International Journal of Food Science + Technology

International Journal of Food Science and Technology 2016, **51**, 2151–2156

Original article

Total polyphenol extraction from red sorghum grain and effects on the morphological structure of starch granules

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(Received 4 April 2016; Accepted in revised form 9 June 2016)

Summary The aim of this study was to propose a treatment to reduce the content of total polyphenols (TPP) and to obtain flour without significant morphological changes in the starch granules. The response surface methodology (RSM) was used to predict the influence of temperature and time on TPP extraction. Research work was conducted using the central composite design methodology. Results showed that the effect of temperature was more significant than that of time. A quadratic fit of data regarding temperature and linear data adjustment as to time was obtained. A scanning electron microscopy analysis of starch showed no morphological change in the granules at the temperature studied. Starch granules presented a size distribution ranging from 6 to 25 μm being 35% of granules <15 μm, and 65% of granules >15 μm in size, respectively.

Keywords Antioxidant activity, flour, nutrient utilisation.

Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth most important cereal crop in the world after wheat, rice, corn and barley (Singh *et al*., 2011), being an excellent source of energy used for both animal and human feed (Carvalho Teixeira *et al*., 2016). The world consumption of this cereal is considerable (Althwab *et al*., 2015) and it is probably due to the ability of the grain to grow over extensive agro-ecological zones (Taylor *et al*., 2014). The cereal has nutrients common to all varieties, including various minerals, vitamins and amino acids (Althwab *et al*., 2015). The presence of polyphenols in the grain is typical in all varieties. The antioxidant level of polyphenols in sorghum is higher than in any other cereal analysed (Dlamini *et al*., 2007). Polyphenol type and amount vary among species. Three basic groups of polyphenols are present in sorghum grain: phenolic acids, flavonoids and condensed tannins. All species have phenolic acids, a considerable number have flavonoids (anthocyanins, catechins and leucoanthocyanidins), and only a few of them have condensed tannins (Taylor *et al*., 2014). Condensed tannins are found in sorghum exocarp and give grains their red-brown colour. In addition to the benefits as an antioxidant, the presence of tannin in sorghum grains provides natural protection against the attack of microorganisms and insects (Chandrashekar & Satyanarayana, 2006).

Despite the interesting antioxidant capacity of the grain, some components like proteins and starch are less available because polyphenols interact with them (Aguiar Moraes *et al*., 2015). The reduction of these components is essential to improve the nutritional quality of the grain and to allow manufacturing flour with light colour for food preparations. The dark colour of the untreated flour conditions the development of certain types of food (Belhadi *et al*., 2013).

It is important to apply treatments that allow the subsequent utilisation of polyphenols to improve the quality of other food (Bröhan *et al*., 2011) and provide flour of a good nutritional value.

While conventional methods for polyphenol extraction are based on the utilisation of organic solvents such as methanol and acetone among others (Barros *et al*., 2013), this study was conducted with water, as it allows reaching both objectives previously set. This solvent was used in several cereals in comparison with others, and results showed that the extract obtained

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doi:10.1111/ijfs.13194

had the highest antioxidant capacity (Tufan *et al.*, 2013).

Flour was obtained applying the wet and dry methods as described by Sun *et al.* (2014). Two methodologies that improve starch quality with no rupture of granules were reported: annealing and heat–moisture treatment. Annealing is the treatment of sorghum grains in excess water (40–60% w/w) at temperatures below gelatinisation temperature, whereas heat–moisture treatment is performed under relatively lower moisture (less than 35% w/w) and higher temperature (usually above gelatinisation temperatures) conditions (Singh *et al.*, 2011).

The aim of this study was to propose a treatment to reduce the content of total polyphenols and to obtain flour without significant morphological changes in the starch granules. Annealing is appropriate to cause not only the desirable modification of the starch with no rupture of granules, but also to achieve polyphenol reduction; hence, it was the treatment adopted in the present work.

Materials and methods

Steeping

Sorghum bicolor (L.) Moench, red variety, from the major cultivars grown under uniform field conditions at Tres Isletas, Chaco Province-Argentina, was selected for this study. Two comparative steeping procedures were selected, depending on their ability to extract polyphenols. Both procedures were conducted at 25 °C during 24 h, one with water and the other with a sodium hypochlorite (NaOCl) solution containing 0.5% (v/v) available chorine. Sorghum grains (50 g) were steeped in 100 mL of water or 100 mL of sodium hypochlorite (NaOCl) solution. The samples were made in duplicate.

Annealing and polyphenol extraction

A solid–liquid extraction of the sorghum samples previously treated with distilled water was performed. The preparation was incorporated into a heat bath at 50, 54, 63, 72 and 75 °C, between 120 and 240 min, as detailed in the experimental design (Table 1). Response surface methodology (RSM) was used to study the simultaneous temperature (*T*) and time (*t*) influence on TPP extraction, as it allowed finding the optimal variation and indentifying the influence of both factors. The 3D response surface was used to determine the individual and cumulative effect of the factors and the mutual interaction between the factors and the dependent variable.

Each factor was set at two different levels (k). The total number of experimental runs (N) was calculated as:

Table 1 CCD: matrix of *T* and *t* along with experimental TPP extraction value

Std order	Run order	Pt type	*T* (°C)	Time (min)	TPP (mg g^{-1} sorghum)
1	6	Factorial	54	138	1.10
2	13	Factorial	72	138	2.65
3	9	Factorial	54	222	1.58
4	4	Factorial	72	222	3.24
5	10	Axial	50	180	1.21
6	12	Axial	75	180	3.62
7	2	Axial	63	120	1.59
8	8	Axial	63	240	2.16
9	5	Central	63	180	1.90
10	1	Central	63	180	1.90
11	11	Central	63	180	1.88
12	7	Central	63	180	1.81
13	3	Central	63	180	1.85

$$N = 2^k + 2 \cdot k + x_0 \quad (1)$$

where x_0 = 3 is the number of central points. Least squares regression methodology was used to obtain estimates for the parameters (Lataza Rovaletti *et al.*, 2014):

$$\mathrm{TPP} = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j + \varepsilon \quad (2)$$

where β_0 is the constant, β_i is the slope or linear effect of the input factor X_i, β_{ii} is the quadratic effect of the input factor X_i, β_{ij} is the interaction effect between the input factors X_i, and ε is the residual error (Montgomery, 2003).

Assays were carried out randomly using the rotatable central composite design (CCD) methodology, with axial values at a distance α = 1.414 from the central point. The resulting responses for each combination of variables are shown in Table 1.

Starch isolation

Five samples of sorghum grain were treated for polyphenol extraction during 240 min at different temperatures: 25, 50, 60, 70 and 75 °C. The maximum temperature applied for polyphenol extraction was set considering the cereal-specific gelatinisation temperature of 75 °C (Ogbonna, 2011). The maximum time applied for the polyphenol extraction in the experimental design was used for starch isolation.

The methodology applied by Belhadi *et al.* (2013) was used for starch isolation. After polyphenol extraction, grains were washed with distilled water and wet milled in a mortar and pestle. The slurry was filtered through a 125-μm, 80-μm and 63-μm mesh sieve. The material remaining on the sieve was rinsed with water. The collected filtrate was allowed to stand for 1 h. The filtrate was centrifuged at 760 ***g*** (Zelian, Tyfon II, Argentina) for 10 min. Centrifugation and washing were repeated several times until the top starch layer became white. The starch isolated for each sample was dried for 24 h at 40 °C. The samples were made in duplicate.

Analysis of SEM images

All five starch samples at the different temperature were analysed through scanning electron microscopy (SEM) analysis with a SEM microscope (LEO, EVO 40, Carl Zeiss Microscopy Ltd., Cambridge, UK) Further details of the methodology are given in Lataza Rovaletti *et al.* (2014).

Six images for each temperature at different magnifications 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 K× were taken. For each image, the maximum length of each starch granule observed was selected and converted from pixels to microns with the scale of each image. The maximum longitudinal size and the perpendicular size of each starch aggregate were determined in the same way that the individual starch granules were determined. The samples were made in duplicate.

Total polyphenol determination

Total polyphenols content was determined using the Folin–Ciocalteu method (Singleton *et al.*, 1999) and expressed as mg gallic acid per g sorghum. Measurements were taken in triplicate.

Statistical analysis

Data points were presented as the mean of the measured values. Variance was analysed, and the Tukey test was performed at 0.05 level of significance. The statistical software Design Expert (Stat-Ease Inc., version 7.0, Minneapolis, MN, USA) was used for the regression analysis and for the estimation of coefficients of the regression equations for the RSM, and Infostat (2002) was used for the analysis of the steeping procedures and starch isolation data.

Results and discussion

Steeping procedures

The extracts obtained with water and NaOCl solution showed an increasing trend with time in polyphenol extraction (Fig. 1). An empirical polynomial of second-order equation was obtained, which explained the behaviour of both treatments with time, eqn 3 for water with ${R_1}^2 = 0.993$ and eqn 4 for NaOCl solution with ${R_2}^2 = 0.999$.

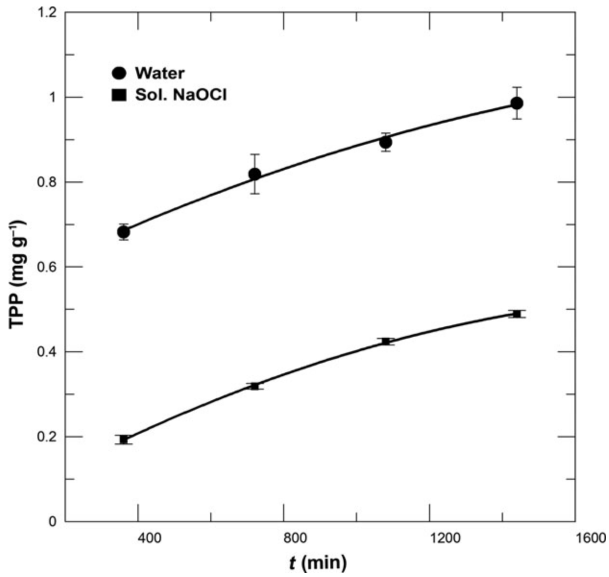

Figure 1 Total polyphenols extraction in the steeping process. Full lines represent eqns (3) and (4) for water and NaOCl solution, respectively.

$$TPP_w = -8.51 \times 10^{-8} \cdot t^2 + 4.27 \times 10^{-4} \cdot t + 5.43 \times 10^{-1} \quad (3)$$

$$TPP_{NaOCl} = -1.17 \times 10^{-7} \cdot t^2 + 4.86 \times 10^{-4} \cdot t + 3.22 \times 10^{-2} \quad (4)$$

However, results showed a significant decrease in the extracts with NaOCl sodium with respect to the water extract. TPP concentration was 0.5 ± 0.1 mg g^{-1} sorghum in the NaOCl while it was 1.0 ± 0.1 mg g^{-1} sorghum in the water extract after 24 h. This means that with a previous steeping process with NaOCl, 50% of the extractable polyphenols of the steeping stage was lost by oxidation. It is important to determine the loss of polyphenols occurring at this stage, as the sanitisation of grains with NaOCl in the wet milling of red sorghum is a common practice (Beta & Dzama, 2013) and its avoidance will improve performance in obtaining polyphenols.

Annealing and TPP extraction

Table 1 lists the matrix of T and t obtained with the CCD methodology, and the experimental TPP value after annealing.

Results showed that TPP in the water extract varied significantly under experimental conditions. A continuous increase was observed for both experimental variables. The increase in T was more significant than t (Fig. 2).

The increase in T allowed a TPP extraction of 3.7 ± 0.5 mg g^{-1} sorghum at 75 °C and 222 min. The result is in accordance with previous studies where polyphenol content ranging from 0.3 to 4.3 mg g^{-1}, determined using the Folin–Ciocalteu reagent (Taylor *et al.*, 2014).

A regression equation using the significant coefficients is represented as follows:

$$\mathrm{TPP} = 0.27 \times T^2 + 0.83 \times T + 0.23 \times t + 1.87 \quad (5)$$

The regression equation had two main effects and one curvature effect.

Significant estimated values of linear regression coefficients for T ($P < 0.0001$), t ($P < 0.0001$) and quadratic regression coefficients for T^2 ($P < 0.0001$) were obtained (Table 2). However, the quadratic regression coefficient for t^2 and the interaction between T and t were not significant.

The low standard deviation value (0.05) between the measurements and the estimates indicated that the equation represents the relationship between the response and the significant variables appropriately.

The high values of R^2 (99.6%) and R^2 (adj) (99.5%) indicated a high dependency and a correlation between the observed values and the predicted response values (Table 3). Furthermore, these values indicate that this model can explain 99.1% of the total variation.

The statistical significance of the ratio of the mean square variation due to regression and mean square residual error was tested using analysis of variance (ANOVA). F values for all regressions were high (823.9, vs. $F_{0.05;\ 5,\ 5} = 5.05$), according to the F-distribution tables for $F_{0.05}$ (Montgomery, 2003), indicating that most of the variations in the response can be explained by the regression equation. The lack of fit with $F = 2.1$ indicated that it is not significant.

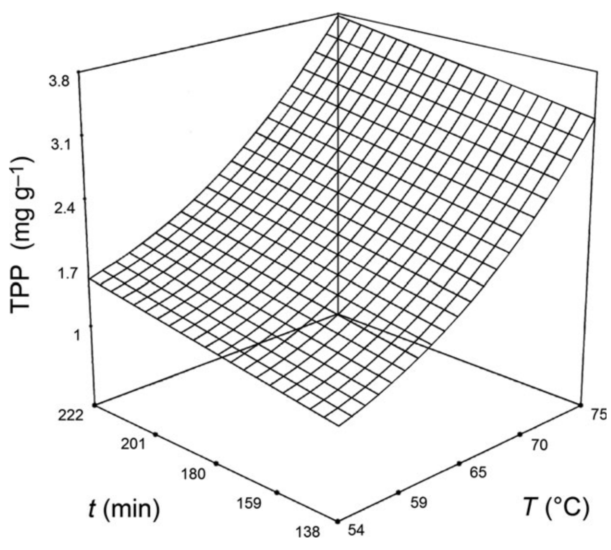

Figure 2 Response surface of total polyphenols vs. T and t.

Table 2 Analysis of variance (ANOVA) for TPP vs. T and t

Source	Sum of squares	df	Mean square	F value	P-value > F	
Model	6.44	3	2.145	823.991	<0.0001	Significant
T	5.48	1	5.475	2103.121	<0.0001	
t	0.44	1	0.441	169.241	<0.0001	
T^2	0.52	1	0.520	199.611	<0.0001	
Residual	0.02	9	0.003			
Lack of fit	0.02	5	0.003	2.100	0.2460	Not significant
Pure error	0.01	4	0.002			
Corrected total	6.46	12				

Table 3 Statistical parameters for the quadratic model of eqn 5

SD	0.05	R-squared (R^2)	0.996
Mean	2.04	Adj R-squared(R^2_{adj})	0.995
CV %	2.50	Pred R-squared(R^2_{pred})	0.991
PRESS	0.06	Adeq precision	88.434

Annealing with increased T may be favourable not only for the modification of the internal structure of starch granules but also for TPP extraction.

Starch isolation

The images obtained by SEM showed that starch granules have pores, holes and irregular shapes with a predominance of spherical and spheroid shapes (Fig. 3). The size distribution of granules of thirty images ranged from 6 to 25 µm with 35% of granules corresponding to sizes <15 µm and 65% corresponding to> 15 µm, of the total number of granules observed. Size distribution agreed with the values reported by Belhadi *et al.* (2013). There was no significant difference in size with the increasing temperature of polyphenol extraction. No breaking or gelatinisation of the granules was observed at high extraction temperature. Results indicated that the morphology of starch granules showed no change when the annealing procedure was applied.

The size of starch aggregates was analysed because other studies postulated the existence of a protein matrix acting as a link among starch granules. This matrix does not allow the easy starch digestibility (Duodu *et al.*, 2003). Aggregates of starch granules whose size was not affected by temperature changes

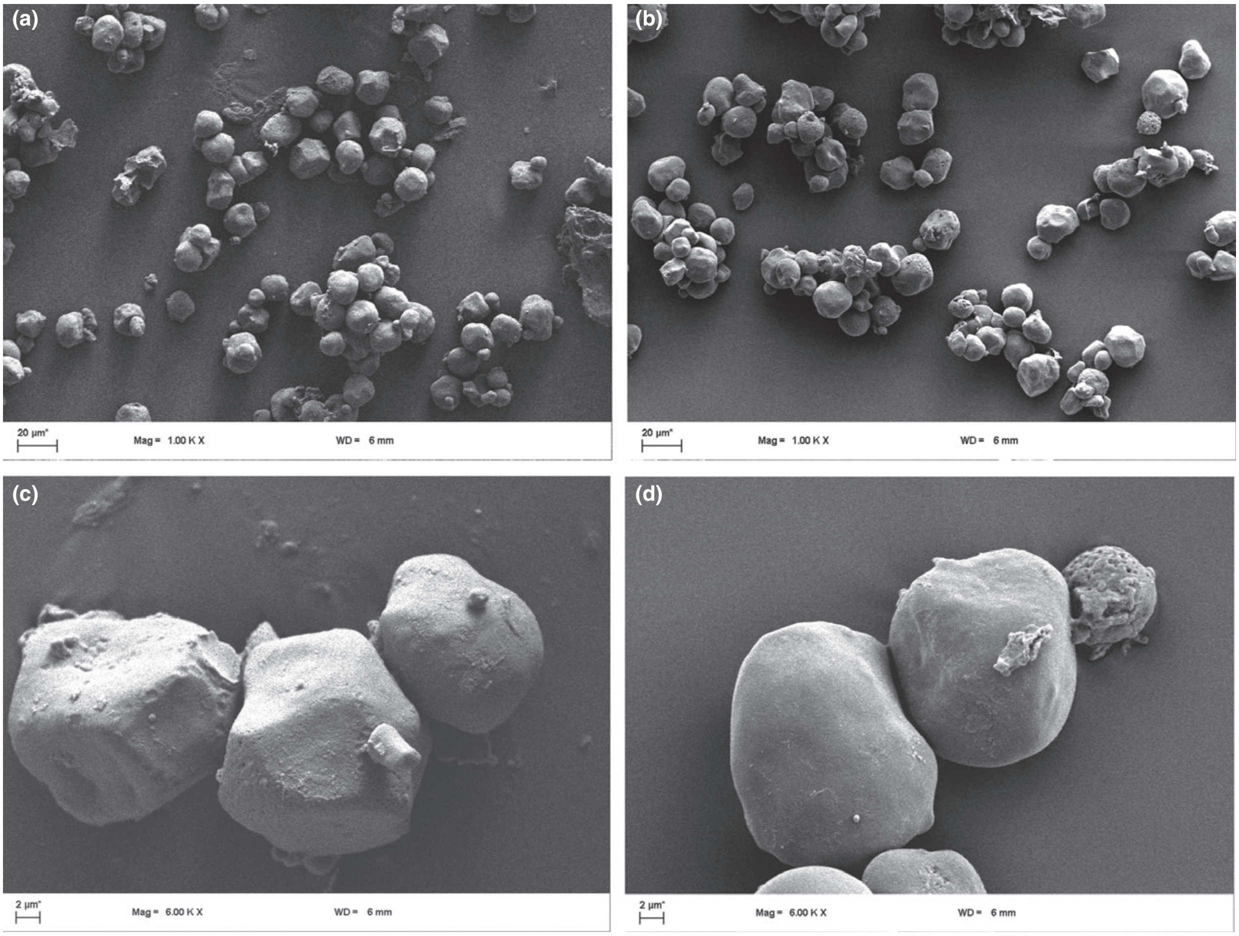

Figure 3 SEM images of starch granules: (a) 25 °C and (b) 75 °C, both with a magnification of 1.00 K×. Scale bar: 20 µm = 63 pixels, (c) 25 °C and (d) 75 °C, both with a magnification of 6.00 K×. Scale bar: 2 µm = 40 pixels.

were observed. The maximum longitudinal size of the aggregates was 102 ± 26 µm and 47 ± 12 µm perpendicular size. Figure 3 shows only the extreme operating temperatures for a better comparison as well as sizes of 1.0 and 6.0 K×.

Conclusions

Results showed that during stepping, it is possible not only to condition grains for milling but also to remove some of the polyphenols present in red sorghum grain. Also, it was possible to estimate the magnitude of the extraction and loss of polyphenols using NaOCl. By annealing, in comparison with steeping, the extraction of a more significant amount of polyphenols without causing structural changes in the starch granules was achieved.

The fact that starch granules do not change their morphology during treatment is a positive development, as it allows (i) recovering polyphenols and (ii) obtaining flour without the loss of starch granules by gelatinisation.

Acknowledgments

The authors thank the *Facultad Regional Resistencia-Universidad Tecnológica Nacional* and the *Consejo Nacional de Investigaciones científicas y Técnicas* (CONICET) for their financial support.

References

Aguiar Moraes, E., Da Silva Marineli, R., Lenquiste, S.A. *et al.* (2015). Sorghum flour fractions: correlations among polysaccharides, phenolic compounds, antioxidant activity and glycemic index. *Food Chemistry*, **180**, 116–123.

Althwab, S., Carr, T.P., Weller, C.L., Dweikat, I.M. & Schlegel, V. (2015). Advances in grain sorghum and its co-products as a human health promoting dietary system. *Food Research International*, **77**, 349–359.

Barros, F., Dykes, L., Awika, J. & Rooney, L.W. (2013). Accelerated solvent extraction of phenolic compounds from sorghum brans. *Journal of Cereal Science*, **58**, 305–312.

Belhadi, B., Djabali, D., Souilah, R., Yousfi, M. & Nadjemi, B. (2013). Three small-scale laboratory steeping and wet-milling procedures for isolation of starch from sorghum grains cultivated in Sahara of Algeria. *Food and Bioproducts Processing*, **91**, 225–232.

Beta, T. & Dzama, K. (2013). *Cereals: Novel Uses and Processes*. Manchester: Springer Science and Business Media.

Bröhan, M., Jerkovic, V. & Collin, S. (2011). Potentiality of red sorghum for producing stilbenoid-enriched beers with high antioxidant activity. *Journal of Agricultural and Food Chemistry*, **59**, 4088–4094.

Carvalho Teixeira, N., Vieira Queiroz, V.A., Rocha, M.C. *et al.* (2016). Resistant starch content among several sorghum (*Sorghum bicolor*) genotypes and the effect of heat treatment on resistant starch retention in two genotypes. *Food Chemistry*, **197**, 291–296.

Chandrashekar, A. & Satyanarayana, K. (2006). Disease and pest resistance in grains of sorghum and millets. *Journal of Cereal Science*, **44**, 287–304.

Dlamini, N., Taylor, J. & Rooney, L. (2007). The effect of sorghum type and processing on the antioxidant properties of African sorghum-based foods. *Food Chemistry*, **105**, 1412–1419.

Duodu, K.G., Taylor, J.R.N., Belton, P.S. & Hamaker, B.R. (2003). Factors affecting sorghum protein digestibility. *Journal of Cereal Science*, **38**, 117–131.

Lataza Rovaletti, M.M., Benítez, E.I., Martinez Amezaga, N.M., Peruchena, N.M., Sosa, G.L. & Lozano, J.E. (2014). Polysaccharides influence on the interaction between tannic acid and haze active proteins in beer. *Food Research International*, **62**, 779–785.

Montgomery, D.C. (2003). *Design and Analysis of Experiments*. New York, NY: Wiley and Sons.

Ogbonna, A.C. (2011). Current developments in malting and brewing trials with sorghum in Nigeria: a review. *Journal of the Institute of Brewing*, **117**, 394–395.

Singh, H., Chang, Y.H., Lin, J.H., Singh, N. & Singh, N. (2011). Influence of heat–moisture treatment and annealing on functional properties of sorghum starch. *Food Research International*, **44**, 2949–2954.

Singleton, V.L., Orthofer, R. & Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin Ciocalteau reagent. *Methods in Enzymology*, **299**, 152–178.

Sun, Q., Han, Z., Wang, L. & Xiong, L. (2014). Physicochemical differences between sorghum starch and sorghum flour modified by heat-moisture treatment. *Food Chemistry*, **145**, 756–764.

Taylor, J.R.N., Belton, P.S., Beta, T. & Duodu, K.G. (2014). Increasing the utilization of sorghum, millets and pseudocereals: developments in the science of their phenolic phytochemicals, biofortification and protein functionality. *Journal of Cereal Science*, **59**, 257–275.

Tufan, A.N., Çelik, S.E., Özyürek, M., Güçlü, K. & Apak, R. (2013). Direct measurement of total antioxidant capacity of cereals: QUENCHER-CUPRAC method. *Talanta*, **108**, 136–142.

