Research article

Development of healthy gluten-free *crackers from white and brown tef* (*Eragrostis tef* Zucc.) flours

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ABSTRACT

This study evaluated the effect of inclusion of two types of tef flours (white and brown) at different levels (25, 50 and 100 %, total flour) on the nutritional (proximal and mineral composition), *in vitro* bioactive (antioxidant capacity and starch digestibility) and sensory properties of rice-tef crackers. The aim was to formulate a gluten-free product with nutritional and healthy benefits, and acceptable for consumers. Results showed than crackers enriched with white tef had a significant ($p \leq 0.05$) higher concentration of all the minerals tested, except for calcium and manganese, compared to brown tef. Iron content of white tef was almost twice that of brown tef, and copper and magnesium increased from 0.12 mg/100 g and 39.2 mg/100 g in control crackers to 0.56 mg/100 g and 197 mg/100 g in white tef crackers (WT 100%), respectively. Moreover, white tef flour and crackers showed significantly higher antioxidant activity than rice or brown tef counterparts. Formulation with tef flour significantly contributed to a reduction of the rapidly available glucose and rapidly digestible starch of crackers.

1. Introduction

According to FAO/WHO (1994), gluten-free products that substitute important basic foods (e.g. flour, bread, pasta) should provide approximately the same amount of vitamins and minerals as the original food replaced. The energy and nutrient content of gluten-free products require attention as the substitution of wheat flour with gluten-free alternatives may result in inadequate intake of important nutrients (Hager et al., 2012).

Bakery is the fastest growing segment of gluten-free products, due to increasing availability of gluten-free flour alternatives. Biscuit formulation with gluten-free flours is technologically easier than other bakery products, since the structure of biscuits does not depend as much on the protein network as it does on the starch gelatinization (Di Cairano et al., 2018). Moreover, commercial availability of several types of gluten-free flours is expected to boost the market revenue growth in near future.

Tef (*Eragrostis tef*) has a big potential as flour ingredient for healthy and functional foods (Belay et al., 2009; Shumoy and Raes, 2017; Mengesha, 1966). One of the main advantages of tef flour is its gluten-free characteristic, which allows its inclusion on celiac disease patients' diets (Hopman et al., 2008); when compared with the two most used flours in gluten-free products, i.e. rice and maize, tef is more nutritive alternative, due to higher content in protein and total and soluble fibre (Hager et al., 2012).

Furthermore, this cereal has a complete profile of essential amino acids, with high levels of lysine in contrast with other cereals (Ketema, 1997; Bultosa et al., 2002). Tef has important folate and polyphenol content, when compared with most consumed cereals, including wheat or oat, and it is relatively high in mineral content, including iron, copper, calcium, magnesium, zinc, manganese and phosphorous (Hager et al., 2012). The high iron content is a very important attribute, since anaemia is common in patients with celiac disease (CD) (Mahadev et al., 2018).

Commercially, tef is principally divided into white and brown cultivars. Scarce information is available regarding varieties since most of commercial flours come from unidentified varieties in Europe. Different cultivars of tef do not have significant variation in their caloric, moisture, protein, carbohydrate, or phosphorus content; however, significant differences have been observed by Ravisankar et al. (2018) on the phenolic

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profiles, overall flavonoids, especially the presence of procyanindins in the case of brown tef, which are not present in white tef varieties. Other differences found are associated to the presence of apigenin glycosides in white tef, while luteolin glycosides are present in brown tef. Shumoy and Raes (2017) observed significant differences in the phenolic profiles of seven varieties studied, although some common characteristics were also reported, such as the important percentage of bound phenolic compounds (over 84%), or the lack of gallic, caffeic and salicylic acids. Its antioxidant properties contribute on the reduction of adipose tissue and inflammation processes among others (Lemecha et al., 2018).

Refined grains tend to have a higher glycacemic index (GI) than non-refined grains (Jenkins et al., 1997; Hager et al., 2013; Brand-Miller et al., 2008). Tef has a high gelatinization temperature, which determines partially its low GI (Shumoy and Raes, 2017).

Although different authors have previously studied bioactive or techno-functional properties of tef flours and its formulated products, the objective of this study is to optimize a biscuit formulation with tef flour, comparing two types of tef (white and brown), covering bioactivity, techno-functional properties, starch digestibility evaluation, and sensory test analyses, in order to optimize final product formulation.

2. Materials and methods

2.1. Chemicals

2,2′-Azinobis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2′-diazobis-(2-amidinopropane)-dihydrochloride (AAPH), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid (GA), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), sodium carboxymethylcellulose (CMC) were obtained from Sigma-Aldrich, Co. (St. Louis, MO, USA). Folin-Ciocalteu (FC) reagent was purchased from Panreac Quimica S.L.U. (Barcelona, Spain).

2.2. Raw material

Commercial tef (*Eragrostis tef* Zucc.) flours (white and brown), both grown in Spain, were provided by Salutef (Palencia, Spain). Rice flour was provided by (Emilio Esteban, S.A). High oleic oil, salt and sucrose was provided by Makro (Valladolid, Spain and Hydroxy-propyl-methylcellulose (HPMC) from Guinama.

2.3. Experimental design

The study evaluated two different tef flours, white and brown, and a mixture of both (1:1). The tef flours were formulated at different levels over total flour content (25, 50 and 100 %). Balance flour content to 100 % was completed with rice flour, and a control of 100 % rice flour was also included. The study was conducted in duplicate.

2.4. Product preparation

Yeast crackers were formulated based on a rice and tef flour mixtures. Dough samples were prepared by mixing 100 g of flour mixture, with 20 g of olive oil, 10 g of sugar, 3 g of salt, and 2 g of hydroxypropyl methyl cellulose (HPMC). Water (70 g 100 g^{-1} flour) was added last, while mixing. After kneading, the doughs were left for fermentation at 18 °C for 24 h. After fermentation, the doughs were laminated to ~2.5 mm, and cut into 3.5 mm-side square pieces. Each batch was baked in a forced-air convection oven at 165 °C for 25 min. The crackers were allowed to cool at room temperature for 1 h before being placed in sealed polyethylene bags for further analyses. All crackers doughs were prepared in triplicate.

2.5. Proximal composition

Protein, fat, moisture, ash and carbohydrate content were determined for the all crackers formulated. Protein content was determined by the Dumas method (AOAC, 2005) through in a CN-2000 elemental analyser (Leco Corp., St. Joseph, MI, USA). Protein was calculated from nitrogen using the conversion factor of 6.25. Fat content was determined using dried samples extracted with petroleum ether (BP 40–60C) during 4 h in an extracting unit Soxtec System 2055 Tecator (FOSS, Hillerød, Denmark) and gravimetrically determined. Moisture was measured by drying at 100 °C (AOAC, 2005). Ash content was determined by heating in a 550 °C furnace for 24 h (AOAC, 2005). Carbohydrates were estimated by difference.

Mineral content (Ca, Cr, Cu, Fe, K, Mg, Mn, P, Zn and Se) of crackers were determined using a Radial Simultaneous inductively coupled plasma optical emission spectrometry (ICP-OES) Varian 725-ES spectrophotometer (Agilent Technologies, Santa Clara, CA, US). Aliquots of tef crackers (0.5 g) were placed in Teflon cups, diluted with 6 mL of 65% HNO$_3$ and 2 mL of 30% H$_2$O$_2$, heated for 6 min up to 200 °C and hold for 15 min at 200 °C for mineralization in a microwave digester (MLS 1200 mega, Milestone, Shelton, CN, US) and finally diluted to 25 mL. The determination was carried out in duplicated.

2.6. Total antioxidant capacity (TAC)

Classical and QUENCHER (Q-) versions of several TAC methodologies were used to assess the potential antioxidant capacity of different flour ingredients and crackers. Total Phenol content (TP), DPPH radical scavenging activity (DPPH), Oxygen Radical Absorbance Capacity (ORAC), FRAP (Ferric Reducing Ability of Plasma) and Trolox Equivalent Antioxidant Capacity (TEAC) were evaluated.

2.6.1. Extract preparation

One gram of each finely ground (mesh size 0.3 mm) sample was extracted with 10 mL of methanol:water (1:1, v/v; acidified to pH = 2 with 0.1M HCl) in a temperature-controlled orbital shaker (25 °C, 250 rpm, 1 h). After centrifugation (25 °C, 3800 g, 10 min), the supernatant was collected, filtered (Whatman paper nº 1), adjusted to 25 mL with extracting solvent added through the filter residue, and stored at -80 °C until further analysis.

2.6.2. Total phenol content (TP)

TPs were measured using the Folin-Ciocalteu method as described by Slinkard and Singleton (1977) with mofidications (Martín-Diana et al., 2017). Extracts were diluted 1/10 (w/v) in methanol. A volume of 140 μL of the sample extract was mixed with 280 μL of Folin-Ciocalteu reagent previously diluted (1:10, v/v) and 980 μL of 42.86 mM sodium carbonate. The mixture was shaken and allowed to stand for 100 min in darkness, following centrifugation at 15,000xg for 3 min. The absorbance was measured at 760 nm with a microplate reader (Fluostar Omega, BMG Ortenberg, Germany). Results were expressed as μmol gallic acid equivalents (GAE) g^{-1} of sample using a calibration curve with Gallic acid as standard (9.8–70 mM).

2.6.3. DPPH (on extracts) and Q-DPPH (on solid samples)

The antioxidant activity of the extracts against DPPH· radical was estimated according to the procedure described by Brand-Williams et al. (1995) with modifications. A total of 0.1 mL of sample in methanol was added to 3.9 mL of 63.4 μM DPPH methanolic solution. Absorbance was measured at 515 nm using a microplate reader (Fluostar Omega, BMG Ortenberg, Germany) after 60 min incubation at room temperature in dark conditions.

The Q-DPPH method was assayed according to the procedure by Serpen et al. (2007), with modifications. Ten milligrams of powdered solid samples (particle size below 300 μm) were mixed with 1.6 mL of DPPH· working solution (50 μM) prepared in methanol. After incubation at 750 rpm for 30 min (Thermomixer Compact, Eppendorf, AG, Hamburg, Germany), samples were centrifuged at 10,000xg for 2 min and the absorbance measured at 515 nm in a microplate reader. Results for both assays were expressed as percentage of inhibition (%).

2.6.4. ORAC

The procedure was based on a previously reported method with slight modifications (Ou et al., 2001). Standard curve of Trolox (15–240 mM) and samples were diluted in phosphate buffer (10 mM, pH 7.4). A volume of 150 μL fluorescein was placed in a 96-well black polystyrene plates, and 25 μL of Trolox standard, sample or phosphate buffer as blank were added, all in duplicates. Samples, standards and blanks were incubated with fluorescein at 37 °C for 3 min before AAPH solution was added to initiate the oxidation reaction. Fluorescence was monitored over 35 min with a microplate reader (Fluostar Omega, BMG, Ortenberg, Germany), using 485 nm excitation and 528 nm emission filters. Results calculated using the areas under the fluorescein decay curves, between the blank and the sample, and expressed as mmol Trolox Equivalent (TE) g^{-1} of sample.

2.6.5. TEAC

TEAC was evaluated following the method first described by Miller et al. (1993), as modified by Martín-Diana et al. (2017). TEAC analysis was used to evaluate the antioxidant capacity of the samples in a direct way, without extraction. Ten milligrams of solid sample was mixed with 160 mL of ethanol:water (50:50, v:v). After that, 1.6 mL of ABTS solution were added to the sample and the mixture incubated at $3 + 0$ °C; 250 rpm for 30 min (Thermomixer Compact, Eppendorf, AG, Hamburg, Germany). After incubation, the sample was centrifuged at 14000 rpm during 2 min. The absorbance was measured at 730 nm with a microplate reader (Fluostar Omega, BMG, Ortenberg, Germany). Results were expressed as μmol Trolox g^{-1} sample.

The Q-TEAC method described by Serpen et al. (2007), as modified in Martín-Diana et al. (2017), was used to evaluate the direct antioxidant capacity of ingredients and/or crackers. Powdered solid samples were diluted 1:4 (w:w) with cellulose. Ten milligrams of diluted sample were mixed with 1.6 mL ABTS^{+} working solution. A volume of 160 μL of methanol:water (50:50, v:v; pH $= 2$) was added to sample assays to equal the final volume present in the calibration curve runs. After incubation at 750 rpm for 30 min at 25 ºC in a thermomixer, samples were centrifuged at 10000xg for 2 min and the absorbance was measured at 730 nm in a microplate reader. Results were corrected for moisture and expressed as μmol Trolox g^{-1} sample.

2.7. Starch fractions analysis

In vitro starch digestibility was analyzed on the minced samples using the method by Englyst et al. (1992), including its latest modifications (Englyst et al., 1999, 2000) as was used by Abebe et al. (2015) and Ronda et al. (2012). The final determination of glucose was performed using the glucose oxidase colorimetric method. The free sugar glucose (FGS) content was also determined through separated test for all the samples following the procedure proposed by Englyst et al. (2000). Hydrolysed glucose at 20 min (G20) and 120 min (G120) and the total glucose (TG) were tested six times for each cracker sample. Once obtained the data, rapidly digestible starch (RDS) = 0.9 * (G20− FGS), slowly digestible starch (SDS) = 0.9 * (G120− G20), resistant starch (RS) = 0.9 * (TG − G120), total starch (TS) = 0.9 * (TG − FGS), rapidly available glucose (RAG) = G20 and the starch digestion rate index (SDRI) which is the amount of RDS in the sample as a percentage of the TS content, were calculated and expressed as the mean and 95% confidence interval on dry matter.

2.8. Sensory analysis

A trained panel of eight panellists aged between 20 and 45 years old recruited from the Staff of the Institute and trained in food product development evaluated texture (hardness), masticability (gumminess), aftertaste (bitterness), flavour and preference for the crackers with better bioactive profile: control (rice), white 50% tef, 50% tef mixture (white-brown), and 100 % white and brown.

A multiple-samples ranking test was used as a quick, simple, and useful tool to assess differences in sensory attribute among multiple products. In this test, each panellist evaluates and ranks a complete set of samples once, generating one vector of multiple dependent data (Carabante and Prinyawiwatkul, 2018). To fit this ordinal dependency, the nonparametric Friedman's test was used. According with the ITACyL normative was not necessary approval by ethical committee. All the sensory analyses were conducted immediately after product preparation to ensure the safety consumption of these crackers.

2.9. Statistical analysis

Principal component analysis (PCA) was employed to qualitatively investigate relationships among the ingredients and bioactive and proximal composition markers. Differences between clusters were established using Least Significant Difference test with a level of significance of 5 %. Correlations among variables were assessed by means of the Pearson's correlation tests ($p < 0.05$). The impact of ingredients on antioxidant and proximal composition properties was evaluated using multifactor ANOVAs. All the statistical analyses performed using Statgraphics Centurion XVI®.

3. Results and discussion

Replacing gluten functionality is a challenge for food industry. The lack of gluten leads to weak cohesion and elastic doughs which results in a crumbling texture, poor colour, and low specific volume. Tef is a cereal with poor functionality. Based on previous experiences of the authors (Del Pino-García et al., 2018; Martín-Diana et al., 2017; Abebe et al., 2015; Ronda et al., 2015a,b) with tef and gluten free cracker at different concentrations with white, brown and mixture were formulated and evaluated their nutritional, healthy and organoleptic behaviour.

3.1. Proximal composition

Nutritional analyses were carried out in order to evaluate the proximal content of the different flour used in the study. The results showed significant differences among rice and tef flours and between two tef flours (white and brown) (Table 1). Tef flour showed significant (p ≤ 0.05) higher levels of ashes regardless of the tef flour type. It is described that tef contains higher levels of minerals than other cereal grains, including wheat, barley and sorghum (Abebe et al., 2007). White tef showed higher levels compared to brown.

Protein is the second most abundant component in tef after starch. It ranges between 8.7 and 11 %, according to the results reported by Bultosa (2007); in our study the flour protein ranged 10.78–11.72 %, thus, the protein content of the tef grain was comparable to other common cereals such as barley, wheat and maize and higher than rice flour (aprox. 8 %) that it was used as control in the study. Brown tef also showed significantly higher protein level than white tef. No differences were observed among tef types on carbohydrates content although were in both cases slightly lower than rice but in all cases higher than 75 % (Table 1).

The study of the nutritional properties of crackers formulated showed significant differences among control and crakers with tef in all the parameters analysed (ash, protein, humidity and carbohydrates) with the exception of fat (Table 1). Ash content showed higher values in tef and higher in white tef *vs* brown tef. The control cracker showed the lowest value of ashes (2.75 %) and the maximum values corresponded to the cracker formulated using 100% of white tef (4.84%). Higher levels of humidity were reported for cracker with 100 % of tef (11.8% for brown and 12.47% for white) meanwhile the crackers formulated with rice showed significant lower values (8.32%) which probably could be associated to the higher fat content of tef flours compared with rice flour, and in tef flour higher protein levels were observed at higher concentration in agreement with the result reported by other authors (Alaunyte

Table 1
Proximal composition (ash. fat. moisture. protein and carbohydrate) of flours and crackers. WT: White tef; BT: Brown tef; MT: Mixture of white and brown tef. 25-, 50- and 100-: Percentage of tef flour over total flour. Values with a letter in common in the same column are not significantly different (p > 0.05). Values with a letter in common in the same column are not significantly different (p > 0.05). Statistic for Flours and Crackers is independent.

Flour-cracker	Ash (%)	Fat (%)	Moisture (%)	Protein (%)	Carbohydrate (%)
RICE	1.35 ± .00a	1.07 ± 0.01a	10.01 ± 0.12a	7.63 ± 0.00a	79.95 ± 0.13b
WT	3.05 ± 0.02c	2.46 ± 0.06c	11.13 ± 0.13b	10.78 ± 0.042b	72.58 ± 0.17a
BT	2.21 ± 0.00b	1.98 ± 0.16b	11.74 ± 0.56b	11.72 ± 0.042c	72.36 ± 0.77a
CONTROL	2.75 ± 0.20a	13.4 ± 0.99b	8.32 ± 0.01b	5.98 ± 0.06a	69.56 ± 0.82d
25-MT	3.23 ± 0.20b	14.55 ± 0.21c	7.16 ± 0.00a	6.60 ± 0.05bc	68.46 ± 0.14d
25-WT	3.46 ± 0.28b	12.35 ± 0.35ab	12.03 ± 0.04e	6.13 ± 0.04b	66.03 ± 0.21c
25-BT	3.24 ± 0.04b	10.47 ± 1.49a	10.47 ± 0.01c	6.28 ± 0.05b	66.06 ± 1.38c
50-MT	3.57 ± 0.05c	14.65 ± 0.49c	7.61 ± 0.06ab	7.16 ± 0.04c	67 ± 0.57cd
50-WT	4.15 ± 0.03d	14.20 ± 0.42c	9.00 ± 0.04b	6.91 ± 0.04bc	65.75 ± 0.38c
50-BT	3.83 ± 0.03cd	15.00 ± 0.14c	11.66 ± 0.01d	7.03 ± 0.04c	62.48 ± 0.15b
100-MT	4.84 ± 0.10f	14.57 ± 0.21c	14.57 ± 0.02f	7.88 ± 0.09d	57.77 ± 0.42a
100-WT	4.25 ± 0.01e	14.50 ± 0.41c	11.8 ± 0.06d	8.44 ± 0.00e	60.31 ± 0.70b
100-BT	2.77 ± 0.01a	14.60 ± 0.70c	10.98 ± 0.12c	8.04 ± 0.00e	70.33 ± 0.70d

Table 2
Total phenols (TP) and antioxidants markers (DPPH. ORAC. Q-TEAC and Q-DPPH) of flours and crackers. WT: White tef; BT: Brown tef; MT: Mixture of white and brown tef. 25-. 50-. and 100-: Percentage of tef flour over total flour. Values with a letter in common in the same column are not significantly different (p > 0.05). Values with a letter in common in the same column are not significantly different (p > 0.05). Statistic for Flours and Crackers is independent.

Flour-cracker	TP (mg GAE 100g^{-1})	DPPH (µmol Eq. Trolox 100g^{-1})	TEAC (µmol Eq. Trolox 100g^{-1})	ORAC (µmol Eq. Trolox 100g^{-1})	Q-DPPH (µmol Eq. Trolox 100g^{-1})	Q-TEAC (µmol Eq. Trolox 100g^{-1})
RICE	21.13 ± 1.45a	23.36 ± 0.37a	1198 ± 34a	102 ± 43a	114 ± 18a	23211 ± 83a
MT	66.20 ± 4.08c	39.90 ± 2.29bc	2336 ± 41bc	5019 ± 40c	1049 ± 10c	24232 ± 37a
WT	74.53 ± 1.48d	43.25 ± 1.10c	2640 ± 42c	4309 ± 62c	641 ± 49b	21197 ± 80a
BT	56.50 ± 2.29b	36.08 ± 2.87b	1979 ± 36b	2157 ± 77b	997 ± 13c	21675 ± 83a
CONTROL	16.91 ± 4.65a	23.09 ± 0.29a	1283 ± 39a	145 ± 11a	88 ± 27a	8298 ± 29a
25-MT	47.19 ± 2.12d	28.78 ± 1.93bcd	1917 ± 39bcd	919 ± 34a b	194 ± 80bc	8562 ± 36a
25-WT	31.02 ± 1.17c	25.74 ± 0.16abc	1555 ± 33ab	656 ± 22ab	163 ± 11abc	13235 ± 16ab
25-BT	23.58 ± 0.93b	25.16 ± 0.48ab	1354 ± 33a	423 ± 43ab	142 ± 17ab	11394 ± 68ab
50-MT	51.40 ± 2.18de	29.47 ± 0.75cd	2051 ± 37bcde	1192 ± 16bc	201 ± 13bc	10629 ± 89ab
50-WT	55.62 ± 3.27ef	29.27 ± 0.47cd	2183 ± 32cde	1786 ± 53cd	181 ± 80bc	16048 ± 70bc
50-BT	38.96 ± 2.37c	26.69 ± 1.02abc	1683 ± 29abc	690 ± 97bc	221 ± 11c	16433 ± 70bc
100-MT	58.26 ± 3.55fg	30.69 ± 0.20d	2274 ± 34de	2093 ± 30d	486 ± 31d	20956 ± 10cd
100-WT	66.98 ± 3.75h	31.53 ± 0.60d	2486 ± 28e	2928 ± 39d	471 ± 64d	21165 ± 82cd
100-BT	63.45 ± 7.72gh	35.77 ± 4.51e	2240 ± 32de	1918 ± 78cd	465 ± 10d	22295 ± 33d

et al., 2012; Hofmanová et al., 2014). Water retention capacity are generally also favours due to the fibre hydration. Moreover, the white tef showed significant higher protein levels than brown tef flour. Carbohydrates showed an inverse relation to the concentration used, the higher values appeared in the control cracker (69, 56 %) and the lower value in the cracker formulated with 100 % of tef (57, 77 %).

The mineral contents are dependent on the genetic and environmental factors (Baye, 2014). As can be seen in Table 2, incorporation of tef flour resulted into significantly higher amount of micro-elements compared to rice control crackers. These results are in agreement with previous studies, which confirmed the high nutritional value of tef flour (Zhu, 2018; Ronda et al., 2015a,b; Hager et al., 2012). A reduction in the dosage of tef flour in the formulation was linked to a proportional reduction in the mineral content in the final product. Crackers with 50% tef flour showed half the mineral content of those made with 100 % tef flour. Significant differences were also observed in cracker micro-element contents depending on the type used. Those made with white tef flour had higher concentration of all the elements tested, except for calcium and manganese, than those made with brown tef. It should be noted that Fe content of white tef crackers was almost twice that of brown tef counterparts. The different ash content of the original tef flours may explain these results. The calcium and iron content of 100% WT crackers was 15 and 10 times respectively higher than control crackers, making tef an excellent source of these important macro-minerals. The Cupper and Magnesium content passed from 0.12 mg/100 g and 39.2 mg/100 g in the control crackers to be 0.56 mg/100 g and 197 mg/100 g respectively for 100%WT crackers, which represents an increase of around 500 %. The Potassium, Manganese, Phosphorous and Zinc content were also incremented by around 300 % compared to control crackers made with rice flour. According to the Regalement EU 1169/2011, the intake of 100 grams of crackers made with 100 % WT could satisfy half of the daily iron requirements. The same would apply to Copper, Magnesium and Phosphorous while Manganese requirements may covered.

3.2. Total antioxidant capacity (TAC)

Total antioxidant capacity (TAC) was evaluated with different *in vitro* antioxidant markers (TP, DPPH, ORAC, Q-DPPH and Q-TEAC) for the different flours and crackers (Table 3).

The results showed significantly (p ≤ 0.05) higher total phenol content on tef flours (56.50–74.53 mg GAE/100 g) compared to rice flours (21.13 mg GAE/100 g). In addition, higher values were observed in white tef, compared to the brown, results in agreement with those found by other authors (Forsido et al., 2013; Salawu et al., 2014; Shumoy and Raes, 2017). These authors reported higher soluble phenolic content in white tef varieties, while brown varieties showed higher bound and total phenolic content. Differences in TP level with previous works may be associated to cultivar variability, or agronomic factors such as fertilisation and climatological conditions (Ronda et al., 2015a,b; Forsido et al., 2013). Regarding antiradical activity against DPPH, rice flour showed significantly (p ≤ 0.05) lower capacity than tef flour, and white had significant higher activity than brown, with mixture flour showing similar values to white flour, which it would be in agreement with total phenol content (Table 2).

It was also observed that tef flours showed significantly higher TEAC and ORAC antiradical capacity than rice flour, and among those, BT flour showed lower values than WT flour, in agreement with the results obtained for DPPH.

Polyphenols in cereal samples are distributed as free, soluble-esterified, and insoluble-bound forms; direct antioxidant methods, Q-DPPH and Q-TEAC, are carried out on solid samples and include therefore activity of free and bound phenolics. The results showed higher Q-DPPH values for tef than those obtained for rice. Opposite results found with the antioxidant methods carried out on extracts (DPPH, ORAC), the

Table 3
Micro element content and starch fractions, FSG, RAG and SDRI of crackers supplemented with 50% or 100% of tef flour of different types (brown or white) in substitution of rice flour. All the values are referred to dry matter except SDRI. Control: 100% rice flour crackers; WT: White tef; BT: Brown tef. Values with a letter in common in the same column are not significantly different (p > 0.05). FSG: Free glucose and sucrose; RAG = rapidly available glucose; RDS = rapidly digestible starch. SDS = slowly digestible starch; TDS: total digestible starch; RS = resistant starch; TS = total starch and SDRI = starch digestion rate index. Values are the mean ± standard deviation. Values with a letter in common in the same row are not significantly different (p > 0.05).

Cracker	Control	50-WT	50-BT	100-WT	100-BT
Ca (mg/100g)	11.9±1e	97.9±2d	117±6c	182±1b	220±2a
Cr (mg/100g)	<0.25	<0.25	<0.25	<0.25	<0.25
Cu (mg/100g)	0.117 ± 0.03e	0.356 ± 0.01d	0.407 ± 0.02c	0.562 ± 0.02a	0.534 ± 0.03b
Fe (mg/100g)	0.80 ± 0.2d	4.05 ± 0.3b	2.67 ± 0.5c	8.07 ± 0.2a	4.4 ± 0.2b
K (mg/100g)	119±4d	231±5b	181±4c	338 ± 30a	242 ± 10b
Mg (mg/100g)	39.2±1e	115±4c	83.6±7d	197±1a	130±2b
Mn (mg/100g)	0.832 ± 0.02e	1.77 ± 0.11d	2.23 ± 0.26c	2.76 ± 0.05b	3.68 ± 0.07a
P (mg/100g)	133±1e	263 ± 25b	194 ± 10d	404±2a	256 ± 13c
Zn (mg/100g)	1.01 ± 0.03d	1.91 ± 0.09b	1.42 ± 0.18c	2.88 ± 0.24a	1.85 ± 0.03b
Se (mg/100g)	<0.004	<0.004	<0.004	<0.004	<0.004
FSG (g/100g)	5.0 ± 0.1c	4.8 ± 0.1b	4.8 ± 0.1b	4.0 ± 0.1a	4.8 ± 0.1b
RAG (g/100g)	60.6 ± 1.9c	57.7 ± 0.5b	57.5 ± 0.4ab	55.6 ± 1.2a	56.0 ± 1.2ab
RDS (g/100g)	50.0 ± 1.7b	47.6 ± 0.4a	47.4 ± 0.3[a]	46.5 ± 1.1a	46.1 ± 1.1a
SDS (g/100g)	7.1 ± 1.2c	3.4 ± 1.3b	6.0 ± 1.8c	2.8 ± 1.1ab	1.2 ± 1.4a
TDS (g/100g)	57.1 ± 2.8d	51.0 ± 1.4bc	53.4 ± 1.5c	49.2 ± 1.6ab	47.3 ± 1.1a
TS (g/100g)	60.7 ± 1.9c	55.6 ± 1.1b	56.2 ± 1.0b	52.6 ± 0.8a	52.3 ± 0.6a
RS (g/100g)	3.5 ± 4.5a	4.6 ± 2.2a	2.8 ± 1.0a	1.5 ± 4.7a	5.0 ± 1.6a
SDRI (g/100g)	80.2 ± 2.4a	85.6 ± 2.3bc	84.4 ± 1.8b	88.4 ± 2.5c	88.1 ± 1.6c

brown type showed higher antiradical capacity (significant in the case of Q-DPPH) than white. This result may be associated to higher content of non-extractable phenols in brown tef compared to white, which would be in agreement with results reported by other authors (Kotásková et al., 2016; Shumoy and Raes, 2017).

The TP content and antioxidant capacity of crackers were also evaluated. Total phenolic compounds (TP, Table 3) in crackers formulated with tef were higher than those formulated solely with rice (control; 16.91 ± 4.65 mg GAE/100g). In agreement with results obtained with flours, white tef crackers showed significantly (p ≤ 0.05) higher antioxidant activity than brown tef crackers.

The antioxidant activity was also evaluated in the extracts and direct methods (QUENCHER). The antioxidant activities of tef crackers were in all cases higher than the control. In agreement to the results observed for flours, and similarly to the results observed in TP content of the crackers, formulating with white tef resulted in higher antiradical activity (TEAC and ORAC), as compared with brown tef crackers. In the case of DPPH values, this trend was not observed in the case of 100% tef crackers (Table 3).

Principal Analysis Components (PCA) (Fig 1) was used to explain the effect of the tef type and its addition level on the antioxidant activity. Two main components were able to explain with an accumulate coefficient of 84.38 the sample variability (Fig 1I). The component 2 separated samples depending on the method used for evaluation TP, TEAC, ORAC, DPPH vs Q-TEAC and Q-DPPH.

The PCA (Fig 1II) shows a clear separation of the tef concentration based on antioxidant activity. Tef flours and crackers with increasing tef concentration in their formulation, showed higher antioxidant activity than rice flour and crackers with lower tef concentration. Particularly, crackers formulated only with tef (100 %) showed a clear separation from those formulated at lower concentration.

It was observed a significant separation between crackers and flours (Fig 1III). White tef flour and crackers had higher antioxidant activity, followed by cracker formulated with mixture (25 % white, 25 % brown) or only brown tef (100 %). In addition, rice crackers and flour (controls) were clearly separated and showed lower antioxidant values, as it was expected.

3.3. Starch fractions and in vitro starch digestibility

Rate of starch hydrolysis and the subsequent nutritionally relevant starch fractions obtained from rice (100 %, considered the control), tef (100 %) and (50 % flour-50 % rice) blended crackers are presented in Table 2. The significant differences (p < 0.05) in free sugar glucose (FSG) contents of crackers cannot be related to the different content of free sugar in the flours (1.5–1.9 % in tef flour versus 0.2% in rice flour) (Abebe et al., 2015). The effect of the addition of sucrose to the formula and the fermentation stage in the cracker making process undoubtedly had a preponderant effect on the final sugar content of the samples. Starch fractions (RDS, SDS and RS), rapidly available glucose (RAG) and starch digestion rate index (SDRI) did not show dependence on tef type (p > 0.05). Amounts of digestible starch (RDS and TDS) of crackers made with 100 % tef were significantly lower than values found for the 100 % rice flour cracker (46 % and 48 % in average versus 50 % and 57 %). This is probably because of the relatively lower starch contents (74–76 % vs 78.8 %) and higher dietary fiber and ash contents in the respective tef flours as compared to the rice one (Abebe et al., 2015). Results are in accordance with the superior total starch content (TS) found in rice crackers (61 %) compared to crackers made with 100% tef, 52 % regardless the type (Table 2). Crackers made with 50 % tef-50 % rice led to starch fractions (RAG, RDS, SDS and TDS) values between those obtained for 100 % rice and 100% tef crackers. All starch fractions except RS decreased significantly (p < 0.05) with the increase of tef flour. Other studies made in pastas with the addition of tef observed also a decrease in the starch digestion when the fibre content of the samples increased (Brennan and Tudorica, 2008; Hager et al., 2013). The addition of tef to wheat flour cracker also had the same effect (Ronda et al., 2015a,b). This means that the addition of tef leads to a lower glycemic response (Englyst and Englyst, 2005; Regand et al., 2009). However the starch digestion rate index (SDRI) of crackers increased significantly (p < 0.05) with tef flour, passing from 80 %, for the control sample (100 % rice flour), to 85 % and 88 % for the crackers made with 50 % tef-50 % rice and 100 % tef respectively. This means that, although the addition of tef contributes to a reduction of the RAG and RDS, the starch digestion rate increased as consequence of tef addition. Opposite effect was observed with the addition of tef to wheat breads, where a slight decrease of SDRI, from 98 % (100 % wheat flour) to 94 % (40 % tef), was reported (Ronda et al., 2015a,b). The different formulation and the particular fermentation process (24 h) used in cracker making could explain the different starch hydrolysis kinetics as result of digestive enzymes action (Gularte and Rosell, 2011).

3.4. Sensory analysis

The effect of the incorporation of tef flour to rice-based crackers was analysed using sensory judges. The formulations with higher antioxidant activities were selected (50 % and 100 % white, brown and mixture tef) and were compared to control cracker. The panel evaluated samples according to the texture, flavour, aftertaste, masticability and preference.
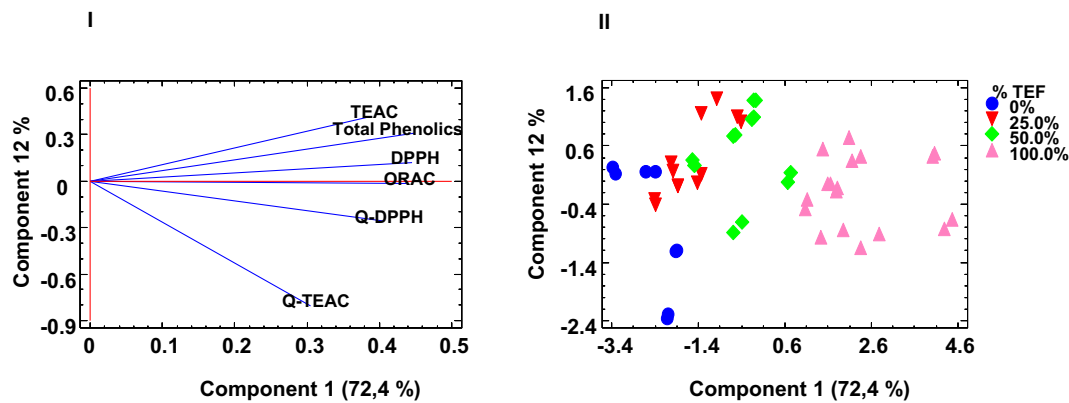
Fig. 1. Principal component analysis (PCA). I: Main components. II: Teff concentration.

Formulation with different tef flours did not affect the spreadability of the crackers, which presented identical width and thickness (Fig. 2), factor which has direct relation to product uniformity, quality, and consumer acceptance (Chauhan et al., 2015).

The panellist observed a similar profile regarding the parameters evaluated among control (rice) and white tef craker at 50 % followed by tef mixture 50 %, meanwhile crackers formulated with 100 % mixture or brown tef showed the biggest differences to control cracker profile (Fig. 3).

Compared to control, crackers at 50 % of white tef or mixture (25 % white and 25 % brown) produced similar textural profiles showing similar heights and spreads (Fig. 2). However, an increase of concentration (100 %) or incorporation of brown tef at concentration higher than 25 % produced a significant (p ≤ 0.05) gumminess difference, reducing the crispness of the product. The incorporation of brown tef modified significantly (p ≤ 0.05) the aftertaste compared to control probably associated to the bitterness produced by tef polyphenols (Duță et al., 2018). Panelists preferred the control and 50% white tef cracker to the other crackers evaluated.

4. Conclusion

The study showed than tef flour enhanced the antioxidant properties of snacks and this property was proportional to the concentration of tef added. White tef flour and crackers showed higher antioxidant activity associated to their higher soluble phenols content, meanwhile brown tef flour and crackers showed higher content of non-extractable phenols. Crackers formulated with white tef may result in products with higher biodisponibility of antioxidant compounds due to their higher solubility. In addition, the incorporation of tef would contribute to reduce the glycemic index of the product regardless of the type used (white or brown) and with a high dependence on the concentration incorporated. The significant increase in the mineral content of tef-enriched crackers, particularly those formulated with white flour, also justifies the nutritional interest of this product. Crackers formulated with 50 % tef flour (white or mixture) showed a sensory profile similar to rice crackers and acceptable preference for consumers.
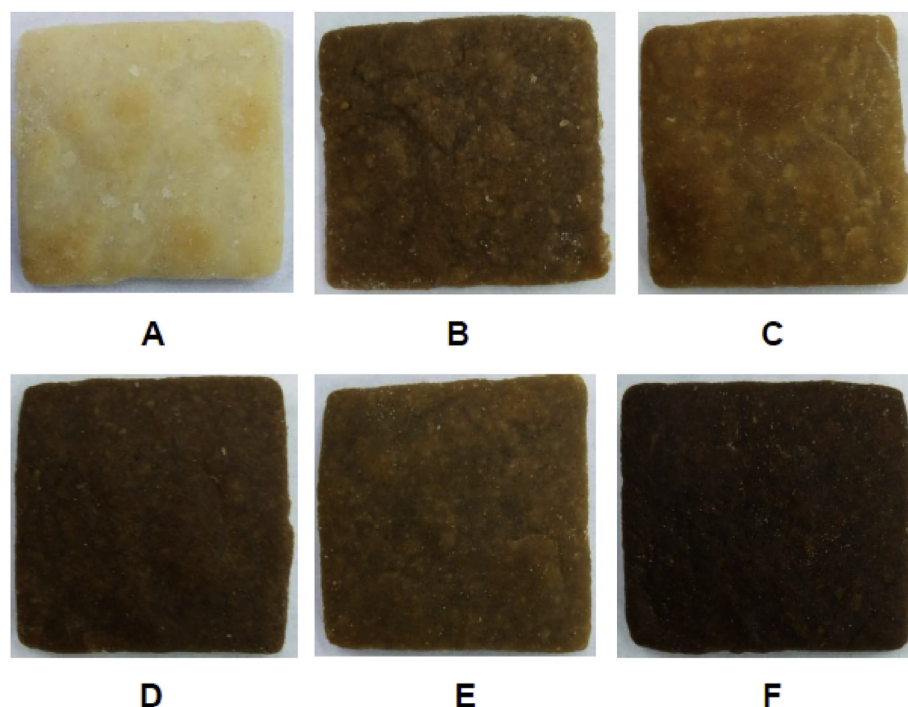

Fig. 2. Crackers produced with different concentrations of tef. A: Control (rice). B: 50 % tef mixture (white and brown). C: 50% White tef. D: 100% tef mixture. E: 100% white tef. and F: 100% Brown tef.
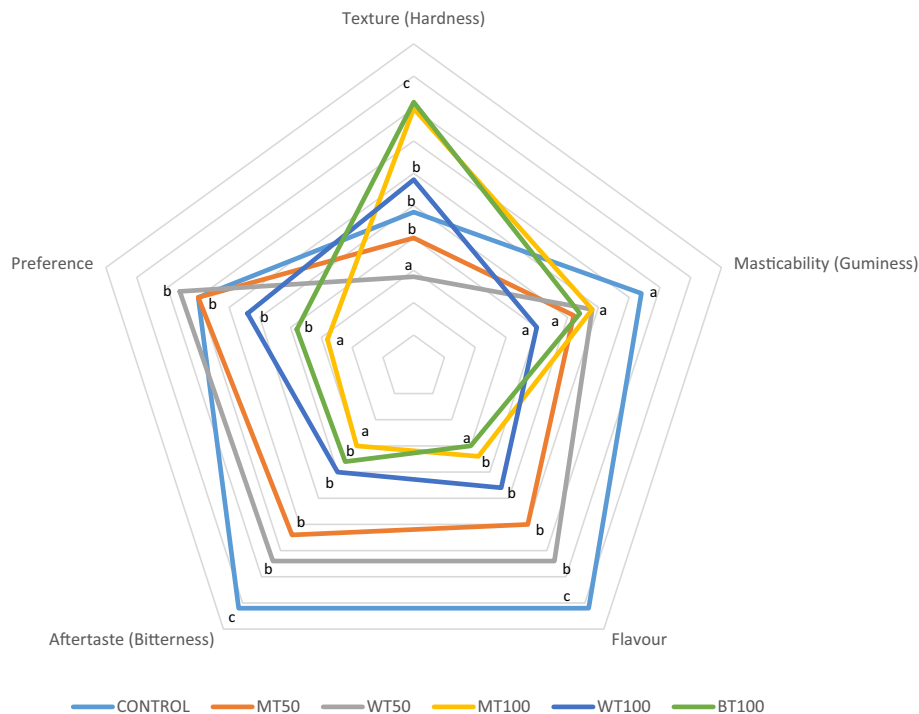
Fig. 3. Consumer preference of crackers supplemented with 50–100 % of tef. CONTROL: Rice flour; MT: Mixture of white and brown tef flour; BT: Brown tef flour; WT: White tef flour. Values with a letter in common in the same attribute are not significantly different (p > 0.05).

Declarations

Author contribution statement

Daniel Rico, Felicidad Ronda, Ana B. Martin-Diana: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Marina Villanueva, Carolina Perez Montero: Performed the experiments; Analyzed and interpreted the data.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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