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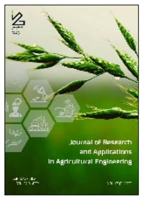

Antioxidant properties of beetroot fortified with iodine

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This study aimed to investigate the use of beetroot as a carrier of potassium iodide (KI) and potassium iodate (KIO_3) at different concentrations (2.3, 0.23, and 0.023 mg kg^{-1}). It was hypothesised that the concentrations and forms of iodine fortification affect the antioxidant activity of beetroot. The results showed a high recovery of the introduced iodine, especially for KIO_3.
However, a relationship it was confirmed between the forms, and concentration of iodine and the free radical scavenging (the $ABTS^{\bullet+}$ and the $DPPH^{\bullet}$) test results. In the systems with the iodine concentration at 0.023; 0.23 mg kg^{-1}, the antioxidant activity of the beetroot did not change. Nevertheless, a statistically significant decrease in free radical scavenging was confirmed for the beetroot fortified with the KIO_3 concentration of 3.9 mg kg^{-1} (2.3 mg kg^{-1} of iodine). Therefore, maximum amount of KIO_3 addition to beetroot should be 0.39 mg kg^{-1}.

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1. Introduction

Food fortification is the most common strategy to alleviate human nutrient deficiencies [1]. Approximately 30% of the world's population remains at risk of iodine deficiency [2]. Programs for food fortification with iodine are carried out in many countries worldwide to minimise the risk of this element's deficiency in the diet [3, 4]. One of the most common fortification strategies is salt iodization. Nonetheless, in 2006, the World Health Organisation recommended limiting salt intake to 5 g/day as it is a risk factor for atherosclerosis and hypertension [5]. Consequently, the iodine supply from this source can be limited [6–8]. It is, therefore, necessary to find new carriers for iodine salts. Preliminary studies showed the possibility of using beetroot as a carrier of iodine salt with the simultaneous design of a cereal product with its addition [9]. The fortification of beetroot may constitute an attractive alternative source of iodine for all consumers, especially vegetarians and vegans. Beetroot is commonly consumed in addition to juice, powder, bread, and gels. They are consumed in boiled, oven-dried, pickled, and pureed forms. Beetroot supplements are also an important source of dietary polyphenols owing to their many health benefits [10]. Many studies also confirm the antioxidant properties of beetroot [11-14]. However, preliminary studies indicate the existence of certain correlations between the antioxidant activity and the iodine content [15]. The antioxidant activity of pumpkin in the presence of iodine was found to be variable. A statistically significant decrease in free radical

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scavenging was confirmed for pumpkin varieties fortified with a KIO_3 concentration of 2.3 mg kg^{-1}. In contrast, fortification with a KI concentration of 2.3 mg kg^{-1} caused no significant changes in the antioxidant activity in the iodine-fortified pumpkin. Also, such an impact was not found at lower concentrations of both iodide and iodate [16].

Therefore, this study aimed to investigate to possibility of using beetroot as a carrier of potassium iodide (KI) and potassium iodate (KIO_3) at different concentrations. It was hypothesised that the concentrations of iodine fortification affect the antioxidant activity of fortified beetroot.

2. Materials and methods

2.1. Material

Beetroot (*Beta vulgaris L. subsp. Vulgaris,* var. Czerwona Kula) was used as the matrix for the iodine. The plant material was from marketplaces in the region of Poland - Wielkopolska. The product contained iodine in the amount below 0.003 mg kg^{-1}. The KI and KIO_3 constituted the sources of iodine (Merck, Germany).

2.1.1. Conditions of impregnation

The samples were steamed (100 °C; 10 min) in a convection oven (Rational, Landsberg am Lech, Germany), drained and homogenised (homogenizer—Foss, Hilleroed, Denmark). The next impregnation stage was soaking the beetroot samples in an aqueous solution of KI/KIO_3. In the research, the model adopted three variable iodine concentrations: 0.023 mg kg^{-1} (0.030 mg kg^{-1} of KI or 0.039 mg kg^{-1} of KIO_3 – low iodine level in food products), 0.23 mg kg^{-1} (0.30 mg kg^{-1} of KI or 0.39 mg kg^{-1} of KIO_3 - natural iodine levels in foods from iodized salt) and 2.3 mg kg^{-1} (3.01 mg kg^{-1} of KI or 3.88 mg kg^{-1} of KIO_3 - fortified matrices for food fortification). The following impregnation conditions were assumed: the degree of hydration in the ratio 1:1 (m/v) and incubation at -76 °C/12 h. Then the impregnated preparations were freeze-dried to a moisture content of 4–5%. Finally, the dried samples were homogenised (homogenizer—Foss, Hilleroed, Denmark).

2.1.2. Storage conditions of iodine sources

The impregnated and freeze-dried beetroot samples were stored in jars (black glass, closed with a screw top, d=7 cm, h=10 cm). The influence of the storage conditions on the stability of KI and KIO_3 was tested during storage during 320 days at 21±1 °C.

2.2. Methods

2.2.1. Stability of iodine

The quantitative changes in the total iodine (after drying and at 1, 60, 120, 180, 240 and 320 days of storage) were determined by means of the macro chemical method with potassium thiocyanate described by Kuhne, Wirth, and Wagner [17], and subsequent colorimetric analysis [18].

2.2.2. Antioxidant activity

All the beetroot samples directly after drying and after 320 days of storage were taken for analysis of antioxidant activity. Ethanol extracts from beetroot were prepared by 2-h maceration of the dried beetroot with 80% ethanol (1:10 (m/v)) [19].

The antioxidant activity of the prepared ethanol extracts of beetroot with iodine was examined based on the free radical scavenging indices — the DPPH scavenging capacity ($DPPH^{\bullet}$) and the ABTS scavenging capability ($ABTS^{\bullet +}$).

The $DPPH^{\bullet}$ scavenging capacity [20, 21] was tested utilising spectrophotometric methods with the DPPH radical.

The $ABTS^{\bullet +}$ scavenging capability [22], was tested by means of spectrophotometric measurement of changes in the concentration of the ABTS radical cation (98%). The DPPH and ABTS scavenging capability was tested with regard to the scavenging capacity of Trolox (97%). The result was expressed as mg Trolox/100 g dry matter of extract.

3. Chemicals

The following chemicals were used: KI and KIO_3 — the sources of iodine (Merck, Germany); DPPH radical (2,2-difenylo-1-pikrylhydrazyl) (Sigma-Aldrich , Saint Louis, Missouri, USA); ABTS radical cation (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) (98%), (Sigma-Aldrich, Saint Louis, Missouri, USA); Trolox (6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) (97%), (Sigma-Aldrich, Saint Louis, Missouri, USA). All the chemicals and solvents used in the tests were of an analytical grade.

4. Statistical analysis

The obtained results were subject to statistical analysis using STATISTICATM PL 13.3 (StatSoft, Cracow, Poland) software. The software was employed to calculate significant differences between the means ($p< 0.05$, analysis of variance ANOVA), and Tukey's mul tiple range test. The iodine content and the antioxidant activity of the tested samples were analysed in 6 samples (2 independent samples and three measurements for each sample) (2 independent samples and three measurements for each sample).

5. Results and discussion

5.1. Iodine stability

Table 1 shows the iodine content (%) of the beetroot samples fortified with iodine KI and KIO_3 directly after drying and after storing. It was found that the differences in the iodine content in were in the range of 83-92% directly after drying, and 61-76% after storage. Previous data on the fortification of protein preparations and vegetables confirm the maximum reproducibility of iodine in fortified matrices at a similar level [16, 23]. High iodine stability in storage was also confirmed. The analysis of variance (One-Way ANOVA test) revealed (Table 2) a statistically significant effect ($p< 0.05$) of the type of iodine compound used for fortification (KI, KIO_3). Both iodine forms exhibited a capacity to accumulate iodine in large amounts, with a higher concentration noted for KIO_3 than KI. Previous studies also confirmed the higher instability of iodine in the form of KI [24].

5.2. Antioxidant activity directly after drying and after 320 days of storage.

The results of our study confirmed the antiradical effect of beetroot with the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods [10, 19].

The statistical analysis (Table 3) confirmed a relationship between the forms of iodine (KI/KIO_3) and the $ABTS^{\bullet+}$ and the $DPPH^{\bullet}$ test results. The strongest relationship (one-way ANOVA test) was confirmed between the iodine concentration in the form KIO_3 and $ABTS^{\bullet+}$ ($F = 10248.00$; $p< 0.05$) and the $DPPH^{\bullet}$ test results ($F = 95024.34$; $p< 0.05$). In the systems with the iodine concentration at 0.023 and 0.23 mg kg^{-1} (0.030 and 0.301 mg kg^{-1} of KI or 0.039 and 0.388 mg kg^{-1} of KIO_3), the free-radical scavenging capacity indices ($ABTS^{\bullet+}$ and $DPPH^{\bullet}$) did not change. This was confirmed for the samples directly after drying and after 320 days of storage. The fortification with KI at 2.3 mg kg^{-1} caused no significant changes in antioxidant activity in the iodine-fortified beetroot.

Nevertheless, in the samples directly after drying containing KIO_3 at 2.3 mg kg^{-1}, the capacity to terminate $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ decreased by 7% compared to the samples without iodine. This was also confirmed for the samples after storage. It was found that the capacity to terminate $DPPH^{\bullet}$ decreased by 29% and $ABTS^{\bullet+}$ by 21%, compared to the samples without iodine.

An earlier study confirmed that KI and KIO_3 have different pro- and antioxidative properties; KI is the reductant, while KIO_3 is the oxidant [25, 26]. An earlier study also confirmed that iodine, especially iodate, may react with oxidizable substances [27]. It was found that lipid oxidation, the degradation of ascorbic acid, and changes in protein functions could be mediated through the presence of iodate. Additionally, it was confirmed that iodate in high concentrations with herbs damages rat thyroid follicular cells less, which may be related to the oxidant activity of iodate and the high antioxidant capacity of these herbs [28].

6. Conclusions

Beetroot can be fortified with iodine with a higher stability obtained using KIO_3. The addition of iodine in the form of KI does not affect the free radical scavenging activity of beetroot. Nonetheless, the addition of KIO_3 at 3.9 mg kg^{-1} (2.3 mg kg^{-1} of iodine) confirmed a statistically significant decrease in free radical scavenging. Therefore, for maximum effectiveness in beetroot free radical scavenging indices, it is suggested to introduce iodine in the form of KI and KIO_3, but in controlled concentrations. The maximum addition of KIO_3 should be 0.39 mg kg^{-1}.

Table 1. Iodine content [%] in beetroot fortified with KIO_3 and KI

Iodine form	Iodine concentration [mg kg^{-1}] 0.023		0.23		2.30	
	%	standard deviation	%	standard deviation	%	standard deviation
directly after drying						
KI	83.27^{bA*}	0.15	83.28^{bB}	0.22	84.21^{bB}	0,11
KIO_3	91.25^{aB}	0.20	92.03^{aB}	0.21	92.10^{aA}	0,14
after storage (320 days)						
KI	61.25^{bA}	0.19	61.45^{bBA}	0.31	61.56^{bB}	0.19
KIO_3	73.58^{aB}	0.22	75.02^{aB}	0.11	75.98^{aA}	0.24

*Mean values (n = 6); different letters (lower case letters in same form of iodine; upper case letters in same concentration of iodine) denote significant difference at $p< 0.05$ (one-way ANOVA, and post hoc Tukey test).

Table 2. Statistical significance of predictors of variance models for changes in iodine content in fortified beetroot directly after drying and after 320 days of storage (one-way ANOVA test)

Predictors	SS	df	MSE	F-value	*p*-value
directly after drying					
Iodine concentration	2.42	2	1.21	0.06	0.94
Iodine form	303.81	1	303.81	1546.41	0.00
after storage (320 days)					
Iodine concentration	13.39	2	6.70	0.09	0.92
Iodine form	4081.20	1	4081.41	110.94	0.00

*SS – statistical significance; df - degrees of freedom; MSE - mean sum of squares.

Table 3. Statistical significance of predictors (iodine concentration) of covariance models for changes in $ABTS^{•+}$ and $DPPH^{•}$ scavenging capacity in iodine-fortified beetroot directly after drying and 320 days of storage (one-way ANOVA test)

Predictors	SS	df	MSE	F-value	*p*-value
$ABTS^{•+}$					
directly after drying					
KIO_3	108.95	3	36.32	421.72	0.00
KI	0.15	3	0.05	264.12	0.00
after 320 days of storage					
KIO_3	204.96	3	68.32	10248.00	0.00
KI	2.43	3	0.81	1298.60	0.00
$DPPH^{•}$					
directly after drying					
KIO_3	105.25	3	35.08	6102.21	0.00
KI	0.22	3	0.07	212.32	0.00
after 320 days of storage					
KIO_3	451.37	3	150.45	95024.34	0.00
KI	1.62	3	0.54	676.78	0.99

*SS – statistical significance; df - degrees of freedom; MSE - mean sum of squares

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