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EFFECT OF PACKAGES ON NITRATES AND NITRITES CONTENTS IN SAUERKRAUTS

WPŁYW OPAKOWANIA NA ZAWARTOŚĆ AZOTANÓW(V) I (III) W KAPUSTACH KISZONYCH, CHODNICZO SKADOWANYCH

Abstract: Nitrites are thought to be ten times more toxic to humans and animals than nitrates. Nitrites are able to form nitrosamines, stable, strongly toxic, mutagenic, teratogenic and carcinogenic compounds. The presented paper investigates the effect of packaging types and length of chilled storage on changes in nitrates and nitrites contents in white and red cabbage sauerkraut. Two types of bags were used for packing the product: one made from low density polyethylene (PE-LD) and the other from the metalized polyethylene terephthalate (PET met/PE). Vegetables were analysed before and after packaging and after 1, 2, 3, and 4 months of chilled storage in two types of packaging. It has been observed in this work that nitrate contents in cold-stored sauerkrauts fluctuated in subsequent four months and the values found were generally significant. A type of packaging did not have a significant effect on the levels of nitrites in the sauerkrauts analyzed.

Keywords: cold stored; nitrates; nitrites; packaging; sour white cabbage, sour red cabbage

Introduction

High atmospheric concentration of nitric oxides consequent to industrial emission can be a cause of elevated concentrations of nitrates and nitrites in plants [1]. Nitrites, and indirectly nitrates, can be hazardous for the health, when consumed in food in too much amounts. Nitrites are thought to be ten times more toxic to humans and animals than nitrates. Nitrites toxicity is caused, among other things, by methemoglobinemia (cyanosis). Nitrite ion formed by nitrate reduction is responsible for the hemoglobin's Fe²⁺ ion oxidation to Fe³⁺ [2, 3]. Nitrites are able to form nitrosamines, stable, strongly toxic, mutagenic, teratogenic and carcinogenic compounds [2, 4].

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On the other hand, several clinical trials are being performed to determine the broad therapeutic potential of increasing nitrite bioavailability on human health and disease, including studies related to vascular aging. Inorganic nitrite, as well as dietary nitrate supplementation, represents a promising therapy for treatment of arterial aging and prevention of age-associated CVD in humans [5, 6].

Nitrates (75–87% of total content) and nitrites (16–43%) in human body derive principally from vegetables [7, 8]. Among vegetables, the Cruciferous are characterized by a medium (white cabbage) or low (cauliflower, Brussels sprouts) accumulation of these compounds, but due to a large mass consumed, they can be a significant component of daily food intake. These are seasonal vegetables. Some of them may be consumed raw or preserved by souring or freezing [9]. The content of nitrates and nitrites in the plant raw material can not reflect the real intake since both pre-treatment (washing, peeling) and culinary and technological treatments may lead to changes in their levels [10, 11].

Biological value of food is a wide notion which includes a nutritive value, sensory attributes, being also significant for the consumer health. Hence, biological value is understood to be not only the content of valuable vitamins, mineral compounds, and pro-health substances but is also considered with regard to the level of contaminants which have a negative effect for the health. Food contaminants include, among other substances, nitrates and nitrites [12].

Cruciferous are abundant in several biologically active substances such as, among other, vitamins C and E, carotenoids, polyphenols, and glucosinolates. They owe their anti-inflammatory and antimutagenic properties to the presence of these substances, which also affect expression of the gene responsible for cell proliferation in the development of cancer through modulation of cell redox homeostasis [13, 14]. Moreover, some polyphenol groups exhibit anti-inflammatory, anti-allergic, anti-clotting, anti-virus, as well as anti-cancerogenic properties [15].

The process of sauerkraut fermentation, known from the ancient times, is one of the biological methods of food preservation based on lacto-fermentation. The products obtained due to this process are characterized by extended shelf life and by micro-biological safety. They are better digestible and their properties are different from the initial material [16, 17].

The raw material used in the sauerkraut fermentation is cabbage both white and red. A main constituent of sauerkraut is lactic acid, the product of sugars' fermentation conducted by such lactic acid bacteria as, *eg Lactobacillus acidophilus*. The identical process runs in the large intestine, where the bacteria colonizing it are employed to ferment probiotics. The lactic acid formed is a constituent of skin protective layer (epidermis), playing also a substantial protective role in the mucous membrane. The consumption of sauerkraut enables easier colonization of the large intestine by the LAB species, which are responsible for crucial functions in human body. They produce vitamins and enzymes, inhibits the formation of putrefactive products, improve the peristalsis of gastrointestinal tract, and are also found to compete with such pathogenic bacteria as, for example, *E. coli* or *Candida albicans* [18].

The level of nitrates in vegetables changes depending on the intensity of fertilization, climatic conditions, plant species, and plant maturity at harvest [19]. The presence of some amounts of nitrates in plants is a consequence of the natural cycle of nitrogen absorbed by the plants to synthesize protein. The remaining quantity is a result of the excessive soil fertilization, infiltration of soil surface layer with sewages or the process of leaching running in lode. Nitrates can also be added as preservatives to several products. The content of nitrates tended to rise in plants at the early stage of growth, inadequate exposure to the sun's rays, acidic pH of soil, low humidity, deficiency of such nutrients as molybdenum and magnesium, and when herbicides were used [20].

The obligatory EC Regulation of 2005 sets maximum acceptable levels for nitrates only in certain vegetables such as fresh, preserved, and frozen spinach, fresh lettuce, and Iceberg-type lettuce. The Regulation of the Minister of Health of 2003, which was obligatory until 30 April 2004, defined the maximum nitrates contamination of cabbage at the level of 750 mg NO₃/kg of fresh weight; the level of nitrites was not set. The human intake of nitrate(V) should not however exceed the acceptable daily intake of 3.7 mg/kg body weight (b.w.) and in the case of nitrates(III) of 0.07 mg NO₂/kg b.w. [20–22].

Vegetables which are self-produced by farmers can be cultivated and sell on the market and there is no an obligation to control their production [23]. In view if the above, a contemporary man is searching for effective methods of food storage to minimize losses occurring during this process. Hence, studies are conducted all over the world to find a method which will be beneficial from both nutritional and economic point of view and the use of which is inexpensive but efficient enough to maintain a natural composition of the stored product.

Therefore, vegetables can contain excessive amounts of nitrates that may have a negative heath effect on a consumer. Food containers are multifunctional and one of their fundamental function is to protect the product against mechanical damages as well as external factors. With regard to the sour products, barrels are commonly used for their storage. On the other hand, characteristic feature of the unit packaging is its small volume and the fact that such packaging should provide specific microclimate for the product to maintain high quality as long as possible in appropriate storage condition [24].

The aim of this paper was to examine and compare sour white and red cabbage, which was chilled stored for four subsequent months, in terms of changes in the nitrates and nitrites content. The experimental material was packed in two ways: in low density polyethylene (PE-LD) bags and in metalized polyethylene terephthalate (PET met/PE) bags.

To our best knowledge this is the first study determining the effect of cold storage in different packaging type (especially innovative is one of presented packaging type – metalized foil made from PET met/PE) on selected contaminants of the chilled stored white and red sauerkraut.

This study aimed also at increasing the consumer knowledge about biological value of the sour cabbage: white and red, particularly with regard to the presence of such contaminants as nitrates and nitrites as well as at contributing in the selection of an optimal packaging intended for the chilled storage of such a product.

Material and methods

Material

The experimental material comprised sour white cabbage and sour red cabbage, which were freshly soured and purchased in 5 selected stands of direct selling in Krakow. Vegetables were analysed before and after packaging and after 1-, 2-, 3- and 4-month periods of chilled storage. First analyses were done immediately before packaging. The remaining material was divided into two batches, one was packed in low density polyethylene (PE-LD) bags with the zipper closure (0.91-0.92 g/cm³; size: 230 x 320 mm) and the other in bags of the similar size made of the laminate: metalized polyethylene terephthalate (PET met/PE) (a polymer from the polyester group, obtained through a polycondensation reaction between dimethyl terephthalate (DMT) and ethylene glycol (GE), CAS number: 25038-59-9, density 1.370 g/cm³) with polyethylene. The representative samples obtained were then stored at chilled conditions (4–5°C) in a fridge for four subsequent months.

Analytical methods

The adequately prepared mean and representative samples of vegetables were analyzed for the levels of contaminants: nitrates and nitrites according to the Polish Standard PN-92/A-75112 [25]. Samples were analyzed prior to their packaging and after the established periods of chilled storage.

Colorimetric method using to determinate this contaminants based on nitrites coloured reaction with Griess I, II reagent. Previously nitrates must be reduced to nitrites.

Nitrates content was assessed using Griess I (sulfanilamide, Sigma-Aldrich) and Griess II (n-(1-Naphtyl)ethylene-diamine dihydrochloride, water solution, Sigma-Aldrich). The principle of this method is to cause a colour reaction of nitrate(III) with n-(1-Naphtyl)ethylene-diamine dihydrochloride in acidic conditions, and to measure absorbance at wavelenght 538 nm. Nitrates had tobe reduced to nitrites before to beginning of colour reaction.

The described research method is recognized and widely used in assays to determine the content of nitrates and nitrites in vegetables.

Statistical analysis

All analyses were carried out in three parallel replications (n = 3) and for all the mean values obtained, standard deviations (SD) were calculated. In the vegetables investigated, single- and two-factor analysis of variance was used to establish the level of significance for the differences in the levels of nitrates and nitrites depending on the

processes applied (chilled storage) and type of packaging. The single-factor analysis of variance was employed to determine how significant were differences between mean parameter values with regard to two different packaging types used for the storage of vegetables. In two-factor analysis of variance, significance of differences was found between values of the parameters evaluated in the chilled stored vegetable depending on the packaging type. The Statistica 9.1. PL program was applied for all the calculations made. The Duncan's multiple range test was used to assess significance of differences at the critical significance level of $p \le 0.05$.

Results

As the dry matter content in the vegetable varies depending on the process applied and the container used, all the results presented below along with conclusions have been discussed basing on the results calculated per the dry matter unit. In consequence, only an effect of the process applied was shown.

Nitrates and nitrites

The nitrate levels in white and red cabbage sauerkraut were expressed as the amount of NO_3^- nitrate ions per kg of dry matter (Tables 1 and 2).

Table 1

The kind of processing	Nitrates [NO ₃ ⁻]		Nitrites [NO ₂ ⁻]		
Before storage	$522^{b} \pm 21$		$0.60^{\mathrm{cd}}\pm0.04$		
	The kind of packaging				
Cool storage	Zipper seal bags (PE-LD)		Bags (PET met/PE)		
	Nitrates	Nitrites	Nitrates	Nitrites	
1 month	$84.9^{e} \pm 4.0$	$0.70^{\text{cd}} \pm 0.11$	$139^{de} \pm 19$	$0.80^{\rm bc}\pm0.00$	
2 months	247 ± 26	$1.10^{b} \pm 0.04$	$520^{b} \pm 15$	$1.80^{\rm a}\pm0.37$	
3 months	$176^{d} \pm 29$	$0.60^{cd}\pm0.04$	$156^d \pm 70$	$1.10^{\rm b}\pm0.04$	
4 months	$576.7^{ab}\pm8.4$	$0.50^{\text{d}} \pm 0.04$	$624^{a} \pm 16$	$0.70^{cd} \pm 0.19$	
Mean value for packaging	$321^{\rm A}\pm200$	$0.70^{\rm A}\pm0.24$	$392^{\rm A}\pm220$	$1.00^{\rm A}\pm 0.51$	

The content of nitrates and nitrites in cold-stored sour white cabbage under the influence of packaging type and storage time [mg/kg d.m.]

Values are presented as mean value \pm standard deviation (n = 3). The values denoted with the same small or capital letters don't differ statistically significantly at p < 0.05.

After 1-, 2- and 3-month chilled storage, the content of these substances in the sauerkrauts stored in the PE-LD bags decreased significantly ($p \le 0.05$) by 83.7, 52.7, and 66.3%. respectively, compared with the values before packaging (Table 1). With regard to the sauerkraut packed in the PET met/PE bags, 1- and 3-months' chilled storage caused statistically significant reductions in the nitrate levels of 73.4 and 70.1%

respectively; after 4-months of storage, a significant increase was noted of 19.6% compared with the unpacked sauerkraut; while after 2 months of storage in such bags, the nitrate level corresponded (p>0.05) to the level determined prior to packaging.

After 1-, 2-, and 3-month chilled storage of the red cabbage sauerkraut, the level of these substances fell significantly ($p \le 0.05$) in the products packed in the PE-LD bags (48.1, 13.1, and 39% respectively) as well as in those kept in the PET met/PE bags (56.7, 19.8, and 49.5% respectively), compared with the product before packaging (Table 2). In comparison with the unpacked sauerkraut, after 4 month of storage there were no statistically significant (p > 0.05) changes in the content of these substances, regardless of the type of packaging applied.

Table 2

The kind of processing	Nitrates [NO ₃ ⁻]		Nitrites [NO ₂ ⁻]		
Before storage	$1042.3^{ab} \pm 0.7$		$12.20^{\circ} \pm 0.42$		
	The kind of packaging				
Cool storage	Zipper seal bags (PE-LD)		Bags (PET met/PE)		
	Nitrates	Nitrites	Nitrates	Nitrites	
1 month	$541^{de} \pm 92$	$13.50^{\rm bc} \pm 0.46$	$451^{e} \pm 11$	$12.20^{\circ} \pm 0.45$	
2 months	$906^{\circ} \pm 33$	$19.90^{a} \pm 0.22$	$836^{\circ} \pm 26$	$14.50^{b} \pm 0.33$	
3 months	$635.3^d\pm 6.5$	$9.00^d\pm0.30$	$525.9^{de}\pm4.5$	$4.90^{\rm e} \pm 0.54$	
4 months	$1151^{a} \pm 29$	$4.70^{e} \pm 0.32$	$958^{bc} \pm 54$	$6.1^{e} \pm 1.2$	
Mean value for packaging	$855^{A} \pm 240$	$11.9^{A} \pm 5.0$	$763^{\rm A} \pm 240$	$10.0^{A} \pm 3.8$	

The content of nitrates and nitrites in cold-stored sour red cabbage under the influence of packaging type and storage time [mg/kg d.m.]

Values are presented as mean value \pm standard deviation (n = 3). The values denoted with the same small or capital letters don't differ statistically significantly at p < 0.05.

A type of the packaging used was found to have no statistically significant (p > 0.05) effect on the level of nitrates in the sauerkrauts stored in chilling conditions.

The nitrite levels in white and red cabbage sauerkraut were expressed as the amount of NO_2^- nitrate ions per kg of dry matter (Tables 1 and 2).

In the case of white cabbage sauerkraut chilled stored in the zipped PE-LD bags, only in the samples stored for 2 months was the content of nitrites significantly higher ($p \le 0.05$) (of 122.4%); whereas, in those kept in the another packaging type, after 2 month and 3 month of chilled storage increased by 293.1% and 138.0% respectively, compared to the levels determined in the vegetables prior to packaging (Table 1).

Two-month chilled storage led to a significant increase ($p \le 0.05$) in the level of nitrites in the red cabbage sauerkraut stored in the zipped PE-LD and PET met/PE bags of 63% and 18.9% respectively; although, after 3- and 4-month periods of storage the significant reductions were observed of respectively 26.2 and 61.5% (PE-LD bags) as well as 59.8 and 50.0% (PET met/PE bags), compared to the unpacked product (Table 2).

It has been proved that a type of the packaging used had no significant effect (p > 0.05) on the level of nitrites in the cold-stored sauerkrauts (Tables 1 and 2).

Discussion

Nitrates and nitrites

The results obtained in this work for the nitrate levels in the sauerkrauts not being packed, generally correspond to the literature data concerning their levels, however, in the raw (not soured) red and white cabbage. Gajewska et al [23] proved that white cabbage harvested in the Spring/Summer season contained 75.0–915.2 mg NaNO₃/kg fresh weight, while the cabbage originated from the Autumn/Winter season had 30.5-655.4 mg NaNO₃/kg fresh weight. The sour white cabbage examined in this work had 50.6 mg nitrates per kg fresh weight, when calculated per NO₃⁻ ions, and 69.3 mg/kg fresh weight, expressing the result as the amount of NaNO₃. The amounts of nitrates reported by Du et al [26] in white cabbage were 259–1250 mg NO₃⁻/kg fresh weight, exceeding the results obtained in the present work.

According to Santamaria [27], vegetables from *Cruciferae* family, depending on species, belong to the vegetable group characterized by either low or moderate level of nitrates, being respectively: 200–500 mg/kg fresh weight in broccoli and cauliflower; and 500–1000 mg/kg fresh weight in cabbage and Savoy cabbage. Compared to the value obtained in this work, the level of nitrates in fresh red cabbage (958.7 mg NO_3^- /kg fresh weight) reported by Wojciechowska et al [28] was lower. Such a large discrepancy in the values reported by various authors may be due to the fact that in the case of vegetables, their ability to accumulate nitrates may result from genetic factors and is a characteristic feature attributed to the specific plant species or individual cultivar [23].

Nitrates level in vegetables is contingent not only on the cultivation conditions but also on biological features of these plants. Different plant parts accumulate different contents of nitrates. They are transported from roots principally to leaves where they undergo biotransformation. For this reason, leafy vegetables usually contain more these compounds than other vegetable types. Nitrates accumulation is the largest in the first days after nitrogen fertilization and also in plants in which photosynthesis is limited. Therefore, nitrates content is higher in the morning than in the afternoon. Nitrates and nitrites can also be formed by transformation of nitrogen compounds during storage of products (vegetables) in coolers and freezers [8].

It has been observed in this work that nitrate contents in cold-stored sauerkrauts fluctuated in subsequent four months and the values found were generally significant. However, after this period of storage, the level of this constituent was similar to the value determined prior to packaging, regardless of the packaging type. Tendencies presented by the authors with regard to several vegetable species are similar and not so clear-cut. Wojciechowska and Rozek [29] noted that red cabbage which was chilled stored for four months had 22.3% more nitrates and these results are much more higher than those reported in this paper. As for the butterhead lettuce stored for 14 days in the PE bags, after 7 days, a great increase (of 36.4%) in these substances was observed and then, after 14-days' storage, a fall of 6.8%, compared to the vegetable not being packed [30]. A large fall in the levels of these substances (from 46 to 49%) was observed by Chew et al [31] in various cold-stored (4°C) Amaranthus species. A reduction in nitrate

content in the stored vegetables could result from their conversion into nitrites. Storing of the plant raw material under inappropriate conditions (in the temperature higher than recommended) along with the lack of oxygen may lead to undesirable biochemical processes, which in turn may affect the levels of nitrates [32].

The results obtained cannot be fully verified by the findings of other authors, since, particularly with regard to the products stored in various types of packaging, there is no data on an effect of chilled storage on changes in nitrate contents.

The nitrite levels determined prior to packaging in the sauerkrauts investigated generally do not agree with the values reported by other authors. A mean value declared by Hou et al [33] in white sour cabbage was over five times higher, being 3.08 mg/kg fresh weight for the packed product; and 6.41 mg/kg fresh weight for the unpacked sauerkraut. The aforementioned authors presented also such values for the marinated cucumber (2.62 mg/kg fresh weight) and turnip (2.68 mg/kg fresh weight).

Nitrite contents in the raw vegetables from Cruciferae family fluctuated from 1.47 mg/kg fresh weight in green cauliflower to 3.49 mg/kg in white cauliflower, as was reported by Leszczynska et al [32]. The amounts reported by Smiechowska [34] (0.2–3.3 mg/kg fresh weight) were similar to those found in this work; although, slightly higher levels of 0.9 mg NaNO₂/kg fresh weight in the Spring/Summer season and 1.1 in the Autumn/Winter season were recorded by Gajewska et al [23]. According to Du et al [26], in white cabbage nitrite content ranged from 0.00 to 0.41 mg NO₂⁻/kg fresh weight, being minimally lower than the value obtained in this work.

Greater amounts of these substances were determined in lettuce and beetroot: in the Spring/Summer season: respectively 2.3 mg NaNO₂/kg and 1.5 mg NaNO₂/kg fresh weight; and in the Autumn/Winter season: respectively 2.9 mg NaNO₂/kg and 1.8 mg NaNO₂/kg fresh weight fresh weight [23]. On the other hand, it has been revealed that tomato, carrot or cucumber possess low tendency to accumulate these substances (about 0.6 mg NaNO₂/kg fresh weight). The authors of certain papers, did not state the presence of nitrites in the vegetables examined, at all; these were findings of Hsu et al [35] with regard to English spinach, Chinese cabbage and Iceberg-type lettuce as well as Huarte-Mendicoa et al [36] referring broccoli.

The nitrite level in fresh healthy and undamaged vegetables which were stored properly is low probably due to retaining the balance between nitrate and nitrite reductases. During the process of fermentation, nitrite concentration rises as a result of microbiological decomposition of nitrates and activity of endogenous nitrate reductase. The level of these substances is affected by a number and type of LAB species. Throughout the process of storage, accumulation of nitrites can be inhibited [33].

Four-month chilled storage resulted in reductions in this parameter in white and red sour cabbage stored in the PE-LD bags of 16.7 and 6.3% respectively, compared to the product not being packed.

In the case of white sour cabbage kept in the PET met/PE bags under chilled conditions, the content of these substances was increasing at each stage of investigation and after 4 months of storage increased by 16.7% compared to the unpacked product. It is worth noting that such tendency was not observed for the sauerkraut obtained from red cabbage, in which a rapid statistically significant decrease of 50% was recorded,

compared to sauerkraut not being packed. Generally, it has been revealed that a type of packaging did not have a significant effect on the levels of nitrites in the sauerkrauts analyzed; however, there is no data in the available literature confirming this thesis.

It has been found that losses noted in this parameter were smaller of 27.5% in Butter-head lettuce, which was chilled stored for 14 days [30]. On the other hand, Chew et al [31] observed an increase in nitrite content within the range 54–70% in the blanched Amaranthus after its 4-day chilled storage. In turn, the presence of packaging had a substantial effect on the levels of the aforementioned substances, as was observed by Hou et al [33]. Packed vegetables, compared to those unpacked, exhibited a 2-fold decrease in their content, which agrees with the findings obtained for the sour red cabbage.

Conclusions

It has been observed in this work that nitrate and nitrite contents in cold-stored sauerkrauts fluctuated in subsequent four months and the values found were generally significant.

Tendencies presented by this paper are not clear-cut, but after this period of storage, the level of nitrates and nitrites (excluding red cabbage sauerkraut) were generally similar to the value determined prior to packaging, regardless of the packaging type.

A type of the packaging used was found to have no statistically significant effect on the level of nitrates and nitrites in the sauerkrauts stored in chilling conditions.

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WPŁYW OPAKOWANIA NA ZAWARTOŚĆ AZOTANÓW(V) I (III) W KAPUSTACH KISZONYCH, CHODNICZO SKADOWANYCH

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Abstrakt: Azotany(III) są dużo bardziej toksyczne dla zdrowia człowieka niż azotany(V). Związki te są prekursorami N-nitrozozwiązków, które charakteryzują się właściwościami kancerogennymi, mutagennymi i embriotoksycznymi. Celem niniejszej pracy było zbadanie wpływu rodzaju opakowania (torebki wykonane z folii polietylenowej o małej gęstości (PE-LD) oraz torebki z metalizowanego politereftalanu etylenu (PET met/PE) i czasu chłodniczego składowania na zmiany zawartości azotanów(V) i azotanów(III) w kapuście kiszonej białej i kapuście kiszonej czerwonej. Analizy były wykonywane w warzywach przed zapakowaniem oraz po 1-, 2-, -3 i 4-miesięcznym okresie chłodniczego składowania w dwóch rodzajach opakowań. W toku pracy zaobserwowano na ogół istotne statystycznie zmiany w zawartości azotanów(V). Badania nie wykazały istotnego statystycznie (p > 0,05) wpływu rodzaju użytego opakowania na zawartość azotanów(III) w przechowywanych chłodniczo kiszonkach.

Słowa kluczowe: przechowywanie chłodnicze; azotany(V), azotany(III), opakowania strunowe z PE-LD, opakowania metalizowane PET/met/PE, kapusta kiszona biała, kapusta kiszona czerwona