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Impact of some domestic processing on Nitrate, Nitrite and Oxalates contents of selected leafy vegetables

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Abstract

The objective of this study was to identify the changes of certain toxic compounds in some leafy vegetables, which commonly consumed in Upper Egypt. Spinach contained the highest concentration (3837.5 ppm) of nitrate while coriander leaves had the lowest value (575.28 ppm). Nitrite content was higher in cabbage leaves (95.85 ppm) than other leafy vegetables. Spinach and chard leaves contained the highest levels of oxalate (40.11 and 42.38 mg/g "DW", respectively) than other fresh vegetables. Ordinary cooking process led to a significant loss of nitrate and nitrite contents of leafy vegetables under study. The losses of nitrite content were 74.12, 35.13 and 74.12% of its initial values in spinach, cabbage and green Jew’s mallow, respectively. However, the loss of oxalate in cooked leafy vegetables varied from 33% in spinach to 100% in cabbage of its initial raw material values. Effect of blanching and freezing on nitrate, nitrite and oxalate contents of some selected leafy vegetables investigated. The loss of nitrate as result of blanching and freezing processes was: 42.3, 49.1 and 54.6% of its initial values in spinach, cabbage and Jew’s mallow, respectively. From the obtained results, it could be observed that the highest loss of oxalate was recorded in blanched frozen cabbage leaves which reaching 100%, followed Jew’s mallow by 58% and spinach by 30% of its initial values.

Keywords:
Nitrate – Nitrite – Oxalate - leafy vegetables.

INTRODUCTION

Vegetables play an important role in human nutrition since they are an outstanding source of vitamins, minerals and biologically active compounds (Kmiecik *et al*., 2004). Leafy vegetables usually consumed in raw and cooked forms. About 87% of the total nitrate concentration in a normal diet believed to be a direct result of vegetable intake (Huarte-Mendicoa *et al.*, 1997). Nitrate is the ionic form, which supplements the essential plant nutrient, nitrogen (Cieslik and Sikora, 1998). Since nitrogen plays a key role in plant growth, the most readily available agricultural fertilizers contain nitrate. It has been discovered that green leafy vegetables (such as lettuce, spinach, radishes, celery, etc.) contain the highest levels of nitrates (MAFF, 1998). Leafy vegetables are known to provide a significant portion of the nitrates in our diet. Approximately 5% of all dietary nitrates reduced to nitrites in saliva and the gastrointestinal tract (Santamaria, 2006). Nitrites being highly unstable, it can be metabolize within the digestive tract to N-nitroso compounds (MAFF, 2001). Nitrosamines produced through acid catalysis of nitrite with certain nitrogen compounds are carcinogenic and volatile (Ezeagu, 1996 and Perez-Olmos *et al*., 1997). Young babies with low stomach acidity may suffer from infantile methemoglobinemia due to excessive nitrates in their diet, where nitrite is substitute for oxygen in hemoglobin and death may occur (Ezeagu, 1996). Benefits of High content of inorganic nitrate, which in concert with symbiotic bacteria in the oral cavity is converted into nitrite, nitric oxide, and secondary reaction products with vasodilating and tissue-protective properties (Lundberg *et al.,* 2006). Nitrite, when added to foods like pure meat, has at least three uses: First, it helps to improve taste. Second, brings in pink-colored meat, and thirdly, inhibits the growth of corrosive bacteria and, most importantly, controls *Clostridium botulinum.* "*C. Botulinum*" grows under anaerobic conditions (Cammack et al., 1999). Studies have shown that nitrate and nitrite content in conventional cooking reduces content by 38.79 - 74.03%. Low nitrate level (Vahed *et al.* 2015). In the vegetables stored frozen for 4 months, previously blanched, generally a decrease was noted in the nitrate, and an increase in the nitrite level compared to the levels in the blanched vegetables (Leszczyn *et al.*, 2009). The nitrite concentrations in fresh, undamaged vegetables are usually very low but under adverse post-harvest storage conditions nitrite concentration can increase in vegetables as a result of bacterial or endogenous nitrate reeducates reducing the nitrate to nitrite (WHO; 2003). Consumption of oxalate containing foods (vegetables) can have two major effects on human health. First, oxalic acid can form insoluble salts by binding to cations (e.g. calcium, iron, magnesium) in the foods and, thus, decrease the bioavailability of these essential minerals (Noonan and Savage, 1999). These insoluble oxalate salts are excreted in the faeces (Marengo and Romani, 2008). Secondly, once absorbed, soluble oxalate cannot be used in the body and needs to be excreted. The soluble oxalic acid in urine may bind to calcium and become insoluble. This calcium oxalate can accumulate in the kidneys and lead to kidney stone formation (Chai and Liebman, 2005). Previous studies have found that oxalate can bind with calcium ions to form calcium oxalate crystals in the mouth (Perera *et al.*, 1990). Thus, the purpose of this study was to investigate the effects of cooking, blanching conditions and frozen storage on the changes of nitrate, nitrite and oxalate contents in some leafy vegetables consumed in Upper Egypt.

MATERIALS AND METHODS

Materials

1. Leafy vegetables

Spinash (*Spinacia oleracea*), Jew's mallow *(Corchorus olitorius)*, Cabbage (*Brassica oleracea var. capitata*), Dill (*Anethum graveolens*), Lettuce (*Lactuca sativa*), Chard (*Beta vulgaris var. cicla*), Parsely (*Petroselinum crispum*), Rocket salad (*Eruca vesicaria ssp. Sativa*) and coriander (*Coriander sativum*). All samples were purchased from local markets in Sohag Governorate. Fresh vegetables were obtained during the season of 2019 and were representative of quality of those available at that season. Vegetable samples were purchased from local markets in Sohag city, Egypt.

2. Chemicals

Aluminium potassium sulphate, glacial acetic acid, sulphanilic acid, 1-naphthol, cadmium sulfate, Zn powder, Potassium permanganate, aqueous methanol, Folin-Ciocalteu and gallic acid were obtained from Sigma (Germany). Other chemicals were purchased from Al-Gomhoria company, Egypt.

Methods

1. Technological Methods

a. Cooking process

Samples prepared for normal consumption and cooked in boiling distilled water (1:2 w/v) for different times according to the type of sample. Leafy vegetables were cooked at 90^{o}C for 5 min, but pulses vegetables were cooked for 10 min. After cooling the samples, nitrates and nitrites are determined directly.

b. Blanching and Freezing of vegetables

Frozen samples were prepared using a deep freezer, blanched in distilled boiled water (90^{o}C) for 1 min to leafy vegetables and for 5 min to pulses vegetables. After cooling the samples, nitrates and nitrites are determined directly and packaged in polyethylene bags, frozen at -18 to -20°C, were stored for 6 months and the analysis was carried out every month.

2. Analytical methods

a. Sample preparation

Fresh samples were cleaned, trimmed, peeled and washed with water and finally cut in small pieces and quickly used for chemical analysis. Cooked and blanched samples were cooled and minced, then used for chemical analysis.

b. Moisture content

Moisture content was determined using an air oven at 105^{o}C for 3 hrs until a constant weight was obtained according to A.O.A.C. (2005).

c. Preparation of extracts

Fresh vegetables were prepared as for normal consumption, then sliced or cut into small pieces. Frozen samples were thawed and minced before analysis. Samples extracted according to the method of Kamm, *et al.* (1965). A 2 g sample mixed with 5 ml of ammonia buffer solution (pH 9.6 - 9.7) and then 50 ml of alumina cream (saturated aqueous solution of aluminium potassium sulphate and ammonium hydroxide) were added, followed by 50 ml of distilled water. The mixture was shaken for 5 min. on a Waring Blinder. Distilled water was added to the mixture until the total volume reached 200 ml and then it was filtered through Whatman No. 4 filter paper. Nitrite and nitrate were determined in the filtrate.

d. Nitrate and nitrite determination

Reagents

(A) *Ammonia buffer solution (pH 9.6 - 9.7):* Twenty milliliters of concentrated HC1 were added to 500 ml of water, then 50 ml of aqueous ammonia solution (sp. gr. 0.880) were added and the solution was diluted to 1 liter.

(B) *Alumina cream:* Saturated aqueous solution of aluminium potassium sulphate and ammonium hydroxide.

(C) *Orange I reagent*: 50 ml of glacial acetic acid and 360 ml of distilled water were warmed to 50 °C and poured into a dark glass reagent bottle (600 ml) containing 0.25 g of powdered sulphanilic acid and shaken until dissolved. Then 0.20 g of 1-naphthol were added and dissolved by shaking. The solution was cooled and 90ml of 10% aqueous ammonia solution were added. The pH should be 4.0 ± 0.05.

Nitrite determination

The nitrite content was determined colorimetrically according to the method described by Abu-Dayeh, *et al.* (2006). Standard curve was prepared using sodium nitrite solution.

Nitrate determination

The nitrate content was determined by reduction nitrate to nitrite using the spongy metallic cadmium and then nitrite was determined as described before. The reduction and determination were carried out according to the method described by Cortesi, *et al.* (2015).

Oxalates determination

Samples were drying according to the method described by Sallam and Anwar (2017). Plant was cut into pieces then dried in oven at 45ºC for 72 h and ground to fine powder. Powder was packed in polyethylene bags and stored at - 4^{o}C, until used for analyses.The titration method (Day and Underwood, 1986) described as follow: 0.1g of food powder sample was weighed into 100ml conical flask. 75ml 3MH_2SO_4 was added and stirred for 1 hr with a magnetic stirrer. This was filtered using a Whatman No 1 filter paper. 25ml of the filtrate was then taken and titrated while hot against 0.05 M $KMnO_4$ solution until a faint pink colour persisted for at least 30 sec. The oxalate content was then calculated by taking 1 ml of 0.05 M $KMnO_4$ as equivalent to 2.2 mg oxalate (Chinma, and Igyor, 2007; Ihekoronye and Ngoddy, 1985).

Statistical analysis

Data were statistically analyzed by SAS statistical software (SAS ver. 9.2, SAS Institute, 2008). The LSD at 5% significant level was calculated according to Petersen (1985), for comparing mean values of studied traits.

RESULTS AND DISCUSSION

Nitrate, nitrite and oxalate in fresh leafy vegetables

The results in Table (1) show nitrate, nitrite and oxalate content in some selected fresh leafy vegetables. The means nitrate content of the nine fresh leafy vegetables ranged from 575.28 to 3837.5 ppm. The results showed that spinach contained the highest concentration of nitrate (3837.5ppm.) followed by Jew's mallow (2837.5ppm.) while coriander leaves had the lowest concentration (575.28ppm.). Cabbage leaves contained the highest concentration of nitrite (95.85ppm), while the lowest level was in rocket salad leaves. It is evident from the in Tables (1) that there is considerable variation of nitrate and nitrite contents between different samples of vegetables. The results are in the line with those reported by Wang *et al.,* (2018). The environmental conditions, nitrogen fertilization regime, temperature, and use of herbicides can also increase the plant nitrate content (Iammarino *et al.*, 2014). The high variability shown is satisfactory since vegetable nitrate content is known to vary extensively as reported in literature (Van der Schee and Speek, 2000). There is a general accord amid researchers that vegetables grown in low irradiance high temperature zones accumulate more nitrate-N (Cardenas-Navarro *et al.,* 1999). On the other hand, spinach and chard leaves contained the highest concentration of oxalate (40.11 and 42.38mg/g, on dry weight basis) compared with other fresh vegetables. The results are in the line with those reported by Wang *et al*., (2018), who found that the concentrations of oxalic acid in raw spinach were 362 ± 18 mg of 100 g of fresh weight. According to the previous literature, the limit values of the content of total oxalates varied from 250 to 1760 mg/100 g fresh matter in spinach (Yamanaka *et al.,* 1983) and from 506 to 1765 mg/100 fresh matter in New Zealand spinach. (Jaworska, 2005; Savage *et al.,* 2000). Grevsen and Kaack (1996) investigated the 12 spinach cultivars in the processing industry, determining the content of 605–806 mg oxalic acid in 100 g fresh matter. The variety of fresh vegetables and environmental factors could affect the biosynthesis and level of oxalate in spinach and other leafy vegetables. Mou (2008) investigated that there were significant differences in oxalate concentration among the genotypes using 11 commercial cultivars of spinach.

Effect of cooking on nitrate, nitrite and oxalates content

This study was conducted to evaluate the nitrate, nitrite and oxalate contents of leafy vegetables under normal cooking conditions and other household treatments. The results in Table (2) indicated that cooking process of leafy vegetables led to a significant loss of their nitrate and nitrite contents. The loss was greater in nitrate content in spinach, reaching (71.63%) of its initial value, while it was lowest in cabbage (52.68%). However, the loss percentage in nitrite content was 74.12, 35.13 and 74.12% in spinach, cabbage and green Jew's mallow of its initial value, respectively after cooking process. These results are in agreement with those reported by Abo Bakr, et al. (1986) and Wang, et al. (2018). On the other hand, the oxalate loss in cooked leafy vegetables ranged between 33% in spinach to 100% of its content in raw material as shown in Table)2(. Savage, et al. (2000) reported that soluble oxalate could be removed by boiling. Chai, et al. (2005) showed that the boiling process markedly reduced the soluble oxalate content more effectively than steaming and baking processes. Wang, et al. (2018) demonstrated that after cooking for 30 seconds, oxalic acid concentrations were significantly reduced at all treated temperatures ($p < 0.05$). This result agrees with that of Izumi, et al. (2005), they report that 50% of oxalic acid can be removed after boiling at 100°C for 1 min with 20 times the amount of spinach weight of boiling water. It was considered that higher temperature and longer cooking time can break down the tissues of spinach and improve the release of oxalate. The results indicated that oxalic acid contents can be removed more easily than nitrate ion contents at the same cooking conditions. For nitrate ion removal, it was required to boil the spinach at 100°C only for 2–3 min (Wang et al., 2018).

Table (1): Nitrate, nitrite and oxalate content in some fresh leafy vegetables (on dry weight basis).

Sample	Moisture (%)	Nitrate (ppm)	Nitrite (ppm)	Oxalates (mg /g)
Spinach	91.06^{b}	3837.5^{a}	34.63^{c}	40.11^{b}
Cabbage	92.06^{a}	2250.51^{d}	95.85^{a}	7.7^{g}
Jew's mallow	81.3^{f}	2882.05^{b}	16.58^{e}	33.95^{d}
Rocket salad	89.43^{c}	2790.85^{c}	10.06^{f}	30.72^{e}
Dill	83.2^{d}	1539.62^{f}	29.61^{d}	28.52^{f}
Chard	92^{a}	2126.45^{e}	14.21^{e}	42.38^{a}
Lettuce	83.03^{ed}	1317.85^{g}	39.67^{b}	29.62^{ef}
Parsley	79.73^{g}	622.95^{h}	13.62^{ef}	37.32^{c}
Coriander	82.53^{e}	575.28^{h}	15.39^{e}	37.18^{c}
$LSD_{0.05}$	**0.59**	**3.77**	**63.55**	**1.59**

Table 2: Effect of cooking on nitrate, nitrite and oxalates contents of some leafy vegetables. (on dry weight basis).

Sample	Treatment	Nitrate (ppm)	Loss (%)	Nitrite (ppm)	Loss (%)	Oxalates (mg /g)	Loss (%)
Spinach	Fresh	3837.50^{a}	71.63	34.63^{a}	44.44	40.11^{a}	33.83
	Cooked	1088.55^{b}		19.24^{b}		26.54^{b}	
	$LSD_{0.05}$	87.43		11.08		1.78	
Cabbage	Fresh	2250.51^{a}	52.68	95.85^{a}	35.13	7.70^{a}	100
	Cooked	1064.79^{b}		62.17^{b}		0.00^{b}	
	$LSD_{0.05}$	78.70		8.78		1.61	
Jew's mallow	Fresh	2882.05^{a}	55	16.58^{a}	74.12	33.95^{a}	60.47
	Cooked	1297.13^{b}		4.29^{b}		13.42^{b}	
	$LSD_{0.05}$	39.32		4.42		1.62	

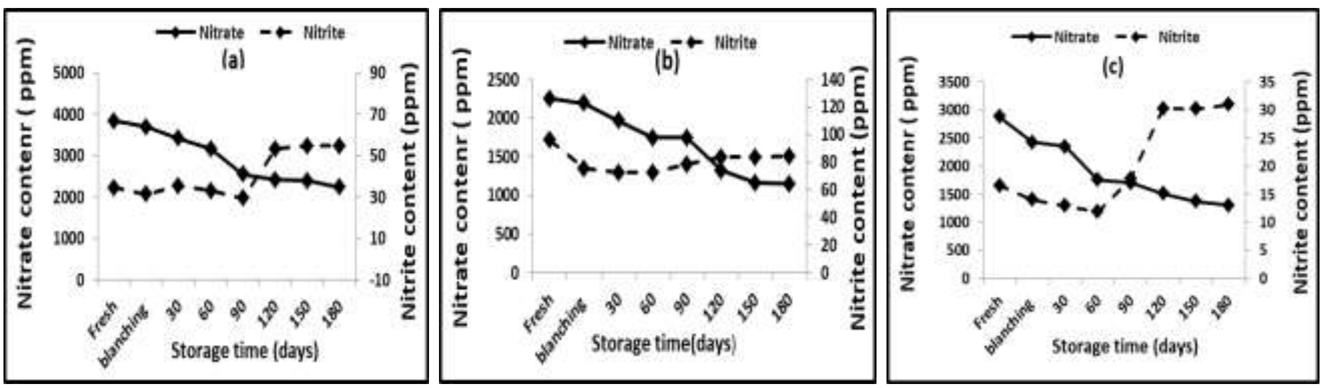

Fig. 1: Effect of blanching, freezing and storage for 180 days on nitrate and nitrite content of leafy vegetables: (a) spinash. (b) cabbage. (c) Jew's mallow.

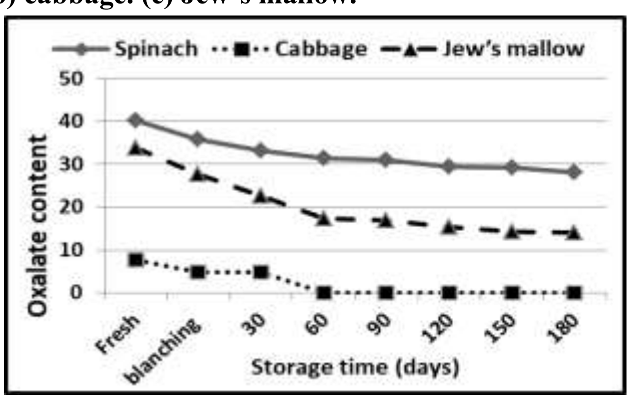

Fig. 2: Effect of blanching, freezing and storage for 180 days on oxalate content of some leafy vegetables (on dry weight basis).

Effect of blanching on nitrate, nitrite and oxalates contents

The effect of blanching process before freezing on nitrate and nitrite and oxalate contents in leafy vegetables under investigation illustrated in Fig 2. Results showed that after blanching, the loss percentage of nitrate was 3.7, 2.6 and 15.8% of its content in raw material of spinach, cabbage and Jews mallow, respectively; with a significant difference ($p \leq 0.05$). These results are in same trend with those reported by Abo Bakr, *et al.* (1986) and Wang, *et al*. (2018). On the other hand, the oxalate content in spinach decreased from 40.11 to 35.86 mg/g, with a decrease of 10.59 %, while, the decrease in both cabbage and green Jew's mallow were 36.23 and 18.55%, respectively. The obtained results are the same trend with those reported by Savage and Klunklin (2018) who found that the losses of total oxalates reached 17–27% after blanching and 24–41% after cooking, always being higher in the case of New Zealand spinach. Blanching in water constitutes an effective tool for controlling the nitrate content in vegetables, by varying the time and temperature of treatment.

Effect of freezing on nitrate and nitrite content

Effect of freezing on the nitrate and nitrite contents of some selected leafy vegetables was studied every 30-day during the storage period (180 days) Fig. 1. The results showed that the nitrate content of frozen vegetables was slightly fluctuated from the original values during storage period. In terms of nitrate content, a declining trend was evident although not anticipated in all the samples of leafy vegetables. The losses of nitrate over the 180 days period in the vegetables were: spinach 42.3%, cabbage 49.1%, and Jew’s mallow 54.6% For fresh leafy vegetables. These results are in agreement with those obtained by Abu-Bakr, *et al.* (1986) and Wang, *et al.* (2018). On the other hand, nitrite content increased due to the reduction of nitrate to nitrite. It tend was observed by Abu-Bakr, *et al.* (1986), who found that when frozen cabbages were stored. The level of nitrate decreased and no nitrite was detected during the first period of storage, then it accumulated to a high level after 3 months' storage.

Effect of freezing on oxalate content

Fresh and blanched samples were periodically analyzed to determine the combined effects of freezing and blanching on the oxalate content of the three selected vegetables as shown in Fig. 2. Through the obtained results, it is appears that the highest percentage of oxalate loss was in frozen cabbage leaves after blanching, reaching 100%, followed Jew’s mallow by 58% and spinach by 30% of its initial value. Analysis of the water used in blanching showed that considerable amount of oxalate was lost in the decanted water. Cleveland and Soleri (1991) reported that boiling or steaming of vegetables and then rinsing them and discarding the water reduced their oxalate content. Our findings are in support of this earlier observation. Ogbadoyi, *et al.* (2006) mentioned that oxalate is a metabolic product in plants and the reducing effect of freezing is probably as a result of the general stoppage of all metabolic reactions at freezing temperatures. For instance, it is known that the activity of the enzyme ascorbase, which converts ascorbic acid to oxalate, increases with storage of vegetables and fruits especially under warm conditions (Ogbadoyi *et al*. 2006). At freezing conditions, the activities of all enzymes are stopped and therefore production and accumulation of oxalate is prevented. This seems unlikely as the time between freezing and analyzing the fresh samples is not much to allow such a difference. However, it is known that freezing of tissues at high moisture content results in the formation of ice crystals within the cells. The sharp edges of the crystals so formed are capable of lacerating the cell membranes resulting in cell leakage (Baker and Gawish, 1997). It is therefore possible that blanching of the fresh leaves might have led to cell leakage leading to considerable loss of oxalate into the blanching water used in blanching the leafy vegetables before freezing.

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الملخص العربى

تأثير بعض عمليات التصنيع المنزلية على محتوى النترات والنيتريت والأكسالات لبعض الخضروات الورقية المختارة

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الهدف من هذه الدراسة هو التعرف على التغيرات في بعض المركبات الخطرة في بعض الخضروات الورقية المنزرعة والتي يتم تناولها بشكل شائع في صعيد مصر ، وقد اظهرت الدراسة احتواء السبانخ الخضراء على أعلى تركيز من النترات (3837.5 جزء في المليون) بينما احتوت أوراق الكزبرة على أقل تركيز (575.28 جزء في المليون) ، وكان محتوى النيتريت أعلى في أوراق الكرنب (95.85 جزء في المليون) مقارنة بالخضروات الورقية الأخرى ، احتوت أوراق السبانخ والسلق على أعلى تركيز من الأكسالات (40.11 و 42.38 ملجم / جم) مقارنة بالخضروات الطازجة الأخرى. وقد أدت عملية الطهي العادية للخضروات الورقية إلى فقد كبير في محتواها من النترات والنيتريت ، و كان الفقد فى محتوى النيتريت 74.12 و 35.13 و 74.12٪ في السبانخ ، الكرنب والملوخية الخضراء على التوالي نتيجة لعملية الطبخ ، وتراوح الفقد فى الأكسالات في الخضروات الورقية المطبوخة بين 33% في الملوخية الخضراء و 100٪ في الكرنب ، ، وعند دراسة تأثير السلق والتجميد على محتوى النترات والنيتريت والأكسالات في بعض الخضروات الورقية اظهرت الدراسة أن نسبة الفقد فى النترات نتيجة لعمليات السلق والتجميد كانت: 42.3 و 49.1 و 54.6٪ في السبانخ، الكرنب والملوخية الخضراء على التوالي ، ومن النتائج التي تم الحصول عليها أيضا يمكن ملاحظة أن أعلى نسبة فقد للأوكسالات كانت في أوراق الكرنب المسلوقة والمجمدة والتي وصلت إلى 100٪ ، تليها الملوخية الخضراء بنسبة 58٪ والسبانخ بنسبة 30٪ من قيمها الأولية فى المادة الطازجة.