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Fibre enrichment of cookies to mitigate acrylamide formation and gastrointestinal bioaccessibility

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ABSTRACT

Acrylamide (AA) is a food contaminant with serious health effects. In this work, the addition of dietary fibre was proposed to mitigate AA in cookies and to reduce bioaccesibility in the gastrointestinal trac. The analytical methodology applied for AA quantification was based on solid-liquid extraction (SLE) followed by gas chromatography (GC) coupled to mass spectrometry (MS). Preliminar results with commercial dietary fibres, such as k-carrageenan, arabinogalactan and pectin, indicated that the highest reduction of AA could be obtained with the addition of 5 g of pectin/100 g of flour to cookies recipe. Thus, different sources of pectin were evaluated: commercial pectin (CP) and three apple pomaces (dehydrated apple pomace (DP), sugar removed lyophilized apple pomace (SRL) and sugar removed lyophilized and powdered apple pomace (SRLP)). The highest AA mitigation was obtained with SRL and SRLP (62% and 48% of inhibition). After *in vitro* digestion, all sources of dietary fibres provided the lowest bioaccessibility results (13–46%) compared with control (>63%).

1. Introduction

Acrylamide (AA) is a food chain contaminant produced during heating processes such as baking, cooking, or roasting. It can be found in a variety of foods as potato chips, meat, bread, cookies, or coffee. The formation of AA in heat-processed foods has been attributed to the Maillard reaction that involves the formation of Schiff bases between asparagine (Asn) and reducing sugars, with or without an oxazolidin-5-one intermediate (Zhu et al., 2020). In recent years, several strategies to reduce or mitigate the AA content in foods (Zhu et al., 2020). Modifications of the recipe ingredients are widely described to mitigate AA formation in cookies, including the replacement of reducing sugars such as honey or inverted sugar syrup by sucrose, maltitol or stevia; the change of wheat flour, the main source of asparagine in the cookie formulation, by others such as rice flour; the modification of the pH in the recipe, since a lower pH can reduce the formation of AA, adding citric acid; the addition of polysaccharides such as chitosan or pectin, which can compete to asparagine in binding with the reducing sugar and induce a pH lowering effect; or adding the enzyme asparaginase which reacts with asparagine (to produce aspartic acid) limiting the formation of AA by the reduction of asparagine content (Champrasert, Orifila & Suwannaporn et al., 2022; Chang, Sung & Chen et al., 2016; Pasqualone et al., 2021; Schouten, Tappi, Rocculi, & Romani, 2022; Sung, Chang, Chou, & Hsiao, 2018; Zeng et al., 2010). This last process is widely employed by the food industry, however it is expensive, and the effectiveness of the process depends on several factors such as concentration, time, incubation temperature, water activity of the food, and the pH at which the asparagine conversion reaction takes place (Pasqualone et al., 2021; Schouten et al., 2022). The toxicological effects of AA on humans are neurotoxicity, genotoxicity, carcinogenicity, and reproductive toxicity, leading to its classification as a Group 2A carcinogen by the International Agency for Research on Cancer (International Agency for Research on Cancer (IARC), 1994). Therefore, mitigation and control of AA formation in foods and establishing reference levels for the reduction of the presence of AA in bakery products (European Commission, 2017) is of major relevance for the food industry to reach the lowest possible

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level below the reference one. Concerning the category "cookies and wafers", the AA benchmark value is 350 μg/kg and cookies are included in foods that must be monitored to identify the AA risk and adopt new reduction measures against this contaminant (European Commission, 2019).

The purpose of this work is the search for promising ingredients that can reduce the formation of AA in cookies and its bioaccessibility after gastrointestinal digestion. In addition to sugar, leavening agents and the type of wheat employed, which has already been studied, fibre addition of fibre is a promising alternative (Champrasert et al., 2021; Torres, Dueik, Carré, & Bouchon, 2019). Dietary fibres present distinct physico-chemical properties (e.g., they are positively or negatively charged and linear or with a variable degree of branching), which may differently impact acrylamide formation mostly considering the variation in the pH during cookies preparation. Therefore, several modifications of cookies recipe were tested to reduce the AA formation by partial replacement of wheat flour with dietary fibres such as pectin, arabinogalactan, k-carrageenan, or apple pomace. AA bioaccessibility was studied by INFOGEST *in-vitro* digestion model. The AA content in cookies and digests was assessed by gas chromatography (GC) coupled to mass spectrometry (MS) after solid-liquid extraction (SLE) and derivatization with xanthydrol.

2. Materials and methods

2.1. Materials and reagents

AA standard (99 g/100 g of purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and $AA^{13}C_3$ internal standard (IS) (99 g/100 g of purity) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA).

For the extraction of the analytes 1,2-dichloroetane (≥99 g/100 g), diethylene glycol (99 g/100 g), xanthydrol (98 g/100 g), hydrochloric acid (HCl) (37 g/100 mL) and methanol (MeOH) HPLC grade were acquired from Sigma-Aldrich. Ethyl acetate (AcOEt) pesticide residue analysis grade was acquired from Fluka, potassium hydroxide (KOH) and sodium chloride (NaCl) from VWR (Leuven, Belgium) and potassium carbonate (K_2CO_3), ammonium bicarbonate (NH_4HCO_3) and sodium bicarbonate ($NaHCO_3$) from Panreac (Barcelona, Spain). Ultrapure water (UPW) (0.054 μS/cm) was supplied by a "Seral" system (SeralPur Pro 90 CN, Germany).

For *in vitro* digestion, porcine α-amylase, pepsin, bile and pancreatin extracts were provided by Sigma-Aldrich. Dietary fibres: k-carrageenan from plant, arabinogalactan from larch wood and pectin from apple (poly-D-galacturonic acid methyl ester) were acquired from Sigma-Aldrich. Apple pomace (Bravo de Esmolfe (*Malus domestica Borkh*)), a byproduct from apple juice production, was kindly provided by Sumol + Compal® and processed in the laboratory as described in section 2.2.

2.2. Apple pomace processing and flour preparation

After defrosting at 4 °C for 24 h, apple pomace was washed with water to remove free sugars and other soluble nutrients, or not washed (Bátori et al., 2017; Gustafsson et al., 2019). Apple pomace was soaked in cold water (to avoid starch dissolution) (4 L of water per 1 kg of pomace). After stirring for 5 min, pomace was filtered on a filter cloth and manually pressed to drain excess water. The residue was frozen at −20 °C and lyophilized for at least 48 h (sugar removal and lyophilized apple pomace (SRL)). The other sample was dehydrated for 12 h (dehydrated apple pomace (DP)) by lyophilization as SRL. SRL and DP were ground in a domestic grinder to obtain homogeneous apple pomace. The pomace obtained was packed air-tight in plastic bags and stored at −20 °C for further use. Moisture and fibres (soluble and total) were analysed according to Martins et al. (Martins Pinto, Almeida, Pinho, & Ferreira et al., 2017) and the sugar content to Santos et al. (Santos et al., 2016)

2.3. Cookies preparation

Ingredients for cookies were purchased from local markets of Porto (Portugal). The recipe was prepared mixing the following ingredients: 500 g of T-45 flour (this amount was reduced for fibre-enriched in the same proportion of fibre added), 2 g of NH_4HCO_3, 9 g of $NaHCO_3$, 136 g of sugar, 4 g of NaCl, 20 g of malt extract, 100 g of glucose-fructose syrup, 120 g of sunflower oil, dietary fibre or pomace (Tables S1 and S2) and water to homogenize. Later, all of them was introduced in a mixer and was mashed continuously during 10 min. After that, the cookies shape were done (approximately 25 g of dough per cookie) employing a circular mould to ensure that all cookies have the same dimensions (4.5 cm diameter and 1 cm height) and baking during 15 min at 190 °C. In each batch (3), 14 cookies were positioned randomly on a baking tray. The baking tray was placed in the oven chamber on the third shelf (out of six) from the top. Finally, the cookies were kept 3 h at ambient temperature, to cool down and after size and color measurements they were crushed, homogenized, and introduced in a plastic pot until the analysis. Moisture and fibre analysis were analysed as described in *Section 2.2*.

2.4. Acrylamide extraction

Acrylamide extraction method was based on the method previously developed by Molina-Garcia et al. (Molina-Garcia et al., 2015). Briefly, 2 g of cookie was weighted into a 50 mL Falcon tube and spiked with 200 μL of $AA^{13}C_3$ at 10 mg/L. After 15 min, 20 mL of water and 5 mL of 1,2-dichloroethane were added. The tube was shaken on a rotatory shaker for 15 min, and centrifuged for 5 min at 5000 rpm (4696 *g*). The supernatant was introduced into a round-bottom flask and 3 mL of diethyl glycol in methanol (10/90, *v/v*) was added. Later it was concentrated in a rotary evaporator until a final volume of 5 mL. Then, the derivatization was carried out employing 1 mL of xanthydrol in methanol (10/90, *v/v*) and 1 mL of HCl (1.5 mol/L) followed by water bath at 40 °C for 50 min. The pH of the sample was adjusted to pH 9.0, by adding 0.7 mL of KOH (2.5 mol/L) and buffered with 30 mg of $NaHCO_3$ and 170 mg of K_2CO_3. Then, 2 g of NaCl and 2 mL of water were added and for the extraction of the xanthyl-AA derivative, 1 mL of ethyl acetate was added twice, being vigorously shaken. Finally, it was centrifuged for 10 min at 5000 rpm (4696 *g*) and 1 mL of the organic layer was transferred to a vial and concentrated to 0.5 mL under a gentle stream of nitrogen, prior to GC-MS analysis.

2.5. In vitro digestion

In vitro cookies digestion procedure was based on the internationally standardised method (INFOGEST) described by Brodkorb et al. (Brodkorb et al., 2019). Each sample was done by triplicate plus one blank sample used to adjust the pH of the digestion and used as blank matrix. Thus, 4 g of cookies sample was mixed with 3.2 mL of simulated salivary fluid (SSF), 0.4 mL of α-amylase solution at 1500 U/mL in UPW, 20 μL of $CaCl_2$ 0.3 mol/L and 0.380 mL of UPW. After keeping the mixture at 37 °C with continuous mixing with a horizontal shaker at 90 rpm for 2 min, 6.4 mL of simulated gastric fluid (SGF), 0.4 mL of pepsin solution at 25000 U/mL in UPW, 4 μL of $CaCl_2$ 0.3 mol/L, 0.4 mL of HCl 1 N (to reach pH 3) and 0.696 mL of UPW were added to perform gastric digestion. The gastric mixture was mixed at 37 °C for 2 h. Finally, intestinal digestion was performed by adding 6.8 mL of simulated intestinal fluid (SIF), 4 mL of pancreatin solution at 800 U/mL in SIF, 2 mL of bile solution at 10 mM in SIF, 32 μL of $CaCl_2$ 0.3 mol/L, 0.1 mL of sodium hydroxide 1 N (to adjust pH 7) and 3.068 mL of UPW. The gastrointestinal mixture was incubated at 37 °C for 2 h in an incubator with horizontal shaker at 90 rpm. After digestion, the samples were cooled in an ice bath for 10 min and centrifuged at 5000 rpm (4696 *g*) for 10 min at 4 °C to obtain the bioaccessible fraction (supernatant) and non-bioaccessible fraction (solid part). Supernatants were extracted

following the procedure described previously using 4 g of supernatant instead 2 g.

2.6. Gas chromatography-mass spectrometry parameters

For the analysis of acrylamide, a gas chromatograph Hewlett Packard HP6890 coupled to an Agilent 5973 single quadrupole mass analyzer with an electron ionization (EI) source (Agilent Technologies, Santa Clara, CA, USA) was used. The GC system was equipped with autosampler COMBI PAL (PAL3 –SI, CTC, Zwingen Switzerland) and an analytical column DB-5ms (30 m × 0.25 mm, 0.25 μm film thickness). The carrier gas was helium at a constant flow of 1 mL/min. The injector temperature was set at 250 °C, and injection volume was 1 μL in splitless mode (pulsed pressure 220 kPa, 60 s). Oven temperature started at 85 °C (hold 1 min) and increased to 280 °C at 18 K/min, and finally hold for 4.17 min. The transfer line temperature was set at 280 °C and the mass spectrometry was worked in electron ionization (EI) mode at energy of 70 eV. The data acquisition was performed using the ChemStation platform in selected ion monitoring (SIM) mode (Molina-Garcia et al., 2015). Table S3 shows the acquisition parameters and retention time for acrylamide analytes. The limits of detection (LOD), quantification (LOQ), and precision in term of relative standard deviation (% RSD) were 4 μg/kg, 25 μg/kg, and <8%, respectively.

2.7. Colour measurement of cookies

The impact of fibre enrichment on cookies colour was performed using the L*, a* b* system, where L* refers to the luminosity or lightness component, a* (intensity of red (+) and green (−)) and b* (intensity of yellow (+) and blue (−)). Cookies were analysed in terms of the referred parameters using a colorimeter (Chroma Meter, CR-400, (Konica Minolta, Tokyo, Japan) previously calibrated with a white standard tile. Color distance (ΔE), a dimensionless parameter that arises from the combination of the L*a* and b* values when pairs of samples are considered, was assessed (Eq (1)). ΔE of sample pairs leads to foreessen whether or not there is a difference in the cookies colour that can be perceived by the human eye according to specific thresholds i.e., $\Delta E < 1$ normally invisible difference; $1 < \Delta E < 2$ very small difference, only obvious to a trained eye; $2 < \Delta E < 3.5$ medium difference, also obvious to an untrained eye; $3.5 < \Delta E < 5$ An obvious difference; $\Delta E > 6$ A very obvious difference (Ashe, 2014). The color data correspond to the average of 16 measurements in 4 cookies for each cookie formulation.

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2} \quad (1)$$

2.8. Statistical analysis

Statistical analysis (analysis of variance, ANOVA) was carried out with IBM SPSS Statistics v23 (Armonk, NY).

3. Results and discussion

3.1. Impact of dietary fibres addition on AA formation and color of cookies

Three different dietary fibres were incorporated in cookies dough as an AA mitigation strategy: commercial pectin, k-carrageenan (negatively charged) and arabinogalactan (neutrally charged). Pectin and arabinogalactan are plant polysaccharides. Pectinis are the linear region from pectic polysaccharides consisting of units of (α1→4)-D-GalpA residues, obtained from apple. Arabinogalactan is a branched biopolymer from larch wood, mainly composed out of a 1,4 linked β-D-Galp backbone with α-L-Ara*f* residues. k-carrageenan is a linear sulfated mucopolysaccharide from the cell walls of the red algae. For the evaluation of these three fibres, the cookie recipes were prepared following the procedure described in *Section 2.3*, adding each dietary fibre to the ingredients prior to the mash step. Two percentages were evaluated (2 g of fibre/100 g of flour (to simplify all subsequent citations g/100 g) and 5 g/100 g of flour content) according to Table S1. The results exposed in Table 1 show that arabinogalactan was not efficient to mitigate the AA content because the concentration was significantly higher compared to the control cookies (about 70% higher) for both fibre percentages (*p*-values = 0.0006 and 0.0013). AA content of k-carrageenan cookies was not significantly different than that of control cookies (*p*-values = 0.2302 and 0.5878) for both, 2 g/100 g and 5 g/100 g addition, being the reduction lower than 10%. Pectin was a promising fibre to reduce the AA content, depending on the percentage added to the cookies. When 2 g/100 g was added, AA formation was 50% higher compared to control cookies (*p*-value = 0.0025), but when a higher percentage of pectin (5 g/100 g) was employed, the reduction was about 30% (*p*-value = 0.0072). Therefore, the amount of pectin needed to be carefully selected when this fibre was used as a mitigation strategy; it can be assumed that the inhibition of AA formation by pectin is conditional and selective (Wang et al., 2022) and directly related to the balance between the amount of reducing sugars and the pH lowering effect (Passos et al., 2018). As Passos et al. (Passos et al., 2018) reveals, the molar ratio of acidic polysaccharides (as pectin) is really critical and only can be achieved with the pectin content of 5 g/100 g. The explanation for the increase in AA content in cookies with 2 g of pectin/100 g is due to that the amount of acidic polysaccharides of pectin was not sufficient to reduce the AA content as described in previous work and also the pH was different in the cookies (7.83 for 2 g of pectin/100 g and 6.68 for 5 g of pectin/100 g), so it can be expected that mitigation effect of pH was more accused in cookies with 5 g/100 g. In addition, it is important to highlight that the benchmark value stablished for AA in cookies (350 μg/kg) only was reached with 5 g of pectin/100 g, using the other fibres the concentrations of AA were higher.

The AA reduction when pectin and k-carrageenan were used as ingredients in cookies could be due to the fact that linear and negative fibres trap the asparagine molecule in an egg-box structure and avoid its reaction with reducing sugars to produce AA. In the case of pectin, it only occurred when the pectin content is enough, for this reason at lower doses (2 g/100 g) the AA content was not reduced. In addition at higher doses of pectin the pH-lowering effect of the repeating units of GalA residues in the polymeric structure of pectin occurred, so the pH of the cookie was slightly lower and as indicated in the introduction, lower pH can reduce the AA formation.

In addition to AA content, the impact of fibre enrichment on cookies size and colour (L*a*b system) was measured, because colour is sometimes associated with the highest amount of AA. Size results indicated that the diameter and height of the cookies differed by just 0.2 cm and 0.1, respectively, so it indicated that the spread of the cookies was

Table 1
Concentration of acrylamide and mitigation obtained in cookies after the evaluation of dietary fibres.

Type of fibber	Concentration of AA (μg/kg) (n = 3) (RSD (%))	AA mitigation according to control	*p*-value[a]
Control	412 (8)	–	
Pectin 2 g/100 g	636 (5)	+54%	0.0025
Pectin 5 g/100 g	296 (10)	−28%	0.0072
Arabinogalactan 2 g/100 g	710 (6)	+72%	0.0006
Arabinogalactan 5 g/100 g	725 (4)	+76%	0.0013
K-carrageenan 2 g/100 g	371 (7)	−10%	0.2302
K-carrageenan 5 g/100 g	397 (4)	−4%	0.5878

Abbreviations: AA: Acrylamide; RSD: Relative standard deviation.
[a] Obtained from an ANOVA statistical analysis (control vs enriched sample).

similar. Colour results (summarized in Table S4) indicate that for cookies containing k-carrageenan, 2 g/100 g and 5 g/100 g, the addition promoted medium differences compared to the control cookies (ΔE between 2.5 and 3.5), while for cookies containing 5 g/100 g pectin the difference of colour was obvious (ΔE = 4.30). The samples containing 5 g/100 g pectin presented lighter colour than the control, so it is expected to present a lower AA content as previously indicated. Fig. 1 shows the cookies placed randomly previous and after baking on the baking tray. As can be observed, not significant deferences were observed for baked cookies in a visual way (Fig. 1), however the L*a*b system indicates the opposite. For this reason, it is important to do this type of test to check the results by experimental way. In addition, if the results of the L* component on the colorimetric scale are related to the AA content, it can be observed that for the case of pectin at 5 g/100 g, the L* component is lower than for arabinogalactan and k-carrageenans (Table S4), indicating that the color is lighter, and directly related to the lower AA content.

3.1.1. *Evaluation of pectin addition from commercial and apple pomace sources*

To deepen the use of pectin as a potential industrial strategy for reducing AA reduction in cookies, apple pomace (a by-product of the juice industry rich in pectic polysaccharides) was tested. Different types of apple pomace were used. Therefore, the impact of adding commercial pectin, DP and SRL as a cookie ingredient on AA reduction was compared. To produce cookies with a similar composition, the pectin content in the apple pomaces was determined, in order to estimate the amount of pomace needed to add 5 g/100 g of pectin to the recipe. Furthermore, the sugar content of the pomaces was measured to adjust the amount of sugars added to the recipe. These results are analysed according to Section 2.2 and summarized in Table S5. The content of soluble fibre (pectin) was higher in the SRL pomace (15.9 g/100 g) than in DP (7.2 g/100 g), so for the DP it was necessary to add more pomace to the recipe. In relation to sugar content, the quantity of sugars in SRL was negligible; meanwhile, in DP the sugar content was high, mainly due to the presence of fructose. Thus, sugar and glucose-fructose syrup were removed from ingredients recipe and replaced by sugars of DP. However, this replacement can be a problem, the richness of apple pomace in reducing sugars (81 g/100 g fructose + glucose vs 21 g/100 g sucrose) can increase the formation of AA. In Table S2, the total amount of each ingredient added to the recipe was indicated fitting the flour content according to the amount of pomace added. In this step, is important to indicate that due to the lack of homogenization of the SRL pomace, it was decided to test that pomace in two versions, powdered (SRLP) and non-powdered (SRL), to check if AA content and visual analysis provided the same results or not.

Once the proportions were determined, the cookies were prepared and, as shown in Fig. 2, differences in cookie dough and colour were observed using the different pomaces. A dark brown colour was noted when DP was employed and a heterogeneous mixture was observed using SRL pomace. Once the cookies were baked (Fig. 2), the colour of the cookies containing the different sources of dietary fibres was lighter than the control cookies. L*a*b colour was measured to check the ΔE (Table S6). Results show that for the cookies added with SRL and SRLP the differences from the control were very obvious (ΔE > 10), being the cookies with pomace lighter compared with the control. For the other mixtures, commercial pectin and DP, the differences were less obvious (ΔE around 5). Size results were similar to those for cookies with fibre enrichment, obtaining values of diameter and height not different from 0.15 cm to 0.10 cm, respectively. So the addition of pomaces did not modify the cookies spread.

To continue, the AA content was measured, as indicated in Table 2, and the results were very promising, especially for SRL and SRLP, in which the percentage of AA reduction was higher, between 50 and 65% compared to commercial pectin which only showed a 20% reduction (p-value s = 0.0004–0.0011), this result can be associated with the colour of the cookies because, as indicated previously, cookies with SRL and SRLP present a lighter colour compared with control (L* component was lower (Table S6)). However, as foreseen, due to the high content of reducing sugars, mainly fructose, the cookies containing DP presented a 386% increase in AA content (p-value = 0.0001) and also as can be seen in Fig. 2, the colour of the cookies DP was darker compared with the other, so they expected to have more AA content.

These results prove that pomace from juice by-products can be valorized by novel applications in cookies industry and provide AA values lower than the benchmark if sugars are washed from pomace.

The total dietary fibre content of the cookies (Table S7) reveals that the cookies with apple pomace are richer in fibre (around 20 g/100 g)

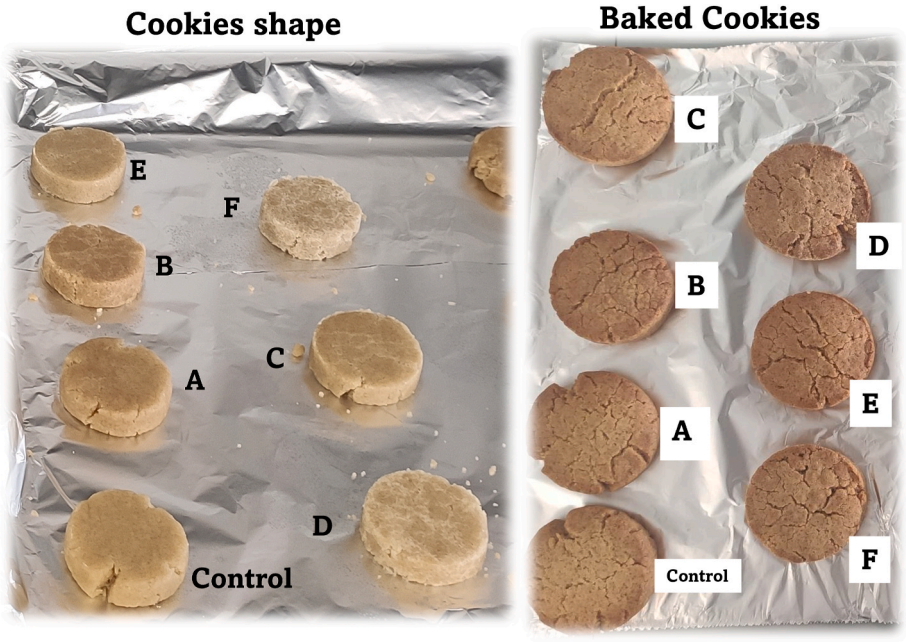

Fig. 1. Cookies shape and baked cookies with fibre addition. (A = pectin 2 g/100 g; B = pectin 5 g/100 g; C = arabinogalactan 2 g/100 g; D = arabinogalactan 5 g/100 g; E = k-carrageenans 2 g/100 g; F = k-carrageenans 5 g/100 g).

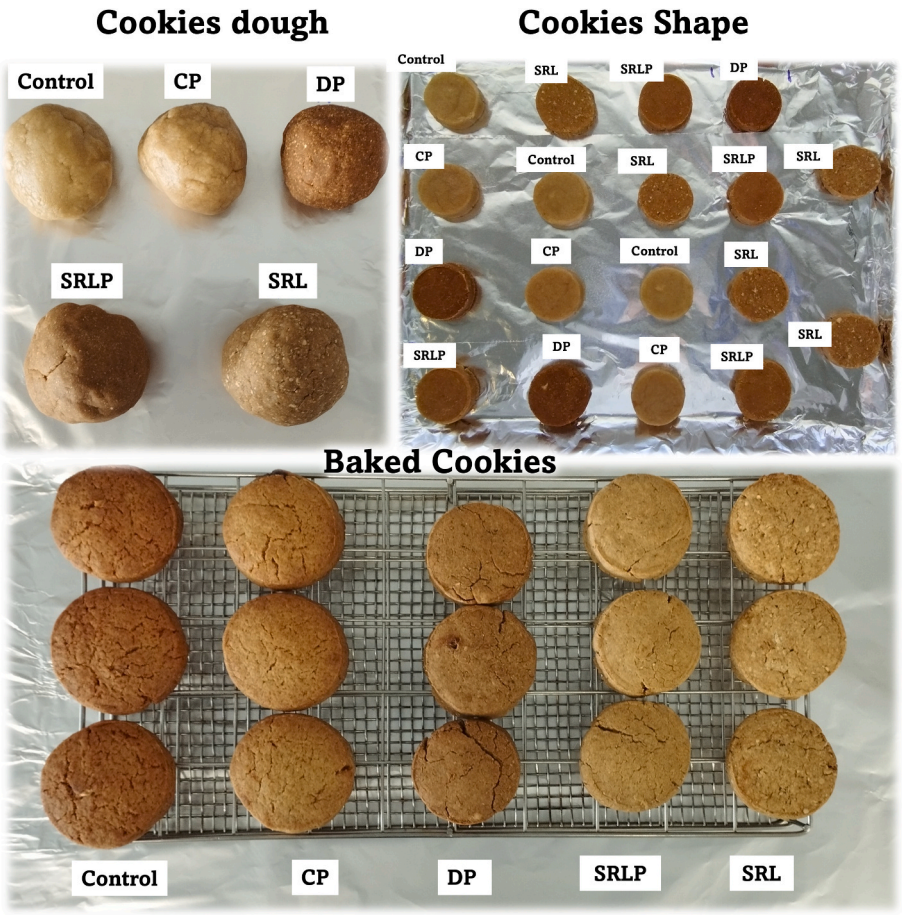

Fig. 2. Cookies dough, shape, and baked cookies with different sources of pectin addition. (CP = commercial pectin; S = apple pomace with sugars; SRL = apple pomace sugar removed and lyophilized; SRLP = apple pomace sugar removed, lyophilized, and powdered).

Table 2
Concentration of acrylamide and mitigation obtained in cookies after evaluation of apple pomace rich in pectin and commercial pectin.

Type of fibber	Concentration of AA (μg/kg) (RSD (%))[a]	AA mitigation according to control (%)	*p*-value[b]
Control	392 (8)	–	–
Commercial pectin (5 g pectin/100 g)	316 (13)	−20%	0.0232
DP (5 g pectin/100 g)	1904 (4)	+386%	0.0001
SRL (5 g pectin/100 g)	150 (6)	−62%	0.0004
SRLP (5 g pectin/100 g)	204 (9)	−48%	0.0011

Abbreviations: AA: Acrylamide; DP: Dehydrated apple pomace; RSD: relative standard deviation; SRL: Sugar removal and lyophilized apple pomace; SRLP: Sugar removal, lyophilized and powdered apple pomace.
[a] n = 3.
[b] Obtained from an ANOVA statistical analysis (control vs enriched sample).

compared with control (5 g/100 g) and commercial pectin (8 g/100 g), and meet the EU conditions to apply the nutritional claim that is a high fibre food (>6 g/100 g) (European Union, 2006). Therefore, the results indicate that SRL or SRLP cookies can be a healthier solution with higher content of fibre and less quantity of AA compared to classic cookies.

3.2. Bioaccessibility of AA

In vitro digestion was also evaluated to verify if the gastrointestinal bioaccessibility of AA changed when dietary fibres were used. It was assessed in pectin cookies, as it was the dietary fibre that provided the best results. Digestion was evaluated in the stomach (gastric digestion) and in the stomach and duodenum (gastrointestinal digestion).

Gastric digestion (Table 3 and Fig. 3) reveal that a reduction of AA bioaccessibility was observed for pectin-containing cookies compared to the control cookies (*p*-values = 0.0026–0.0123). The lowest percentages of bioaccessibility were observed for cookies that contain SRL pomace (30% of bioaccessibility) and those with SRLP, the second that provided

Table 3
Concentrations of acrylamide obtained after cookies *in vitro* digestion.

Type of fibre	Concentration of AA (μg/kg) in gastric phase (RSD (%))[a]	*p*-value[b]	Concentration of AA (μg/kg) in gastrointestinal phase (RSD (%))[a]	*p*-value[b]
Control	292 (3)	–	245 (7)	–
Commercial pectin (5 g pectin/100 g)	150 (8)	0.0123	49 (12)	0.0013
SRL (5 g pectin/100 g)	46 (10)	0.0026	22 (11)	0.0013
SRLP (5 g pectin/100 g)	89 (13)	0.0059	40 (16)	0.0025

Abbreviations: AA: Acrylamide; RSD: relative standard deviation; SRL: Sugar removal and lyophilized apple pomace; SRLP: Sugar removal, lyophilized and powdered apple pomace.
[a] n = 3.
[b] Obtained from an ANOVA statistical analysis (control vs enriched sample).

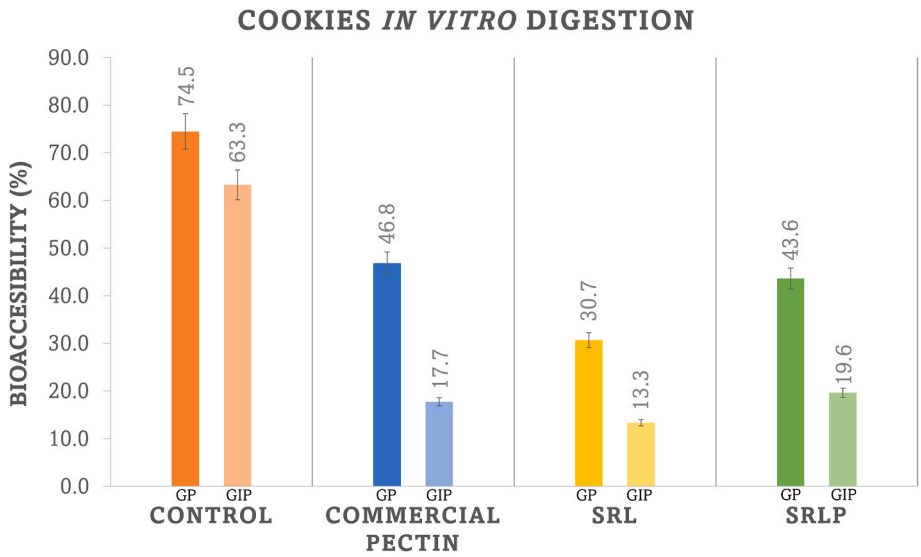

Fig. 3. Bioaccessibility of AA after *in vitro* digestion at gastric phase (GP) and gastrointestinal phase (GIP) in the different cookies evaluated (SRL = apple pomace sugar removed and lyophilized; SRLP = apple pomace sugar removed, lyophilized, and powdered).

lower results (43%) statistically significant differences were observed (p-value = 0.034).

For gastrointestinal digestion, the bioaccessibility of AA in pectin-containing cookies was statistically different compared with the control cookies (*p*-values <0.0025). However, the differences between commercial pectin, SRL and SRLP were not statistically different (p-value >0.19). In general, the addition of fibre to cookies reduces AA bioaccessibility compared with control cookies (13–20% compared to 63%) (Fig. 3). The pectin added to the cookies acts as an AA binder (mitigation agent) during digestion and promotes a relevant reduction in the bioaccessibility of AA compared with control cookies.

4. Conclusions

The present paper provides novel knowledge about AA mitigation strategies in cookies using dietary fibres. The addition of 5 g/100 g of dietary fibres, especially different sources of pectin, provided excellent results in the reduction of AA, SRL and SRLP the best option, with a reduction greater than 50%, obtaining concentrations values lower than the benchmark value (350 μg/kg) for AA in cookies. The *invitro* digestion results also indicate that the addition of dietary fibres promotes lower AA bioaccessibility compared to control samples. Our results provided useful information for the food industry, as new strategies to mitigate AA content in foods and the use of apple pomace as a cheap and bio-circular economy product because it can be obtained as a subproduct from apple juice. In addition, introducing dietary fibres in cookies could help us to obtain cookies with higher fibre content, which is nutritionally better than the normal recipe.

CRediT authorship contribution statement

Rosalía López-Ruiz: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, preparation, Visualization. **Jesús Marin-Saez:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing – original draft, preparation, Visualization. **Sara C. Cunha:** Conceptualization, Writing – review & editing, Formal analysis, Visualization, Resources, Funding acquisition. **Ana Fernandes:** Methodology, Formal analysis, Visualization, Resources, Writing – review & editing, Project administration, Funding acquisition. **Victor de Freitas:** Formal analysis, Visualization, Resources, Writing – review & editing, Project administration, Funding acquisition. **Olga Viegas:** Methodology, Formal analysis, Visualization, Resources, Writing – review & editing. **Isabel M.P.L.V.O. Ferreira:** Formal analysis, Visualization, Resources, Writing – review & editing, Project administration, Funding acquisition.

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 will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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