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Use of Coffee Silverskin and Stevia to Improve the Formulation of Biscuits

Esther Garcia–Serna[1], Nuria Martinez–Saez[1], Marta Mesias[2], Francisco J. Morales[2], M. Dolores del Castillo[1]*

*[1]Institute of Food Science Research (CSIC–UAM), C/ Nicolás Cabrera, 9,
Campus de Cantoblanco, Universidad Autónoma de Madrid, 28049 Madrid, Spain, www.cial.uam–csic.es
[2]Institute of Food Science and Technology and Nutrition (ICTAN–CSIC), C/ José Antonio Novais, 10,
28040 Madrid, Spain, www.ictan.csic.es*

Decreasing the amount of sugar added to biscuits is a good strategy to obtain a healthy product. However, a reduction in the quantity of sugar may affect its nutritional value and quality. The feasibility of the combined use of stevia and coffee silverskin for achieving healthier, nutritious and good quality biscuits has been investigated. Ten wheat flour biscuit formulations were designed. Sucrose, maltitol and stevia were used as sweeteners and coffee silverskin was used as a natural colouring and as a source of dietary fibre. The quality of the biscuits was evaluated by measuring their moisture, thickness, breaking force and colour. Acrylamide (ACR) and hydroxymethylfurfural (HMF) contents were also determined in the interest of food safety. The quality and safety of the innovative biscuits was obtained by an analysis of the sugars, proteins, free amino acids, chlorogenic acid, overall antioxidant capacity and acrylamide after *in vitro* digestion. Only the stevia biscuits and those added with coffee silverskin extract and the solid residue recovered from the extraction process, were selected for that study. A comparison of the stevia formulated biscuits, with the stevia formula added with silverskin, showed that the added biscuits had a good nutritional quality and improved texture and colour.

INTRODUCTION

Nowadays, food industry is searching for solutions to reduce the levels of certain ingredients in their products, such as salt, fats and sugars, in order to produce healthier foods that meet the consumers' expectations. High sucrose levels are associated with health problems such caries, obesity, type II diabetes, high blood cholesterol and coronary diseases. During the baking process complex, biochemical and physicochemical reactions occur, which can affect the quality and safety of the biscuits. Sucrose is the main sugar used in the biscuit industry, and it plays an important role in the manufacturing process and in the final quality of the product. Sugar determines the gelatinisation of starch, gluten mobility, biscuit spread, crispness and the surface characteristics of baked biscuits. Consequently, sugar affects the flavour, dimensions, colour, hardness, and surface of the final product [Laguna *et al.,* 2013]. On the other hand, contaminants may be formed during food processing, *e.g.* Maillard reaction products such as acrylamide (ACR) and hydroxymethylfurfural (HMF) [Kukurová *et al.,* 2013; Morales, 2009; Pedreschi *et al.,* 2014]. Therefore, decreasing the quantity of sugar added to biscuits might be a good strategy of obtaining a healthy and a low sugar product.

Sugar replacement is a challenge for the food industry because of the many functions it has in biscuit manufacture. Polyols like maltitol are suitable as sugar replacements [Martinez–Cervera *et al.,* 2014] because they do not affect the sensory properties or mitigate the formation of HMF [Courel *et al.,* 2009; Morales, 2009]. However, no data regarding the effect of maltitol on acrylamide formation are available.

Stevia, in particular its steviol glycosides, have beneficial effects on human health such as a low calorific content, antioxidant properties, an anti–diabetic capacity [Sharma *et al.,* 2012] as well as renal protective characteristics [Shivanna *et al.,* 2013]. Steviol glycosides are an authorised food additive (EU Regulation 1131/2011) and their popularity is rapidly increasing. Substituting sucrose with stevia (*Stevia rebaudiana Bertoni*) inhibits acrylamide formation and has an effect on dough rheology and the baking attributes of biscuits [Abdel–Shafi *et al.,* 2011]. Substitution by more than 30% of the sugar with stevia also alters quality attributes such as moisture content, colour development, spread ratio, breaking force, and consequently the sensory acceptance of the biscuits.

Coffee silverskin can be used in the preparation of functional beverages [Martinez–Saez *et al.,* 2014] and bakery products [Pourfarzad *et al.,* 2013] with acceptable sensorial quality. Adding dietary fibre to the biscuit formulation has an impact on its rheological properties, water activity, viscosity, sensorial quality and nutritional properties [Popov–Raljic

* Corresponding Author:
  Tel. +3491 0017900 Ext. 953; Fax: +3491 0017905;
  E–mail: mdolores.delcastillo@csic.es (Dr. M. Dolores del Castillo)

et al., 2013]. Coffee silverskin, is a by–product of roasting coffee, rich in dietary fibre, which makes it a good candidate for improving the overall quality of stevia biscuits. No studies regarding the impact of coffee silverskin addition on the HMF and ACR content, colour and textural parameters of biscuits are available.

The present research aims to evaluate the usefulness of coffee silverskin and stevia for obtaining healthier, safer, and high quality dietary biscuits. The effect of replacing sucrose with maltitol on the formation of acrylamide has also been studied. The quality properties of the novel food, the maltitol and sucrose biscuits, have been compared. The bio–accessibility of the nutrients, ACR and the functional components has been determined in order to evaluate the balance between the risks and benefits of the new formulation.

MATERIALS AND METHODS

Reagents

Pepsin, α–amylase, pancreatin, bile extract, bovine serum albumin (BSA), starch, potassium persulfate, chlorogenic acid (CGA, Reference C–3878), 6–hydroxy2,5,7,8–tetrametilhylchroman–carboxilicoylic acid (Trolox), and N–α–acetyl––L–lysine, were supplied by Sigma–Aldrich. Ethanol (96% v/v) and formic acid were supplied by Panreac S.A. (Spain). 2,2'–azino–bis (3–ethylbenzothiazoline–6–sulphonic acid (ABTS)), reagent o–phtaldialdehyde (OPA) and sodium dodecyl sulfate (SDS) were supplied by Fluka and β–mercaptoethanol by Merck (Germany). Bio–Rad Laboratories S.A provided Bradford reagent. The ACR marker ($^{13}C_3$ – acrylamide) (isotopic purity 99 %) was provided by Cambridge Isotope Labs (USA). Methanol and acetonitrile (HPLC grade) were provided by Scharlau (Spain). Cartridges for solid phase extraction – Oasis HLB (30 mg, 1 mL) from Waters (USA) and fibre kit from Megazyme International Ireland Ltd. D–glucose, mannose and fructose kit were supplied by Megazyme (Ireland). All other chemicals and reagents were of analytical grade.

Food ingredients

All the basic ingredients were purchased at specialised and certified food markets. Food grade soy lecithin was provided by Manuel Riesgo SA (Spain) and maltitol was supplied by a national food company. The commercial stevia sweetener powder which contained 3% steviol glycosides, was supplied by Gerblé (Spain).

Arabica coffee silverskin was provided by Fortaleza S.A. (Spain). The extract, which contained natural colouring as well as other bioactive compounds, was obtained using the method described by del Castillo *et al.* [2013].

Briefly, 3.3 g of silverskin was treated with 100 mL boiling water for 10 min. The solid residue from the extraction process was recovered by filtration using gravimetric paper filters and dried at room temperature for 24 h. The dried solid was used as natural source of dietary fibre. Addition of raw coffee silverskin was also carried out. The total dietary fibre of coffee silverskin was determined by the enzymatic gravimetric method using the Total Dietary Fibre Assay Kit (Megazyme International Ireland, Ireland).

Preparation of biscuits

A total of ten biscuit formulations were prepared as described in Table 1. The dough was prepared by mixing salt, baking powder and sugar or its substitutes. Mineral water at room temperature was added to the dry mixture and thoroughly blended to obtain a homogenous mixture. In a separate bowl, lecithin and oil were mixed and then added to the mixture. Finally, the flour was added gradually to the mixture and the dough was kneaded to obtain a homogeneous, elastic and slightly sticky dough. The dough was allowed to rest for 30 min, and shaped into discs with a diameter of 6.4 cm and a thickness of 0.8 cm. The surface of the biscuits was punctured several times using a fork to prevent puffing. In those formulations with coffee silverskin or the solid residue from the extraction process, these were combined with the flour and added as described above. Coffee silverskin extract was used as a substitute for water in the biscuit formulation.

The biscuits were baked at 190°C for 20 min with air recirculation (30% power) in a Memmert GmbH (Schwabach, Germany) UNE–400 model oven. Two sets of 4 biscuits were baked in duplicate (n=8). The biscuits were placed in the centre of the tray forming a square, in order to reduce process variability during baking. The temperature of processing was controlled and monitored by internal sensors (2007 Celsius v8.0, Memmert GmbH) and externally using temperature controller (type K, 0.1 mm, 0.1°C accuracy), located at tray height. A temperature measurement was recorded every second in a data logger (Delta OHM, Model HD–2178–2, Italy) throughout the process.

Quality attributes

Moisture

Prior to analysis, the capsules were heated at 110°C for 15 min, cooled in a desiccator at room temperature. The moisture content was determined by a gravimetric method as described in AOAC–925.10. Crushed biscuits (n=4) were weighed accurately (~1 g) into a test tube and they were dried until constant weight in an oven at 105°C. Results were expressed as percentage (%).

Thickness

After cooling, the thickness of a half of four biscuits from each set of samples (n=8) was measured using a calliper. The results were expressed in cm.

Texture (breaking force)

The texture measurements for biscuit hardness were performed using a Texture Analyser (TA–TXPlus Texture analyser, Texture Technologies Corporation, USA) equipped with a 50 kg load cell, a probe (Warner–Bratzcer, HDP / BSK knife model) with a speed at 1 mm/s and a distance prolongation of 10 mm. The force at the first major drop in force–deformation curve (Fmax) and deformation at maximum force were obtained for 4 replicates per sample. The results of hardness are expressed as N (Newton).

Colour

The colour parameters were expressed according to CIE L*a*b* scale [CIE Colorimetric Committee, 1974; McLaren

TABLE 1. Biscuit formulations: A, sucrose; B, maltitol; C, 15% stevia; D, 30% stevia; E, 60% stevia; F, 100% stevia; G, 100% stevia and coffee silverskin (CS) extract; H, 100% stevia and CS extract plus solid residue; I, 100% stevia and coffee silverskin (1.33 g); and J, 100% stevia and coffee silverskin (3.33 g).

Ingredients (g)	Biscuit formulations									
	A	B	C	D	E	F	G	H	I	J
Wheat flour	56.00	54.27	57.28	58.63	61.51	65.84	65.84	65.03	64.96	60.77
Water	20.00	19.38	20.46	20.94	21.97	23.51	0	0	23.20	24.70
Sunflower oil	7.75	7.51	7.93	8.11	8.51	9.11	9.11	9.00	8.99	9.57
Baking powder	0.55	0.53	0.56	0.58	0.60	0.65	0.65	0.64	0.64	0.68
Salt	0.37	0.36	0.38	0.39	0.41	0.43	0.43	0.43	0.43	0.46
Lecithin	0.33	0.32	0.34	0.35	0.36	0.39	0.39	0.38	0.38	0.41
Sucrose	15.00	0	13.04	10.99	6.59	0	0	0	0	0
Maltitol	0	17.62	0	0	0	0	0	0	0	0
Stevia	0	0	0.01	0.02	0.04	0.07	0.07	0.07	0.07	0.07
Coffee silverskin	0	0	0	0	0	0	0	0	1.33	3.33
Extract	0	0	0	0	0	0	23.51	23.23	0	0
Solid residue	0	0	0	0	0	0	0	1.22	0	0
TOTAL	100	100	100	100	100	100	100	100	100	100

& Riggs, 1976]. The measurements were made using a HunterLab Spectrophotometer CM–3500D colorimeter (Hunter Associated laboratory, USA). Four independent measurements of a*(redness), b*(yellowness) and L*(lightness) parameters, were carried out on different areas of the biscuit (top and bottom). The impact of the baking process on the colour of the biscuits was estimated as $\Delta E = E_{biscuit} - E_{dough}$. E index was calculated according to the equation: $E = (L^2 + a^2 + b^2)^{1/2}$.

Processing chemical contaminants

Acrylamide

The presence of ACR was determined by liquid chromatography coupled with tandem mass spectrometry and quantified by isotopic dilution, based on the method of Arribas–Lorenzo et al. [2009]. The ACR was quantified using a linear calibration with standard solutions (0.1 to 100 g/L) of ACR standard containing same concentration of labelled ACR. The ACR content in the samples (n=4) was expressed as μg/kg dry weight.

Hydroxymethylfurfural

The HMF was determined by liquid chromatography coupled with a diode array detector as described by Rufián–Henares et al. [2006]. Sample preparation was performed in duplicate. The results were expressed as mg of HMF/kg dry weight.

Bioaccesibility

Preparation of in vitro digests

Abiotic digestion *in vitro* was carried out on three stages (oral, gastric and intestinal) as described by Hollebeeck et al. [2013]. Briefly, the salivary step was performed at pH 6.9, 37°C, 5 min of incubation, 3.9 units alpha–amylase/mL under aerobic conditions. The gastric step was carried out at pH 2, 37°C 90 min of incubation, 71.2 units pepsin/mL under anaerobic conditions while the abiotic duodenal step was run at pH 7, 37°C 150 min, 9.2 mg pancreatin and 55.2 mg bile extract/mL under anaerobic conditions. The final mixture was centrifuged at 5000 r.p.m. for 40 min at 4°C. The soluble fraction obtained by centrifugation was frozen at – 20°C and freeze–dried. The resulting powder was stored at – 20°C until analysis.

Controls containing BSA, starch and sunflower oil and wheat flour were also hydrolysed. This process was performed in duplicate. Subsequent analyses to characterise the digests, were performed in triplicate.

Glycaemic sugars

Glucose, mannose and fructose were determined using the Megazyme kit according to the manufacturer's instructions (Megazyme K–MANGL 04/13, Ireland) adapted to micro–method format. A BioTek Power WaveTM XS microplate reader (BioTek Instruments, USA) was used to measure the samples absorbance at 340 nm. D–Mannose, D–glucose, and D–fructose were used as standards at a concentration range of 0.15–0.4 mg/mL. The concentration of D–glucose, D–mannose, and D–fructose in each sample was expressed as g glucose, mannose and fructose/kg digest.

Water soluble proteins

A Bio–Rad Protein Assay, based on the method of Bradford in micro–method format was used to determine the protein concentration. All reagents were prepared according to the manufacturer's instructions (Bio–Rad Laboratories, SIG 093094). A sample blank and a reagent blank were also analysed. A calibration curve was constructed using BSA (0.05–0.5 mg/mL). The results were expressed as mg BSA/kg digest.

Free amino groups

An OPA assay was employed to determine the content of free amino groups [Michalska *et al.*, 2008]. Quantitative analysis was performed by the external standard method, using a calibration curve of Nα–acetyl–l–lysine which ranged from 10 to 250 μmol/L. The results were expressed as mol lysine/kg digest.

Chlorogenic acid

This procedure was carried out using capillary electrophoresis, according to the method of del Castillo *et al.* [2002]. The level of chlorogenic acid in the samples was quantified using a calibration curve in the range of 0.15–2.5 mmol/L chlorogenic acid. The results were expressed as μg CGA/ kg digest.

Overall antioxidant capacity

An ABTS decolourisation assay was performed according to the method of Oki *et al.* [2006]. A Trolox calibration (0.15–2 mmol) was used to calculate the overall antioxidant capacity. The results were expressed as mol Trolox/kg digest.

Statistical analysis

Data were expressed as the mean ± standard deviation. The T–test (independent samples, 2 groups) was applied to determine the differences between means (F test of Snedecor––Fisher). For the comparison of multiple means (cluster analysis) an analysis of variance (one–way ANOVA) and multiple Bonferroni test were applied. Differences were considered to be statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Quality attributes

The quality attributes of the biscuits for each of the formulations are shown in Table 2.

Moisture

No significant differences ($p > 0.05$) in the moisture levels were found between the biscuits made with sucrose (6.88±0.2) and maltitol (7.54±1.7) (Table 2). Laguna *et al.* [2013] found similar results when using sucrose and maltitol in short dough biscuits.

Substituting 100% of the sucrose with stevia significantly ($p < 0.05$) increased the percentage moisture of the biscuits (Table 2). These results agree with those obtained by Abdel––Shafi *et al*. [2011]. The authors have associated the increasing levels of moisture observed in the higher levels of sugar substitution with a higher gluten development and water retention in the dough. Higher moisture content will make the biscuits softer and as a consequence lower texture acceptability by consumers. In addition, this factor may also affect the shelf–life of the product.

The moisture content of the stevia biscuits was significantly reduced ($p < 0.05$) by adding coffee silverskin to the formulation. No significant differences ($p > 0.05$) in the moisture content were found between biscuits made with sucrose (A), maltitol (B) and stevia plus coffee silverskin (G, H, I). Addition of coffee silverskin may enhance the quality of dietary biscuits made with stevia.

Thickness

Sugar substitution ($>60\%$) and the addition of coffee silverskin had significant ($p < 0.05$) effects on the thickness of the biscuits (Table 2). The biscuits made with sucrose (A) were the thickest. Eliminating sucrose from the biscuit formulation caused a significant reduction ($p < 0.05$) in the thickness.

No significant differences ($p > 0.05$) were detected between the thickness of the maltitol (B) and stevia biscuits (C, E, F, H, I, J). The thinnest biscuits were those which were prepared using stevia and coffee silverskin in their formulation (G–J). The values recorded for these biscuits were significantly lower ($p < 0.05$) than those corresponding to 100% sucrose (A). These results may be explained by containing less air inside the dough, or a reduction in the capacity of the dough to retain air of the biscuits made with maltitol, stevia and coffee silverskin, when compared with sucrose biscuits. These results concur with those reported by Martinez–Cervera *et al.* [2014], who found that muffins prepared using sucrose were significantly higher than those prepared using maltitol.

Texture

Table 2 shows data for the hardness of the different biscuits. The use of maltitol as a sugar replacement did not significantly affect the hardness of the biscuits. Similar results were found by Laguna *et al.* [2013] who explained this finding on the basis of the similar solubility of maltitol and sucrose in water.

The results also show a trend similar to that observed by Abdel–Shafi *et al.* [2011]. The use of 15% (C) and 60% (E) of stevia to replace sucrose, significantly decreased the breaking properties of the biscuits. The breaking force of the biscuits prepared using sucrose (A) or 100% stevia (F) did not differ significantly. Sucrose causes the formation of a weak gluten network and disperses proteins and starch, which makes the biscuit fragile [Laguna *et al.,* 2013]. The elimination of sucrose and the addition of coffee silverskin in our formulations did not significantly affect ($p > 0.05$) the breaking properties of the biscuits. This suggests that in terms of texture and crispness, stevia is as suitable as maltitol to be used as a sucrose replacer.

Colour

The sucrose (A) and maltitol (B) biscuits showed similar L* and b* values indicating the usefulness of this polyol as a sucrose replacer. The substitution of sucrose by 100% stevia (F) significantly reduced ($p < 0.05$) the three parameters (Table 2). Decreased a* and b* values indicate the absence of the typical brown colour characterised by the biscuits made with sucrose. This might make the stevia biscuits unacceptable to consumers.

The addition of coffee silverskin extract to the biscuits enhanced the colour profile of the stevia biscuits. The values for a* and b* of the stevia biscuits with coffee silverskin extract (G) did not differ significantly ($p > 0.05$) from those made with sucrose (A). This finding supports the validity of using coffee silverskin extract as a natural colouring. The addition of coffee dietary fibre to the biscuits also affected their colour. The values for L* agree with those obtained by Popov–Raljic *et al.* [2013] for dietary biscuits. The lowest L* value found

TABLE 2. Quality properties of biscuit samples: A, sucrose; B, maltitol; C, 15% stevia; D, 30% stevia; E, 60% stevia; F, 100% stevia; G, 100% stevia and coffee silverskin (CS) extract; H, 100% stevia and CS extract plus solid residue; I, 100% stevia and coffee silverskin (1.33 g); and J, 100% stevia and coffee silverskin (3.33 g).

Biscuits	Moisture (%)	Thickness (cm)	Hardness (N)	Colour		
				L*	a*	b*
A	6.88±0.22[a]	1.11±0.11[f]	200.05±66.04[b]	72.13±1.99[f]	4.00±1.12[d,e]	23.97±1.08[e,f]
B	7.54±1.75[a]	0.84±0.05[b,c,d]	146.39±42.31[a,b]	71.94±1.86[f]	3.15±0.77[b]	24.10±1.53[f]
C	6.70±0.01[a]	0.98±0.09[d,e,f]	102.26±37.39[a]	71.40±1.32[e,f]	3.86±0.95[c,d,e]	23.37±1.18[e,f]
D	8.83±0.01[a,b]	1.06±0.15[f]	204.8±52.28[b]	69.98±2.01[e]	3.06±0.78[b]	22.16±1.47[d,e]
E	6.91±0.09[a]	0.77±0.05[a,b,c]	112.81±18.46[a]	71.07±1.05[e,f]	3.28±0.57[b,c]	23.31±0.88[e,f]
F	10.55±0.12[b]	0.89±0.10[c,e]	121.33±10.82[a,b]	66.93±1.41[d]	2.31±0.55[a]	20.80±0.97[c,d]
G	7.26±0.03[a]	0.70±0.00[a]	131.92±11.78[a,b]	65.87±1.32[c,d]	4.09±0.46[e]	22.36±1.08[d,e]
H	7.75±0.10[a]	0.74 ± 0.05[a,b]	139.25±11.94[a,b]	64.41±2.14[b,c]	3.22±0.69[b,c]	20.09±1.43[b,c]
I	7.16±0.03[a]	0.74±0.05[a,b]	129.16±10.50[a,b]	63.56±1.39[b]	3.20±0.44[b,c]	18.64±0.89[a,b]
J	8.61±0.01[a,b]	0.75±0.04[a,b,c]	130.49±10.36[a,b]	61.70±1.24[a]	3.30±0.25b[c,d]	16.89±0.8[a]

Each value represents the mean ± standard deviation. The different letters denote significant differences (p<0.05) between samples.

in this study (J) is of the same order of magnitude as that reported for biscuits formulated using inulin and oligofructose as a source of fibre, which are very acceptable to consumers. The parameters a* and b* may be further improved by changing the concentration of coffee silverskin extract or adjusting the baking conditions.

Figure 1 shows the appearance and development of the colour throughout the baking process (ΔE) of sucrose, maltitol, stevia and stevia with coffee silverskin biscuits. The colour differences can be categorised as: imperceptible differences (0–0.5), slight differences (0.5–1.5), just noticeable differences (1.5–3.0), marked differences (3.0–6.0), extremely marked dif-
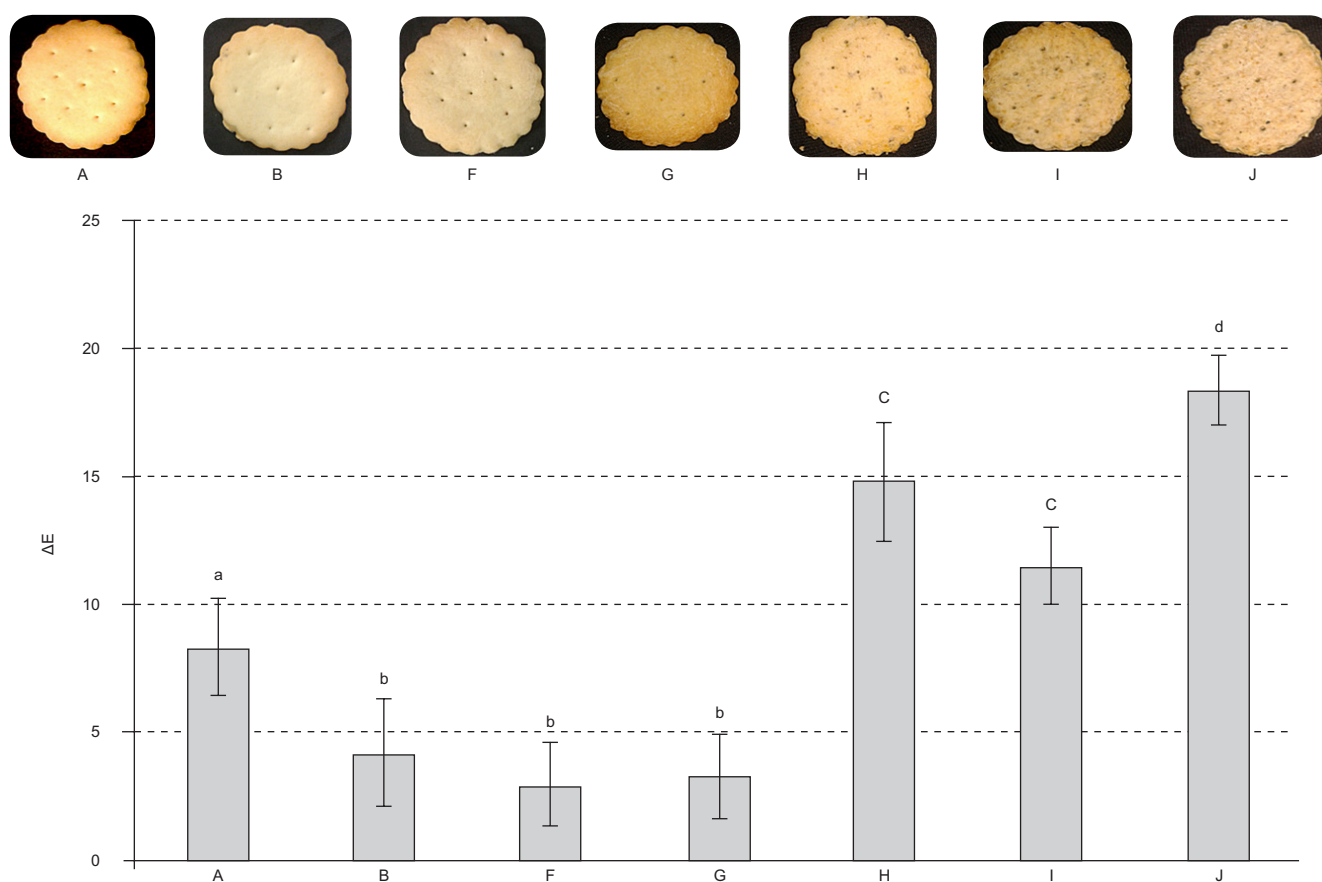

FIGURE 1. Appearance of biscuits and changes in dough colour due to baking. Changes in colour signify the difference between the biscuit colour and the dough colour (ΔE). A, sucrose; B, maltitol; F, 100% stevia; G, 100% stevia and coffee silverskin (CS) extract; H, 100% stevia and CS extract plus solid residue; I, 100% stevia and coffee silverskin (1.33 g); and J, 100% stevia and coffee silverskin (3.33 g). Bars represent the mean values and the error bars denote standard deviations. The different letters indicate significant differences (p<0.05) between the means of the samples.
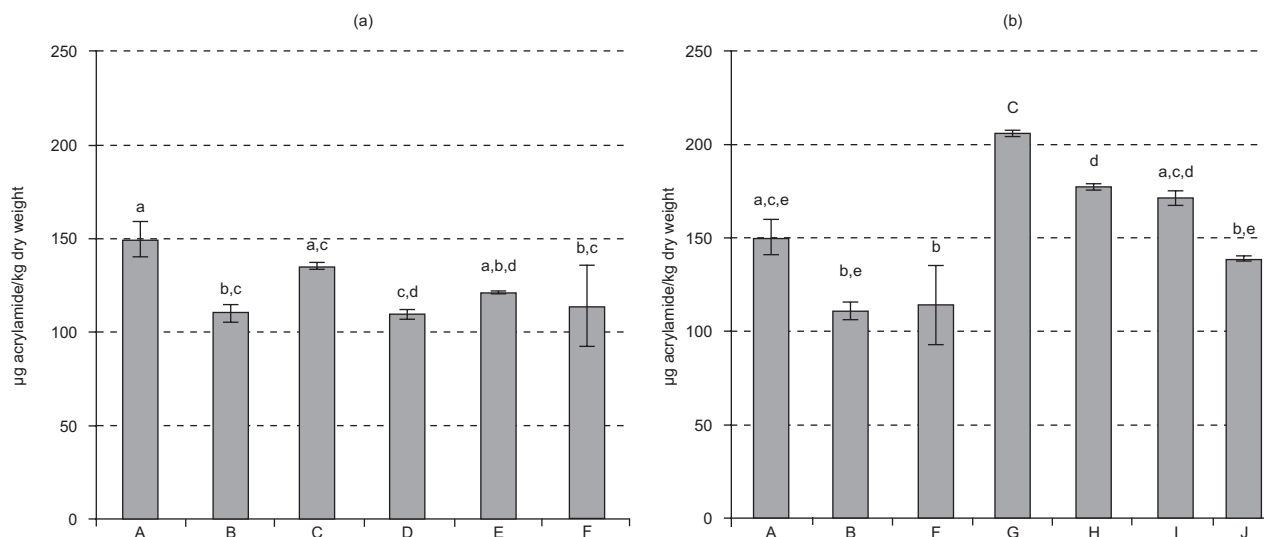
FIGURE 2. ACR content of the biscuit samples, expressed as μg acrylamide/kg dry weight. (2a) A, sucrose; B, maltitol; C, 15% stevia; D, 30% stevia; E, 60% stevia; F, 100% stevia. (2b) A; B; F; G, 100% stevia and coffee silverskin (CS) extract; H, 100% stevia and CS extract plus solid residue; I, 100% stevia and coffee silverskin (1.33 g); and J, 100% stevia and coffee silverskin (3.33 g). Bars represent the mean values and the error bars the standard deviation. The different letters indicate significant differences (p<0.05) between the means of the samples.

ferences (6.0–12.0) and colours of different shades (above 12.0) [Popov–Raljic *et al.,* 2013]. The baking process caused noticeable differences in the colour of the maltitol, stevia, and stevia with coffee silverskin extract biscuits. It caused extremely marked differences in the sucrose biscuits and a completely different colour or different shade in those with coffee silverskin fibre.

The substitution of sugar and the addition of solid ingredients, rich in dietary fibre, significantly affected (p<0.05) the colour of the biscuits (Figure 2). The poor colour development of sucrose free biscuits can be explained by a reduction in the progress of non–enzymatic browning reactions during baking. The stevia biscuits and those corresponding to the biscuits with coffee silverskin extract showed no significant differences (p>0.05) in colour development (ΔE). All the results (Table 2 and Figure 1) regarding the colour of the biscuits containing coffee silverskin extract support its feasibility as a natural colouring which will provide the typical golden colour expected of this type of baked products. In addition, stevia biscuits containing coffee silverskin presented an increase of the thermal impact (ΔE) compared with the sugar, maltitol and stevia formulation. The constituents of the coffee dietary fibre seem to have the biggest influence on the development of colour during baking. The coffee silverskin added to the biscuits contains 67.12% dietary fibre. Biscuits with coffee by–products usually contain up to 3.1% of dietary fibre. Recently Popov–Raljic *et al.* [2013] studied the sensory and colour properties of dietary biscuits prepared using different sources of fibre. The authors found that the fibre present in the dough of dietary biscuits has an impact on their rheological properties, water activity, viscosity and sensory characteristics, especially that of colour. According to our results, used in combination of stevia, coffee extract and coffee silverskin dietary fibre enables the production of dietary biscuits with acceptable quality properties. Coffee silverskin is an excellent natural source of dietary fibre because it contains both soluble and insoluble fibre (6.6% and 60.5%, respectively).

Processing chemical contaminants

Acrylamide

Figure 2 shows the acrylamide content of the biscuits. Sucrose and maltitol biscuits produced values of 149.89±8.96 and 110.29±4.39 μg/kg dry weight, respectively. A comparison of the sucrose biscuits with the maltitol formulation showed a significant (p<0.05) acrylamide mitigation (26.4%). In addition, the ACR content was significantly (p<0.05) reduced by substituting 30% and 100% sucrose with stevia. These results are in agreement with those obtained by Abdel––Shafi *et al.* [2011].

The addition of coffee silverskin did not inhibit ACR formation. The ACR content of the sucrose biscuits and those containing solid silverskin ingredients was not significantly different (p>0.05). Biscuits with coffee silverskin extract had an ACR content of 205.93±0.63 μg/kg dry weight which was significantly higher (p<0.05) than that found in the sucrose biscuits. Coffee silverskin extract contained 11.42 μg/L ACR which is approximately 10 times lower than that reported in coffee beverages. The FDA gives values for ACR in coffees of 175–263 μg/L [FDA, www.fda.gov]. The European Commission recommends indicative ACR values of 450 μg/kg for roast coffee (dry) and 900 μg/kg for instant (soluble) coffee (Commission Recommendation 2013/647/EU).

Rufián–Henares *et al.* [2007] reported values of ACR in commercial biscuits of 423 μg/kg which is in line with the indicative ACR value recommended by the EU for biscuits (500 μg/kg) (Commission Recommendation 2013/647/EU). The highest ACR values detected in our biscuits were 59% lower than the EU indicative values for biscuits.

Hydroxymethylfurfural

The HMF value of the sucrose formulation was significantly (p<0.05) higher (6.51±0.71 mg/kg dry weight) than that of the maltitol biscuits (0.83±0.07 mg/kg dry weight).
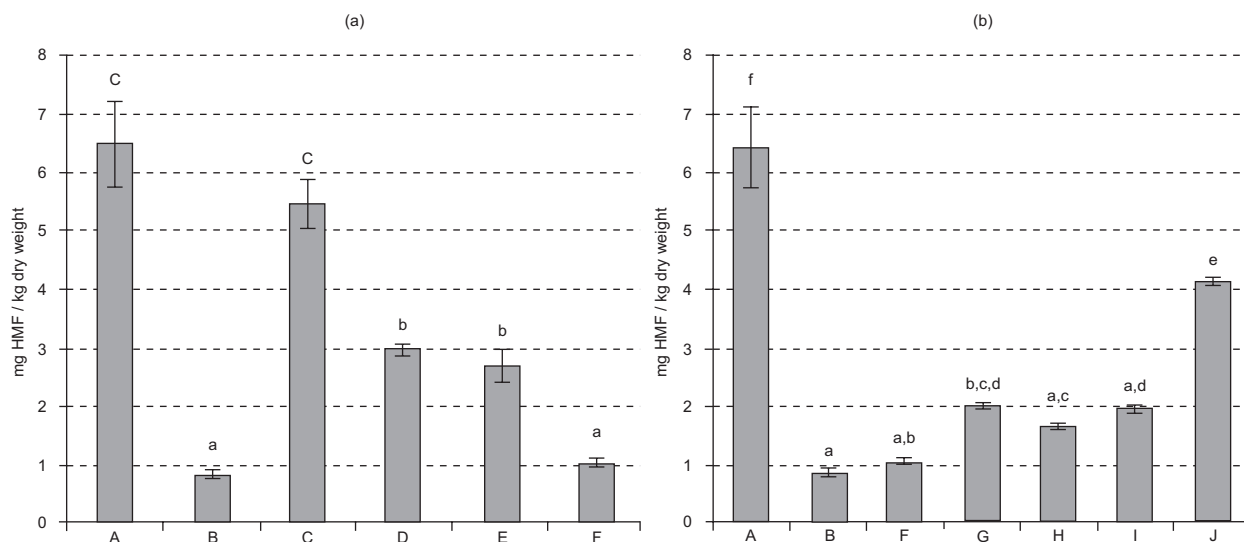
FIGURE 3. HMF content of the biscuit samples, expressed as mg HMF/kg dry weight. (3a) A, sucrose; B, maltitol; C, 15% stevia; D, 30% stevia; E, 60% stevia; F, 100% stevia. (3b) A; B; F; G, 100% stevia and coffee silverskin (CS) extract; H, 100% stevia and CS extract plus solid residue; I, 100% stevia and coffee silverskin (1.33 g); and J, 100% stevia and coffee silverskin (3.33 g). Bars represent the mean values and the error bars the standard deviation. The different letters indicate significant differences (p<0.05) between the means of the samples.

This large difference agrees with that described by Delgado––Andrade *et al.* [2009]. Replacing more than 30% sucrose with stevia significantly reduced the HMF content of the biscuits (Figure 3a). These results clearly show the effect of both maltitol and stevia on reducing HMF in these biscuits.

The biscuits with coffee silverskin (Figure 3b) produced HMF values significantly lower (p<0.05) than that found in the sucrose biscuits. The HMF content of the biscuits containing in their formulation coffee by–products ranged from 1.65±0.05 to 4.18±0.05 mg/dry weight. The highest HMF value corresponded to the biscuits that contain the highest concentration of silverskin, although this was still 35.79% lower than that found in the sucrose biscuits.

In 2005 the EFSA established a reference range for HMF in biscuits of between a minimum of 5 mg/kg and a maximum of 25 mg/kg. Delgado–Andrade *et al.* [2009] described a wide range of HMF in Spanish biscuits from 3.1 to 182.5 mg/kg with an average of 14.4 mg/kg. The combined use of stevia and coffee silverskin enables the production of biscuits with low levels of HMF (<2 mg/kg).

Bioaccesibility of food components and nutritional properties of biscuits

The chemical composition and nutritional properties of the digests of stevia biscuits (F) and those containing coffee silverksin extract plus solid residue (H) are shown in Table 3.

Glycaemic sugars

The glucose content of stevia biscuit digests (66.28±17.65 g glucose/kg) was not significantly different (p>0.05) to that of the biscuits with coffee silverskin (56.22±3.42 g glucose/kg) (Table 3). This means that a serving of four biscuits would provide less than 3 g sucrose. The fructose and mannose content was undetectable in both digests (Table 3). Consequently, these innovative foods may be included in the category of low

TABLE 3. Chemical composition and nutritional properties of biscuit digests, F (100% stevia) and H (100 % stevia with coffee silverskin extract plus solid residue).

Measurements	Digests	
	F	H
Glucose (g glucose/kg digest)	66.28±17.65[a]	56.22±3.42[a]
Fructose (g fructose/kg digest)	n.d.	n.d.
Mannose (g mannose/kg digest)	n.d.	n.d.
Total soluble proteins (mg BSA/kg digest)	129.05±8.81[a]	140.31±5.29[b]
Free amino groups (mol lysine/kg digest)	46.93±7.83[a]	40.48±3.76[a]
Acrylamide (μg acrylamide/ kg digest)	n.d.	n.d.
Chlorogenic acid (μg CGA/ kg digest)	n.d.	n.d.
Total antioxidant capacity (mol trolox/kg digest)	5.04±0.02[a]	5.07±0.02[a]

Each value represents the mean ± standard deviation. The different letters denote significant differences (p<0.05) between samples and n.d means non-detected.

glycaemic foods which may be suitable for diabetics or people who want to lose weight.

Total soluble proteins

The content of soluble proteins surviving the digestion process was significantly different (p<0.05) (Table 3). Although most of the protein in the biscuits comes from wheat flour, coffee silverskin also contains proteins [Martinez–Saez *et al.,* 2014]. The digestibility of the proteins in flour and coffee silverskin may be different. This could explain the differences in the protein content found in the control (F) and biscuits containing coffee by–products (H).

Free amino groups

Similar levels of free amino acids were released by digestion in the control and the biscuits with coffee by–products (Table 3). According to the results of the soluble proteins and free amino acid content, coffee silverskin dietary fibre, did not appear to affect the digestibility of the flour proteins. Therefore, the addition of coffee wastes did not have a negative effect on the nutritional status of the food. Coffee silverskin fibre may be used in the formulation of dietary biscuits such as those made using apple, lemon and wheat [Bilgicli *et al.,* 2007].

Chlorogenic acid

No chlorogenic acid was detected in the digests from biscuits containing coffee by–products. This suggests that although coffee silverskin is a natural source of this compound [del Castillo *et al.,* 2013; Martinez–Saez *et al*., 2014] it may not survive the digestion process. Vallejo *et al.* [2004] observed an 80% reduction in the total hydrocinnamic acids, including chlorogenic acid, after *in vitro* gastrointestinal digestion of broccoli.

Acrylamide

Acrylamide was not detected in the digests. In theory, acrylamide may be present at a concentration of 0.004 and 0.007 μg/mL digest in control sample (F) and biscuits containing coffee wastes (H). Berger *et al.* [2011] found a delayed acrylamide liberation and/or absorption from food compared with drinking water in the upper gastrointestinal tract indicating the influence of the food matrix on the bioavailability of acrylamide. Because acrylamide was not bioaccessible in the biscuits digests this suggests that a healthy biscuit formulation was achieved.

Total antioxidant capacity

No significant differences ($p > 0.05$) were found between the digests from the control and the biscuits with coffee by––products in their formulation (Table 3). Because chlorogenic acid was not detected, the antioxidant power may be ascribed to the gluten peptides released during the digestion process [Pastoriza *et al.,* 2011].

CONCLUSIONS

The complete replacement of sucrose by stevia affected the moisture content of the biscuits. However, the addition of coffee silverskin improved this and other related physical parameters. HMF was greatly reduced and no bioaccesible acrylamide was detected in the digests of the new innovative biscuits. The nutritional value of the biscuits also improved. Our results indicate the feasibility of using coffee silverskin as a natural colouring and as source of dietary fibre, in order to achieve a healthier, nutritious, and an acceptable quality biscuit.

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