Heliyon 7 (2021) e07441

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Effect of novel sequential soaking treatments on Maillard reaction products in potato and alternative vegetable crisps

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HIGHLIGHTS

- Vegetable crisps contain more acrylamide than the benchmark for potato crisps.
- Vegetable crisps contain significant levels of HMF, GO and MGO than potato crisps.
- Wash additives effect on potato, are variable on vegetable.
- Mitigation strategies for the reduction of acrylamide are vegetable specific.

ARTICLE INFO

Keywords:
Acrylamide
5-hydroxymethylfurfural
Glyoxal
Methylglyoxal
Vegetable crisps

ABSTRACT

Frying leads to the formation of numerous food contaminants through the Maillard reaction (MR). In this paper, commercially available vegetable crisps were analysed for and established to have high levels of acrylamide. Consequentially, the capability of two novel sequential pre-frying treatments were applied to potato, beetroot and parsnip snacks to inhibit the formation of acrylamide, 5-hydroxymethylfurfural (HMF), glyoxal (GO) and methylglyoxal (MGO) was investigated. Data revealed that immersion in cold tap water for 2 min followed by blanching at 70 ± 2 °C for 2 min (Cold soak, hot soak, (CSHS)) as well as soaking in a 0.01M $CaCl_2$ solution for 2 min followed by blanching at 70 ± 2 °C in 0.1M citric acid for 2 min were both effective pre-treatments for potato crisps, simultaneously decreasing acrylamide concentration under the benchmark level of 750 μg/kg and lowering GO content by 55.19 and 54.67% and MGO concentration by 39.17% and 81.62%, respectively. CSHS was the only efficient treatment for concurrent mitigation of acrylamide (-41.64%) and HMF (-88.43%) with little GO and MGO development in beetroot. Sequential cold soak in 0.01M calcium chloride and hot soak in a 0.1M citric acid solution has been effective in decreasing acrylamide in alternative crisps. However, this led to an increase in HMF, 30 and 20-fold respectively from the initial concentration. Data reveal that the tested mitigation strategies are vegetable specific.

1. Introduction

Frying, one of the main processing techniques used in cooking (Krokida et al., 2001), imparts appealing sensorial attributes comprising taste, flavour and colour to food (Mariotti et al., 2015). However, some toxic compounds resulting from the Maillard Reaction (MR) are formed during frying. MR products (MRPs) including acrylamide, 5-hydroxymethylfurfural (HMF) and 1,2-dicarbonyl compounds, namely glyoxal (GO) and methylglyoxal (MGO) (Lund and Ray, 2017).

Acrylamide is generated from the interaction between asparagine and carbonyl groups of reducing sugars at temperatures >120 C (Zyzak et al., 2003). It is currently classified by the International Agency for Research on Cancer (IARC) as a Group 2A probable carcinogen to humans (IARC, 1994).

HMF is an intermediate compound in the MR is a furan ring with both an aldehyde and an alcohol substituent. It is formed via non-enzymatic dehydration of sugars under acidic conditions during thermal treatments (Kroh, 1994). Several studies have demonstrated that HMF is

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https://doi.org/10.1016/j.heliyon.2021.e07441
Received 21 April 2021; Received in revised form 8 June 2021; Accepted 25 June 2021

implicated in upper respiratory issues, eyes irritations and skin conditions. Furthermore, it may be carcinogenic, hepatotoxic, nephrotoxic, or lead to neoplastic transformation (Pastoriza de la Cueva *et al.*, 2017; Shapla et al., 2018).

GO and MGO are reactive carbonyl species derived from reducing sugars and are pivotal in the formation of advanced glycation end-products by reacting with lysine and arginine side chains on proteins causing oxidative stress and tissue damage (Lund and Ray, 2017). However, some MRPs have been subjected to widespread controversy regarding their toxicity and carcinogenicity. In fact, some studies have reported positive effects of HMF such as antioxidant activity and protection against acute hypobaric hypoxia (Zhao et al., 2013; Li et al., 2011), inhibition of alcoholic liver oxidative injury (Li et al., 2015), cardioprotective effects (Wolkart et al., 2017) and a mitigating function in the lipopolysaccharide-induced inflammatory response in cells (Kong et al., 2019). Moreover, de Bari et al. (2021) suggested that a transient increase in MGO formation and metabolism through the glyoxalase system could signal oxidative stress and be actively involved in cellular antioxidant response and restoration of normal redox conditions.

Potato crisps have often been considered as a major source of acrylamide (Powers et al., 2017). In order to limit human exposure to acrylamide, a benchmark level of 750 μg/kg for potato crisps has been established by The Commission Regulation (EU) 2017/2158 ([EC] European Commission, 2017). In response, numerous mitigation strategies have been developed by the food industry to reduce acrylamide levels in potato crisps. Some of these methods are based on the reduction of acrylamide precursors levels in the raw potatoes. For instance, sugars were leached out by soaking in water at room temperature (Pedreschi et al., 2004) resulting in the reduction of acrylamide levels. Blanching at temperatures ranging between 50 and 90 °C was demonstrated to be also effective in lowering asparagine concentrations pre-frying (Pedreschi et al., 2010). Furthermore, blanching has been used in combination with other pre-treatments including immersion in solutions containing monovalent (Pedreschi et al., 2010) and divalent cations (Ou et al., 2008) leading to a reduction in acrylamide formation. This mitigation mechanism is initiated with the protonation of the asparagine amino group. This prevents the nucleophilic addition of asparagine to a carbonyl compound, inhibiting the formation of the corresponding Schiff base, a key intermediate in the MR and in the formation of acrylamide (Gökmen and Şenyuva, 2007). The use of organic acids such as citric, acetic and L-lactic acids has been also effective in reducing acrylamide, due to the pH decrease, by a mechanism similar to that of salt additives (Mestdagh et al., 2008). However, the effect of soaking pre-treatments on HMF and dicarbonyl compounds has yet to be extensively studied.

In recent years, the growing demand for quality and healthy snacks has led the food industry to develop new food products, based on novel pseudo-cereals, vegetables and legumes (Mesias et al., 2019). Increasingly popular, root vegetables such as beetroot, carrots and parsnips are fried in the same manner as potato and are commonly sold as a vegetable crisp mixture (Elmore et al., 2019). Nonetheless, data reported for acrylamide in vegetable crisps is scarce and reported acrylamide values exceed the benchmark value for acrylamide in potato crisps. In addition, vegetable crisps are not included in the current list of foods for which benchmark levels exist (Elmore et al., 2019).

The aim of this study was to assess the formation of acrylamide, HMF, GO and MGO in commercial vegetable crisps (a parsnip and beetroot mixture). Moreover, the efficiency of two novel sequential soaking treatments as mitigation strategies for acrylamide, HMF, GO and MGO was investigated.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile and hexane (HPLC grade), methanol (LC-MS and HPLC grade), pyridine (99%), magnesium sulphate ($MgSO_4$, 97%), citric acid monohydrate (99%), calcium chloride ($CaCl_2$, 95%), sodium chloride (NaCl, 99.5%) and acrylamide (98%) were purchased from Fisher Scientific (Loughborough, UK). Primary Secondary Amine sorbent (PSA) was purchased from Agilent Technologies (CA, USA). N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA, 99%) was purchased from Fluorochem (Glossop, UK). Formic acid (LC-MS grade), [2,3,3-d_3]-acrylamide (Internal standard, IS, 98%), cycloleucine (97%), carrez I and II reagent kits, methoxyamine hydrochloride (98%), disodium hydrogen phosphate (99%) and sodium dihydrogen phosphate (99 %), o-phenylenediamine dichloride (O-PD, 99%) and ethylenediaminetetraacetic acid (EDTA, 99%) were purchased from Merck (Darmstadt, Germany). All solutions were prepared using ultra-pure water.

2.2. Food material

Five different commercially available vegetable crisps were purchased from local supermarkets. Taurus variety potatoes were sourced from a nearby crisp manufacturer; beetroot and parsnip were purchased from a local organic grocer in Dundee, UK. Palm oil (RSPO Palm RD Oil) was purchased from Kerfoot Oil Specialists (Northallerton, UK).

2.3. Pre-treatment and frying

Vegetables were sliced using a HOBART food processor to a thickness of 2 mm for potatoes and 3 mm for parsnip and beetroot. A 30 mm diameter cutter was used to obtain identical discs. Two 30 g of each samples were treated under conditions as follows: i) Control (C): samples were not subjected to any treatment prior to frying; ii) Cold soak (CS): soaking in 2 L of cold tap water for 2 min; iii) Hot soak (HS): soaking in 2 L of tap water at 70 ± 2 °C for 2 min; iv) CS followed by HS (CSHS): soaking in 2L of cold tap water for 2 min, followed by soaking in 2 L of tap water at 70 ± 2 °C for 2 min, v) soaking in 2 L of a 0.01M $CaCl_2$ solution for 2 min followed by blanching at 70 ± 2 °C in 0.1M citric acid for 2 min. After each treatment, samples were immersed in 1L of cold tap water, immediately withdrawn and the excess water removed using compressed air.

Prior to frying, one 30 g portion of each sample was retained for metabolomic analysis. Samples were freeze-dried using a Micro Modulyo RV3 Edwards (CA, USA), pulverised using a Waring blender and stored at -18°C until analysis.

Frying in palm oil was performed in duplicate at 175 ± 2°C in a 3 L Selection Magimix professional deep fat fryer (UK). Commercial processing condition were adopted from Bartlett et al. (2020) with some modification in particular frying times were 4, 4.5 and 5.5 min for parsnip, potato and beetroot, respectively (frying times were minimized to reduce lipid oxidation while maintaining vegetable crisp quality). The oil temperature was monitored by an external probe (E.T.I food check thermometer, Sussex, UK). Samples were removed from the fryer and excess oil removed from samples, samples were pulverised and stored at -18°C until analysis.

2.4. Acrylamide quantification

Acrylamide was quantified by liquid chromatography tandem-mass spectrometry (LC-MS/MS) as described by Ledbetter et al. (2020) with some modifications. Briefly, 1 g of fried crisps powder was combined with [2,3,3-d_3]-acrylamide (10 μL, 0.2 mg/mL in water), 10 mL of water, 10 mL of acetonitrile, 5 mL of hexane, 4 g of $MgSO_4$ and of 0.5 g of NaCl. The mixture was vigorously shaken for 1 min and then centrifuged (9000 g for 10 min (Z 323 K Centrifuge, Hermle, Germany)). One mL of the acetonitrile layer (middle layer) was transferred to a 2 mL Eppendorf tube containing 50 mg of PSA and 175 mg of $MgSO_4$. The mixture was vortexed for 1 min and centrifuged (1000 g for 1 min). The supernatant was transferred to a HPLC vial for analysis by LC-MS/MS. The column, 4 μm Synergi RP column (150 mm × 2 mm, 80 Å pore size) (Phenomenex, UK), was maintained at 30 °C. Mobile phase A was water containing 0.1%

v/v formic acid and mobile phase B was methanol containing 0.1% v/v formic acid. The gradient was 2% B at 0.2 mL/min for 3.5 min, the flow rate increased to 0.3 mL/min and 75% B over 2 min and held for 2 min before re-equilibration to initial conditions for 16.7 min. The mass spectrometer was equipped with an electrospray ionisation (ESI) source and was operated in positive ionisation mode. Multiple reaction monitoring (MRM) transitions were m/z 72.07→55.1 and 44.0 for acrylamide and 75.2→58.0 and 44.0 for 2,3,3-d_3-acrylamide (IS) with a dwell time of 100 ms. The MS source conditions were spray voltage 3500 kV, capillary temperature 270 °C, nitrogen was used as a nebulizer gas. Acrylamide and the internal standard eluted from the column at 2.8 min. Acrylamide was quantified using a linear calibration with a 1/x fitting with a range 10-10 000 ng/mL ($R^2 > 0.99$), The limit of detection (LOD) was 15 ng/mL, and the limit of quantification (LOQ) was 50 ng/mL with a method detection limit (MDL) of 26.7 ppb (equivalent to 26.7 ng/mL). The analysis was performed in duplicate.

2.5. HMF quantification

HMF determination was based on the method described by Troise et al. (2018) with some modifications. Briefly, 9 mL of 0.1% formic acid water solution and 0.5 mL each of Carrez I and Carrez II were added to 0.5 g of fried vegetable powder. Samples were vortexed for 30 s then centrifuged at 7000 g (Z 323 K Centrifuge, Hermle, Germany) for 10 min at 4 °C. Supernatants were collected and the procedure was repeated twice by adding 5 mL of formic acid in water (0.1% v/v) each time. The supernatant was shaken then an aliquot transferred to a 2 mL Eppendorf tube and centrifuged (Centrifuge 5417 R, Eppendorf, Germany) at 20800 g for 10 min at 4 °C. Samples were filtered through a 0.22 μm nylon filter into HPLC vials for injection. The high performance-liquid chromatography (HPLC) system consisted of a Thermo Scientific (Germany) Ultimate 3000 Pump, Dionex DDA-100 diode array detector, and a Dionex autosampler ASI-100 (San Jose, CA). Twenty μL were injected onto a Synergy 4 μm Hydro-RP, 80 Å 250 mm × 4.6 mm column (Phenomenex, USA). The mobile phase was methanol in water (10% v/v). The chromatographic run was isocratic at a flow rate of 0.8 mL/min at 20 °C with a run time of 20 min. Chromatograms were recorded at 280 nm and quantification was carried out by an external calibration in the range 1–50 μg/mL; $R^2 = 0.99$. LOD was 0.050 μg/mL, and LOQ was 0.150 μg/mL and MDL was 0.109 μg/mL. The analysis was performed in duplicate.

2.6. Dicarbonyl compounds quantification

Glyoxal and methylglyoxal were determined as described by previous work (Troise et al., 2020) with modifications. Briefly, 3 mL of a 0.1% formic acid solution was added to 0.5 g of fried vegetable powder. After being vortexed for 1 min, samples were agitated (Thermomixer Comfort, Eppendorf, Germany) at 500 rpm for 1 h at 25 °C. After adding 3 mL of methanol, samples were briefly vortexed then kept at -18 °C for 1 h. Samples were then withdrawn from the freezer and centrifuged at 4000 rpm for 20 min at 4 °C. Supernatants were transferred to 2 mL Eppendorf tubes and centrifuged at 14000 rpm, 4 °C for 5 min for absolute clarity. Phosphate buffer solution (0.4 M, pH 7.0) (150 μL) and 0.2% O-PD in 9.6 mM EDTA solution (150 μL) were added to 0.5 mL of the supernatant in order to derivatise MGO and GO into 2 -methylquinoxaline (2-MQ) and 1-quinoxaline (1- Q), respectively. The samples were then incubated at 37 °C for 3 h. Aliquots were transferred to a HPLC vials for analysis. Analysis was performed on the HPLC instrument detailed above. Chromatographic separation was carried out onto a 5 μm Eclipse Plus C18 column (150 mm × 4.6 mm) (Agilent, USA). The flow rate was 0.4 mL/min and the injection volume was 20 μL. A binary solvent system gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was used as follows: 0–2 min: 2% B, 2–8 min: 20% B, 8–10 min: 20–40% B, 10–13 min: 40–95% B, 13–14 min: 95-2% B and 14–17 min: 2% B. Chromatograms were recorded at 313 nm. 1-Q and 2-MQ were eluted at 13.01 and 12.55 min, respectively. Peaks were identified by comparison of the retention times with those of the standards. Quantification was carried out by an external calibration in the range (0.1–40 μg/mL); $R^2 = 0.99$; LOD LOQ and MDL were 0.010, 0.030 μg/mL 0.019 μg/mL, respectively for both 1-Q and 2-MQ. The analysis was performed in duplicate.

2.7. Metabolomic analysis of raw material

Relevant amino acids and reducing sugars in the raw vegetables were quantified using the method of de Falco et al. (2018) with minor modifications. Briefly, 3 mL of 60:40 methanol/water solution (v/v) was added to 0.1 g of dried powdered raw material. Samples were vortexed for 1 min, agitated for 30 min at 1000 rpm (Thermomixer Comfort, Eppendorf, Germany) then centrifuged for 10 min at 4000 rpm. Into 2 mL Eppendorf tubes, 0.25 mL of supernatant was transferred and 10 μL of internal standard, cycloleucine (1 mg/mL in water), was added. Samples were briefly vortexed then evaporated to dryness in a vacuum centrifuge (Concentrator 5301, Eppendorf, Germany) for 4 h. Beetroot samples were dried for an additional hour. To each sample, 0.15 mL of methoxyamine hydrochloride (20 mg/mL in pyridine) was added, and the mixture was incubated at 60°C for 3 h in an oven (Loading model 100–800, Memmert, Germany). Following incubation, 150 μL of MSTFA was added to the mixture and samples were vortexed and incubated (Orbital Incubator SI50, Stuart, UK) at 45 °C for 45 min. An aliquot was transferred to a HPLC vial with insert for analysis. GC-MS analysis was performed on an Agilent-7820 GC System with 5977E MSD operating in positive EI mode at 70 eV. The system was equipped with a 30 m × 0.25 mm ID fused-silica capillary column with 0.25 μm HP-5MS stationary phase (Agilent technologies, UK). Sample (1 μL) was injected in pulsed splitless mode. The injection temperature was set at 270 °C. Helium was used as carrier gas at a constant flow rate of 1.0 mL/min. Inlet temperature was at 220 °C and the splitless mass spectrometric detector (MSD) transfer line temperature was at 280 °C. The oven temperature gradient started at 70 °C held for 2 min, then increasing at 5 °C/min to 260 °C with no hold, then increasing at 15 °C/min to 290 °C and held for 5 min.

The mass spectrum ionization source temperature was 230 °C and the MS quadrupole temperature 150 °C. All spectra were recorded in the mass range 50–500 m/z. Quantification of cycloleucine was carried out in selected ion monitoring (SIM) mode using m/z 156.1 (cycloleucine 2TMS) with a dwell time of 200 ms. Peak areas of compounds of interest were compared to that of cycloleucine.

2.8. Statistical analysis

IBM SPSS (version 25.0, Armonk, NY) was used to apply one-way Analysis of Variance (ANOVA) and the Tukey test for significant differences between samples at $p < 0.05$ confidence level. The average was calculated using the results of the thermal treatment replicates and the technical replicates (four observations per sample).

3. Results and discussion

3.1. MRPs contents in commercial crisps

Table 1 summarises the measured values of the different MRPs in commercial snacks. Acrylamide concentrations ranged between 1848.27 ± 153.39 and 3762.00 ± 153.23 μg/kg in beetroot crisps and between 168.71 ± 18.34 and 3742.06 ± 208.03 μg/kg in parsnip crisps. Except parsnip crisps of brand 2, these values significantly exceed the benchmark level of acrylamide permissible in potato products. These high amounts could be due to the high reducing sugar content in root vegetables that promote the formation of acrylamide (Mesias et al., 2019). The data are in agreement with the findings of Hamlet et al. (2014) that reported values ranging between 962 and 2795 μg/kg of acrylamide in different commercial crisps mixtures containing beetroot and parsnip.

Table 1. MRPs levels in commercial alternative crisps. Concentrations are expressed in μg/kg for acrylamide and in mg/kg for HMF, GO and MGO. Different uppercase letters in the same row indicate significant differences ($p < 0.05$). Different lowercase letters indicate significant differences in a given MRP in different samples of the same type of crisps. Results are expressed as mean ± SD for n = 2.

MRPs	Brand	Beetroot crisps	Parsnip crisps
Acrylamide	1	2275.74 ± 307.00Ab	1696.61 ± 283.44Ab
	2	3762.00 ± 153.23Aa	168.71 ± 18.34Bd
	3	2599.36 ± 163.93Ab	1869.18 ± 144.00Bb
	4	2125.64 ± 279.48Bb	3742.06 ± 208.03Aa
	5	1848.27 ± 153.39Ab	1103.40 ± 92.03Bc
HMF	1	55.23 ± 8.14Bd	80.99 ± 9.30Aa
	2	91.57 ± 10.24Ac	28.77 ± 6.15Bb
	3	198.93 ± 34.90Aa	28.41 ± 1.26Bb
	4	133.69 ± 15.52Ab	26.85 ± 1.36Bb
	5	63.60 ± 4.65Ad	29.21 ± 1.89Bb
GO	1	6.08 ± 1.22Aa	4.31 ± 0.33Ad
	2	6.09 ± 0.54Aa	4.73 ± 0.28Bcd
	3	7.94 ± 0.62Aa	6.93 ± 0.45Ab
	4	7.14 ± 0.59Aa	7.71 ± 0.42Aa
	5	5.37 ± 0.38Aa	5.29 ± 0.35Ac
MGO	1	7.15 ± 1.40Aa	5.43 ± 0.75Bb
	2	5.93 ± 0.34Ab	3.71 ± 0.23Bc
	3	9.88 ± 1.61Aa	7.34 ± 0.59Aa
	4	9.58 ± 0.85Aa	2.37 ± 0.88Bc
	5	6.45 ± 1.33Ab	7.09 ± 0.73Aa

Apart from brand 1, beetroot crisps showed a significantly higher HMF concentration (from 55.23 ± 8.14 to 198.93 ± 34.90 mg/kg) compared to parsnip crisps (from 26.85 ± 1.36 to 80.99 ± 9.30 mg/kg). This is reported to be due to a higher sugar content in beetroot than in parsnip which leads to increased HMF formation (Mesias et al., 2019). Regarding dicarbonyls, the obtained concentrations are higher than those reported in the literature for potato fries where MGO was not detected (Degen et al., 2012). These results reveal the necessity for a mitigation strategy of MRPs in both beetroot and parsnip crisps.

3.2. Soaking in water

3.2.1. Effect on acrylamide formation

Acrylamide reduction varied between vegetables and treatments. All immersions led to an acrylamide level under the benchmark value for potato crisps (Figure 1A). The most significant decrease was observed after HS with 67.03% reduction while CSHS and CS were comparably effective with 63.32 and 59.67% decrease, respectively. These results are in agreement with previous works (Pedreschi et al., 2007). Depending on the raw material (potato variety and field location) and the production process variables (for instance, blanching conditions and frying temperatures), Jung et al. (2003) reported that resting potato strips in distilled water for 1hr induced almost 25% acrylamide reduction while blanching for 2.5 min at 82 °C was found to lower acrylamide concentration by about 60% in potato chips (Haase et al., 2003).

Asparagine and glucose followed similar decreasing trends comparing the pre- and post-soaking levels. In fact, their most notable reductions were observed after HS (47.42 and 69.06% reduction, respectively) followed by CSHS (34.18 and 44.11% reduction, respectively) (Table 2). This concurs with results from the literature demonstrating that soaking induced significant reductions in glucose and asparagine contents as the temperature of blanching increased leading to fried potatoes with less acrylamide content (Pedreschi et al., 2007).

Despite the comparable asparagine levels observed across the vegetables analysed, control beetroot and parsnip samples showed notably higher acrylamide levels than in control potato crisps with values of 2053.12 ± 359.83 and 5070.24 ± 754.07 μg/kg, respectively (Figures 1B and 1C). Although acrylamide is mostly derived from asparagine (Mottram et al., 2002), it can also form in the presence of acrylic acid that is produced directly from amino acids, such as glutamine, arginine, threonine and aspartic acid leading to significant acrylamide formation in model systems (Daniali et al., 2018). Furthermore, acrylamide can be indirectly generated via the interaction of acrylic acid formed from different sources with ammonia. Additionally, acrylic acid can be derived from pyruvic acid originating from amino acids cysteine and serine (Yaylayan et al., 2004). The high total amino acid content found in parsnip and beetroot samples support the formation of elevated acrylamide levels compared to potatoes. For beetroot crisps the amino acid glutamine may be significant in acrylamide formation and supports findings of Elmore et al. (2019). For parsnip crisps, aspartic acid may be the significant amino acid in the formation of acrylamide (Table 2). Regarding the soaking operation, none of the treatments has significantly reduced acrylamide levels post-frying except CSHS for beetroot bringing acrylamide concentration to 1198.12 ± 270.73 μg/kg (a reduction of 58%), supporting the importance of total amino acid content in acrylamide formation. However, this value exceeds the benchmark level of acrylamide in potatoes (Figures 1B and 1C).

When compared to potato crisps, these results suggest that immersion in water is not effective in mitigating acrylamide for parsnip crisps. This might be due to insufficient leaching of reducing sugars and asparagine (Table 2). This can be explained by differences in the tissue structure compared to potatoes (van der Sman et al., 2020; Mohr, 1974; Van der Sman, 2020).

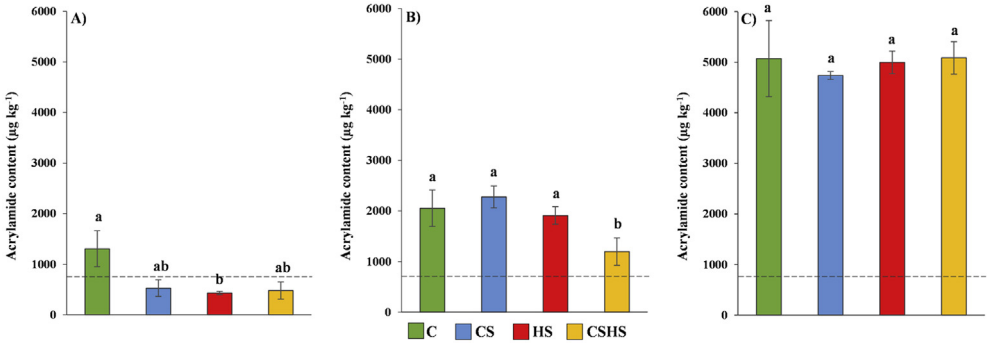

Figure 1. Acrylamide content (μg/kg) in (A) potato, (B) beetroot and (C) parsnip crisps after different soaking treatments. Different letters indicate significant differences between treatments within the same vegetable ($p < 0.05$). Results are expressed as mean ± SD for n = 2.

Table 2. Concentrations of MRPs precursors (μg/mg cycloleucine equivalent) after different soaking treatments. Different letters in the same row indicate significant differences ($p < 0.05$). Results are expressed as mean ± SD for n = 2. n.d.: not detected.

Vegetable	Treatment / Compounds	C	CS	HS	CSHS
Potato	Asparagine	4.22 ± 0.57a	3.88 ± 0.87ab	2.26 ± 0.64c	2.29 ± 0.30bc
	Glutamine	n.d.	n.d.	n.d.	n.d.
	Aspartic acid	n.d.	n.d.	n.d.	n.d.
	Threonine	1.12 ± 0.03a	0.76 ± 0.01b	0.50 ± 0.01c	0.43 ± 0.05c
	Serine	0.09 ± 0.03b	n.d.	0.45 ± 0.04a	0.08 ± 0.05b
	Total amino acids	**16.56 ± 5.18a**	**12.48 ± 1.38a**	**5.00 ± 0.52b**	**5.29 ± 0.36b**
	Glucose	8.50 ± 0.73a	7.90 ± 0.99a	2.07 ± 0.23b	3.77 ± 1.09b
	Fructose	10.62 ± 0.98a	6.64 ± 0.57b	2.33 ± 1.23c	3.80 ± 0.56c
	Total reducing sugars	**32.00 ± 2.39a**	**20.86 ± 8.33b**	**6.41 ± 1.24c**	**11.16 ± 0.59c**
Beetroot	Asparagine	3.15 ± 0.16a	2.50 ± 0.67ab	1.73 ± 0.63b	1.83 ± 1.08b
	Glutamine	8.38 ± 2.35a	10.89 ± 5.05a	10.20 ± 9.27a	6.49 ± 3.23a
	Aspartic acid	n.d.	n.d.	n.d.	n.d.
	Threonine	2.91 ± 0.32a	2.81 ± 0.45ab	3.00 ± 0.78a	1.79 ± 0.13b
	Serine	21.51 ± 0.90a	22.20 ± 3.38a	17.84 ± 7.80ab	11.44 ± 0.97b
	Total amino acids	**52.87 ± 0.95a**	**55.06 ± 7.71a**	**55.00 ± 2.00a**	**25.48 ± 4.75b**
	Glucose	121.11 ± 25.48a	114.93 ± 26.06 a	99.46 ± 19.18a	49.90 ± 8.43b
	Fructose	50.58 ± 2.64a	54.92 ± 2.00a	44.39 ± 4.78a	23.42 ± 4.57b
	Total reducing sugars	**298.79 ± 37.81a**	**298.53 ± 27.99a**	**251.06 ± 30.69a**	**238.70 ± 79.57a**
Parsnip	Asparagine	5.47 ± 0.78a	4.40 ± 0.68ab	3.17 ± 0.81bc	2.26 ± 0.42c
	Threonine	2.95 ± 0.10bc	3.09 ± 0.17b	3.46 ± 0.07a	2.70 ± 0.08c
	Glutamine	n.d.	n.d.	n.d.	n.d.
	Aspartic acid	3.78 ± 0.50a	5.09 ± 0.69a	4.64 ± 1.10a	4.51 ± 0.53a
	Serine	2.68 ± 0.00b	n.d.	3.94 ± 0.31a	2.42 ± 0.06b
	Total amino acids	**34.56 ± 1.02a**	**36.02 ± 3.43a**	**34.44 ± 2.23a**	**32.76 ± 5.29a**
	Glucose	92.37 ± 7.36a	81.87 ± 10.47ab	64.72 ± 8.58b	72.51 ± 12.17ab
	Fructose	66.63 ± 19.29a	55.80 ± 8.44ab	38.88 ± 1.74b	47.44 ± 1.62ab
	Total reducing sugars	**276.69 ± 57.29a**	**235.88 ± 28.50a**	**231.21 ± 16.06a**	**246.65 ± 35.31a**

3.2.2. Effect on HMF formation

HMF concentration in potato crisps was found to be below the LOD for both control and CS samples. Low levels were observed in HS and CSHS, 6.96 ± 0.33 and 6.20 ± 0.73 mg/kg, respectively (Figure 2A). These results suggest that blanching of raw potatoes can lead to a slight increase in HMF post frying.

Untreated beetroot and parsnip crisps showed a higher HMF content than untreated potato samples with values of 176.87 ± 25.59 and 44.66 ± 8.04 mg/kg, respectively (Figures 2B and 2C). These results correlate with those for reducing sugars, the main contributor to the formation of HMF, which showed total reducing sugar values of 298.79 ± 37.81 and 276.69 ± 57.29 μg/mg cycloleucine equivalent in beetroot and parsnip, respectively (Table 2). All soaking treatments were effective in reducing HMF levels in beetroot. CSHS was the most effective treatment lowering HMF by 88.44% followed by HS (66.72%) and CS (49.50%). CS and CSHS were able to notably lower HMF in parsnip crisps by 48.68 and 34.56%, respectively. On the other hand, HS promoted HMF formation for parsnip, increasing it by 78.7% (Figure 2C).

With these exceptions, HMF amounts in vegetable crisps after soaking treatments fall into the lower limit of the HMF concentration range found for other processed foods such as breakfast cereals (6.6–240.5 mg/kg) (Rufián-Henares et al., 2006) and biscuits (3.1–182.5 mg/kg) (Delgado-Andrade et al., 2009).

3.2.3. Effect on dicarbonyl compound formation

Untreated potato crisps contained less GO than untreated beetroot and parsnip crisps (2.21 ± 0.35, 8.61 ± 0.30 and 7.25 ± 0.62 mg/kg, respectively). GO levels varied between vegetable type and treatment

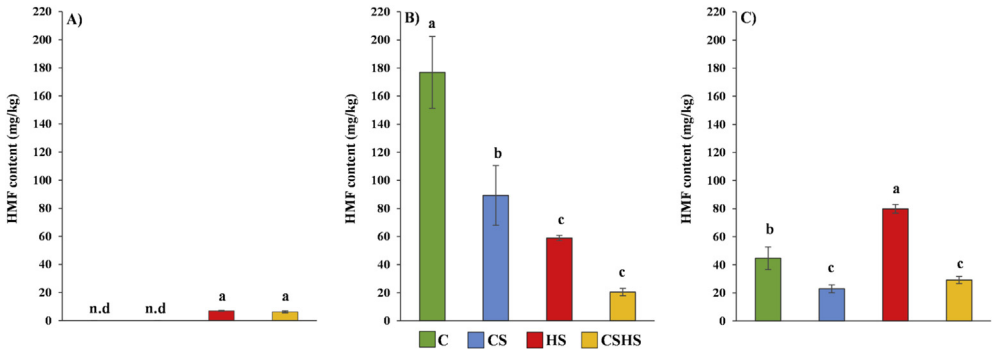

Figure 2. HMF content (mg/kg) in (A) potato, (B) beetroot and (C) parsnip crisps after different soaking treatments. Different letters indicate significant differences between treatments within the same vegetable ($p < 0.05$). Results are expressed as mean ± SD for n = 2. n.d: not detected.

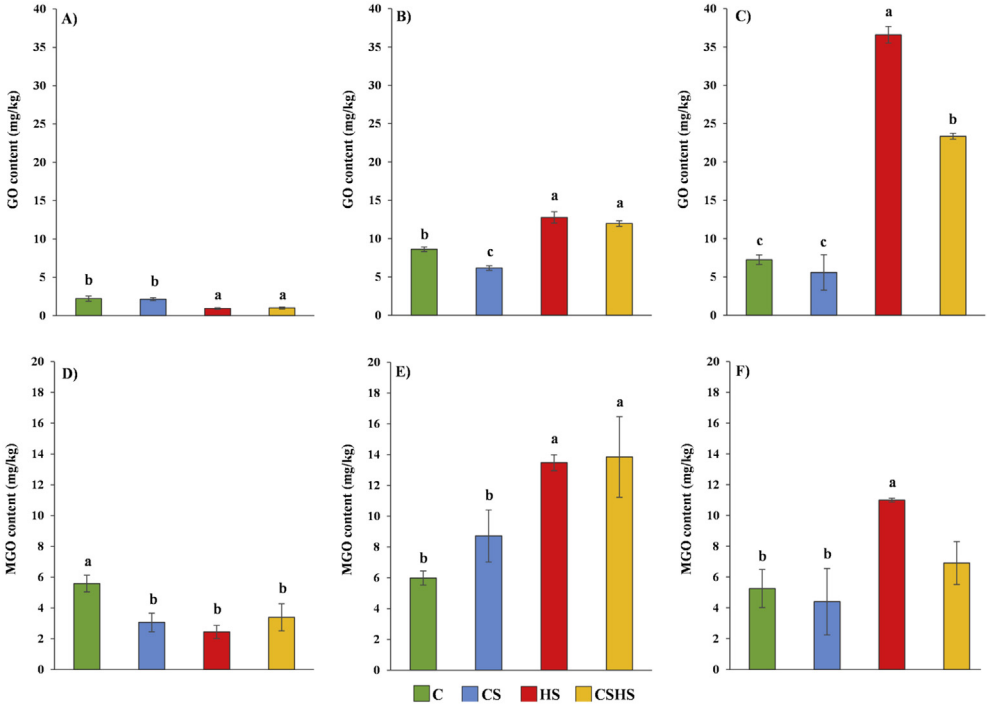

Figure 3. GO and MGO contents (mg/kg) in (A, D) potato, (B, E) beetroot and (C, F) parsnip crisps after different soaking treatments Different letters indicate significant differences between treatments within the same vegetable and the same dicarbonyl compound ($p < 0.05$). Results are expressed as mean ± SD for n = 2.

strategy. HS and CSHS decreased GO levels in potatoes by 57.93 and 55.19% while the same treatments saw an increase for beetroot and parsnip of 48.17 and 404.70%, respectively. Additionally, a similar trend was observed after CSHS with increases of 39.02 and 222.24%, respectively (for beetroot and parsnip). After CS, GO slightly decreased in beetroot (28.32%) whilst no significant change in potato and parsnip crisps was observed (Figures 3A, B and C).

MGO concentrations were comparable in untreated samples. However, they varied among treatments and vegetables. In potato crisps, MGO levels similarly diminished after all soaking treatments. In beetroot crisps, HS and CSHS caused an increase of 125.44 and 131.62% reaching 13.48 and 13.85 mg/kg, respectively. In parsnip crisps, HS increased MGO levels significantly compared to the control. Similar to GO, CS had minimal effect on MGO formation in both vegetable crisps (Figures 3E and F).

3.3. Soaking in additives solutions

3.3.1. Effect on acrylamide formation

The sequential use of immersion in a 0.01M $CaCl_2$ solution at ambient temperature followed by blanching in 0.1M citric acid had a different effect on acrylamide levels of the tested crisps. A significant decrease has been observed for all vegetables, compared to the relative controls. Most significant reduction was beetroot crisps (89.83%), followed by potato and parsnip crisps (52.03 and 54.40%, respectively) (Figure 4). For both potato and beetroot crisps, the treatment reduced acrylamide concentration below the benchmark level of 750 μg/kg. A significant reduction in asparagine was also observed (potato (79.86%) parsnip (55.21%) and beetroot crisps (100%) (Table 3). These findings are in line with those of other works (Gökmen and Şenyuva, 2007; Kita et al., 2004).

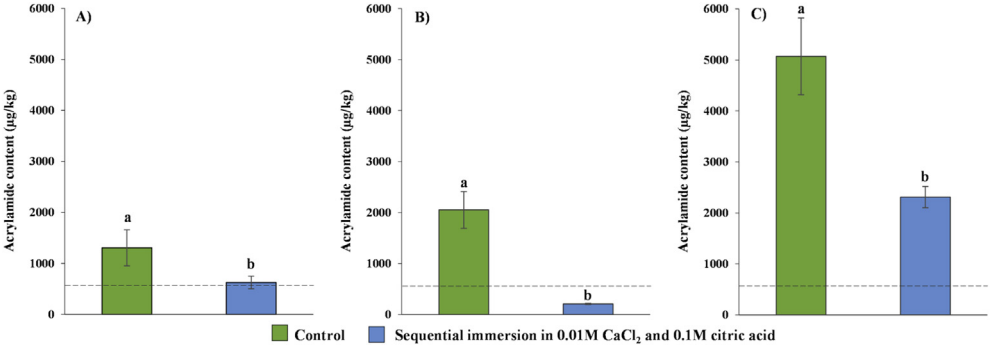

Figure 4. Acrylamide content (μg/kg) in (A) potato, (B) beetroot and (C) parsnip crisps without (control) and with sequential immersion in 0.01M $CaCl_2$ and 0.1M citric acid. Different letters indicate significant differences between treatments within the same vegetable ($p < 0.05$). Results are expressed as mean ± SD for n = 2.

Table 3. Concentrations of MRPs precursors (μg/mg cycloleucine equivalent) before and after successive immersions in 0.01 M $CaCl_2$ and 0.1 M citric acid solutions. Different letters in the same row indicate significant differences for a given vegetable ($p < 0.05$). Results are expressed as mean ± SD for n = 2.

Sample	Potato Pre	Potato Post	% reduction	Beetroot Pre	Beetroot Post	% reduction	Parsnip Pre	Parsnip Post	% reduction
Compound									
Asparagine	4.22 ± 0.57a	0.85 ± 0.08b	79.86	3.15 ± 0.16a	n.d	100.00	5.47 ± 0.78a	2.45 ± 0.23b	55.21
Glucose	28.06 ± 3.33a	24.03 ± 1.63a	14.36	121.11 ± 9.48a	55.30 ± 3.12b	54.34	92.37 ± 7.36a	73.71 ± 14.21a	20.20
Fructose	10.62 ± 0.98a	21.40 ± 3.41b	-101.51	50.58 ± 2.64a	37.50 ± 1.19b	25.86	66.63 ± 19.29a	36.24 ± 14.91b	45.61
Sucrose	6.28 ± 0.49a	3.73 ± 0.91b	40.61	294.41 ± 9.26a	185.49 ± 5.42b	37.00	125.31 ± 9.48a	82.70 ± 18.54b	34.00
Reducing sugars	67.51 ± 5.90a	69.56 ± 8.84a	-3.04	298.79 ± 7.81a	124.96 ± 7.47b	58.18	276.69 ± 57.29a	198.51 ± 6.50b	28.26

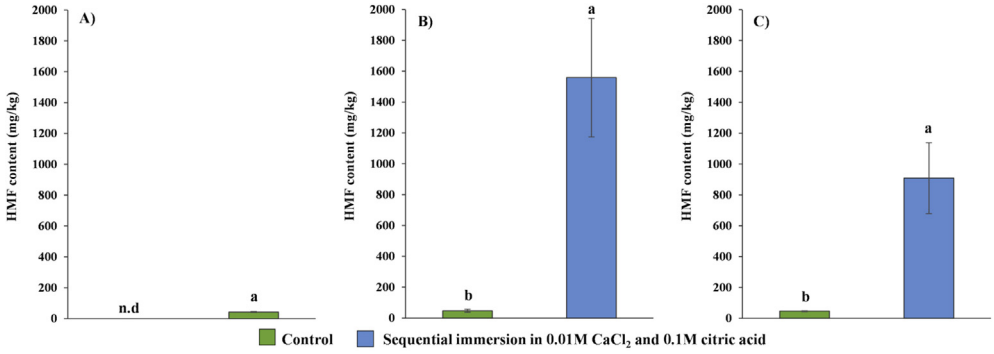

Figure 5. HMF content (mg/kg) in (A) potato, (B) beetroot and (C) parsnip crisps without (control) and with sequential immersion in 0.01M $CaCl_2$ and 0.1M citric acid. Different letters indicate significant differences between treatments within the same vegetable ($p < 0.05$). Results are expressed as mean ± SD for n = 2. n.d: not detected.

3.3.2. *Effect on HMF formation*

The same strategies applied in 3.3.1 were found to increase HMF levels in all vegetable crisps to different extents. HMF in the control potatoes was under the LOD, whilst in the treated samples this increased to 42.46 ± 2.50 mg/kg. HMF for treated beetroot and parsnip fried crisps has shown significant increases of ~30 and ~20 times respectively when compared to their corresponding controls, reaching 1558.20 ± 384.08 and 908.53 ± 229.32 mg/kg (Figure 5). The data support the findings of Gökmen et al. (2007) who demonstrated that the low pH of bread dough caused a reduction of acrylamide content and promoted HMF formation. The increase in HMF level could also be attributed to the catalysing effect of calcium on sugars (Kocadaglı & Gokmen, 2016). This can occur by

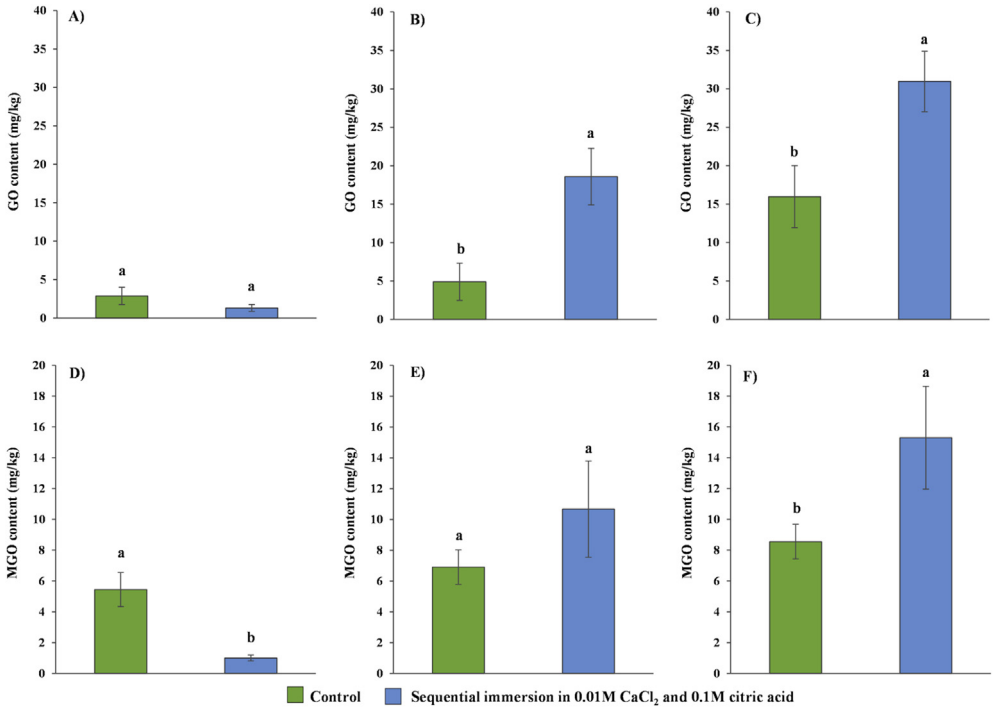

Figure 6. GO and MGO contents (mg/kg) in (A, D) potato, (B, E) beetroot and (C, F) parsnip crisps without (control) and with sequential immersion in 0.01M $CaCl_2$ and 0.1M citric acid. Different letters indicate significant differences between treatments within the same vegetable and the same dicarbonyl compound ($p < 0.05$). Results are expressed as mean ± SD for n = 2.

glucose ring opening and consecutive dehydration via open-chain intermediates (mainly 3-deoxyglucosone and 3,4-dideoxyglucosone), or by glucose ring opening and isomerization to fructose and consecutive dehydration via fructofuranose ring to HMF (Antal et al., 1990) or via cleavage of sucrose glycosidic bond directly yields a cyclic fructofuranosyl cation, which dehydrates to HMF (Mayes et al., 2014). Table 3 shows a significant sucrose reduction post-treatment in all 3 vegetable types. This indicates that degradation of sucrose to form fructofuranosyl cation could be a significant pathway for HMF formation in this case.

3.3.3. Effect on dicarbonyl compounds formation

Soaking in additive solutions altered dicarbonyls differently in the tested crisps (Figure 6). It promoted the formation of GO in beetroot and parsnip crisps by 279.36 and 93.99%, respectively, whilst a reduction of 54.67% relative to the control was observed in the potato crisp.

Regarding MGO, immersion considerably lowered its concentration in potato samples from 5.44 ± 1.11 to 1.00 ± 0.19 mg/kg (Figure 6), representing a decrease of 81.62% comparing to the control, supporting the findings of Yu et al. (2017) that demonstrated that a lower pH slows the formation of MGO in different glucose–amino acid model system. Yet, this treatment was less effective in beetroot snacks and even promoted MGO formation in parsnip crisps from 8.55 ± 1.12 to 15.29 ± 3.33 mg/kg, an increase of 78.83% (Figure 6).

4. Conclusion

Considered as an alternative healthy choice to potato crisps, commercial vegetable snacks contain high amounts of acrylamide, HMF, GO and MGO indicating the necessity of applying mitigation strategies in order to lower the concentration of these compounds to an acceptable and safe level.

The outcomes of this current research suggest that the sequential soaking in $CaCl_2$ and citric acid solutions at ambient and high temperatures seems to be suitable for raw potatoes as it reduced acrylamide concentration in a similar way to other methods in the literature. It also lowered GO and MGO levels and developed little HMF. For the alternative vegetable crisps, CSHS was the only promising treatment in simultaneously reducing acrylamide (yet the benchmark level set for potato products is still exceeded) and HMF with in beetroot crisps without forming considerable amounts of dicarbonyl compounds.

Soaking in additive solutions was proven to be effective in lowering acrylamide in all tested crisps. However, it significantly increased HMF levels in beetroot and parsnip crisps implying that this approach is unsuitable for the simultaneous mitigation of MRPs in alternative vegetable snacks.

Finally, tested mitigation strategies have shown to be vegetable specific. More research is yet to be done to find a more effective approach for the reduction of MRPs reduction in vegetable crisps.

Declarations

Author contribution statement

Moira Ledbetter: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Slim Blidi, Stefania Ackon: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Francesca Bruno: Performed the experiments; Analyzed and interpreted the data.

Keith Sturrock, Alberto Fiore: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Nicoletta Pellegrini: Conceived and designed the experiments; Wrote the paper.

Funding statement

This work was supported by Abertay University Structural Funding provided by the School of Applied Science.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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