Dynamics of nitrate and nitrite content during storage of homemade and small-scale industrially produced raw vegetable juices and their dietary intake.

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Dynamics of nitrate and nitrite content during storage of homemade and small-scale industrially produced raw vegetable juices and their dietary intake

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Abstract

The influence of storage conditions on nitrate and nitrite contents, pH and total viable bacterial count of raw vegetable juices was studied. Three different types of juices from an Estonian small-scale producer and five different types of homemade juices were analyzed. Analyzes were performed immediately after opening the commercial juice packages and immediately after preparation of a homemade juice. Additionally, samples were taken after open storage of a juice at the refrigerator and ambient temperatures during 24 and 48 hours. The biggest changes in nitrate and nitrite contents were found during the storage of carrot, beetroot and radish juices. During 48 hours of storage at ambient temperature the mean increase of nitrite content in homemade carrot, beetroot and radish juices were from 0.1 to 187, from 2.1 to 578 and from 0.5 to 259, respectively. In the case of commercial lightly pasteurized products, the biggest increase of nitrite content, from 3.2 to 11 mg l⁻¹ was found in red beetroot juice. After 48 h of storage at refrigerator temperature the changes of nitrite and nitrate were smaller. In the case of consumption of 300 ml homemade carrot juice, with nitrate and nitrite content of 64 and 110 mg l⁻¹, respectively, stored for 24 h at ambient temperature, the average intake is 8 and 846% of ADI of nitrates and nitrites, respectively. After consumption of 50 ml of the same carrot juice by children (1-2 years) the average intake of nitrates and nitrites is 7.0% and 733% of ADI, respectively.

Keywords: raw vegetable juice, nitrate, nitrite, storage, intake, ADI

Introduction

Interest in the dietary intakes of nitrates and nitrites has arisen due to the concern about their possible adverse effect on health (Belitz et al. 2004; Thomson et al. 2007, EFSA, 2008). Nitrate has a low level of acute toxicity but may be transformed into nitrite, which may lead to the formation of carcinogenic nitrosoamines (Walker, 1990, Kolb et al. 1997) and the clinical symptoms of methaemoglobinemia (WHO, 1995; Sanchez-Echaniz et al. 2001; Santamaria, 2006). Infant methemoglobinemia has been shown to be a result of consumption of high doses of nitrates by the infant foods and drinking water (Ezeagu, 1996, Coss et al.

2003, Thomson et al. 2007). The acceptable daily intake (ADI) for nitrate is from 0 to 3.7 mg/kg body weight, and for nitrite from 0 to 0.06 mg/kg body weight, established by EU Scientific Committee for Food (EU Scientific Committee, 1995) and reconfirmed by JECFA in 2002.

Vegetable juices are an important source of essential nutrients for humans. During the last years the vegetable-based raw juices have become very popular. The popularity has arisen due to public interest in variable diets and alternative nutrition. Nitrate is naturally present in all vegetables and vegetable products that are substantial sources of nitrates in human intake. Some vegetables, such as carrot, pumpkin and cabbage, and particularly red beetroot and radish, can accumulate high levels of nitrates. Consumption of 150 ml of red beetroot juice containing 2000 mg l⁻¹ nitrate thus would already result an intake greater than ADI value for a 70 kg adult (Kolb et al. 1997).

Nitrite content of most fresh, frozen or canned vegetables is generally low and usually in the range of 0 to 2 mg/kg (Meah et al. 1994, Pennington 1998, Chung et al. 2003, Belitz et al. 2004, Hsu et al. 2009). Reduction of nitrate to nitrite may take place in the presence of bacteria or enzyme nitrate reductase (Phillips, 1968, Chung et al. 2004) and after improper storage, especially of homemade vegetable products like purees and juices (Heisler et al. 1974; Kolb et al. 1997, Sanchez-Echaniz et al. 2001). It has been estimated that from 5 to 8% of the nitrate from the diet may be reduced to nitrite by the microflora in the oral cavity (Mensinga et al. 2003) and up to 20% for individuals with a high rate of conversion (WHO 1995).

If food contains high levels of nitrate, it is a potential risk if the conditions during storage or processing are conducive to conversion to nitrite (Hill 1996, EFSA, 2008). Most critical are vegetables that have been damaged, poorly stored, or stored for extended periods, pickled and fermented vegetables as well as raw vegetables juices. In such circumstances, nitrite levels of up to 400 mg kg⁻¹ have been found (WHO, 1995). In fermented maize on storage in water at room temperature over period of 8 days, nitrate levels decreased 80% in average while the nitrite content increased about 200% in average of its initial level (Ezeagu, 1996).

There is very limited up-to-date scientific information available on nitrate and nitrite levels and dynamics in the raw vegetable juices. According to the results of the earlier studies (Phillips, 1968, Heisler et al. 1974) the initial nitrate present in fresh vegetable juices was significantly reduced to nitrite under storage at ambient temperature. In 1994 Nabrzyski and Gajewska reported the decrease of nitrate from 261 to 46 mg kg⁻¹ with consequent increase of

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3 nitrite from 0.14 to 83.0 mg kg⁻¹ for juices prepared from blanched carrot and stored at
4 ambient temperatures.

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6 During storage of homogenized leafy vegetables (spinach, crown daisy, etc.) at ambient
7 temperature, nitrate levels in the vegetables dropped significantly (mean 87.4%) from the
8 third day while nitrite levels increased dramatically in the range of 1857 to 3617 mg kg⁻¹.
9 Refrigerated storage did not lead to significant changes in nitrate and nitrite levels in the
10 homogenized leafy vegetables (Chung et al. 2004). Phillips (1968) reported that after storage
11 of fresh homogenized spinach at ambient temperatures nitrate content was reduced to
12 approximately 64% of its initial level.

13
14 Considerable public interest has lately been focused on the consumption of raw vegetable
15 juices (Yabsley and Cross, 2001; Kroom, 2008). Different methods, like food supply data,
16 published tables of the mean consumption of dietary items, duplicate portion analysis, dietary
17 survey among the individuals, probabilistic and worse case screening models are used for
18 estimation of the nitrate and nitrite intake from food and for calculations of the acceptable
19 daily intakes (Ellen et al. 1990; Penttilä et al. 1990; Murata et al. 2002). Depending on the
20 method used, intake estimates can vary to a large extent (Kroes et al. 2002).

21
22 According to the Scientific Opinion of the Panel on Contaminants in the Food Chain of the
23 European Food Safety Authority, adopted on 10 April 2008, there is a need for further
24 research into the factors that influence nitrate and nitrite concentrations and alterations during
25 production, storage and processing of the foodstuffs (EFSA, 2008).

26
27 The aim of the present work was to study the changes of nitrate and nitrite levels in selected
28 raw vegetables juices under refrigerated and ambient temperature storage conditions for a
29 period of 2 days. The assessment of dietary nitrate and nitrite intake by consumption of raw
30 vegetable juices in accordance with acceptable daily intakes (ADI) was the second objective
31 of the present work.

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49 **Materials and methods**

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53 **Juices**

54 All juices were analysed in five replications.

55 Series 1 analyses were performed with lightly pasteurized raw juices of carrot, cabbage and
56 red beetroot, produced at a small-scale local enterprise and purchased at retail market. Light
57 pasteurization is directed at vegetative microorganisms and is usually conducted at about
58 65 °C during 10 seconds (Sadler et al., 1992). The series 2 consisted of homemade raw juices
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prepared in the laboratory following the traditional raw juice preparation at homes without any pasteurization. Briefly, samples of five species of vegetables, i.e. carrot, cabbage, red beetroot, radish and pumpkin were collected from different outlets of Tartu, Estonia. The vegetables were washed with warm water, mechanically brushed and peeled. Raw juices were produced with commercially available squeezer (Stollar Juice Fountain).

All the raw juices were stored at two different temperatures, refrigerated (4-6 °C) and ambient (20-22 °C) for two days. The nitrate and nitrite contents, total viable counts, coliforms counts, yeast and moulds counts and pH were determined after opening the commercial juice packages and immediately after preparation of the homemade juices as well as after 24 and 48 hours of storage.

HPLC analysis of nitrate and nitrite ions

HPLC analyses were performed by a liquid chromatograph (Agilent Technologies Model 1100, Palo Alto, CA, USA) equipped with a fluorescence detector. Chromatographic separation of samples and standards was carried out at a reversed phase column Agilent 300SB-C₈ (2,1x150mm, 5µm; Agilent Technologies, USA) using gradient elution with 0.1% formic acid and acetonitrile eluent at 35 °C with a speed of 0.2 ml/min. Fluorescence was monitored with excitation at 355 nm and emission at 420 nm.

Nitrate ions were converted to nitrite enzymatically by nitrate reductase and further to the highly fluorescent 2,3-naphthotriazole (NAT) with 2,3-diaminonaphthalene (DAN) as described in literature (Misko et al. 1993; Li et al. 2000). Obtained solution was used directly for chromatographic analysis. Conversion rate of nitrate to nitrite was 98%, as detected with known amounts of nitrate and nitrite standards. All chemicals were purchased from Sigma, except sodium nitrate and potassium nitrite which were obtained from Riedel-de Haën. Limit of quantification (LOQ) of nitrate was 0.23 mg l⁻¹ and of nitrite 0.10 mg l⁻¹.

Bacteriological analyses

All juices were examined microbiologically for total viable counts, coliform bacteria, yeasts and moulds using the four plate method. Decimal dilution series were prepared in accordance with EVS-EN ISO 6887-1:2001. Plate Count Agar LAB 115 was used for isolation of total viable counts at 30 °C for 72 h (EVS-EN ISO 4833:2006). Coliforms were grown in the Violet Red Bile Agar LAB 31 (V.R.B.A.) at 37 °C for 24 h (NMKL 44 5th edition 2001). Yeast extract-dextrose-chloramphenicol-agar medium LAB 119-B5 was used for isolation of

yeast and moulds at 25 °C for 5 days (EVS-EN ISO 7954:1999). Microbiological counts were expressed as a number of colony forming units (cfu) per milliliter (ml).

Determination of pH-values of vegetable juices

The pH of the juices was determined by pH meter Consort C 833, according to the instructions of European Standard EVS-EN 1132:2000.

Methods of intake estimation

The consumption amounts of raw vegetable juices were calculated in accordance with dietary recommendations for average intake of 65 kg body weight of adult by Kroom (2008). Intake of nitrates and nitrites by infants and young children was estimated in accordance with the nutrition recommendations of Estonian paediatricians (Kroom, 2008).

Statistical analysis

All individual laboratory results of nitrate and nitrite content were recorded and the statistical analyses were performed using MS Excel 2003 software (Microsoft Corporation; Redmond, WA, USA) T-test was used to reveal the statistical significance of differences in measured variables at different time points. The results with values $p < 0.05$ were considered statistically significant. Correlation analyses were used to study relationships between the pH and total viable count of aerobic bacteria (log cfu/ml) as well as between nitrate/nitrite content and total viable count of bacteria (log cfu/ml).

Results and discussion

The range and mean values of initial nitrate and nitrite contents in the studied vegetable-based juices are shown in Table 1. The highest mean initial values of nitrate were obtained for the homemade juices of beetroot and radish, 2625 and 4615 mg l⁻¹, respectively. Significantly lower values were determined for the carrot juices ($p < 0.05$). The mean nitrite content for all juices was below 3.2 mg l⁻¹.

[Insert Table 1 about here]

Nitrates, nitrites and pH value.

Storage experiments were performed at ambient (20-22 °C) and refrigerator (4-6 °C) temperatures during 24 and 48 hours.

Series 1. The changes in nitrate and nitrite mean contents in a small-scale company produced slightly pasteurized juices during open storage are shown in Table 2. Nitrate content decreased in all three juices during storage at both temperatures. The biggest decrease in nitrate content were found during 48 hours of storage for carrot, cabbage and beetroot juices at the ambient temperature, 47% (87 to 46 mg l⁻¹), 39% (250 to 152 mg l⁻¹), 57% (1708 to 739 mg l⁻¹), respectively. After 48 hours of storage at refrigerated temperatures smaller decreases in nitrate contents was found, 11-30% in comparison with the initial contents. The biggest increase of nitrites, from 3.2 to 11.1 mg l⁻¹ and from 3.2 to 6.8 mg l⁻¹ was found for raw beetroot juice during storage both at ambient and refrigerated temperatures, respectively (Table 2). The pH dynamics of the juice samples is shown in Table 2. In the carrot juice, stored during 48 h at ambient temperatures, the highest pH decrease, from 6.3 to 4.1, was measured. The pH changes were lower in juices, stored at refrigerator temperatures.

[Insert Table 2 about here]

Series 2. The changes in nitrate and nitrite mean contents in the homemade carrot, cabbage, radish and pumpkin raw juices are shown in Figure 1. During the storage the nitrate contents in juices were decreasing and conversely, the nitrite contents were increasing. Changes in nitrate and nitrite contents depend on the juice type and storage conditions. Storage of the homemade raw juices for 24 h at ambient temperatures is clearly unacceptable, because the nitrite contents in carrot, beetroot and radish juices increases significantly ($p < 0.05$) (Figure 1 A, B, D). Also, there was statistically significant increase of nitrite content (Figure 1 E) in cabbage juices ($p < 0.05$). At the same time the nitrate contents decreased during 24 hour storage in the range of 26-66% of the initial contents. The biggest decrease in nitrate content was found after 24 h storage at ambient temperatures for carrot and beetroot juices, from 163 to 64 mg l⁻¹ and from 2625 to 692 mg l⁻¹, respectively (Table 4, Figure 1 A, B). Nitrate contents decreased during 48 h of storage at ambient temperatures for carrot, beetroot and radish raw juices, from 163 to 6.7 mg l⁻¹, 2625 to 523 mg l⁻¹ and 4615 to 1772 mg l⁻¹, respectively (Figure 1 A, B, D). At the same time, there was a considerable increase in nitrite content from 0.1 to 187 mg l⁻¹, from 2.1 to 578 mg l⁻¹ and from 0.5 to 259 mg l⁻¹ was

registered, respectively (Figure 1 A, B, D). For example, during 48 hours of storage of the cabbage juice at the ambient temperature the nitrate contents decreased from 116 to 44 mg l⁻¹, at the same time the nitrite contents stayed below 2.3 mg l⁻¹ (Figure 1 C). In general, during 48 h of storage at refrigerated temperatures the nitrate contents decreased. The biggest decreases, from 163 to 108 mg l⁻¹ and from 2625 to 1486 were found for carrot and beetroot raw juices, respectively (Figure 1 A, B). At the same time, during the 48 h storage at refrigerated temperatures, nitrite contents increased mostly for carrot juice, 47 mg l⁻¹ and less for beetroot and radish juice, 4.9 and 11.2 mg l⁻¹, respectively (Figure 1).

In an earlier study, Heisler et al. (1974) reported a considerable decrease in nitrate and increase of nitrite content during 24 hours at ambient temperature for the beetroot and spinach raw juices. The nitrite content of beetroot and spinach juices increased up to 540 and 900-1000 mg kg⁻¹, respectively. The nitrate content decreased from 3800 to 2800 and from 1500 to 350 mg kg⁻¹, respectively (Heisler et al. 1974).

The pH dynamics of the raw juice samples is illustrated in Figure 1. The pH of the juices did not change considerably during the storage at refrigerated temperatures. Differently, at ambient temperatures, the highest decrease of pH value from initial 6.4 to 4.2 was observed in radish juice. The pH of the raw juice samples was measured at 0, 24 and 48 h of storage. Generally, during the storage studies, the pH decreased and nitrite contents and total viable counts increased (Figure 1). Since both nitrite concentration and total viable microbial counts increased during storage it may be concluded that microbial activity was the main factor in nitrate reduction process. Better correlations between the nitrite content and total viable counts were found in vegetable juices after storage at ambient temperatures ($r = 0.9$ to 0.58) It can be explained by microbiological reduction of nitrite from nitrate ion. The negative correlations between nitrate content and total viable counts were found from homemade carrot juice and homemade radish juice ($r = -0.84$ to -0.81).

It is recommended to use only fresh-prepared homemade carrot juices for babies. Raw material used for the preparation of juices should have as low initial nitrate content as possible.

[Insert Figure 1 about here]

Figure 1. The changes of nitrate and nitrite content during storage in raw homemade vegetables juices – carrot juice A, beetroot juice B, cabbage juice C, radish juice D, pumpkin juice E

Microbiological count and pH value.

All the products were analyzed for total viable bacterial, coliform bacteria, yeasts and moulds counts. In the series 1, three different vegetable-based small-scale industry produced raw juices and in series 2, five different homemade vegetable-based raw juices were microbiologically analyzed. During the storage, all the species of the microorganisms showed the increase in counts (Table 3 and Figure 1), while pH showed decrease tendency (Table 2 and Figure 1). Between the pH and total viable counts (log cfu/ml) clear negative correlations were determined ($r=-0.95$ to -0.75 , $p<0.05$). The decrease of pH is caused by the microorganisms' vital activity products, as indicated by the increase of microorganism multiplicity. As reported by Akin et al. (2008), pH decrease was assumed to correlate with the nitrogen consumption of the studied microorganism strains. The nitrogen concentration did not influence the pH itself but during the fermentation, the consumption of nitrogen by yeasts produces H^+ ions (Castrillo et al. 1995). In Table 3 it is shown that compared to initial microorganism counts, after 48 hours of storage at ambient temperature the average percentage in counts of microorganisms in homemade raw juices were higher than for commercially produced juices, 173 and 161%, respectively. During the storage experiment of the industrial lightly pasteurized raw juices at ambient temperatures for 48 h the bacterial total counts increased from 5.03-5.33 log cfu/ml to 7.81-8.85 log cfu/ml. During the storage of the homemade juices at ambient temperature for 48 h the total bacterial counts increased from 5.14-5.39 log cfu/ml to 8.84-9.19 log cfu/ml, with the average increase 3.8 log cfu/ml. During storage of raw juices at ambient temperatures, the total counts of coliform bacteria and moulds and yeast increased significantly ($p<0.05$). Higher increase of microorganism counts was detected in the homemade juices.

[Insert Table 3 about here]

Intake calculation of nitrate and nitrite

Adults. The intake of nitrate and nitrite has been calculated in accordance with the recommendations given by Estonian and other dietitians (Heinerman, 1994, Yabsley and Gross, 2001, Kroom, 2008). The recommended consumption data of the homemade vegetable based raw juices and calculated ADI percentages are shown in Table 4. In calculations of nitrate and nitrite daily intakes, 65 kg was used as the average body weight of Estonian adults.

The average consumption amounts of vegetable raw juices in accordance with dietitians' recommendations were 100 to 300 ml dependent on the juice variety. The daily doses of nitrate and nitrite depend on initial contents of NO_3^- and NO_2^- in vegetable based raw juices (Table 4). Additionally, it depends on consumption amounts and storage conditions of the vegetable-based raw juices. The daily doses are exceeded by adults even by consumption of freshly made beetroot and radish juices as well as by using the same juices after 24 h of storage at refrigerated temperatures, 164%, 193% and 109%, 172% of ADI, respectively (Table 4). Daily doses of nitrites when juices are stored at same conditions are generally low and in range of 0.8% to 16% of ADI, exceptional was a carrot juice with 30% of ADI. Nitrite daily doses increased significantly ($p<0.05$) when the juices were stored at ambient temperatures for 24 h, at the same time the nitrate doses decreased. Nitrite doses in the case of consumption of 300 ml raw carrot juice, 150 ml raw beetroot juice and 100 ml of raw radish juice exceeded ADI values 846%, 346% and 167%, respectively. Beetroot and radish juices could be mixed with other vegetable based raw juices as recommended by dietitians (Heinerman, 1994; Kroom, 2008). Industrially produced raw juices are lightly pasteurized and therefore the nitrate and nitrite changes (Table 2) are lower compared to the homemade juices (Table 4). Consequently, the nitrate and nitrite daily doses are also considerably lower and will not exceed the ADI values.

[Insert Table 4 about here]

Infants and young children. According to our data obtained from questionnaires, filled in by parents, the most popular raw vegetable juices given to young children were carrot based raw juices. Generally, carrot juices contain nitrates in low concentrations. In our study the carrot raw juices produced by Estonian small-scale industry and in homemade juices contained nitrates 87 and 163 mg l^{-1} , respectively (Table 2 and 4).

Recommended consumption of raw carrot juices and related nitrates and nitrites daily doses are shown in Table 5. In accordance with our data, it is obvious that only freshly made carrot raw juices can be used for infants because the ADI values of nitrate and nitrite were 12.9% and 0.5%, respectively. Consumption of the fresh and 24 h at refrigerated temperatures stored homemade carrot raw juices increased nitrite daily intakes to 19.7% of ADI. During 24 h of storage at ambient temperatures of the homemade carrot raw juices the nitrite contents were higher compare to storage in refrigerator which resulted with the mean values of 110 mg l^{-1}

(Table 4). Consumption of a juice containing 110 mg l^{-1} of nitrite by 10-12 months-old infants 30 ml as the maximum amount recommended by dieticians and 50 ml for 1-2 years old young children could be harmful for health because the mean nitrite intake is 540% and 733% of ADI, respectively (Table 5). According to the calculations by Phillips (1968), one dose of raw vegetable juice in amount of 30 ml could result in cyanosis for a 1-year-old child.

Lightly pasteurized carrot raw juice (Table 2) made in small-scale industry could be given for young child even after 24 h of storage at refrigerated temperatures because as shown in Table 2 the nitrate and nitrite contents had only minor changes, 87 mg l^{-1} to 80 mg l^{-1} and 0.1 mg l^{-1} to 0.2 mg l^{-1} , respectively. In the present case the mean daily nitrate dose for infants in result of consumption of carrot raw juices will stay below 10% of ADI and nitrite dose will not exceed 2% of ADI.

[Insert Table 5 about here]

Conclusions

The initial contents and changes in the dynamics of nitrates and nitrites were determined after 24 and 48 hours of storage at ambient and refrigerated temperatures of homemade (carrot, beetroot, cabbage, radish, pumpkin) and commercially produced raw vegetable based juices (carrot, beetroot, cabbage). A significant decrease ($p < 0.05$) in nitrate and a significant increase ($p < 0.05$) in nitrite contents in homemade carrot, beetroot and radish raw juices after 48 hours of storage at ambient temperatures was found. Nitrite contents of carrot, beetroot and radish raw juices were high already after 24 h of storage at ambient temperatures. Therefore, the consumption of the homemade vegetable based raw juices is recommended only shortly after preparation. The industrially produced lightly pasteurized raw juices can be openly stored at refrigerated temperatures for a maximum of 24 hours. In conclusion, very high nitrite intakes from vegetable based raw juices can occur when juices are stored for a long time at an improper e.g. at ambient temperatures. Acceptable daily intakes can be exceeded 5-8 fold for young children and 2-8 fold for the adults.

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Figure 1. The changes of nitrate and nitrite content during storage in raw home made vegetables juices carrot juice A, beetroot juice B, cabbage juice C, radish juice D, pumpkin juice E

The changes of nitrate and nitrite content during storage in raw homemade vegetables juices – carrot juice A, beetroot juice B, cabbage juice C, radish juice D, pumpkin juice E

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Table 1. Nitrate and nitrite initial contents of raw vegetable juices, [5 replications](#)

Vegetable juice	Nitrate mg l ⁻¹		Nitrite mg l ⁻¹	
	Range	Mean	Range	Mean
Commercial juices				
Carrot juice	47-139	87	LOQ-0.4	0.1
Red beetroot juice	1097-2257	1707	0.23-10.2	3.2
Cabbage juice	171-415	250	LOQ-2.7	0.6
Homemade juices				
Carrot juice	40-311	163	LOQ-0.2	0.1
Red beetroot juice	130-6479	2625	LOQ-4.2	2.1
Cabbage juice	38-299	116	LOQ-0.9	0.23
Radish juice	3714-6553	4615	0.23-0.9	0.5
Pumpkin juice	148-520	255	LOQ-1.4	1.0

LOQ – limit of quantification

NO₃⁻: LOQ 0.23 mg l⁻¹

NO₂⁻: LOQ 0.10 mg l⁻¹

Table 2. The changes of mean nitrate and nitrite content during storage of commercial raw vegetable juice (5 replications) at ambient (20-22°C) and refrigerated (4-6°C) temperature

Type of juice	Storage period (hours)	Nitrate mg l ⁻¹		Nitrite mg l ⁻¹		pH	
		20-22°C	4-6°C	20-22°C	4-6°C	20-22°C	4-6°C
Carrot juice	0	87	87	0.1	0.1	6.3	6.3
	24	63	80	0.4	0.2	5.3	6.3
	48	46	62	2.7	1.5	4.1	6.2
Cabbage juice	0	250	250	0.6	0.6	6.0	6.0
	24	192	236	1.3	0.6	5.0	5.7
	48	152	188	1.5	1.2	4.2	5.5
Red beetroot juice	0	1708	1708	3.2	3.2	5.9	5.9
	24	1158	1606	7.1	4.7	5.0	5.8
	48	739	1517	11.1	6.8	4.2	5.7

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Table 3. Effect of ambient (20-22 °C) and refrigerated (4-6 °C) storage conditions to raw vegetables juices (5 replications) microbiological counts

Type of juice	Storage time	Total microbial count log cfu/ml*		Coliforms log cfu/ml*		Molds and Yeast log cfu/ml*	
		20-22 °C	4-6 °C	20-22 °C	4-6 °C	20-22 °C	4-6 °C
		I ^a /H ^b	I ^a /H ^b	I ^a /H ^b	I ^a /H ^b	I ^a /H ^b	I ^a /H ^b
Carrot juice	0	5.13/5.17	5.13/5.17	2.97/4.43	2.97/4.43	4.18/3.76	4.18/3.76
	24 h	6.56/7.13	5.65/5.44	4.94/6.15	4.03/4.63	5.01/4.37	4.78/4.01
	48 h	8.85/8.84	6.73/7.33	5.64/6.95	4.96/5.83	5.9/5.73	4.97/4.73
Cabbage juice	0	5.33/5.14	5.33/5.14	4.69/4.54	4.69/4.54	4.11/3.58	4.11/3.58
	24 h	7.24/7.50	5.88/5.38	5.97/5.09	4.78/4.91	5.25/3.8	4.29/3.07
	48 h	8.29/9.19	6.37/6.59	6.59/5.82	5.53/5.24	6.46/4.2	4.77/3.82
Red beetroot juice	0	5.03/5.39	5.03/5.39	3.38/3.85	3.38/3.85	2.94/2.74	2.94/2.74
	24 h	6.88/7.55	5.39/5.72	5.57/6.07	4.69/4.34	3.61/3.98	2.89/3.30
	48 h	7.81/9.03	6.77/6.74	6.14/6.70	5.59/5.07	4.53/4.65	3.57/3.73

* Arithmetic mean value of 5 replicate results
I^a - Industrial juices
H^b - Home-made juice

Table 4. The mean content of nitrate and nitrite and changes during storage in raw home-made vegetable juices and daily intake by Estonian adults

Vegetable juice	Nitrate content mg l ⁻¹	Nitrite content mg l ⁻¹	Recommended mean intake of juice per day, ml*	Expected nitrate intake mg day ⁻¹	% of ADI	Expected nitrite intake mg day ⁻¹	% of ADI
Raw juices before storage							
Carrot	163	0.1	300	49.0	20.4	0.03	0.8
Cabbage	116	0.23	200	23.0	9.7	0.05	1.2
Red beetroot	2625	2.1	150	394.0	164.0	0.32	8
Radish	4615	0.5	100	462.0	193.0	0.05	1.3
Pumpkin	255	1.0	200	51.0	21.3	0.2	5.1
Raw juices after storage at refrigeration temperature (4-6 °C) within 24 h							
Carrot	152	3.9	300	45.6	19.0	1.17	30
Cabbage	100	0.4	200	20.0	8.3	0.08	2.1
Red beetroot	1745	2.5	150	262.0	109.0	0.38	9.6
Radish	4126	1.5	100	413.0	172.0	0.15	3.8
Pumpkin	230	3.1	200	46.0	19.2	0.62	16
Raw juices storage at ambient temperature (20-22 °C) within 24 h							
Carrot	64	110.0	300	19.0	8.0	33.0	846
Cabbage	65	1.2	200	13.0	5.4	0.24	6.2
Red beetroot	692	90.0	150	103.8	43.3	13.4	346
Radish	2633	65.1	100	263.0	109.7	6.5	167
Pumpkin	167	14.3	200	33.4	13.9	2.86	73

* calculated by the basis of the recommended by dietitians.

Table 5. Estimated mean nitrate and nitrite intake by infants and young children after consumption of raw home-made carrot juice

Storage condition	NO ₃ ⁻ content mg l ⁻¹	NO ₂ ⁻ content mg l ⁻¹	Mean NO ₃ ⁻ intake mg per day	% of ADI	Mean NO ₂ ⁻ intake mg per day	% of ADI
Fresh raw juice	163	0.1	4.9 ^a 8.2 ^b	12.9 ^a 17.8 ^b	0.003 ^a 0.005 ^b	0.5 ^a 0.7 ^b
Stored 24 h at 4-6°C	152	3.9	4.6 ^a 7.6 ^b	12.0 ^a 16.5 ^b	0.12 ^a 0.20 ^b	19.7 ^a 26.6 ^b
Stored 24 h at 20-22°C	64	110	1.9 ^a 3.2 ^b	5.0 ^a 7.0 ^b	3.3 ^a 5.5 ^b	540 ^a 733 ^b

^a 10 - 12 months infants, mean body weight 10.2 kg – recommended consumption of raw carrot juice 30 ml per day

^b 1 - 2 year young children, mean body weight 12.5 kg – recommended consumption of raw carrot juice 50 ml per day