Risk/benefit evaluation of traditional and novel formulations for snacking: Acrylamide and furfurals as process contaminants

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ABSTRACT

Novel alternatives for snacking contain ingredients such as pseudo-cereals, roots or legumes, which offer health related benefits to consumers. Alternative technological processes have been introduced within the snack industry that encompass valued sensorial characteristics and improve nutritional quality of the new formulations. However, the impact of the novel formulations on the exposure to process contaminants must also be carefully evaluated. The present study investigated the occurrence of acrylamide, hydroxymethylfurfural (HMF) and furfural in novel formulations and compared this with that seen in traditional snacks. Some novel snacks presented high levels of acrylamide, specifically chips with a potato-based dough (1252–1393 µg/kg) and chips made from other vegetables (carrot chips: 958 µg/kg; beet chips: 908 µg/kg). These samples demonstrated concentrations that exceeded those observed in traditional snacks (44–671 µg/kg). The average values of HMF and furfural in the novel formulations were also significantly higher than traditional ones (approximately 2.5- and 3-fold, respectively). The snack manufacturing industry should consider the nutritional and sensorial improvements gained through the application of alternative technological process or the addition of health-promoting ingredients in the design of innovative snacks. This must be considered alongside toxicological aspects which reduce the content of process contaminants.

KEYWORDS: Traditional snacks, Acrylamide, Hydroxymethylfurfural, Furfural, Exposure, Food analysis, Food composition

1. INTRODUCTION

The consumption of snacks has increased in recent decades, becoming part of the dietary habits and lifestyle of many consumers (MAPAMA, 2017). Consumer demand for novel products that satisfy convenience needs and sensory and health trends has led food companies to modify the composition of traditional snacks. Such snacks now offer a healthier formulation that is low-calorie, low-fat, low-carbohydrate, low-sodium and rich in fiber and vitamins. Novel products are formulated with alternative ingredients such as pseudo-cereals, roots, legumes, and others. Many such products have emerged recently from the food market with the aim of increasing nutritional value and improving health-promoting properties of foodstuffs (Niva, 2007). Their formulation combines the complexity of satisfying the changing tastes and expectations of different consumers, with the search for unique products that provide nutritional characteristics and greater market value (Bartelme, 2016). The development of these types of snacks is, therefore, an emerging and innovative aspect of the sector.

Extrusion, frying and baking are the main processing techniques used worldwide in the development of snack products. Extrusion has been used extensively in the production of snacks due to its ease of operation and ability to produce a variety of textures and shapes which appeal to consumers (Brennan et al., 2013). Frying imparts flavor, taste, color and crispness in the fried foods (Farkas et al., 1996; Krokida et al., 2001), whereas baking produces valuable sensorial characteristics with a crisp texture but a lower fat content (Han et al., 2010). Further alternative methodologies to be employed by the snack industry include ultrasound-osmotic dehydration prior to frying (Dehghannya and Abedpour, 2018), vacuum frying (Dueik et al., 2010),

oven toasting (Nath and Chattopadhyay, 2007) and injection with carbon dioxide gas during the extrusion process (Masatcioglu et al., 2014). These alternatives enable the properties of the novel formulations for snacking to be modified, obtaining new textural and sensory properties.

Novel formulations and innovative technologies have the potential to improve the nutritional profile and the sensorial quality of snacks. However, the use of novel ingredients within the recipe and the application of alternative technological processes may also affect the formation of process contaminants including acrylamide and emerging process contaminants, such as hydroxymethylfurfural (HMF) and furfural. Process contaminants, and more precisely heat-induced process contaminants, form as a result of the thermal treatments applied during processing. These compounds are essentially produced by the Maillard reaction, initiated by carbohydrates but also by carbonyls generated from the lipid oxidation (Zamora and Hidalgo, 2011). Acrylamide is generated as a result of the reaction between asparagine and reducing sugars, which are its main precursors. The European Food Safety Agency (EFSA) has identified the presence of acrylamide in food as a possible public health concern due to its relation with different types of cancer. This obviates the need for continued efforts to reduce exposure to this metabolite (EFSA, 2015). HMF and furfural are formed as intermediate products of the Maillard reaction. Furthermore, HMF is generated from the caramelization of sugars at high temperatures (Morales, 2008). With regards to HMF, animal studies have identified its biotransformation into the genotoxic and mutagenic metabolite, sulphoxymethylfurfural (Høie et al., 2015; Pastoriza de la Cueva et al., 2017). Further, furfural may lead to hepatotoxicity (EFSA, 2005).

The Commission Regulation (EU) 2017/2158 establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food considers French fries (ready-to-eat), potato crisps from fresh potatoes and from potato dough, and other potato products from potato dough (European Commission, 2017). However, other snack formulations are not considered by the regulation. The objective of the present work was to evaluate the levels of acrylamide, HMF and furfural in novel snack formulations, or so-called healthy snacks, and to compare these with traditional snack formulations. This prospective study aims to consider the risk/benefit of these innovative products, assessing if they are a healthier option relative to traditional ones, not only in nutritional but also in toxicological terms.

2. MATERIALS AND METHODS

2.1 Reagents and chemicals

Potassium hexacyanoferrate (II) trihydrate (98%, Carrez-I) and zinc acetate dehydrate (>99%, Carrez-II) were obtained from Sigma (St. Louis, USA). $^{13}\text{C}_3$ -labelled acrylamide (99% isotopic purity) was obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Formic acid (98%), D(+)-Glucose and methanol (99.5%) were acquired from Panreac (Barcelona, Spain). Deionized water was obtained from a Milli-Q Integral 5 water purification system (Millipore, Billerica, MA, USA). Reversed-phase Oasis-HLB cartridges (30 mg, 1 mL) were purchased from Waters (Milford, MA, USA). Syringe filter units (0.45 μm , cellulose) were purchased from Análisis Vínicos (Tomelloso, Ciudad Real, Spain). All other chemicals, solvents and reagents were of analytical grade.

2.2 Samples

Snacks (n=77) were purchased from various Spanish supermarkets in May 2017. Samples were classified as traditional snacks (n=46) or novel formulation snacks (n=31). Classification was based on commercial claims regarding the health profile of the product and the producers description of its composition. Sampling included the largest number of alternatives found in the market and avoided the duplication of products belonging to the same category, for instance, savory snacks made from the same formulation. The aim was

to evaluate a wide and representative scenario of snacking choices and identify the degree of variability in their formulations and processing. The sample examined is representative in the context of the Spanish industry but could also be extrapolated to the European market since most of the commercial brands are present in EU countries. Samples were grouped according to their main ingredient such as cereal, root or tuber, legume and others. The major and innovative ingredients for each sample are detailed in the Supplementary Material (Annex 1, 2) (quinoa, oatmeal, spelt, soybean, carrot, parsnip, beet, cassava, lentil, chickpea, banana and seaweed). The presence of innovative ingredients provided the criteria by which snacks were classified as novel formulations. For each sample, the whole bag was mixed and grounded to assure a homogeneous distribution of potential hotspots. Then a portion of 50–100 g was vacuum packaged and stored under light protection at 4 °C until analysis.

2.3 LC-ESI-MS-MS determination of acrylamide

Acrylamide was determined as described by Mesias and Morales (2015). The accuracy of this method for potato crisps has been recently demonstrated in three proficiency tests launched by the Food Analysis Performance Assessment Scheme (FAPAS) program, yielding a z-score of 0.2 (Test 3065, May-June 2016), -0.2 (Test 3071, Feb-March 2017) and -0.3 (Test 3080, Feb-March 2018). Precision (reproducibility) was lower than 10%, and recovery between 84 and 109%. The limit of the quantitation was set at 20 µg/kg. Analyses were performed in duplicate and results were expressed as µg/kg of product.

2.4 Determination of HMF and furfural

HMF and furfural determinations were based on the method previously reported by Rufián-Henares et al. (2009). Quantitation was conducted with a Shimadzu HPLC system, as previously described by Mesias et al. (2015). Chromatographic separation was carried out on a Mediterranean Sea ODS-2 (250×4.0 mm, 5 µm, Tecknokroma, Barcelona, Spain). The mobile phase was an acetonitrile and water mix (5 mL/100 mL), with a flow rate of 1 mL/min under isocratic conditions. The total running time was 20 min, the UV detector was set at 280 nm and 20 µL of the extract was injected. HMF and furfural were quantified using the external standard. The limit of quantification was set at 0.3 and 0.2 mg/kg for HMF and furfural, respectively. Analyses were done in duplicate and results were expressed as mg/kg sample.

2.5 Food consumption data and exposure

Dietary exposure to acrylamide, HMF and furfural within the different categories of snacks was estimated by considering data for total per capita consumption of snack (6.43 kg/person/year), as established by the Spanish Association of snack manufacturers (AFAP, 2015) and the percentage of consumption assigned to each type of product (22% of the total, excluding French fries and nuts). An average adult body weight of 70 kg was assumed.

2.6 Statistical Analysis

Statistical analyses were performed using SPSS version 23 (SPSS, Chicago, IL). Data were expressed as mean±standard deviation (SD). Student's t-test was used to identify the overall significance of differences between the two groups. Analyses were performed of the process contaminants identified in the tested snacks (traditional group versus novel formulations). Differences were considered to be significant at $p < 0.05$. Relationships between the different measured parameters were evaluated using Spearman correlation coefficients at the $p < 0.05$ confidence level. Correlations were also performed between the aforementioned parameters and the nutrient content (as provided on the product labels).

3. RESULTS AND DISCUSSION

3.1 Nutritional composition of snacks

Nutritional composition of traditional (Table 1) and novel (Table 2) snacks was described according to information provided by the manufacturer. The energy contained in traditional snacks (393–567 kcal/100 g) and novel formulations (364–508 kcal/100 g) was not found to be significantly different. The protein, carbohydrate and sugar content was similar between groups, with two exceptions: i) pork rinds contained the highest level of protein (70 g/100 g) but was void of carbohydrate, and ii) root-based chips contained the highest level of sugars (32 g/100 g). Novel snacks presented a higher fiber content (with values up to 14.3 g/100 g) and a lower fat content (with values up to 29 g/100 g), when compared to traditional snacks (values up to 6.9 g/100 g for fiber and up to 35.5 g/100 g for total fat). Differences in the composition of snacks were more evident with regards to saturated fatty acid content, with novel snacks not exceeding 4 g/100 g, except in the case of salty banana chips (11.3 g/100 g), and traditional snacks displaying values up to 18 g/100 g. Similarly, novel formulations presented lower salt content (0.08–3.1 g/100 g) (Table 2) in comparison to traditional snacks (0.8–4.5 g/100 g) (Table 1). In this sense, the formulation of novel snacks provides a nutritional tool for achieving public health recommendations and population salt reduction strategies aimed at addressing the associated burden of chronic disease (Jaenke et al., 2017).

3.2. Acrylamide levels in snacks

Acrylamide levels in traditional snacks ranged from 44 to 671 µg/kg, with a mean value of 194 µg/kg (Table 3, Fig. 1). These levels are in concordance with those reported by the Food and Drug Administration (FDA, 2006), EFSA (EFSA, 2015), and other authors when analyzing similar categories of snacks (Takatsuki et al., 2003; Zubeldia Lauzurica and Gomar Fayos, 2007; Daniali et al., 2010; Cheng et al., 2012; Komthong et al., 2012; Shamla and Nisha, 2014). The lowest acrylamide content in the traditional sample group was observed in cheese leaf chips (44 µg/kg) and cheese puffs (48 µg/kg). On the other hand, the highest acrylamide values were found in ketchup flavored snacks (671 µg/kg) and in cheese and ketchup flavored snacks (660 µg/kg) (Table 3). Most of the traditional snacks are cereal-based (mainly corn and wheat) and may or may not be mixed with potato. While the specific technological process applied to each product is unknown, traditional methods in the snack industry typically include deep-frying, extrusion, drying and baking (Riaz, 2016). The high temperatures applied during deep-frying and baking promote the formation of process contaminants. This has been well reported in cereal and potato-based products (Açar and Gökmen, 2009; Gökmen et al., 2006). In the case of extrusion, foods are directly cooked in the extruder at 140–180°C. Upon leaving the extruder they are dried and shaped due to sudden decompression. Finally, they are toasted at temperatures as high as 330°C (Fast, 2001).

The acrylamide content of the alternative snacks ranged from none detected to 1393 µg/kg. The mean content (263 µg/kg) was higher than that of traditional snacks (194 µg/kg), though differences were not statistically significant. Four samples displayed very high values for acrylamide (Fig. 1): cereal, potato and soy snack (1393 µg/kg), potato sticks with carrot (1252 µg/kg), carrot chip (958 µg/kg) and beet chip (908 µg/kg) (Table 4). These findings support the need to examine contemporary formulations of food products using risk/benefit considerations. The search for healthier and more nutritious products to meet consumer demand has led snack manufacturers to use alternative technologies. In this respect, innovative treatments such as vacuum frying (Dueik et al., 2010), ultrasound-osmotic dehydration combined with the frying process (Dehghannya and Abedpour, 2018) and oven toasting at optimum process conditions (Nath and Chattopadhyay, 2007), have proven to be efficient in producing reduced-fat products. In addition, these novel snack formulations exhibit desirable quality attributes, preserve nutritional value and color properties, and improve the crispness and overall acceptability of products. However, modifications in the composition of the foodstuffs and the different processing conditions may also contribute to the generation of high

amounts of process contaminants (Cheng et al., 2012; Stadler and Studer, 2017). An example of this is given by Mesias et al. (2016) who incorporated chia flour into biscuits. While nutritional properties of the biscuits were improved, acrylamide content was also higher in the new formulation. A higher acrylamide formation has also been reported in fried potato crisps embellished with dragon fruit extract (Cheng et al., 2010). Despite improved antioxidant properties of the crisps, functional properties should be carefully considered alongside the risk/benefit, whereby increasing a benefit (antioxidant) should not increase a risk (process contaminants).

In June 2015, EFSA concluded that the presence of acrylamide in foods potentially increased the risk of developing certain types of cancer in all age groups (EFSA, 2015). Due to uncertainty surrounding the human health risk, exposure to acrylamide is a public health concern and a priority for the National Food Safety Authorities. Recently, the Directorate General for Health and Food Safety of the European Commission has established mitigation measures and updated benchmark levels for the reduction of acrylamide in food (European Commission, 2017). The benchmark levels target the main contributors of acrylamide in food. These include fried potato products, cereals, coffee and coffee substitutes, baby foods, and processed cereal based foods intended for infants and young children. The Commission Regulation established specific mitigation measures referring to French fries (ready-to-eat), potato crisps made from fresh potatoes and from potato dough, and other potato products made from potato dough. In the case of mixed dough, the reference value for potato dough is considered when potato is the major ingredient. The regulation does not yet include specific benchmark levels for snacks that are not potato dough-based. Reference values for cereal-based products (maximum 400 µg/kg excluding ginger bread) and potato products (750 µg/kg) are available that are based on their composition. In the traditional snack group, all samples formulated with potato exhibited acrylamide concentrations lower than the reference value. In contrast, two samples containing potato and pertaining to the novel snack group also showed levels higher than 750 µg/kg. As previously mentioned, these samples were potato sticks with carrot (1252 µg/kg) and snacks formulated with cereal, potato and soy (1393 µg/kg). Several variables directly affect acrylamide levels in potato products. These include the content of precursors in the fresh tuber (asparagine and reducing sugars), processing conditions and the final moisture (Elmore et al., 2005). Potatoes are a rich source of free asparagine (2010–4250 mg/kg) and reducing sugars (97–2550 mg/kg). Reducing the sugar content is a limiting factor of acrylamide formation in potato products (Becalski et al., 2004; Williams, 2005). Vegetables other than potatoes have a similar sugar and asparagine composition. Equivalent acrylamide formation during the thermal treatment could therefore be promoted. For example, levels of 515 µg/kg in fried pumpkin chips and 225 µg/kg in fried carrot chips (no other ingredients were present) have been recently reported by Mesias et al. (2018). In the present study, the acrylamide content in some of the vegetable chips (beet and carrot chips) were similar to those reported in French fries (Mesias and Morales, 2015) (Table 4).

Cereals contain lower levels of precursors of acrylamide compared with potato. Consequently levels of acrylamide are usually lower in cereal-based products (Granby et al., 2008). According to Cheng et al. (2012), the progression of acrylamide content among typically used bases for snack foods is rice flour < corn flour < wheat flour < wheat/potato mixed flour < rice/potato mixed flour. In the present study, six samples in the traditional snacks group and two samples in the novel formulations group presented acrylamide concentrations higher than 400 µg/kg. This exceeds the reference values recommended for all cereal-based products, apart from spices bread (European Commission, 2017). However, these concentrations are not only a product of the kind of cereal used, but result from the combination of all ingredients together with the processing conditions, which can also affect the formation of the contaminant.

Amongst the innovative snacks, those including legumes in their formulation showed levels of acrylamide ranging from none detected to 401 $\mu\text{g/kg}$ (mean value: 142 $\mu\text{g/kg}$) (Table 4). On the other hand, the concentration observed in seaweed chips was very low (43 $\mu\text{g/kg}$), corroborating results reported by Cheng et al. (2012). These authors stated that the low level of acrylamide in this foodstuff was due to the absence of reducing sugars and asparagine.

Exposure to acrylamide within the Spanish population has been estimated according to data on total per capita consumption of snacks as established by the Spanish Association of snack manufacturers (AFAP, 2015). In 2015, snack consumption was 6.43 kg/person/year, with 22% being snacks that did not include nuts and potato chips. Assuming an average adult body weight (bw) of 70 kg, exposure to acrylamide through the different groups of snacks ranges from 2.43 to 37.10 ng/kg bw/day for traditional snacks (mean: 10.77 ng/kg bw/day) and from 0.55 to 77.01 ng/kg bw/day for novel formulations (mean: 17.22 ng/kg bw/day) (Table 5). Both of these ranges are similar to those previously reported from potato crisps and potato chips in adult populations (Mojska et al., 2010; Sirot et al., 2012; Pugajeva et al., 2014; Mesias and Morales, 2015). Levels lower than 40 $\mu\text{g/kg}$ bw/day are considered to describe tolerable daily intake (TDI) for neurotoxicity of acrylamide, while levels lower than 2.6–16 $\mu\text{g/kg}$ bw/day have been associated with the development of some types of cancer (Tardiff et al., 2010). However, due to the carcinogenic properties of acrylamide, no safe levels can be established for this compound (FAO/WHO, 2010). The margin of exposure (MOE) for acrylamide in the different groups of snacks has also been calculated. MOE values for acrylamide were 28,783 and 18,002 for traditional and novel formulations, respectively, using the established benchmark doses (BMDL10) (310 $\mu\text{g/kg}$ bw/day) found for mammary tumors in female rats. Similarly, MOE values of 16,716 (traditional snacks) and 10,452 (novel formulations) were obtained when comparing with the established BMDL10 (180 $\mu\text{g/kg}$ bw/day) for Harderian gland tumors in male mice. According to the EFSA Scientific Committee on Contaminants in the Food Chain (CONTAM Panel), an MOE above 10,000 for a compound that is both genotoxic and carcinogenic is considered to be of low health concern (EFSA, 2015).

3.3. HMF and Furfural levels in snacks

Content of HMF and furfural in the individual snacks is presented in Table 3 (traditional snacks) and Table 4 (novel snacks). An overall comparison between both groups is provided in Fig. 2 (HMF content) and Fig. 3 (furfural content). Results showed a high variability between traditional and novel snacks, and amongst the various categories (cereal-based, root/tubers-based, legumes, and other). HMF levels ranged from none detected to 91.30 mg/kg in traditional snacks (Table 3) and from 0.40 to 94.70 mg/kg in novel formulations (Table 4) (Fig. 2). Mean HMF content in traditional snacks (7.00 mg/kg) was significantly lower than that found in the innovative formulations (18.52 mg/kg) ($p=0.042$). Differences were more pronounced with regards to furfural content, where novel formulations exhibited significantly higher concentrations (2.53 mg/kg) than traditional snacks (0.86 mg/kg) ($p=0.004$) (Fig. 3). To the best of our knowledge, the HMF and furfural content in novel snack formulations has not been discussed previously in the scientific literature.

As previously discussed for acrylamide, HMF and furfural formation depends greatly on the presence of precursors in the recipe and the conditions of the thermal process used. The addition of sugar in the caramelized bar shaped Japanese snack could therefore justify the high levels of HMF found in this snack (91.3 mg/kg) (Table 3). Similarly, the high reducing sugar content of roots could promote the higher formation of HMF in vegetable chips (Table 4). In fact, root-based novel formulations also demonstrated a raised acrylamide concentration, suggesting that the presence of sugar could be a key factor for the generation of both of these processing contaminants. The composition of cereals could also contribute to the different levels of HMF and furfural found in the snack formulations. The highest levels were found in

formulations containing rye and spelt, which concurs with results reported previously. Capuano et al. (2009) reported higher values for HMF in rye bread model systems relative to wheat bread. This might be explained by the higher free amino acids and protein content found in cereal. Spelt is also reported to have higher protein content than common wheat (Kohajdová and Karovicová, 2008), promoting the formation of HMF. This reiterates the importance of conducting risk/benefit analysis when introducing formulations for new food products. In this respect, the incorporation of chia flour in the formulation of biscuits has been reported to be desirable from a nutritional and health perspective, despite the associated increase in the content of process contaminants such as HMF and furfural (Mesias et al., 2016).

Taking a similar approach, exposure to HMF and furfural within the Spanish population through the consumption of the various snacks was estimated. In the case of HMF, traditional snacks were estimated to provide a mean of 0.38 µg/kg bw/day (range: 0.01–5.05 µg/kg bw/day) and novel formulations a mean of 1.02 µg/kg bw/day (range: 0.02–5.24 µg/kg bw/day) (Table 5). In the case of furfural, contributions were estimated to be 0.05 µg/kg bw/day (range: 0.01–0.21 µg/kg bw/day) and 0.14 µg/kg bw/day (range: 0.01–0.57 µg/kg bw/day) for traditional and novel snacks groups, respectively. Rufián-Henares and de la Cueva (2008) reported a mean exposure to HMF of 10 mg/day within the Spanish population. Using this data it would follow that traditional snacks and novel formulations contribute 0.27% and 0.71%, respectively, to overall HMF exposure.

It is well known that the formation of products through the Maillard reaction directly depends on the content of amino acids and reducing sugars, pH, moisture, and water activity of the food (Friedman, 1996). Despite parameters concerning the raw matters being unknown, the present study conducted statistical analyses of the relationships between the content of process contaminants and the nutritional composition of snacks, as declared by manufacturer. Analysis of Spearman correlations for non-parametric variables uncovered a significant relationship between HMF and sugar content ($\rho=0.389$, $p=0.000$). No significant correlations were observed with regards to furfural. On the other hand, correlations between the measured process contaminants were significant, as follows: acrylamide and HMF ($\rho=0.293$, $p=0.010$), acrylamide and furfural ($\rho=0.366$, $p=0.001$), and HMF and furfural ($\rho=0.465$, $p=0.000$).

4. CONCLUSIONS

The present study summarizes the presence of acrylamide, HMF and furfural in different categories of traditional and novel snacks. Results highlight that the reformulation of traditional snacks does not meet objectives for mitigating processing contaminants, despite being aimed towards healthier nutritional profiles, reducing fat and salt and increasing fiber content. Levels of acrylamide were higher in certain novel snacks, specifically those made from mixed dough, potato and/or other vegetables. These snacks even exceeded the reference value of 750 µg/kg established for acrylamide in potato products by the recent European regulation for potato-based snacks. Novel formulations for snacks also promoted to an even greater extent the formation of HMF and furfural. In this case, significant differences were found between traditional and novel snacks. Importantly, the present study reveals that the snack industry should not only consider the health-promoting benefits and sensorial characteristic modifications of products. It must also take into account the toxicological aspects of the process contaminants when designing novel products for release into the food market. To this end, it is recommended to include a risk/benefit evaluation of the control of process contaminants when designing novel snack formulas. The present study, therefore, urges food safety authorities to consider including a subgroup of non potato dough-based snacks when developing strategies for the control of acrylamide in foods. This will ensure that “healthy snacking habits” are also included within the global strategy for acrylamide mitigation.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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FIGURES AND TABLES

Table 1. Nutritional composition of traditional snacks per 100 g of sample. Information provided by the manufacturer

Sample	Energy (Kcal)	Total fat (g)	Saturated fat (g)	Carbohydrates (g)	Sugars (g)	Fiber (g)	Protein (g)	Salt (g)
Cereal based								
Cheese flavored corn snack	556	35.5	11.3	53.5	2.3	na	5.5	1.5
Corn strip	567	33	17	57	2.2	4.5	5	1.5
Corn cake	407	2.6	0.3	85.6	1	2	9.2	2.2
Corn chip	512	26.7	10.2	60.6	2.1	4.2	5.5	1.2
Spicy rolled corn snack	533	28	8.1	62	9	5.4	5.6	2.8
Puffcorn bars	467	17.4	2.2	71.1	1	na	6.6	2
Puffcorn	474	18.6	2.4	71	1.5	na	5.6	1.8
Corn snack cones	512	28	3.8	57.3	5.1	3.1	6.3	2.4
Polynesian corn flavored chip	497	25	3	58	3.3	6.9	6.1	1.9
Corn and onion chip	524	26.5	8.7	61.9	3.7	6	6.5	0.8
Ring shaped corn snack	530	24	11.1	71.1	0.5	na	5.7	3.7
Corn snack with barbecue flavor	543	32.2	14.7	54.9	4.8	4.6	6.1	1.5
Corn based snack	533	28	3.1	63	0.5	3.1	5	1.5
Cheese puff	503	27	3.8	59.2	6.3	1.2	5.1	2.1
Cheese and ketchup flavor snack	518	29.3	4.2	56.2	4.8	2.7	6	1.7
Sunflower seed stick	526	32	4.5	48	4	na	10	2.03
Cracker with puffed rice	428	9.5	1.4	74	2.2	2.5	1	1.3
Pretzel stick	393	5.9	0.8	71	3.4	4	12	4.5
Salty cracker	473	18	1.6	70	6	na	6.7	2
Salty cookie	508	26	9.9	62	9	na	6	2
Spicy and salty cookie	414	10	3.8	69	3.8	4	10	3
Salty cookie with sesame	414	10	3.8	69	3.8	4	10	3
Brezel cracker	414	10	3.8	69	3.8	4	10	3
Brezel mini stick	414	10	3.8	69	3.8	4	10	3

Puff pastry sticks	503	27	18	49	3.5	na	14	1.8
Cheese leaf chip	473	23.7	6.4	49.4	0.9	3.7	13.5	3.7
Wheat tortilla	484	31	3.2	47	3.6	5.6	5.4	1.4
Cracker	460	18	8.6	65	3	3	8	2.3
Wheat rind	501	26.1	3.4	55.6	1	3.7	9	3.3
Cocktail snack*	466	23	2.5	56	2.5	na	3.6	2.2
Japanese snack**	418	7.5	1.9	78.5	4.9	na	8	1.4
Root or tuber based								
Potato long chip	526	29	3	58.8	0.9	na	6.7	1.5
Potato based snack	453	17	2	69	0.4	3.3	5.1	2.3
Potato based snack with onion	506	32	3.5	51	3.1	2.7	4	1.5
Potato based triangles	478	22	2.5	66	0.5	3.1	3.6	2.5
Potato based snack with cheese	480	23.1	3.3	60.3	1.8	4.1	5.7	1.3
Others								
Pork rinds	487	23	7.7	0.0	0.0	na	70	3.7

na: not available. *Cocktail snack: onion flavored ring, grill snack, bacon strip snack, potato based ring, ketchup flavored snack. ** Japanese snack: Loop shaped, triangle shaped, flower shaped, bar shaped, caramelized bar shaped, ball shaped.

Table 2. Nutritional composition of novel formulations of snacks per 100 g of sample. Information provided by the manufacturer.

Sample	Energy (Kcal)	Total fat (g)	Saturated fat (g)	Carbohydrates (g)	Sugars (g)	Fiber (g)	Protein (g)	Salt (g)
Cereal based								
Corn cake with cheese and basil	489	18	2.2	73	4.3	1.7	7.8	1.1
Cereal, potato and soy snack	454	17	2	68	2.9	3.6	5.4	2.6
Cereal sticks with sunflower seeds	505	27	2.5	52	1.8	5	11	2.1
Bread sticks with olive and rosemary	499	27	2.9	54	3	4	9.5	1.7
Crusty bread roll with seeds and quinoa	507	29	3.3	48	1.7	7.2	12	1.5
Rice and oatmeal cake	389	3	0.6	78.8	3.6	5.5	8.8	0.08
Rice and quinoa cake	380	3	0.6	77	2.7	5.6	8.5	0.56
Rice and seven cereals cake	382	3.4	0.6	76.4	3.5	6.1	8.2	0.7
Rice cake	385	2	0.5	82.6	0.4	2.8	7.7	0.6
Rice crisp with sesame	431	7.3	2.1	83	0.5	1.3	7.6	1.3
Rice and whole flax toast	377	4.31	1.1	74.46	0.23	4.89	7.71	1
Rice and rye cake	364	1.6	0.3	75.2	3.0	8.1	8.0	0.13
Spelt cake	375	3.1	0.5	67.36	4.4	7.4	15.9	0.035
Quinoa snack with tomato and basil	460	17	2.0	65	2.6	5.8	8.4	2.2
Root or tuber based								
Carrot chip	432	25	2.8	40	32	14	5.4	0.9
Parsnip chip	432	25	2.8	40	32	14	5.4	0.9
Sweet potato chip	432	25	2.8	40	32	14	5.4	0.9
Beet chip	432	25	2.8	40	32	14	5.4	0.9
Potato sticks with tomato, carrot and spinach	454	15.6	1.4	75.3	3.7	1.4	2.4	2.38
Prawn snack (with cassava flour)	494	24.6	3.2	66.2	4.6	na	2.1	1.8
Legumes								
Pea chip	392	16	1.8	60	3.6	14.3	17.8	1.8
Mixed with cereal or potato								
Chickpea chip	508	28.4	4	51.8	1.4	4.7	8.8	2.5

Lentil chip	474	21.5	2.5	56.2	1.1	3.9	12.2	2.7
Pea snack with cheese and dill	463	18	2.2	60	3.9	4.8	13	2
Chickpea, corn and rice ring	463	15.7	2.2	73.5	3.3	2.1	5.8	0.92
Hummus snack with basil and parsley	459	18	2	61	2.9	6.2	11	2.1
Lentil snack with cheese and spices	471	20	2.3	56	5.2	6.8	15	1.9
Others								
Salty banana chip	497	24.5	11.3	63	2.7	7.3	2.5	0.48
Seaweed chip	403	8.2	1	78.6	5.2	2.7	5	3.1

na: not available.

Table 3. Acrylamide, HMF and Furfural content in traditional snacks.

Sample	Acrylamide ($\mu\text{g/kg}$)	HMF (mg/kg)	Furfural (mg/kg)
Cereal based			
Cheese flavored corn snack	73 ± 0.3	0.6 ± 0.0	nd
Corn strip	279 ± 4.2	4.6 ± 0.3	1.0 ± 0.1
Corn cake	105 ± 9.6	11.0 ± 1.0	3.8 ± 0.2
Corn chip	646 ± 11.8	2.0 ± 0.0	nd
Spicy rolled corn snack	74 ± 2.1	2.5 ± 0.0	0.5 ± 0.0
Puffcorn bars	146 ± 4.7	2.2 ± 0.3	0.7 ± 0.0
Puffcorn	108 ± 2.7	1.9 ± 0.1	1.3 ± 0.1
Corn snack cones	270 ± 7.8	0.7 ± 0.0	0.6 ± 0.0
Polynesian corn flavored chip	114 ± 0.9	3.7 ± 0.2	1.1 ± 0.0
Corn and onion chip	536 ± 8.6	2.9 ± 0.3	0.7 ± 0.1
Ring shaped corn snack	560 ± 2.9	0.8 ± 0.1	nd
Corn snack with barbecue flavor	76 ± 0.9	2.4 ± 0.0	nd
Corn based snack	120 ± 0.5	0.8 ± 0.1	0.7 ± 0.1
Cheese puff	48 ± 0.2	2.5 ± 0.2	0.7 ± 0.1
Cheese and ketchup flavor snack	660 ± 80.6	4.2 ± 0.0	2.9 ± 0.1
Sunflower seed stick	96 ± 1.9	6.4 ± 0.7	0.9 ± 0.0
Cracker with puffed rice	69 ± 3.8	1.0 ± 0.3	0.6 ± 0.0
Pretzel stick	144 ± 8.1	4.1 ± 3.3	1.7 ± 0.0
Salty cracker	68 ± 1.2	3.4 ± 0.4	nd
Salty cookie	272 ± 2.4	4.5 ± 0.1	nd
Spicy and salty cookie	267 ± 5.8	3.0 ± 0.1	nd
Salty cookie with sesame	233 ± 4.9	2.6 ± 0.1	nd
Brezel cracker	114 ± 0.1	1.8 ± 0.0	1.5 ± 0.0
Brezel mini stick	111 ± 1.9	2.2 ± 0.1	1.5 ± 0.0
Puff pastry sticks	181 ± 6.1	9.9 ± 0.1	0.2 ± 0.0
Cheese leaf chip	44 ± 2.2	1.1 ± 0.0	2.7 ± 0.2
Wheat tortilla	102 ± 2.7	1.2 ± 0.1	2.0 ± 0.1

Cracker	262 ± 2.7	1.9 ± 0.1	nd
Wheat rind	145 ± 7.1	12.7 ± 0.6	nd
Onion flavoring rings	335 ± 7.3	4.0 ± 0.1	0.6 ± 0.1
Grill snack	491 ± 3.1	1.7 ± 0.0	2.7 ± 0.1
Bacon strip snack	86 ± 0.4	8.8 ± 0.3	1.1 ± 0.1
Potato based ring	272 ± 29.5	1.3 ± 0.1	2.1 ± 0.0
Ketchup flavored snack	671 ± 0.7	2.7 ± 0.2	3.1 ± 0.2
Loop shaped Japanese snack	78 ± 1.9	69.4 ± 6.6	nd
Triangle shaped Japanese snack	70 ± 0.8	23.7 ± 0.2	nd
Flower shaped Japanese snack	72 ± 1.6	15.2 ± 0.0	nd
Bar shaped Japanese snack	75 ± 0.1	0.7 ± 0.1	nd
Caramelized bar shaped Japanese snack	63 ± 0.3	91.3 ± 1.5	nd
Ball shaped Japanese snack	75 ± 8.8	2.1 ± 0.1	0.7 ± 0.1
Root or tuber based			
Potato long chip	76 ± 2.6	0.4 ± 0.0	0.6 ± 0.0
Potato based snack	180 ± 3.2	0.9 ± 0.0	0.6 ± 0.1
Potato based snack with onion	74 ± 3.5	0.4 ± 0.0	0.3 ± 0.0
Potato-based triangles	123 ± 1.0	0.6 ± 0.0	nd
Potato based snack with cheese	246 ± 5.1	0.4 ± 0.0	nd
Others			
Pork rinds	53 ± 0.1	nd	0.4 ± 0.0

nd: not detected.

Table 4. Acrylamide, HMF and Furfural content in novel formulations of snacks.

Sample	Acrylamide ($\mu\text{g/kg}$)	HMF (mg/kg)	Furfural (mg/kg)
Cereal based			
Corn cake with cheese and basil	143 ± 1.6	10.4 ± 0.1	3.0 ± 0.1
Cereal, potato and soy snack	1393 ± 65.2	9.4 ± 0.2	2.5 ± 0.1
Cereal sticks with sunflower seeds	106 ± 5.5	5.8 ± 0.0	1.4 ± 0.0
Bread sticks with olive and rosemary	112 ± 4.2	10.5 ± 0.4	2.2 ± 0.1
Crusty bread roll with seeds and quinoa	78 ± 2.4	15.6 ± 0.1	1.5 ± 0.0
Rice and oatmeal cake	253 ± 9.1	25.4 ± 2.7	4.1 ± 0.3
Rice and quinoa cake	191 ± 1.6	30.3 ± 2.5	4.5 ± 0.2
Rice and seven cereals cake	178 ± 20.6	10.5 ± 1.2	2.5 ± 0.2
Rice cake	147 ± 10.0	14.6 ± 0.1	2.9 ± 0.1
Rice crisp with sesame	69 ± 1.1	1.3 ± 0.0	0.4 ± 0.0
Rice and whole flax toast	52 ± 0.4	0.4 ± 0.0	0.4 ± 0.0
Rice and rye cake	341 ± 28.6	73.2 ± 6.1	10.3 ± 0.8
Spelt cake	594 ± 11.2	63.5 ± 2.1	9.8 ± 0.6
Quinoa snack with tomato and basil	32 ± 0.8	1.8 ± 0.2	1.0 ± 0.1
Root or tuber based			
Carrot chip	958 ± 73.4	92.1 ± 3.4	9.4 ± 0.3
Parsnip chip	235 ± 13.7	94.7 ± 6.3	2.3 ± 0.2
Sweet potato chip	265 ± 4.6	53.2 ± 0.5	6.4 ± 0.2
Beet chip	908 ± 32.0	27.5 ± 0.7	3.7 ± 0.0
Potato sticks with tomato	684 ± 1.1	2.2 ± 0.1	1.1 ± 0.0
Potato sticks with carrot	1252 ± 15.6	13.3 ± 0.9	2.7 ± 0.2
Potato sticks with spinach	354 ± 6.0	1.3 ± 0.1	nd
Prawn snack (with cassava flour)	36 ± 0.4	1.4 ± 0.1	nd
Legumes			
Pea chip	nd	1.5 ± 0.0	0.8 ± 0.0
Mixed with cereal or potato			
Chickpea chip	401 ± 7.5	1.2 ± 0.1	0.9 ± 0.1

Lentil chip	317 ± 0.3	1.0 ± 0.0	nd
Pea snack with cheese and dill	64 ± 0.7	0.6 ± 0.0	nd
Chickpea, corn and rice ring	102 ± 3.7	1.1 ± 0.1	1.1 ± 0.1
Hummus snack with basil and parsley	55 ± 0.4	3.7 ± 0.1	0.9 ± 0.1
Lentil snack with cheese and spices	45 ± 0.0	1.2 ± 0.0	nd
Others			
Salty banana chip	240 ± 20.3	3.9 ± 0.4	1.7 ± 0.1
Seaweed chip	43 ± 1.0	1.4 ± 0.0	nd

nd: not detected.

Table 5. Exposure to acrylamide (ACR), hydroxymethylfurfural (HMF) and furfural through the consumption of snacks.

	Mean $\pm$ SD	Minimum	Maximum	P25	P75	P95
ACR (ng/kg bw/day)						
Traditional	10.77 $\pm$ 17.22 A	2.43	37.10	4.13	14.8	36.22
Novel formulations	17.22 $\pm$ 20.14 A	0.55	77.01	3.54	19.57	72.33
HMF (μg/kg bw/day)						
Traditional	0.38 $\pm$ 0.92 A	0.01	5.05	0.05	0.23	2.95
Novel formulations	1.02 $\pm$ 1.51 B	0.02	5.24	0.07	1.40	5.15
Furfural (μg/kg bw/day)						
Traditional	0.05 $\pm$ 0.52 A	0.01	0.21	0.01	0.06	0.17
Novel formulations	0.14 $\pm$ 0.16 B	0.01	0.01	0.02	0.16	0.55

Different letters indicate statistically significant differences ($p < 0.05$) between the groups of traditional snacks ($n = 46$) and novel formulations of snacks ($n = 31$).

Figure 1. Box-and-whisker plot of acrylamide content in traditional and novel formulations of snacks. Differences were not found significant ($p > 0.05$). Mean values: 194 $\mu\text{g/kg}$ (traditional), 263 $\mu\text{g/kg}$ (novel).

Figure 2. Box-and-whisker plot of hydroxymethylfurfural content (HMF) in traditional and novel formulations of snacks. * Differences were found significant ($p < 0.05$). Mean values: 7.00 mg/kg (traditional), 18.52 mg/kg (novel).

Figure 3. Box-and-whisker plot of furfural content in traditional and novel formulations of snacks. * Differences were found significant ($p < 0.05$). Mean values: 0.86 mg/kg (traditional), 2.53 mg/kg (novel).

Supplementary Material

Annex I. Major ingredients in the traditional snacks.

Sample	Major ingredients
Cereal based	
Cheese flavoured corn snack	Corn flour, cheese
Corn strip	Corn flour
Corn cake	Corn flour
Corn chip	Corn flour
Spicy rolled corn snack	Corn flour
Puffcorn bars	Corn flour
Puffcorn	Corn flour
Corn snack cones	Corn flour
Cheese puff	Corn flour, cheese
Polynesian corn flavoured chip	Corn flour
Corn and onion chip	Corn flour, onion
Ring shaped corn snack	Corn flour
Corn snack with barbecue flavour	Corn flour, barbecue sauce
Corn based snack	Corn and potato flour
Cheese and ketchup flavour snack	Corn, wheat and potato flour, cheese, ketchup
Sunflower seed stick	Wheat flour, sunflower seeds
Cracker with puffed rice	Wheat flour, rice
Pretzel stick	Wheat flour
Salty cracker	Wheat flour
Salty cookie	Wheat flour
Spicy and salty cookie	Wheat flour
Salty cookie with sesame	Wheat flour, sesame seeds
Brezel cracker	Wheat flour
Brezel mini stick	Wheat flour
Puff pastry sticks	Wheat flour, butter, cheese, skim milk powder
Cheese leaf chip	Wheat flour, cheese

Wheat tortilla	Wheat flour
Cracker	Wheat flour
Wheat rind	Wheat flour
Cocktail snack*	Wheat, rye, corn and potato flour
Japanese snack**	Wheat, cassava and corn flour, soybean sauce, seaweed
Root or tuber based	
Potato long chip	Potato and wheat flour
Potato based snack	Potato
Potato based snack onion	Potato flour, sour cream, onion
Potato-based triangles	Potato and corn flour
Potato based snack with cheese	Potato, wheat and corn flour, cheese
Others	
Pork rinds	Pork rind

*Cocktail snack: onion flavoured ring, grill snack, bacon strip snack, potato based ring, ketchup flavoured snack. **Japanese snack: Loop shaped, triangle shaped, flower shaped, bar shaped, caramelized bar shaped, ball shaped.

Supplementary Material

Annex 2. Major and singular ingredients of novel snacks.

Sample	Major and singular ingredients
Cereal based	
Corn cake with cheese and basil	Corn flour, cheese, basil
Cereal, potato and soy snack	Corn and potato flour, soybean, cheese, tomato, aromatic plants
Cereal sticks with sunflower seeds	Wheat
Bread sticks with olive and rosemary	Wheat flour, black olives, tomato, rosemary
Crusty bread roll with seeds and quinoa	Wheat and quinoa flour, sesame seeds
Rice and oatmeal cake	Rice and oatmeal flour
Rice and quinoa cake	Rice and quinoa flour
Rice and seven cereals cake	
Rice cake	Rice flour
Rice crisp with sesame	Rice flour, sesame seeds
Rice and whole flax toast	Rice and flax flour
Rice and rye cake	Whole rice and rye flour
Spelt cake	Spelt flour
Quinoa snack with tomato and basil	Whole quinoa and rice flour, potato, tomato, basil
Root or tuber based	
Carrot chip	Carrot
Parsnip chip	Parsnip
Sweet potato chip	Sweet potato
Beet chip	Beet
Potato sticks with tomato, carrot and spinach	Potato flour, tomato, carrot, spinach
Prawn snack (with cassava flour)	Cassava flour, prawn, egg, sugar
Legumes	
Pea chip	Pea
Mixed with cereal or potato	
Chickpea chip	Chickpea and potato flour

Lentil chip

Lentil and potato flour

Pea snack with cheese and dill

Rice and pea flour, cheese, dill

Chickpea, corn and rice ring

Corn, chickpea and rice flour, cheese

Hummus snack with basil and parsley

Rice and whole chickpea flour, onion, basil, parsley

Lentil snack with cheese and spices

Whole lentil and rice flour, milk, cheese, spices

Others

Salty banana chip

Banana

Seaweed chip

Seaweed, wheat flour