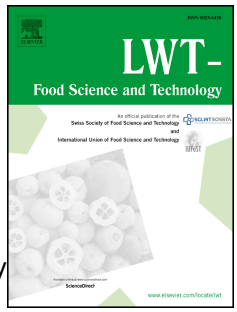

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- **Performed the experiments**: Moira Ledbetter, Francesca Bruno.
- **Analyzed and interpreted the data**: Moira Ledbetter, Francesca Bruno, Keith Sturrock.
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Effect of post-harvest anti-sprouting treatment with spearmint essential oil on acrylamide formation in potato crisps

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Abstract

The control of sprouts is essential to ensure quality of stored potatoes destined for the processing market. This paper investigates the effects of post-harvest treatment of tubers with spearmint essential oil (MEO) as sprouts suppressant, on both precursors of and acrylamide formation in potato crisps. Two trials were designed using two varieties of potato cultivars, to investigate the effect of a single MEO application over time and of one and two MEO applications compared to controls. A lower amount of reducing sugars was found in treated potatoes from both varieties after one day from treatment (Lady Claire: -26.8 %; Taurus: -59.5 %), which for Taurus tubers corresponded to lower acrylamide content in the crisps (-72.8 %). Lower acrylamide levels were quantified in Lady Claire treated twice with MEO (-70.2 %) and in controls (-59.6 %) compared with potatoes treated once. Both trials demonstrate that treatment with MEO has no overall negative impact on acrylamide formation. Furthermore, no substantial change can be observed in the amino acidic and sugar profile of the tubers, which cannot be attributed to variability among tubers. These findings support the effective and safe use of MEO to control sprouting of potatoes destined to the processing market.

Keywords

Acrylamide, crisps, potato tubers, sprouting, storage

List of Abbreviations

ANOVA	Analysis of Variance
CIE	Commission on Illumination
CIPC	Chlorpropham (isopropyl *N*-(3-chlorophenyl) carbamate
ESI	Electrospray Ionisation
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer

44	JHI	James Hutton Institute
45	LC-MS	Liquid Chromatography-Mass Spectrometry
46	MEO	Spearmint Essential Oil
47	MRM	Multiple Reaction Monitoring
48	MSD	Mass Spectrometric Detection
49	MSTFA	N-Methyl-N-(trimethylsilyl) trifluoroacetamide
50	PSA	Primary Secondary Amine
51	SIM	Selected Ion Monitoring
52	SNFA	Swedish National Food Administration
53	TMS	Trimethylsilyl

1. Introduction

Acrylamide has been classified as a probable human carcinogen from the International Agency for Research on Cancer (IARC) based on its carcinogenicity in rodents (IARC, 1994). In April 2002 acrylamide presence in food was first reported by the Swedish National Food Administration (SNFA) and the University of Stockholm after finding significant levels of the contaminant in heat treated starch-rich foods (Tareke et al., 2000, 2002). Acrylamide is formed during the Maillard Reaction when foods rich in amino acids and reducing sugars are cooked at high temperatures, typically during baking frying or roasting (Mottram et al., 2002; Stadler et al., 2002; Zyzak et al., 2003). Potato tubers contain high amounts of acrylamide precursors asparagine and reducing sugars (glucose and fructose), therefore processed potato products (such as potato crisps and French Fries) are one of the main contributors to dietary acrylamide intake, next to coffee (Capuano & Fogliano, 2011; WHO, 2011)

To allow potato availability throughout the year, long-term storage (up to 9 months) is essential and sprout control during storage enables potato quality to be maintained minimizing potato loss and damage (Giri et al., 2020; Pinhero et al., 2009). Sprouting is associated with weight loss, softening, and reduced airflow which increases disease problems (Sonnewald & Sonnewald, 2014). Low temperature storage delays sprout development, but it also causes cold-induced sweetening, due to storage temperatures below 8-10 °C promoting the conversion of starch into sugars. Reducing sugars accumulation is highly undesirable for potatoes destined for the processing market because it enhances acrylamide formation (Biedermann-Brem et al., 2003; Burton, 1989; Coffin et al., 1987; Gökmen et al., 2007; Rosen et al., 2018; Teper-Bamnolker et al., 2010).

Chlorpropham (isopropyl *N*-(3-chlorophenyl) carbamate; CIPC) has been the primary sprout suppressant used on potatoes for more than 40 years (Paul et al., 2016; Smith & Bucher, 2012). However due to its negative impact on health and the environment, in June 2019 the European

Union legislated for the non-renewal of approval of CIPC adopting Commission Implementing Regulation (EU) 2019 and establishing the 2019-2020 season as last storage season for which Chlorpropham use was permitted.

Natural essential oils derived from plants (herbs and spices) and their active monoterpenes have been investigated for years as sprout control alternatives to CIPC and they have demonstrated to be effective in several studies (Gómez-Castillo et al., 2013; Şanlı & Karadoğan, 2019; Song et al., 2008; Teper-Bamnolker et al., 2010; Vokou et al., 1993). Spearmint essential oil (MEO) from *Mentha spicata* L. has been registered in the UK as BIOX-M since 2012 as an alternative sprout inhibitor to CIPC; the active component is *R*-(-)-carvone, one of the two enantiomers of carvone. S-(+)-carvone, extracted from caraway and dill seed oil is also commercially marketed as sprout suppressant (Gómez-Castillo et al., 2013; Şanlı & Karadoğan, 2019). MEO inhibits sprouting by causing local necrosis in the bud meristems, without damaging the tuber skin; Teper-Bamnolker et al. (2010) hypothesized that *R*-(-)-carvone affects the cell membrane, being a lipophilic molecule (Morcia et al., 2016; Teper-Bamnolker et al., 2010). A study by Rentzsch et al. (2012) shows how monoterpenes from peppermint oil at high concentrations inhibit dormancy release and initiation of sprouting by interacting with the production of plant signalling hormones gibberellins, through the mevalonate pathway (Oosterhaven et al., 1993, 1995; Rentzsch et al., 2012; Song et al., 2008).

Considering the current extensive use of MEO and its active components as main anti sprouting alternative to CIPC, we intended to confirm MEO is safe to use on potatoes destined to the processing market. In this study we investigate for the first time the effects of MEO anti-sprouting treatment on potato tubers metabolomic profile, reducing sugars content, and subsequent acrylamide formation in potato crisps.

2. Materials and methods

2.1. Chemicals and reagents

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 ($MgSO_4$, 97%) was 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), and 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

Lady Claire and Taurus variety potatoes were grown at James Hutton Institute (JHI) in Dundee, UK. Palm oil (RSPO Palm RD Oil) was purchased from Kerfoot Oil Specialists (Northallerton, UK). Spearmint essential oil (*Mentha spicata* L.) was purchased from Essential Oil Direct (Royton, Oldham, Gtr Manchester, UK).

2.3. Anti-sprouting treatment

Lady Claire and Taurus have been selected as potato varieties for this study, being widely used in crisps manufacturing.

For anti-sprouting treatment with MEO, potato tubers were placed in nylon fire bags (Crime Scene Investigation Equipment, Milton Keynes, UK) of 24 potatoes and MEO was applied by placing a cottonwool ball with 4 mL of essential oil (1 mL/6 tubers, without MEO in the case of control tubers), for 24h treatment at 8-10 °C; the bags were then opened, and the tubers

stored in the dark at 8-10 °C, 73 ± 2 % humidity. Precautions were taken to avoid contact between tubers and the cottonwool ball with essential oil.

Two studies have been carried out. In the first trial (Trial 1) the acrylamide levels of potato crisps generated on the day of the treatment (T=0 control), the day the bags were opened (T=1), and after 7, 14, 28 and 56 days from MEO treatment (M) have been determined and compared to controls (C1). The sample size is equivalent to six tubers for each time point, for both controls and MEO treated potatoes. The second trial (Trial 2) compared the acrylamide levels of potato crisps generated after application of one MEO treatment (M1) or two MEO treatments (M2) and controls (C2). The second treatment was applied 30 days after the initial treatment, as per general practice in the industry sector; potato tubers from all three conditions were fried 56 days after the second treatment. The sample size is equivalent to six tubers for C2, M1 and M2. For both studies, potato sprouting, and weight loss were measured weekly throughout the experiment; prior to frying 20 g of raw potato (pre-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.

2.4. 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. Potato slices (20 g) were soaked in 2 L of distilled water for 2 mins at room temperature, then immersed in 2 L distilled water at 78 °C for 3 mins. Samples were then immediately withdrawn and excess water drained.

Samples were fried in palm oil at 173 ± 2 ℃ in a 3 L Selection Magimix professional deep fat fryer (Godalming, UK). Commercial processing conditions were adopted from (Bartlett *et al.*, 2020) with some modification, frying time was 4.5 mins. The oil temperature was monitored

149 by an external probe (E.T.I food check thermometer, Sussex, UK). Potato crisps were
150 pulverised and stored at -18℃ until analysis.

151 **2.5. Acrylamide quantification**

152 Acrylamide was quantified by liquid chromatography tandem-mass spectrometry (LC-
153 MS/MS) using a three-phase extraction method as described by Ledbetter et al. (2021, 2020).
154 Briefly, approximately 1.000 g of fried crisps (ground) was accurately weighed then combined
155 with [2,3,3-d_3]-acrylamide (10 µL, 0.2 mg/mL, Internal standard), 10 mL water, 10 mL
156 acetonitrile and 5 mL hexane, 4 g $MgSO_4$ and 0.5 g NaCl. The mixture was then shaken
157 vigorously for 1 min, then centrifuged (2683 rcf for 10 mins; Hermle GmbH Z 323 K,
158 LaborTechnik, Düsseldorf, Germany). An aliquot (1 mL) of the acetonitrile layer (middle
159 layer) transferred to a 2 mL Eppendorf tube containing premixed PSA (50 mg) and $MgSO_4$
160 (175 mg), this was vortexed and centrifuged (9300 rcf for 1 min; Microcentrifuge 5415R,
161 Eppendorf, Hamburg, Germany). Supernatant was transferred to a vial for analysis by LC-
162 MS/MS.
163 Acrylamide quantification was performed on a Thermos Fisher Scientific LC-MS/MS (San
164 Jose, CA, USA) consisting of a degasser, a quaternary pump, a thermostatic autosampler, a
165 column oven and a TSQ Mass spectrometer. Chromatographic separation was achieved with
166 ultra-pure water containing 0.1% formic acid (mobile phase A) and methanol containing 0.1 %
167 formic acid (mobile phase B). The gradient was 98% A at 200µl/min for 3.5 mins, the flow
168 rate increased to 300 µL/min and 75% B over 2 mins and held for 2 mins before re-equilibration
169 to initial conditions for 16.7 mins. Each sample (10 µL) was injected on a Synergi Hydro RP
170 column (250 mm x 4.6 mm x 4 µm, 80 Å pore size) (Phenomenex, Macclesfield, UK).
171 The mass spectrometer was equipped with an electrospray ionisation (ESI) source and was
172 operated in positive ionization 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 (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 mins. 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 method detection limit of 26.7 ppb (equivalent to 267 µg/kg).

2.6. Metabolomic analysis of raw material

The metabolomic profile of the raw tubers was determined using the method of de Falco et al. (2018) with minor modifications; acrylamide precursors (asparagine and reducing sugars) and other amino acids 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 mins at 1000 rpm (Thermomixer Comfort, Eppendorf, Hamburg, Germany) then centrifuged for 10 mins 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 ℃ 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 SI50, Cole-Parmer, St. Neots,UK) at 45 °C for 45 mins. 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 x 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 mins, 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 mins.

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.

2.7. Colour analysis

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):

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

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

222 ### 2.8. Statistical analysis

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

230 ## 3. Results and discussion

231 ### 3.1. Sprouting and weigh loss

232 No growth in sprouts length has been observed until week 5 (day 35) in Trial 1, in controls and
233 treated tubers for both varieties. In Lady Claire tubers from Trial 2 first sprouts > 1 mm
234 appeared in week 4 (day 28) for controls and M1, in week 5 (day 35) for M2. In Taurus tubers
235 from Trial 2 sprouts started to grow in length during week 5 (day 35) in controls, week 4 (day
236 28) in M1 and week 6 (day 42) in M2. Potato tuber eyes were counted when sprouts length was
237 < 1 mm. The maximum sprout length measured in Trial 1 (at T=56) was 5 mm for controls and
238 3 mm for M in Lady Claire tubers, 3 mm for controls and 4 mm for M in Taurus variety. The
239 maximum sprout length measured in Trial 2 was 7.5 mm for controls, M1 and M2 in Lady
240 Claire potatoes; 6 mm, 9 mm and 6.5 mm for controls, M1 and M2 respectively in Taurus
241 tubers.

Figures 1 and 2 show the weekly increase in number of eyes/sprouts for Trial 1 and Trial 2, respectively. No significant difference was observed in sprouts number between different groups for Lady Claire potatoes for both Trial 1 and Trial 2. In tubers of the Taurus variety from Trial 1, a higher number of sprouts was found in the treated group compared to controls for week 1, 2 and 3; no difference was observed in the following weeks. This shows how in the treated group, starting with a higher average sprouts number from day 1, MEO has an effect in delaying sprouting over 6 weeks compared to the control group, where sprouting is faster. Taurus control tubers from Trial 2 showed higher number of sprouts compared to M1 potatoes in weeks 3, 5, 6, 7 and compared to both M1 and M2 treated tubers in week 8. Tubers of both cultivars used for the study have been harvested in October and the trials started in January of the following year; overall, no considerable sprouting had occurred in either Trial 1 or Trial 2 with a maximum sprout length measured as 9 mm. This is most likely why the anti-sprouting effect of MEO treatment in this study is less evident compared to previous studies, which were designed specifically to determine its activity as a sprout suppressant only.

No significant weight loss was measured in potato tubers from both varieties during storage in both experiments; additionally, no difference was observed in weight loss between varieties, conditions, and experiments (Trial 1 – Figure 3; Trial 2 – Figure 4). Figure 3 shows how in Trial 1 after an initial decrease during the first two weeks, the weight loss of the Lady Claire and Taurus tubers stabilizes to reach a constant weight with no further losses in the following weeks. The same trend can be observed in Trial 2 for both Lady Claire and Taurus (Figure 4); however, here we can see a subsequent loss in weight during the last week, which follows weeks of stability where no changes were observed. This is shown for all three conditions in both varieties and can be explained by the longer duration of this experiment (12 weeks) compared to Trial 1, where the last tubers were fried at the end of week 9.

3.2. Effect on acrylamide formation in potato crisps

Table 1 summarises the measured acrylamide values in potato crisps from Trial 1. Acrylamide concentrations ranged between 243.92 ± 56.93 and 438.89 ± 87.39 µg/kg for control crisps, and between 245.23 ± 119.89 and 410.98 ± 56.21 µg/kg in M for Lady Claire crisps with no significant difference in acrylamide content observed between controls and M for all the time points. In Taurus crisps acrylamide values varied between 668.98 ± 397.03 and 1999.29 ± 2029.44 µg/kg for controls, and between 337.00 ± 155.65 and 2209.58 ± 1355.49 µg/kg for M; a lower amount of acrylamide was found in potato crisps treated with MEO compared to controls for T=1 (-72.8%), no difference in acrylamide content was observed between M and controls for all the other time points for Taurus crisps.

Table 2 shows acrylamide content of potato crisps from Trial 2. A lower amount of acrylamide was measured for M2 (156.53 ± 61.10 µg/kg), and controls (214.41 ± 50.87 µg/kg) crisps compared to M1 (530.70 ± 312.52 µg/kg) for Lady Claire crisps. No difference in acrylamide levels was observed between treatments for the Taurus variety, with 752.38 ± 596.01 µg/kg acrylamide content in controls, 567.58 ± 428.06 µg/kg in M1 and 547.63 ± 406.30 µg/kg in M2.

The overall calculated average of acrylamide content in Lady Claire crisps was 318.63 ± 133.67 µg/kg in Trial 1 and 305.61 ± 249.53 µg/kg in Trial 2, in all the samples the measured acrylamide was below the set benchmark level of 750 µg/kg (Commission Regulation, 2017); potato crisps from the Taurus variety showed significantly higher acrylamide levels compared to Lady Claire in both trials, with a total calculated average of 1244.45 ± 1139.31 µg/kg in Trial 1 and 622.53 ± 458.38 µg/kg in Trial 2. These findings are in line with data from KP snacks potato crisps factory line (Bartlett et al., 2020) as well as with data from other studies conducted within our research laboratories (data not published); tubers from the Taurus cultivar contain higher reducing sugars levels, which generate higher acrylamide levels in the crisps as

observed here, as well as a higher variability reflected in the standard deviation of the data, when compared to the Lady Claire cultivar. Moreover, Lady Claire crisps generally show higher oil absorption during frying (higher fat content, factory data not showed); considering that acrylamide is formed in the solid portion of the potato, this could also be a factor explaining why crisps from this cultivar often show lower levels of the contaminant.

3.3. Effect on acrylamide precursors and metabolomic profile of raw material

Tables 3 (3a, 3b) and 4 show the amino acid and sugar content of potato tubers from Trial 1 and Trial 2 respectively, from GC-MS metabolomic analysis of raw material samples collected prior to frying. Following results from the quantification of acrylamide in the crisps, analysis of raw material from Trial 1 has been conducted on three selected timepoints, this decision has been made considering the overall similar acrylamide content between treated and control groups. Timepoints have been chosen as first (T=1), middle (T=14) and last (T=56) in order to have a complete picture of eventual changes in sugars and amino acids levels over the duration of the study. In the first trial tubers for both varieties from the treated group show a lower amount of reducing sugars compared to controls for T=1, in Lady Claire variety sucrose content was lower in MEO treated tubers from T=1. No significant difference was observed in asparagine content for both varieties at any timepoints. In the case of the Taurus variety, the higher reducing sugars content in T=1 controls also correspond to a higher amount of acrylamide in the crisps as indicated above (Table 1).

Correlations between reducing sugars and acrylamide content were investigated for the two varieties together and separately. A significant correlation was found overall ($r = 0.815$, $p < 0.001$) with a coefficient of determination $R^2 = 0.640$ and considering the individual cultivars in Taurus tubers ($r = 0.775$, $p < 0.001$); no correlation was found when considering data from Lady Claire tubers alone.

315 Other differences in sugars and amino acids content can be observed from Table 3. In the Lady
316 Claire variety, for T=1 control tubers have higher lysine content compared to treated tubers;
317 for T=14, no glycine is detected in treated potatoes (glycine content in controls is 0.37 ± 0.08
318 mg/g cycloleucine equivalent); for T=56 MEO treated tubers have a higher aspartic acid
319 content compared to controls. In the Taurus variety treated potatoes have a higher amount of
320 sucrose compared to controls for T=56; no alanine has been detected in control tubers from
321 T=14 and T=56 (alanine content in MEO tubers is 0.41 ± 0.21 and 0.25 ± 0.01 mg/g
322 cycloleucine equivalent respectively); a higher content in tyrosine has been detected in treated
323 tubers from T=56 compared to controls.
324 In the second trial Lady Claire tubers with one MEO treatment show higher reducing sugars
325 content compared to controls (Table 4), which corresponds to a higher acrylamide formation
326 in the crisps produced (Table 2). Potatoes from M1 of the Lady Claire variety also show a
327 higher asparagine, serine and threonine content compared to tubers treated twice with MEO
328 (Table 4), this again corresponds to an increased acrylamide formation in the crisps (Table 2).
329 In Taurus potatoes that received two MEO treatments a higher amount of glucose, sucrose,
330 asparagine, valine, serine, threonine, and aspartic acid was observed compared to controls and
331 tubers treated once (Table 4). However, no corresponding variation in acrylamide levels had
332 been observed in the resulting crisps.
333 A significant correlation between reducing sugars and acrylamide content was found in the
334 Trial 2 as well ($r = 0.428$, $p < 0.05$); however, the coefficient of determination was $R^2 = 0.289$
335 and no correlation has been observed in the two individual cultivars separately. There was also
336 a strong significant correlation when considering all the data together from both experiments
337 and tuber varieties, between acrylamide and reducing sugars ($r = 0.753$, $p < 0.001$; with $R^2 =$
338 0.459), acrylamide and glucose alone ($r = 0.677$, $p < 0.001$), acrylamide and fructose alone (r
339 $= 0.801$, $p < 0.001$), and between glucose and fructose ($r = 0.914$, $p < 0.001$). A correlation

between acrylamide content and asparagine in the raw material was only found when considering data from Trial 1 among Lady Claire tubers ($r = 0.446$. $p < 0.01$); this result is in line with previous findings, where asparagine levels measured in tubers did not always correlate with acrylamide concentration in potato products (Amrein et al., 2003; Halford et al., 2012; Vinci et al., 2012).

3.4. Colour analysis

The colour of potato crisp as a result of the Maillard reaction represents an important parameter to control during manufacturing. The chromatic component a* of fried potato products, which indicates redness, has been found to change considerably during frying and correlates to acrylamide levels (Gökmen & Şenyuva, 2006). The values of colour parameters (L*, a*, b* and ΔE) obtained from the analysis of fried potato crisps from Trial 1 and Trial 2 are reported in Table 5. As for the analysis of the raw material, measurement of colour development on crisps from the first trial has been conducted on selected timepoints (T=1, T=14 and T=56). No difference in L*, a*, b* values observed between the control and treated group in Trial 1 for both varieties at all time points analysed. In Trial 2 potato crisps from the cultivar Taurus treated twice with MEO showed a higher b* value (blue to yellow) compared to controls. The ΔE value has been calculated to compare the overall colour difference of the treated groups from the control, used as standard value. Generally, low ΔE values can be observed, confirming that application of MEO on potato tubers during storage has no influence on the colour of fried potato crisps. A strong linear correlation was found between the chromatic component a* (redness) and acrylamide levels ($r = 0.869$, $p < 0.001$; $R^2 = 0.892$) for data from both cultivars and trials together. No correlation was found for lightness with acrylamide levels. The chromatic component b* not always correlated with acrylamide levels, no correlation was shown when considering all data together. These findings are in line with previous studies on

364 colour development during frying and correlation with acrylamide formation during the
365 Maillard reaction (Pedreschi et al., 2005, 2006).

366 **4. Conclusions**

367 Since the most abundantly used sprout suppressant CIPC was banned from the European Union
368 in 2019, due to its negative effects on human health and environment, natural alternatives such
369 as MEO and its active compound have been widely implemented across Europe and the UK to
370 control sprouts development (Pedreschi et al., 2006) and ensure quality of stored potatoes. The
371 outcomes of this study show that MEO has no negative effect on raw potatoes amino acid and
372 reducing sugar profile when applied as sprout suppressant during storage. Moreover, it has no
373 overall influence on colour development and formation of acrylamide during frying of potato
374 crisps. For these reasons MEO and its active compounds can be considered safe and suitable
375 for use on potato tubers, including when destined to the processing market.

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379 through the MCAP2 project.

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Table 1. Trial 1. Acrylamide levels in potato crisps. Concentrations are expressed in µg/kg. Different letters in the same row indicate significant differences ($p < 0.05$) between tubers treated with mint essential oil (M) and controls (C1). Results are expressed as mean ± SD for $n = 6$. † Indicates outliers have been excluded following Grubbs' test.

Variety	Lady Claire		Taurus	
Days after treatment	**C1**	**M**	**C1**	**M**
0	304.25 ± 68.73	-	1691.98 ± 1108.36	-
1	438.89 ± 87.39a	410.98 ± 56.21a	1954.42 ±1245.91b	532.55 ± 272.08c
7	282.38 ± 82.07a	245.23 ± 119.89a	1999.29 ± 2029.44b	2209.58 ± 1355.49b
14	347.00 ± 231.08a	381.04 ± 200.87a †	1506.21 ± 1062.27b	960.57 ± 724.71b
28	243.92 ± 56.93a	323.46 ± 165.66a	694.40 ± 308.86b	982.75 ± 882.16b
56	251.84 ± 95.20a	279.88 ± 104.69a †	668.98 ± 397.03b	337.00 ± 155.65b †

Table 2. Trial 2. Acrylamide levels in potato crisps. Concentrations are expressed in µg/kg. Different letters in the same row indicate significant differences ($p < 0.05$) between tubers treated with mint essential oil once (M1), twice (M2) and controls (C2). Results are expressed as mean ± SD for $n = 6$. † Indicates outliers have been excluded following Grubbs' test.

Variety	**C2**	**M1**	**M2**
Lady Claire	214.41 ± 50.87a †	530.70 ± 312.52b	156.53 ± 61.10a
Taurus	752.38 ± 596.01a †	567.58 ± 428.06a †	547.63 ± 406.30a †

Table 3a. Trial 1: Lady Claire. Sugar and amino acidic profile of raw potato tubers treated with mint essential oil (M) and controls (C1). Concentrations are expressed in µg/mg cycloleucine equivalent. Different letters in the same row indicate significant difference ($p < 0.05$) between treated samples and controls from the same timepoint. Results are expressed as mean ± SD for $n = 6$. n.d.: not detected.

Treatment	C1			M		
Days after treatment	1	14	56	1	14	56
Glucose	3.36 ± 0.42a	4.35 ± 2.20a	2.76 ± 0.26a	2.57 ± 0.58b	4.85 ± 2.55a	3.47 ± 0.80a
Fructose	1.20 ± 0.25a	1.04 ± 0.69a	0.74 ± 0.25a	0.77 ± 0.20b	0.80 ± 0.41a	0.66 ± 0.12a
Total reducing sugars	**4.56 ± 0.50a**	**5.39 ± 2.89a**	**3.42 ± 0.34a**	**3.34 ± 0.77b**	**4.88 ± 2.31a**	**4.14 ± 0.83a**
Sucrose	10.75 ± 1.97a	6.72 ± 1.89a	4.02 ± 0.54a	7.69 ± 0.73b	7.81 ± 1.77a	5.02 ± 1.48a
Asparagine	1.50 ± 0.49a	0.93 ± 0.18a	1.13 ± 0.74a	1.01 ± 0.33a	0.94 ± 0.29a	1.15 ± 0.66a
Alanine	0.43 ± 0.08a	0.51 ± 0.20a	n.d.	0.42 ± 0.10a	0.38 ± 0.13a	n.d
Valine	2.21 ± 0.34a	2.71 ± 0.52a	3.12 ± 1.27a	1.97 ± 0.33a	2.74 ± 0.56a	3.25 ± 1.27a
Isoleucine	0.82 ± 0.38a	0.60 ± 0.31a	1.09 ± 0.33a	0.67 ± 0.27a	0.53 ± 0.34a	0.77 ± 0.51a
Glycine	0.36 ± 0.03a	0.37 ± 0.08	n.d.	0.56 ± 0.25a	n.d.	n.d.
Serine	0.71 ± 0.09a	0.58 ± 0.21a	0.44 ± 0.17a	0.65 ± 0.15a	0.49 ± 0.05a	0.58 ± 0.19a
Threonine	0.92 ± 0.17a	1.14 ± 0.41a	1.06 ± 0.33a	0.82 ± 0.19a	1.02 ± 0.12a	1.16 ± 0.33a
Aspartic acid	1.61 ± 1.08a	3.37 ± 2.15a	1.54 ± 1.96a	1.60 ± 1.61a	2.94 ± 0.91a	3.95 ± 1.26b
Glutamic acid	2.07 ± 0.23a	1.50 ± 0.25a	1.66 ± 0.30a	1.79 ± 0.30a	1.46 ± 0.37a	1.81 ± 0.38a
Phenylalanine	n.d.	0.59 ± 0.05a	n.d.	n.d.	0.84 ± 0.24a	n.d.
Glutamine	0.27 ± 0.05a	0.53 ± 0.67a	n.d.	0.27 ± 0.03a	0.13 ± 0.07a	n.d.
Lysine	1.02 ± 0.26a	0.99 ± 0.18a	1.23 ± 0.56a	0.73 ± 0.18b	0.90 ± 0.17a	1.28 ± 0.51a
Tyrosine	1.54 ± 0.28a	1.78 ± 0.91a	1.98 ± 0.50a	1.33 ± 0.20a	1.80 ± 0.28a	1.90 ± 0.52a
Tryptophan	0.75 ± 0.27a	0.86 ± 0.30a	0.98 ± 0.59a	0.55 ± 0.30a	0.63 ± 0.31a	0.79 ± 0.38a
Total amino acids	**13.68 ± 2.16**	**14.28 ± 2.85**	**13.41 ± 6.99**	**11.93 ± 2.82**	**13.29 ± 2.15**	**15.12 ± 5.33**

Table 3b. Trial 1: Taurus. Sugar and amino acidic profile of raw potato tubers treated with mint essential oil (M) and controls (C1). Concentrations are expressed in µg/mg cycloleucine equivalent. Different letters in the same row indicate significant difference ($p < 0.05$) between treated samples and controls from the same timepoint. Results are expressed as mean ± SD for $n = 6$. n.d.: not detected.

Treatment	C1			M		
Days after treatment	1	14	56	1	14	56
Glucose	8.93 ± 4.80a	9.94 ± 5.47a	4.67 ± 1.58a	4.14 ± 1.36b	10.41 ± 9.48a	4.65 ± 2.53a
Fructose	7.96 ± 4.91a	8.90 ± 7.38a	3.15 ± 1.97a	2.70 ± 1.32b	8.09 ± 6.62a	3.10 ± 2.16a
Total reducing sugars	**16.89 ± 9.63a**	**18.25 ± 12.81a**	**7.82 ± 3.49a**	**6.84 ± 2.65b**	**18.50 ± 15.99a**	**7.75 ± 4.62a**
Sucrose	12.99 ± 3.05a	10.64 ± 2.21a	5.61 ± 0.92a	11.49 ± 2.40a	10.39 ± 2.56a	7.70 ± 1.86b
Asparagine	1.47 ± 0.61a	2.21 ± 1.57a	1.92 ± 0.45a	1.84 ± 0.66a	2.11 ± 1.01a	2.02 ± 0.50a
Alanine	0.37 ± 0.22a	n.d.	n.d.	0.47 ± 0.21a	0.41 ± 0.21	0.25 ± 0.01
Valine	2.12 ± 0.37a	2.73 ± 0.85a	2.43 ± 0.35a	2.41 ± 0.72a	2.89 ± 0.81a	3.06 ± 0.62a
Isoleucine	0.99 ± 0.26a	1.29 ± 0.53a	1.23 ± 0.17a	1.25 ± 0.37a	1.47 ± 0.45a	1.49 ± 0.26a
Glycine	0.39 ± 0.08a	0.57 ± 0.46a	0.72 ± 0.28a	0.41 ± 0.07a	0.58 ± 0.16a	0.54 ± 0.25a
Serine	0.46 ± 0.12a	0.49 ± 0.20a	0.53 ± 0.18a	0.56 ± 0.12a	0.49 ± 0.12a	0.59 ± 0.19a
Threonine	1.16 ± 0.26a	1.68 ± 0.61a	1.77 ± 0.33a	1.47 ± 0.39a	1.79 ± 0.57a	1.99 ± 0.38a
Aspartic acid	2.93 ± 1.46a	4.07 ± 0.90a	3.46 ± 0.21a	3.88 ± 1.53a	3.08 ± 1.91a	4.03 ± 0.82a
Glutamic acid	1.73 ± 0.36a	2.16 ± 0.82a	1.88 ± 0.29a	1.86 ± 0.38a	2.67 ± 0.63a	1.64 ± 0.08a
Phenylalanine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Glutamine	0.32 ± 0.19a	0.33 ± 0.20a	0.46 ± 0.29a	0.32 ± 0.20a	0.34 ± 0.09a	0.43 ± 0.14a
Lysine	0.88 ± 0.25a	1.11 ± 0.50a	1.07 ± 0.22a	1.16 ± 0.37a	1.28 ± 0.58a	1.39 ± 0.30a
Tyrosine	1.10 ± 0.18a	1.67 ± 0.70a	1.38 ± 0.33a	1.53 ± 0.45a	1.86 ± 0.78a	1.88 ± 0.39b
Tryptophan	0.74 ± 0.48a	1.18 ± 0.82a	1.08 ± 0.33a	1.21 ± 0.49a	1.57 ± 0.94a	1.47 ± 0.54a
Total amino acids	**13.81 ± 4.30**	**18.02 ± 8.18**	**16.55 ± 1.87**	**17.13 ± 5.60**	**19.97 ± 7.21**	**19.72 ± 3.60**

Table 4. Trial 2. Sugar and amino acidic profile of raw potato tubers treated once (M1) or twice (M2) with mint essential oil, and controls (C2). Concentrations are expressed in µg/mg cycloleucine equivalents. Different letters in the same row indicate significant difference ($p < 0.05$) between treatments among the same variety. Results are expressed as mean ± SD for $n = 6$. n.d.: not detected.

Variety	Lady Claire			Taurus		
Treatment	C2	M1	M2	C2	M1	M2
Glucose	1.90 ± 0.93a	3.73 ± 1.09b	2.47 ± 0.22ab	4.16 ± 3.00a	4.25 ± 1.37a	11.21 ± 7.07b
Fructose	0.80 ± 0.33a	2.03 ± 1.12b	1.14 ± 0.58ab	3.69 ± 2.61a	3.76 ± 1.67a	8.00 ± 5.15a
Total reducing sugars	**2.70 ± 0.89a**	**5.77 ± 2.09b**	**3.39 ± 0.32ab**	**7.86 ± 5.58a**	**8.01 ± 3.02a**	**19.21 ± 11.54a**
Sucrose	3.29 ± 0.71a	2.62 ± 0.96a	3.02 ± 0.57a	4.61 ± 0.96a	5.07 ± 0.87a	13.84 ± 3.95b
Asparagine	1.05 ± 0.39ab	1.63 ± 0.68a	0.84 ± 0.35b	1.10 ± 0.41a	1.73 ± 0.44a	5.89 ± 2.48b
Alanine	0.48 ± 0.18a	0.49 ± 0.22a	0.34 ± 0.12a	n.d.	n.d.	n.d.
Valine	1.93 ± 0.27a	2.38 ± 0.82a	1.75 ± 0.30a	1.99 ± 0.39a	2.07 ± 0.35a	3.77 ± 1.14b
Isoleucine	0.89 ± 0.19a	1.41 ± 0.48a	n.d.	0.95 ± 0.18a	1.15 ± 0.12ab	1.52 ± 0.48b
Proline	0.45 ± 0.16a	0.52 ± 0.07a	0.48 ± 0.08a	0.53 ± 0.22a	0.55 ± 0.12a	0.80 ± 0.20a
Glycine	n.d.	1.07 ± 0.24	n.d.	n.d.	n.d.	n.d.
Serine	0.67 ± 0.15ab	0.82 ± 0.25a	0.53 ± 0.13b	0.43 ± 0.10a	0.54 ± 0.12a	0.84 ± 0.29b
Threonine	1.31 ± 0.15ab	1.72 ± 0.46a	1.19 ± 0.32b	1.33 ± 0.33a	0.71 ± 0.18a	2.84 ± 0.57b
Aspartic acid	2.99 ± 1.28a	3.77 ± 0.95a	2.75 ± 2.01a	3.19 ± 0.96a	3.65 ± 0.36a	6.05 ± 1.90b
Phenylalanine	n.d.	n.d.	n.d.	n.d.	0.66 ± 0.04a	1.14 ± 0.84a
Glutamic acid	2.03 ± 0.12	n.d.	n.d.	n.d.	n.d.	2.92 ± 0.40
Glutamine	0.15 ± 0.02	n.d.	n.d.	0.23 ± 0.15a	0.23 ± 0.11a	n.d.
Lysine	0.94 ± 0.05a	1.38 ± 0.43a	1.00 ± 0.31a	0.84 ± 0.14a	0.93 ± 0.03a	0.74 ± 0.50a
Tyrosine	1.57 ± 0.41a	1.82 ± 0.60a	1.47 ± 0.23a	1.13 ± 0.27a	1.17 ± 0.09a	1.57 ± 0.59a
Tryptophan	0.62 ± 0.38a	2.24 ± 1.45b	0.70 ± 0.40a	0.37 ± 0.14a	0.73 ± 0.32ab	1.28 ± 0.65b
Total amino acids	**13.79 ± 2.94**	**18.21 ± 6.42**	**11.56 ± 4.51**	**11.28 ± 3.10**	**14.90 ± 0.89**	**27.38 ± 7.37**

Table 5. Trial 1. Colour measurement on potato crisps treated with mint essential oil (M) and controls (C1). Trial 2. Colour measurement on potato crisps treated with mint essential oil once (M1), twice (M2), and controls (C2). Results are expressed as mean ± SD for $n = 3$.

Trial	**Variety**	Lady Claire				Taurus			
S1		**L***	**a***	**b***	**Δ*E***	**L***	**a***	**b***	**Δ*E***
T=1	**C1**	42.33 ± 5.60	3.24 ± 0.51	21.17 ± 0.17	/	41.34 ± 0.82	7.39 ± 1.49	24.63 ± 1.14	/
	M	38.44 ±6.42	3.22 ± 0.21	20.30 ± 1.15	3.99	43.27 ± 5.11	4.89 ± 1.00	22.66 ± 0.60	3.72
T=14	**C1**	35.57 ±7.83	4.04 ± 1.59	19.72 ± 2.56	/	34.31 ± 9.32	6.57 ± 1.98	19.68 ± 3.93	/
	M	35.62 ± 2.83	3.41 ± 0.24	19.86 ± 0.97	0.65	35.20 ± 6.48	6.10 ± 1.79	20.41 ± 3.06	1.24
T=56	**C1**	43.66 ± 7.40	2.51 ± 0.55	21.00 ± 1.56	/	36.23 ± 3.98	3.42 ± 1.21	18.95 ± 0.85	/
	M	40.90 ± 3.91	2.52 ± 0.17	20.36 ± 0.83	2.84	34.91 ± 4.60	2.67 ± 0.44	17.54 ± 2.28	2.07
S2									
	C2	34.76 ± 1.02	2.94 ± 0.67	19.60 ± 1.27	/	27.35 ± 4.74	4.08 ± 3.42	12.87 ± 1.47	/
	M1	39.51 ± 9.44	2.80 ± 0.33	21.02 ± 3.00	4.96	34.87 ± 5.34	3.01 ± 1.14	17.16 ± 3.25	8.72
	M2	34.59 ± 5.04	2.11 ± 0.25	18.59 ± 2.54	1.32	34.54 ± 4.69	4.52 ± 1.56	19.94 ± 2.43	10.09

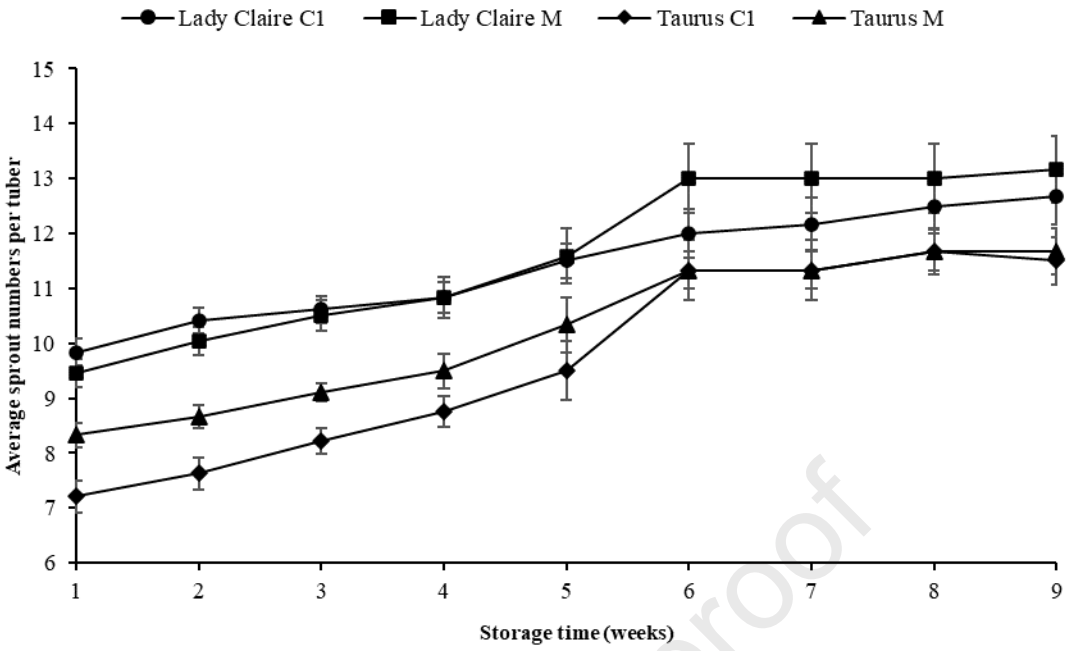

Figure 1. Sprout number of potato tubers treated with mint essential oil (M) and controls (C1) up to 9 weeks storage. Results are expressed as mean ± SD; week 1 and 2 (n = 24); week 3 (n = 18); week 4 and 5 (n = 12); week 6 to week 9 (n = 6).

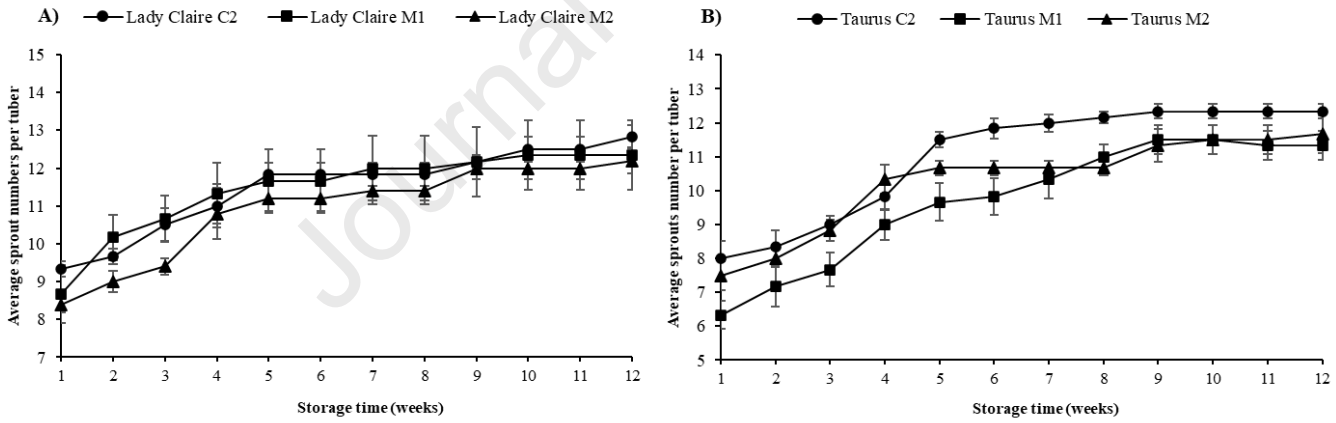

Figure 2. Sprout number of Lady Claire (A) and Taurus (B) potato tubers treated once (M1) or twice (M2) with mint essential oil and controls (C2) for 12 weeks storage. Results are expressed as mean ± SD for n = 6.

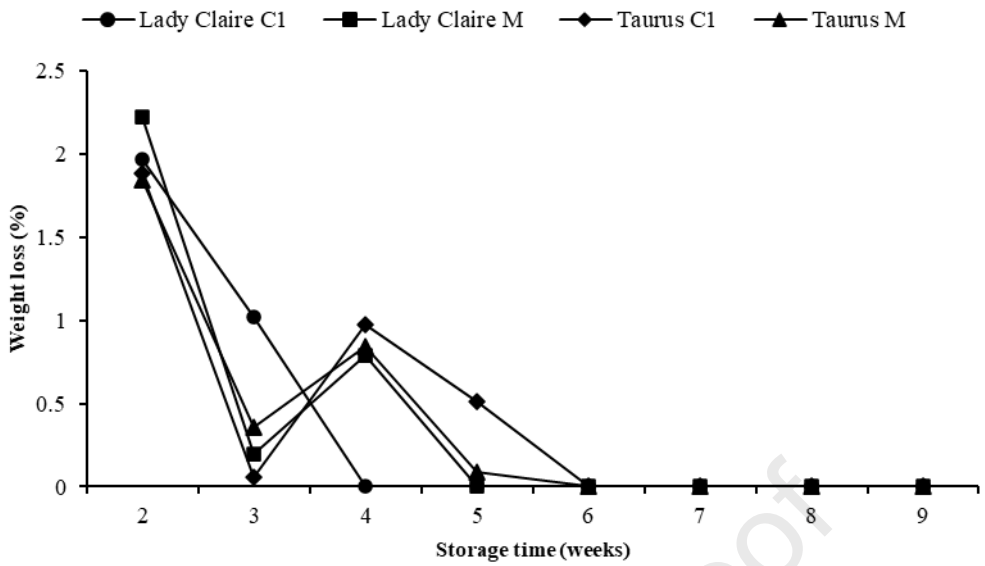

Figure 3. Weight loss (%) of potato tubers treated with mint essential oil (M) and controls (C1) up to 9 weeks storage. *n* = 24 week 2; *n* = 18 week 3; *n* = 12 week 4 and 5; *n* = 6 week 6 to 9.

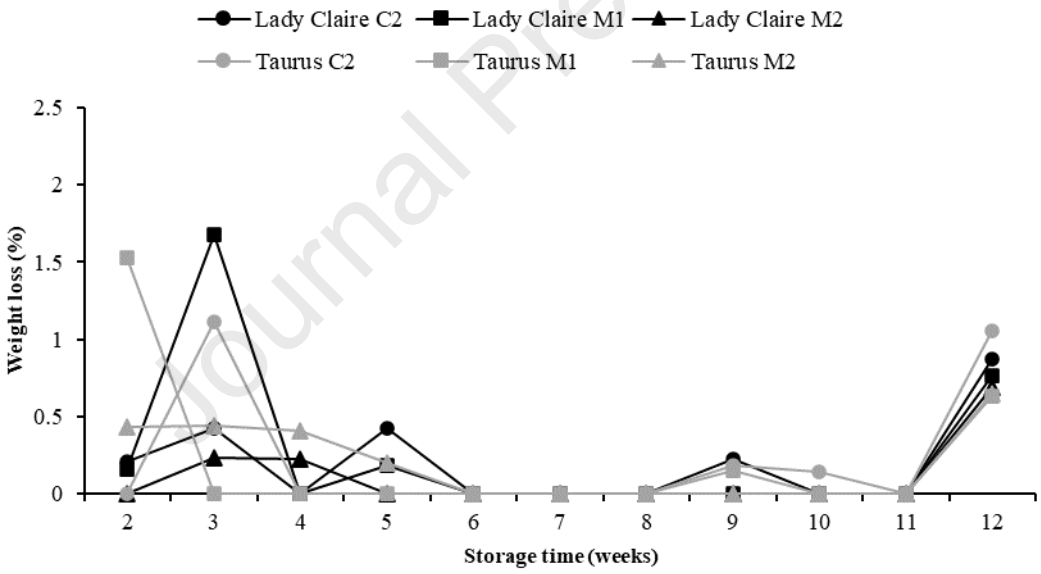

Figure 4. Weight loss (%) of potato tubers treated once (M1) or twice (M2) with mint essential oil and controls (C2) for 12 weeks storage; *n* = 6.

Highlights:

- Mint essential oil anti-sprouting treatment does not affect acrylamide formation in potatoes.
- Mint essential oil application does not increase reducing sugars content of potatoes.
- Mint essential oil application has no negative effect on potatoes metabolomic profile.
- Mint essential oil is safe to use for sprout control in potatoes destined for the processing market.

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: