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ORIGINAL PAPER

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THE EFFECT OF IODINE FORTIFICATION ON – THE ANTIOXIDANT ACTIVITY OF CARROTS AND CAULIFLOWER

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ABSTRACT

Introduction. In many countries worldwide, food fortification programs with iodine are carried out to minimise the risk of a deficiency of this element in the diet. However, preliminary studies have shown correlations between antioxidant activity and iodine content.
Aim. The aim of this study was to examine the use of cauliflower and carrots as matrices for potassium iodide (KI) and potassium iodate (KIO_3) at different concentrations. It was hypothesised that iodine compounds and their concentrations affect the antioxidant activity of fortified cauliflower and carrots.
Material and methods. The study tested variable iodine concentrations: 0.023; 0.23; 0.77; 1.30; 2.30 and 3.0 mg KIO_3 or KI/100 g. These iodine concentrations were applied to two varieties of carrot and two varieties of cauliflower. After the fortification process and 60, 120, 180, 240, and 320 days of storage, the iodine content was determined. Additionally, after 320 days of storage, the antioxidant activity of all vegetable samples was analysed (based on two free radicals scavenging indices, the DPPH scavenging capacity ($DPPH^•$) and the ABTS scavenging capability ($ABTS^{•+}$)).
Results. Covariance between the iodine compound (KI/KIO_3) and the $ABTS^{•+}$ and $DPPH^•$ test results of fortified carrots and cauliflower was noted. For the samples of dried carrots and cauliflower with iodine concentrations from 0.23 to 3.0 mg kg^{-1} of KI or 0.23 to 1.30 mg kg^{-1} of KIO_3, the free-radical scavenging capacity indices were similar to those for samples not fortified with iodine. However, for the samples of both carrots and cauliflower with KIO_3 at 2.30 to 3.0 mg kg^{-1}, the capacity to terminate $ABTS^{•+}$ and $DPPH^•$ was lower than in samples without iodine. This was especially true after storage.
Conclusions. Cauliflower and carrots can be good iodine matrices. However, to maximise free radical scavenging indices, iodine should be fortified at concentrations of up to 2.30 mg kg^{-1}.

Keywords: iodine fortification, cauliflower, carrot, antioxidant activity

INTRODUCTION

Vegans eliminate both fish and dairy products from their diet, making them particularly vulnerable to iodine deficiency. Additionally, the iodine content in plant products is related to the concentration of this element in the soil (Hatch-McChesney and Lieberman, 2022). The primary plant source of iodine may be algae, as they are rich in this element (Hatch-McChesney and Lieberman, 2022). However, seaweed is not customarily consumed in the Western diet, although its popularity is increasing. This also applies to the diet in Poland (Zaremba et al., 2023a). In addition, legal restrictions limit the potential to enhance food with algae (Cruz and Vasconcelos, 2024). Plant-based dairy and meat analogues are becoming increasingly

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popular, but because they are naturally low in iodine, they cannot be thought of as nutritionally equivalent to milk. In many countries, iodine fortification programs are implemented to minimise the possibility of dietary iodine deficiency (Chanthilath et al., 2009).

The fortification of salt with iodine is one of the most common strategies for preventing iodine deficiency. However, in 2006, the World Health Organization recommended limiting salt intake to 5 g/day (WHO, 2007). Consequently, the supply of iodine from this source may be limited (WHO, 2022). This necessitates the use of new and stable carriers for iodine. Numerous studies indicate the effectiveness of iodine biofortification of plant products, including vegetables (Krzepiłko et al., 2015). Preliminary research has confirmed that vegetables such as pumpkins and beets are good matrices for iodine (Jankowska et al., 2023; Zaremba et al., 2023b). The high effectiveness of enriching bread with beets fortified with iodine has been confirmed. Our preliminary research has also confirmed that there is no change in the taste (Zaremba et al., 2022). Enriching other vegetables could diversify the diets of consumers and also be an effective alternative source of iodine. This may especially apply to both overweight people and vegetarians, particularly vegans. Carrot is often eaten as an addition (dried juice or gel) to ice cream, pasta, cereal snacks and other cereal products (Dziki, 2021). Also, the enrichment of pasta and bread with cauliflower could be thought of as a support to increase vegetable consumption without seriously changing the population's eating habits (Nartea et al., 2023; Shamshad et al., 2023). Products containing cauliflower and carrot are also an important source of polyphenols (Nartea et al., 2023), and many studies have confirmed their antioxidant properties. However, preliminary studies have shown some correlations between antioxidant activity and iodine content (Iwan et al., 2021). The variable antioxidant activity of pumpkin dried, fortified with iodine, has also been observed. It depended on the pumpkin variety and the amount and type of iodine compound used (Zaremba et al., 2023b). Therefore, the aim of this study was to investigate using cauliflower and carrots as a matrix for potassium iodide (KI) and potassium iodate (KIO_3) at different concentrations. It was hypothesised that iodine compounds and their concentrations may influence the antioxidant properties of fortified cauliflower and carrot.

MATERIALS AND METHODS

Materials

Cauliflower (*Brassica oleracea* var. Botrytis L., var. David and Bora) and carrot (*Daucus carota* L.) were used as matrices for the iodine. The products, in a ripe state, were bought at a retail store (Poznań, Poland) in August and September. KI and KIO_3 were used as iodine compounds (Merck, Darmstadt, Germany).

Methods

Conditions of preparation

Washed and peeled (carrot) vegetables were heat treated (100°C; 10 min) in a convection oven (Rational, Landsberg am Lech, Germany), drained and homogenised (homogeniser Foss, Hilleroed, Denmark). Then, the carrot or cauliflower homogenates were soaked in the aqueous solution of KI/KIO_3 (1:1 (m/v)) at 18 ±2°C (Zaremba et al., 2022). Variable iodine concentrations were adopted: 0.023; 0.23; 0.77; 1.30; 2.30; and 3.0 mg/100 g. The homogenates of carrot and cauliflower with iodine were subjected to the freeze-drying process (4–5% of the moisture content). The samples of dried carrots and cauliflower fortified with iodine were stored in glass containers for 320 days at 21 ±1°C.

Stability of iodine

The total iodine content in all samples after 60, 120, 180, 240 and 320 days of storage was determined with a macro chemical method using potassium thiocyanate (Kuhne et al., 1993; Moxon and Dixon, 1980).

Antioxidant activity

The antioxidant activity of dried carrots and cauliflower fortified with iodine was analysed immediately after freeze-drying and after 320 days of storage. Extracts from carrot or cauliflower were prepared by 2-h maceration of dried vegetables with 80% ethanol (1:10 (m/v)) (Gudiño et al., 2022).

The antioxidant activity of dried carrots and cauliflower fortified with iodine was analysed based on two free radical scavenging indices: the DPPH scavenging capacity ($DPPH^•$) (Chu et al., 2000; Nuutila et al., 2003) and the ABTS scavenging capability ($ABTS^{•+}$) (Re et al., 1999). The $DPPH^•$ scavenging capacity was analysed by the spectrophotometric method using the

DPPH radical (2,2-difenylo-1-pikrylhydrazyl) (Sigma-Aldrich, Saint Louis, Missouri, USA) at 517 nm of absorbance. The $ABTS^{•+}$ scavenging capability was analysed by spectrophotometric measurement of changes in the concentration of the ABTS radical cation (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)) (98%), (Sigma-Aldrich, Saint Louis, Missouri, USA) at 734 nm of absorbance.

Statistical methods

An analysis of the statistical dependencies between the means of antioxidant activity, iodine content and iodine compound ($p < 0.01$) was performed with Tukey's multiple range test and PCA analysis. These analyses were calculated using the software STATISTICA PL 13.3 (StatSoft, Cracow, Poland). The iodine content was analysed in 6 samples (3 measurements / 2 independent series). Hypotheses were tested at $\alpha = 0.01$. To predict the dynamics of losses of iodine in dried carrots and cauliflower fortified with iodine throughout storage, the $T_{25\%}$ value was used. This calculation indicates the time in which the losses will amount to 25% (Szymandera-Buszka et al., 2021).

RESULTS AND DISCUSSION

Iodine stability after the fortification process and storage

Our results confirmed that all types of dried carrots and cauliflower (both varieties) were suitable as iodine fortification matrices (Fig. 1). The iodine reproducibility content was found to be 68–80% directly after drying. Higher iodine stability was confirmed for cauliflower samples (post hoc Tukey test; $p < 0.05$). Earlier research on the enrichment of protein preparations and pumpkin with iodine confirms the reproducibility of iodine at similar values (Zaremba et al., 2022). The statistical analysis (one-way ANOVA test) confirmed the significance of iodine compound (KI, KIO_3) (F-value = 81.70) and vegetable type (F-value = 5.40) as predictors of changes in iodine content (Table 1). A higher concentration of iodine was confirmed in cauliflower than in carrots. Our research confirmed that both forms of iodine exhibited high iodine stability after the freeze-drying process, with a higher concentration noted for KIO_3 than for KI. Previous studies also found a higher stability of iodine in the form of KIO_3 (Waszkowiak and Szymandera-Buszka, 2007).

High iodine stability during storage was also confirmed. The statistical analysis (one-way ANOVA test) also revealed a statistically significant effect ($p < 0.05$) of the type of iodine compound used for fortification (KI, KIO_3) (F-value = 26.45) and vegetable type (F-value = 23.89) (Table 1). A higher concentration of iodine was noted for KIO_3 than for KI. The highest stability of iodine after 320 days of storage was found for KIO_3 applied to cauliflower and the lowest stability for KI added to carrot. In future research, this topic should be further developed by examining, for example, the

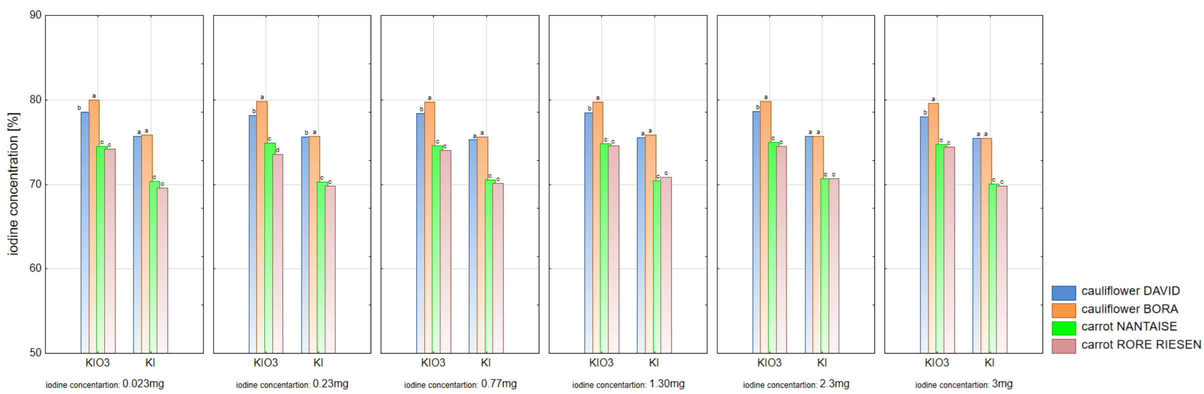

Fig. 1. The concentration of iodine (%) in dried carrots and cauliflower fortified with KI or KIO_3 in concentrations from 0.023 to 3.0 mg/100 g. Different letters signify a significant difference at $p < 0.05$ (tests of one-way ANOVA and post-hoc Tukey test) in the same concentration and type of iodine; values of the mean (n = 6)

Table 1. Statistical analysis of losses in iodine content for dried carrots and cauliflower (both varieties) fortified with iodine after the fortification process and after 320 days of storage (test of one-way ANOVA)

Predictors	SS	df	MSE	F-value	*p*-value
After drying					
Iodine concentration	1.50	5	0.30	0.10	0.99
Vegetables type	35.80	3	11.90	5.40	0.00
Iodine form	85.30	1	85.30	81.70	0.00
After 320 days of storage					
Iodine concentration	1.10	5	0.20	0.02	1.00
Vegetables type	301.10	3	100.40	23.89	0.00
Iodine form	177.40	1	177.40	26.45	0.00

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

possibility of iodine interactions with other vegetable ingredients and the related impact on iodine stability. Particular attention should be paid to analysing the different carbohydrate profiles and degree of ripeness of vegetables, especially for KI.

The analysis of the dynamics of losses in iodine content ($T_{25\%}$) confirmed a slower pace for KIO_3 (by 14–19%) than for KI (Table 2). The dynamics of iodine changes during storage were at a similar level for all iodine concentrations. However, the type of vegetable influenced these dynamics. Iodine applied to carrots had a statistically significantly faster pace of iodine loss. For cauliflower, the pace was slower by 14 to 20%.

Antioxidant properties of dried carrots and cauliflower enriched with iodine

Our research results confirmed (using the $DPPH^{•}$ and $ABTS^{•+}$ methods) the antioxidant effect of dried carrots and cauliflower (Blando et al., 2021; Kapusta-Duch et

Table 2. The dynamics of changes in iodine content (mg kg^{-1}) for the storage (320 days) of dried carrots and cauliflower fortified with iodine at variable concentrations and in different vegetable types

Parameters fortifications		Dynamics of change in iodine content during 320 days of vegetable storage									
Vegetable variety	Iodine concentration mg kg^{-1}	$T_{25\%}$ days	R^2	*RMSE*	k	A_0^*	$T_{25\%}$ days	R^2	*RMSE*	k*	A_0^*
		KIO_3					KI				
1	2	3	4	5	6	7	8	9	10	11	12
Cauliflower DAVID	0.23	379.07	0.99	0.00	0.000	1.02	330.13	0.99	0.00	0.000	1.02
	0.2.3	373.95	0.98	0.00	0.000	1.26	327.65	0.98	0.00	0.000	1.26
	0.77	379.66	0.99	0.01	–0.001	2.18	324.98	0.98	0.01	–0.001	2.18
	1.30	377.38	0.99	0.01	–0.001	3.72	326.95	0.98	0.01	–0.001	3.73
	2.30	377.14	0.99	0.01	–0.002	10.14	330.08	0.99	0.02	–0.002	10.29
	3.00	371.06	0.98	0.02	–0.002	20.82	323.80	0.98	0.04	–0.002	21.15
Cauliflower BORA	0.023	386.51	0.97	0.00	0.000	1.02	323.14	0.98	0.00	0.000	1.02
	0.23	388.36	0.96	0.00	0.000	1.26	325.01	0.97	0.00	0.000	1.26
	0.77	379.21	0.95	0.01	–0.001	2.19	319.19	0.97	0.01	–0.001	2.18
	1.30	385.64	0.96	0.02	–0.001	3.76	325.07	0.97	0.02	–0.001	3.72
	2.30	386.42	0.96	0.03	–0.002	10.43	323.63	0.98	0.03	–0.002	10.33
	3.00	379.30	0.95	0.04	–0.002	21.50	323.10	0.99	0.04	–0.002	20.78

Table 2 – cont.

1	2	3	4	5	6	7	8	9	10	11	12
Carrot NANTAISE	0.023	316.28	0.99	0.00	0.000	1.02	276.61	0.99	0.00	0.000	1.02
	0.23	318.65	0.98	0.00	0.000	1.26	274.60	0.99	0.00	0.000	1.26
	0.77	315.18	0.98	0.01	–0.001	2.19	276.08	0.99	0.01	–0.001	2.18
	1.30	316.97	0.99	0.01	–0.001	3.73	274.44	0.99	0.01	–0.001	3.75
	2.30	319.44	0.99	0.02	–0.002	10.34	276.47	0.99	0.03	–0.002	10.43
	3.00	314.53	0.98	0.04	–0.003	21.19	270.96	0.99	0.03	–0.003	19.47
Carrot RORE RIESEN	0.023	313.65	0.99	0.00	0.000	1.02	271.05	0.99	0.00	0.000	1.02
	0.23	310.59	0.98	0.00	0.000	1.26	274.46	0.99	0.00	0.000	1.26
	0.77	310.81	0.98	0.01	–0.001	2.18	275.12	0.99	0.00	–0.001	2.19
	1.30	316.27	0.99	0.01	–0.001	3.74	277.65	0.99	0.01	–0.001	3.75
	2.30	315.69	0.98	0.02	–0.002	10.40	278.90	0.99	0.02	–0.002	10.4
	3.00	313.46	0.99	0.00	–0.003	21.10	270.96	0.98	0.00	–0.003	20.95

* A_0 – the initial content (immediately after drying) of iodine, k – decay constant (Szymandera-Buszka et al., 2020).

al., 2017). However, there were differences between the different varieties of vegetable. The highest anti-radical activity was observed in cauliflower (David variety) and the lowest in carrots (Nantaise variety).

The statistical analysis also indicated a relationship between the type of iodine compound (KIO_3/KI) and the $ABTS^{•+}$ and the $DPPH^{•}$ test results (Table 3). The

Table 3. Statistical analysis of changes in $ABTS^{•+}$ and $DPPH^{•}$ scavenging capacity in dried carrots and cauliflower varieties fortified with iodine (KIO_3/KI) after the fortification process and after storage (320 days) (one-way ANOVA test)

Predictors	SS	df	MSE	F-value	*p*-value
1	2	3	4	5	6
$ABTS^{•+}$					
	After drying, fortified with KIO_3				
Iodine concentration	263.93	6	43.99	433.00	0.00
Vegetable variety	0.39	3	0.13	1.26	0.32
	After drying, fortified with KI				
Iodine concentration	0.03	3	0.02	0.57	0.58
Vegetable variety	0.73	6	0.121	4.415	0.06
	After 320 days of storage, fortified with KIO_3				
Iodine concentration	271.96	6	45.33	25.64	0.00
Vegetable variety	0.40	3	0.10	0.01	1.00
	After 320 days of storage, fortified with KI				
Iodine concentration	0.39	6	0.06	0.45	0.83
Vegetable variety	39.52	3	13.17	22.47	0.00
$DPPH^{•}$					
	After drying, fortified with KIO_3				
Iodine concentration	163.76	6	27.29	42.98	0.00
Vegetable variety	4.09	3	1.36	2.15	0.13
	After drying, fortified with KI				
Iodine concentration	3.54	6	0.59	1.35	0.29
Vegetable variety	0.80	3	0.27	0.61	0.62
	After 320 days of storage, fortified with KIO_3				
Iodine concentration	291.38	6.00	48.56	51.15	0.00
Vegetable variety	17.29	3.00	5.76	0.47	0.71
	After 320 days of storage, fortified with KI				
Iodine concentration	0.65	6	0.11	0.48	0.81
Vegetable variety	10.91	3	3.64	16.18	0.00

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

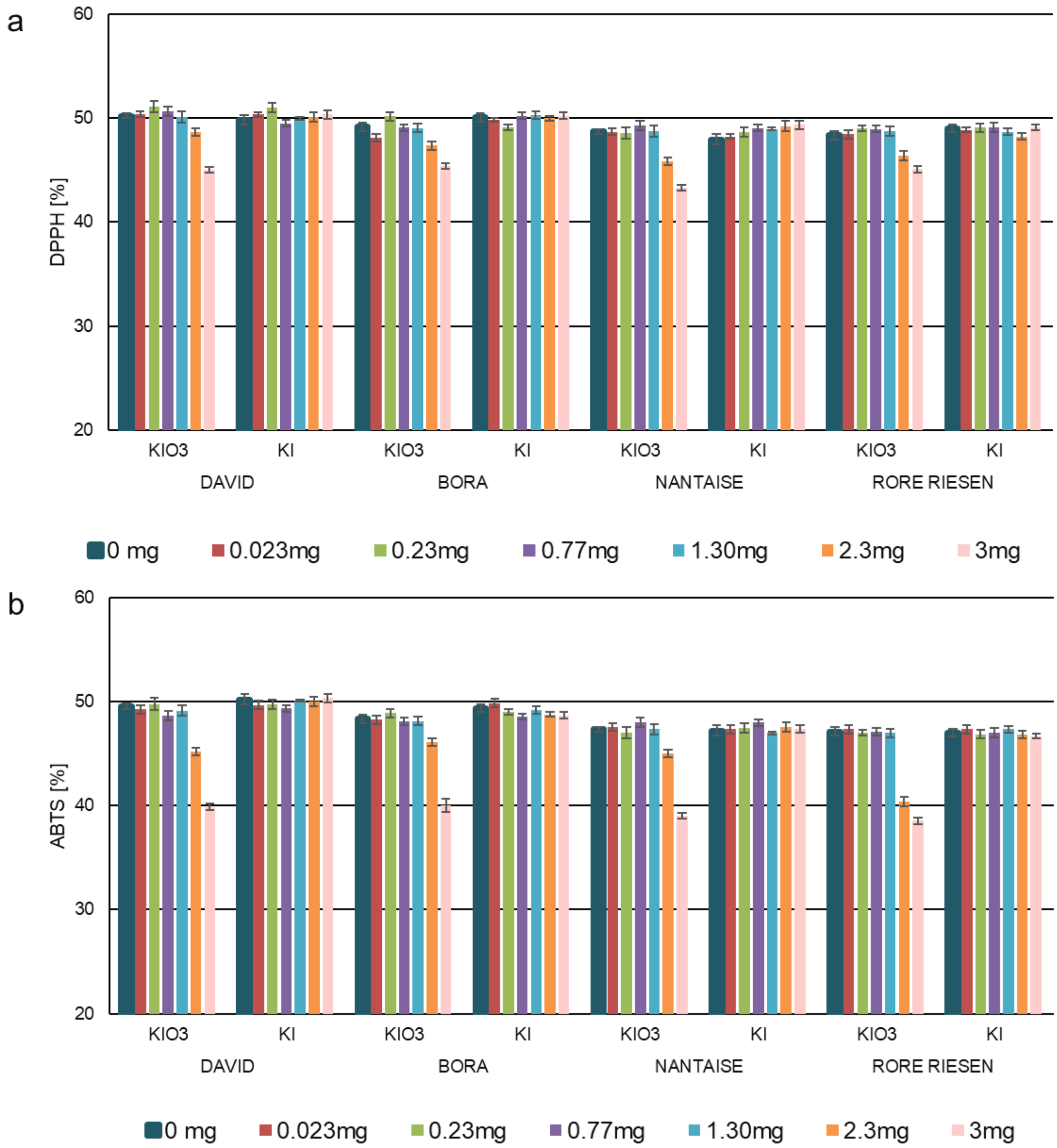

Fig. 2. The DPPH• (a) and ABTS•+(b) free radical scavenging capability of carrots and cauliflower enriched with KI and KIO_3 at levels from 0.023 to 3.0 mg/100 g after 320 days, compared to non-iodised samples

strongest relationship (one-way ANOVA test) was between the content of iodine in the compounds KIO_3 and the results of the DPPH• (F = 291.38; $p < 0.05$) and ABTS•+ tests (F = 271.00; $p < 0.05$). In the samples of dried carrots and cauliflower enriched with iodine at levels of 0.023 and 0.23 mg kg^{-1} (for both iodine compounds), the capacity indices derived from the ABTS•+ and DPPH• methods did not change (Figure 2a and 2b). Similarly, fortification with iodine in the form of potassium iodide at a level of 2.3 mg kg^{-1} resulted in no significant changes in the antioxidant properties of the enriched carrots and cauliflower. This relationship was found after both the drying and storage processes. In the samples containing KIO_3 at 2.3 mg kg^{-1}, on the other hand, the capacity to terminate ABTS•+ and DPPH• was decreased. This relationship was found both for the carrot and cauliflower samples. For these enriched varieties, the indicators of DPPH$^{\bullet}$ decreased by 9% and ABTS$^{\bullet+}$ by 6–9% immediately after drying, compared to those without iodine.

This trend was also found after storage. The indicators of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ decreased by 8–9% compared to the non-iodised samples.

An earlier study verified that KIO_3 and KI can have different pro- and antioxidative properties. The research results also indicate that iodine, especially in the form of iodate, has strong chemical activity and is also prooxidative (Li et al., 2020). Lipid oxidation, the variable stability of ascorbic acid, and changes in protein function could be mediated by the presence of iodate. The results of other authors have demonstrated the variable impact of applying iodine in its iodide (I^-) and potassium iodate (IO_3^-) forms on antioxidant concentration in tomato seedlings (Medrano-Macías et al., 2016).

CONCLUSIONS

Cauliflower and carrots can be fortified with iodine, and iodine-fortified carrots and cauliflower can be stored for a long time. Iodine stability after 320 days of storage was 70–80%, with a higher stability obtained using KIO_3. However, to maximise free radical scavenging indices, iodine should be fortified at concentrations of up to 2.30 mg/100 g.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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