

Original article

Valorisation of cold plasma-treated onion skin waste in chocolate productionBerna Senguler^{1,2} & Celale Kirkin^{1*}

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Summary Onion skin is generated in high amounts as food waste that is rich in bioactive components. Thus, the valorisation of onion skin waste in food production following suitable methods of treatment can be regarded as a sustainable strategy in terms of nutrition, economy, and environment. Skin waste from onions (*Allium cepa*) was ground and exposed to dielectric barrier discharge cold plasma (DBDCP) at 40 kV for either 0 or 30 min. Then the cold plasma-treated onion skin powder (OSP) was added to chocolate at a concentration of 0%, 1%, or 2% (w/w). The DBDCP-induced changes in the microbial load, colour, water content, total phenolic content (TPC), and antioxidant activity of OSP were assessed. The TPC, antioxidant activity, and sensory properties of the chocolate samples added with OSP were also monitored. Cold plasma treatment increased the TPC and antioxidant activity of OSP, but it did not affect the water content, colour, and microbial load. The antioxidant activity and TPC of the chocolate samples incorporated with DBDCP-treated OSP at a level of 2% were higher than the samples with or without untreated OSP. However, the samples with 2% DBDCP-treated OSP demonstrated lower overall acceptability scores compared to samples without OSP. It can be said that cold plasma can be utilised in the treatment of onion skin waste and valorisation of cold plasma-treated onion skin waste in the production of chocolate to improve its antioxidant properties. Future studies can focus on the enhancement of the sensory properties of onion skin-added chocolate products.

Keywords Antioxidant activity, chocolate, onion skin, quality, total phenolic content.

Introduction

The generation of food waste and subsequently the carbon footprint of the food industry has been increasing (Scherhauer *et al.*, 2018; Tonini *et al.*, 2018). Food waste and loss has been defined as the reduction in the amount of food and quality (FAO, 2019). Food waste is produced during retail and consumption, while food waste occurs before retail (FAO, 2019). It is estimated that annually 1.3 billion tons of food is lost or wasted (FAO, 2019). Thus, the reduction of food waste is necessary for economic and environmental sustainability.

In this regard, onion (*Allium cepa*) has been one of the most frequently consumed agricultural products worldwide (Benítez *et al.*, 2011; Stoica *et al.*, 2023). As a consequence, the level of onion waste that is generated is considerable (Benítez *et al.*, 2011). Approximately 60% of onion waste is made up of skin waste (Sagar &

Pareek, 2020), and it was claimed that the annual generation of onion skin waste by the European Union was above 500 000 tons (Choi *et al.*, 2015). Several researchers have suggested that valorisation of onion waste in food production is a sustainable way of waste reduction (Benítez *et al.*, 2011; Sharma *et al.*, 2016; Stoica *et al.*, 2023). Onion waste is rich in total phenolics, flavonoids, and flavanols and shows good antioxidant activity (Sharma *et al.*, 2016). As a result, it can be employed as a food additive or a source of bioactive compounds for use in food production.

The major part of onion waste is made up of onion skins. It was stated that onion skin powder had a high content of flavonoids and phenolics, quercetin being the main flavanol (Sagar *et al.*, 2020). Thanks to its good bioactive properties (Sagar *et al.*, 2020), onion skin waste has been valorized as a food ingredient in several studies (Gawlik-Dziki *et al.*, 2015; Sagar & Pareek, 2020, 2021; Michalak-Majewska *et al.*, 2020a, 2020b; Kirkin *et al.*, 2021).

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There have been several technologies that can be used for the treatment of food waste, including anaerobic digestion, incineration, landfill, composting, and heat-moisture reaction (Gao *et al.*, 2017). In addition, several emerging technologies, such as, pulsed electric fields, ultrasonication, and cold plasma can be used for the purpose of extracting bioactive components from food waste (Galanakis, 2012). Cold plasma processing is a new non-thermal technology that can be exploited for decontamination and pre-treatment (Laroque *et al.*, 2022). Cold plasma is composed of ionised gas components, including electrons, ions, atoms, free radicals, and molecules (Niemira, 2012; Bourke *et al.*, 2017). It can be generated at atmospheric pressure and temperature (Niemira, 2012; Bourke *et al.*, 2017). Cold plasma can inactivate microorganisms by the degradation of DNA, cell membrane, and other cell components (Moisan *et al.*, 2002). This technology can also provide enzyme inactivation, seed germination, starch modification, etc. (Thirumdas *et al.*, 2015). Cold plasma can be regarded as an environmentally friendly technology for some reasons, such as, being efficient at low temperatures, not requiring the use of toxic solvents, and causing less damage to the product in comparison to conventional methods (Thirumdas *et al.*, 2015; Heydari *et al.*, 2023). It has been stated that this technology can be used to improve the extraction and bioavailability of bioactive compounds (Bezerra *et al.*, 2023). The effect of cold plasma on the enhancement of extraction mainly depends on the damage to the cell structure, reduction of the surface tension and facilitating the release of hydrophilic compounds, and disruption of the covalent bonds (Bezerra *et al.*, 2023; Heydari *et al.*, 2023). Cold plasma-induced increase in the antioxidant activity and phenolic content of various food products, such as, dandelion root (Elcik & Kirkin, 2024), grape pomace (Bao *et al.*, 2020), green tea leaves (Keshavarzi *et al.*, 2020), haskap (Li *et al.*, 2023), and peppermint (Kashfi *et al.*, 2020) has been reported before. On the other hand, the influence of cold plasma on the extraction of bioactive compounds can be different depending on several factors, including power, voltage, time, gas, etc. (Heydari *et al.*, 2023).

Thus, cold plasma technology can be utilised in the treatment of onion skin waste before adding it to food recipes, but more studies are required to predict the effects on the bioactive properties well. Previous studies focused on the enrichment of savoury products, such as, bread (Gawlik-Dziki *et al.*, 2015), pasta (Michalak-Majewska *et al.*, 2020a), and pizza base (Sagar & Pareek, 2020) with onion skin or its extracts, except for one study in which only the effects on the sensory properties of chocolate truffles were evaluated (Kirkin *et al.*, 2021). This study aimed to investigate the effects of (i) cold plasma treatment on the colour, water content, microbial load, total phenolic content

(TPC), and antioxidant activity of onion skin waste and (ii) the addition of cold plasma-treated onion skin waste on the TPC, antioxidant activity, and sensory properties of chocolate.

Materials and methods

Preparation of the onion skin powder

Skin waste of yellow onion (*A. cepa*) bulbs was collected as waste produced after a culinary course at Gelisim University, Istanbul. The onion skin was then washed under tap water three times, dried in an oven, and pulverised using a coffee grinder (Bosch, Istanbul, Türkiye). A total amount of 100 g of onion skin powder (OSP) was obtained for the study. Three gram of the OSP was weighed in a 10-cm glass dish. The glass dish was placed on the bottom electrode of a dielectric barrier discharge cold plasma (DBDCP) reactor and subjected to cold plasma at 40 kV for 0 min (CP0) or 30 min (CP30). The setup of the DBDCP system was explained before (Elcik & Kirkin, 2024).

Preparation of the chocolate samples

Chocolate samples were prepared by melting bitter chocolate callets (No 811; Callebaut, Istanbul, Türkiye) in a bain-marie. The melted chocolate was tempered with the help of a spatula until its temperature dropped to 27–28 °C. The tempered chocolate was substituted at a level of 1% or 2% with DBDCP-treated (CP30-1 and CP30-2) or untreated OSP (CP0-1 and CP0-2). Then, the chocolate mixtures were poured into silicone moulds and incubated at approximately 17 °C for 30 min. The samples without the incorporation of OSP were used as the control.

Colour

A colorimeter (CR400; Konica-Minolta, Tokyo, Japan) was employed to measure the CIE L^* , a^* , and b^* values of the OSP samples. The hue angle (h°) and chroma (C^*) values were determined using eqns (1) and (2), respectively.

$$h^\circ = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

Water content

The water content of the OSP samples was tested using a moisture analyser (IR 35; Denver Instrument,

Denver, Colorado, USA). One gram OSP was weighed into the measurement plate and heated to 105 °C until constant weight.

Microbiological analysis

One gram of OSP was inoculated on the plate count agar (PCA) or dichloran rose bengal agar (DRBC) diluted in peptone water for the total viable bacterial count and total yeast and mould count, respectively. The inoculated plates were incubated for 48 h at 37 °C and 3–5 days at 25 °C for total viable and yeast-mould count, respectively.

Extraction

The methanolic extracts of the OSP samples were prepared as described before (Kirkin & Gunes, 2018). The extraction of the chocolate samples was conducted according to Gültekin-Özgüven *et al.* (2016). The chocolate samples were defatted by washing 1 g of the samples with 10 mL n-hexane for 5 min at 30 °C in an ultrasonic bath (Isolab, Eschau, Germany). Centrifugation at 3000 *g* for 10 min and air drying for 24 h at room temperature were employed to remove the hexane. The procedure was repeated twice. One gram of OSP or defatted chocolate sample was homogenised in 25 mL methanol (80%, v/v) in the ultrasonic bath for 1 h at 40 °C and centrifuged at 2575 *g* for 10 min. The supernatants were collected and stored at −18 °C until analysis.

Total phenolic content

The Folin–Ciocalteu method was employed to evaluate the TPC of the extracts of the OSP and chocolate samples (Viuda-Martos *et al.*, 2010). The TPC of the samples was obtained as mg gallic acid equivalents (GAE) g^{−1} using a standard calibration curve.

Antioxidant activity

The antioxidant activity of the OSP and chocolate extracts was investigated by 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric-reducing antioxidant power (FRAP) assays (Viuda-Martos *et al.*, 2010; Kirkin & Gunes, 2018). The antioxidant activity was expressed in terms of mg Trolox equivalents (TE) g^{−1} using a standard calibration curve.

Sensory analysis

The sensory evaluation of the chocolate samples was done with 12 experienced panellists. The panellists were served 15 chocolate samples coded with three-digit

random numbers and asked to evaluate the odour, colour, taste, bitterness, oiliness, texture, and overall acceptability. A 9-point hedonic scale (1: dislike extremely, 9: like extremely) was used for the scoring.

Statistical analysis

This study was conducted according to a full factorial design. All treatments were replicated three times. Data were analysed by variance of analysis and Fisher's least significant difference using Minitab® 20.1 Statistical Software (Minitab Inc., State College, USA).

Results

DBDCP treatment of onion skin waste

Colour and water content

No significant effects of DBDCP treatment on the colour and water content of onion skin waste were observed ($P > 0.05$) as demonstrated in Table 1.

Similarly, no effects of cold plasma on the colour of different food items have been reported previously (Bermúdez-Aguirre *et al.*, 2013; Ziuzina *et al.*, 2016). On the other hand, cold plasma caused colour changes in products, such as, thyme, cumin, and dandelion root (Shokoohi *et al.*, 2022, 2024; Elcik & Kirkin, 2024). The variations in the effects of cold plasma on colour values reported previously can be related to the cold plasma equipment set-up, power, treatment time, gas, food product, etc. (Elcik & Kirkin, 2024).

Contrary to the present study, the water content of brown rice (Thirumdas *et al.*, 2016) and dried walnut kernels (Ahangari *et al.*, 2021) was decreased by increasing the treatment time and cold plasma power. Also, Chen *et al.* (2020) utilised cold plasma to dehydrate fresh wet noodles without compromising freshness. It should be noted that the OSP samples used in the present study were in dried form which could have limited the influence of cold plasma on the water content.

Microbial count

The total viable bacteria and yeast-mould counts of the OSP samples were not significantly changed by the DBDCP treatment ($P > 0.05$), as given in Table 2.

It has been stated by several researchers that cold plasma technology could be used for microbial decontamination of food (Niemira, 2012; Mandal *et al.*, 2018; Ansari *et al.*, 2022). However, Kim *et al.* (2014) observed only a 1-log reduction in the total aerobic microorganism count after cold plasma treatment of red pepper powder. Moreover, it was stated that cold plasma-induced microbial inactivation can be lower in products with lower water activity (Kim *et al.*, 2014; Lee *et al.*, 2015). Thus, the negligible influence of the

Table 1 Colour and water content of OSP as affected by DBDCP treatment

Treatment time (min)	L*	a*	b*	h°	C*	Water content (%)
0	64.98 ± 1.31 ^a	11.39 ± 0.42 ^a	21.22 ± 0.79 ^a	1.08 ± 0.01 ^a	24.09 ± 0.87 ^a	14.87 ± 0.83 ^a
30	65.58 ± 0.48 ^a	12.17 ± 0.59 ^a	22.44 ± 0.59 ^a	1.07 ± 0.02 ^a	25.53 ± 0.64 ^a	14.91 ± 0.41 ^a

Values are mean ± standard deviation of three replications and different if labelled with different superscript letters in the same column.

Table 2 Microbial load of OSP as affected by DBDCP treatment

Treatment time (min)	Total viable bacterial count (log cfu g ⁻¹)	Yeast and mould (log cfu g ⁻¹)
0	4.15 ± 0.35 ^a	4.23 ± 0.07 ^a
30	3.87 ± 0.27 ^a	4.14 ± 0.08 ^a

Values are mean ± standard deviation of three replications and different if labelled with different superscript letters in the same column.

cold plasma treatment on the microbial load of OSP could be related to the low water content.

Total phenolic content and antioxidant activity

The TPC and antioxidant activity as assessed by the DPPH and FRAP assays of the OSP samples were increased by the 30-min DBDCP treatment ($P < 0.05$) as exhibited in Fig. 1.

Similar to our findings, the antioxidant activity and TPC of various food products were improved after cold plasma treatment (Hou *et al.*, 2019; Illera *et al.*, 2019; Li *et al.*, 2019; Bao *et al.*, 2020; Keshavarzi *et al.*, 2020; Farias *et al.*, 2022). It has been claimed that cold plasma-generated UV-radiation and reactive species can improve the release of bioactive compounds by causing the breakdown of the cell membrane and covalent bonds (Herceg *et al.*, 2016; Hou *et al.*, 2019; Bezerra *et al.*, 2023). However, opposite

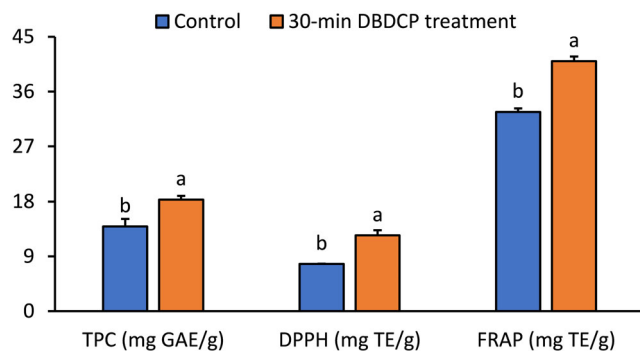


Figure 1 Total phenolic content and antioxidant activity of OSP as affected by DBDCP treatment. Values labelled with different letters are significantly different ($P < 0.05$).

findings were also observed previously (Pankaj *et al.*, 2017; Hemmati *et al.*, 2021). The TPC and antioxidant activity can be influenced by cold plasma in different directions depending on factors, such as, equipment, power, gas, gas flow rate, exposure time, and food product (Elcik & Kirkin, 2024).

The TPC and antioxidant activity were determined by two different methods (DPPH and FRAP) followed a similar trend upon cold plasma treatment of the OSP samples. Several other researchers also reported a similar finding (Illera *et al.*, 2019; Li *et al.*, 2019; Farias *et al.*, 2022). A relationship between TPC and antioxidant activity was also reported before (Tawaha *et al.*, 2007; Saeed *et al.*, 2012; Muhammad *et al.*, 2018).

Incorporation of DBDCP-treated onion skin waste into chocolate

Total phenolic content and antioxidant activity

The effect of cold plasma-treated OSP incorporation on the TPC of chocolate samples is shown in Fig. 2. The TPC of the chocolate samples added with DBDCP-treated OSP at a concentration of 2% was higher than that of the other samples ($P < 0.05$). The DPPH and FRAP test-based antioxidant activity of the chocolate samples after OSP addition are demonstrated in Fig. 3. The addition of DBDCP-treated OSP increased the DPPH radical scavenging activity of the chocolate samples compared to the rest of the samples ($P < 0.05$). Also, the chocolate samples added with DBDCP-treated OSP at a level of 2% demonstrated higher FRAP values than the control and samples with untreated OSP ($P < 0.05$).

The increase in the antioxidant activity and TPC cold plasma-treated OSP added (2%) chocolate samples was expected, as the antioxidant activity of the OSP samples increased after the treatment. Similarly, several other studies reported an increase in phenolic content and antioxidant capacity of several food products after fortification with onion skin. For instance, Gawlik-Dziki *et al.* (2013) recommended onion skin addition at a concentration of 2–3% to improve the antioxidant properties of bread. Moreover, the antioxidant activity and phenolic content of pasta were increased after substitution of semolina with 2.5–7.5% of OSP (Michalak-Majewska *et al.*, 2020b). The

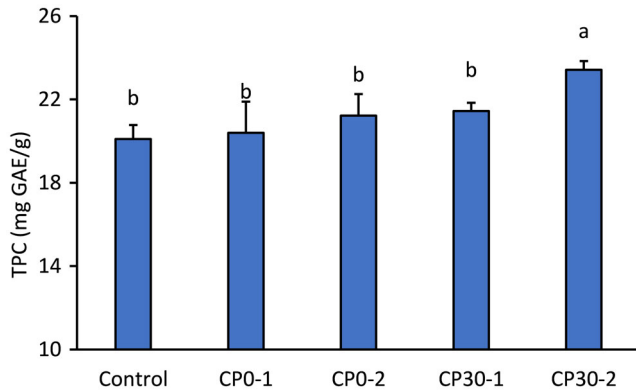


Figure 2 Total phenolic content of chocolate as affected by OSP addition. Values labelled with different letters are significantly different ($P < 0.05$). CP0-1: samples incorporated with untreated OSP at a level of 1%, CP0-2: samples incorporated with untreated OSP at a level of 2%, CP30-1: samples incorporated with DBDCP-treated OSP at a level of 1%, CP30-2: samples incorporated with DBDCP-treated OSP at a level of 2%.

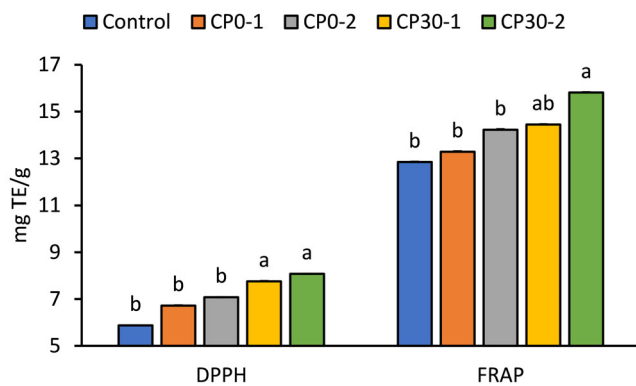


Figure 3 Antioxidant activity of chocolate as affected by OSP addition. Values labelled with different letters are significantly different ($P < 0.05$). CP0-1: samples incorporated with untreated OSP at a level of 1%, CP0-2: samples incorporated with untreated OSP at a level of 2%, CP30-1: samples incorporated with DBDCP-treated OSP at a level of 1%, CP30-2: samples incorporated with DBDCP-treated OSP at a level of 2%.

antioxidant activity and TPC of bean paste with onion skin phenolics were also increased (Sęczyk *et al.*, 2015). Also, the antioxidant activity and TPC of bread (Sagar & Pareek, 2021) and pizza base (Sagar & Pareek, 2020) were increased by increasing the amount of onion skin addition.

Sensory properties

The effects of DBDCP-treated OSP incorporation on the sensory properties of chocolate are shown in Table 3. The odour scores of CP0-1 and CP0-2 were lower than those of the control ($P < 0.05$); however,

the addition of cold plasma-treated OSP (CP30-1 and CP30-2) maintained the odour of the samples ($P > 0.05$). The colour scores of OSP-added samples were below that of the control ($P < 0.05$). The control and CP0-1 had higher taste scores than CP0-2 and CP30-1 ($P < 0.05$). The bitterness and oiliness scores of samples added with untreated OSP at a level of 2% were lower than those of the other samples ($P < 0.05$). Also, the texture scores of CP0-2, CP30-1, and CP30-2 were less than those of the control and CP0-1 ($P < 0.05$). The overall acceptability of samples incorporated with cold plasma-treated OSP was lower compared to that of the control ($P < 0.05$), but the addition of untreated OSP did not affect the overall acceptability of the samples ($P > 0.05$).

It should be noted that a decrease in the overall acceptability of food products, such as, bread (Gawlik-Dziki *et al.*, 2013), chicken meat patties (Kurt *et al.*, 2019), pasta (cooked and uncooked) (Michalak-Majewska *et al.*, 2020a), peanut butter (Kirkin *et al.*, 2021), and pizza base (Sagar & Pareek, 2020) with the increasing concentration of onion skin was reported before. In addition, it was observed in a previous study that the incorporation of chocolate truffles with 5–10% OSP decreased the overall acceptability scores from 8 to 4.5 on a 9-point hedonic scale (Kirkin *et al.*, 2021). On the other hand, improvement in the sensory properties of bread after the addition of 3% OSP was reported (Sagar & Pareek, 2021). In the present study, the overall acceptability was affected by only the addition of cold plasma-treated OSP. Onion skin is rich in phenolic, such as, quercetin, quercetin 3,4'-diglucoside, kaempferol, and luteolin (Albishi *et al.*, 2013; Sagar *et al.*, 2020). It is possible that the decrease in the sensory scores was associated with the existence of these compounds, as the total amount of phenolics was increased after the cold plasma treatment. The score for all sensory attributes ranged between 6 and 7.9 on the 9-point scale, thus all of the samples can be regarded as acceptable.

Conclusions

The utilisation of cold plasma for onion skin waste treatment and the impact of adding onion skin that has been cold plasma-treated on chocolate's TPC, antioxidant activity, and sensory qualities were investigated. Cold plasma treatment of OSP for 30 min at 40 kV increased the TPC and antioxidant activity based on the DPPH and FRAP methods. Also, the addition of cold plasma-treated OSP into chocolate samples at a level of 2% (w/w) improved the TPC and antioxidant activity, while sensory overall acceptability was lowered. However, the samples were still acceptable. The findings of this study suggest that onion skin waste has the potential to be a sustainable

Table 3 Sensory properties of chocolate as affected by OSP addition

Sample	Odour	Colour	Taste	Bitterness	Oiliness	Texture	Overall acceptability
Control	7.44 ± 0.04 ^a	7.92 ± 0.02 ^a	7.31 ± 0.01 ^a	7.00 ± 0.07 ^a	6.86 ± 0.05 ^a	7.39 ± 0.04 ^a	7.53 ± 0.02 ^a
CP0-1	7.08 ± 0.05 ^b	7.56 ± 0.02 ^b	7.03 ± 0.01 ^a	6.83 ± 0.03 ^a	6.94 ± 0.02 ^a	7.17 ± 0.06 ^a	6.94 ± 0.02 ^{ab}
CP0-2	6.64 ± 0.05 ^b	7.36 ± 0.03 ^b	6.75 ± 0.03 ^b	6.50 ± 0.06 ^b	6.00 ± 0.04 ^b	6.53 ± 0.02 ^b	6.81 ± 0.04 ^{ab}
CP30-1	7.28 ± 0.05 ^a	7.56 ± 0.01 ^b	6.69 ± 0.04 ^b	6.69 ± 0.01 ^a	6.56 ± 0.01 ^a	6.78 ± 0.04 ^b	6.75 ± 0.07 ^b
CP30-2	7.14 ± 0.06 ^{ab}	7.50 ± 0.07 ^b	6.86 ± 0.08 ^{ab}	6.81 ± 0.04 ^a	6.56 ± 0.06 ^a	6.56 ± 0.01 ^b	6.78 ± 0.04 ^b

Values are mean ± standard deviation of three replications and different if labelled with different superscript letters in the same column.

environmentally-friendly ingredient of food formulations that improve the antioxidant properties. Future studies will focus on the effects of cold plasma treatment on the bioavailability of the bioactive compounds and the improvement of sensory properties.

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Author contributions

Berna Senguler: Investigation; writing – original draft; formal analysis. **Celale Kirkin:** Conceptualization; writing – review and editing; supervision; methodology.

Conflict of interest

The authors declare no conflict of interest.

Peer review

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/ijfs.17582>.

Data availability statement

Data will be made available upon request.

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These papers were crucial for this study, as they encouraged us to investigate the valorisation of onion skin waste in chocolate formulations.

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