JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Okara Promoted Acrylamide and Carboxymethyl-lysine Formation in Bakery Products

Mariantonella Palermo,[†] Alberto Fiore,[‡] and Vincenzo Fogliano*[,‡]

[†]Department of Agricultural Engineering and Agronomy, University of Naples Federico II, via Università 100, I-80055 Portici (Naples), Italy

[‡]Department of Food Science, University of Naples Federico II, Via Università 133, Parco Gussone Edificio 84, I-80055 Portici (Naples), Italy

ABSTRACT: Soybeans are widely used in bakery products because of their technological advantages and, recently, soybean-containing products have been marketed as functional foods thanks to several health benefits. Okara is a soybean-based ingredient obtained after elimination of the water-soluble component from ground soybeans. In this paper the effect of okara addition to bakery products on the formation of some potentially harmful Maillard reaction products was evaluated. Cookies obtained by replacing 15% of wheat flour with okara showed a visible browning increase and a more intense Maillard reaction development as shown by higher concentrations of 5-hydroxymethyl-2-furaldehyde (HMF) (+100%), acrylamide (+60%), and carboxymethyl-lysine (CML) (+400%) with respect to the control. This phenomenon could be related to the presence in okara of about 50% of insoluble dietary fiber: the fiber reduces water activity during cooking, thus promoting Maillard reaction. To confirm this hypothesis, cookies obtained by replacing 7% of wheat flour with three different types of dietary fiber (cellulose, chitosan, and pea fiber) were prepared: these experimental cookies showed higher Maillard reaction product concentration with respect to the control and, in particular, HMF and CML values were directly related to the fiber water-holding capacity (WHC). To extend the observation to the food market, a sampling of soybean-containing commercial bakery products was analyzed by comparing the concentrations of Maillard reaction products with those of similar bakery products without soy. Soybean-containing samples showed higher concentrations of acrylamide and CML than corresponding controls.

KEYWORDS: *soybean, okara, acrylamide, dietary fiber, carboxymethyl lysine*

INTRODUCTION

Soybean flour and soybean protein isolates are widely used in bakery products because of technological advantages such as water binding, dough conditioning, crust coloration, and protein supplementation.[1] Recently, many soybean-containing products have been designated functional foods thanks to the several health benefits attributed to soybeans, such as hypocholesterolemic activity, lowering the risk of coronary heart disease, and reducing prostate and breast cancer risks.[2]

Vegetarians or vegans are heavy consumers of soy products,[3] and they have lower mean body mass index, mean plasma total cholesterol concentration, mortality from ischemic heart disease, risk for some other diseases such as constipation, diverticular disease,[4] and metabolic syndrome risk factors associated with microinflammation than omnivores.[5]

Maillard reaction products (MRPs) are formed during the heat treatment of food,[6] and a diet rich in heated processed foods (baked, roasted, and fried products) implies the intake of several grams of MRPs, which are named dietary advanced glycation end products (AGEs).[7]

A significant correlation has been found between ingested and circulating AGEs in humans,[8] and several studies have suggested that dietary MRPs are implicated in the development of glycation and inflammation associated diseases such as renal failure, diabetes, and Alzheimer's disease.[9−11] Interestingly, higher levels of plasma AGEs were found in vegetarian people than in omnivores, and this phenomenon has not yet been well explained.[2]

Bakery products are among the pillars of the human diet,[13] and the Maillard reaction is the main chemical event occurring in this kind of foodstuff during cooking.[14] In bakery products the principal markers of Maillard reaction development are 5-hydroxymethyl-2-furaldehyde (HMF), acrylamide, and carboxymethyl-lysine (CML). Bakery products containing soybean derivatives are widely used, in particular, by vegetarian people to achieve the recommended protein daily intake.

The aim of this study was first to evaluate the effects of the addition to bakery product formulations of okara, which is a soybean product obtained after extraction of the water-soluble component, on the formation of potentially harmful MRPs. Cookies prepared by partially replacing wheat flour with okara were analyzed measuring acrylamide, CML, and HMF formation.

As a second step, a sampling of commercial soybean-containing bakery products was analyzed aiming at verifying if there is a relationship between the soybean addition and the amount of dietary MRPs.

MATERIALS AND METHODS

Materials. All chemicals of analytical grade were obtained from Sigma-Aldrich (St. Louis, MO, USA), unless mentioned otherwise.

Received: June 26, 2012
Revised: September 12, 2012
Accepted: September 21, 2012
Published: September 21, 2012

dx.doi.org/10.1021/jf302750q | J. Agric. Food Chem. 2012, 60, 10141−10146

$^{13}C_3$-Acrylamide (isotopic purity = 99%) was from Cambridge Isotope Laboratories (Andover, MA, USA). Oasis-HLB cartridges (30 mg, 1 mL) were supplied by Waters (Milford, MA, USA). Soybean seeds, wheat flour, palm oil, and sugar were purchased from a local market. Cellulose was from Chimpex Industriale Spa (Italy), chitosan from A.CEF Spa (Italy), and cortical pea fiber from I.T.ALI. Srl (Italy).

Nine different types of bakery products (with or without soy addition) were analyzed: crispbread, breakfast cereals, cracker, toasted bread, cereal bars, shortbread, wholemeal cookies, cream cookies, dry cookies. Partially defatted and extruded soy flour was analyzed, too. All bakery products were purchased from supermarkets in Italy.

Preparation of Okara. According to the method of Palermo et al.,[15] soybean seeds were soaked in water (ratio 1:10 w/v) at room temperature for 24 h, and soaked soybeans were milled with the same amount of water. The obtained paste was boiled for 30 min and the material filtered to separate soy milk from residue pulp. Okara was freeze-dried and finely ground. One hundred grams of dried okara contained 33.60 g of protein, 9.40 g of fat, and 48.78 g of dietary fiber.

Preparation of Cookies. The model cookies were prepared according to a recipe described in an American Association of Cereal Chemists (AACC) method. Soy effect was evaluated by replacing 15% of the flour content with dried okara, and fiber effect was evaluated by replacing 7.5% of the flour content with dietary fiber to obtain the same fiber amount of cookies with okara; the compositions of model systems are reported in Table 1.

Table 1. Ingredients of Model System Cookies Containing Okara or Different Dietary Fiber As Detailed in the Text

	control cookies	soy cookies	fiber cookies
wheat flour (g)	80.0	68.0	74.1
okara (g)		12.0	
dietary fiber (cellulose or pea fiber or chitosan) (g)			5.9
shortening (palm oil) (g)	20.0	20.0	20.0
sucrose (g)	35.0	35.0	35.0
$NaHCO_3$ (g)	0.8	0.8	0.8
NaCl (g)	1.0	1.0	1.0
NH_4HCO_3 (g)	0.4	0.4	0.4
water (mL)	17.6	19.3	18.4

Shortening, sugar, salt, and $NaHCO_3$ were mixed 1 min + 1 min + 1 min. NH_4HCO_3 dissolved in water was added to the mixer for 20 s + 20 s + 20 s. Flour was added as the last ingredient and mixed for 10 s + 10 s + 10 s (total = 30 s). Dough was kept for 30 min in the refrigerator (4 °C) because palm oil melts very easily at room temperature and leaks from the dough, but before rolling and molding, the dough was kept at room temperature for 5 min to soften it.

Dough was shaped in a small cylinder having a diameter of 3 cm and thickness of 0.3 cm. Each time the same amount of dough (~20 g) was put in a laboratory oven set at 200 °C for 14 min.

Cookies were freeze-dried and finely ground.

Chemical Parameters. Moisture was determined by oven-drying at 105 ± 1 °C (AOAC, 1995).

Quantitative determination of reducing sugar was performed by using a Fehling solution.[16]

For water-holding capacity (WHC) determination, 1 g of fiber was mixed with 50 mL of distilled water vigorously for 1 min and then centrifuged for 15 min at 10000*g* at 20 °C. The supernatant was discarded, and the tube was kept inverted for 10 min; results were expressed as grams of holding water per gram of fiber.[17]

All determinations were performed three times for each sample.

Instrumental Measurement of Color. The color was measured by the CIE $L^*a^*b^*$ system using a Minolta CM2600d (KonicaMinolta, Japan). Six measurements representing the cookies were taken from each sample over a measuring area of 8 mm. The instrument was standardized using standard white plates. Browning is inversely related with b^* values.

Asparagine Determination. Asparagine content was determined in duplicate according to a modified method suitable for use with the commercially available Ez:Faast amino acid kit.[18] The freeze-dried and milled okara sample (0.5 g) was extracted by 2 mL of water and sonicated at room temperature for 10 min. The mixture was then centrifuged at 4000*g*, and 100 μL of the supernatant was subjected to solid phase extraction and derivatization steps using the Ez:Faast technology and kit. Chromatographic separation was performed using an HPLC apparatus equipped with two micropumps, series 200 (PerkinElmer, Shelton, CT, USA), and an Ez:Faast column (250 mm × 3 mm, particle size = 4 μm) (Phenomenex, Torrance, CA, USA). The eluents were (A) water containing 10 mM ammonium formiate and (B) methanol containing 10 mM ammonium formiate. The gradient program was as follows, at a constant flow of 0.5 mL/min: 0.00 min, 68% B; 13.00 min, 83% B; 13.01 min, 68% B; 17.00 min, 68% B. The LC flow was split, and 200 μL/min was sent to the mass spectrometry. MS analysis was performed on an API 2000 triple-quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with an electrospray ionization working in the positive ion mode.

Acrylamide Determination. The samples were prepared for acrylamide analysis using a procedure described elsewhere[19] with some modifications. Two grams of sample was dispersed into 9 mL of water, d_3-acrylamide (100 μL of 20 ppm solution) was added, and the mixture was homogenized for 10 min in a vortex mixer. The coextracted colloids were precipitated by adding 1 mL of a 0.68 M potassium hexacyanoferrate(II) trihydrate solution (Carrez I) and 1 mL of a 2 M zinc sulfate heptahydrate solution (Carrez II). The mixture was centrifuged at 5000 rpm for 10 min; centrifugation was performed at 4 °C to separate fat at the top as a solid layer. Aqueous extract was transferred into a tube, and the solid residue was extracted twice again with 5 mL of water. After centrifugation, the clear supernatants were collected, and the extract obtained was eluted through a preconditioned Oasis MCX cartridge at a rate of 1 drop/s. The extraction procedure was repeated twice for each sample.

LC-MS/MS analysis was performed using an API 2000 triple-quadrupole mass spectometer (Applied Biosystem Sciex), with an electrospray interface, coupled to HPLC binary micropumps (Perkin-Elmer series 200). Analytical separation was achieved with an Inertsil ODS-3 column (25 × 0.46 cm, 5 μm) (GLC-Sciences, Tokyo, Japan) using isocratic elution with a mobile phase of 0.2% formic acid in water at a flow rate of 0.8 mL min^{-1}. Quantification was carried out in multiple reaction monitoring (MRM) mode at m/z ratios of 72.1 and 75.1 for acrylamide and [2,3,3-d_3]-acrylamide, respectively. Moreover, m/z 55.1 and 44.0 and m/z of 58.0 and 44.0 corresponding to specific molecular fragments of acrylamide and [2,3,3-d_3]-acrylamide, respectively, were monitored. The ions were obtained through fragmentation by specific collision energy of a selected ion precursor, applying a voltage of 4.5 kV.

A delay time of 3 min was selected to avoid the introduction of coextracted matrix components into the MS/MS instrument prior to acrylamide elution. The needle and cone voltages were set at 3.0 kV and 100 V, respectively. Nitrogen was used as nebulizer gas (12.0 L h^{-1}), and the source temperature was set at 350 °C. Acrylamide was quantified using a linear calibration function that was established with standard solutions of acrylamide and [2,3,3-d_3]-acrylamide dissolved in Milli-Q water (25−1000 μg/L). Acrylamide contents in sample extracts were calculated from the calibration curve and intercept value, taking into account the recovery calculated by means of the [2,3,3-d_3]-acrylamide curve. Two injections were performed for each extract.

HMF Determination. The analysis of HMF was performed as described by García-Villanova et al.[20] with slight modifications. The extraction protocol was the same as the acrylamide extraction protocol, but after the third centrifugation, samples were filtered (0.45 μm) and analyzed by HPLC (Shimadzu, Kyoto, Japan). The mobile phase was a mixture of acetonitrile in water (5% v/v) at a flow rate of 1 mL/min under isocratic conditions and a Synergy 4 μm Hydro-RP 80A, 25 × 4.6 cm (Phenomenex) column. The UV detector was set at 280 nm, and HMF was quantified using the external standard. Two injections were performed for each extract.

Table 2. Acrylamide Content, HMF Content, CML Content, and b^* Value in Cookies with Okara and with Fiber (Mean Value ± SE)[a]

	acrylamide (μg/kg dry basis)	HMF (mg/kg dry basis)	CML (mg/kg dry basis)	b^*
control cookies (wheat flour only)	361.88 ± 19.97 c	34.81 ± 2.02 b	6.32 ± 0.45 d	27.33 ± 0.47 a
okara cookies	588.84 ± 27.54 a	67.48 ± 1.35 a	22.84 ± 0.30 a	24.40 ± 0.42b
cellulose cookies	513.76 ± 7.75 b	32.89 ± 3.52 b	9.98 ± 0.30 b	27.86 ± 0.31 a
pea fiber cookies	540.56 ± 7.84 ab	28.13 ± 2.80 b	9.09 ± 0.47 b	24.41 ± 0.52 b
chitosan cookies	544.64 ± 8.56 ab	14.93 ± 0.40 c	7.66 ± 0.14 c	24.17 ± 0.58 b

[a]Different letters within the same column indicate significant differences at $P < 0.05$.

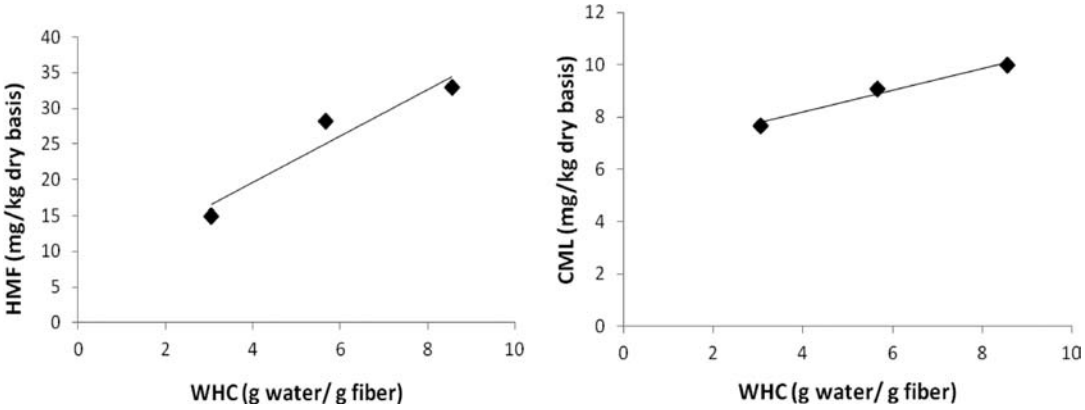

Figure 1. Correlation between fiber WHC and the concentration of Maillard reaction products: (left) HMF; (right) CML.

CML Determination. CML extraction was performed as described by Charissou et al.[21] with slight modifications.

Ten milligrams of ground samples was reduced overnight at room temperature by adding 100 μL of 500 mM $NaBH_4$ in 0.2 M borate buffer, pH 9.2. After the addition of the internal standard (100 μL of 1 ppm solution), the reduced samples were hydrolyzed in 5 mL of 6 M HCl at 110 °C for 24 h. Five hundred microliters was dried under vacuum (Speed Vac concentrator, Savant, Farmingdale, NY, USA) and further dissolved in distilled water (500 μL), filtered (nylon 0.22 μm), and dried again. The extraction procedure was repeated twice for each sample.

LC analysis was performed as described Schettgen et al.[22] with some modifications, with Micro HPLC series 200 (PerkinElmer) equipment including a binary gradient pump, degasser, and autosampler. Chromatographic separation was performed on a Tosoh Bioscience TSK Gel Amide-80 column (250 mm × 2 mm, particle size = 5 μm).

The solvents used to prepare the mobile phase were 5 mmol L^{-1} aqueous ammonium formiate buffer, and these conditions were kept for a run of 10 min.

The mobile phase flow rate was constant at 0.2 mL min^{-1}. Mass spectrometric analysis was performed using a Sciex API 2000 tandem mass spectrometer (MS/MS) with an electrospray ionization (ESI) source working in the positive ion mode. Instrument control, data acquisition, and evaluation were performed with Analyst 1.3.2 software from PerkinElmer. The operating conditions were as follows: applied ESI needle potential, +5000 V; nitrogen as nebulizer gas, pressure = 12 psi; as turbo heater gas, 500 °C; and as collision gas; collision gas and curtain gas were set at 10 and 8 instrument units, respectively. Two injections were performed for each extract.

Statistical Analysis. Differences among model system cookies were determined by analysis of variance and Duncan's multiple-range test ($P \leq 0.05$).

Differences between products with and without soybean addition were determined by Student's t test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Formation of Maillard Reaction Products in Cookies with Added Soybean. In the first set of experiments the effects of the okara addition to a cookie model system formula were evaluated. The okara ingredient used in this study contains about 50% DF and >30% proteins. According to previous works,[23,24] the main constituent in okara is dietary fiber, but it also presents high protein content, so it is useful to fortify bakery products, particularly those intended for vegetarians and vegans.[25]

Okara samples used in this study had an asparagine concentration of 0.12 g kg^{-1}, which is a value in line with those usually present in wheat flour.[26,27] On the other hand, okara is richer in reducing sugar than wheat flour: our okara sample has 3.83 g/100 g of reducing sugar, whereas the average content of reducing carbohydrates in wheat flour is 1.7 g/100 g.[28]

In Table 2 MRP content and b^* colorimetric values in model cookies are shown. During cookie baking, the water in the dough was completely removed (final moisture was below 0.5%), so there were no differences in terms of the final relative humidity and in terms of water activity among model system cookies MRP concentration data showed that okara-containing cookies had higher browning and significantly higher values of HMF (+100%), acrylamide (+60%), and CML (+400%) with respect to the control: the MRP content was influenced only by the flour replacement and not by the moisture.

Formation of Maillard Reaction Products in Cookies with Added Dietary Fiber. To explain the increased presence of MRPs in the okara-containing cookies, other recipes replacing okara with different types of dietary fiber were prepared.

The okara sample used in this study had 48.8% of dietary fiber, and previous work indicates that okara polysaccharides contain predominantly galactan, arabinan, arabinogalactan, xylogalacturonan, rhamnogalacturonan, xylan, xyloglucan, and cellulose:[29] these polysaccharides bind water, so, during baking of cookies, they reduce water availability and for this reason they have the potential to speed the Maillard reaction.

Table 3. Acrylamide Concentrations Determined in a Sampling of Commercial Soybean-Containing Commercial Samples (Mean Value ± SE) and Acrylamide Concentration Reported by the European Food Safety Agency (EFSA, 2006)

	concn (μg/kg dry basis) in commercial samples			
	soy-containing	conventional (control)	signif[a]	concn (μg/kg dry basis) in EFSA ref
crispbread	198.36 ± 40.50	nd		5−2838
shortbread	341.25 ± 22.57	189.04 ± 7.30	*	5−2949
wholemeal cookies	496.33 ± 7.96	169.58 ± 16.20	*	15−499
toasted bread	48.45 ± 3.66	nd		10−1430
breakfast cereals	64.42 ± 5.93	90.04 ± 5.39	*	10−440
crackers	97.27 ± 4.89	nd		5−830
dry cookies	63.16 ± 1.86	272.14 ± 23.81	*	10−4256
butter cookies	743.78 ± 8.06	62.22 ± 7.96	*	5−3324
cereal bars	112.04 ± 1.49	85.47 ± 3.71	*	96
extruded soy flour	118.77 ± 18.26			

[a]* indicates significant difference between soybean-containing and non-oybean-containing samples at $P < 0.05$.

Results of Table 2 demonstrated that the addition of insoluble dietary fiber to cookies increased the concentration of acrylamide and CML with respect to the control. The dietary fibers used in the formulas have different WHC values: cellulose showed the highest value of WHC, namely, 8.55 g water/g fiber. Cortical pea fiber and chitosan had WHC values of 5.66 and 3.05, respectively.

Interestingly, the type of fiber did not significantly modify the acrylamide content, whereas dietary fiber differently influenced HMF and CML values with respect to okara: in the case of chitosan cookies, the HMF concentration was below the control, whereas all dietary fiber increased the CML to a much lesser extent with respect to okara.

As shown in Figure 1 significant correlation was found between the fiber WHC and HMF concentration in cookies (R^2 = 0.9157) and between fiber WHC and CML content in cookies (R^2 = 0.9735). This figure indicated that the greater the fiber WHC, the faster the Maillard reaction development as the water activity decreased.

Previous works studied the addition of polysaccharides in bakery products (bread, in particular) and reported a higher browning related to the development of the Maillard reaction. Anil[30] added 5−10% of hazelnut testa to wheat flour bread and found differences in terms of crust color. Similar results were also reported by Gómez et al.:[31] they analyzed bread amended with several types of dietary fiber. In general, no significant color differences were observed between the control bread and the 2% fiber-supplemented bread, but breads with 5% of fiber produced a darker crumb. A good correlation between Maillard product content and browning development has been repeatedly reported.[32,33]

The possible reason for the dramatic increased concentration of CML in soybean-containing cookies could be related to the okara lipid composition. The okara fatty acid profile presents polyunsaturated fatty acids such as linoleic acid (54% of the total fatty acids content) and linolenic acid (9% of the total fatty acids content),[34] and many papers[35−37] have demonstrated that the presence of polyunsaturated fats can markedly contribute to MRP formation, which is higher than in the system containing fats less easil oxidized.

A greater Maillard reaction in the presence of soy-added products was also reported by Guerra-Hernandez et al.[38] Their analysis of infant cereal foods reported a higher furosine concentration in those containing soybean.

Maillard Reaction Product Concentrations in Commercial Cookies Containing Soybean. Table 3 shows acrylamide concentrations determined in a sampling of soybean-containing commercial samples and correlates them with data on acrylamide concentration reported by the European Food Safety Agency:[39] the EFSA database reported a large number of samples for each type of bakery product, but no products with soybean added.

Data reported in Table 3 for wheat-based products demonstrated that our data are quite in line with the literature, being well within the range reported by EFSA for each product category. Interestingly, looking at the comparison between conventional and soybean-containing products in most of the cases, those containing soybean showed higher acrylamide content. This is even more intriguing considering that in all cases but one the water content is higher in soybean products, and it is known that the lower the water concentration of the bakery products, the higher the formation of acrylamide. On the other hand, the sample designated "dry cookies" behaved as expected, having a lower water content than the corresponding soybean product (2.62 ± 0.09 vs 4.05 ± 0.05), and also showed a higher acrylamide concentration (272.14 ± 23.81 vs 63.16 ± 1.86). A similar behavior was also observed for breakfast cereal product: lower water content and higher acrylamide concentration in the conventional products.

A very high acrylamide concentration was found in butter cookies with soy addition: this feature is also related to the fact that in this product sucrose was replaced by rice syrup, which is a mixture of reducing sugars (glucose, maltose, and maltotriose).[40]

Table 4 shows HMF and CML concentrations and correlates them with proximate composition.

Unlike what was observed for acrylamide, in most of the analyzed foods, HMF content was higher in products without soy than in similar products with soy. For example, crispbread, shortbread, and butter cookie samples without soybean showed higher values than samples with soy addition. This could be explained by the higher sugar content in the analyzed products without soybean as HMF is formed from the degradation of sugars at high temperatures.[41] HMF was not detected in wholemeal cookies and breakfast cereals with soybean addition. Toasted bread with soy addition showed not only higher acrylamide content but also higher HMF content than toasted bread without soybean addition. Literature data for HMF content in bakery products are in line with those here obtained: dry cookie values ranged from 0.5 to 182.5 mg/kg;[42−44] breakfast cereal values ranged from 3.7 to 193 mg/kg;[43] and toasted bread values range from 11.8 to 90 mg/kg.[43,45]

Table 4. MRP Concentration and Proximate Composition in Commercial Samples (Mean Value ± SE)

	HMF (mg/kg dry basis)			CML (mg/kg dry basis)			water content (%)			reducing sugar content (%/dry basis)		
	soy products	conventional products (control)	signif[a]	soy products	conventional products (control)	signif[a]	soy products	conventional products (control)	signif[a]	soy products	conventional products (control)	signif[a]
crispbread	4.53 ± 0.28	12.39 ± 0.41	*	18.2 ± 0.19	1.32 ± 0.27	*	2.09 ± 0.19	1.06 ± 0.02	*	2.76 ± 0.01	3.94 ± 0.00	*
shortbread	3.23 ± 0.31	6.07 ± 1.79	*	1.13 ± 0.26	1.43 ± 0.11	ns	2.08 ± 0.09	1.07 ± 0.24	*	21.65 ± 0.02	24.86 ± 0.06	*
wholemeal cookies	nd	4.73 ± 0.16		4.14 ± 0.51	1.73 ± 0.60	*	6.13 ± 0.18	2.81 ± 0.04	*	20.24 ± 0.04	15.43 ± 0.01	*
toasted bread	35.88 ± 0.31	20.43 ± 0.62	*	0.12 ± 0.05	2.75 ± 0.10	*	1.34 ± 0.02	6.63 ± 0.20	*	1.64 ± 0.00	4.71 ± 0.01	*
breakfast cereals	nd	23.41 ± 0.77		8.68 ± 0.37	1.22 ± 0.14	*	7.20 ± 0.06	2.70 ± 0.05	*	3.34 ± 0.00	16.44 ± 0.01	*
crackers	1.43 ± 0.02	nd		11.31 ± 0.36	2.96 ± 0.37	*	3.13 ± 0.03	1.91 ± 0.05	*	5.78 ± 0.00	3.06 ± 0.00	*
dry cookies	3.61 ± 0.32	3.30 ± 0.17	ns	6.22 ± 0.34	0.11 ± 0.04	*	4.05 ± 0.05	2.62 ± 0.09	*	18.34 ± 0.01	19.00 ± 0.02	*
butter cookies	9.54 ± 0.59	23.74 ± 0.08	*	3.08 ± 0.10	3.94 ± 0.09	ns	6.56 ± 0.03	3.08 ± 0.29	*	8.88 ± 0.00	18.37 ± 0.06	*
cereal bars	2.69 ± 0.06	13.01 ± 0.23	*	4.54 ± 0.16	3.45 ± 0.19	ns	12.37 ± 0.28	6.62 ± 0.21	*	26.93 ± 0.09	26.56 ± 0.06	ns
extruded soy flour	4.02 ± 0.45			3.84 ± 0.99			5.57 ± 0.29			11.65 ± 0.04		

[a]* and ns indicate significant and nonsignificant differences between soybean-containing and nom-soybean-containing samples at $P < 0.05$.

In almost all analyzed products with soybean, CML content was much higher than in similar products without soy. This is probably due to the presence of unsaturated fats, which can be thermo-oxidized during baking and promote CML formation. One significant exception occurs with toasted bread: although HMF and acrylamide contents were higher in the toasted bread with soybean addition, in toasted bread without soybean addition the Maillard reaction was more advanced with a higher CML value. Very few data are reported in the literature about the content of CML in bakery products, and they are not always in agreement with our finding. Hull et al.[45] reported similar values in crackers (1.1 mg/kg) but higher values in shortbread and breakfast cereal (61.8 and 54.2 mg/kg, respectively).

In summary, okara is considered to be a health-promoting and technologically interesting food ingredient because of its macronutrient composition and for the high presence of insoluble dietary fiber. On the other hand, the data of this paper highlight that okara addition in bakery products promoted the formation of some MR products. In particular acrylamide and CML are present at significantly higher concentration in soybean products than in the conventional ones in almost all commercial products. This trend is related to the water-holding capacity of the okara fiber, but it is also related to the protein and lipid moiety of this ingredient.

Within this framework, the addition of soy to bakery products could raise some awareness regarding the possible negative effects to human health. It is well established that plasma concentration of advanced MRPs and, in particular, acrylamide and CML is correlated to their dietary intake.[46] The findings here reported could explain the evidence that people consuming a vegetarian or vegan diet have higher concentrations of plasma AGEs than omnivores[12] because as vegetarians they are heavy consumers of soybean-containing products.

Finally, the data in this paper contribute to improve the knowledge of MRP concentration in commercial foods. Nowadays, the European Food Safety Agency database reports acrylamide content in a large numbers of foodstuffs; however, it does not take into account soybean-based products.

AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AGEs, advanced glycation end products; CML, carboxymethyllysine; DF, dietary fibers; HMF, 5-hydroxymethyl-2-furaldehyde; MRPs, Maillard reaction products; WHC, water-holding capacity.

REFERENCES

(1) Stauffer, C. E. *Soy Protein in Baking*, 2nd ed.; Technical Foods Consultants, ASA (American Soybean Association): Cincinnati, OH, 2005.

(2) Messina, M. Insights gained from 20 years of soy research. *J. Nutr.* **2010**, *140*, 22289S−2295S.

(3) Divi, R. L.; Chang, H. C.; Doerge, D. R. Anti-thyroid isoflavones from soybean. Isolation, characterization and mechanism of action. *Biochem. Pharmacol.* **1997**, *54*, 1087−1096.

(4) Key, T. J.; Davey, G. K.; Paul, N.; Appleby, P. N. Health benefits of a vegetarian diet. *Proc. Nutr. Soc.* **1999**, *58*, 271−275.

(5) Šebeková, K.; Boor, P.; Valachovičová, M.; Blažíček, P.; Parrák, V.; Babinská, K.; Heidland, A.; Krajčovicová-Kudlcáčková, M.

Association of metabolic syndrome risk factors with selected markers of oxidative status and microinflammation in healthy omnivores and vegetarians. *Mol. Nutr. Food Res.* **2006**, *50*, 858−868.
(6) Maillard, L. C. Action des acides aminés sur les sucres. Formation des mélanoides par voi méthodique. *C.R. Acad. Sci. Ser.* **1912**, *145*, 66−68.
(7) Van Nguyen, C. Toxicity of the AGEs generated from the Maillard reaction: on the relationship of food-AGEs and biological-AGEs. *Mol. Nutr. Food Res.* **2006**, *50*, 1140−1149.
(8) Vlassara, H. Advanced glycation in health and disease. Role of the modern environment. *Ann. N.Y. Acad. Sci.* **2005**, *1043*, 452−460.
(9) Koschinsky, T.; He, C.; Mitsuhashi, T.; Bucala, R.; Liu, C.; Buenting, C.; Heitmann, K.; Vlassara, H. Orally absorbed reactive glycation products (glycotoxins): a potential risk factor in diabetic nephropathy. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6474−6479.
(10) Riviere, S.; Birlouez-Aragon, I.; Vellas, B. Plasma protein glycation in Alzheimer's disease. *Glycoconjugate J.* **1998**, *15*, 1039−1042.
(11) Vlassara, H.; Cai, W.; Crandall, J.; Goldberg, T.; Oberstein, R.; Dardaine, V.; Peppa, M.; Rayfield, E. J. Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15596−15601.
(12) Šebeková, K.; Krajčovicová-Kudlcáčková, M.; Schinzel, R.; Faist, V.; Klvanová, J.; Heidland., A. Plasma levels of advanced glycation end products in healthy, long-term vegetarians and subjects on a western mixed diet. *Eur. J. Nutr.* **2001**, *40*, 275−281.
(13) Smith, J. P.; Daifas, D. P.; El-Khoury, W.; El-Khoury, J.; El-Khoury, A. Shelf life and safety concerns of bakery products. A review. *Crit. Rev. Food Sci.* **2004**, *44*, 19−55.
(14) Borrelli, R. C.; Mennella, C.; Barba, F.; Russo, M.; Russo, G. L.; Krome, K.; Erbersdobler, H. F. Characterization of coloured compounds obtained by enzymatic extraction of bakery products. *Food Chem. Toxicol.* **2003**, *41*, 1367−1374.
(15) Palermo, M.; Paradiso, R.; De Pascale, S.; Fogliano, V. Hydroponic cultivation improves the nutritional quality of soybean and its products. *J. Agric. Food Chem.* **2012**, *60*, 250−255.
(16) Quisumbing, F. A.; Thom, W. Conditions affecting the quantitative determination of reducing sugars by Fehling solution. Elimination of certain errors involved current in methods. *J. Am. Chem. Soc.* **1921**, *43*, 1503−1526.
(17) Chen, H.; Rubenthaler, G. L.; Leung, H. K.; Baranowski, J. D. Chemical, physical and baking properties of apple fibre compared with wheat and oat bran. *Cereal Chem.* **1998**, *65*, 244−247.
(18) Phenomenex. *Ez-Faast Easy-Fast Amino Acid Sample Testing Kit Manual*; Phenomenex USA: Torrance, CA, 2001.
(19) Gökmen, V.; Şenyuva, H. Z.; Acar, J.; Sarıoğlu, K. Determination of acrylamide in potato chips and crisps by high performance liquid chromatography. *J. Chromatogr.* **2005**, *1088*, 193−199.
(20) García-Villanova, B.; Guerra-Hernández, E.; Martínez Gòmez, E.; Montilla, J. Liquid chromatography for the determination of 5-(hydroxymethyl)-2-furaldehyde in breakfast cereals. *J. Agric. Food Chem.* **1993**, *41*, 1254−1255.
(21) Charissou, A.; Ait-Ameur, L.; Birlouez-Aragon, I. Evaluation of a gas chromatography/mass spectrometry method for the quantification ofcarboxymethyllysine in food samples. *J. Chromatogr.* **2007**, *1140*, 189−194.
(22) Schettgen, T.; Tings, A.; Brodowsky, C.; Müller-Lux, A.; Musiol, A.; Kraus, T. Simultaneous determination of the advanced glycation end product Nε-carboxymethyllysine and its precursor, lysine, in exhaled breath condensate using isotope-dilution−hydrophilic-interaction liquid chromatography coupled to tandem mass spectrometry. *Anal. Bioanal. Chem.* **2007**, *387*, 2783−2791.
(23) O'Toole, D. K. Characteristics and use of okara, the soybean residue from soy milk productions. A review. *J. Agric. Food Chem.* **1999**, *47*, 363−371.
(24) Bunde, M. C.; Osundahunsi, F. O.; Akinoso, R. Supplementation of biscuits using rice bran and soybean flour. *Afr. J. Food Agric. Nutr. Dev.* **2010**, *10*, 4047−4059.
(25) Mashayekh, M.; Mahmoodi, M. R.; Enterazzi, M. H. Effect of fortification of defatted soy flour on sensory and rheological properties of wheat bread. *Int. J. Food Sci. Technol.* **2008**, *43*, 1693−1698.
(26) Noti, A.; Biedermann-Brem, S.; Biedermann, M.; Grob, K.; Albisser, P.; Realini, P. Storage of potatoes at low temperature should be avoided to prevent increased acrylamide formation during frying or roasting. *Mitt. Lebensm. Hyg.* **2003**, *94*, 167−180.
(27) Springer, M.; Fischer, T.; Lehrack, A.; Freund, W. Acrilamidbildung in Backwaren (Development of acrylamide in baked products). *Getreide Mehl Brot* **2003**, *57*, 274−278.
(28) Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (INRAN). Tabelle di composizione degli alimenti; http://www.inran.it/646/tabelle_di_composizione_degli_alimenti.html.
(29) Li, B.; Fei, L.; Nan, H.; Liu, Y. Isolation and structural characterisation of okara polysaccharides. *Molecules* **2012**, *17*, 753−761.
(30) Anil, M. Using of hazelnut testa as a source of dietary fiber in breadmaking. *J. Food Eng.* **2007**, *80*, 61−67.
(31) Gómez, M.; Ronda, F.; Blanco, C. A.; Caballero, P. A.; Apesteguía, A. Effect of dietary fibre on dough rheology and bread quality. *Eur. Food Res. Technol.* **2003**, *216*, 51−56.
(32) Capuano, E.; Ferrigno, A.; Acampa, I.; Ait-Ameur, L.; Fogliano, V. Characterization of Maillard rection in bread crisps. *Eur. Food Res. Technol.* **2008**, *228*, 311−319.
(33) Capuano, E.; Ferrigno, A.; Acampa, I.; Serpen, A.; Açar, Ö.Ç.; Gökmen, V.; Fogliano, V. Effect of flour type on Maillard reaction and acrylamide formation during toasting of bread crisp model systems and mitigation strategies. *Food Res. Int.* **2009**, *42*, 1295−1302.
(34) Mateos-Aparicio, I.; Redondo-Cuenca, A.; Villanueva-Suárez, M. J.; Zapata-Revilla, M. A.; Tenorio-Sanz, M. D. Pea pod, broad bean pod and okara, potential sources of functional compounds. *Food Sci. Technol.* **2010**, *43*, 1467−1470.
(35) Peñalvo, J. L.; Castilho, M. C.; Silveira, M. I. N.; Matallan, M. C.; Torij, M. E. Fatty acid profile of traditional soymilk. *Eur. Food Res. Technol.* **2004**, *219*, 251−253.
(36) Ehling, S.; Hengel, M.; Shibamoto, T. In *Chemistry and Safety of Acrylamide in Food*; Friedman, M., Mottram, D. S., Eds.; Springer: New York, 2005; pp 223−233.
(37) Arribas-Lorenzo, G.; Fogliano, V.; Morales, F. J. Acrylamide formation in a cookie system as influenced by the oil phenol profile and degree of oxidation. *Eur. Food Res. Technol.* **2009**, *228*, 311−319.
(38) Guerra-Hernandez, E.; Corzo, N.; Garcia-Villanova, B. Maillard reaction evaluation by furosine determination during infant cereal processing. *J. Cereal Sci.* **1999**, *29*, 171−176.
(39) European Commission Joint Research Centre's Institute for Reference Materials and Measurements. *Acrylamide Monitoring Database*; http://www.irmm.jrc.be/html/activities/acrylamide/database.htm.
(40) Mottram, D. S.; Wedzicha, B. L.; Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **2002**, *419*, 448−449.
(41) Kroh, L. W. Caramelisation in food and beverages. *Food Chem.* **1994**, *51*, 373−379.
(42) Delgado-Andrade, C.; Rufian-Henares, J. A.; Morales, F. J. Hydroxymethylfurfural in commercial biscuits marketed in Spain. *J. Food Nutr. Res.* **2009**, *48* (1), 14−19.
(43) Ramirez-Jimenez, A.; Garcia-Villanova, B.; Guerra-Hernandez, E. Hydroxymethylfurfural and methylfurfural content of selected bakery products. *Food Res. Int.* **2000**, *33* (10), 833−838.
(44) Ait-Ameur, L.; Mathieu, O.; Lalanne, V.; Trystram, G.; Birlouez-Aragon, I. Comparison of the effects of sucrose and hexose on furfural formation and browning in cookies baked at different temperatures. *Food Chem.* **2007**, *101*, 1424−1433.
(45) Hull, L. J.; Woodside, J. V.; Ames, J. M.; Cuskelly, G. J. *N*-(Carboxymethyl)lysine content of foods commonly consumed in a Western style diet. *Food Chem.* **2012**, *13*, 170−174.
(46) Somoza, V. Five years of research on health risks and benefits of Maillard reaction products: an update. *Mol. Nutr. Food Res.* **2005**, *49*, 663−672.