

Effect of ultrasound and additives treatment as mitigation strategies to reduce acrylamide formation in potato crisps on industrial scale

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ABSTRACT

The aim of this work was to examine the applicability on large scale of additives and ultrasound treatments during soaking of potatoes before frying to mitigate the formation of acrylamide in potato crisps. Calcium chloride and citric acid were applied at laboratory scale in various concentrations and orders during washing before frying, to establish optimum conditions which were scaled up to pilot plant. Up to 91.0% reduction in acrylamide was obtained at laboratory scale. Both concentration and order of additives influenced the extent of the mitigation observed, with a higher concentration of additive in the second wash being beneficial. When upscaled to factory pilot plant, the reduction observed was not consistent across the three trials, with a 33.4% reduction in the first trial but no significant reduction in following studies. A 2-min ultrasound treatment was applied in two trials to test various powers and amplitudes, and washing combinations respectively. Up to 67.1% of acrylamide reduction was recorded after 2 min of ultrasound treatment in the cold wash followed by hot wash; however, ultrasound treatment was not effective in reducing acrylamide or its precursors when solely applied or when followed by cold wash under the tested conditions of duration and power.

1. Introduction

Acrylamide is a chemical compound classified as probable carcinogen (IARC, 1994). Its presence in foods was first detected in 2002 by the Swedish National Food Administration (SNFA) (Tareke et al., 2000, 2002). Similar findings of the contaminant presence in cooked foods have been reported in other countries (Joint & FAO/WHO Codex Alimentarius Commission, 2004). Following the identification of unexpected levels of acrylamide in fried or baked food products, a significant body of research has been undertaken to further the understanding of its formation as part of the Maillard Reaction (MR) (Amrein et al., 2003; Matthäus et al., 2004; Mottram et al., 2002; Serpen & Gökmen, 2009; Stadler et al., 2002). The MR encompasses a wide array of non-enzymatic browning reactions occurring at temperatures over 120 °C in foods containing reducing sugars, (predominantly glucose and fructose) and amino acids (principally asparagine in the case of

potatoes), resulting in Advanced Glycation End Products (AGEs) and Maillard Reaction Products (MRP) (Knol et al., 2005; Mottram et al., 2002). Since potato tubers contain high amounts of acrylamide precursors, potato products, including crisps and chips, remain a major dietary source of acrylamide, especially in the western diet (Capuano & Fogliano, 2011; Keramat et al., 2011; Maan et al., 2020; Nehlig & Cunha, 2020; Timmermann et al., 2021). The European Commission has set product specific benchmarks based on both the occurrence of acrylamide and the state of the art for its monitoring and control. The benchmark set for acrylamide level in potato crisps is 750 µg/kg (Commission Regulation, 2017). A toolbox describing methods to reduce acrylamide in food and industrial best practices was published by FoodDrinkEurope to give guidelines to food manufacturers (Food-DrinkEurope, 2019).

Potato is one of the most studied food matrices for acrylamide formation and mitigation (Zhang & Zhang, 2007). The main strategies to

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control acrylamide formation involve reducing the precursors, controlling the frying conditions and trapping acrylamide formation (Bartlett et al., 2020; Keramat et al., 2011; Ledbetter et al., 2020; Maan et al., 2022; Palermo et al., 2016; Stadler, 2005; Vinci et al., 2012). Storage conditions are also a very important factor to monitor, since low storage temperatures (below 8–10 °C) can promote the conversion of starch into sugars (Biedermann-Brem et al., 2003; Burton, 1989; Coffin et al., 1987; Gökmen et al., 2007).

During the industrial processing of potatoes into fried crisps, potatoes are washed, peeled and sliced. Slices then undergo a cold wash and eventually a hot wash followed by a final rinse prior to frying, with the purpose of reducing glucose and fructose content in the tubers (Bartlett et al., 2020; FoodDrinkEurope, 2019; Zhang et al., 2018). Many studies have been performed on the effects of mitigation strategies applied during the washing steps before frying; among these, promising results were reported when using additives or ultrasound treatments (Antunes-Rohling et al., 2018; Gökmen & Şenyuva, 2007; Mestdagh et al., 2008; Ostermeier et al., 2021; Pedreschi et al., 2010, 2021).

The addition of additives to the washing steps serves several purposes: i) removal of sugars and asparagine from the raw material; ii) changing the pH of the matrix, thus affecting pathways of acrylamide formation; iii) promoting competitive reactions to the formation of acrylamide.

Citric acid significantly decreases the pH of the water, causing the protonation of the amino groups of asparagine, thus blocking the pathway to acrylamide formation (De Vleeschouwer et al., 2006). Kita et al. (2004) reported a 50% reduction in acrylamide for potato slices blanched in 0.05 M citric acid for 3 min at 70 °C, however a slight sour taste was reported. Significant reductions (~70%) in acrylamide were reported by Pedreschi et al. (2004), following 30 min immersion in 10 and 20 g/L citric acid.

The addition of cation ions, particularly divalent cations, limits acrylamide development by interacting with asparagine preventing the formation of the Schiff base intermediary. Gökmen and Şenyuva (2007) demonstrated up to 95% reduction in acrylamide content for chips soaked in 0.1 M CaCl₂ for 1 h. Mestdagh et al. (2008) found a complete inhibition of acrylamide generation when blanching for 5 min at 65 °C with both citric acid and CaCl₂ (0.1 M); however, these concentrations resulted in unwanted sensorial changes in the crisps.

Low frequency and high intensity ultrasound treatment (UST) improves extraction of intracellular components from vegetable matrices. The application of such approach in the wash waters can accelerate leaching of sugars and can lead to a reduction in acrylamide formation during frying (Antunes-Rohling et al., 2018; Dourado et al., 2019).

Ultrasound-assisted extraction is a widespread “green” technology that uses ultrasonic frequencies in the range 20–150 kHz to accelerate heat and mass transfer processes (Awad et al., 2012; Chemat et al., 2011; Picó, 2013). The main mechanism associated with the extraction is the formation of cavitation bubbles, voids created when a sound wave passes through a liquid medium as a result of the displacement of particles. When cavitation bubbles are created close to plant material and collapse, a microjet is directed towards the plant matrix disrupting the cell walls of the plant, due to high pressure and temperature involved, thus resulting in the release of its content into the medium (Antunes-Rohling et al., 2018; Chemat et al., 2011; Tao & Sun, 2015). Ultrasonic frequency is inversely proportional to the size of cavitation bubbles, with large bubbles created when low frequencies are applied; high ultrasonic power (>1 W/cm²) is preferable since it increases extraction yields (Antunes-Rohling et al., 2018; Soria & Villamiel, 2010; Zou et al., 2010).

Antunes-Rohling et al. (2018) obtained up to 50% reduction of acrylamide content when applying UST of 35 kHz and 92.5 W/kg at 42 °C for 30 min compared to controls only soaked in water. Similarly, Pedreschi et al. (2021) achieved a reduction of acrylamide level up to 95% with UST at 70 °C for 15 min. Ostermeier et al. (2021) applied UST at 1000 W power for 3 min during frying of potato chips, which led to

34% reduction of acrylamide concentration compared to controls, within a frying regime that did not involve washing steps. The reduction achieved was even higher (66%) when UST was preceded by Pulse Electric Field (PEF) treatment.

The application of a treatment involving long time exposure (15–30 min) to elevated temperatures would be incompatible with food production requirements in terms of costs and applicability. Furthermore, there is a range of well-established industrial approaches such as the combination cold wash-hot wash that were proven to maintain acrylamide content in the crisps around the required benchmark level (Bartlett et al., 2020).

This paper explores the use of additives (CaCl₂ and citric acid) and the application of UST in the wash waters during the production of potato crisps, to reduce precursors levels and acrylamide formation at both laboratory and pilot plant scale. The current study investigates novel experimental conditions potentially applicable within a potato crisp manufacturing line, considering time and costs constraints, as well as overall impact of the treatments on the sensorial attributes of the end product.

2. Materials and methods

2.1. Chemicals

Methanol (LC-MS grade), water (LC-MS grade), acetonitrile (HPLC grade), hexane (HPLC grade), sodium chloride (NaCl, 99.5%) and pyridine anhydrous (99.5%) were purchased from Fisher Scientific (Loughborough, UK). Magnesium sulphate (MgSO₄, 97%), citric acid monohydrate (99.5%), and calcium chloride (CaCl₂, 96%) were purchased from Acros Organics (Geel, Belgium). Primary Secondary Amine sorbent (PSA) was purchased from Agilent Technologies (Santa Clara, CA, USA). Acrylamide (98%) was purchased from Fluka (Buchs, Switzerland). [2,3,3-*d*₃]-acrylamide (98%), formic acid (LC-MS grade), cycloleucine (97%) were purchased from Sigma Aldrich (Gillingham, UK). N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) (100%) was purchased from Fluorochem (Hadfield, UK).

2.2. Food material

Potatoes from Lady Claire and Taurus varieties were grown at James Hutton Institute (JHI) (Dundee, UK) or provided by KP snacks (Billingham, UK). Palm oil (RSPO Palm RD Oil) was purchased from Kerfoot Oil Specialists (Northallerton, UK).

2.3. Crisp production

Potatoes were washed, manually sliced to a proprietary slice of varying thickness using FAM cutting Urschel slicer blades (0.212 v-cut) with a 0.80 mm shim (Leicester, UK), a 30 mm disc was taken from the slices. Washing protocols and pre-treatments applied before frying are detailed in sections 2.4 and 2.5 for the additives trials conducted in the laboratory of Abertay University (AU) and KP Snacks pilot plant respectively, and in section 2.6 for the UST trial conducted within AU processing plant.

Samples were fried in palm oil at 173 ± 2 °C in a 3 L Selection Magimix professional deep fat fryer (Godalming, UK). Commercial processing condition were adapted from Bartlett et al. (2020) with some modifications, frying time was 4.5 min. The oil temperature was monitored by an external probe (E.T.I food check thermometer, Sussex, UK). Samples were removed from the fryer, drained to remove the excess oil and cooled down; they were then pulverised and stored at –18 °C until analysis.

2.4. Laboratory additives trial

Potato slices (60g) were soaked twice in 2 L of distilled water

containing additives: i) 1st wash for 2 min at ambient temperature (cold wash); ii) 2nd wash for 3 min at 78 °C (hot wash) or at ambient temperature. Control samples were soaked twice in distilled water following the washing regime temperature of the corresponding additive treatment (cold wash - hot wash or double cold wash). All samples were manually agitated during the washes to mimic the agitation experienced by the rotating drum on the production line. Following soaking, samples were rinsed in 1 L of fresh distilled water and excess water removed with compressed air. Two additives (CaCl₂, citric acid) and two concentrations (0.01 M, 0.1M) were tested.

2.5. Pilot plant trial

The pilot plant at KP snacks included an automated FAM cutting Urschel slicer, blades (0.212 v-cut) with a 0.80 mm shim (Leicester, UK), two cold washes of 150 L and a 9 L Valentine V600 fryer (Reading, UK) filled with sunflower oil.

Potato slices (200 g) were added to a first cold wash for a set residence time of 2 min and a second cold wash of 3 min, they were manually agitated to mimic the agitation experienced by the rotating drum on the production line; slices were then rinsed in fresh water for 20 s and dried to remove excess water before entering the oil.

Following results from the laboratory trial and sensory analysis (see section 3.1, Table 1, Fig. 1 the additives order and concentrations chosen to be tested at pilot plant scale were 0.01 M for CaCl₂ in the 1st wash and 0.05 M for citric acid in the 2nd wash.

2.6. Ultrasound trial

The processing plant at Abertay University included a UIP2000hdT (20 kHz, up to 2000 W) ultrasonic processor (Hielscher Ultrasonics, Teltow, Germany) implemented in a 30 L cold wash, hot wash at 78 °C and fryer (see section 2.3). In a first trial, conducted on Taurus cultivar, UST for 2 min was tested at two powers (450 W, 1500 W) and two amplitudes (50% A, 100% A) in the cold wash followed by 3 min of hot wash in distilled water; in addition to a control with no UST, a positive control was trialled where UST at 1500 W, 100% A was applied for 15

min. Following results from the first trial (see section 3.3, Fig. 3) a second trial was designed where a 450 W, 100% A UST was applied for 2 min in three soaking conditions before frying: i) cold wash with UST, ii) cold wash with UST followed by a second 3-min cold wash, iii) cold wash with UST followed by 3 min of hot wash at 78 °C. Control condition were 2 min of cold wash followed by 3 min of hot wash at 78 °C.

Two sets of Lady Claire potatoes were trialled: i) tubers stored from September to March at 5 °C at JHI, with expected high reducing sugars content; ii) tubers provided by KP snacks stored from September to March at 8–10 °C, with expected low reducing sugars content. Prior to frying, 20 g of raw potato pre-wash and 20 g of raw potato post-wash for each sample were retained for metabolomic analysis. Samples were freeze-dried using a Micro Modulyo RV3 Edwards (San Jose, CA, USA), then ground in a coffee grinder.

2.7. Acrylamide and precursors analysis

Acrylamide was quantified by liquid chromatography tandem-mass spectrometry (LC-MS/MS) using a three-phase extraction method as described by Bruno et al. (2023).

Briefly, approximately 1.000 g of fried crisps (ground) was accurately weighed then combined with [2,3,3-*d*₃]-acrylamide (10 µL, 0.2 mg/mL, Internal standard), 10 mL water, 10 mL acetonitrile and 5 mL hexane, 4 g MgSO₄ and 0.5 g NaCl. The mixture was then shaken vigorously for 1 min, then centrifuged (2683 rcf for 10 min; Hermle GmbH Z 323 K, LaborTechnik, Düsseldorf, Germany). An aliquot (1 mL) of the acetonitrile layer (middle layer) transferred to a 2 mL Eppendorf tube containing premixed PSA (50 mg) and MgSO₄ (175 mg), this was vortexed and centrifuged (9300 rcf for 1 min; Microcentrifuge 5415R, Eppendorf, Hamburg, Germany). Supernatants were transferred to HPLC vials for LC-MS/MS analysis.

Acrylamide quantification was performed on a Thermo Fisher Scientific LC-MS/MS (San Jose, CA, USA) consisting of a degasser, a quaternary pump, a thermostatic autosampler, a column oven and a TSQ Mass spectrometer. Chromatographic separation was achieved with ultra-pure water containing 0.1% formic acid (mobile phase A) and methanol containing 0.1 % formic acid (mobile phase B). The gradient

Table 1

Acrylamide levels of potato crisps, effect of adding additives to the 1st and 2nd washes of potatoes before frying. Concentrations are expressed in µg/kg. Letters denote grouping from one-way ANOVA with Tukey post hoc test ($p < 0.05$). One way ANOVA was applied to groups separated by variety and 2nd wash temperature. Results are expressed as mean ± SD for $n = 6$. A negative % reduction indicates an increase in acrylamide compared to the control.

Variety	Crop year	2nd wash	1st wash additive	2nd wash additive	1st additive conc. (M)	2nd additive conc. (M)	Acrylamide (µg/kg)	% reduction
Taurus stored	2018	Hot	CaCl ₂	Citric	0.01	0.01	568.7 ± 185.56 ^b	79.9
					0.01	0.1	305.44 ± 124.24 ^a	89.2
					0.1	0.01	896.82 ± 279.75 ^c	68.3
					0.1	0.1	227.7 ± 101.17 ^a	91.9
					0.01	0.01	2567.54 ± 901.77 ^{bc}	9.2
					0.1	0.01	2986.48 ± 801.76 ^c	-5.7
			Citric	CaCl ₂	0.01	0.1	1552.31 ± 375.24 ^{ab}	45.1
					0.1	0.1	1792.16 ± 1358.49 ^{abc}	36.6
					-	-	2826.62 ± 712.58 ^{bc}	0.0
					-	0.1	844.07 ± 330.79 ^a	70.1
					-	0.1	399.2 ± 223.69 ^{ab}	16.0
					-	0.1	809.66 ± 568.11 ^{ab}	-70.1
Taurus fresh	2019	Cold	None	Citric	-	0.1	339.37 ± 200.53 ^a	28.7
					-	0.01	476.73 ± 337.06 ^{ab}	-0.2
					-	0.01	637.68 ± 692 ^b	-34.0
					-	-	475.87 ± 306.47 ^{ab}	0.0
					-	0.1	142.65 ± 35.48 ^a	24.9
					-	0.1	118.21 ± 38.2 ^a	37.7
			CaCl ₂	Citric	0.01	0.1	105.82 ± 24.68 ^a	44.3
					-	-	189.89 ± 115.05 ^a	0.0
					-	0.1	260.22 ± 125.1 ^b	-39.2
					-	0.1	33.5 ± 6.73 ^a	82.1
					0.01	0.1	104.32 ± 162.06 ^{ab}	44.2
					-	-	186.96 ± 79.4 ^{ab}	0.0
Lady Claire fresh	2019	Cold	None	CaCl ₂	-	0.1	142.65 ± 35.48 ^a	24.9
					-	0.1	118.21 ± 38.2 ^a	37.7
					0.01	0.1	105.82 ± 24.68 ^a	44.3
					-	-	189.89 ± 115.05 ^a	0.0
					-	0.1	260.22 ± 125.1 ^b	-39.2
					-	0.1	33.5 ± 6.73 ^a	82.1
		Hot	None	CaCl ₂	0.01	0.1	104.32 ± 162.06 ^{ab}	44.2
					-	-	186.96 ± 79.4 ^{ab}	0.0

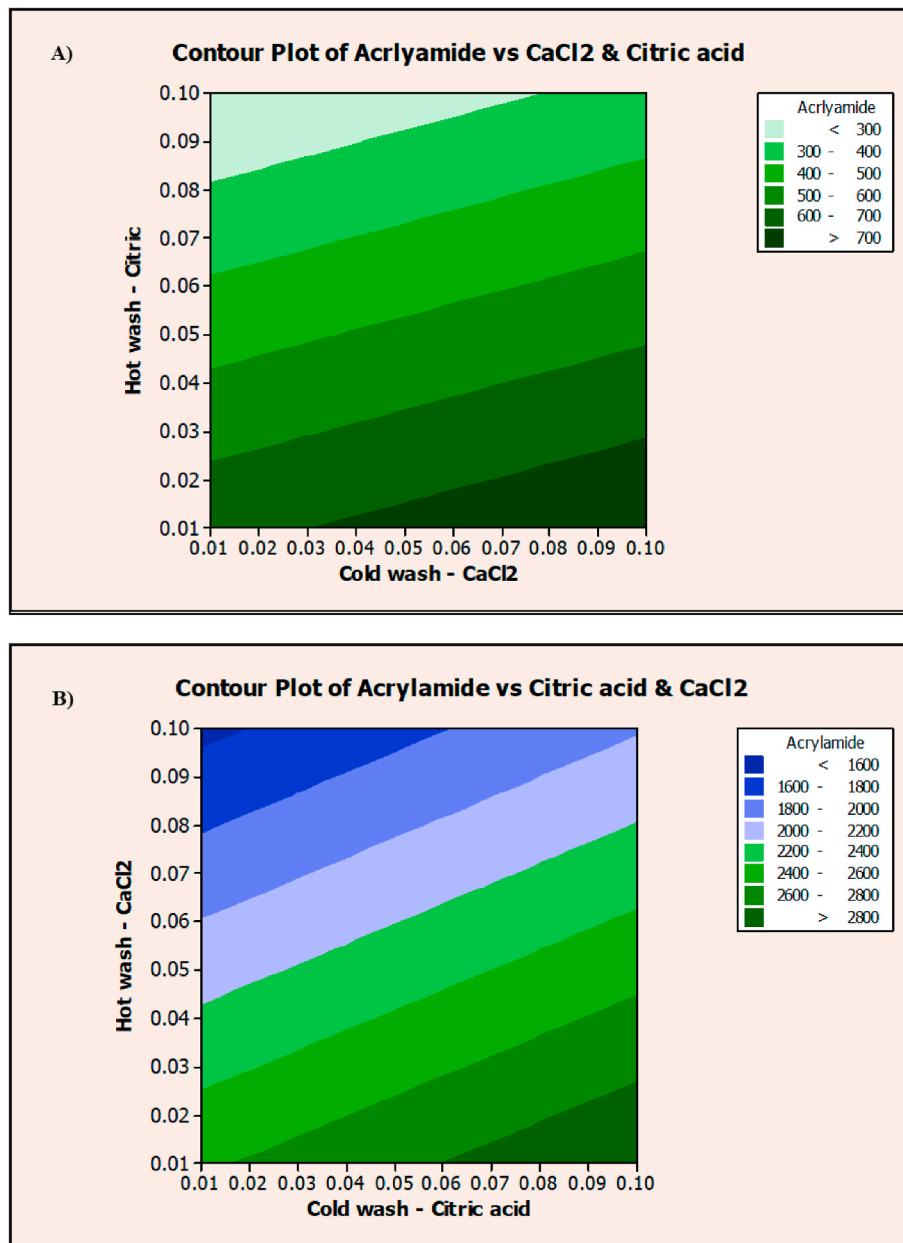


Fig. 1. Contour plots for the prediction of acrylamide formation based on the concentration and order of additives implemented in the washes. Data from first experiment, crop year 2018, Taurus variety. A) CaCl₂ in the 1st wash (cold wash), citric acid in the 2nd wash (hot wash). B) Citric acid in the 1st wash (cold wash), CaCl₂ in the 2nd wash (hot wash).

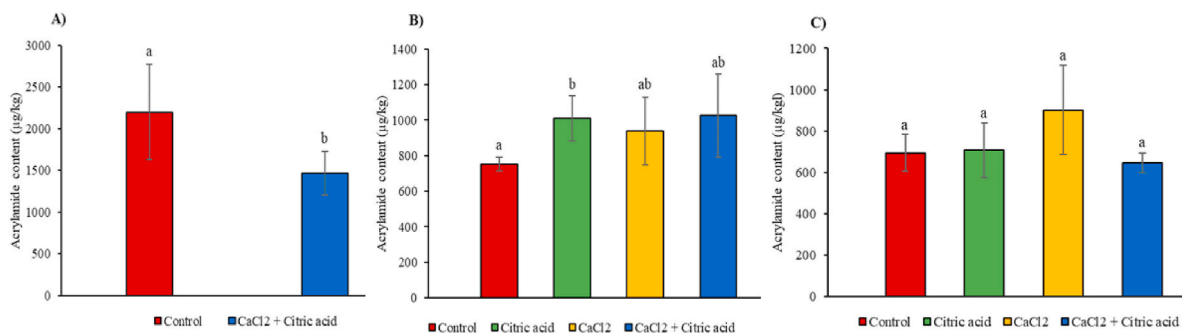


Fig. 2. Pilot plant trials (letters A, B, C, correspond to 1st, 2nd, 3rd trial). Acrylamide levels of potato crisps, effect of adding additives to the 1st and 2nd washes of potatoes before frying. Variety: A) Taurus; B) Lady Claire; C) Lady Claire. Concentrations are expressed in µg/kg. Letters denote grouping from one-way ANOVA with Tukey post hoc test ($p < 0.05$). Results are expressed as mean \pm SD for A) $n = 9$; B) $n = 3$; C) $n = 3$.

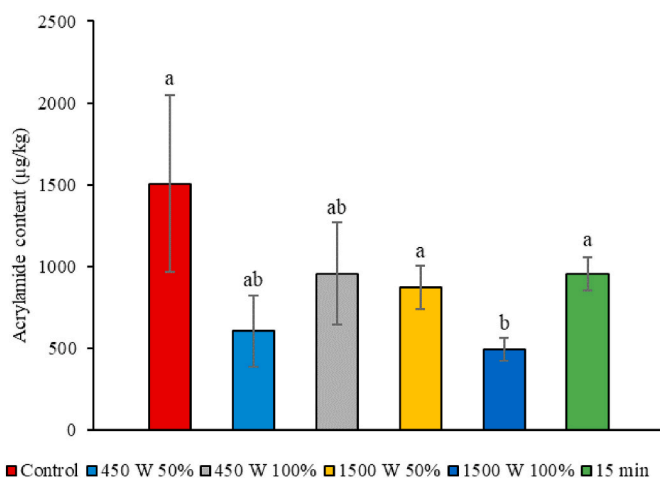


Fig. 3. UST 1st trial. Variety: Taurus, stored at 8–10 °C. Acrylamide levels of potato crisps, effect of ultrasound treatment (UST) in the cold wash followed by hot wash before frying. “15 min” corresponds to a positive control where UST was applied at 1500 W 100% A for 15 min. UST was applied for 2 min in all the others samples. Concentrations are expressed in µg/kg. Letters denote grouping from one-way ANOVA with Tukey post hoc test ($p < 0.05$). Results are expressed as mean \pm SD for $n = 3$.

was 98% A at 200 µL/min for 3.5 min, the flow rate increased to 300 µL/min and 25% A over 2 min and held for 2 min before re-equilibration to initial conditions for 16.7 min. Each sample (10 µL) was injected on a Synergi Hydro RP column (250 mm \times 4.6 mm \times 4 µm, 80 Å pore size) (Phenomenex, Macclesfield, UK).

The mass spectrometer was equipped with an electrospray ionization (ESI) source and was operated in positive ionization mode. Multiple reaction monitoring (MRM) transitions were m/z 72.07 \rightarrow 55.1 and 44.0 for acrylamide and 75.2 \rightarrow 58.0 and 44.0 for 2,3,3- d_3] acrylamide (internal standard) 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 10–1000 ng/mL ($R^2 > 0.99$), with a limit of detection (LOD) of 8.25 ppb (equivalent to 82.5 µg/kg of crisps), limit of quantification (LOQ) of 25 ppb (equivalent to 250 µg/kg of crisps).

The metabolomic profile of the raw tubers (2nd UST trial) was determined using the method described by Bruno et al. (2023) by gas chromatography tandem-mass spectrometry (GC-MS); the main acrylamide precursors (glucose, fructose and asparagine) were quantified. Briefly, 3 mL of 60:40 methanol/water solution (v/v) was added to approximately 0.100 g of dried powdered raw material. Samples were vortexed for 1 min, agitated for 30 min at 1000 rpm (Thermomixer Comfort, Eppendorf, Hamburg, Germany) then centrifuged for 10 min at 4180 rcf (Hermle Z 206 A, LaborTechnik, Düsseldorf, Germany). 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. To each sample, 150 µL 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, Büchenbach, Germany). Following incubation, 150 µL of MSTFA was added to the mixture and samples were vortexed and incubated (Orbital Incubator S150, Cole-Parmer, St. Neots, UK) at 45 °C for 45 min. An aliquot was transferred to a HPLC vial 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 \times 0.25 mm ID fused-silica capillary column with 0.25 µm HP-5MS stationary phase (Agilent technologies,

Cheadle, Cheshire, UK). Each 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. The analysis was performed in duplicate.

2.8. Colour analysis

Colour analysis was conducted on potato crisps from the 2nd UST trial. The colour of the fried crisps samples was evaluated using a colorimeter PCE-CSM 5 (PCE Instruments, Meschede, Germany). The colorimeter was calibrated using the provided white calibration tile and a black calibration box. The instrument evaluates the colour of the samples using the $L^*a^*b^*$ colour space defined by the International Commission on Illumination (CIE). Ground potato crisps were used for the analysis in order to have a homogeneous sample colour. L^* (Lightness), a^* (green to red) and b^* (blue to yellow) were measured for every sample in triplicate. Three samples per condition, each corresponding to approximately 1.000 g of grounded crisp originated from one raw potato, were analysed and the mean values for L^* , a^* and b^* were calculated. Using the means the ΔE value was calculated, to determine total colour differences between control and treated groups, using equation (1) (Pedreschi et al., 2005):

$$\Delta E = \sqrt{\left((L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2 \right)} \quad (1)$$

The $L_0^*a_0^*b_0^*$ values correspond to the control group while the $L^*a^*b^*$ values correspond to the treated group.

2.9. Statistical analysis

Statistical analysis was conducted on IBM SPSS (version 26.0, Armonk, NY). Shapiro-Wilk test was used to check normality of the data with α value at 0.05 for significance. Independent sample t -test and one-way ANOVA were performed to show significant differences between samples at $p < 0.05$ confidence level. Tukey Post Hoc was performed with one-way ANOVA to identify differences between groups. The Pearson correlation test and scatter plots were used to correlate acrylamide content with colour parameters. Grubbs' test was used to identify outliers.

3. Results and discussion

3.1. Laboratory additives trial

The first study was conducted on stored potato tubers of the Taurus cultivar investigating both order of additives and two concentrations (0.1 M, 0.01 M) (Table 1). Stored potatoes are expected to have higher reducing sugars levels than fresh ones. Therefore, a cold wash – hot wash regime was applied, following indications from Bartlett et al. (2020). Table 1 shows the effect of additives in the washes on acrylamide formation in the crisps. The reductions observed clearly demonstrate that both the concentration and the order of additives are important. $CaCl_2$ followed by citric acid led to a greater reduction of acrylamide formation. The combination 0.1 M $CaCl_2$ – 0.1 M citric acid gives the greatest

reduction in acrylamide content compared to controls (-91.0 %), followed by 0.01 M CaCl₂ – 0.1 M citric acid (-89.2 %). When scaling up to a factory line, the costs associated with using the higher concentration of 0.1 M CaCl₂ would not be justified, therefore, 0.01 M CaCl₂ – 0.1 M citric acid was identified as the most effective treatment and was selected for the next trials. Considering these findings, the addition of 0.1 M citric acid only, in the second wash, has also been trialled, which resulted in 70.1 % reduction of acrylamide formation compared to the control.

Fig. 1 shows contour plots for CaCl₂ followed by citric acid in the 1st and 2nd washes (A) and citric acid followed by CaCl₂ in the 1st and 2nd washes (B), respectively. Both contour plots show similar trends which indicates that a higher concentration of additive in the 2nd wash is beneficial in the reduction of acrylamide formation; moreover, citric acid in the 2nd wash is more effective for acrylamide reduction.

The groupings based on Tukey post hoc test from one-way ANOVA analysis on stored Taurus tubers, initially demonstrate that the effect of the addition of 0.1 M citric acid in the hot wash was significant on acrylamide reduction compared to 0.01 M citric acid. Moreover, the sole addition of 0.1 M citric acid was also significant at reducing acrylamide compared to the control (Table 1).

A subsequent study was then designed based on the results discussed above, and conducted on fresh potatoes of the Taurus cultivar where a double cold wash regime was implemented (Bartlett et al., 2020), and on fresh potatoes of the variety Lady Claire where both cold wash – hot wash and double cold wash combinations were tested (Table 1). It can be observed that, irrespective of the variety and wash temperature, the addition of additives to the wash does not reduce acrylamide to a statistically significant level in fresh, low sugar potatoes (Table 1).

Samples from each laboratory additives trial were sent for sensory analysis conducted at KP Snacks in which triangle tests and comparative profiles were carried out (data not shown). Both flavoured and unflavoured samples were analysed for appearance, aroma, texture, and flavour differences.

In the triangle test, all samples with 0.1 M citric acid failed, a strong taste to the product was noted, which would be unacceptable to the consumer.

3.2. Pilot plant additives trial

A first pilot plant trial was planned based on the outcome of the laboratory trials, and was conducted at KP Snacks (Billingham, UK). Based on the results of the sensory analysis, the concentration of citric acid in this trial was lowered from 0.1 M to 0.05M. As shown in Fig. 1a, the contour plot revealed that the predicted acrylamide content of the generated crisps would still be lower than benchmark levels, with values ranging between 400 and 500 µg/kg, when starting from Taurus stored potatoes with an expected high content of precursors.

In the first trial, the addition of 0.01 M CaCl₂ in the first cold wash followed by a second cold wash with 0.05 M citric acid resulted in a significant reduction of acrylamide formation compared to controls (-33.4 %) (controls: 2199.8 ± 574.6 µg/kg; additives: 1465.6 ± 263.1 µg/kg) (Fig. 2a). Two further trials were conducted on Lady Claire potato tubers to confirm these findings and to investigate whether the use of a single additive was sufficient in reducing the contaminant formation. However, these studies did not exhibit a similar mitigation effect. As shown in Fig. 2b and c, no significant reduction in acrylamide levels was observed for any of the tested conditions compared to controls. Regarding acrylamide levels, we can observe that crisps from the first trial (Fig. 2a) have an overall higher acrylamide content while crisps from the second and third trials (Fig. 2b and c) show contaminant concentrations around or slightly higher than the benchmark level. Considering these results in parallel with those from the laboratory trial that indicated high mitigation effects in stored potatoes, it is hypothesised that the studied additives are more effective in reducing acrylamide in crisps with a higher starting reducing sugars concentration in the tubers, in this case due to their accumulation during storage.

We can also comment that the mitigation of acrylamide achieved in the pilot plant trials is less than anticipated from results obtained within laboratory trials. It is important to observe that higher concentrations of additives, which might be effective in reducing acrylamide also in crisps from fresh potato tubers and/or at pilot plant scale, resulted not feasible due to their impact on the taste of the end consumer product.

3.3. Ultrasound trial

Fig. 3 shows the acrylamide content of crisps from the first UST trial, conducted on tubers from Taurus cultivar. This pilot study was conducted to investigate the effect of the short-time application of UST in the washes on acrylamide formation during frying of crisps, and to establish the minimum power required to achieve a possible mitigation effect. Ultrasonic powers of 450W and 1500W were trialed at amplitudes of 50% and 100% for 2 min, corresponding to the duration of the cold wash. In addition to a control with no UST, a positive control where maximum power and amplitude were applied for 15 min was also tested, to confirm a causal relationship between efficacy and duration of treatment in the event of no mitigation effect observed within 2 min of treatment.

The highest acrylamide levels were found in the control group with an average of 1507.4 ± 541.5 µg/kg, with all the UST conditions tested showing inhibitions of the contaminant formation between 36.5% (for the tested conditions of 450W 50% amplitude and 15 min ultrasound treatment) and 67.1% (for the tested condition of 1500W 100% amplitude). The acrylamide level of crisps from tubers treated with 1500W 100% A (495.5 ± 69.4 µg/kg) was found to be significantly lower than the control group (no UST), 1500W 50% A UST and positive control (15 min UST) (Fig. 3).

A second trial was designed and conducted on Lady Claire tubers, to confirm these results and to investigate further whether UST could effectively reduce the formation of acrylamide: a) when solely applied during a cold wash or when followed by a second cold wash instead of hot wash; b) when applied on tubers with both high and low reducing sugars levels. Since no direct relation between increasing UST power and amplitude and higher reduction in acrylamide content was established, and considering factory costs constrains, 450W at 100% A was chosen as UST for the second trial.

Levels of acrylamide precursors (glucose, fructose, and asparagine) were measured in the raw material before and after soaking with UST and are reported in Table 2.

A one-way ANOVA was conducted to compare the starting levels of main precursors in pre-wash samples which would undergo different treatments. Potatoes from KP showed similar levels of precursors, with no difference observed in the content of glucose, fructose, and asparagine. In the tubers from JHI comparable levels of glucose and fructose were found; however, the starting content of asparagine showed some differences with significantly lower asparagine concentration in the UST + hot wash samples compared to those in controls and UST + cold wash. As expected, levels of both glucose and fructose were found to be significantly higher in JHI potatoes, which were stored in cold storage at 5 °C, than in the tubers from KP (stored between 8 and 9 °C). On the other hand, asparagine content in KP pre-wash samples was higher than in the JHI ones.

A two-sample *t*-test was used to study the reduction in precursors content between pre and post washes in samples within the same treatment. No significant reduction in asparagine and fructose was found for KP tubers following any of the applied UST or in the controls. Glucose content was significantly reduced after UST + hot wash, while it was unchanged for the other conditions. For the JHI potatoes, UST + hot wash was effective in reducing all main precursors. Asparagine was also found to be significantly lower in the post-wash control group.

Fig. 4 shows the percentage decrease of reducing sugars (glucose + fructose) between pre- and post-wash of tubers (Fig. 4a) and the acrylamide content of potato crisps (Fig. 4b) from the second UST trial. As

Table 2

Precursors content of raw potato tubers from the 2nd ultrasound treatment (UST) trial. Variety: Lady Claire. Concentrations are expressed in $\mu\text{g}/\text{mg}$ cycloleucine equivalent. Different uppercase letters in the same column within the same precursor denote significant difference according to one-way ANOVA with Tukey post hoc test ($p < 0.05$). Different lowercase letters in the same row within the same potato lot (KP or JHI) indicate significant differences ($p < 0.05$) between pre and post wash according to independent sample t -test. Results are expressed as mean \pm SD for $n = 3$.

	Treatment	KP		JHI	
		Pre-wash	Post-wash	Pre-wash	Post-wash
Asparagine	UST	10.77 \pm 3.48Aa	9.32 \pm 5.02Aa	6.95 \pm 1.80Aba	5.58 \pm 0.78Aa
	UST – Cold wash	12.80 \pm 2.98Aa	7.49 \pm 1.74Aa	6.43 \pm 0.94Aa	5.72 \pm 1.39Aa
	UST – Hot wash	7.69 \pm 3.69Aa	4.82 \pm 3.18Aa	4.47 \pm 0.41Ba	2.54 \pm 0.70Bb
	Control	8.10 \pm 1.35Aa	5.76 \pm 0.98Aa	7.39 \pm 0.56Aa	4.34 \pm 0.38Ab
Glucose	UST	7.22 \pm 0.49Aa	6.59 \pm 1.28Aba	53.55 \pm 10.05Aba	51.61 \pm 11.10ABCa
	UST – Cold wash	7.22 \pm 1.35Aa	6.26 \pm 0.21Aa	53.94 \pm 1.30Ba	51.87 \pm 1.85Aa
	UST – Hot wash	6.75 \pm 0.50Aa	5.28 \pm 0.44Bb	52.68 \pm 2.90Aba	44.81 \pm 1.26Bb
	Control	7.10 \pm 0.41Aa	5.67 \pm 0.81Aba	42.58 \pm 6.96Aa	33.00 \pm 5.80Ca
Fructose	UST	0.51 \pm 0.03Aa	0.45 \pm 0.12Aa	19.83 \pm 3.54Aba	21.55 \pm 7.17ABCa
	UST – Cold wash	0.51 \pm 0.09Aa	0.50 \pm 0.18Aa	19.59 \pm 0.55Ba	19.39 \pm 1.69Aa
	UST – Hot wash	0.57 \pm 0.17Aa	0.59 \pm 0.23Aa	20.36 \pm 2.23Aba	15.71 \pm 1.31Bb
	Control	0.57 \pm 0.10Aa	0.61 \pm 0.08Aa	15.47 \pm 2.48Aa	10.75 \pm 1.92Ca

expected, tubers stored at 5 °C (JHI potatoes) and therefore containing high levels of reducing sugars, resulted in crisps with overall significantly higher acrylamide content ($3449.4 \pm 1262.5 \mu\text{g}/\text{kg}$) compared to KP tubers stored at 8–10 °C ($148.2 \pm 23.0 \mu\text{g}/\text{kg}$).

The lowest acrylamide content for both JHI and KP potatoes was found in the controls (1930.8 ± 247.9 and $139.4 \pm 9.7 \mu\text{g}/\text{kg}$ respectively), where a cold wash followed by hot wash were applied, and in UST followed by hot wash ($2896.0 \pm 626.8 \mu\text{g}/\text{kg}$ for JHI; $141.8 \pm 45.7 \mu\text{g}/\text{kg}$ for KP). Both controls and UST + hot wash samples of JHI tubers showed significantly lower acrylamide content compared to UST + cold wash, with controls also having significantly lower contaminant levels

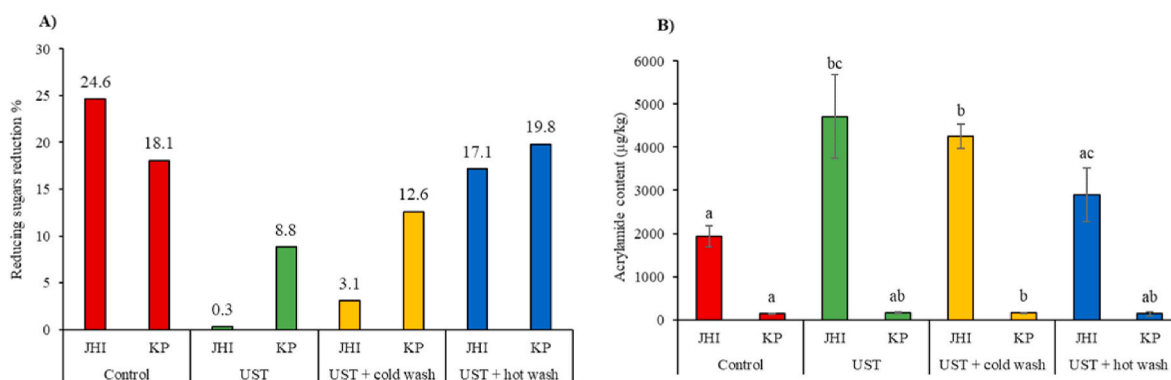


Fig. 4. UST 2nd trial. Variety: Lady Claire. Effects of ultrasound treatment (UST) application on high sugar level (JHI) and low sugar level (KP) potatoes. A) Reduction (%) in total reducing sugar content (glucose and fructose) between pre and post washes. B) Acrylamide levels of potato crisps. Concentrations are expressed in $\mu\text{g}/\text{kg}$. Letters denote grouping from one-way ANOVA with Tukey post hoc test ($p < 0.05$) among samples from the same storage condition (KP or JHI). Results are expressed as mean \pm SD for $n = 3$.

than UST samples (Fig. 4b). Similarly, in the KP samples controls significantly less acrylamide was found compared to UST + cold wash (Fig. 4b). However, all the measured acrylamide values in the KP crisps are below the limit of quantification of $250 \mu\text{g}/\text{kg}$, which makes the significance of the observed differences limited.

Regarding the decrease percentage of reducing sugars (Fig. 4b), the same trend can be observed, with the greatest reductions being in the controls (24.6 % and 18.1 % reduction) and in UST + hot wash (17.1 % and 19.8 % reduction) for both JHI and KP potatoes respectively.

These findings, in parallel with those from the analysis of the corresponding precursors reductions in the raw material suggest that, in the studied conditions, the hot wash appears to be the main contributor in reducing precursors levels, consequently leading to a lower acrylamide content in the crisps, while the only use of UST or the combination UST – cold had little to no effect.

3.4. Colour analysis

Colour analysis was carried out on crisps samples generated from the 2nd UST trial to study the influence of UST on colour development during frying.

The colour of potato crisps is a result of the MR and represent an important quality parameter which is highly monitored during manufacturing.

Previous studies have shown that colour can be correlated with acrylamide content of fried potato products; the strongest correlation is observed with the a^* colour parameter which indicates redness and is positively correlated to acrylamide content, particularly when the contaminant levels are high (Bruno et al., 2023; Gökmen & Şenyuva, 2006). Additionally, a study of Mesias et al. (2021) highlighted that a^* emerged as the optimal predictor for segregating acrylamide levels either exceeding or falling below the benchmark levels stipulated by the EU regulation for fried potatoes ($500 \mu\text{g}/\text{kg}$) during the domestic preparation of French fries from fresh potatoes, irrespective of the employed frying practices. The L^* parameter, which corresponds to lightness, is also often correlated with acrylamide, with crisps with high acrylamide content being darker. The yellowness (b^* , blue to yellow) of fried potato products is the parameter that shows less correlation with the contaminant levels, crisps with lower acrylamide occasionally have higher b^* values (Pedreschi et al., 2005, 2006).

The values of colour parameters L^* , a^* , b^* and ΔE are reported in Table 3. As expected, JHI crisps which have considerably higher acrylamide content, are darker (lower L^* values) and show consistently higher a^* and overall lower b^* values compared to KP crisps.

The ΔE value indicates the overall colour difference between crisps from treated groups and controls, used as standard value. Low ΔE values

Table 3

Colour measurements on potato crisps treated with UST and controls. Lady Claire. Results are expressed as mean \pm SD for $n = 3$. Different letters in the same column indicate significant difference ($p < 0.05$) between treatments.

Treatment	KP			ΔE	JHI			ΔE
	L*	a*	b*		L*	a*	b*	
UST	49.44 \pm 5.19a	2.74 \pm 1.49 ab	25.20 \pm 0.61a	9.41	22.33 \pm 3.05a	14.28 \pm 1.74a	14.29 \pm 5.41a	14.38
UST – Cold wash	46.07 \pm 1.33a	2.76 \pm 0.72 ab	22.86 \pm 0.87b	5.66	24.24 \pm 1.35a	14.13 \pm 1.05a	18.96 \pm 1.94a	9.65
UST – Hot wash	47.94 \pm 3.68a	1.93 \pm 0.25a	23.17 \pm 1.01b	7.59	28.81 \pm 0.92b	11.54 \pm 1.37 ab	24.54 \pm 0.35b	2.02
Control	40.42 \pm 0.35b	2.73 \pm 0.21b	22.50 \pm 0.34b	/	30.30 \pm 3.11b	11.18 \pm 0.58b	25.86 \pm 2.25b	/

were observed for both KP and JHI samples, which confirms that UST does not influence the overall colour of potato crisps.

It is worth noting that KP control crisps are darker than treated crisps and show higher redness values than UST + hot wash samples. However, no correlation between the colour parameters and the acrylamide content could be found, which is in line with previous findings, where fried potato products with low acrylamide content rarely show a correlation between colour and contaminant levels (Bethke & Bussan, 2013).

A significant negative correlation has been observed in JHI crisps between acrylamide content and L* ($r = -0.765$, $p < 0.01$), and acrylamide content and b* ($r = -0.691$, $p < 0.05$), while a significant positive one was noted with a* ($r = 0.810$, $p < 0.01$). However, the coefficients of determination found were $R^2 = 0.585$ for L*, $R^2 = 0.655$ for a* and $R^2 = 0.478$ for b*, indicating that colour parameters would not be suitable for predicting acrylamide content even if a correlation is present.

4. Conclusions

Various approaches to mitigate acrylamide formation during frying of potato crisps were reported in literature. While some of these strategies were effective on a laboratory scale, they could have a limited application on a large scale due to high costs or time-consuming processes.

It is also important to point out that to justify implementing a new mitigation measure within a factory line, it is often required to replicate trials at pilot plant scale enough times to ensure the reliability of the results obtained and to validate the transferability of laboratory-scale outcomes.

This study considered the scaling up of two mitigation strategies, the use of additives and ultrasound treatment, that in previous studies, have proven effective in reducing acrylamide formation in fried potato products and could potentially be easily implemented within a crisps production line.

From the outcome of the additives studies, we observed that addition of CaCl₂ and citric acid in the wash waters before frying is more effective in mitigating acrylamide formation in stored potatoes compared to fresh ones.

UST demonstrated to be effective in reducing acrylamide formation, even for short-time treatments (2 min), when applied in the cold wash followed by hot wash. However, the efficacy was not confirmed when only UST treatment was applied or when it was followed by a second cold wash.

The precision and accuracy which characterize laboratory trials are difficult to control during scaling up and this might affect the reproducibility and repeatability of the observed results. This is valid for both the pilot plant additives trials and the processing plant ultrasound trials, where reductions in acrylamide formation observed in a first study were not always confirmed in the following ones.

CRediT authorship contribution statement

Francesca Bruno: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maira Ledbetter:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Ben Davies:** Writing – review & editing, Resources,

Methodology, Formal analysis, Conceptualization. **Lena Riedinger:** Investigation, Formal analysis. **Slim Bliidi:** Writing – review & editing, Methodology, Formal analysis. **Keith Sturrock:** Writing – review & editing, Supervision, Resources, Project administration. **Ged McNamara:** Supervision, Resources, Funding acquisition. **Gary Montague:** Writing – review & editing, Project administration, Funding acquisition. **Alberto Fiore:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data supporting this study are provided in full in the 'Results' section of this paper

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Appendix A. Supplementary data

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