

Optimization of Gluten Free Cookies from Red and White Sorghum Flours

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Abstract The aim of this study was to evaluate the use of red/white sorghum flours in the development of gluten free cookies. A Box Behnken design involving guar gum (GG), baking powder (BP) and water added to a given formulation and red/white sorghum flours as categorical variable, was used to obtain the optimal gluten free cookie formulation. Resulting cookies were subjected to sensory evaluation being flavour, fragility and crispiness evaluated responses. After that, the optimal formulation was obtained being GG and BP significant variables whereas sorghum flour did not showed statistical significance. Also proximal composition, total phenols and antioxidant activity as 1,1-diphenyl-2-picrylhydrazyl (DPPH) sequestering activity were evaluated in optimized red (RSC) and white (WSC) sorghum cookies. In spite of being total polyphenols contents of RSC (280 ± 1.83 mg tannic acid equivalents./100 g) higher than that from WSC (73 ± 1.91 mg tannic acid equivalents /100 g), evaluated DPPH scavenging activity was important in both cookies. Consuming a portion (30g) of them would provide a DPPH scavenging capacity of 572 mg and 544 mg ascorbic acid equivalent from RSC and WSC, respectively, which could be compared to the one provide from a 100 g portion of strawberries. Resulting sorghum cookies would contribute to a more diverse and nutritious diet for celiac people.

Keywords: sorghum, gluten free cookies, celiac disease, antioxidant activity, total polyphenols, melanoidins

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1. Introduction

Among the cereals without gluten used in food, sorghum has been characterized as a staple food for more than half a billion people in at least thirty countries [1]. It is the fifth most important cereal crop worldwide, with more than 63 million tons produced from approximately 47 million ha of land in 2007 [2]. Also, sorghum outperforms other cereals under various abiotic stresses and is thus generally more economical to produce being an important crop in Argentina [2]. Argentina is an important producer and the second world exporter of sorghum but it is underutilized in our country being consumed mainly by animals in spite of its known importance in human nutrition [3,4]. Sorghum has been utilized for human food along centuries, especially in Africa and Asia, being known to be rich in health promoting compounds such as phenolic acids, tannins, phytosterols, and policosanols [4,5]. Earlier studies have shown that sorghum grains possess antioxidant activity and anticarcinogenic effects and can reduce the risk of cardiovascular disease [4].

It is important to point out that celiac disease is a pathology increasingly detected not only in Argentina but all over the world. Celiac patients suffer a syndrome

characterized by damage of the small intestine mucose caused by ingestion of prolamine of wheat and the related cereals: oats, rye and barley being the only certain remedy the total omission of gluten from diet [6]. Sorghum flour, being devoid of gluten, may be replaced for wheat flour in gluten-free products such as cakes, breakfast cereals, breads, biscuits and pasta [2,7,8,9,10,11].

The present study aimed to the development of gluten-free sorghum cookies for celiac individuals using different guar gum, baking powder and water concentrations, and red or white sorghum flour as gluten replacement.

2. Materials and Methods

2.1. Red and White Sorghum Flours

Red and white condensed tannins free sorghum seeds were obtained from the INTA (Instituto de Tecnología Agropecuaria), Paraná, Argentina. Seeds were conditioned and milled by a roller mill (Buhler Miag, tipo MLGV, Germany). The maximum particle size was governed by the sieve mesh of 420 μ .

2.2. Gluten Free Sorghum Cookies

Method of cookies preparation was adapted from Erben et al. [12] using the following ingredients: sorghum flour

(100 g), shortening (25 g), sugar (25 g), salt (0.8 g) and also guar gum, baking powder and water were added to the dough at levels stated by the chosen statistical design. The dough was kneaded and sheeted to a uniform thickness of 0.2 cm and cut into circular shapes of 4 cm diameter. Baking was carried out at 220°C for 8 min. After baked, cookies were allowed to reach room temperature, removed from the baking sheet and packaged in polypropylene bags.

2.3. Proximate Analysis

Moisture, ash, fat and protein were determined according to Association of Official Analytical Chemists methods [13], in red/white sorghum flours and optimized sorghum cookies. Moisture was evaluated at 100- 105°C and ash in muffle at 550°C. Also, fat was extracted with petroleum ether by Soxhlet. For proteins determination, nitrogen content was measured by the semimicro-Kjeldahl method and converted to protein by using a factor of 6.25.

2.4. Total Phenols Analysis

Phenols were analyzed by Folin Denis method [14]. A 350 mg subsample of red/white sorghum flours or optimized cookies were added to 10 mL methanol 1% HCl (v/v) [2,15]. Samples were agitated at 225 rpm on shaker at room temperature for 20 min and filtrated. Aliquots of 1 mL were taken from the filtrate or its dilutions to which 0.5 mL of Folin Denis reagent and 1 mL of saturated Na₂CO₃ were added and diluted to 10mL with water. After that samples were incubated during 30 min. The absorbance was determined at 640 nm. A standard curve was established and results were expressed as mg tannic acid equivalents (eq.) per 100g.

2.5. DPPH Assay

The free radical scavenging activity of red and white sorghum flours and optimized cookies were evaluated by measuring the decrease in absorbance of methanolic DPPH (1,1-diphenyl-2-picrylhydrazyl) solution at 517 nm in the presence of acid methanolic extracts (1% conc HCl in methanol, v/v) [16]. The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for 30 min. IC₅₀ the amount of sample extracted into 1 mL solution necessary to decreased by 50 % the initial DPPH concentration was derived from the % disappearance vs. concentration plot. Results were expressed as mg/mL.

Also, the results are also expressed as ascorbic acid (AA) equivalent antioxidant capacity (AEAC) [17].

$$AEAC = (IC_{50(AA)} / IC_{50(sample)}) \times 10^5$$

IC_{50(AA)} value was determined to be 2.88±0.38 x 10⁻³ mg/mL based on ten determinations.

2.6. Sensory Evaluation

Cookies were evaluated by an expert panel of 3 individuals with more than 20 years of experience in baked products, the day after being baked, being stored in air tight containers. Samples were presented in a random order and judges evaluated the flavour, fragility and crispiness using a 10-point scale being scored from 1

(least favourable) to 10 (most favourable). Commercial wheat cookies were used as reference.

2.7. Experimental Design and Optimization

In order to optimize a sorghum cookie formulation a Box Behnken design was used. Three responses were measured for runs: flavour, fragility and crispiness. The independent variables chosen were guar gum (GG), baking powder, (BP), water, (W) and sorghum flour (SF). SF was a categorical variable which varied between white sorghum flour (WSF) and red sorghum flour (RSF). The design consisted of 30 experiences, 15 with RSF and 15 with WSF.

For each run, GG (0.5 g, 1.0g, 1.5g), BP (1.0 g, 2.0 g, 3.0 g) and W (45.0 mL, 50.0 mL, 55.0 mL) (Table 1) were added to the formulation at levels stated by the design. The selection of extreme levels was based on previous studies which had been done by the authors. Finally, with the aim to obtain the highest sensory quality cookie, an optimization process was employed using the Derringer desirability function [18].

2.8. Statistical Analysis

Means and standard deviations were calculated for proximate composition, polyphenols and antioxidant activity.

A software package Design Expert® 7.0.0 (Statease Inc., Minneapolis, USA) was used to fit the models and generate response surfaces. Tests to verify that the residues satisfy the assumptions of normality, independence and randomness were also evaluated. Significance was accepted at $p \leq 0.05$.

3. Results

3.1. Red and White Sorghum Flours

Fat contents for RSF was 2.62 ± 0.51 g/100g whereas for WSF was 2.73 ± 0.16g/100g. For protein contents in RSF 10.5 ± 0.5 g/100g were found and 9.80 ± 0.83 g/100g in WSF. Also moisture and ash were analyzed in sorghum flours, the first was 7.48 ± 0.19g/100g and the latter 1.04 ± 0.08 g/100g for WSF where for RSF both parameters were 7.79 ± 0.13 g/100g and 1.14 ± 0.01 g/100g, respectively. There were not significant differences between the analyzed parameters of WSF and RSF ($p \leq 0.05$).

The analysis of phenolic contents in different samples is influenced by the polarity of extracting solvents and the solubility of these compounds in the solvent used for the extraction process [19]. Therefore, it is hard to select an appropriate solvent for the extraction of phenolic contents from all samples. In the present study, methanol 1% HCl was used to extract sorghum phenolics as is usually employed in literature for these type of samples [2,15]. Total phenols proved to be 508±19 mg tannic acid eq./100g for RSF and 81.1±5.14 mg mg tannic acid eq./100g for WSF. For red and white sorghum varieties, similar features as those reported here for total phenols, have been published [2].

Antioxidant activity expressed as AEAC was higher in RSF than WSF being 1206±50 and 787±75 mg/100g, respectively. The latter could be expected because of their different phenols contents. It can be mentioned that

Ragaa S. et al, concluded, comparing DPPH sequestering activities from different cereals, that sorghum was exceptionally high in antioxidant activities followed by millet and barley [3].

3.2. Gluten Free Sorghum Cookies Optimization

Table 1 shows the Box Behnken experiment design arrangements and responses for the established points.

Table 1. Variables and their levels for Box Behnken design and responses

Run	Independent variables			Sensorial Responses			
	GG (g)	BP (g)	Water (mL)	Sorghum	Flavour	Fragility	Crispiness
1	0.50	2.00	55.0	W	7.33	7	6.66
2	1.50	1.00	50.0	R	5	5	4.66
3	1.00	1.00	45.0	R	5.66	5	4.66
4	1.00	2.00	50.0	R	7	7	6.33
5	0.50	1.00	50.0	R	6.66	6.33	5.66
6	1.00	1.00	45.0	W	7.66	7.33	6.33
7	1.50	1.00	50.0	W	7	6.66	5
8	1.00	2.00	50.0	W	6.66	5.66	5.66
9	0.50	1.00	50.0	W	5.66	6	5.33
10	1.50	3.00	50.0	R	6.33	5.33	5.66
11	1.50	2.00	55.0	W	6	5.66	5.33
12	0.50	2.00	45.0	W	7.33	7	6
13	1.00	3.00	55.0	R	7.33	7	6.66
14	1.00	2.00	50.0	R	6	6	5.66
15	1.00	2.00	50.0	R	7.66	7.33	7.66
16	1.00	3.00	45.0	R	7.33	7.66	7
17	0.50	3.00	50.0	R	7	7.33	7
18	0.50	2.00	45.0	R	7	6.33	6
19	1.00	1.00	55.0	W	6.66	6.66	5.66
20	0.50	2.00	55.0	R	7.66	7	6.66
21	1.50	2.00	45.0	W	5.66	6	5.33
22	1.50	3.00	50.0	W	7	7	6.33
23	1.00	2.00	50.0	W	6.66	6.66	5.66
24	1.00	1.00	55.0	R	6.66	5.33	4.33
25	1.00	3.00	55.0	W	8	7.66	8
26	1.00	2.00	50.0	W	6.33	6.33	6
27	1.00	3.00	45.0	W	7.33	8.33	8
28	0.50	3.00	50.0	W	8.33	8	8.66
29	1.50	2.00	45.0	R	7	6.33	5.33
30	1.50	2.00	55.0	R	6.33	6	5.66

GG: Guar gum, BP: baking powder, R: red sorghum, W: white sorghum.

The assumptions underlying the ANOVA test were investigated. Normal probability plot of residuals against estimated values for the response and plot of residuals against random order of runs revealed that the residuals satisfied the assumptions of normality, independence and randomness. Therefore, the ANOVA (Table 2) was performed and the lack of fit was evaluated to find whether the model appeared to fit the data or not. As it can be seen lack of fit was not significant so the chosen model was adequate for our purpose.

Table 2. Results of analysis of variance for flavour, fragility and crispiness

Source	DF	Valor p		
		Flavour	Fragility	Crispiness
Model	4	0.0091	0.0009	< 0.0001
GG	1	0.0145	0.0120	0.0037
Water	1	0.6960	0.5244	0.9098
BP	1	0.0055	0.0007	< 0.0001
Sorghum	1	0.3963	0.0599	0.2509
Lack of fit	21	0.5207	0.4988	0.7707
R ²		0.41	0.52	0.64

GG: guar gum, BP: baking powder, R²: determination coefficient. Statistical significance: p ≤ 0.05.

Analyses of variance for flavour, fragility and crispiness (Table 2) show that the linear model was significant and without lack of fit. For flavour, the model explain 41% of the results variability and for fragility and crispiness 52% and 64%, respectively. The analysis of variance shows that water was not a significant variable and that guar gum and baking powder were significant at p ≤ 0.05.

In this study, GG was used to replace the elastic and extensible properties of gluten in cookie preparation because sorghum flour is gluten free as it was mentioned above. As can be seen in Figure 1, higher amounts of GG reduce flavour, fragility and crispiness scores.

Also, BP addition was evaluated in this study taking into account that sorghum cookies have harder texture [15]. As can be seen in Figure 1, BP higher amount increases not only flavour but fragility and crispiness scores as well. It is known that BP is used for increasing the volume and almost invariably gives a more tender bite. Also a cookie with a larger volume is more appealing to the eye which is a factor consumer oriented. In our study, better scores were obtained at high levels of BP [20].

Besides explaining the behavior of variables by the response surface plots, the models fitted in this study may apply for optimization using the Derringer desirability

function [18]. Thus, the latter procedure was conducted to maximize the flavour, fragility and crispiness simultaneously. The formulation with the highest overall desirability (0.37) was selected. The optimum variables levels obtained were GG: 0.5 g, BP: 3 g, water 50 mL, which have to be added to the formulation mentioned in 2.2 in order to obtain the optimized cookies, being the same for WSF or RSF.

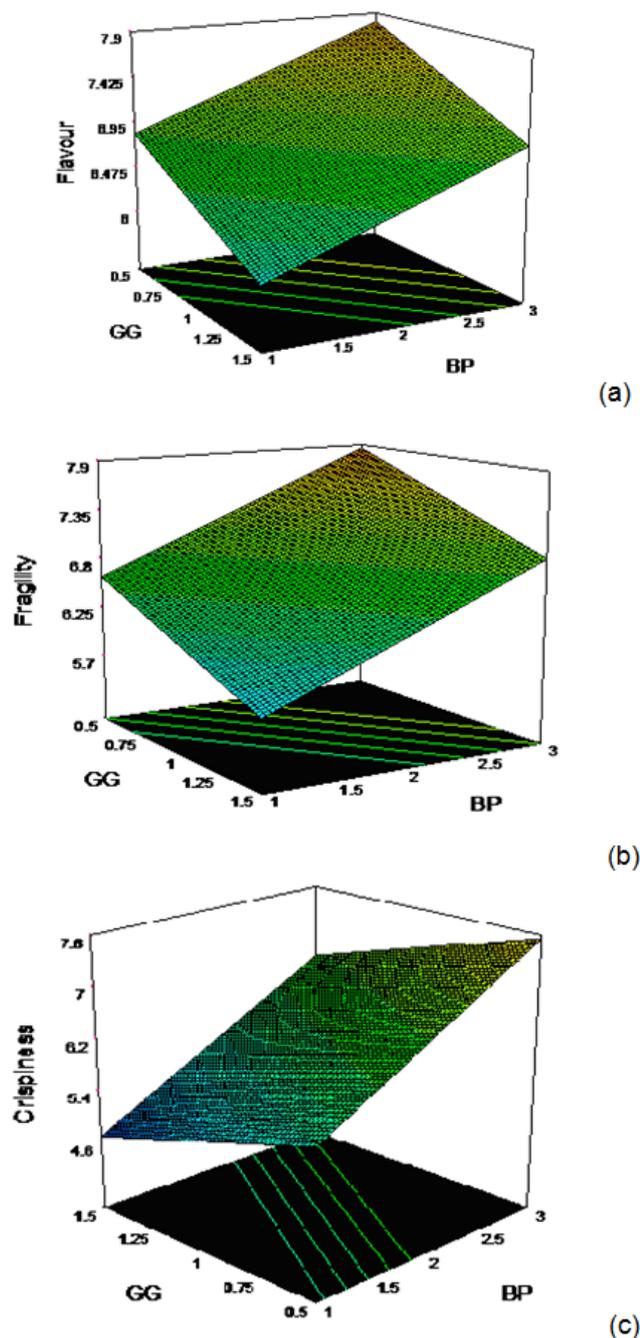


Figure 1. Response surfaces for evaluated responses: Flavour (a), Fragility (b) and Crispiness (c) against Guar gum (GG) and Baking powder (BP). Water was fixed at 50 mL. Surfaces correspond for white sorghum flour

3.3. Characteristics of Optimized Gluten Free Sorghum Cookies

Moisture, protein, ash, fat, total phenols and antioxidant activity of optimized gluten free cookies prepared with RSF and WSF are shown in Table 3. There were not

significant differences between protein and fat contents of optimized red sorghum cookie (RSC) and white sorghum cookie (WSC) ($p \leq 0.05$).

Table 3. Proximal composition, total polyphenols and antioxidant activity from red (RSC) and white sorghum (WSC) cookies

Parameters	Cookie	
	RSC	WSC
Moisture (g/100g)	5.54 ± 0.07	5.39 ± 0.08
Ash (g/100g)	1.83 ± 0.09	2.01 ± 0.08
Proteins (g/100g)	6.74 ± 0.37	6.67 ± 0.42
Lipids (g/100g)	16.21 ± 0.13	16.47 ± 0.16
Total phenols (mg tannic acid eq./100g)	280.01 ± 1.83	73.45 ± 1.91
Antioxidant activity (IC ₅₀ , mg/mL)	0.166 ± 0.01	0.180 ± 0.05

Media ± Standard deviation.

It can be seen in Table 3 that, differences in total phenols content were found for RSC and WSC being 280 ± 1.83 mg tannic acid eq./100g for the first one and 73 ± 1.91 mg tannic acid eq./100g for the latter which was related with the total phenols content previously found in RSF and WSF. Some phenols could be solubilized during dough processing for WSC.

Cookies antioxidant activities expressed as mg ascorbic acid equivalents (AEAC) were 1734 ± 20 mg/100g for RSC and 1650 ± 39 mg/100g for WSC. If the flour component in the cookie is considered (62% sorghum flour in WSC or RSC formulation), the antioxidant activities of cookies were higher than that of flours.

4. Discussion

The aim of this study was to evaluate the use of red/white sorghum flours in the development of gluten free cookies with adequate sensory characteristics, using a Box Behnken design, taking into account the fact that sorghum is devoid of gluten and its health promoting compounds. Thus, an optimal formulation was obtained and, as there was no sensorial statistical difference between WSF cookies and RSF ones, WSC and RSC were analyzed.

As was mentioned and can be seen in Table 3 WSC and RSC have similar contents of protein and fat. Regarding proteins in sorghum, it was reported that there is no difference in protein digestibility between red and white condensed tannins free sorghums in spite of its total polyphenols contents [21].

RSC and WSC could give 92mg tannic acid eq. and 24mg tannic acid eq. per portion (30g), respectively. As the health effects of polyphenols like prevention of cardiovascular diseases and cancers, depend on the amount consumed, RSC could give an important amount per portion (30 g) [22]. It can be mentioned that fruits like banana or orange contain 51mg and 75mg of total phenolics, respectively in a 100g portion [17].

As was mentioned above, RSC or WSC could give similar antioxidant activities per portion (30g) which can be 572 mg AEAC and 544 mg AEAC, respectively. It could be interesting if they are compared with the DPPH scavenging activity of a 100g portion of some fruits such as strawberries which was found to be 472 ± 92.9 mg AEAC/100g [23].

Chiremba C. et al, evaluating sorghum cookies did not find enhanced antioxidant activity after cooking as in our study which could be due to different recipes and baking conditions as oven temperature [15]. Patrignani et al, found higher DDPH sequestering activity when biscuits where baking at more elevated temperatures [24]. The important antioxidant activity found in optimized sorghum cookies, higher than it was expected taking into account their WSF and/or RSF contents, could be due to Maillard reactions products developed during cooking specially melanoidins (MLD) [25,26]. MLD are described as nitrogenous compounds with high molecular weight, high reducing potential and an intense brown colour [27]. MLD have antiradical, antimutagenic, antimicrobial, antihypertensive, antiallergenic, antioxidant and cytotoxic activities and could contribute for the DPPH sequestering activity in evaluated optimized sorghum cookies [24,28]. Also, it is important to mention that MLD are known to be very sensitive to DPPH [29].

5. Conclusions

An optimal formulation for red or white sorghum cookies was obtain using a Box Behnken design and evaluating sensory characteristics by trained judges. Also there was no statistical differences in protein content between both cookies and they have higher protein content comparing with those usually consumed by celiac patients. Besides, red and white sorghum cookies posses an interesting antioxidant activity because of their phenols and Maillard reaction products contents, specially red sorghum ones. Sorghum cookies would contribute to a more diverse and nutritious diet for celiac people. It could be advisable, in our opinion, to study its acceptability among not only celiac people but doing a test of general consumers as well, in order to expand a broader market for them.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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