

EFFECT OF STORAGE TEMPERATURE ON THE ASCORBIC ACID CONTENT, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY IN LETTUCE (*LACTUCA SATIVA* L.)

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ABSTRACT

The effect of storage temperature (0, 2, 4 and 6 °C) and time (0, 3, 6, 9, 12 and 15 days) on ascorbic acid content, total phenolic content and antioxidant activity of three lettuce cultivars (Roderick, Markies and Locarno) was studied to determine optimal storage conditions. All parameters present in this study – ascorbic acid content, total phenolic content and antioxidant activity – were studied by spectrophotometric assays. The Folin-Ciocalteu assay was used to determine total phenolic content, while antioxidant activity was tested using ABTS assay. After 15 days of storage ascorbic acid content ranged from 6.47 to 2.93 mg / 100 g fresh weight (fw) total phenolic content ranged from 3.83 to 2.27 mg (GAE)/g fw and antioxidant activity ranged from 432.03 to 158.53 μM TEAC/g fw. The optimal storage conditions were between 0 °C and 2 °C up to 6 days. A correlation between temperature and duration of storage was found among cultivars. Statistical analyses showed specific variations for each cultivar analysed.

Key words: *Lactuca sativa* L., ascorbic acid content, total phenolic content, antioxidant activity, storage, temperature.

INTRODUCTION

Postharvest losses in relation to quality and quantity of food are a major problem all over the world. Apart from appearance, texture, flavour and nutritive value or handling procedures, quality parameters also include storage time and temperature. With optimal storage time and temperature, the losses of different bioactive compounds can be minimal and therefore the product's shelf-life can be increased Khan *et al.* (2012); Lamikanra (2002).

Postharvest storage time and temperature can have an influence on ascorbic acid (AA) content, antioxidant activity and total phenolic content (TPC) Lamikanra (2002).

AA is known to be one of the most labile food constituents and its retention has an effect on the nutritional quality of vegetables Lee *et al.* (2000). Independently of AA, TPC and antioxidant activity are also important parameters that can influence the quality of fruit and vegetables, because of their ability to scavenge the free radicals of fatty acids and oxygen. Measuring antioxidant activity could be one of the ways to assess the quality of the product biologically and nutritionally Kyzlink (1990).

Lettuce and other leafy vegetables are perceived to be healthier by consumers, resulting in increased consumption Hertog *et al.* (1993); Altunkaya *et al.* (2008). The aim of this research was therefore to monitor

the change in TPC and AA content along with the antioxidant activity over time for three different cultivars of lettuce, namely Roderick, Markies and Locarno, during storage at different temperatures. This would allow a determination of optimal storage temperature and duration to ensure the retention of antioxidant activity.

MATERIALS AND METHODS

Reagents: Trichloroacetic acid, bathophenanthroline (4,7-diphenyl-1,10-phenanthroline), dithiothreitol, cysteine, citric acid, ascorbic acid, oxalic acid, sodium carbonate, Folin-Ciocalteu reagent, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), potassium persulphate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and methanol were purchased from Sigma-Aldrich (Munich, Germany), and gallic acid was acquired from Merck (Darmstadt, Germany).

Plant material: Three green lettuce (*Lactuca sativa* L.) varieties (Roderick, Markies and Locarno) were grown under the same conditions in a greenhouse. These lettuce cultivars were provided by Rijk Zwaan Company (De Lier, Netherlands). At the time of the analysis these types of cultivars were encountered most widely on the market. The temperature was set between 15 and 20 °C. Heads of lettuce were selected in a random order when harvested at the stage of commercial maturity. After harvesting, the lettuce was transported in a refrigeration environment to

the laboratory, where the samples were analysed. Each lettuce head (approximately 150 g tissue) was placed in a low-density polyethylene (LDPE) bag (360 mL volume) (Carl Roth, Karlsruhe, Germany) and stored at respectively 0, 2, 4 and 6 °C (humidity 95%) for 15 days in conditioned Climacell 111 chambers (MMM Group, München, Germany). Because LDPE bags were used, modified atmosphere (MA) was not generated. On days 0, 3, 6, 9, 12 and 15 three samples were taken from each storage temperature for analysis. After the outside leaves were removed, the next six non-damaged leaves were carefully removed and 4 x 4 cm midrib segments were excised starting 4 cm from the base of the leaf. Randomised samples of at least three midrib segments were used as replicates in each experiment.

Ascorbic acid content: AA content was determined spectrophotometrically using a UV-VIS spectrophotometer (T80+, PG Instruments, Wibtoft, England). Briefly, samples (3 g) were mixed in cold 5 % trichloroacetic acid and cold mortar and then centrifuged (Hettich Universal 320R, Hettich, Frankenberg, Germany) at 12.100g for 15 min. AA was measured in the supernatant in line with the method described by Spinardi *et al.* (2012) with some modifications. A standard curve with a range from 0 to 10 mg AA was used. AA content was reported as mg of ascorbic acid / 100 g fw.

Total phenolic content: TPC was established spectrophotometrically, using Folin-Ciocalteu's phenol reagent similar to the method reported by Altunkaya *et al.* (2008). Practically, samples (3 g) were homogenised in 9 mL of a solution consisting of distilled water, AA (0.5 %), citric acid (0.05 %), oxalic acid (0.05 %) and cysteine (0.05 %), using an Ultra-Turrax T25 tissue homogeniser (IKA, Staufen, Germany). Samples were taken subsequently at 0, 0.25, 0.5, 1, 2, 4 and 6 h, followed by centrifugation at 15.000g for 15 min. Immediately after this, 100 mL of clear liquid was mixed with 4 mL saturated Na₂CO₃ (75 g/L). After adding 5 mL of 0.2 N Folin-Ciocalteu reagent, the mixture of the reaction was maintained for 30 min at 50 °C in a Binder ED 115 (Binder, Tuttlingen, Germany) ahead of the absorbance measurement at 765 nm using a UV-VIS spectrophotometer (T80+, PG Instruments, Wibtoft, England). TPC was reported as mg gallic acid equivalents/g lettuce (mg GAE/g lettuce) using a calibration curve of gallic acid.

Antioxidant activity: The antioxidant activity of the lettuce samples was determined in accordance with the method described by Altunkaya *et al.* (2008), with some modifications. Samples (3 g) were homogenised in 9 mL of a solution made up of distilled water, AA (0.5 %), citric acid (0.05 %), oxalic acid (0.05 %) and cysteine (0.05 %). Samples were taken from the mixture after 0,

0.25, 0.5, 1, 2, 4 and 6 h. The mixture was centrifuged at 15.000g for 15 min in a centrifuge Hettich Universal 320R (Hettich, Frankenberg, Germany). The supernatant was utilised for the measurement of antioxidant activity. The ABTS concentration was diluted appropriately with distilled water and adjusted to obtain an absorbance reading of 0.70 ± 0.02 at 734 nm and at 30 °C. After 2.95 mL of the diluted ABTS solution was added to 5 µL Trolox standards in ethanol or antioxidant compounds, the absorbance was assessed at 30 °C using a UV-VIS spectrophotometer T80+, after mixing for 6 min using an Ultra-Turrax T25 tissue homogeniser. Solvent blanks were run with each test. The absorbance was read at 734 nm and was plotted and calculated as a function of concentration of antioxidants and Trolox for the standard reference data. The results were recorded as µM Trolox equivalent antioxidant activity/g lettuce (µM TEAC/g lettuce).

Statistical analyses: Factorial analysis of variance (ANOVA) was conducted with cultivar, time and temperature as independent variables. Only two-way interactions were estimated in ANOVA models. All data were described as the means \pm standard error (SE). Analysis of variance denotes 0.95 confidence intervals. Different letters denote significant differences, while means defined by the same letters are not significantly different at $P < 0.05$. At day 0 only one measurement was taken. Therefore, day 0 was not included in statistical analyses but was shown on the graphs. Statistical analysis was measured using STATISTICA v. 11 (Version, 2007).

RESULTS AND DISCUSSION

Ascorbic acid content: AA content levels ranged between 6.47 to 3.80 mg / 100 g fw (Roderick), 5.79 to 4.11 mg / 100 g fw (Locarno) and 4.12 to 2.93 mg / 100 g fw (Markies).

There was no significant difference between 0 and 2 °C for the cultivars, while between 2 and 4 °C, and 4 and 6 °C Roderick and Locarno showed a significant decrease ($P < 0.05$) in AA content, as seen in Figure 1a. AA content was close to the values found for other cultivars of lettuce Nicolle *et al.* (2004); Llorach *et al.* (2008). It is accepted that the content of AA decreases during storage of vegetables, depending on the temperature Ferrante *et al.* (2007).

Figure 1b illustrates the decline of AA content during storage time for all cultivars analysed. Locarno and Markies registered losses of 15.37 % and 17.47 % respectively in the first 6 days, and after 15 days of storage losses were 29.01 % and 28.88 %, respectively. Roderick showed losses of 15.61 % after 6 days, but a more pronounced decrease of AA content, especially from day 6 to day 15 ($P < 0.05$), i.e. 41.27 %.

Similar results were found by Spinardi *et al.* (2012) regarding the AA content of lettuce, which decreased significantly during the first 5 days of storage. Storage determined a reduction in the total AA content, especially at temperatures between 2 and 6 °C.

Total phenolic content: TPC of lettuce cultivars throughout the 15 days of storage ranged from 3.83 to 2.27 (Roderick) mg GAE/ g fw, 3.21 to 2.49 mg GAE/ g fw (Markies), and 2.72 to 2.37 mg GAE/ g fw (Locarno).

As seen in Figure 2a, for Roderick there was a significant difference between 0 and 2 °C and up to 6 °C, while for Markies the difference was significant only between 2 and 4 °C. Locarno showed a significant difference between 0 and 2 °C and 4 and 6 °C.

TPC of the cultivars analysed was close in value compared to those found in studies for other cultivars of lettuce Heimler *et al.* (2007); Llorach *et al.* (2008). Total phenolic content of samples stored between 0 and 2 °C was higher throughout the storage period than for temperatures of 4 to 6 °C, probably because between 0 and 2 °C temperatures reduced plant biochemical processes, such as production of ethylene, respiration or enzyme activity. Even though polyphenol oxidase (PPO) primarily degrades polyphenol compounds, these compounds can also be degraded by peroxidase (POD) Boo *et al.* (2011).

Figure 2b illustrates the decline of TPC during storage time for all cultivars analysed. The progression over time and storage temperature was highly dependent on cultivar ($P < 0.01$), indicating that in all cultivars analysed there was a decrease of phenolic content during storage. Locarno and Markies registered losses of 4.14 % and 9.55 % in the first 6 days, but after 15 days losses ranged from 12.77 % to 22.43 %, while Roderick showed losses of 29.07 % after 6 days, but a more pronounced decrease of total phenolic content of 40.97 %, especially from day 6 to day 15.

This reduction in TPC was explained through research that showed that polyphenols were used as substrates for the PPO enzyme Janovitz-Klapp *et al.* (1990). The reduction in TPC could also be explained by the conversion between free and bound phenolic substances Ferrante *et al.* (2007). Other studies showed an increase in TPC during storage, especially in the early days of storage, when lettuce samples were shred or cut Ferrante *et al.* (2009); Chisari *et al.* (2010).

Antioxidant activity: Antioxidant activity of lettuce cultivars throughout 15 days of storage ranged from 432.03 to 159.28 μM TEAC/g lettuce fw (Roderick), 262.03 to 158.53 μM TEAC/g lettuce fw (Markies), and 390.36 to 246.16 μM TEAC/g lettuce fw (Locarno).

For the Locarno and Roderick cultivars significant differences were observed between 0 and 2 °C and up to 6 °C, while for Markies, although the difference was significant between 0 and 6 °C, it was not

significantly different between 2, 4 and 6 °C ($P < 0.01$), as seen in Figure 3a.

The results of antioxidant activity determined during storage in this study were close in value to those determined for other cultivars of lettuce Llorach *et al.* (2008).

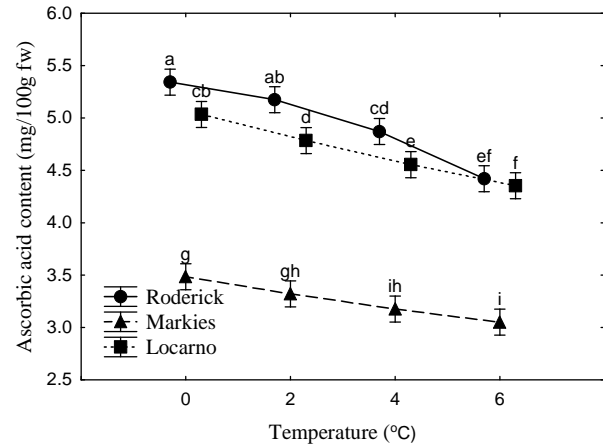


Fig. 1a. Variation of AA content under different storage temperatures determined with analysis of variance (ANOVA). The bars signify the mean of 3 replicates with SE. Different letters denote significant differences and means indicated with identical letters are not significantly different at $P < 0.05$.

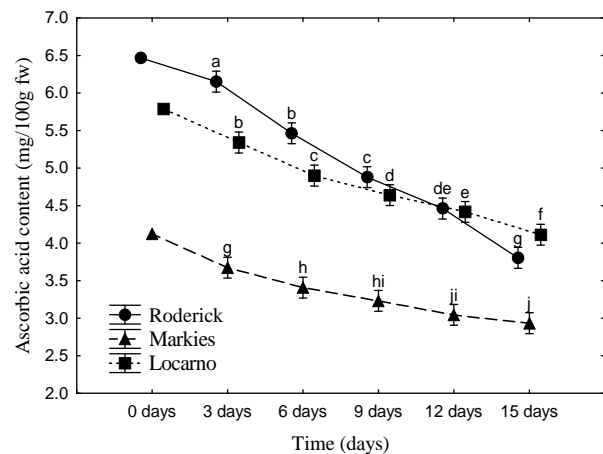


Fig. 1b. Variation of AA content obtained at different days determined with analysis of variance (ANOVA). The bars signify the mean of 3 replicates with SE. Different letters denote significant differences and means indicated with identical letters are not significantly different at $P < 0.05$.

Figure 3b illustrates the decline in antioxidant activity during storage time for all cultivars analysed. Locarno and Markies registered losses between 17.48 % and 21.46 % in the first 6 days, but after 15 days losses ranged from 36.94 % up to 39.5 %, while Roderick showed losses of 31.29 % after 6 days, but a more

pronounced decrease of antioxidant activity of 63.15 %, especially from day 6 to day 15.

The decrease of the content of antioxidants during storage was also reported for other cultivars of lettuce Llorach *et al.* (2008); Dupont *et al.* (2000); Altunkaya *et al.* (2008).

The antioxidant activity analysed for different cultivars of lettuce stored at different temperature conditions could be the result of the synergistic influence of overall phenolic composition and it decreased simultaneously with TPC.

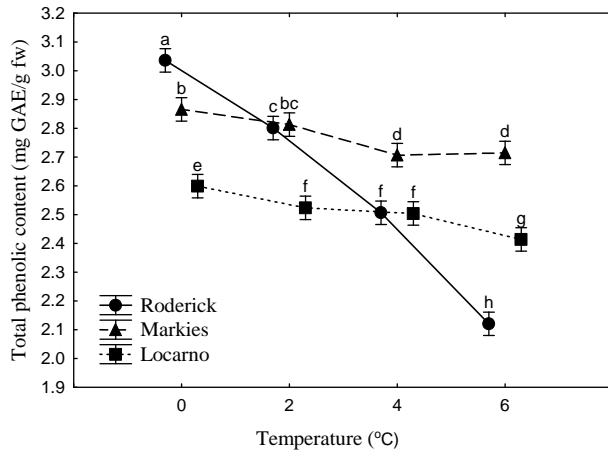


Fig. 2a. Variation of TPC under different storage temperatures determined with analysis of variance (ANOVA). The bars signify the mean of 3 replicates with SE. Different letters denote significant differences and means indicated with identical letters are not significantly different at $P < 0.05$.

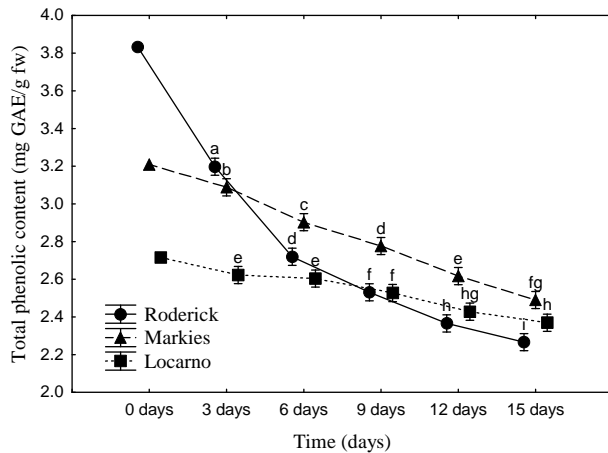


Fig. 2b. Variation of TPC obtained at different days determined with analysis of variance (ANOVA). The bars signify the mean of 3 replicates with SE. Different letters denote significant differences and means indicated with identical letters are not significantly different at $P < 0.05$.

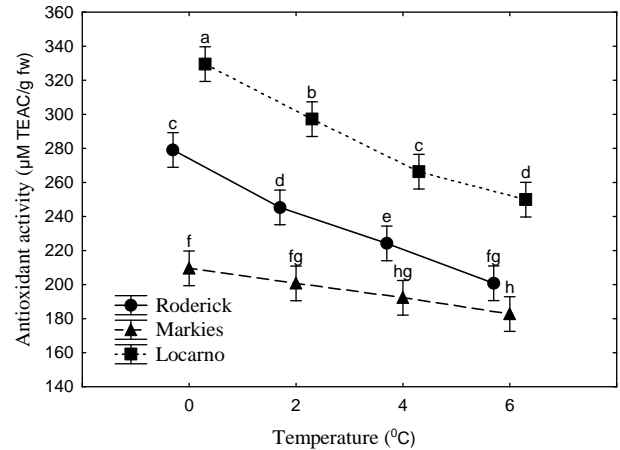


Fig. 3a. Variation of antioxidant activity under different storage temperatures determined with analysis of variance (ANOVA). The bars signify the mean of 3 replicates with SE. Different letters denote significant differences and means indicated with identical letters are not significantly different at $P < 0.05$.

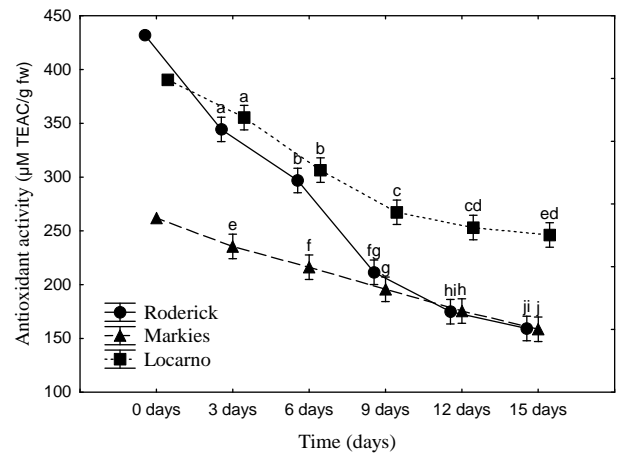


Fig. 3b. Variation of antioxidant activity obtained at different days determined with analysis of variance (ANOVA). The bars signify the mean of 3 replicates with SE. Different letters denote significant differences and means indicated with identical letters are not significantly different at $P < 0.05$.

Conclusion: Locarno and Markies displayed good resistance to storage of AA content, TPC and antioxidant activity. Although Roderick initially had higher values of AA content, after 15 storage days TPC and antioxidant activity showed a pronounced decrease compared to Locarno and Markies.

The importance of this study is that it determines the optimal conditions for storage of lettuce with respect to the effect on the AA content, TPC and antioxidant activity. AA, TPC and antioxidant activity of the cultivars analysed were dependent not only on temperature, but

were also significantly affected by the duration of storage. Major losses were recorded after 6 days and for temperatures above 2 °C for all cultivars.

The outcomes of this study indicate that storage conditions resulting in minimum losses, for AA content, TPC and antioxidant activity, were between 0 and 2 °C and up to 6 days of storage. Similar results were obtained with lamb's lettuce, when the highest value of AA content was obtained within 5 days of storage at 4 °C Ferrante *et al.* (2009). Comparable results were also reported by Gross *et al.* (2004), who recommended that lettuce should be quickly cooled and kept near to 0 °C as possible. The selection of the genotype with the minimum decrease in AA content at storage could be a significant factor in choosing varieties to grow.

Appropriate -storage time and temperature might prevent loss of TPC and a decline of antioxidant activity.

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RERERENCES

- Altunkaya, A. and V. Gökmen (2008). Effect of various inhibitors on enzymatic browning, antioxidant activity and total phenol content of fresh lettuce (*Lactuca sativa*). *Food Chem.*, 107(3): 1173-1179.
- Boo, H. O., B. G. Heo, S. Gorinstein and S. U. Chon (2011). Positive effects of temperature and growth conditions on enzymatic and antioxidant status in lettuce plants. *Plant Sci.*, 181(4): 479-484.
- Chisari, M., A. Todaro, R. N. Barbagallo and G. Spagna (2010). Salinity effects on enzymatic browning and antioxidant capacity of fresh-cut baby Romaine lettuce (*Lactuca sativa* L. cv. *Duende*). *Food Chem.*, 119(4): 1502-1506.
- Dupont, M. S., Z. Mondin, G. Williamson and K. R. Price (2000). Effect of variety, processing, and storage on the flavonoid glycoside content and composition of lettuce and endive. *J. Agric. Food Chem.*, 48(9): 3957-3964.
- Ferrante, A. and T. Maggiore (2007). Chlorophyll *a* fluorescence measurements to evaluate storage time and temperature of *Valeriana* leafy vegetables. *Postharvest Biol. Technol.*, 45(1): 73-80.
- Ferrante, A., L. Martinetti and T. Maggiore (2009). Biochemical changes in cut vs. intact lamb's lettuce (*Valerianella olitoria*) leaves during storage. *Int. J. Food Sci. Tech.*, 44(5): 1050-1056.
- Gross, K. C., C. Y. Wang and M. Saltveit (2004). The commercial storage of fruits, vegetables, and florist and nursery stocks. *Agric. Handbook*, 66, Maryland, (USA) 98 p.
- Heimler, D., L. Isolani, P. Vignolini, S. Tombelli and A. Romani (2007). Polyphenol content and antioxidative activity in some species of freshly consumed salads. *J. Agric. Food Chem.*, 55(5): 1724-1729.
- Hertog, M. G. L., E. J. M. Feskens, D. Kromhout, P. Hollman and M. Katan (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet*, 342(8878): 1007-1011.
- Janovitz-Klapp, A. H., F. C. Richard, P. M. Goupy and J. J. Nicolas (1990). Inhibition studies on apple polyphenol oxidase. *J. Agric. Food Chem.*, 38(4): 926-931.
- Khan, A. S., N. Ahmad, A. U. Malik and M. Amjad (2012). Cold storage influences the postharvest pericarp browning and quality of litchi. *Int. J. Agric. Biol.*, 14(3): 389-394.
- Kyzlink, V., (1990). Principles of food preservation Elsevier; Prague, Czech Republic.
- Lamikanra, O., (2002). Fresh-cut fruits and vegetables: science, technology, and market. CRC Press LLC; Florida, USA.
- Lee, S. K. and A. A. Kader (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biol. Technol.*, 20(3): 207-220.
- Llorach, R., A. Martínez-Sánchez, F. A. Tomás-Barberán, M. I. Gil and F. Ferreres (2008). Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole. *Food Chem.*, 108(3): 1028-1038.
- Nicolle, C., A. Carnat, D. Fraisse, J. L. Lamaison, E. Rock, H. Michel, P. Amouroux and C. Remesy (2004). Characterisation and variation of antioxidant micronutrients in lettuce (*Lactuca sativa folium*). *J. Sci. Food Agric.*, 84(15): 2061-2069.
- Spinardi, A. and A. Ferrante (2012). Effect of storage temperature on quality changes of minimally processed baby lettuce. *J. Food, Agriculture and Environment*, 10(1): 38-42.
- Version, E., (2007). StatSoft, Inc. (2011). Electronic Statistics Textbook. Tulsa, OK: StatSoft. WEB: <http://www.statsoft.com/textbook>.