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Impact of Iodine Biofortification on Tomato Bioaccessibility through Cooking, Soaking and Simulated Digestion

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ABSTRACT

Background: Even though iodine is not an essential micronutrient for the plant, it is very much needed for a person's mental and physical development. Due to the presence of negative charge on iodine it is highly susceptible to leaching. Further iodine is also highly prone to volatilization loss due to biochemical and physiochemical properties of soil leading to iodine deficiency. Agronomic biofortification of iodine is one of the ways to address iodine deficiency globally. Numerous studies have focused on the process by which plants absorb iodine from the soil, but there is still paucity of knowledge on stability of biofortified iodine in fruits.

Methods: In our work, we assessed iodine bioavailability (cooking, soaking and digestion) in tomato fruit from different sources of chitosan and potassium iodate alone and combinations using main and residual crop trials. The field experiment was carried out in Thondamuthur block of Viraliyur village at Coimbatore district of Tamil Nadu in 2021. Potassium iodate and chitosan were applied in the form of soil, foliar and chitosan iodate complex at different stages of plant growth.

Result: The results suggested that combination and potassium iodate and chitosan complex has increased the iodine stability in fruits of main crop and also retained the iodine in residual crop. As electrostatic interaction between chitosan and iodate prevents volatilization and gradually stabilizes the bioavailability of iodine in fruits. Our findings offer more details on iodine mobility and behaviour in fruits when it is used alone and combination with chitosan at different rates.

Key words: Bioavailability chitosan, Biofortification, Iodine, Tomato.

INTRODUCTION

The earth's iodine (as iodide) is extensively spread but unevenly distributed because iodine is a rare element that is primarily found as a salt, it is referred to as iodide rather than iodine. Iodine is found in soils in both inorganic and organic forms [Iodate-(IO_3^-) and Iodide- (I^-)]. Iodine is an essential micronutrient for a person's mental and physical development (Antonyak *et al*., 2018). Iodine is a component of thyroid hormone, which is essential for human health and plays an indispensable function in metabolism (Sorrenti *et al*., 2021). The recommended daily allowance (RDA) of iodine is 120 µg for children 6 to 12 years old, 150 µg for adults over 12 years old and 200 µg for pregnant and nursing women, according to the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF).

The thyroid hormone will stop acting if its iodine requirements are not met. Low thyroid hormone levels in the blood caused a slew of functional and developmental problems known as iodine deficiency disorder (IDDs) (Snart *et al*., 2019). The universal fortification of salt with iodine is one technique to treat iodine deficiency disorder (IDD). Over the last few decades, numerous countries have embraced this technique, which has drastically reduced the prevalence of iodine deficiency around the world (Abdurrahim *et al*., 2023). This strategy, however, will not be sufficient to treat iodine deficiency disorder. This is due to the fact that the iodine in table salt is unstable and exposed to increased volatilization (Dávila-Rangel *et al*., 2019). The concentration of iodine in salt, on the other hand, is highly variable and depends on the level of iodization at the time of manufacture, as well as future losses due to cooking or inappropriate storage (Desta *et al*., 2019).

Over the last few decades, fortification has helped to lower the prevalence of iodine deficiency around the world.

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Adding iodine-containing salts or iodine-rich organic materials (*e.g.*, seaweed) to soils can help crops absorb and concentrate this trace element. Iodine deficiency can be avoided by biofortifying commonly consumed crops with iodine (Dijck-Brouwer *et al*., 2022). Potassium iodide (KI), Potassium iodate (KIO_3) and potassium per iodate (KIO_4) are some of the common salts used for biofortification study. Among them potassium iodate salt is more stable when compared to potassium iodide as it is oxidized readily and lost by evaporation (Iwan *et al*., 2019).

Chitosan (Cs) is a biological polymer that acts as a metal and trace metal complexing agent and it is biodegradable (Gamage *et al*., 2023). Chitin is a polysaccharide-rich fibrous substance found in the exoskeletons of shellfish such as shrimp, lobsters and crabs as well as the cell walls of fungi (Ali *et al*., 2022). If Iodine is applied as a chitosan-iodate complex, the absorption of the iodine will be boosted. Hence in the present study potassium iodate along with chitosan is preferred over potassium iodide. The objective of the present study was to assess the potential of biofortified iodine in retaining the iodine content in the biofortified crop through some bioavailability experiments.

MATERIALS AND METHODS

To know the effect of biofortified iodine in improving the bioavailability of iodine in tomato two field experiment was carried out in summer and *kharif* season during the year 2021 in Viraliyur village of Thondamuthur block of Coimbatore district of Tamil Nadu (GPS value: 10°.9′99.284″N; 76.7′ 82.652″E). Iodine was biofortified through soil, foliar and chitosan iodate complex mechanism for the main crop. The experiments were performed in randomized block design with three replications in palaviduthi soil series using hybrid tomato “Shivam”. The treatments were T_1- KIO_3-Soil Application (SA)-5 kg ha^{-1}, T_2- KIO_3-SA- 10 kg ha^{-1}, T_3- Chitosan-$SKIO_3$ Complex-5 kg ha^{-1},T_4- Chitosan-KIO_3 Complex-10 $Kgha^{-1}$, T_5- Foliar Application (FA)-KIO_3-0.2% @ 60 and 90 DAT, T_6- FA-KIO_3-0.3% @ 60 and 90 DAT, T_7- KIO_3- SA- 5 $Kgha^{-1}$ +FA-KIO_3- 0.2% @ 60 and 90 DAT, T_8- KIO_3-SA- 10 kg ha^{-1} + FA-KIO^-_3- 0.2% @ 60 and 90 DAT, T_9- Chitosan-KIO_3 Complex-5 $Kgha^{-1}$ +FA-KIO_3-0.2% @ 60 and 90 DAT, T_{10}-Chitosan-KIO_3 Complex-10 Kg ha^{-1} + FA-KIO_3-0.2% @ 60 and 90 DAT, T_{11}- KIO_3- SA- 5 Kg ha^{-1} + FA-KIO_3- 0.3% @ 60 and 90 DAT, T_{12}- KIO_3- SA- 10 Kg ha^{-1} + FA-KIO_3- 0.3% @ 60 and 90 DAT. T_{13}- Chitosan-KIO_3 Complex-5 Kg ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT, T_{14}- Chitosan-KIO_3 Complex-10 Kg ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT, T_{15}- Chitosan Spraying (control) and T_{16}- Water spraying (Absolute Control). After two days of transplanting potassium iodate and the chitosan iodate complex solution was fertilized into the soil. The plants were grown in medium black clay loam soil which is low in nitrogen and medium in organic carbon, phosphorous and potassium. The soil is neutral in pH and non saline in EC. Fruit samples were collected during green; pink and red ripen harvest stages of summer (main) and kharif season tomato (residual) crop to conduct cooking, soaking and digestion bioavailability experiments (Table 1). The iodine concentration was measured using inductively coupled plasma-optical emission spectrometry by following (Knapp *et al*., 1998) procedure. The data obtained were subjected to one-way ANOVA. The programme IBM SPSS ® Statistics, version 25 was used to run all statistical tests.

RESULTS AND DISCUSSION

Effect of potassium iodate and iodine chitosan complex on soaking

Cleaning of vegetables is a necessary provision before going for cooking. It is standard practice in the kitchen to always wash the vegetables initially and then slice them, as compared to the reverse. Food can lose important nutrients if it is chopped first and then washed. The soaking experiment has been conducted in three stages to determine the loss of iodine in the fruits of tomato. During the first stage (0-8 hours) the loss of iodine from the biofortified fruits accounted for about 8-24% in main crop and 16-35% in residual crop. During second stage (8-16 hours), the iodine loss increased significantly and reached a maximum of 47% in main crop and 78% in residual crop. During third stage (16-24 hours), the iodine loss was only 52% and 87% and it almost attained equilibrium in both the crops. Since the cleaning process for vegetables only takes a matter of minutes, very little iodine is lost during washing of biofortified vegetables. Irrespective of the treatments in all the stages the loss of iodine in biofortified fruits was higher in the KIO_3 foliar application alone and combined KIO_3 soil and foliar application treatments. The amount of iodine retained in the biofortified fruits was higher in combined Cs-KIO_3 and FA-KIO_3 treatments in all the soaking stages (Table 2 and 3).

Table 1: Bioavailability of Iodine in tomato.

Experiment	Procedure	Authors
Soaking experiment	A 10 g of Iodine biofortified tomato fruits was soaked in 100 mL of deionized water for different durations	Li *et al*., 2018
Cooking experiment	A 10 g of Iodine biofortified and nonbiofortified tomato fruits was boiled in 200 mL of deionized water and 5 mL of saline solution at 100°C for different minutes	Li *et al*., 2018
Digestion experiment	A 40 g of tomato fruits was mixed with 100 mL of simulated gastric juice and sealed with a rubber stopper. An anaerobic condition was created by pumping the air outside	Liard *et al*., 2007

Table 2: Effect of soaking on bioaccesibility of biofortified iodine (mg kg^{-1}) at different harvest stages of main crop.

Treatments	Stage-I (8 hours)	Stage-II (16 hours)	Stage-III (24 hours)	Treatment mean
T_1- KIO_3-SA- 5 kg ha^{-1}	0.090^{i}	0.059^{h}	0.056^{h}	0.068
T_2- KIO_3-SA- 10 kg ha^{-1}	0.096^{i}	0.067^{h}	0.065^{h}	0.076
T_3- Chitosan-KIO_3 Complex-5 kg ha^{-1}	0.262^{g}	0.187^{f}	0.134^{f}	0.194
T_4- Chitosan-KIO_3 Complex-10 Kg ha^{-1}	0.374^{f}	0.274^{e}	0.227^{e}	0.292
T_5- FA-KIO_3-0.2% @ 60 and 90 DAT	0.104^{hi}	0.076^{h}	0.071^{h}	0.084
T_6- FA-KIO_3-0.3% @ 60 and 90 DAT	0.144^{h}	0.108^{g}	0.103^{g}	0.118
T_7- KIO_3- SA- 5 kg ha^{-1} +FA-KIO- 0.2% @ 60 and 90 DAT	0.457^{e}	0.337^{d}	0.255^{d}	0.350
T_8- KIO_3-SA-10 kg ha^{-1} +FA-KIO_3-0.2% @ 60 and 90 DAT	0.577^{c}	0.406^{c}	0.358^{c}	0.447
T_9- Chitosan-KIO_3 Complex-5 kg ha^{-1} + FA-KIO_3-0.2% @ 60 and 90 DAT	0.615^{c}	0.418^{c}	0.396^{c}	0.476
T_{10}-Chitosan-KIO_3 Complex-5 kg ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT	0.704^{b}	0.486^{b}	0.432^{b}	0.541
T_{11}- KIO_3- SA- 5 kg ha^{-1} + FA-KIO_3- 0.2% @ 60 and 90 DAT	0.525^{d}	0.363^{d}	0.310^{d}	0.399
T_{12}- KIO_3 SA-10 kg/ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT	0.617^{c}	0.425^{c}	0.359^{c}	0.467
T_{13}- Chitosan-KIO_3 Complex-5 kg ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT	0.718^{b}	0.494^{b}	0.447^{b}	0.553
T_{14}- Chitosan-KIO_3 Complex-10 kg ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT	0.856^{a}	0.607^{a}	0.562^{a}	0.675
T_{15}- Chitosan spraying	0.0011^{j}	0.0014^{i}	0.0016^{i}	0.0013
T_{16}- Water spraying	0.0010^{j}	0.0011^{i}	0.0012^{i}	0.0011
Mean	0.383	0.269	0.236	0.296
S.Ed	0.020	0.014	0.013	
C.D (0.05)	0.042	0.030	0.026	

Table 3: Effect of soaking on bioaccesibility of biofortified iodine (mg kg^{-1}) at different harvest stages of residual crop.

Treatments	Stage-I (8 hours)	Stage-II (16 hours)	Stage-III (24 hours)	Treatment mean
T_1- KIO_3-SA- 5 kg ha^{-1}	0.039^{gh}	0.010^{ijk}	0.007^{hi}	0.018
T_2- KIO_3-SA- 10 kg ha^{-1}	0.044^{gh}	0.017^{hij}	0.01^{hi}	0.023
T_3- Chitosan-KIO_3 Complex-5 kg ha^{-1}	0.060^{fg}	0.032^{gh}	0.014^{gh}	0.035
T_4- Chitosan-KIO_3 Complex-10 kg ha^{-1}	0.079^{f}	0.044^{efg}	0.019^{fgh}	0.047
T_5- FA-KIO_3-0.2% @ 60 and 90 DAT	0.032^{h}	0.012^{ijk}	0.007^{hi}	0.017
T_6- FA-KIO_3-0.3% @ 60 and 90 DAT	0.052^{gh}	0.024^{hi}	0.017^{fgh}	0.031
T_7- KIO_3- SA- 5 kg ha^{-1} +FA-KIO- 0.2% @ 60 and 90 DAT	0.163^{e}	0.057^{def}	0.027^{ef}	0.082
T_8- KIO_3-SA-10 kg ha^{-1} +FA-KIO_3-0.2% @ 60 and 90 DAT	0.198^{d}	0.042^{fg}	0.024^{fg}	0.088
T_9- Chitosan-KIO_3 Complex-5 kg ha^{-1} + FA-KIO_3-0.2% @ 60 and 90 DAT	0.401^{b}	0.188^{c}	0.156^{c}	0.248
T_{10}- Chitosan-KIO_3 Complex-5 kg ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT	0.408^{b}	0.302^{b}	0.216^{b}	0.308
T_{11}- KIO_3- SA- 5 kg ha^{-1} + FA-KIO_3- 0.2% @ 60 and 90 DAT	0.205^{cd}	0.060^{de}	0.047^{d}	0.104
T_{12}- KIO_3 SA-10 kg/ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT	0.224^{c}	0.064^{d}	0.038^{de}	0.109
T_{13}- Chitosan-KIO_3 Complex-5 kg ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT	0.411^{b}	0.314^{b}	0.228^{b}	0.318
T_{14}- Chitosan-KIO_3 Complex-10 kg ha^{-1} + FA-KIO_3-0.3% @ 60 and 90 DAT	0.598^{a}	0.442^{a}	0.317^{a}	0.452
T_{15}- Chitosan spraying	0.0007^{i}	0.0006^{jk}	0.0005^{i}	0.0006
T_{16}- Water spraying	0.0006^{i}	0.0004^{k}	0.0003^{i}	0.0004
Mean	0.1822	0.1004	0.0704	0.1176
S.Ed	0.012	0.008	0.006	
C.D (0.05)	0.024	0.016	0.012	

As chitosan tend to form electrostatic interaction with iodate and thereby preserves the loss of iodine from the fruits (Grande-Tovar *et al*., 2018). The loss of iodine for the treatments Chitosan-KIO_3 Complex-10 kg ha^{-1} + FA-KIO_3-0.3% at 60 and 90 DAT and Chitosan-KIO_3 Complex-10 kg ha^{-1} + FA-KIO_3-0.2% at 60 and 90 DAT at stage-I (8% and 10%), stage-II (34% and 38%) and stage-III (38% and 43) of main crop and at stage-I (16% and 19%), stage-II (38% and 40%) and stage-III (55% and 57) of residual crop further confirmed the above findings.

Effect of potassium iodate and iodine chitosan complex on cooking

The cooking experiment has been conducted to determine the volatilization loss of iodine in the fruits of tomato at three stages. The biofortified tomato was boiled with non iodized salt at 100°C and in contrary to that the non biofortified tomato was boiled with iodized salt at same temperature for 2,5 and 30 minutes. The initial iodine concentration after addition of iodized salt in non biofortified tomato was 0.0075 mg kg^{-1} in main crop and 0.0068 mg kg^{-1} in residual crop which decreased to 0.0052 mg kg^{-1}, 0.0038 mg kg^{-1} and 0.0012 mg kg^{-1} in main crop and 0.0045 mg kg^{-1}, 0.0030 mg kg^{-1} and 0.0003 mg kg^{-1} in residual crop after 2, 5 and 30 minutes of boiling respectively (Fig 1 and 2). The iodine concentration in the biofortified tomato was 0.904-0.099 mg kg^{-1} and 0.683-0.047 mg kg^{-1} in main and residual crop after boiling for 2 minutes. After boiling for 5 minutes, the concentration of iodine in the biofortified tomato of main and residual crop was reduced to 0.895-0.093 mg kg^{-1} and 0.672-0.034 mg kg^{-1}. The concentration was further reduced to 0.857-0.075 mg kg^{-1} and 0.624-0.011 mg kg^{-1} in main and residual crop after 30 minutes of boiling. The concentration of iodine in the tomato decreases in both the crops with increasing boiling time, whether the tomato is biofortified or

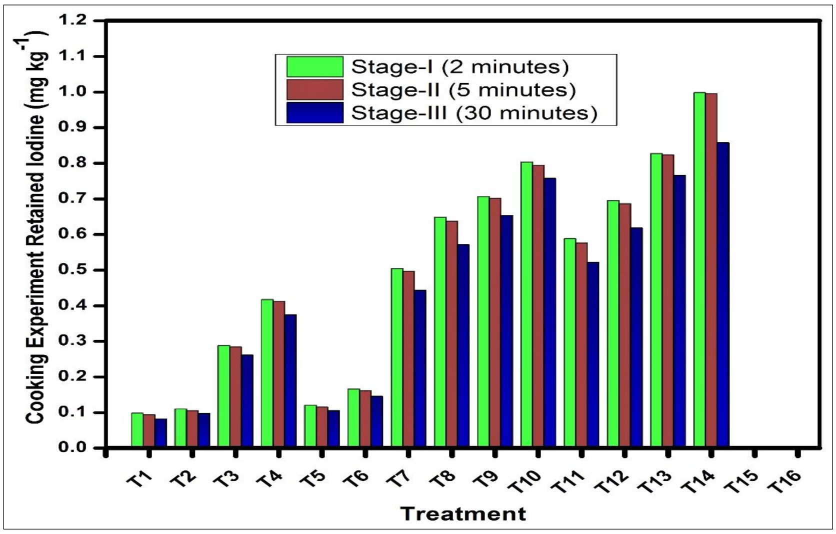

Fig 1: Effect of cooking on bioaccesibility of biofortified iodine content (mg kg^{-1}) at different stages of main crop.

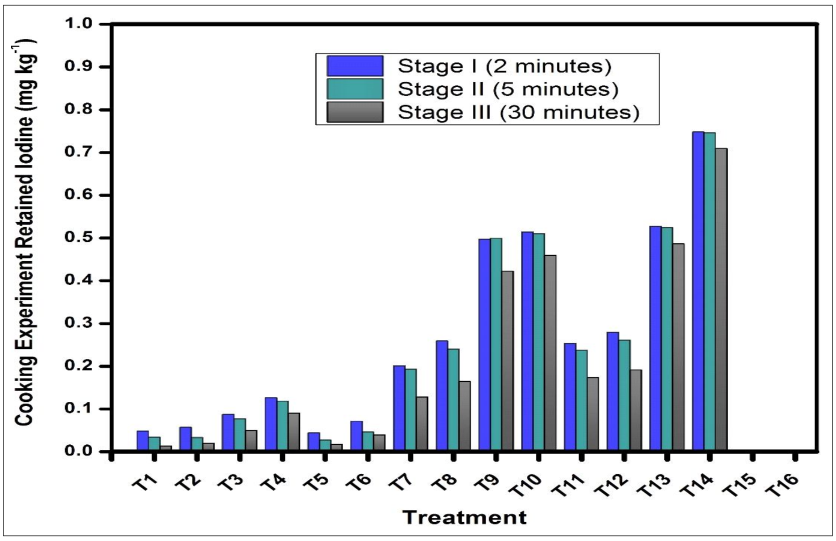

Fig 2: Effect of cooking on bioaccesibility of biofortified iodine content (mg kg^{-1}) at different stages of residual crop.

not. This is because only a very small amount of iodine seeped into tomato fruit after the iodine in the soup was volatilized with water. But the decrease rate was more in non biofortified tomato when compared to biofortified one. This is due to the fact that the chitosan-potassium iodate iodine is more stable when compared to the iodized salt inorganic iodine used during cooking (Li *et al*., 2017). The results clearly revealed that application of combined Cs-KIO_3 and FA- KIO_3 treatments has retained more iodine in fruits and less iodine is lost through the tomato soup in all the stages of cooking when compared to other treatments in main as well as residual crop. Additionally, the iodine loss rate was much lower accounting for about 2.79%, 3.76% and 7.84% in main crop and 3.8%, 5.35% and 12.11% in residual crop respectively after cooking for 2, 5 and 30 minutes. While the corresponding iodine loss rate for the non-biofortified tomato + iodized salt treatment was 30.6%, 49.3% and 84% in main crop and 33.8%, 55.8% and 95.5% in residual crop. During 2, 5 and 30 minutes of boiling with non-iodized salt, the biofortified tomato can retain 97.2%, 96.2% and 92.15% and 96.1%, 94.6% and 87.8% of its initial iodine in main and residual crop.

Effect of potassium iodate and iodine chitosan complex on digestion

Iodine in food forms complex compounds with other substances. After digestion, the iodine present in the solid component of the vegetable is regarded as inaccessible to the human body. Iodine bioaccessibility refers to the physiologically available iodine in the vegetable prior to digestion (Li *et al*., 2018). The bioaccessible iodine (BI) in the biofortified tomato increased with increasing duration of digestion from 30 minutes to 90 minutes in main and residual crop. Irrespective of the treatments in all the stages of

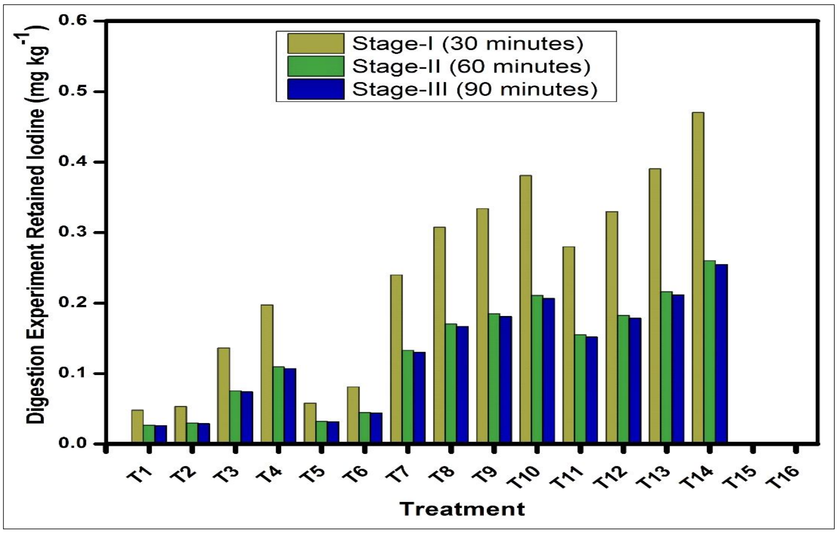

Fig 3: Effect of digestion on bioaccesibility of biofortified iodine content (mg kg^{-1}) at different stages of main crop.

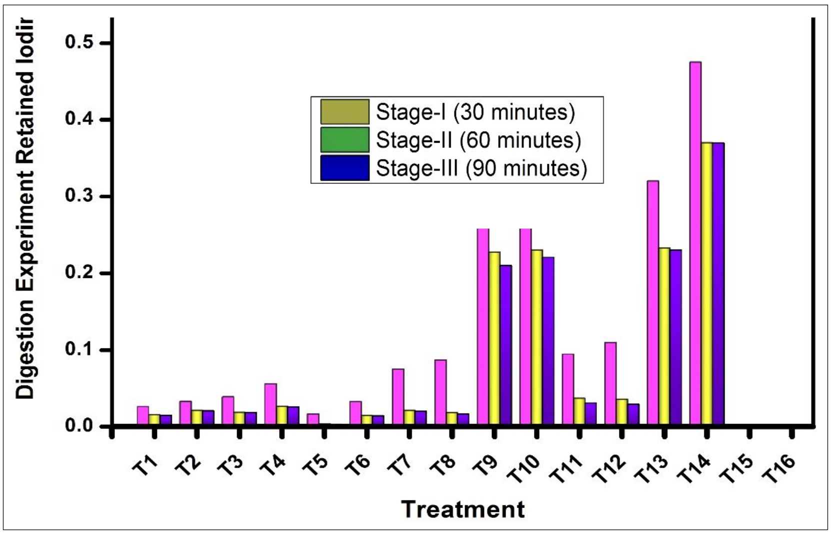

Fig 4: Effect of digestion on bioaccesibility of biofortified iodine content (mg kg^{-1}) at different stages of residual crop.

digestion the lower bioaccessible iodine in biofortified fruits was found in the KIO_3 soil and foliar application alone and chitosan alone treatments. The amount of BI in the biofortified fruits was higher in combined Cs-KIO_3 and FA- KIO_3 treatments followed by SA-KIO_3 and FA- KIO_3 in all the stages of digestion in main and residual crop (Fig 3 and 4). The bioavailable iodine of the treatment Chitosan-KIO_3 Complex-10 kg ha^{-1} + FA-KIO_3-0.3% at 60 and 90 DAT in the main crop reached 47.32%, 26.34% and 25.80% at 30, 60 and 90 minutes of digestion. Similarly for residual crop it reached 43.7%, 25.35% and 24.9% at 30, 60 and 90 minutes of digestion. When the digesting time exceeds 90 minutes, the BI becomes relatively constant, indicating that the iodine exchange between the tomato fruits and simulated stomach fluid has achieved a state of equilibrium.

Previous research also revealed that digestion and absorption are dynamic processes that occur concurrently (Thambiliyagodage *et al*., 2023). The digestion experiment clearly revealed that the most of the iodine was stored as soluble cellular substances in tomato.

CONCLUSION

The findings of bioavailability experiment showed that the absorbed iodine in tomato fruits is stable after harvest. The use of combined Cs-KIO_3 and FA-KIO_3 treatments preserved the iodine content and reduced iodine losses in the fruits. The iodine loss of the biofortified tomato during soaking was very low at 8% in the main crop and 15% in the residual crop. During cooking, the iodine in the biofortified tomato was more stable than the iodized salt. After 30 minutes of cooking, about 96% and 94% of the main and residual crop iodine are still maintained in the fruit. The majority of the iodine absorbed by the tomato is kept in the solid part of the fruits. After 30 minutes of digestion, the bioaccesibility of iodine in biofortified tomato accounted for approximately 48% and 44% in the main and residual crop, respectively. Because of the strong electrostatic connection between iodate and chitosan, the fruits do not loss iodine. Furthermore, iodine loss is greater in the residual crop and less in the main crop and also the iodine bioavailability is greater in the main crop and less in the residual crop. This is due to the lack of iodine application to the residual crop in comparison to the main crop.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Data availability

All datasets generated or analyzed during this study are included in the manuscript.

Ethics statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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