JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

TRACKING CHANGES IN CHLOROPHYLL AND CAROTENOIDS IN THE PRODUCTION PROCESS OF FROZEN SPINACH PURÉE

Andrea Mendelová[1], Ľubomír Mendel[2], Peter Czako[1], Ján Mareček[1]*

Address(es): Andrea Mendelová,
[1]Slovak University of Agriculture in Nitra, Faculty of Biotechnology and Food Sciences, Department of Plant Processing and Storage, Tr. A. Hlinku 2, 949 76 Nitra, Slovak Republic.
[2]Plant Production Research Center Piešťany, Bratislavská 122, 921 68 Piešťany, Slovak Republic.

*Corresponding author: andrea.mendelova@uniag.sk

ARTICLE INFO

Received 24. 10. 2013
Revised 26. 11. 2013
Accepted 9. 1. 2014
Published 1. 2. 2014

Regular article

OPEN ACCESS

ABSTRACT

Spinach is in the professional and general public considered highly nutritious vegetable with many beneficial effects on human health. It is a rich source of antioxidant active substances, especially chlorophyll, carotenoids, flavonoids and minerals especially zinc and copper. This work studies the changes of chlorophyll and carotenoids that occur after mass production technology of freezing at -37 °C. Before freezing was used blanching operation. In this work we used a variety Boeing, Boa, Beaver, Hudson and Chica. The highest content of all monitored parameters are found in fresh leaves of sampled Hudson. We found that within the processing decreases chlorophyll in 16.6%, 13.8% of chlorophyll b and carotenoids of 6.15%. This decrease was in all cases statistically significant.

Keywords: spinach, freezing, chlorophyll, carotenoids

INTRODUCTION

Chlorophylls and carotenoids are often commonly occurring pigments that give color to many kinds of vegetables. Darker colors tend to mask the chlorophyll carotenoid colors of pale color (**Heaton *et al.*, 1996**). The presence of ohlorophyll derivatives in vegetables is associated with their antioxidant and anti-mutagenic effects (**Ferruzzi and Blakeslee, 2007**). Vegetables high in chlorophyll are used in the prevention of coronary heart disease, certain cancers, diabetes and cataract (**Kitts, 1997**). A similar protective effect against many degenerative diseases also have carotenoids (**Van Der Berg *et al.*, 2000; Cinar, 2004**). The most commonly are associated with a reduced risk of chronic eye diseases, including cataracts and age-related macular degeneration (**Johnson *et al.*, 2000**).
Spinach is in the professional and general public considered highly nutritious vegetable (**Dehkharghanian *et al.*, 2010**). As reported by **Singh *et al.* (2001)** Spinach in all is a rich source of antioxidant active substances, such as carotenoids, flavonoids, chlorophyll and minerals particularly zinc and copper. **Kidmose *et al.* (2001)** shows that lutein, β-carotene, violaxanthin and 9'-(Z)-neoxanthin are the major carotenoids in fresh and processed spinach. **Reif *et al.* (2012)** suggest that the synthesis of carotenoids lutein and β-carotene in spinach is closely associated with the synthesis of chlorophyll and nitrogen uptake and sulfur from the soil. **O'Neill *et al.* (2001)** reported that spinach with parsley leaves belong to the major sources of lutein and β-carotene for the population in many European countries.
Spinach is consumed in the form of fresh leaves or after partial cooking in the form of frozen spinach purée. Frozen spinach purée is primarily used by the majority of consumers. For compliance with proper storage conditions, has a long shelf life and to consumer it is available throughout the year **(Bergquist *et al.*, 2006)**. As reported by several authors (**Pandrang and Laborde, 2004; Choe *et al.*, 2001; Bunea *et al.*, 2008**) even frozen spinach purée as well as fresh Spinach leaves is a rich source of the carotenoid and chlorophyll. Carotenoids during processing have higher stability than chlorophyll (**Calvo and Guillermo, 2008**). It is reported that the production of food operations such as mixing, chopping, freezing, blanching and cooking, can release carotenoids from the food matrix, thus increasing their usefulness in the body (**Hedren *et al.*, 2002**). On the other side, chlorophyll is very susceptible to degradation during processing and storage. Mainly due to higher processing temperatures and acidic pH, takes place as supplement of magnesium from the the chlorophyll molecule and loss of green color. During storage in turn leads to intensive oxidation chlorophyll and carotenoids (**Puuponen-Pimia *et al.*, 2003**).

The aim of this work was to monitor the content of chlorophyll *a* and *b* and total carotenoids in leaves of Spinach and change in content of chlorophyll *a*, chlorophyll *b* and carotenoiof the frozen spinach purée.

MATERIAL AND METHODS

In this work, we used five varieties of Spinach (*Spinacia oleracea* L.). Variety Boeing and Hudson are the early spring varieties with short growing season, characterized by resistance to bolting and flowering with dark extremely green leaves. Variety Chica, Boa and Beaver have a longer growing season, are used for the spring and autumn sowings, as well as the characteristic resistance to bolting and flowering with extremely green leaves.
Samples of plant material were Spinach grown in Želiezovce area. They were harvested at optimum ripeness technology. At the stage of harvesting the crop was fully engaged, balanced, without damaging pests. Plants had developed 7-8 true leaves of different sizes depending on the location and variety. The leaves were vibrant, dark green color. The crop was harvested by single way harvester and was subsequently transported to the processing plant. Leaves were analyzed in the fresh state and after processing frozen spinach purée.
Spinach purée was produced in large-scale manufacturing enterprise. Leaves after the collection and transport to the company were put on the revenue table, followed by sorting, washing, sorting, blanching at 96 °C for 6 minutes, cutting, straining, bottling and freezing in tunnel freezers at -37 °C. Storage of the final product was at a temperature of -18 °C.
The total content of carotenoids were assessed by spectrofotometric device Jenway UV-VIS according to the methodology STN 12136 - Determination of total carotenoids and individual carotenoid fractions. Homogenized samples of spinach and spinach purée are extracted in acetone and then carotenoids were captured in the light petroleum solution. Carotenoids density of the solution was measured at a wavelength of 445 nm. The content of chlorophyll *a* and chlorophyll *b* we determined according to the methodology Environmental Sciences Section 150.1 ESS Chlorophyl spectrofotometric. Chlorophyll dyes are extracted in acetone and then was measured the absorbance of acetone solution at wavelengths between 664 nm and 649.5 nm.
The results were processed by the statistical program Statistica 8.0. Influence of variety and processing of Spinach on carotenoids and chlorophyll content were followed by one-and two-factor analysis of variance (ANOVA) and relative differences between varieties and forms of spinach were tested with Fisher's LSD test.

RESULTS AND DISCUSSION

The values of chlorophyll *a* in samples spinach leaves ranged from 0.398 mg.g^{-1} in a sample of spinach Boa from 0.596 mg.g^{-1} in a sample of Hudson. The content of chlorophyll *a* in samples decreased in the order of Hudson> Chica> Beaver> Boeing> Boa (Figure 1). Chlorophyll *b* content in the samples decreased in the order of Hudson> Chica> Beaver> Boeing> Boa (Figure 2).

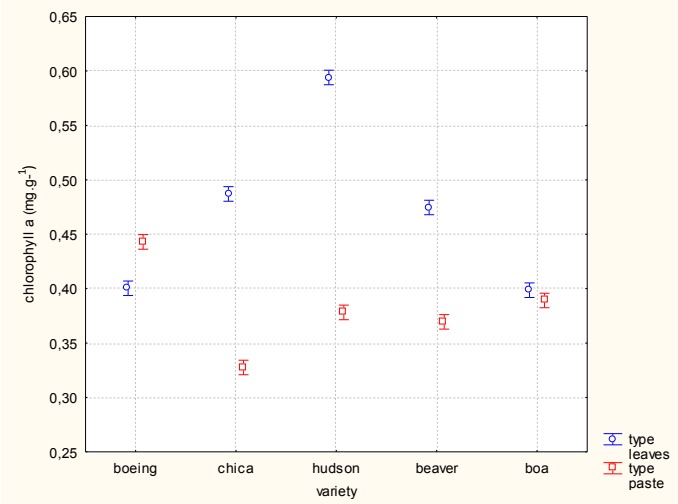

Figure 1 Comparison of marginal averages of chlorophyll *a* in leaves of fresh spinach and spinach purée

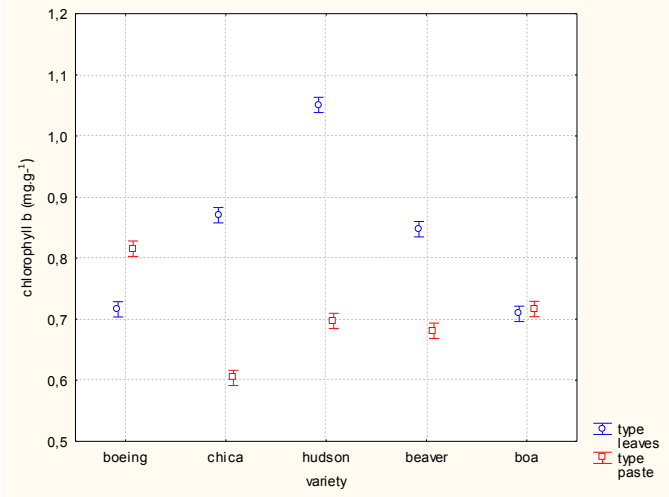

Figure 2 Comparison of marginal averages of chlorophyll *b* in fresh spinach and spinach purée

Andrejiová, Mendelová (2012) investigated the content of chlorophyll *a*, *b* in samples of Spinach varieties Ballet F1, Bolero, Emilia, Matador, Monores, Orbita F1, Rembour F1, F1 Rembrandt and Winterriesen from years 2011 and 2012. Higher values were found in the year 2011, from 0.514 to over 0.636 mg.g^{-1}, the values were slightly higher than we found in our work. In 2012, the chlorophyll *a* content from 0.315 to 0.428 mg.g^{-1}. As I mentioned pinach from the year 2011 the authors indicate chlorophyll *b* content from 0.923 to 1.142 mg.g^{-1} The content of chlorophyll *b* in Spinach year from the 2012 was 0.59 mg.g^{-1} in the Orbit F1 to 0.747 mg.g^{-1} in the a variety Monores, which are lower than the values we found in our work**. Larsen and Christensen (2005)** show that high levels chlorophyll in the parsley and cabbage in all kinds of vegetables. **Kopsell *et al.* (2003)** found that chlorophyll *a* content in fresh spinach is 2.70 to 4.88 mg.g^{-1} dry weight, depending on the variety and the year of production. Chlorophyll *a* content in our samples of spinach to a dry weight basis was from 4.144 mg.g^{-1} dry matter (Boeing) to 6.620 mg.g^{-1} dry matter (Hudson). Chlorophyll *b* was 7.369 mg.g^{-1} dry matter (Boeing) to 11.708 mg.g^{-1} dry matter (Hudson).
The content of total carotenoids in samples of Spinach leaves ranged from 18.005 mg.100 g^{-1} in the sample Boa to 37.734 mg.100 g^{-1} in the sample Hudson. The content of carotenoids in samples decreased in the order of Hudson> Beaver> Chica> Boeing> Boa (Figure 3).

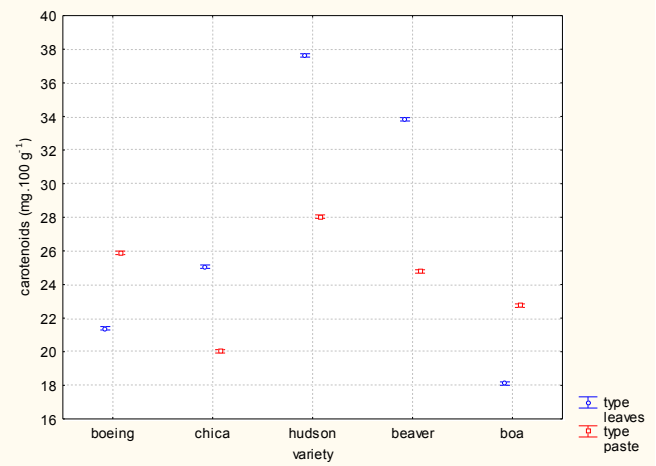

Figure 3 Comparison of marginal averages of carotenoids content in fresh spinach and spinach purée

The issue of evaluation of carotenoids content in the different genotypes of spinach divided into groups by color also addressed **Singh *et al.* (2001)**. They found that the highest content of carotenoids was in spinach with dark green foliage, an average of 22.63 mg.100 g^{-1} of fresh matter. The lowest number of genotypes were analyzed in the the lightest green leaves and an average of 17.66 mg.100 g^{-1}. These values correspond well with our findings in samples Boa, Boeing and Chica. In samples Beaver and Hudson, we analyzed above values of carotenoids. The authors investigated the content of carotenoids in these kinds of spinach genotypes and found that the content of β-carotene, lutein and neoxantínu was significantly highest in the dark green genotypes.
Bunea *et al.* (2008) analyzed the content of total carotenoids in leaves of Spinach by gas chromatography and spectrophotometry. By spectrophotometric method carotenoid content was found in the fresh samples of 11.9 mg.100 g^{-1} and by HPLC method it was 12.7 mg.100 g^{-1}. For the analysis of samples was used the same plant material. The authors also observed content of carotenoids and found that in the most spinach is lutein (52.0 mg.100 g^{-1}), β-carotene (31.5 mg.100 g^{-1}), violaxantine (26.6 mg.100 g^{-1}) and neoxantine (15.3 mg.100 g^{-1}).
Two-factor analysis of variance, we clearly confirmed the influence of varieties on the contents of all endpoints. Testing Fisher's LSD test ($p < 0.05$) we have in the case of chlorophyll *a* and chlorophyll *b* just found out their differences in copper pursued in all varieties with the exception of the varieties Boeing and Beaver. When evaluating the differences in the content of carotenoids with Fisher's LSD test ($p < 0.05$) we found statistically significant differences between all assessed varieties (Table 1).

Table 1 The average values of chlorophyll *a*, *b* and of carotenoids in leaves of Spinach based on Fisher's LSD test

Variety	chlorophyll *a* (mg.g^{-1})	chlorophyll *b* (mg.g^{-1})	carotenoids (mg.100 g^{-1})
boa	0.39a	0.71a	20.43a
chica	0.41b	0.74b	20.54b
boeing	0.42c	0.77c	23.64c
beaver	0.42c	0.76c	29.30d
hudson	0.49d	0.87d	32.84e

means indicated by the same letter are insignificantly different at $p<0.05$

The most common product of canning Spinach is frozen spinach purée. The key measures of the quality of the product in addition to hygiene and safety, high nutritional value also good sensory properties. Sensory properties of the emphasis placed primarily on the desired intense dark green color, which is proportionally dependent on the content of chlorophyll (**Lisiewska, *et al.*, 2004; Ferrante *et al.*, 2004**).
The content of chlorophyll a and frozen spinach puree of our samples ranged from 0.327 mg.g^{-1} following 0.443 mg.g^{-1}. The highest content was measured in a variety Boeing and lowest in variety Chica (Figure 1). Chlorophyll *b* content in the samples was 0.604 mg.g^{-1} to 0.816 mg.g^{-1} (Figure 2). The highest content just had a variety of Boeing and lowest in variety Chica. The content of chlorophyll *a* was higher than chlorophyll *b*, as in the case of fresh samples. This finding is consistent with the work of **Kidmose *et al.* (2001)**, which followed the contents of carotenoids, chlorophyll and flavonoids in samples of frozen spinach.
Carotenoid content in our samples of frozen spinach puree ranged from 19.968 mg.100 g^{-1} in the sample of Chica to 28.007 mg.100 g^{-1} in the sample of Hudson. The content of carotenoids in samples decreased in the order of Hudson> Boeing> Beaver> Boa> Chica (Figure 3).
One aim of the study was to monitor changes in the content of chlorophyll *a* and chlorophyll *b* following large-scale treatment freezer. As reported by **Heaton *et***

***al.* (1996)** Chlorophyll is very susceptible to degradation during processing and storage, the intensity of decomposition depends on many factors including the temperature of processing, the change in pH of the medium, the processing time, the impact of enzymes, oxygen and light.

We found that after the freezing, the mean decrease in chlorophyll *a* was in 16.6% and a decrease in chlorophyll *b* was in 13.78%. The decline occurred in all varieties with the exception of the variety Boeing, in which we found that after the blanching there was increase in chlorophyll.

Statistical processing of the data obtained on the content of chlorophyll *a* and chlorophyll *b* in Spinach leaves and spinach puree by two-factor analysis of variance, we found statistically significant effect forms of Spinach, fresh leaves and frozen purée on the contents of chlorophyll *a* and also chlorophyll *b* (Table 2).

Table 2 The average values of chlorophyll *a*, *b* and of carotenoids in leaves of Spinach and spinach purée based on Fisher's LSD test

Form	chlorophyll a ($mg.g^{-1}$)	chlorophyll b ($mg.g^{-1}$)	carotenoids ($mg.100\ g^{-1}$)
Leaves	0.47a	0.84a	27.21a
purée	0.38b	0.70b	24.29b

means indicated by the same letter are insignificantly different at $p<0.05$

Bahceci *et al.* (2005) indicate that the processing of blanching vegetables may disable particular enzymes that break phytochemicals depending on the duration and temperature used. However, during blanching may occur also undesirable discoloration, altered taste, texture and nutritional quality.

Choe *et al.* (2001) found that the rapid and gentle spinach blanching in an aqueous solution for 2 minutes may result in increase in chlorophyll content in spinach by an average of 8%. The authors attribute this increase in chlorophyll release of bonds after the thermal denaturation of the protein components of dry matter, which is bound to chlorophyll. The authors further state that the increase in blanching for 20 minutes or more may cause degradation of chlorophyll by up to more than half of the initial quantity. Inappropriate as a method of blanching before freezing as regards the stability of chlorophyll stated blanching identified by autoclaving at 121 °C, when there was a decrease of up to 62%.

Our findings of small losses of chlorophyll after the freezing in most of the samples correspond to the the findings of **Jaworska and Kmiecik (2000)** and **Jaworska *et al.* (2001)**, who point out that technically flawless freezing Spinach and also New Zealand Spinach does not cause significant analytical and free eye observable loss of chlorophyll.

Changes in chlorophyll content during freezing and in other vegetables were also addressed by other authors. **Kidmose and Kaack (1999)** observed changes in the asparagus, **Korus (2012)** in the kohlrabi and sprouts. Even these authors came to the same conclusion that freezing is a method of processing very friendly to the maintenance of chlorophyll. **Lisiewska *et al.* (2004)** state that for preservation of chlorophyll and carotenoids during refrigerated storage positively affect the proper way of blanching vegetables before freezing. In their work they monitor changes in chlorophyll content in the dill fragrant during refrigerated storage. In blanching sample detected chlorophyll loss during storage of frozen product only 4-9%.

After blanching and freezing in our samples was preserved in average 94,85% of total carotenoids. The decline was recorded in content of the samples Chica, Hudson and Beaver. An increase was observed in samples Boa and Boeing. As is well known carotenoids are relatively stable due to the heat treatment and on the other hand sensitive to oxidation with oxygen.

Statistical processing of the data obtained the carotenoid content in fresh leaves of Spinach and frozen spinach puree by two-factor analysis of variance, we found statistically significant effect of forms of Spinach, ie Fresh leaves and frozen purée on contents of carotenoids. Fisher's LSD test similarly to the chlorophylls found that fresh Spinach leaves have a statistically significantly higher content of total carotenoids (Table 2).

As reported by **Kim *et al.* (2003)** carotenoids are in comparison to chlorophyll more stable after the heat treatment. **Choe *et al.* (2001)** investigated and compared the changes in the content of the most important colors of spinach after the freezing and after the blanching in different ways. The most appropriate way of blanching with regard to preservation of the carotenoid selected blanching in an autoclave at 121 °C, when in the samples was preserved to 8.48% β-carotene and lutein 97.45%. To preserve chlorophyll, this method was determined to be the least appropriate. As at least appropriate method of heat treatment prior to freezing to maintain β-carotene authors identified blanching in hot steam for 5 minutes when there was a decrease in β-carotene 58.2%. High losses authors ascribe to the effect of severe oxidative action of steam, which is used for heat treatment.

Korus (2012) found that after the freezing of cabbage vegetables there are changes in the content of total carotenoids by an average of 10-11%. **Dutta *et al.* (2005)** point out, however, that the loss of the carotenoid usually does not occur during manufacturing, freezing and freezer storage, but just in wrong time of slow defrosting before final processing of culinary vegetables, spinach not excepting.

Changes in cartenoid content in spinach leaves during cold storage were followed by **Pandrang and Laborde (2004)** and they found that storage at 10 °C for 6 days, results in degradation of the carotenoid up to 50%. In this respect, we can thus clearly identify the production of purée and freezing as a more appropriate method for extend of the shelf life of spinach.

CONCLUSION

Spinach is one of the most nutritionally important species of leafy vegetables. The bulk of the production of spinach leaves are processed by freezing for spinach purée. The aim of this work was to identify changes in the content of chlorophyll *a* and chlorophyll *b* and total carotenoids in the samples of five varieties of Spinach. Spinach was grown in Želiezovce and was treated by freezing at -37 °C after the previous blanching. In this work, we found the highest content of all monitored components (chlorophyll *a*, chlorophyll *b*, total carotenoids) in variety Hudson. During processing comes to decomposition and subsequent decrease in chlorophyll content. The average decrease in chlorophyll *a* was 16.6% and 13.8% of chlorophyll *b*. The decline we recorded in all varieties with the exception of the variety Boeing. In this work we have also detected a decrease in the content of carotenoids, an average of 6.15% compared to fresh samples. The decrease was found in varieties Chica, Hudson and Beaver. An increase was observed in the samples of Boa and Boeing.

Acknowledgments: This study was supported by KEGA Cultural and Educational Grant Agency no. 024SPU-4/2013.

REFERENCES

ANDREJIOVÁ, A., MENDELOVÁ, A. 2012. Effect of variety on the chlorophyll conten in fresh leaves of spinach (*Spinacia oleracea* L.) and spinach purée. *Horticulture Nitra 2012. International reviewed proceedings of scientifi papers*, Nitra : SUA, p. 23-27.

BAHCECI, K. S., SERPEN, A., GOKMEN, V., ACAR, J. 2005. Study of lipoxygenase and peroxidase as indicator enzymes in green beans: Change of enzyme activity, ascorbic acid and chlorophylls during frozen storage. *Journal of Food Engineering*, 66(2), 187–192. ISSN 0260-8774 http://dx.doi.org/10.1016/j.jfoodeng.2004.03.004

BERGQUIST, S. A. M., GERTSSON, U. E., OLSSON, M. E. 2006. Influence of growth stage and postharvest storage on ascorbic acid and carotenoid content and visual quality of baby spinach (*Spinacia oleracea* L.). *Journal of the Science of Food and Agriculture*, 86(3), 346-355. ISSN 1097-0010 doi 10.1002/jsfa.2373

BUNEA, A., ANDJELKOVIC, M., SOCACIU, C., BOBIS, O., NEACSU, M., VERHE´ R., VAN CAMP, J. 2008. Total and individual carotenoids and phenolic acids content in fresh, refrigerated and processed spinach (*Spinacia oleracea* L.). *Food Chemistry,* 108(2), 649-656. ISSN 0308-8146 http://dx.doi.org/10.1016/j.foodchem.2007.11.056

CALVO, M. M.,GUILLERMO, S. M. 2008. Effect of illumination and chlorophylls on stability of tomato carotenoids. *Food Chemistry,* 107(4), 1365–1370. ISSN 0308-8146 http://dx.doi.org/10.1016/j.foodchem.2007.09.059

CINAR, I. 2004. Carotenoid pigment loss of freeze-dried plant samples under different storage conditions. *LWT. Food Science and Technology,* 37(3), 363–367. http://dx.doi.org/10.1016/j.lwt.2003.10.006

DEHKHARGHANIAN M, HERVE A, MOOKAMBESWARAN A. V. 2010. Study of flavonoids in aqueous spinach extract using positive electrospray ionisation tandem quadrupole mass spectrometry. *Food Chemistry*, 121(3), 863-870. ISSN 0308-8146 http://dx.doi.org/10.1016/j.foodchem.2010.01.007

DUTTA, D., CHAUDHURI, U. R., CHAKRABORTY, R. 2005. Structure, health benefits, antioxidant property and processing and storage of carotenoids. In *African Journal of Biotechnology*, **4(13),** 1510–1520. ISSN 1684-5315

FERRANTE, A., INCROCCI, L.,MAGGINI, R., SERRA, G., TOGNONI, F. 2004. Colour changes of fresh-cut leafy vegetables during storage. *Journal Food, Agriculture and Environment*, 2(3-4), 40-44. ISSN:1459-0255

FERRUZZI, M. G., BLAKESLEE, J. 2007. Digestion, absorption, and cancer preventative activity of dietary chlorophyll derivatives. *Nutrition Research*, 27(1), 1-12. ISSN: 0271-5317 http://dx.doi.org/10.1016/j.nutres.2006.12.003

HEATON, J., W., LENCKI, R., W., MARANGONI, A., G. 1996. Kinetic model for chlorophyll degradation in green tissue. *Journal of Agricultural and Food Chemistry,* 44(2), 399-402. ISSN 0021-8561. doi 10.1021/jf950448x

HEDREN, E., DIAZ, V., SVANBERG,U. 2002. Estimation of carotenoid accessibility fromcarrots determined by an in vitro digestionmethod. *European Journal of clinical nutrition*, 56(5), 425-430. ISSN: 0954-3007 PMID:12001013

CHOE, E. O., LEE, J., PARK, K., LEE, S. 2001. Effects of heat pretreatment on lipid and pigments of freeze-dried Spinach. *Food Chemistry and toxicology*, 66(8), 1074-1080. ISSN: 0278-6915

JAWORSKA, G., KMIECIK,W. 2000. Comparison of the nutritive value of frozen spinach and New Zealand spinach. *Polish Journal of Food and Nutrition Sciences*, 3(9), 79-84. ISSN 1230-0322

JAWORSKA, G., KMIECIK,W. MACIEJASZEK, I. 2001.Comparison of the quality of canned spinach (*Spinacia oleracea* L.) and New Zealand spinach (*Tetragonia expansa* Murr.). In *Polish Journal of Food and Nutrition Sciences*,

4(2), dostupné na: http://www.ejpau.media.pl/volume4/issue2/food/art-04.html ISSN 1505-0297

JOHNSON, E. J.,HAMMOND, B. R., YEUM, K. J., QIN, J.,WANG, X. D., CASTANEDA, C., SNODDERLY, D. M., RUSSELL, R. M. 2000. The relationship among serum, tissue and macular pigment concentrations of lutein and zeaxanthin. *American Journal Clinical of Nutrition*, 71(6), 1555-1562. ISSN: 1938-3207 PMID:10837298

KIDMOSE, U., KAACK, K. 1999. Changes in texture and nutritional quality of green asparagus spears (*Asparagus offìcinalis* L.) during microwave blanching and cryogenic freezing. *Acta Agriculturae Scandinavica B,* 49(2), 110-116.

KIDMOSE, U., KNUTHSEN, P., EDELENBOS, M., JUSTESEN, U., HEGELUNG, E. 2001. Carotenoids and flavonoids in organically grown spinach genotypes after deep frozen storage. *Journal of the Science of Food and Agriculture,* 81(9), 918-923. ISSN: 1097-0010 doi 10.1002/jsfa.902

KIM M., J., LEE, J., Y., CHOE, E., O. 2003. Effects of the number of fryings on pigment stability in frying oil and fried dough containing spinach powder. *Journal of Food Science*, 68(3), 866–869. DOI: 10.1111/j.1365-2621.2003.tb08257.x

KITTS, D. D. 1997. An evaluation of themultiple effects of the antioxidant vitamins. *Trends in Food and Science Technology*, 8(6), 198-203. http://dx.doi.org/10.1016/S0924-2244(97)01033-9

KOPSELL, D. E., KOPSELL, D. A., RANDLE, W. M., COOLONG, T. W., SAMS, C. E., CURRAN-CELENTANO, J. 2003. Kale carotenoids remain stable while flavor compounds respond to changes in sulfur fertility. *Journal of Agricultural and Food Chemistry,* 51(18), 5319-5325. ISSN 0021-8561 doi 10.1021/jf034098n

KORUS, A. 2012. Effect of preliminary and technological treatments on the content ofchlorophylls and carotenoids in kale (*Brassica oleracea* L. var. *acephala*). *Journal of Food Processing and Preservation,* 36(3), 1-10. ISSN: 1745-4549 doi 10.1111/j.1745-4549.2011.00653.x

LARSEN, E., CHRISTENSEN, L. 2005. Simple saponification method for the quantitative determination of carotenoids in green vegetables. *Journal of Agricultural and Food Chemistry*, 53(17), 6598–6602. ISSN 0021-8561 PMID: 16104772

LISIEWSKA, Z., KMIECIK,W., SLUPSKI, J. 2004. Contents of chlorophylls and carotenoids in frozen dill: Effect of usable part and pre-treatment on the content of chlorophylls and carotenoids in frozen dill (*Anethum graveolens* L.), depending on the time and temperature of storage. *Food Chemistry*, 84,(4), 511-518. ISSN 0308-8146 http://dx.doi.org/10.1016/S0308-8146(03)00265-6

O´NEILL, M. E., CARROLL, Y., CORRIDAN, B., OLMEDILLA, B.,80 GRANADO, F., BLANCO, I., BERG, H., HININGER, I., ROUSELL, A., M., CHOPRA, M., SOUTHON, S., THURNHAM, D. I. 2001. European carotenoid database to assess carotenoid intakes and its use in a five comparative study. *British Journal of Nutrition*, 85(4), 499-507. ISSN: 0007-1145. PMID: 11348565

PANDRANG, I. S., LABORDE, L. F. 2004. Optimization of microbiological assay of folic acid and determination of folate content in spinach. *International Journal of Food Science and Technology*, 39, 525–532. doi:10.1111/j.1365-2621.2004.00812.x

PUUPONEN-PIMIA, R., HAKKINEN, S. T., AARNI, M., SUORTTI, T., LAMPI, A. M., EUROLA, M., PIIRONEN, V., NUUTILA, A. M., OKSMAN-CALDENTEY, K.-M. 2003. Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. *Journal of the Science of Food and Agriculture*, 83(14), 1389-1402. ISSN: 1097-0010 doi 10.1002/jsfa.1589

REIF, C., ARRIGONI, E., NEUWEILER, R., BAUMGARTNER, D., NYSTRÖM, L., HURRELL, R. F. 2012. Effect of Sulfur and Nitrogen Fertilization on the Content of Nutritionally Relevant Carotenoids in Spinach (*Spinacia oleracea*). *Journal of Agricultural and Food Chemistry*, 60(23), 5819–5824. ISSN 0021-8561 doi 10.1021/jf301114p

SINGH, G., KAWATRA, A., SEHGAL, S. 2001. Nutritional composition of selected green leafy vegetables, herbs and carrots. *Plant food human nutrition*, 56(4), 359-364. ISSN: 1573-9104 PMID: 11678441

VAN DER BERG, H., FAULKS, R.,GRANADO, F.H., HIRSCHBERG, J., OLMEDILLA, B., SANDMANN, G., SOUTHON, S., STAHL,W. 2000. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *Journal of the Science of Food and Agriculture*, 80(7), 890-912. doi 10.1002/(SICI)1097-0010(20000515)80:7<880::AID-JSFA646>3.0.CO;2-1