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Effect of dry-heat treatments on the nutritional value of maize germ

RUNNING TITLE: Quality of heat-treated maize germ

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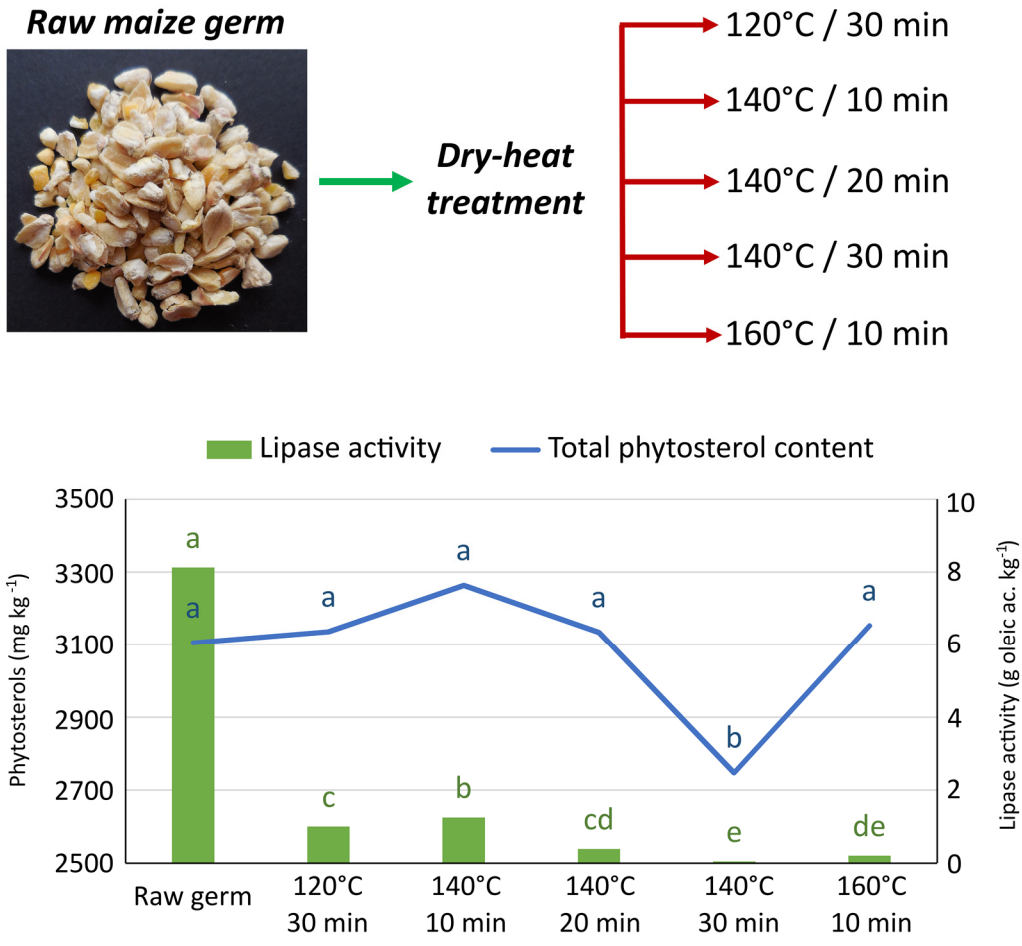
KEYWORDS: maize germ, dry-heat treatments, lipase activity, phytosterols, vitamins, colour.

16 **GRAPHICAL ABSTRACT**



17

ABSTRACT

Maize germ is a by-product of the maize milling process that is characterized by a high nutritional value. Currently, heat treatments are employed to prevent full-fat maize germ from spoilage. The aim of this research was to study the effect of five dry-heat treatments on the nutritional value of full-fat maize germ. The results confirmed that after each dry-heat treatment the lipase activity decrease but the use of high temperatures could be detrimental for phytosterol and thiamine concentrations. The main negative effects have been observed after treatments at 140°C for 30 min and 160°C for 10 min. No significant difference has been observed for protein, ash or fatty acid contents. The treatment at 140°C for 20 min resulted an optimal combination between temperature and heating time in order to inactivate lipase without altering deeply the nutritional value and the colour of maize germ.

INTRODUCTION

Cereals play an important role in human nutrition. Maize (Zea mays L.) is the main cereal grain, as far as raw production is concerned, but ranks third as a staple food, after wheat and rice.

The maize kernel is composed of four main parts: endosperm, pericarp, germ and tip cap. The germ portion, or embryo, constitutes about 6-12% of the total weight of the maize kernel, depending on the cultivar. Dried full-fat maize germ is a by-product of maize milling that is generally used for oil production and as a diet supplement in animal feed formulations due to its high density of nutrients, and in particular lipids, proteins, and fibre (Gwirtz and Garcia-Casal, 2014). Maize germ is also rich in vitamins of the B complex and antioxidants, such as tocopherols. Moreover, several studies have analysed the nutritional content of maize oil and have demonstrated that it is rich in essential fatty acids, such as linoleic acid (Ostlund *et al.*, 2002). Phytosterols are another important class of functional phytochemicals that have been identified in maize germ and its derived products (Ostlund *et al.*, 2002; Harrabi *et al.*, 2008). Phytosterols may have important effects on the reduction in serum cholesterol levels, thus acting on the prevention of cardiovascular diseases (Ostlund *et al.*, 2002; Noakes *et al.*, 2005). Therefore, the food industry is interested in the identification of plant matrices rich in these compounds in order to produce functional foods enriched in phytosterols, such as yoghurts and milk (Lagarda *et al.*, 2006). For these reasons, maize germ can be considered a potential complementary food supplement, as well as an excellent raw source for the preparation of highly nutritious foods, such as bread (Siddiq *et al.*, 2009),

cookies (Nasir *et al.*, 2010; Barnwal *et al.*, 2013) and pasta (Masoumikhah & Zagari, 2013).

The increase in maize production for food, together with the high germ percentage in the maize kernel could provide a good source for the expanded use of full-fat germ as food. Unfortunately, the presence of a large amount of unsaturated fatty acids, as well as oxidative and hydrolytic enzymes, leads to poor storage stability and renders maize germ highly susceptible to rancidity, thus posing a major limitation to its utilization. The defatting process has an important impact on the nutritional quality of maize germ because it leads to the removal of lipophilic compounds, such as fatty acids and phytosterols, which are collected in maize germ oil.

Currently, heat treatments are employed in order to prevent full-fat maize germ from spoilage. Moreover, roasted maize germ has been proposed as a non-allergenic substitute for nuts in numerous foods, such as cereal bars, because of its typical "halzenut" taste and appearance. Despite its effectiveness, thermal stabilization may not be completely effective and it may negatively affects the nutritional value of maize germ. Although the effect of thermal stabilization procedures has already been investigated on wheat and rice germ (Kwon *et al.*, 2004; Marti *et al.*, 2014), to the best of the authors' knowledge no information is available, in the scientific literature, on the effect of high-temperature treatments on the nutritional value of full-fat maize germ. Therefore, the aim of this study was to determine the effect of five different dry-heat treatments on full-fat maize germ in order to identify a combination between temperature and heating time that will sufficiently inactive enzymes responsible of its

74 poor storage stability and will not deeply modify its colour and concentration of
75 nutritional compounds.

MATERIALS AND METHODS

Sample preparation

An homogenous commercial maize batch, collected from one maize cultivar (Pioneer P1758), was obtained in NW Italy in the 2013 growing season and processed in an industrial mill in order to produce maize flour. An optical selector was used to obtain a pure germ fraction after degermination. The collected germ was stored at 4°C and used for dry-heat treatments within a few days.

A natural ventilation oven (FALC, Treviglio, Italy) was used to perform dry-heat treatments of maize germ monolayers. Starting from information reported in previous studies employed on wheat and rice germ (Kim *et al.*, 2002; Kwon *et al.*, 2004; Srivastava *et al.*, 2007; Marti *et al.*, 2014), and considering that maize germ is about 30 times heavier than wheat and rice germ, an initial screening was performed in order to select the highest temperature to test. Given that treatments for 10 minutes at temperatures higher than 160°C caused an excessive browning of the maize germ, the last was set as the highest temperature to test and five dry-heat stabilization processes were compared: 120°C for 30 min, 140°C for 10 min, 140°C for 20 min, 140°C for 30 min and 160°C for 10 min. Three replicates were carried out for each treatment. The rate of temperature increase was set at 4°C min^{-1} and the time control was started when the desired temperature was reached. After each treatment, samples were equilibrated to room temperature, milled using a laboratory centrifugal mill (ZM-100; Retsch, Haan, Germany) with a 1-mm opening, and stored at -25°C until chemical analyses were performed.

Chemical analyses

Proximate composition analysis

The moisture, protein and ash content were determined on ground germ samples. The moisture content, determined in order to express the results on a dry matter (dm) basis, was obtained using a Sartorius MA30 thermo-balance (Sartorius AG, Goettingen, Germany). The total nitrogen content and total protein content (conversion factor of 6.25) was obtained according to the Dumas method. After the combustion of the sample in a high temperature chamber in presence of oxygen, nitrogen was measured using a thermal conductivity detector. The ash content was determined in a muffle furnace according to the AOAC (1990) procedure.

Lipase activity determination

Lipase activity was measured by means of the titrimetic method reported by El Amrani *et al.* (2003). Samples were incubated for 17 hours at 70°C. The results were expressed as g oleic acid kg^{-1} produced after incubation.

Fatty acid composition analysis

Fatty acids were extracted and analysed as described by UNI EN ISO 12966-2:2011 and UNI EN ISO 5508:1998. Methyl esters of fatty acids were analysed with a gas chromatograph (Agilent 7890A) and separation was carried out using a 100 m x 0.25 mm, 0.2 µm film thickness, CP-Sil 88 column (Agilent Technologies, Santa Clara, California).

Phytosterols analysis

Phytosterols were extracted according to the method reported by Harrabi *et al.* (2008). TMS-ether derivatives were analysed with a gas chromatograph (Agilent 7890A). Separation was carried out using a capillary (5%-phenyl)-methylpolysiloxane, 30 m x 0.32 mm, 0.25 μm film thickness, HP-5 column (Agilent Technologies).

Vitamins E, thiamine and riboflavin analysis

Vitamin E was extracted as reported by Commission Regulation (EC) No 152/2009 of 27 January 2009 and subsequently analysed with a high performance liquid chromatograph (Agilent 1290 Infinity LC) equipped with a fluorescence detector (Agilent 1200 Series). Separation was carried out using a 150 mm x 2.1 mm i.d., 1.7 μm, KINETEX PFP column (Phenomenex, Torrance, California).

Thiamine and riboflavin were extracted as reported in the UNI EN 14663:2006 and UNI EN 14152:2004 and subsequently analysed with an Acquity UPLC (Waters, Milford, Massachusetts) equipped with a API 4000 mass spectrometer (Applied Biosystem, Foster City, California). Separation was carried out using a 150 mm x 2.1 mm, 1.8 μm i.d., Acquity UPLC HSS T3 column (Waters).

Catechins

Catechins were extracted by adding a solution of acetonitrile with trifluoroacetic acid 0.2% v/v. Samples were sonicated for 10 minutes and subsequently analysed with a Acquity UPLC (Waters) equipped with a API 4000 mass spectrometer (Applied

144 Biosystem). Separation was carried out using a 150 mm x 2.1 mm i.d., 1.7 µm, Acquity
145 UPLC BEH C18 column (Waters).
146
147 *Germ flour colour*
148 The chromatic characteristics of the maize germ flour were determined using a Minolta
149 Chroma Meter reflectance spectrophotometer (Model CR-400, Minolta Co., Osaka,
150 Japan). A 45 mm diameter Petri dish was loosely filled with a subsample of germ flour.
151 The dish was tapped gently until the flour was levelled and no gaps were apparent
152 through the base of the dish and the colour values of *L**, *a** and *b**, were determined
153 directly by the instrument.
154
155 *Statistical analysis*
156 One-way analysis of variance (ANOVA) was performed to compare the effect of
157 treatments on the nutritional and technological quality of maize germ. The residual
158 normal distribution was assessed with the Shapiro-Wilk test, while the homogeneity of
159 the variance was checked with the Levene test. When ANOVA assumptions were not
160 verified, data were rank-transformed (Conover and Iman, 1981). The REGW-Q post-
161 hoc test was performed for multiple comparisons. All the tests were carried out with
162 SPSS for Windows statistical package, Version 22.0 (SPSS Inc., Chicago, Illinois)
163 using a 0.05 threshold for the rejection of the null hypothesis.

RESULTS AND DISCUSSION

Chemical characterization of raw full-fat maize germ

The proximate composition of raw full-fat maize germ fell into the range defined in previous studies (Ostlund *et al.*, 2002; Parris *et al.*, 2006; Saoussem *et al.*, 2009). Raw maize germ was characterized by a moisture, protein, fat and ash content of 11.57%, 15.79%, 25.03% and 8.13% dm, respectively. Polyunsaturated fatty acids (54.25% of total fatty acids) were the main fatty acid component, followed by monounsaturated fatty acids (26.76%) and saturated fatty acids (14.65%). Similarly to previous studies (Ostlund *et al.*, 2002; Saoussem *et al.*, 2009) the dietetic value of the maize germ fats was high, since the ratio between total unsaturated fatty acids and total saturated fatty acids was 5.5. As reported by Saoussem *et al.* (2009), linoleic acid (18:2 *n-6*; 65.70% of the total unsaturated fatty acids) and oleic acid (18:1 *n-9*; 32.65%) were the most concentrated unsaturated fatty acids. α-linolenic acid (18:3 *n-3*; 1.15%) and 11-eicosenoic acid (20:1 *n-9*; 0.28%) were also detected. On the contrary palmitoleic (16:1 *n-7*), vaccenic (18:1 *n-7*), γ-linolenic (18:3 *n-6*), stearidonic (18:4 *n-3*), eicosadienoic (20:2 *n-6*), eicosatrienoic (20:3 *n-3*), dihomo- γ-linolenic (20:3 *n-6*), arachidonic (20:4 *n-6*), eicosapentaenoic (20:5 *n-3*), erucic (22:1 *n-9*), docosadienoic (22:2 *n-6*), docosatetraenoic (22:4 *n-6*), docosapentaenoic (22:5 *n-3*), docosahexaenoic (22:6 *n-3*) and nervonic (24:1 *n-9*) acids were not detected in the raw maize germ samples.

Full-fat maize germ was characterized by a high phytosterol content (3105.9 mg kg^{-1} dm). β-sitosterol was the main sterol (63.84%), followed by campesterol (20.97%), stigmasterol (5.41%) and Δ-5-avenasterol (1.85%). Lower concentrations were

observed for clerosterol, 24-methylencholesterol, Δ-5,24-stigmastadienol, Δ-7-avenasterol, Δ-7-stigmastenol, Δ-7-campesterol and Δ-5,23-stigmastadienol. Phytostanols, the fully saturated subgroup of phytosterols, represented 4.98% of the total phytosterols. The concentration of sitostanol (3.56%) was higher than the one of campestanol (1.42%). Brassicasterol and Δ-7,9-stigmastadienol were not detected. The concentration of phytosterols and phytostanols observed was lower than values detected by Harrabi *et al.* (2008), but as previously demonstrated it may depend on the maize genotype considered.

Among the analysed vitamins, tocopherols were detected at a concentration of about 61.7 mg kg^{-1} dm, while thiamine and riboflavin were detected at lower concentrations, that was at 14.9 mg kg^{-1} dm and 3.9 mg kg^{-1} dm respectively. Catechins were not detected in raw maize germ samples.

Changes in the nutritional value and technological characteristics of full-fat maize germ after dry-heat treatments

Full-fat maize germ could be used as a functional ingredient because of its high nutritional value, but the major limitation to its utilization is its susceptibility to rancidity. Treatments at high temperatures could be employed in order to prevent maize germ from spoilage. In this research, five dry-heat treatments, characterized by different combinations of temperature and heating time, were compared in order to evaluate their effect on the nutritional and technological quality of maize germ. After treatments at 120°C for 30 min and at 140°C for 10 min, the moisture decrease on average to

209 2.6%, while after treatments at 140°C for 20 min, 140°C for 30 min and at 160°C for
210 10 min it decreased to 1.2%.
211 The measurement of lipase activity was previously proposed as a rapid germ shelf life
212 prediction tool (Rose & Pike, 2006; Brunschwiler *et al.*, 2013). In this study, the lipase
213 activity was measured in order to evaluate the effectiveness of the dry-heat treatments
214 in the inactivation of the main enzymes responsible of the poor storage stability of
215 maize germ. Results confirmed that dry-heat treatments could be useful to decrease
216 lipase activity and consequently to increase the storage stability of full-fat maize germ
217 in agreement with other studies on wheat germ (Srivastava *et al.*, 2007; Marti *et al.*,
218 2014). The raw full-fat maize germ had an initial lipase activity of 8.1 g oleic acid kg^{-1}
219 and after all heat treatments a significant decrease of the lipase activity was observed
220 ($P<0.001$, Figure 1). Heat treatments at 140°C for 30 min and 160°C for 10 min
221 determined the most significant reduction of lipase activity. In fact, after these
222 treatments, the lipase activity was more than 40-fold lower than that of the raw germ.
223 Even though the use of high temperatures could increase maize germ storage stability
224 because of a decrease of the lipase activity, it could also lead to detrimental effects on
225 some of the nutritional compounds and to undesirable browning of the maize germ.
226 Therefore, it is important to find an optimal combination between temperature and
227 heating time that will sufficiently inactivate lipase and will not decrease the
228 concentration of nutritional compounds. Different heat treatments had no significant
229 effects on the protein content ($P=0.255$), whose values ranged between 15.79 and
230 16.30% dm. The ash content in the raw as well as in the heat-treated germ samples
231 remained around 8.34 ± 0.12% dm ($P=0.253$). Similarly, the content of fats ($P=0.177$)

232 and the total saturated (P=0.395), monounsaturated (P=0.126) and polyunsaturated
233 (P=0.176) fatty acids did not change significantly after all heat treatments; no
234 significant effect was observed also on the unsaturated fatty acid profile (P>0.05), as
235 reported in other studies made on waxy maize (Kim *et al.*, 2009), rice germ oil (Kim *et*
236 *al.*, 2002) and rice germ (Kwon *et al.*, 2004).
237 The variations in the amounts of phytosterols after different dry-heat treatments are
238 shown in Table 1. The concentration of total phytosterols decreased significantly after
239 the treatment at 140°C for 30 min (P<0.001). This reduction was mainly due to the
240 decrease in the concentrations of β-sitosterol, campesterol and stigmasterol, which
241 were the main phytosterols detected in the raw maize germ. The greatest reduction
242 was observed for β-sitosterol, whose concentration, after the treatment at 140°C for
243 30 min decreased by about 15% compared to the raw germ. A lower effect was
244 observed for campesterol and stigmasterol, whose concentrations only decreased by
245 about 10%. Other sterols, such as 24-methylencholesterol and Δ7-campesterol, which
246 were only present in small concentrations, showed the highest detrimental effect after
247 the treatment at 160°C for 10 min. In comparison to the raw germ their concentrations
248 decreased by about 30% and 40%, respectively. Similar results were observed for rice
249 germ after roasting at 200°C (Kwon *et al.*, 2004). Several studies have shown that the
250 degradation of phytosterol standards occur at high temperatures, thus giving rise to
251 fragmented phytosterols molecules, oligomers and volatile compounds. The
252 degradation effects observed in this study were lower than the ones observed in other
253 studies performed on standards solutions, probably because of a matrix-protection-

254 effect (Kwon *et al.*, 2004; Rudzińska *et al.*, 2009; Struijs *et al.*, 2010; Barriuso *et al.*,
255 2012).
256 Thiamine resulted more heat-unstable and more temperature-sensitive than riboflavin
257 and tocopherols as demonstrated in other studies (Barna *et al.*, 1997; Choe *et al.*,
258 2005). Its concentration was significantly reduced by 17% ($P<0.01$) only after the
259 treatment at 160°C for 10 min (Table 2). On the contrary no detrimental effect was
260 observed for tocopherols ($P=0.095$) and riboflavin ($P=0.187$) after each heat treatment.
261 The increase in tocopherol content observed after the treatment at 160°C for 10 min
262 could be related to a heat-induce break of bonds that link tocopherols to proteins,
263 phosphate or phospholipids (Moreau *et al.*, 1999).
264 ANOVA showed significant differences in the *L**, *a** and *b** values for the maize germ
265 after different dry-heat treatments (Table 3). The treatment at 120°C for 30 min and
266 140°C for 10 min resulted in a significant increase in the *L** (lightness) value ($P<0.05$),
267 but an increase in the temperature or in the time of the treatment caused a reduction
268 of this value. The lowest values were observed after treatments at 140°C for 30 min
269 and at 160°C for 10 min. Meanwhile, a significant increase in the *a** (redness) value
270 was observed after the treatment at 140°C for 20 min ($P<0.05$) and the highest values
271 were observed after the treatment at 140°C for 30 min and at 160°C for 10 minutes.
272 Thus, treatments at 140°C for 30 min and 160°C for 10 min lead to a product
273 characterized by a lower lightness and a higher redness values. As observed in a
274 similar study performed on rice germ oil (Kim *et al.*, 2002), the browning of the maize
275 germ resulted probably from Maillard-type nonenzymatic reactions between reducing

sugars and free amino acids or amides. Less significant changes were found for the blue-yellow component (*b**).

In conclusion, dry-heat treatments could be used in order to obtain full-fat maize germ characterized by a high nutritional value and storage stability suitable for food purposes. The choice of a specific thermal treatment could have an effect on the nutritional value of maize germ as far the content of phytosterols and thiamine is concerned, depending on the combination of temperature and heating time. The phytosterol content seems to be affected mainly by the heating time, unlike thiamine, which seems to be affected mainly by the temperature of the treatment. In order to obtain a stabilized maize germ, with technologically optimized functional and nutritional attributes, it is important to choose the best compromise between temperature and time of treatment. The treatment at 140°C for 20 min allows to inactivate lipase without altering deeply both the nutritional value and the colour of the maize germ. The nutritional and technological properties of food products enriched with dry heated full-fat maize germ may be of interest for future researches.

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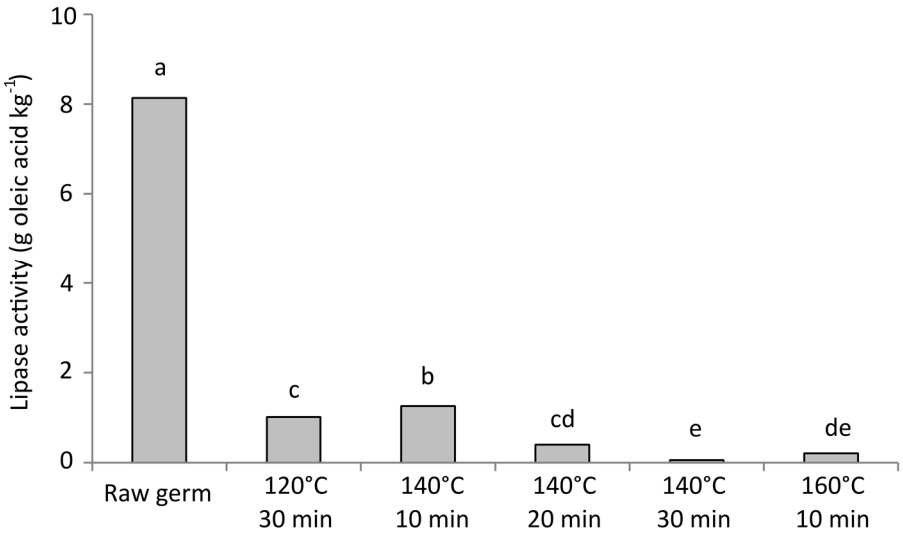
370

FIGURE

Figure 1. Lipase activity in raw and heat treated full-fat maize germ. The reported data are the means of three values. Data were analysed after rank transformation. Values with different letters differ significantly (P<0.05) according to the REGW-Q test.



376 **TABLES**

377 **Table 1.** Phytosterols in the raw and heat treated full-fat maize germ.

	Phytosterols (mg kg^{-1} dm)					
Heat treatment	Total phytosterols	β-sitosterol	Campesterol	Stigmasterol	24-methylencholesterol	Δ7-campesterol
Raw germ	3105.94 a	1982.68 a	651.34 a	168.11 a	18.66 a	8.97 ab
120°C for 30 min	3135.89 a	2017.62 a	658.63 a	165.18 ab	16.96 a	10.12 a
140°C for 10 min	3264.23 a	2092.81 a	687.45 a	176.40 a	16.58 a	8.46 abc
140°C for 20 min	3135.03 a	2002.09 a	661.50 a	170.45 a	18.19 a	7.41 bcd
140°C for 30 min	2748.00 b	1692.66 b	587.37 b	152.41 b	18.04 a	6.81 cd
160°C for 10 min	3153.36 a	2030.29 a	655.63 a	166.60 a	13.22 b	5.71 d

378 The reported data are means of three values. Means followed by different letters differ significantly ($P<0.05$) according to

379 the REGW-Q test.

Table 2. Tocopherol, riboflavin and thiamine contents in the raw and heat treated full-fat maize germ.

Heat treatment	Tocopherol (mg kg^{-1} dm)	Riboflavin (mg kg^{-1} dm)	Thiamine (mg kg^{-1} dm)
Raw germ	61.67 a	3.92 a	14.85 a
120°C for 30 min	60.39 a	3.80 a	14.23 a
140°C for 10 min	59.67 a	4.01 a	14.59 a
140°C for 20 min	59.72 a	3.89 a	14.81 a
140°C for 30 min	58.37 a	4.10 a	14.36 a
160°C for 10 min	62.70 a	3.98 a	12.31 b

The reported data are means of three values. Means followed by different letters differ significantly ($P<0.05$) according to the REGW-Q test.

Table 3. Colour values in the raw and heat treated full-fat maize germ.

Heat treatment	*L**	*a**	*b**
Raw germ	80.57 cd	-2.76 c	26.28 a
120℃ for 30 min	82.75 a	-2.93 d	24.40 de
140℃ for 10 min	82.40 ab	-2.81 cd	24.02 e
140℃ for 20 min	81.01 bc	-1.95 b	24.98 cd
140℃ for 30 min	80.28 d	-1.43 ab	25.30 bc
160℃ for 10 min	79.27 d	-0.56 a	26.07 ab

The reported data are means of three values. Means followed by different letters differ significantly ($P<0.05$) according to the REGW-Q test. Data were analysed after rank transformation.