above average values. Three genotypes of type Batavia, Sierra (code: Bas), Nevada (code: Ban), La Brillante (code: Bal) had the lowest value of total flavonoids among all types of lettuce.

3.4.2.2 Total Phenolics

The total phenolic concentrations at the ten-leaf stage averaged 11257 $\mu\text{g g}^{-1}$ DM and ranged from 9980 to 13405 $\mu\text{g g}^{-1}$ DM at Saint-Jean-sur-Richelieu and averaged 16683 $\mu\text{g g}^{-1}$ DM, ranged from 11839 to 24098 $\mu\text{g g}^{-1}$ DM at Sainte-Anne-de-Bellevue (Table 3.3). The concentration of total phenolics at the maturity stage averaged 16386 $\mu\text{g g}^{-1}$ DM and ranged between 10352 and 16386 $\mu\text{g g}^{-1}$ DM at Saint-Jean-sur-Richelieu; and averaged 15911 $\mu\text{g g}^{-1}$ DM, ranged between 10045 and 32142 $\mu\text{g g}^{-1}$ DM at Sainte-Anne-de-Bellevue. There was significant genetic variation ($P < 0.001$) for total flavonoids concentration among the lettuce genotypes evaluation (Table 3.3).

The ranking of genotypes in terms of total phenolic concentrations along the average environment coordination is illustrated in Figure 3.5. Wild type *L. saligna* [e.g., PI 509525 (code: W25) and PI 490999 (code: W99)] had the highest value of total phenolics among all the genotypes of lettuce evaluated. Among the major types of cultivated lettuce, red leaf type lettuce - Ruby Sky (code: Res), Lolla Rossa 'Sanguine' (code: Rel) and New Red Fire (code: Ren) had the highest concentration of total phenolics, while the crisphead types great lakes-lthaca (code: Cit) and QSJ-09 (Champlain) (code: Cqs) and crisphead type vanguard-Eldorado (code: Cel) had the lowest concentrations.

3.4.2.3 FRAP (Ferric-Reducing Antioxidant Power)

FRAP values in lettuce at the ten-leaf stage averaged $47.5 \mu\text{M g}^{-1}$ DM and ranged from 25.4 to $70.0 \mu\text{M g}^{-1}$ DM in Saint-Jean-sur-Richelieu, and averaged $105.5 \mu\text{M g}^{-1}$ DM from 54.9 to $186.70 \mu\text{M g}^{-1}$ DM in Sainte-Anne-de-Bellevue (Table 3.4). At the maturity stage values averaged $97.0 \mu\text{M g}^{-1}$ DM across all 38 genotypes and ranged from 39.2 to $268.1 \mu\text{M g}^{-1}$ DM in Saint-Jean-sur-Richelieu, and averaged $89.9 \mu\text{M g}^{-1}$ DM, from 24.5 to $248.1 \mu\text{M g}^{-1}$ DM in Sainte-Anne-de-Bellevue. There was significant genetic variation for FRAP values among the lettuce genotypes evaluation (Table 3.4).

The ranking of genotypes along the average environment coordination for FRAP values is illustrated in Figure 3.6. Wild types - *L. saligna* [e.g. PI 509525 (code: W25)] and *L. virosa* [e.g. PI 273597 (code: W97)] had the highest FRAP value among all genotypes evaluated, while red leaf type lettuce - Ruby Sky (code: Res), green leaf lettuce - Royal Oak Leaf (code: Gro) and butterhead type - Rhapsody (code: Bur) were the top three among cultivated types. The Batavia type - Nevada (code: Ban), green leaf type - Lollo Bionda (code: Glb) and Crisphead Empire - Emperor (code: Cem) had the lowest FRAP values.

3.4.2.4 Caffeic Acid

The concentration of caffeic acid at the maturity stage averaged $52.5 \mu\text{g g}^{-1}$ DM across the 38 genotypes evaluated, ranging from 12.9 to $293.0 \mu\text{g g}^{-1}$ DM at Saint-Jean-sur-Richelieu, and averaged $50.8 \mu\text{g g}^{-1}$ DM, ranged from 10.2 to $239.0 \mu\text{g g}^{-1}$ DM at Sainte-Anne-de-Bellevue (Table 3.5). Wild type lettuces overall had significantly

higher concentration of caffeic acid than other types of lettuce at both sites (averaging 123.1 $\mu\text{g g}^{-1}$ DM and 112.0 $\mu\text{g g}^{-1}$ DM respectively, Table 3.5). Three genotypes of wild type lettuce, namely two *L. virosa*, PI 273597 (code: W97), PI 274375 (code: W75) and one *L. serriola*, PI 491181 (code: W81) had by far the highest concentration of caffeic acid among all tested genotypes. Among major types of cultivated lettuce, stem lettuce Da Ye Wo Sun (code: Sdy) had highest concentration of caffeic acid in both site (averaging 70.9 $\mu\text{g g}^{-1}$ DM and 113.3 $\mu\text{g g}^{-1}$ DM respectively); most common types of lettuce used in North America had below average caffeic acid concentrations (Figure 3.7).

3.4.2.5 Chicoric Acid

Concentrations of chicoric acid at the maturity stage averaged 5211 $\mu\text{g g}^{-1}$ DM across the 38 genotypes evaluated and ranged from 1037 to 31001 $\mu\text{g g}^{-1}$ DM in Saint-Jean-sur-Richelieu; and averaged 4759 $\mu\text{g g}^{-1}$ DM, ranged from 619 to 14362 $\mu\text{g g}^{-1}$ DM in Sainte-Anne-de-Bellevue (Table 3.6). Again, highest concentrations were observed in two wild type *L. virosa* genotypes PI 273597 (code: W97) and PI 274375 (code: W75) (Figure 3.8). Among major types of cultivated lettuce, three cultivars of red leaf type had the highest concentrations of chicoric acid, they were Ruby Sky (code: Res), New Red Fire (code: Ren) and Lolla Rossa ‘Sanguine’ (code: Rel) (Figure 3.8).

3.5 Discussion and Conclusion

The current study confirms results from previous studies on concentration of health-beneficial compounds in a range of lettuce genotypes, reporting significant variation of specific compounds. Total flavonoid and total phenolics concentrations, and antioxidant capacity values differed among the 38 lettuce genotypes evaluated herein.

Concentrations of total flavonoids, total phenolics, and FRAP differed significantly among lettuce genotypes evaluated. The ranking of lettuce genotypes in terms of concentrations of total flavonoids, total phenolics, and FRAP were not stable at the two sampling stages investigated; suggesting that selection of genotypes with high concentrations must be done at the optimal (i.e., maturity) stage. Harvest at an earlier stage to speed-up the selection process would not be desirable as the performance of specific cultivars is not stable as plants develop and mature. Results obtained for all variables at the two sites were, however, highly correlated (Figures 3.1, 3.2, and 3.3) and thus selection at one site may be sufficient, although this would need to be validated by testing in a larger number of environments. It should also be noted that our plants were grown in greenhouses with environment control, thus this observation should be also validated for field grown plants.

One-, six- and seven-fold differences were observed between lettuce types for total phenolics, total flavonoids and FRAP, respectively. Among the most commonly cultivated types, red leaf lettuces have the highest total flavonoids concentration at the maturity stage, followed by butterhead, and green leaf, while crisphead and

batavia had the lowest concentrations. Total phenolics concentration and FRAP value generally followed the same rank order as total flavonoids among different types of lettuce. Concentrations of caffeic acid and chicoric acid also significantly differed among lettuce types and genotypes. Red leaf has the highest concentration of chicoric acid among major cultivated lettuce types, followed by butterhead and green leaf, while crisphead has the lowest concentration. Overall, the concentration of caffeic acid is not as high as that of chicoric acid. Stem lettuce has the highest concentration of caffeic acid while crisphead got the lowest.

Overall wild lettuce species and genotypes of cultivated red-leaf varieties had consistently the highest concentrations of all health-beneficial compounds evaluated. Previous studies have also reported that crisphead and butter leaf lettuces contained low concentrations of important health-beneficial compounds including flavonoids and phenolics, while high concentrations have previously been reported in dark green and red-leaf types (Baslam et al., 2013; DuPont et al., 2000; Tomás-Barberán and Espin, 2001). The higher concentrations in flavonoid and phenolic compounds of certain lettuce types were often associated with a concurrent higher antioxidant capacity as determined by the FRAP assay; with again wild species and red-leaf types often having the highest antioxidant capacity among genotypes evaluated. An association between total phenolics concentration and antioxidant capacity was also previously reported among red leaf, green leaf, romaine, crisphead and butterhead lettuce (Liu

et al., 2007). The high antioxidant capacity of red-leaf lettuce might be due to the presence of anthocyanins, one of the main phenols in leaves of red-leaf lettuce (Caldwell, 2003; DuPont et al., 2000).

Considerable variation is present for all traits investigated; selection of lettuce with high concentrations of health-beneficial compounds at the maturity stage should thus be possible.

Table 3.1: List of 38 lettuce genotypes evaluated

No.	Genus &Species	Cultivar	Type	Code	No.	Genus &Species	Cultivar	Type	Code
1	<i>L. sativa</i>	Estival	C	Ces	20	<i>L. sativa</i>	Ruby	RL	Rer
2	<i>L. sativa</i>	Salinas	C	Csa	21	<i>L. sativa</i>	New Red Fire	RL	Ren
3	<i>L. sativa</i>	Eldorado	C	Cel	22	<i>L. sativa</i>	Buttercrunch	BH	Bub
4	<i>L. sativa</i>	Ithaca	C	Cit	23	<i>L. sativa</i>	Rhapsody	BH	Bur
5	<i>L. sativa</i>	Great Lakes 659	C	Cgl	24	<i>L. sativa</i>	Caliente	BH	Buc
6	<i>L. sativa</i>	Hochelaga	C	Cho	25	<i>L. sativa</i>	Batavia reine des Glaces	B	Bab
7	<i>L. sativa</i>	QJ-09 (Champlain)	C	Cqs	26	<i>L. sativa</i>	La Brillante	B	Bal
8	<i>L. sativa</i>	Emperor	C	Cem	27	<i>L. sativa</i>	Nevada	B	Ban
9	<i>L. sativa</i>	Green Tower	R	Rog	28	<i>L. sativa</i>	Sierra	B	Bas
10	<i>L. sativa</i>	Parris Island Cos	R	Rop	29	<i>L. sativa</i>	Little Gem	L	Llg
11	<i>L. sativa</i>	Romora	R	Ror	30	<i>L. sativa</i>	Amadeus	L	Lam
12	<i>L. sativa</i>	Tall Guzmane	R	Rot	31	<i>L. sativa</i>	Kendo	LR	Lrk
13	<i>L. sativa</i>	Salad Bowl	GL	Gsb	32	<i>L. sativa</i>	Da Ye Wo Sun	S	Sdy
14	<i>L. sativa</i>	Grand Rapids	GL	Ggr	33	<i>L. serriola</i>	PI 491181	W	W81
15	<i>L. sativa</i>	Tropicana	GL	Gtr	34	<i>L. serriola</i>	PI 491239	W	W39
16	<i>L. sativa</i>	Lollo Bionda	GL	Glb	35	<i>L. saligna</i>	PI 490999	W	W99
17	<i>L. sativa</i>	Royal Oakleaf	GL	Gro	36	<i>L. saligna</i>	PI 509525	W	W25
18	<i>L. sativa</i>	Lolla 'Sanguine' Rossa	RL	Rel	37	<i>L. virosa</i>	PI 273597	W	W97
19	<i>L. sativa</i>	Ruby Sky	RL	Res	38	<i>L. virosa</i>	PI 274375	W	W75

C crisphead, R romain, GL green leaf, RL red leaf, BH butterhead, B batavia, L latin, LR latin/red sucrine, S stem, W wild

Table 3.2: Total flavonoid concentration of 38 lettuce genotypes harvested at two stages ('ten-leaf stage' and 'maturity' stage) and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).

Genotype	Type	Total Flavonoids ($\mu\text{g g}^{-1}$ DM)			
		Ten-Leaf		Marketable	
		St-Jean	Ste-Anne	St-Jean	Ste-Anne
Estival	Crisphead	10768	9982	6662	3767
Salinas	Crisphead	11232	11889	7568	3559
Eldorado	Crisphead	10473	11618	9785	4081
Ithaca	Crisphead	10316	10782	7509	4007
Great Lakes 659	Crisphead	13166	12083	8096	4462
Hochelaga	Crisphead	11131	10960	6935	3580
Q SJ-09 (Champlain)	Crisphead	11101	10537	5811	3282
Emperor	Crisphead	11139	9976	6981	3362
Green Tower	Romaine	10693	13978	9588	8277
Parris Island Cos	Romaine	11007	11739	10922	7863
Romora	Romaine	7940	10875	8539	5292
Tall Guzmanine	Romaine	10714	14664	10310	8952
Salad Bowl	Green leaf	9005	12607	13442	11610
Grand Rapids	Green leaf	6839	11309	8798	8080
Tropicana	Green leaf	11908	11499	12842	9909
Lollo Bionda	Green leaf	7451	11047	8210	6624
Royal Oakleaf	Green leaf	13808	16882	13050	11177
Lolla Rossa 'Sanguine'	Red leaf	8633	12910	12946	10879
Ruby Sky	Red leaf	12256	17220	16812	13009
Ruby	Red leaf	8209	11073	11125	10914
New Red Fire	Red leaf	8668	12412	13188	11982
Buttercrunch	Butterhead	13655	12530	9612	9538
Rhapsody	Butterhead	13746	14489	10123	11618

Table 3.2 continued

Genotype	Type	Total Flavonoids ($\mu\text{g g}^{-1}$ DM)			
		Ten-Leaf		Maturity	
		St-Jean	Ste-Anne	St-Jean	Ste-Anne
Caliente	Butterhead	13590	13781	11144	11995
Batavia reine des Glaces	Batavia	10769	12681	6210	3487
La Brillante	Batavia	8286	9813	8287	5928
Nevada	Batavia	6725	10500	7045	5413
Sierra	Batavia	7612	9139	7983	5663
Little Gem	Latin	8484	12612	8382	7314
Amadeus	Latin	10722	12872	8368	7275
Kendo	Latin/red sucrine	13184	15836	14443	8374
Da Ye Wo Sun	Stem	10868	11564	10118	8399
PI 491181	<i>L. serriola</i>	14982	15609	14961	16389
PI 491239	<i>L. serriola</i>	14862	13846	10797	16849
PI 490999	<i>L. saligna</i>	15173	14648	17703	18882
PI 509525	<i>L. saligna</i>	13377	13926	20350	21114
PI 273597	<i>L. virosa</i>	13092	15144	18950	15296
PI 274375	<i>L. virosa</i>	14590	13908	15584	11631
Significance ^y		***	***	***	***
Mean		11057	12604	10768	8943
Maximum		15173	17220	20350	21114
Minimum		6725	9139	5811	3282
LSD _{0.05} ^z		1851	2743	2802	2636

^z Least significant differences at $P < 0.05$

^y *** indicate significance at $P < 0.001$

Table 3.3: Total phenolics concentration of 38 lettuce genotypes harvested at two stages ('ten-leaf stage' and 'maturity' stage) and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).

Genotype	Type	Total Phenolics ($\mu\text{g g}^{-1}$ DM)			
		Ten-Leaf		Maturity	
		St-Jean	Ste-Anne	St-Jean	Ste-Anne
Estival	Crisphead	10765	13901	11868	10722
Salinas	Crisphead	11081	15370	11568	10674
Eldorado	Crisphead	10510	15037	12335	10938
Ithaca	Crisphead	10279	11839	12160	10373
Great Lakes 659	Crisphead	13145	12938	12405	10652
Hochelaga	Crisphead	11385	13864	12419	10351
Q SJ-09 (Champlain)	Crisphead	11578	12953	10352	10045
Emperor	Crisphead	10734	14327	12631	10235
Green Tower	Romaine	10284	18107	15002	13402
Parris Island Cos	Romaine	10924	16807	15577	13382
Romora	Romaine	10276	17352	13044	11135
Tall Guzmaine	Romaine	10708	18051	15670	15032
Salad Bowl	Green leaf	10833	19249	20336	19602
Grand Rapids	Green leaf	9980	18209	16320	14559
Tropicana	Green leaf	11053	15330	17439	16399
Lollo Bionda	Green leaf	11030	15244	13274	14086
Royal Oakleaf	Green leaf	11211	21465	16522	18775
Lolla Rossa 'Sanguine'	Red leaf	11603	18389	22497	22030
Ruby Sky	Red leaf	11375	24098	26533	20938
Ruby	Red leaf	11806	17365	14509	20516
New Red Fire	Red leaf	11231	17410	21680	21502
Buttercrunch	Butterhead	11966	16922	13717	14321
Rhapsody	Butterhead	11296	21572	15548	18406

Table 3.3 continued

Genotype	Type	Total Phenolics ($\mu\text{g g}^{-1}$ DM)			
		Ten-Leaf		Maturity	
		St-Jean	Ste-Anne	St-Jean	Ste-Anne
Caliente	Butterhead	10878	21308	15311	20291
Batavia reine des Glaces	Batavia	11746	14293	12683	10879
La Brillante	Batavia	10648	13513	13916	11892
Nevada	Batavia	10689	14993	12892	12208
Sierra	Batavia	11638	13003	14581	12150
Little Gem	Latin	10831	17263	12100	14039
Amadeus	Latin	11000	15995	13642	13140
Kendo	Latin/red sucrine	11101	17183	17954	15531
Da Ye Wo Sun	Stem	11108	17116	13833	15929
PI 491181	<i>L. serriola</i>	11567	18504	17963	20503
PI 491239	<i>L. serriola</i>	11099	14191	12596	20406
PI 490999	<i>L. saligna</i>	13150	19314	20240	28378
PI 509525	<i>L. saligna</i>	13405	19463	37049	32142
PI 273597	<i>L. virosa</i>	11116	17807	30962	24319
PI 274375	<i>L. virosa</i>	12728	14221	23548	14725
Significance ^y		***	***	***	***
Mean		11257	16683	16386	15911
Maximum		13405	24098	37049	32142
Minimum		9980	11839	10352	10045
LSD _{0.05} ^z		1390	5397	4786	4737

^z Least significant differences at $P < 0.05$

^y *** indicate significance at $P < 0.001$

Table 3.4: FRAP values of 38 lettuce genotypes harvested at two stages ('ten-leaf stage' and 'maturity' stage) and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).

Genotype	Type	FRAP ($\mu\text{M g}^{-1} \text{DM}$)			
		Ten-Leaf Stage		Marketable Stage	
		St-Jean	Ste-Anne	St-Jean	Ste-Anne
Estival	Crisphead	54.7	77.8	41.1	29.2
Salinas	Crisphead	52.1	94.1	58.7	46.6
Eldorado	Crisphead	41.5	86.2	54.0	30.4
Ithaca	Crisphead	37.1	65.4	50.0	38.4
Great Lakes 659	Crisphead	65.9	81.2	51.1	40.4
Hochelaga	Crisphead	44.1	77.0	55.6	26.3
Q SJ-09 (Champlain)	Crisphead	43.0	72.2	39.2	31.9
Emperor	Crisphead	37.2	78.7	39.4	26.5
Green Tower	Romaine	44.1	125.7	110.1	77.5
Parris Island Cos	Romaine	47.9	107.8	82.0	69.1
Romora	Romaine	37.7	107.6	50.4	40.9
Tall Guzmaine	Romaine	50.0	133.7	79.0	88.9
Salad Bowl	Green leaf	41.2	129.3	126.9	139.2
Grand Rapids	Green leaf	29.1	111.2	90.2	83.4
Tropicana	Green leaf	50.2	91.1	99.9	96.7
Lollo Bionda	Green leaf	27.2	78.9	45.8	62.3
Royal Oakleaf	Green leaf	61.7	163.7	122.8	124.0
Lolla Rossa 'Sanguine'	Red leaf	42.6	116.0	164.2	157.5
Ruby Sky	Red leaf	51.6	186.7	177.1	144.7
Ruby	Red leaf	37.9	96.6	114.3	140.4
New Red Fire	Red leaf	38.9	114.4	152.3	155.9
Buttercrunch	Butterhead	60.1	113.4	65.7	70.5
Rhapsody	Butterhead	66.1	147.5	100.8	112.1

Table 3.4 continued

Genotype	Type	FRAP ($\mu\text{M g}^{-1}\text{ DM}$)			
		Ten-Leaf Stage		Marketable Stage	
		St-Jean	Ste-Anne	St-Jean	Ste-Anne
Caliente	Butterhead	52.6	156.9	92.0	132.3
Batavia reine des Glaces	Batavia	44.1	88.5	54.1	24.5
La Brillante	Batavia	35.4	70.0	51.1	44.5
Nevada	Batavia	25.4	81.7	65.2	50.3
Sierra	Batavia	36.6	54.9	69.3	54.6
Little Gem	Latin	43.3	105.4	48.0	62.4
Amadeus	Latin	46.7	109.0	60.0	61.3
Kendo	Latin/red sucrine	53.3	117.7	141.8	81.8
Da Ye Wo Sun	Stem	47.1	112.1	80.9	97.2
PI 491181	<i>L. serriola</i>	58.2	124.7	158.8	130.3
PI 491239	<i>L. serriola</i>	70.0	86.9	67.8	149.2
PI 490999	<i>L. saligna</i>	56.3	131.8	151.9	196.0
PI 509525	<i>L. saligna</i>	60.6	119.5	230.3	248.1
PI 273597	<i>L. virosa</i>	55.1	135.5	268.1	156.7
PI 274375	<i>L. virosa</i>	58.7	59.0	174.7	88.9
Significance ^y		***	***	***	***
Mean		47.5	105.5	97.0	89.8
Maximum		70.0	186.7	268.1	248.1
Minimum		25.4	54.9	39.2	24.5
LSD _{0.05} ^z		13.7	55.7	73.2	49.1

^z Least significant differences at $P < 0.05$

^y *** indicate significance at $P < 0.001$

Table 3.5: Caffeic acid concentration of 38 lettuce genotypes harvested at the 'maturity' stage and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).

Genotype	Type	Caffeic Acid	
		$\mu\text{g g}^{-1}$ DM	
		Maturity	
		St-Jean	Ste-Anne
Estival	Crisphead	34.8	14.8
Salinas	Crisphead	12.9	10.2
Eldorado	Crisphead	19.8	33.5
Ithaca	Crisphead	14.2	25.5
Great Lakes 659	Crisphead	41.3	22.0
Hochelaga	Crisphead	25.7	18.0
Q SJ-09 (Champlain)	Crisphead	26.4	28.2
Emperor	Crisphead	17.4	12.6
Green Tower	Romaine	72.1	48.8
Parris Island Cos	Romaine	66.4	35.7
Romora	Romaine	19.7	17.9
Tall Guzmaine	Romaine	62.1	53.7
Salad Bowl	Green leaf	54.5	50.8
Grand Rapids	Green leaf	36.3	58.6
Tropicana	Green leaf	87.4	62.7
Lollo Bionda	Green leaf	28.4	33.7
Royal Oakleaf	Green leaf	32.2	48.0
Lolla Rossa 'Sanguine'	Red leaf	35.2	65.4
Ruby Sky	Red leaf	64.5	37.1
Ruby	Red leaf	46.1	44.7
New Red Fire	Red leaf	48.4	47.1
Buttercrunch	Butterhead	22.5	24.4

Table 3.5 continued

Genotype	Type	Caffeic Acid	
		$\mu\text{g g}^{-1}$ DM	
		Maturity	
		St-Jean	Ste-Anne
Rhapsody	Butterhead	36.9	43.8
Caliente	Butterhead	29.4	51.1
Batavia reine des Glaces	Batavia	35.9	19.1
La Brillante	Batavia	19.6	13.1
Nevada	Batavia	18.7	37.4
Sierra	Batavia	52.3	42.4
Little Gem	Latin	41.2	39.6
Amadeus	Latin	29.6	41.7
Kendo	Latin/red sucrose	54.6	62.2
Da Ye Wo Sun	Stem	70.9	113.3
PI 491181	<i>L. serriola</i>	130.1	155.7
PI 491239	<i>L. serriola</i>	30.5	136.2
PI 490999	<i>L. saligna</i>	36.8	30.3
PI 509525	<i>L. saligna</i>	23.9	17.4
PI 273597	<i>L. virosa</i>	293.0	239.0
PI 274375	<i>L. virosa</i>	224.1	93.6
Significance ^y		***	***
Mean		52.5	50.8
Max		293.0	239.0
Min		12.9	10.2
LSD _{0.05} ^z		43.7	39.2

^z Least significant differences at $P < 0.05$

^y *** indicate significance at $P < 0.001$

Table 3.6: Chicoric acid concentration of 38 lettuce genotypes harvested at the 'maturity' stage and grown at two sites (St-Jean-sur-Richelieu and Ste-Anne-de-Bellevue).

Genotype	Type	Chicoric Acid	
		$\mu\text{g g}^{-1}$ DM	
		Maturity stage	
		St-Jean	Ste-Anne
Estival	Crisphead	1993	1218
Salinas	Crisphead	1469	928
Eldorado	Crisphead	1832	1298
Ithaca	Crisphead	1737	1627
Great Lakes 659	Crisphead	2538	1685
Hochelaga	Crisphead	1734	992
Q SJ-09 (Champlain)	Crisphead	1037	1572
Emperor	Crisphead	1550	1194
Green Tower	Romaine	4986	3977
Parris Island Cos	Romaine	4847	3480
Romora	Romaine	2762	1634
Tall Guzmanine	Romaine	5385	4866
Salad Bowl	Green leaf	8106	7849
Grand Rapids	Green leaf	5371	6606
Tropicana	Green leaf	6116	5693
Lollo Bionda	Green leaf	2171	4764
Royal Oakleaf	Green leaf	5435	8149
Lolla Rossa 'Sanguine'	Red leaf	8792	11786
Ruby Sky	Red leaf	11491	6127
Ruby	Red leaf	6266	8858
New Red Fire	Red leaf	9893	9371
Buttercrunch	Butterhead	3062	3369

Table 3.6 continued

Genotype	Type	Chicoric Acid	
		$\mu\text{g g}^{-1}$ DM	
		Maturity stage	
		St-Jean	Ste-Anne
Rhapsody	Butterhead	7032	7909
Caliente	Butterhead	5837	9444
Batavia reine des Glaces	Batavia	1636	1443
La Brillante	Batavia	2046	2119
Nevada	Batavia	3576	2541
Sierra	Batavia	2900	2900
Little Gem	Latin	1213	3741
Amadeus	Latin	3531	2929
Kendo	Latin/red sucrine	8391	5959
Da Ye Wo Sun	Stem	2421	5426
PI 491181	<i>L. serriola</i>	6375	7937
PI 491239	<i>L. serriola</i>	1152	10230
PI 490999	<i>L. saligna</i>	2066	1793
PI 509525	<i>L. saligna</i>	1925	619
PI 273597	<i>L. virosa</i>	31001	14362
PI 274375	<i>L. virosa</i>	18341	4453
Significance ^y		***	***
Mean		5211	4759
Maximum		31001	14362
Minimum		1037	619
LSD _{0.05} ^z		3979	3696

^z Least significant differences at $P < 0.05$

^y *** indicate significance at $P < 0.001$

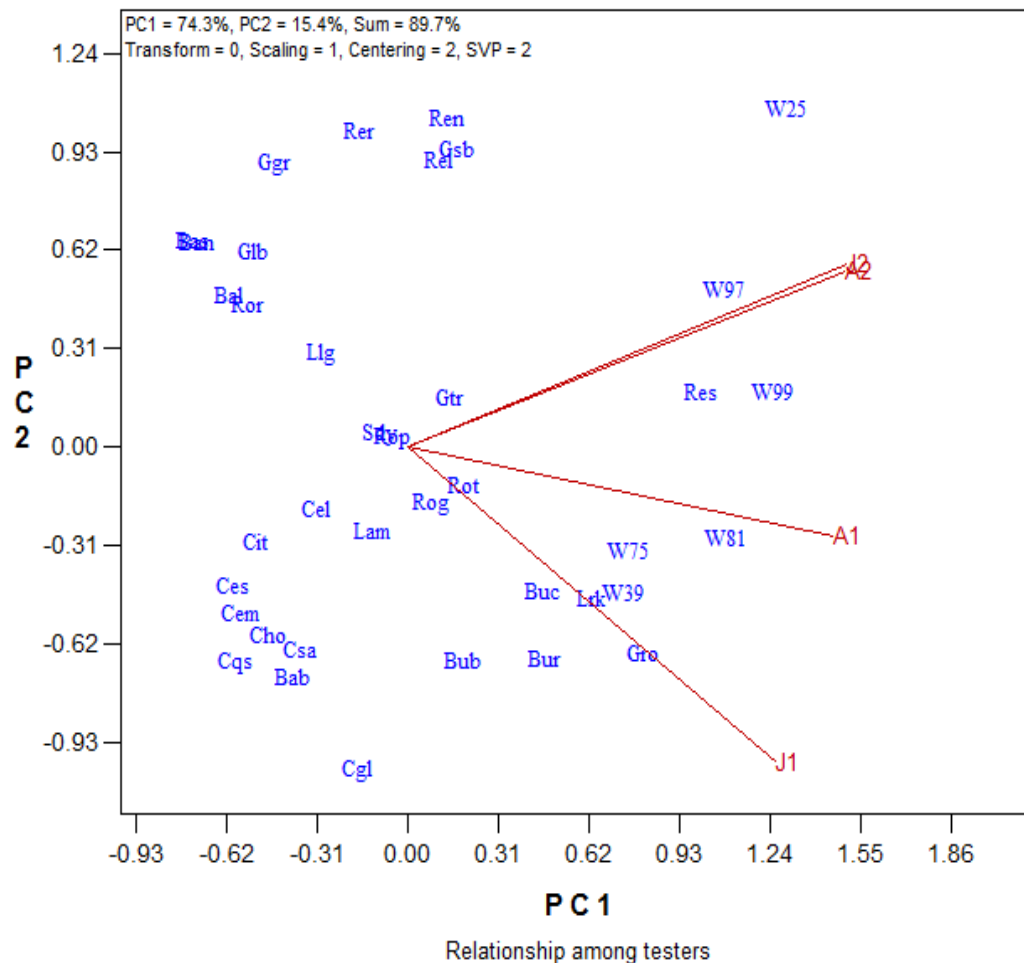


Figure 3.1: The environment-vector view of GGE biplot illustrating the relationship among tested environments for total flavonoids concentrations in 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).

Table 3.7: Correlation matrix for total flavonoids concentrations among 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).

(N = 38; r for P < 0.05 is 0.326; r for P < 0.01 is 0.420)

	A1	A2	J1	J2
A1	1	0.664	0.655	0.703
A2	0.664	1	0.524	0.877
J1	0.655	0.524	1	0.5
J2	0.703	0.877	0.5	1

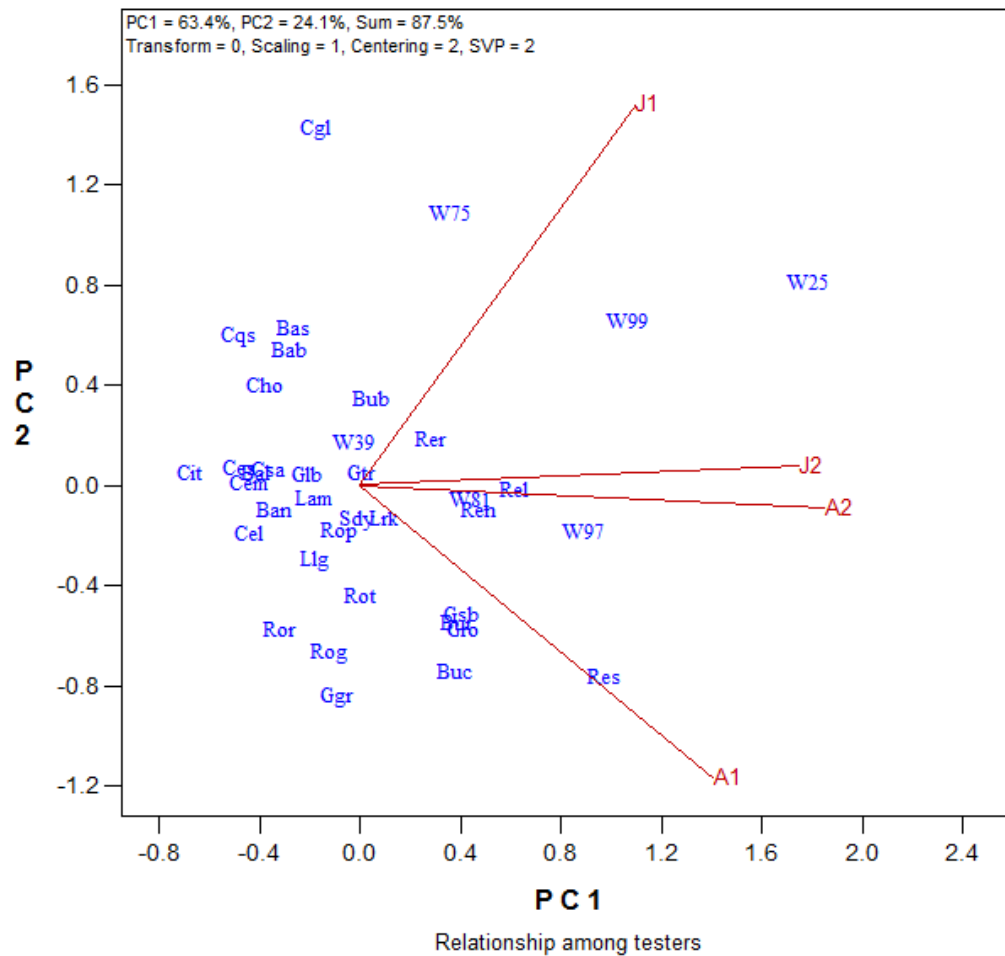


Figure 3.2: The environment-vector view of GGE biplot illustrating the relationship among tested environments for total phenolic concentrations in 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).

Table 3.8: Correlation Matrix for total phenolics concentrations among 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).

(N = 38; r for P < 0.05 is 0.326; r for P < 0.01 is 0.420)

	A1	A2	J1	J2
A1	1	0.658	0.041	0.513
A2	0.658	1	0.468	0.803
J1	0.041	0.468	1	0.438
J2	0.513	0.803	0.438	1

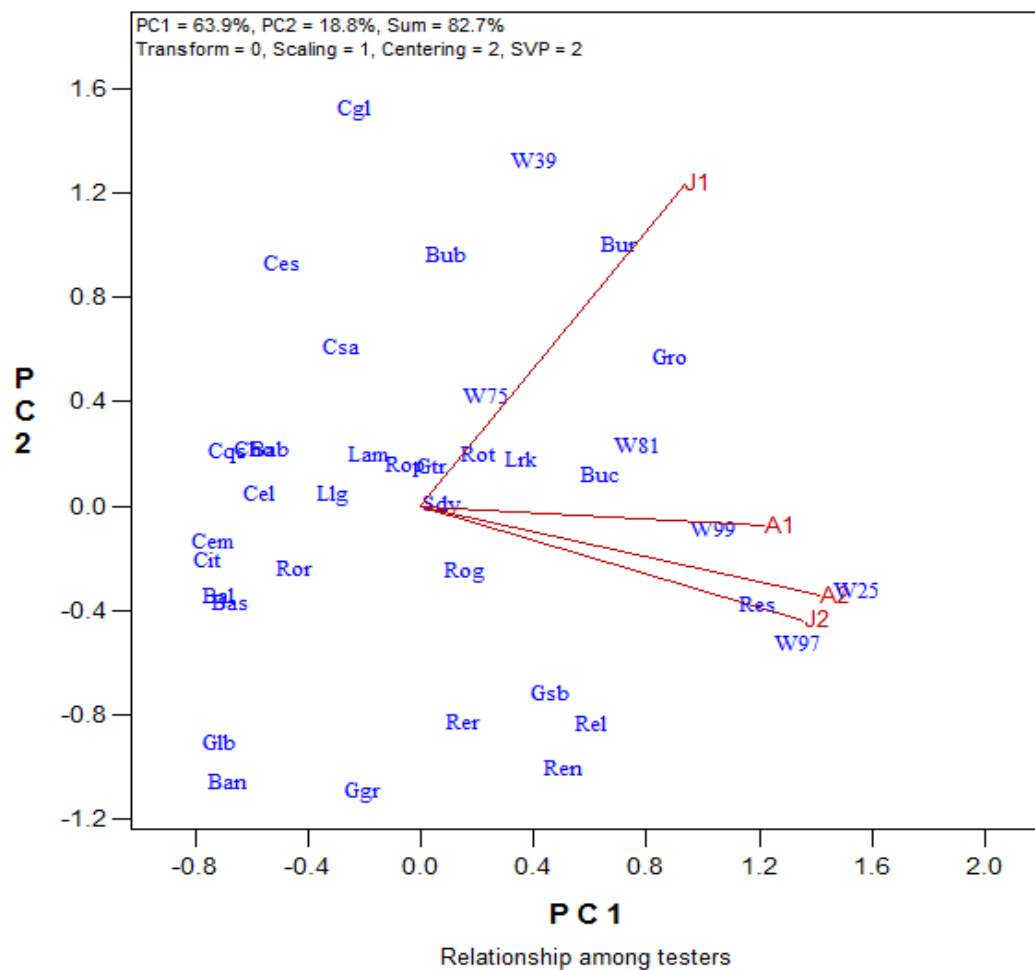


Figure 3.3: The environment-vector view of GGE biplot illustrating the relationship among tested environments for antioxidant activity (FRAP value) in 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).

Table 3.9: Correlation Matrix for antioxidant activity (FRAP value) among 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).
(N = 38; r for P < 0.05 is 0.326; r for P < 0.01 is 0.420)

	A1	A2	J1	J2
A1	1	0.599	0.363	0.524
A2	0.599	1	0.389	0.817
J1	0.363	0.389	1	0.341
J2	0.524	0.817	0.341	1

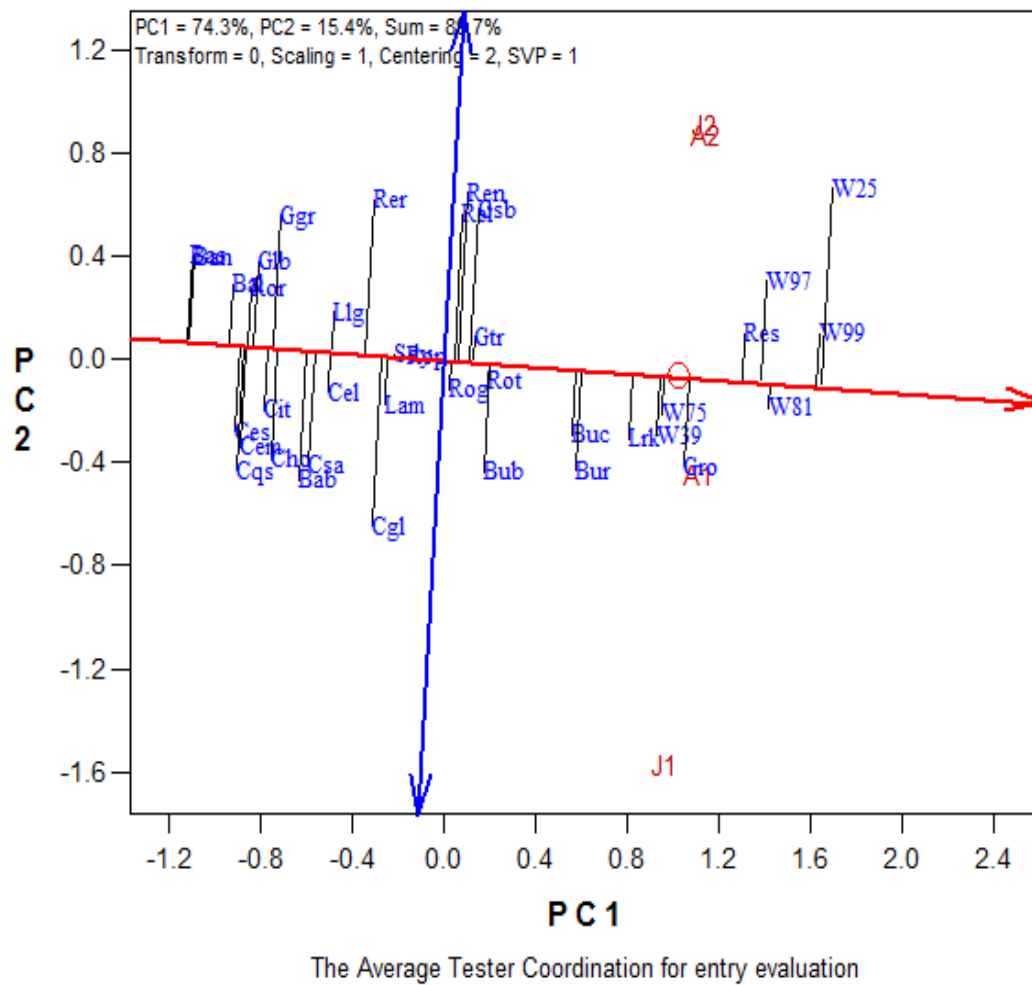


Figure 3.4: Mean-stability ranking for total flavonoid concentrations of 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).

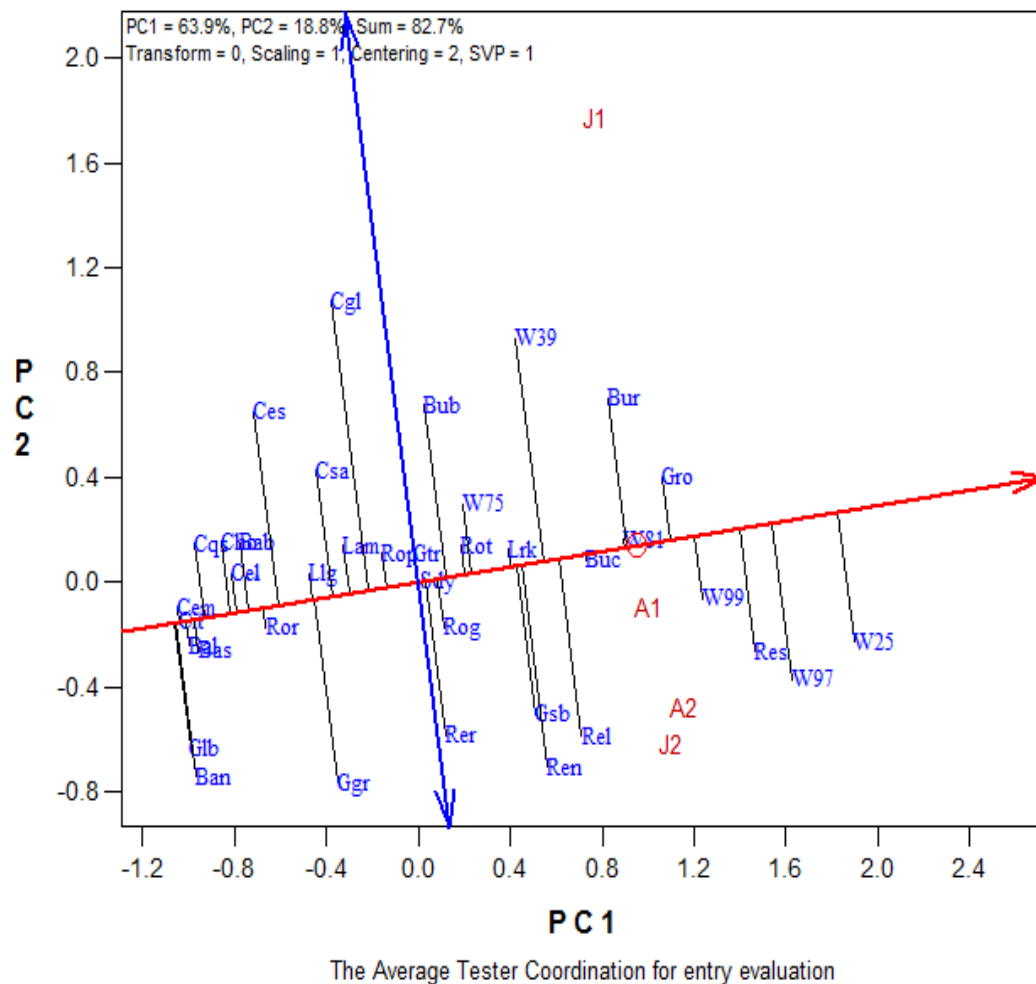


Figure 3.6: Mean-stability ranking for antioxidant activity (FRAP value) of 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).

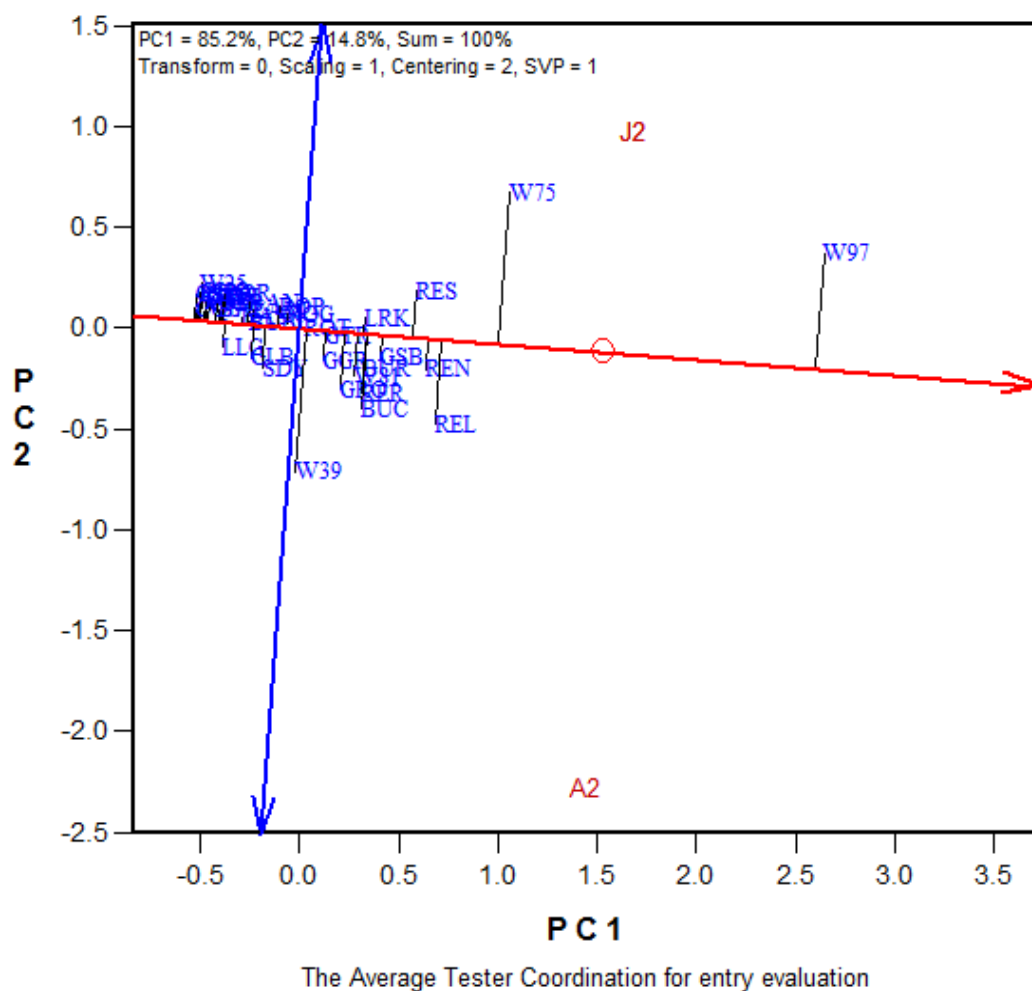


Figure 3.8: Mean-stability ranking for chicoric acid concentrations of 38 lettuce genotypes grown at two sites (A, Sainte-Anne-de-Bellevue; B, Saint-Jean-sur-Richelieu) and harvested at two stages (1, ten-leaf stage; 2, maturity stage).

Chapter IV Heat Stress Effects on the Concentration of Health-Beneficial Compounds in Lettuce

4.1 Abstract

The concentration of health-beneficial compounds (i.e. flavonoids and phenolics) in lettuce plants has previously been reported to be affected by temperature in a range of crops including lettuce. In this experiment, six cultivars of lettuce, including three red-leaf and three crisphead cultivars with contrasted concentrations of specific health-beneficial compounds and contrasted previously reported heat-tolerance were submitted to two temperature regime to assess the response of different lettuce cultivars to heat stress. Plants were grown in grow chambers with high (20°C night/28°C day) or control (14°C night/18°C day) temperatures and plants were harvested at two stages. Concentrations of total flavonoid, total phenolics, chlorogenic acid, and chicoric acid concentrations, as well as antioxidant activity (FRAP value) were determined. The cultivars evaluated differed in their response to heat stress and the response of specific cultivars depended on the stage at which plants were harvested. At the maturity stage heat stress consistently resulted in an increase of antioxidant activity and health-beneficial compounds such as phenolics, flavonoids when a response was observed. There was no association between the concentration of health-beneficial compounds in lettuce and previously reported lettuce heat tolerance.

4.2 Introduction

Lettuce is a cool season annual crop, preferring uniformly cool temperatures (optimum from 18 to 21°C) for germination (Peirce, 1987), and the optimum temperature for growth is from 7°C to 24°C, with an average of 18°C (Lorenz and Maynard, 1980). There are several health-beneficial compounds in lettuce, such as flavonoids, phenolic contents, and other antioxidants which play a role in a complex defense mechanism against stresses (Blokina et al., 2003; Dixon and Paiva, 1995). These compounds also have putative health-beneficial properties in humans (Block et al., 1992; Steinmetz and Potter, 1996; van Poppel et al., 1999). Environmental factors, such as temperature, soil fertility, salt stress, light duration and quality, have been reported to affect the concentrations of health-beneficial compounds in lettuce (Coria-Cayupán et al., 2009; Kim et al., 2008; Li et al., 2010; Oh et al., 2009; Zhou et al., 2009). Previous studies have reported temperature to be one of the most important environmental factor affecting concentrations of health-beneficial compounds in lettuce. For example, lettuce has been reported to have higher antioxidant and anthocyanin contents when grown at the lowest of four temperature regimes (i.e., 13/10°C, 20/13 °C, 25/20°C, and 30/25°C day/night) (Boo et al., 2011). In another study conducted with five-week-old lettuce plants grown in growth chambers, plants subjected to specific temperature stresses (heat shock at 40°C for 10 minutes and chilling at 4°C for 1 day) had higher a higher antioxidant capacity and higher

concentrations of two major phenolic compounds (i.e., chicoric acid and chlorogenic acid) than control plants. A marked accumulation of other specific compounds such as quercetin-3-O-glucoside and luteolin-7-O-glucoside have been reported in response to temperature stresses in other studies (Oh et al., 2009).

The objective of this study was to determine how heat stress may affect specific health-beneficial compounds in lettuce and to determine if there is a link between concentration of certain health-beneficial compounds and heat stress tolerance.

4.3 Material and Methods

4.3.1 Plants Selection and Treatments and Grow Conditions Description

An experiment was conducted in two grow chambers at the Horticulture Research and Development Centre of Agriculture and Agri-Food Canada (AAFC) located in Saint-Jean-sur-Richelieu, Quebec, Canada. Based on the results of a previous experiment (Chapter 3), six cultivars of lettuce including three cultivars with high concentration of total flavonoids and total phenolics and three cultivars with low concentrations were selected. These six cultivars also included three cultivars previously reported to be heat-tolerant. The six cultivars selected included three 'red leaf' cultivars, which were 'Ruby Sky' (not tolerant to heat stress), 'New Red Fire' (tolerant to heat stress) and

'Lolla Rossa 'Sanguine'' (not tolerant to heat stress) and were previously reported to have high concentrations of total flavonoids and total phenolics. The other three cultivars, 'Salinas' (not tolerant to heat stress), 'Estival' (tolerant to heat stress) and 'QJ-09 (Champlain)' (tolerant to heat stress) were 'crisphead' type with previously reported lower concentrations of total flavonoids and total phenolics.

All plants were initially seeded in a growth chamber at the Horticulture Research and Development Centre of AAFC in Saint-Jean-sur-Richelieu on March 21, 2012. Ten seeds of the six selected cultivars of lettuces were sown in plastic transplanting trays (200 cells). The growth chamber was maintained at 18/16°C day/night temperatures with a 14h/10h day/night photoperiod. Plants were then watered with tap water every day. Each cultivar was replicated three times at each growth chamber.

On 23 April, 2012, twenty-five days after seeding, when most of the plants were at the four to five leaves stage, plants were transplanted into six-inch pots filled with commercial peat-based medium (Promix BX, Premier Horticulture, Rivière-du Loup, Quebec). Two different ranges of temperature were set in two growth chambers. Nine plants (three for each replicate) of each cultivar were grown in the control growth chamber at 18/14°C day/night temperatures; and the other three were in the growth chamber with heat stress at 28/20°C day/night

temperatures until harvest. Plants were fertilized with a nutrient solution of 20-8-20 at a rate of 150 ppm of N three times a week and only with water on the other days. Watering was conducted on an as needed basis.

The number of leaves per plant was determined every week. Plants were harvested at two sampling stages, 'ten-leaf stage' and 'maturity stage'. At the 'ten-leaf' stage, all the cultivars were harvested at the same time when the plants had on average 10 leaves. At the 'maturity stage', plants were harvested when they had reached a size that would correspond approximately when they would be commercially harvested before starting to bolt, and thus harvest dates differed among types of lettuces and treatments. 'Red leaf' type lettuce were harvested around 10 days earlier than the cultivars of the 'crisphead' type, and plants in heat stress growth chamber were harvested around 5 days earlier than the ones in control chamber. One plant per treatment and replicate was harvested at 'ten-leaf' stage and the other two for each replicate were harvested at 'maturity stage' (Table 4.1). At the 'ten-leaf' sampling stages, the whole plant without the core but including leaves and ribs were sampled. At the 'maturity stage', base-leaves and wrapper-leaves were removed from crisphead lettuce plants when harvested; six leaves from different parts of the plants were taken, two leaves of the outer part, two of the middle and two of the inside part, to avoid the effect of different concentrations of different parts of the plant (Hohl et al., 2001). Leaves were grinded into small pieces in liquid nitrogen, freeze

dried for forty-eight hours then grinded again into fine powder and stored in a -80°C fridge before use in laboratory analyses.

4.3.2 Laboratory Analyses

4.3.2.1 Extraction of Total Flavonoids, Total Phenolics and FRAP

Total flavonoids, total phenolics and FRAP (Ferric-Reducing Antioxidant Power) assays were extracted and/or analyzed following the procedure of Norris et al. (1995) with minor modifications. First, 0.1 g of freeze-dried lettuce fine powdered sample was placed in 15 mL centrifugal tubes, then 2 mL of 95% methanol was added and the mixture was vortexed well. The samples were then sonicated in a cold room and in the dark for 30 min, centrifuged at 10,000 g at 4°C for 10 min. The supernatant was collected in a new 15mL centrifugal tube. One mL of 95% methanol was added to the pellet and the extraction and centrifugation steps were repeated. The extract was stored at -20°C in the dark before analyses (Norris et al., 1995).

4.3.2.2 Determination of Total Flavonoids

The total flavonoids content was measured using a colorimetric method described by (Chang et al., 2002) with minor modifications. Plant extracts were diluted 10 times before analysis. Aliquots (0.5 mL) of diluted lettuce extract or standard solutions were pipetted into 15 mL polypropylene conical tubes containing 2.8 mL of double-distilled water and mixed with 1.5 mL of 95%

methanol, 0.1 mL of 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ solution and 0.1 mL of 1M CH_3COOK solution. The reaction solution was vortexed and kept at room temperature for 40 min. The absorbance was determined at 415 nm. The total flavonoids content was calculated using a standard curve (5-300 mg L^{-1} quercetin) and expressed as equivalent in μg of quercetin g^{-1} DW.

4.3.2.3 Determination of Total Phenolics

Total phenolics were determined using Folin-Ciocalteu assay (Kang and Saltveit, 2002). Two mL of distilled water and 200 μL of 2N Folin-Ciocalteu-reagent were added to 100 μL of extract; the mixture was then vortexed and incubated at room temperature for 30 minutes. One mL of 20% sodium bicarbonate solution was then added to the mixture, which was vortexed, and absorbance was measured at 765 nm after incubating the mixture at room temperature for 1 hour. The total phenolics content was calculated using a standard curve (5-1000 mg L^{-1} gallic acid) and expressed as equivalent in μg of gallic acid g^{-1} DW.

4.3.2.4 FRAP (Ferric-Reducing Antioxidant Power) assay

The FRAP assay was conducted followed the procedure of (Nilsson et al., 2005) with minor modifications. The principle of the FRAP assay is that the yellow ferric tripyridyl triazine (Fe^{3+} -TPTZ) complex is reduced to the blue Fe^{2+} -TPTZ complex by electron donating substances under acidic conditions. The FRAP

reagent was made from a mixture of 0.1 mol L⁻¹ acetate buffer (pH 3.6)/10 mM TPTZ solution/2 mM FeCl₃ solution/distilled water (50:5:5:6 v:v:v:v); the mixture was then incubated at 37°C for 10 min before use. An aliquot of 10 µL of diluted lettuce extract was added to 30 µL of distilled water and 200 µL of FRAP reagent into a micro plate; mixed 10 s and incubated at room temperature for 8 min before the absorbance reading (593 nm). Calibration was made against a standard curve using freshly prepared FeSO₄ (0.1-1 nM).

4.3.3 HPLC (High-Performance Liquid Chromatography)

Caffeic acid and chicoric acid were extracted and analyzed by HPLC, using the procedure of Ferreres et al. (1997) with minor modifications (Ferreres et al., 1997). First, 0.1g of freeze-dried lettuce fine powdered plant material was placed in 15mL centrifugal tubes. Three mL of mixture of methanol/water/formic acid (25:24:3 v:v:v) was added to the tube and vortexed well and centrifuged twice at 10,000 g.

Separation of caffeic acid and chicoric acid were carried out using a Varian system (Walnut Creek, CA, USA) equipped with a Prostar 210 solvent delivery system, a model 410 autosampler and a Prostar 330 photodiode array detector (PDA). One mL of the extract was used for analyses. The mobile phases were water with 5% formic acid (A) and methanol (B) with a solvent flow rate of 1 ml min⁻¹, in a gradient program starting with 5% B in A, reaching 40% B at 25 min,

and then remaining isocratic for 5 min. The UV chromatograms were recorded at 520 nm. The identification of compounds was based on their UV spectra and retention times. Calibration curves were prepared using chlorogenic acid (Sigma, Saint Louis, MO, USA), caffeic acid (Sigma, Saint Louis, MO, USA), chicoric acid (Sigma, Saint Louis, MO, USA) and luteolin-7-O-glucoside (Sigma, Saint Louis, MO, USA) as standards. Concentrations of all the compounds detected were expressed on a dry matter (DM) basis.

4.3.4 Data analysis - Statistical Analyses

Treatments (cultivars) were replicated three times and assigned to a Randomly Complete Block Design (RCBD). All data were subjected to an analysis of variance (ANOVA) using the general linear model (GLM) procedure in SAS (2012) to identify significant treatment effects and interactions. When cultivar x temperature treatment interactions were significant, data were also analyzed using all cultivar-by-temperature combinations. Comparisons between means were determined using SNK ($P < 0.05$) when ANOVA indicated that both model and treatment effects were significant. Only significant effects ($P < 0.05$) are later discussed.

4.4 Results

4.4.1 Plant Growth

All six cultivars responded to heat stress having a different appearance and growth pattern compared to the plants grown in 'control' conditions. Heat stressed plants developed faster than the controlled plants. One week after applying the temperature treatment, the number of leaves was significantly greater for heat-stressed plants than for control plants (Figure 4.1). Plants grown in heat stress treatments bolted 25 days after transplantation on average while plants in control growth chambers had not reached the maturity size by that time (Figure 4.2). Heat-stressed 'crisphead' type cultivars started bolting without heading. Both heat tolerant plants and not heat tolerant ones responded to the heat stress.

4.4.2 Health-beneficial Compounds at the Ten-leaf Stage

At the ten-leaf sampling stage, total flavonoids concentration differed among cultivars, but was not affected by temperature treatments, nor was there an interaction between both factors (Table 4.2). A similar response was also observed for total phenolics, and caffeic acid concentrations, as well as the antioxidant capacity as determined by the FRAP assay. Total flavonoid, total phenolics, and caffeic acid concentrations ranged between 9881 and 21256 $\mu\text{g g}^{-1}$ DM, 6135 and 25152 $\mu\text{g g}^{-1}$ DM, and 15.4 and 195.4 $\mu\text{g g}^{-1}$ DM, respectively among cultivars. The antioxidant capacity (i.e., FRAP) also only differed among cultivars, the FRAP values ranging between 46.9 and 276.6 $\mu\text{M g}^{-1}$ DM among cultivars.

Concentrations of chlorogenic acid, chicoric acid, and luteolin-7-O-glucoside were all affected by the temperature as well as cultivars. In addition, the cultivar by temperature treatment interaction was significant for chlorogenic acid and luteolin-7-O-glucoside, reflecting that cultivars responded differently to the heat-stress treatment. Heat-stress increased the concentration of chlorogenic acid and luteolin-7-O-glucoside by 15 and 108%, respectively. The interaction revealed that this response differed in cultivars, some cultivars not responding to heat-stress. For example, cultivars Estival, QSJ-09 (Champlain), New Red Fire, Salinas and Ruby Sky were unresponsive to heat-stress, only cultivar Lolla Rossa 'Sanguine' had a 52% increase to heat-stress compared to the control for chlorogenic acid. For concentration of luteolin-7-O-glucoside, there was a 180% increase in cultivar Lolla Rossa 'Sanguine' compared to the control. In contrast, heat-stress decreased the concentration of chicoric acid in cultivar Lolla Rossa 'Sanguine' by 21% compared to the control. The interaction revealed that this response differed in cultivars, some cultivars not responding to heat stress. For example, cultivars New Red Fire, Salinas and Ruby Sky were unresponsive to heat-stress, but cultivars Estival, QSJ-09 (Champlain) and Lolla Rossa 'Sanguine' had a 27, 67 and 33% increase due to heat-stress respectively compared to the control for chicoric acid.

4.4.3 Health-beneficial Compounds at the 'Maturity' Stage

At the maturity stage, total flavonoid, total phenolics, chlorogenic acid, and luteolin concentrations, as well as antioxidant capacity were all affected by temperature, cultivars, and a significant interaction between these two factors was also observed (Table 4.3). Caffeic acid only differed between cultivars, while temperature and cultivar main effects were observed for chlorogenic acid concentration.

Total flavonoids concentration in lettuce grown in control conditions ranged between 4291 and 16142 $\mu\text{g g}^{-1}$ DM among the 6 cultivars; while this ranged between 12889 and 29242 $\mu\text{g g}^{-1}$ DM in heat-stressed plants (Figure 4.3). The interaction illustrated that while all cultivars had higher total flavonoid concentrations in heat-stress conditions (i.e., 103% on average), this increase was not significant for two red-leaf cultivars (i.e., New Red Fire and Ruby Sky). Two of the cultivars previously classified as being heat tolerant [i.e., Estival and QSJ-09 (Champlain)] had higher total flavonoids in heat-stress conditions, while the other heat tolerant cultivar (i.e., New Red Fire) did not respond.

Total phenolic concentrations in lettuce grown in control conditions ranged between 6113 and 23849 $\mu\text{g g}^{-1}$ DM, while the range was between 7819 and 34142 $\mu\text{g g}^{-1}$ DM in heat-stressed plants (Figure 4.4). The interaction illustrated that one cultivar (i.e., Lolla Rossa 'Sanguine') had significantly higher total phenolic concentrations in heat-stress conditions (i.e., 42%), while the others

did not respond to heat-stress. All cultivars previously classified as being heat tolerant [i.e., Estival, QSJ-09 (Champlain) and New Red Fire] did not respond to heat stress. In the meanwhile, one of the cultivars previously classified as being not heat tolerant (i.e., Lolla Rossa 'Sanguine') had significantly increased concentrations of total phenolics, while the other not heat tolerant cultivars did not respond.

Antioxidant activity (i.e., FRAP value) in lettuce grown in control conditions ranged between 30.3 and 228.1 $\mu\text{M g}^{-1}$ DM among the 6 cultivars; while this ranged between 64.4 and 352.3 $\mu\text{M g}^{-1}$ DM in heat stressed plants (Figure 4.5). The interaction illustrated that one cultivar (i.e., Lolla Rossa 'Sanguine') had higher antioxidant activities (FRAP value) in heat-stress conditions (i.e., 52%), while the others did not respond to heat-stress. One of the cultivars previously classified as being not heat tolerant (i.e., Lolla Rossa 'Sanguine') had significantly increased in antioxidant activities (FRAP value), while the other not heat tolerant cultivar did not respond.

Chlorogenic acid concentration in lettuce grown in control conditions ranged between 213 and 6074 $\mu\text{g g}^{-1}$ DM, while the range was between 780 and 10424 $\mu\text{g g}^{-1}$ DM in heat-stressed plants (Figure 4.6). The interaction illustrated that two cultivars (i.e., Lolla Rossa 'Sanguine' and Ruby Sky) had significantly higher chlorogenic acid concentration in heat-stress conditions (i.e., 116% on

average), while the others did not respond to heat-stress. All of the cultivars previously classified as being heat tolerant did not respond. Meanwhile, two of the cultivars previously classified as being not heat tolerant (i.e., Lolla Rossa 'Sanguine' and Ruby Sky) had significantly increased in chlorogenic acid concentration.

Caffeic acid concentration in lettuce grown in control conditions ranged between 16.0 and 79.3 $\mu\text{g g}^{-1}$ DM, while the range was between 19.4 and 144.0 $\mu\text{g g}^{-1}$ DM in heat stress plants (data not shown). The significant cultivar main effect illustrated that cultivars differed in their caffeic acid concentration, concentrations being greater in the red-leaf cultivars. Temperature, however, did not significantly affect concentrations.

Chicoric acid concentration in lettuce grown in control conditions ranged between 1513 and 15955 $\mu\text{g g}^{-1}$ DM, while the range was between 3695 and 20591 $\mu\text{g g}^{-1}$ DM in heat stress plants (data not shown). The significant cultivar main effect illustrated that cultivars differed in their chicoric acid concentration, concentrations being again greater in the red-leaf cultivars than in crisphead cultivars. Temperature similarly affected chicoric acid concentrations heat-stress increasing concentrations across cultivars.

Finally, luteolin-7-O-glucoside concentration in lettuce grown in control conditions ranged between 108 and 472 $\mu\text{g g}^{-1}$ DM, while the range was

between 74 and 13640 $\mu\text{g g}^{-1}$ DM in heat stress plants (data not shown). The significant cultivar by temperature interaction illustrated that cultivars responded differently to heat-stress. The cultivar Lolla Rossa 'Sanguine' had a 645% increase in luteolin-7-O-glucoside concentration due to heat-stress conditions, while the other cultivars did not respond to heat-stress. Luteolin-7-O-glucoside could not be detected in cultivars Estival and Salinas in control conditions but however could be detected in heat-stress condition.

4.5 Discussion and Conclusion

Significant differences were observed at both stage of harvest in the concentration of all health-beneficial compounds studied herein between cultivars. Differences between lettuce cultivars for variables including flavonoids and phenolics concentrations, as well as antioxidant power have previously been reported (DuPont et al., 2000; Ferreres et al., 1997; Liu et al., 2007; Mou, 2005; Tomás-Barberán et al., 1997). Health-beneficial compounds studied herein were all affected by heat stress, although the response was greater at the second of two harvest stages (i.e., 'maturity' stage). The presence of a significant cultivar by treatment interaction for most variables illustrate that not all cultivars responded to the heat stress. At the maturity stage heat stress always resulted in an increase of health-beneficial compounds when a response was observed; the concentration of phenolics and flavonoids being increased by heat stress. These results are in accordance with previous studies

which also reported on the impact of temperature on health-beneficial compounds in lettuce grown in the field and in a greenhouse (Romani et al., 2002). Results from the present study under controlled environment also confirm that the antioxidant capacity (FRAP value) may increase in response to heat stress; other studies have reported that antioxidants accumulated in response to environmental stresses including temperature stress. It was hypothesized that this response is due to the fact that antioxidants are part of a complex defense mechanism in plants against stresses (Blokhina et al., 2003; Dixon and Paiva, 1995).

The present study failed to see an association between flavonoids, phenolics and antioxidant capacity (FRAP) response in lettuce and previously reported heat tolerance or concentrations of these health-beneficial compounds. Although heat-stress was able to increase the concentration of health-beneficial compounds in lettuce, it is important to note that the growth of cultivars was negatively affected by the imposed stress treatment. For example, crisphead lettuce cultivars started bolting instead of heading. This result is in accordance with other studies, which reported that environmental stresses could increase health-beneficial compounds accumulation, but with a concurrent adverse effects on crop growth and yield (Jenni, 2005; Mou and Ryder, 2002; Oh et al., 2009).

Table 4.1: Harvest date of six lettuce cultivars grown at two different temperature regimes in 2012. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown 28/20°C day/night.

Cultivar	Type	Heat Tolerance	Seeding	Transplantation	Ten-leaf Stage Harvest	Maturity Stage Harvest (control)	Maturity Stage Harvest (heat stress)
Estival	C	YES	03.22	04.23	05.11	06.12	06.07
Salinas	C	NO	03.22	04.23	05.11	06.12	06.07
Q SJ-09 (Champlain)	C	YES	03.22	04.23	05.11	06.12	06.07
Lolla Rossa	R	NO	03.22	04.23	05.11	06.03	05.28
Ruby Sky	R	NO	03.22	04.23	05.11	06.03	05.28
New Red Fire	R	YES	03.22	04.23	05.11	06.03	05.28

C crisphead, R red leaf

Table 4.2: Analysis of Variance of Concentrations Table of Total Flavonoid, Total Phenolics, FRAP, Chlorogenic Acid, Caffeic Acid,

Chicoric Acid and Luteolin-7-O-Glucoside at 'Ten-Leaf Stage'

Source	DF	Total Flavonoid	Total Phenolics	FRAP	Chlorogenic Acid	Caffeic Acid	Chicoric Acid	Luteolin-7-O- Glucoside
Temperature	1	0.7630	0.8472	0.9062	0.0467	0.8048	0.0016	0.0027
Cultivar	5	<0.0001	<0.0001	<0.0001	<0.0001	0.0015	<0.0001	<0.0001
Cultivar× Temperature	5	0.7195	0.7116	0.3876	0.0024	0.4608	0.2452	0.0015
CV%		7.4	13.7	10.9	19.6	94.6	10.5	26.6

Table 4.3: Analysis of Variance of Concentrations Table of Total Flavonoid, Total Phenolics, FRAP, Chlorogenic Acid, Caffeic Acid, Chicoric Acid and Luteolin-7-O-Glucoside at 'Maturity stage'

Source	DF	Total Flavonoid	Total Phenolics	FRAP	Chlorogenic Acid	Caffeic Acid	Chicoric Acid	Luteolin-7-O-Glucoside
Temperature	1	<0.0001	0.0014	0.0060	0.0014	0.2502	0.0192	0.0175
Cultivar	5	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Cultivar×Temperature	5	0.0370	0.0219	0.0102	0.0059	0.3747	0.1408	0.0065
CV%		18.6	15.3	21.3	30.1	73.7	21.1	87.6

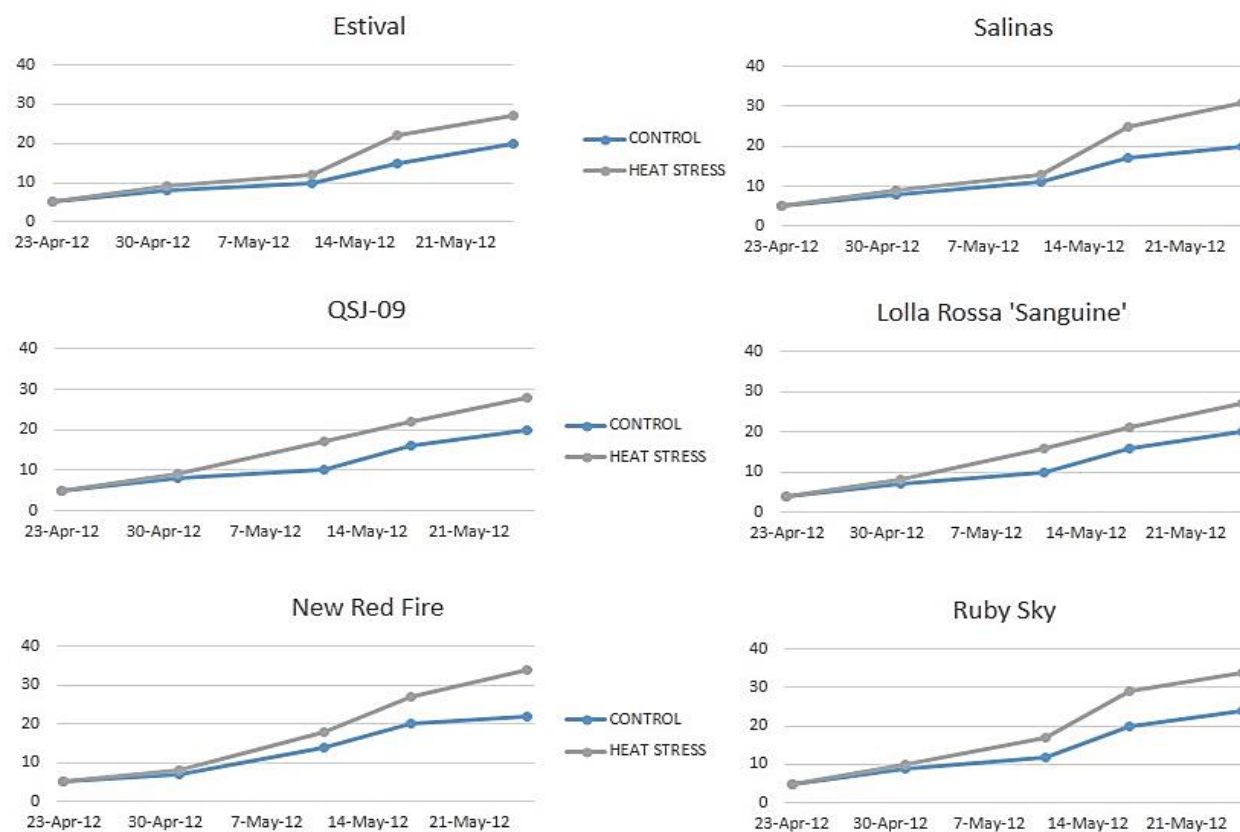


Figure 4.1: Number of leaves in six lettuce cultivars grown at two different temperature regimes. Control plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night.



Figure 4.2: Appearances of six lettuce cultivars grown at two different temperature regimes. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown 28/20°C day/night. Heat tolerant indicates cultivars that have been previously reported to be tolerant to heat-stress. Photos were taken 25 days after transplantation

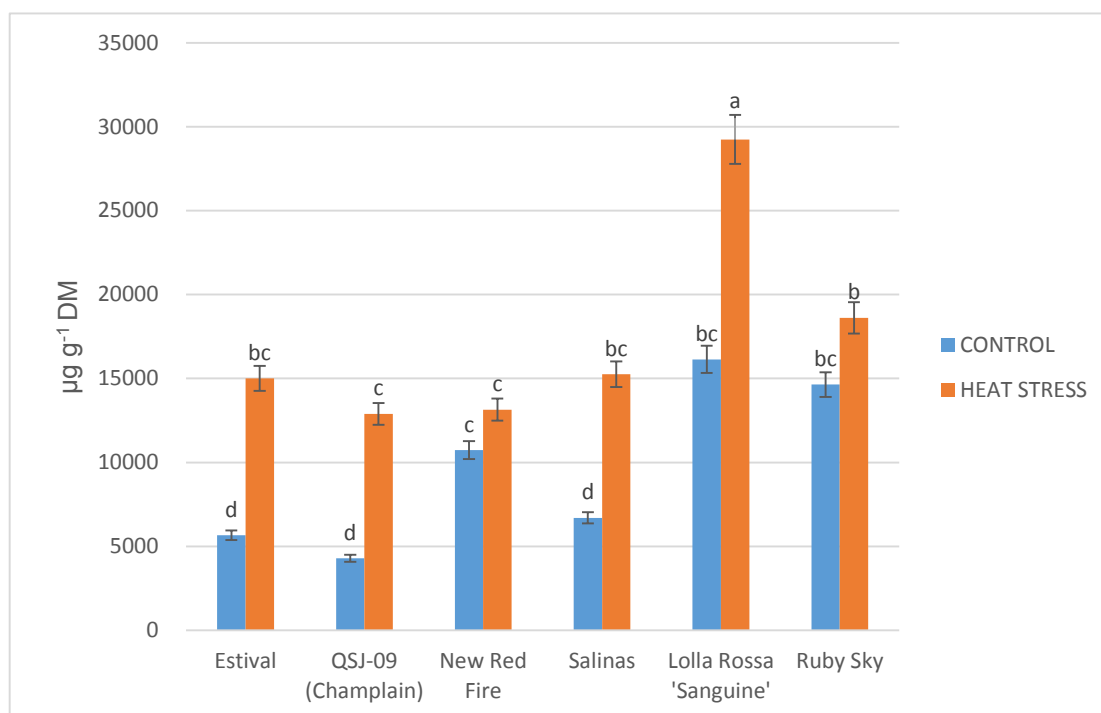


Figure 4.3: Effect of heat stress on total flavonoid concentration in lettuce sampled at the 'maturity' stage. Control plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night. Estival, QSJ-09 (Champlain) and New Red Fire have previously been classified as being heat tolerant cultivars, the others were not. New Red Fire, Lolla Rossa 'Sanguine' and Ruby Sky are red-leaf cultivars, while the other three cultivars are green-leaf cultivars. Treatment means followed by different letters are significantly different ($P < 0.05$). Vertical bars indicate standard deviation.

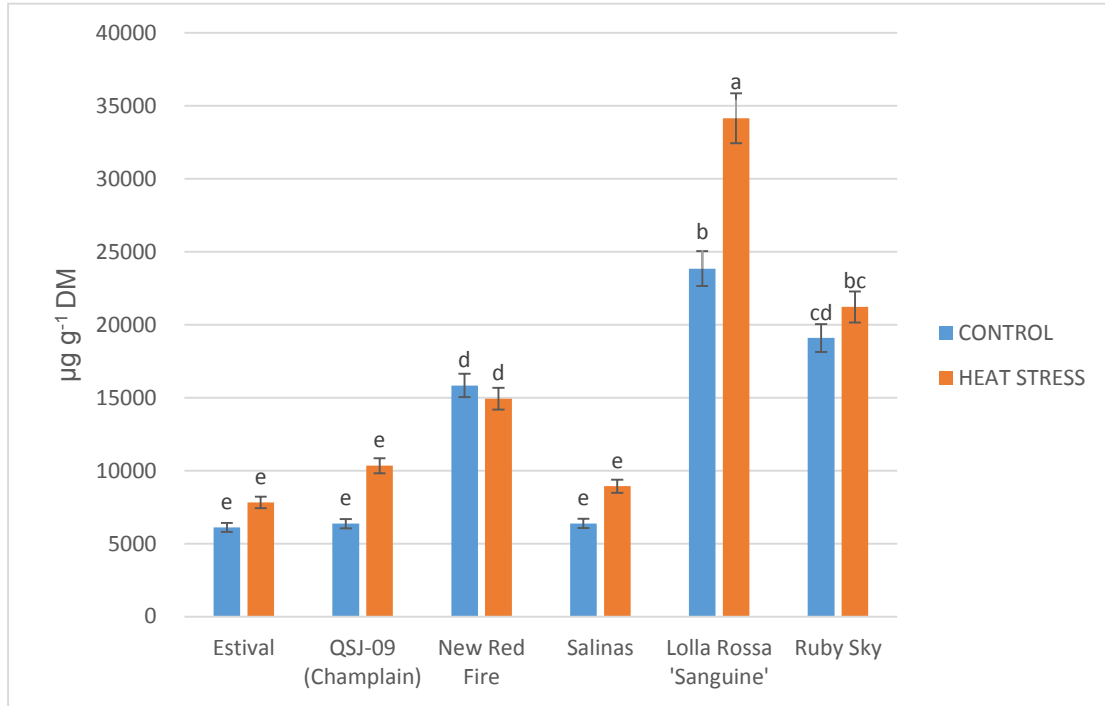


Figure 4.4: Effect of heat stress on total phenolic concentration in lettuce sampled at the 'maturity' stage. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night. Estival, QSJ-09 (Champlain) and New Red Fire have previously been classified as being heat tolerant cultivars, the others are not. New Red Fire, Lolla Rossa 'Sanguine' and Ruby Sky are red-leaf cultivars, while the other three cultivars are green-leaf cultivars. Treatment means followed by different letters are significantly different ($P < 0.05$). Vertical bars indicate standard deviation.

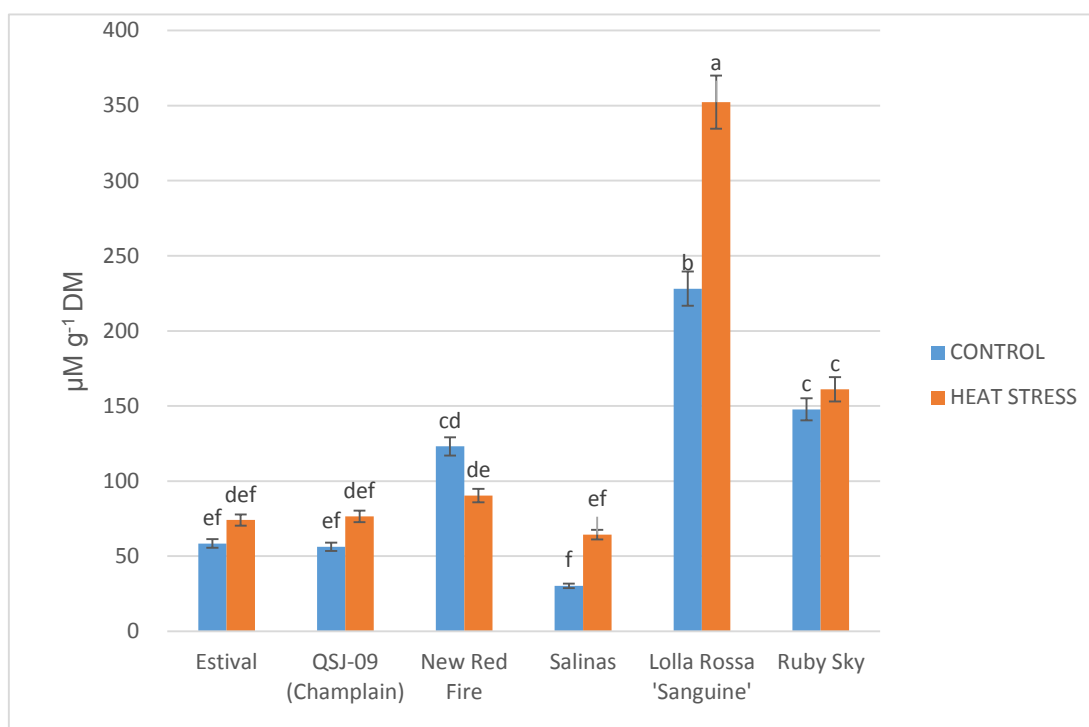


Figure 4.5: Effect of heat stress on antioxidant activity (FRAP value) in lettuce sampled at the 'maturity' stage. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night. Estival, QSJ-09 (Champlain) and New Red Fire have previously been classified as being heat tolerant cultivars, the others are not. New Red Fire, Lolla Rossa 'Sanguine' and Ruby Sky are red-leaf cultivars, while the other three cultivars are green-leaf cultivars. Treatment means followed by different letters are significantly different ($P < 0.05$). Vertical bars indicate standard deviation.

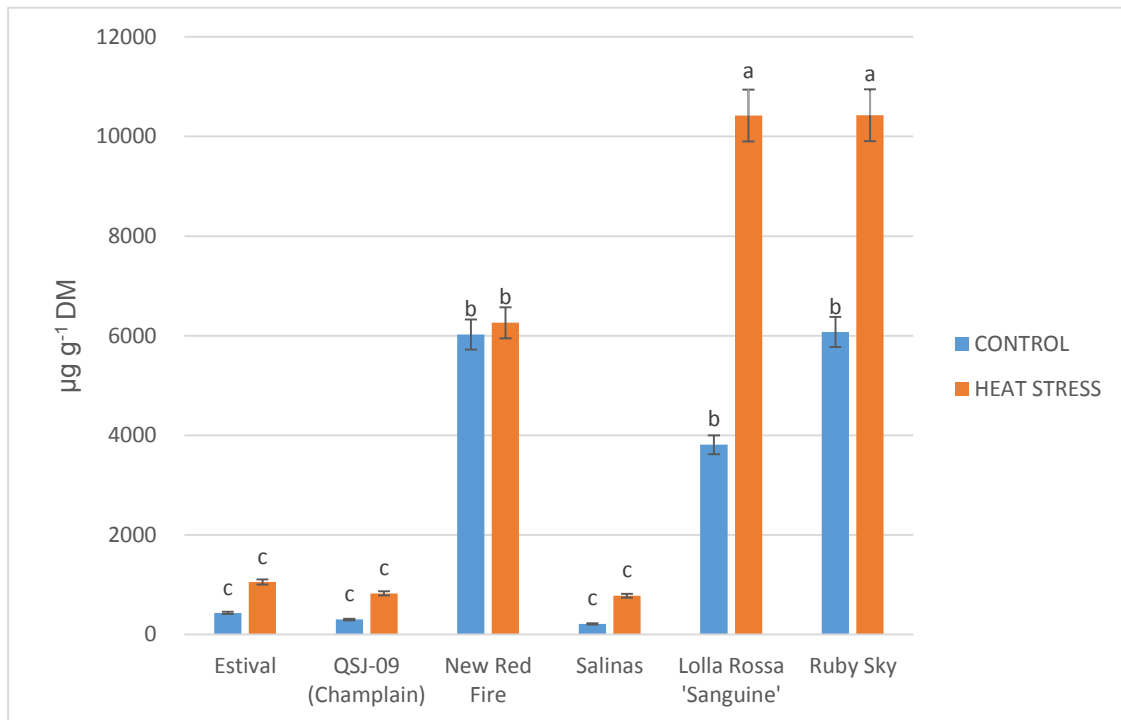


Figure 4.6: Effect of heat stress on chlorogenic acid concentration in lettuce sampled at the 'maturity' stage. Control, plants were grown at 18/14°C day/night from transplant to harvest; Heat Stress, plants were grown at 28/20°C day/night. Estival, QSJ-09 (Champlain) and New Red Fire have previously been classified as being heat tolerant cultivars, the others are not. New Red Fire, Lolla Rossa 'Sanguine' and Ruby Sky are red-leaf cultivars, while the other three cultivars are green-leaf cultivars. Treatment means followed by different letters are significantly different ($P < 0.05$). Vertical bars indicate standard deviation.

Chapter V General Conclusions

The first objective of this study was to determine the concentrations of health-beneficial compounds in different cultivars and species of lettuces as a function of growing sites and harvest stages. Results indicated that there were considerable variations in concentration of health-beneficial compounds among lettuce types and cultivars. The ranking of lettuce cultivars in terms of concentrations of total flavonoids, total phenolics, FRAP value, and also concentration of chlorogenic acid, caffeic acid and chicoric acid were not the same at the two sampling stages investigated; thus suggesting that selection of cultivars with high concentrations must be done at the optimal maturity stage. Traits were stable across environments thus selection should be possible in only one site. Red leaf lettuces had the highest total flavonoids concentration at the maturity stage among cultivated types, followed by butterhead, and green leaf, while crisphead and batavia have the lowest concentrations. Concentration of total phenolics and FRAP almost followed the same order as total flavonoids.

The second objective of this chapter was to find out how a given environmental stress (i.e. heat stress) affects health-beneficial compounds in lettuce and to determine if there is a link between concentration of certain health-beneficial compounds and reported heat stress tolerance. Results did demonstrate that

health-beneficial compounds could be affected by heat stress in lettuce.

Different cultivars of lettuce however responded differently to the heat stress

but response is not associated with documented heat-stress tolerance.

Chapter VI Suggestions for Future Research

1. In the current study, plants were all grown in greenhouses and grow chambers with a set temperature. It is suggested to repeat experiments in the field in future studies to determine if similar response will be observed in field-grown plants.
2. In this study, only heat stress was given as an environmental effects to test, more environmental effects should be tested (e.g. drought, fertilizer, etc.) in the future.
3. It has been reported that health-beneficial compounds such as antioxidants were part of a complex defense mechanism in plants against stresses. Thus a study of the response of different cultivars at the molecular level (e.g. gene expression response to the temperature) is suggested.

Chapter VII Contributions to Knowledge

1. This is the first report of the concentration of major flavonoids, and phenolics in 38 different cultivars of lettuces including wild species.
2. This is the first effort to study how stable the concentration of the specific health-beneficial compounds studied is across environments and harvest stages. Environmental factors and genotype x environment (GxE) interaction affect concentration of health-beneficial compounds in lettuce.
3. This is the first report on the response to heat-stress of certain lettuce types (i.e. red leaf and crisphead) at harvested at two stages.
4. This research can ultimately contribute to the development of recommendations for the production of lettuce with higher concentrations of health-beneficial compounds.

References

Ahuja, V., and Ahuja, A. (2011). Apitherapy—a sweet approach to dental diseases. Part II: propolis. *Journal of Academy of Advanced Dental Research* **2**, 1-8.

Arai, Y., Watanabe, S., Kimira, M., Shimoi, K., Mochizuki, R., and Kinae, N. (2000). Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *The Journal of Nutrition* **130**, 2243-2250.

Bagchi, D. (2008). Nutraceutical and functional food regulations in the United States and around the world. Access Online via <http://www.sciencedirect.com/science/book/9780123739018>.

Baslam, M., Morales, F., Garmendia, I., and Goicoechea, N. (2013). Nutritional quality of outer and inner leaves of green and red pigmented lettuces (*Lactuca sativa* L.) consumed as salads. *Scientia Horticulturae* **151**, 103-111.

Birt, D. F., Hendrich, S., and Wang, W. (2001). Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacology & Therapeutics* **90**, 157-177.

Block, G., Patterson, B., and Subar, A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutrition and Cancer* **18**, 1-29.

Blokhina, O., Virolainen, E., and Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: a review. *Annals of Botany* **91**, 179-194.

Boo, H.-O., Heo, B.-G., Gorinstein, S., and Chon, S.-U. (2011). Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. *Plant Science* **181**, 479-484.

Bravo, L. (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* **56**, 317-333.

Brower, V. (1998): Nutraceuticals: Poised for a healthy slice of the healthcare market?. *Nature Biotechnology* **16**, 728-732.

Caldwell, C. R. (2003). Alkylperoxyl radical scavenging activity of red leaf lettuce (*Lactuca sativa* L.) phenolics. *Journal of Agricultural and Food Chemistry* **51**, 4589-4595.

Chandan, R. C., and Shah, N. P. (2006). Functional foods and disease prevention. *Manufacturing Yogurt and Fermented Milks*, 311.

Chang, C.-C., Yang, M.-H., Wen, H.-M., and Chern, J.-C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods.

Journal of Food and Drug Analysis **10**, 178-182.

Coria-Cayupán, Y. S., Sánchez de Pinto, M. a. I., and Nazareno, M. n. A. (2009).

Variations in bioactive substance contents and crop yields of lettuce (*Lactuca sativa* L.) cultivated in soils with different fertilization treatments. *Journal of Agricultural and Food Chemistry* **57**, 10122-10129.

Crozier, A., Lean, M. E., McDonald, M. S., and Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal of Agricultural and Food Chemistry* **45**, 590-595.

Decoteau, D. R. (2000). Vegetable crops. *Prentice Hall*.

Dickinson, A., Boyon, N., and Shao, A. (2009). Physicians and nurses use and recommend dietary supplements: report of a survey. *Nutr J* **8**, 29.

Dixon, R. A., and Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *The Plant Cell* **7**, 1085.

DuPont, M. S., Mondin, Z., Williamson, G., and Price, K. R. (2000). Effect of variety, processing, and storage on the flavonoid glycoside content and

composition of lettuce and endive. *Journal of Agricultural and Food Chemistry* **48**, 3957-3964.

Dussi, M. C., Sugar, D., and Wrolstad, R. E. (1995). Characterizing and quantifying anthocyanins in red pears and the effect of light quality on fruit color. *Journal of the American Society for Horticultural Science* **120**, 785-789.

Ferreres, F., Gil, M. I., Castañer, M., and Tomás-Barberán, F. A. (1997). Phenolic metabolites in red pigmented lettuce (*Lactuca sativa* L.). Changes with minimal processing and cold storage. *Journal of Agricultural and Food Chemistry* **45**, 4249-4254.

García-Macías, P., Ordidge, M., Vysini, E., Waroonphan, S., Battey, N. H., Gordon, M. H., Hadley, P., John, P., Lovegrove, J. A., and Wagstaffe, A. (2007). Changes in the flavonoid and phenolic acid contents and antioxidant activity of red leaf lettuce (Lollo Rosso) due to cultivation under plastic films varying in ultraviolet transparency. *Journal of Agricultural and Food Chemistry* **55**, 10168-10172.

Gregory, J., Foster, K., Tyler, H., and Wiseman, M. (1990). The dietary and nutritional survey of British adults. *HMSO Publications Centre*.

Halliwell, B., Rafter, J., and Jenner, A. (2005). Health promotion by flavonoids, tocopherols, tocotrienols, and other phenols: direct or indirect effects? Antioxidant or not? *The American Journal of Clinical Nutrition* **81**, 268S-276S.

Harborne, J. B. (1998). Phytochemical methods A Guide to modern techniques of plant analysis. *Springer*.

Harborne, J. B., and Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry* **55**, 481-504.

Hasler, C. M. (1998). Functional foods: their role in disease prevention and health promotion. *Food Technology* **52**, 63-147.

Hayashi, E., You, Y., Lewis, R., Calderon, M. C., Wan, G., and Still, D. W. (2012). Mapping QTL, epistasis and genotype× environment interaction of antioxidant activity, chlorophyll content and head formation in domesticated lettuce (*Lactuca sativa* L.). *Theoretical and Applied Genetics* **124**, 1487-1502.

He, F., Nowson, C., Lucas, M., and MacGregor, G. (2007). Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. *Journal of Human Hypertension* **21**, 717-728.

Heim, K. E., Tagliaferro, A. R., and Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry* **13**, 572-584.

Hertog, M. G., Feskens, E. J., Kromhout, D., Hollman, P., and Katan, M. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet* **342**, 1007-1011.

Hertog, M. G., Hollman, P. C., and Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry* **40**, 2379-2383.

Hohl, U., Neubert, B., Pforte, H., Schonhof, I., and Böhm, H. (2001). Flavonoid concentrations in the inner leaves of head lettuce genotypes. *European Food Research and Technology* **213**, 205-211.

Hu, F. B. (2003). Plant-based foods and prevention of cardiovascular disease: an overview. *The American Journal of Clinical Nutrition* **78**, 544S-551S.

Huang, M.-T., and Ferraro, T. (1992). Phenolic compounds in food and cancer prevention. *ACS symposium series* **507**, pp. 8-8. ACS Publications.

Institute of Food Technologists (IFT) report 2012

Jang, M., Cai, L., Udeani, G. O., Slowing, K. V., Thomas, C. F., Beecher, C. W., Fong, H. H., Farnsworth, N. R., Kinghorn, A. D., and Mehta, R. G. (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **275**, 218-220.

Jenni, S. (2005). Rib discoloration: A physiological disorder induced by heat stress in crisphead lettuce. *HortScience* **40**, 2031-2035.

Kalra, E. K. (2003). Nutraceutical-definition and introduction. *Aaps Pharmsci* **5**, 27-28.

Kang, H.-M., and Saltveit, M. E. (2002). Antioxidant capacity of lettuce leaf tissue increases after wounding. *Journal of Agricultural and Food Chemistry* **50**, 7536-7541.

Kaur, C., and Kapoor, H. C. (2001). Antioxidants in fruits and vegetables—the millennium's health. *International Journal of Food Science & Technology* **36**, 703-725.

Kim, H.-J., Fonseca, J. M., Choi, J.-H., and Kubota, C. (2007). Effect of methyl jasmonate on phenolic compounds and carotenoids of romaine lettuce (*Lactuca sativa* L.). *Journal of Agricultural and Food Chemistry* **55**, 10366-10372.

Kim, H.-J., Fonseca, J. M., Choi, J.-H., Kubota, C., and Kwon, D. Y. (2008). Salt in irrigation water affects the nutritional and visual properties of romaine lettuce (*Lactuca sativa* L.). *Journal of Agricultural and Food Chemistry* **56**, 3772-3776.

Lee, S. J., Yan, W., Ahn, J. K., and Chung, I. M. (2003). Effects of year, site, genotype and their interactions on various soybean isoflavones. *Field Crops Research* **81**, 181-192.

Li, Z., Zhao, X., Sandhu, A. K., and Gu, L. (2010). Effects of exogenous abscisic acid on yield, antioxidant capacities, and phytochemical contents of greenhouse grown lettuces. *Journal of Agricultural and Food Chemistry* **58**, 6503-6509.

Liu, X., Ardo, S., Bunning, M., Parry, J., Zhou, K., Stushnoff, C., Stoniker, F., Yu, L., and Kendall, P. (2007). Total phenolic content and DPPH^{< sup>} radical scavenging activity of lettuce (*Lactuca sativa* L.) grown in Colorado. *LWT-Food Science and Technology* **40**, 552-557.

Llorach, R., Martínez-Sánchez, A., Tomás-Barberán, F. A., Gil, M. I., and Ferreres, F. (2008). Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chemistry* **108**, 1028-1038.

López-Berenguer, C., Martínez-Ballesta, M. a. d. C., Moreno, D. A., Carvajal, M., and García-Viguera, C. (2009). Growing hardier crops for better health: salinity tolerance and the nutritional value of broccoli. *Journal of Agricultural and Food Chemistry* **57**, 572-578.

Lorenz, O. A., and Maynard, D. N. (1980). Knotfs Handbook for Vegetable Growers. *Cambridge Univ Press*.

Luthria, D. L., and Mukhopadhyay, S. (2006). Influence of sample preparation on assay of phenolic acids from eggplant. *Journal of Agricultural and Food Chemistry* **54**, 41-47.

Luthria, D. L., Mukhopadhyay, S., and Krizek, D. T. (2006). Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum* Mill.) fruits as influenced by cultivar and solar UV radiation. *Journal of Food Composition and Analysis* **19**, 771-777.

Mou, B. (2005). Genetic variation of beta-carotene and lutein contents in lettuce. *Journal of the American Society for Horticultural Science* **130**, 870-876.

Mou, B., and Ryder, E. J. (2002). Relationship between the nutritional value and the head structure of lettuce. *XXVI International Horticultural Congress: Advances in Vegetable Breeding*. pp. 361-367.

Mulabagal, V., Ngouajio, M., Nair, A., Zhang, Y., Gottumukkala, A. L., and Nair, M. G. (2010). Evaluation of red and green lettuce (*Lactuca sativa* L.) for functional food properties. *Food Chemistry* **118**, 300-306.

Nicolle, C., Carnat, A., Fraisse, D., Lamaison, J. L., Rock, E., Michel, H., Amouroux, P., and Remesy, C. (2004). Characterisation and variation of antioxidant micronutrients in lettuce (*Lactuca sativa* folium). *Journal of the Science of Food and Agriculture* **84**, 2061-2069.

Nilsson, J., Pillai, D., Önning, G., Persson, C., Nilsson, Å., and Åkesson, B. (2005). Comparison of the 2, 2'-azinobis-3-ethylbenzotiazoline-6-sulfonic acid (ABTS) and ferric reducing anti-oxidant power (FRAP) methods to assess the total antioxidant capacity in extracts of fruit and vegetables. *Molecular Nutrition & Food Research* **49**, 239-246.

Norris, S. R., Barrette, T. R., and DellaPenna, D. (1995). Genetic dissection of carotenoid synthesis in *Arabidopsis* defines plastocyanin as an essential component of phytyl desaturation. *The Plant Cell Online* **7**, 2139-2149.

Oh, M.-M., Trick, H. N., and Rajashekar, C. B. (2009). Secondary metabolism and antioxidants are involved in environmental adaptation and stress tolerance in lettuce. *Journal of Plant Physiology* **166**, 180-191.

Ordidge, M., García-Macías, P., Battey, N. H., Gordon, M. H., Hadley, P., John, P., Lovegrove, J. A., Vysini, E., and Wagstaffe, A. (2010). Phenolic contents of lettuce, strawberry, raspberry, and blueberry crops cultivated under plastic films varying in ultraviolet transparency. *Food Chemistry* **119**, 1224-1227.

Paiva, S. A., and Russell, R. M. (1999). β -carotene and other carotenoids as antioxidants. *Journal of the American College of Nutrition* **18**, 426-433.

Peirce, L. C. (1987). Vegetables. Characteristics, production, and marketing. *John Wiley and Sons*.

Peterson, C., Johnson, V., and Mattern, P. (1986). Influence of cultivar and environment on mineral and protein concentrations of wheat flour, bran, and grain. *Cereal Chemistry* **63**, 183-186.

Pietta, P.-G. (2000). Flavonoids as antioxidants. *Journal of Natural Products* **63**, 1035-1042.

Ransley, J. K. (2001). The rise and rise of food and nutritional supplements—an overview of the market. *Food and Nutritional Supplements*. pp. 1-16. Springer.

Rice-Evans, C. A., Miller, N. J., and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine* **20**, 933-956.

Robbins, R. J. (2003). Phenolic acids in foods: an overview of analytical methodology. *Journal of Agricultural and Food Chemistry* **51**, 2866-2887.

Romani, A., Pinelli, P., Galardi, C., Sani, G., Cimato, A., and Heimler, D. (2002). Polyphenols in greenhouse and open-air-grown lettuce. *Food Chemistry* **79**, 337-342.

Scalbert, A., and Williamson, G. (2000). Dietary intake and bioavailability of polyphenols. *The Journal of Nutrition* **130**, 2073S-2085S.

Shahidi, F., and Naczk, M. (1995). *Food phenolics: sources, chemistry, effects, applications*. Technomic Publishing Company Lancaster.

Steinmetz, K. A., and Potter, J. D. (1996). Vegetables, fruit, and cancer prevention: a review. *Journal of the American Dietetic Association* **96**, 1027-1039.

Tanwar, B., and Modgil, R. (2012). Flavonoids: Dietary occurrence and health benefits. *Journal on Complementary Medicine and Drug Discovery* **2**, 59-68.

Tomás-Barberán, F. A., Gil, M. I., Castañer, M., Artés, F., and Saltveit, M. E. (1997). Effect of selected browning inhibitors on phenolic metabolism in stem tissue of harvested lettuce. *Journal of Agricultural and Food Chemistry* **45**, 583-589.

Tomás-Barberán, F. A., and Espin, J. C. (2001). Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. *Journal of the Science of Food and Agriculture* **81**, 853-876.

UN Food and Agriculture Organization (2011). *Production of Lettuce and Chicory by Countries*.

United States Institute of Food Technologists (2012). *Functional Food Trends 2012: Consumers Want Nutrients from Food, Not Supplements*

Van Duyn, M. A. S., and Pivonka, E. (2000). Overview of the health benefits of fruit and vegetable consumption for the dietetics professional: selected literature. *Journal of the American Dietetic Association* **100**, 1511-1521.

Van Poppel, G., Verhoeven, D. T., Verhagen, H., and Goldbohm, R. A. (1999). Brassica vegetables and cancer prevention. *Advances in Nutrition and Cancer* **2**. pp. 159-168. Springer.

Wildman, R. E. (2006). Handbook of nutraceuticals and functional foods. *CRC press*.

Wittwer, S. H., and Honma, S. (1979). Greenhouse tomatoes, lettuce and cucumbers. *Michigan State University Press*.

Yan, W. (2001). GGEbiplot—a Windows application for graphical analysis of multienvironment trial data and other types of two-way data. *Agronomy Journal* **93**, 1111-1118.

Yao, L. H., Jiang, Y., Shi, J., Tomas-Barberan, F., Datta, N., Singanusong, R., and Chen, S. (2004). Flavonoids in food and their health benefits. *Plant Foods for Human Nutrition* **59**, 113-122.

Zhou, Y.-H., Zhang, Y.-Y., Zhao, X., Yu, H.-J., Shi, K., and Yu, J.-Q. (2009). Impact of light variation on development of photoprotection, antioxidants, and nutritional value in *Lactuca sativa* L. *Journal of Agricultural and Food Chemistry* **57**, 5494-5500.

Appendix

Table 1: Harvest Date of Lettuce Evaluated

Cultivar	Type	Saint-Jean-sur-Richelieu		Sainte-Anne-de-Bellevue	
		Ten-Leaf	Maturity	Ten-Leaf	Maturity
Estival	C	2011.03.15	2011.04.19	2011.03.22	2011.05.04
Salinas	C	2011.03.15	2011.04.19	2011.03.22	2011.05.04
Eldorado	C	2011.03.15	2011.04.19	2011.03.22	2011.05.04
Ithaca	C	2011.03.15	2011.04.19	2011.03.22	2011.05.04
Great Lakes 659	C	2011.03.15	2011.04.19	2011.03.22	2011.05.04
Hochelaga	C	2011.03.15	2011.04.19	2011.03.22	2011.05.04
Q SJ-09 (Champlain)	C	2011.03.15	2011.04.19	2011.03.22	2011.05.04
Emperor	C	2011.03.15	2011.04.19	2011.03.22	2011.05.04
Green Tower	R	2011.03.15	2011.04.13	2011.03.22	2011.04.29
Parris Island Cos	R	2011.03.15	2011.04.13	2011.03.22	2011.04.29
Romora	R	2011.03.15	2011.04.27	2011.03.22	2011.05.04
Tall Guzmanine	R	2011.03.15	2011.04.13	2011.03.22	2011.04.29
Salad Bowl	GL	2011.03.15	2011.03.31	2011.03.22	2011.04.15
Grand Rapids	GL	2011.03.15	2011.03.31	2011.03.22	2011.04.15
Tropicana	GL	2011.03.15	2011.03.31	2011.03.22	2011.04.29
Lollo Bionda	GL	2011.03.15	2011.04.06	2011.03.22	2011.04.29
Royal Oakleaf	GL	2011.03.15	2011.04.06	2011.03.22	2011.04.29
Lolla Rossa 'Sanguine'	RL	2011.03.15	2011.04.06	2011.03.22	2011.04.29
Ruby Sky	RL	2011.03.15	2011.04.06	2011.03.22	2011.04.15
Ruby	RL	2011.03.15	2011.04.06	2011.03.22	2011.04.15
New Red Fire	RL	2011.03.15	2011.04.06	2011.03.22	2011.04.15
Buttercrunch	BH	2011.03.15	2011.04.12	2011.03.22	2011.04.29
Rhapsody	BH	2011.03.15	2011.04.12	2011.03.22	2011.04.29
Caliente	BH	2011.03.15	2011.04.12	2011.03.22	2011.04.29
Batavia reine des Glaces	B	2011.03.15	2011.04.19	2011.03.22	2011.05.04
La Brillante	B	2011.03.15	2011.04.12	2011.03.22	2011.05.04
Nevada	B	2011.03.15	2011.04.12	2011.03.22	2011.05.04
Sierra	B	2011.03.15	2011.04.06	2011.03.22	2011.04.29

Table 1 continued

Cultivar	Type	Saint-Jean-sur-Richelieu		Sainte-Anne-de-Bellevue	
		Ten-Leaf	Maturity	Ten-Leaf	Maturity
Kendo	LR	2011.03.15	2011.04.06	2011.03.22	2011.04.29
Da Ye Wo Sun	S	2011.03.15	2011.04.27	2011.03.22	2011.05.13
PI 491181	W	2011.03.15	2011.04.06	2011.03.22	2011.04.15
PI 491239	W	2011.03.15	2011.04.27	2011.03.22	2011.05.04
PI 490999	W	2011.03.15	2011.04.06	2011.03.22	2011.04.15
PI 509525	W	2011.03.15	2011.04.06	2011.03.22	2011.04.29
PI 273597	W	2011.03.15	2011.04.29	2011.03.22	2011.05.06
PI 274375	W	2011.03.15	2011.04.29	2011.03.22	2011.05.06

C crisphead, R romain, GL green leaf, RL red leaf, BH butterhead, B batavia,

L latin, LR latin/red sucrine, S stem, W wild

Table 2: Means of total flavonoids, total phenolics, FRAP, chlorogenic acid, caffeic acid, chicoric acid and luteolin-7-O-glucoside concentration harvested at 'ten-leaf stage'. Plants were grown at control (18/14°C day/night) and heat stress (28/20°C day/night) from transplant to harvest.

Cultivar	Type	Total Flavonoids		Total Phenolics		FRAP		Chlorogenic Acid		Caffeic Acid		Chicoric Acid		Luteolin-7-O-glucoside	
		µg g-1 DM		µg g-1 DM		µM g-1 DM		µg g-1 DM		µg g-1 DM		µg g-1 DM		µg g-1 DM	
		Con	Trt	Con	Trt	Con	Trt	Con	Trt	Con	Trt	Con	Trt	Con	Trt
Estival	C	12470	12290	5851	6420	53.8	60.3	488	349	25	16	2512	1828	-	-
QSJ-09	C	10984	10453	5943	5040	52.5	41.4	317	119	24	10	3151	1018	-	-
New Red Fire	R	10181	9581	10121	9105	82.9	75.8	5897	3880	55	32	8375	6164	88	48
Salinas	C	11466	12116	7040	6870	63.6	53.9	409	405	34	17	3316	2221	-	-
Lolla Rossa	R	21004	21509	24446	25858	270.1	283.0	8383	12754	142	248	17031	16977	1117	3136
Ruby Sky	R	14548	15318	14076	14793	111.9	117.7	5174	6649	56	43	9602	7447	386	464
C crisphead, R redleaf; Con control, Trt temperature treatment															
Data are means for three replications.															

Table 3: Means of total flavonoids, total phenolics, FRAP, chlorogenic acid, caffeic acid, chicoric acid and luteolin-7-O-glucoside concentration harvested at 'maturity stage'. Plants were grown at control (18/14°C day/night) and heat stress (28/20°C day/night) from transplant to harvest.

Cultivar	Type	Total Flavonoids		Total Phenolics		FRAP		Chlorogenic Acid		Caffeic Acid		Chicoric Acid		Luteolin-7-O-glucoside	
		µg g-1 DM		µg g-1 DM		µM g-1 DM		µg g-1 DM		µg g-1 DM		µg g-1 DM		µg g-1 DM	
		Con	Trt	Con	Trt	Con	Trt	Con	Trt	Con	Trt	Con	Trt	Con	Trt
Estival	C	5656	15005	6113	7819	58.5	74.1	432	1053	16.0	19.4	2315	3695	-	75
QSJ-09	C	4291	12889	6370	10345	56.3	76.5	300	825	22.7	28.2	2274	4349	108	274
New Red Fire	R	10737	13146	15842	14939	123.1	99.0	6022	6259	20.6	33.5	10962	9831	177	291
Salinas	C	6691	15249	6385	8940	30.3	64.4	213	780	31.3	26.9	1513	4949	-	281
Lolla Rossa	R	16142	29242	23849	34142	228.1	352.3	3809	10422	79.3	144.0	15955	20591	1828	13640
Ruby Sky	R	14636	18614	19094	21222	147.8	161.1	6074	10424	21.4	31.1	9953	10120	473	923
C crisphead, R redleaf; Con control, Trt temperature treatment Data are means for three replications															
Data are means for three replications.															
