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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.
**GRAPHICAL ABSTRACT**

*Raw maize germ*

![Geometrical diagram showing the effects of dry-heat treatment on lipase activity and total phytosterol content.](image)

- **Lipase activity**
  - Raw germ
  - 120°C / 30 min
  - 140°C / 10 min
  - 140°C / 20 min
  - 140°C / 30 min
  - 160°C / 10 min

- **Total phytosterol content**
  - Raw germ
  - 120°C / 30 min
  - 140°C / 10 min
  - 140°C / 20 min
  - 140°C / 30 min
  - 160°C / 10 min

Legend:
- a, b, c, d, e, de
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.
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
poor storage stability and will not deeply modify its colour and concentration of 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 titrimetric 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...
Separation was carried out using a 150 mm x 2.1 mm i.d., 1.7 µm, Acquity UPLC BEH C18 column (Waters).

**Germ flour colour**

The chromatic characteristics of the maize germ flour were determined using a Minolta Chroma Meter reflectance spectrophotometer (Model CR-400, Minolta Co., Osaka, Japan). A 45 mm diameter Petri dish was loosely filled with a subsample of germ flour. The dish was tapped gently until the flour was levelled and no gaps were apparent through the base of the dish and the colour values of $L^*$, $a^*$ and $b^*$, were determined directly by the instrument.

**Statistical analysis**

One-way analysis of variance (ANOVA) was performed to compare the effect of treatments on the nutritional and technological quality of maize germ. The residual normal distribution was assessed with the Shapiro-Wilk test, while the homogeneity of the variance was checked with the Levene test. When ANOVA assumptions were not verified, data were rank-transformed (Conover and Iman, 1981). The REGW-Q post-hoc test was performed for multiple comparisons. All the tests were carried out with SPSS for Windows statistical package, Version 22.0 (SPSS Inc., Chicago, Illinois) 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-stigmasterol, 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
2.6%, while after treatments at 140°C for 20 min, 140°C for 30 min and at 160°C for 10 min it decreased to 1.2%.

The measurement of lipase activity was previously proposed as a rapid germ shelf life prediction tool (Rose & Pike, 2006; Brunschwiler et al., 2013). In this study, the lipase activity was measured in order to evaluate the effectiveness of the dry-heat treatments in the inactivation of the main enzymes responsible of the poor storage stability of maize germ. Results confirmed that dry-heat treatments could be useful to decrease lipase activity and consequently to increase the storage stability of full-fat maize germ in agreement with other studies on wheat germ (Srivastava et al., 2007; Marti et al., 2014). The raw full-fat maize germ had an initial lipase activity of 8.1 g oleic acid kg⁻¹ and after all heat treatments a significant decrease of the lipase activity was observed (P<0.001, Figure 1). Heat treatments at 140°C for 30 min and 160°C for 10 min determined the most significant reduction of lipase activity. In fact, after these treatments, the lipase activity was more than 40-fold lower than that of the raw germ.

Even though the use of high temperatures could increase maize germ storage stability because of a decrease of the lipase activity, it could also lead to detrimental effects on some of the nutritional compounds and to undesirable browning of the maize germ. Therefore, it is important to find an optimal combination between temperature and heating time that will sufficiently inactivate lipase and will not decrease the concentration of nutritional compounds. Different heat treatments had no significant effects on the protein content (P=0.255), whose values ranged between 15.79 and 16.30% dm. The ash content in the raw as well as in the heat-treated germ samples remained around 8.34 ± 0.12% dm (P=0.253). Similarly, the content of fats (P=0.177)
and the total saturated (P=0.395), monounsaturated (P=0.126) and polyunsaturated (P=0.176) fatty acids did not change significantly after all heat treatments; no significant effect was observed also on the unsaturated fatty acid profile (P>0.05), as reported in other studies made on waxy maize (Kim et al., 2009), rice germ oil (Kim et al., 2002) and rice germ (Kwon et al., 2004).

The variations in the amounts of phytosterols after different dry-heat treatments are shown in Table 1. The concentration of total phytosterols decreased significantly after the treatment at 140°C for 30 min (P<0.001). This reduction was mainly due to the decrease in the concentrations of β-sitosterol, campesterol and stigmasterol, which were the main phytosterols detected in the raw maize germ. The greatest reduction was observed for β-sitosterol, whose concentration, after the treatment at 140°C for 30 min decreased by about 15% compared to the raw germ. A lower effect was observed for campesterol and stigmasterol, whose concentrations only decreased by about 10%. Other sterols, such as 24-methylencholesterol and Δ7-campesterol, which were only present in small concentrations, showed the highest detrimental effect after the treatment at 160°C for 10 min. In comparison to the raw germ their concentrations decreased by about 30% and 40%, respectively. Similar results were observed for rice germ after roasting at 200°C (Kwon et al., 2004). Several studies have shown that the degradation of phytosterol standards occur at high temperatures, thus giving rise to fragmented phytosterols molecules, oligomers and volatile compounds. The degradation effects observed in this study were lower than the ones observed in other studies performed on standards solutions, probably because of a matrix-protection-
effect (Kwon et al., 2004; Rudzińska et al., 2009; Struijs et al., 2010; Barriuso et al., 2012).

Thiamine resulted more heat-unstable and more temperature-sensitive than riboflavin and tocopherols as demonstrated in other studies (Barna et al., 1997; Choe et al., 2005). Its concentration was significantly reduced by 17% (P<0.01) only after the treatment at 160°C for 10 min (Table 2). On the contrary no detrimental effect was observed for tocopherols (P=0.095) and riboflavin (P=0.187) after each heat treatment. The increase in tocopherol content observed after the treatment at 160°C for 10 min could be related to a heat-induce break of bonds that link tocopherols to proteins, phosphate or phospholipids (Moreau et al., 1999).

ANOVA showed significant differences in the $L^*$, $a^*$ and $b^*$ values for the maize germ after different dry-heat treatments (Table 3). The treatment at 120°C for 30 min and 140°C for 10 min resulted in a significant increase in the $L^*$ (lightness) value (P<0.05), but an increase in the temperature or in the time of the treatment caused a reduction of this value. The lowest values were observed after treatments at 140°C for 30 min and at 160°C for 10 min. Meanwhile, a significant increase in the $a^*$ (redness) value was observed after the treatment at 140°C for 20 min (P<0.05) and the highest values were observed after the treatment at 140°C for 30 min and at 160°C for 10 minutes. Thus, treatments at 140°C for 30 min and 160°C for 10 min lead to a product characterized by a lower lightness and a higher redness values. As observed in a similar study performed on rice germ oil (Kim et al., 2002), the browning of the maize 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.
REFERENCES


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

<table>
<thead>
<tr>
<th>Heat treatment</th>
<th>Total phytosterols</th>
<th>β-sitosterol</th>
<th>Campesterol</th>
<th>Stigmasterol</th>
<th>24-methylcholesterol</th>
<th>Δ7-campesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw germ</td>
<td>3105.94 a</td>
<td>1982.68 a</td>
<td>651.34 a</td>
<td>168.11 a</td>
<td>18.66 a</td>
<td>8.97 ab</td>
</tr>
<tr>
<td>120 °C for 30 min</td>
<td>3135.89 a</td>
<td>2017.62 a</td>
<td>658.63 a</td>
<td>165.18 ab</td>
<td>16.96 a</td>
<td>10.12 a</td>
</tr>
<tr>
<td>140 °C for 10 min</td>
<td>3264.23 a</td>
<td>2092.81 a</td>
<td>687.45 a</td>
<td>176.40 a</td>
<td>16.58 a</td>
<td>8.46 abc</td>
</tr>
<tr>
<td>140 °C for 20 min</td>
<td>3135.03 a</td>
<td>2002.09 a</td>
<td>661.50 a</td>
<td>170.45 a</td>
<td>18.19 a</td>
<td>7.41 bcd</td>
</tr>
<tr>
<td>140 °C for 30 min</td>
<td>2748.00 b</td>
<td>1692.66 b</td>
<td>587.37 b</td>
<td>152.41 b</td>
<td>18.04 a</td>
<td>6.81 cd</td>
</tr>
<tr>
<td>160 °C for 10 min</td>
<td>3153.36 a</td>
<td>2030.29 a</td>
<td>655.63 a</td>
<td>166.60 a</td>
<td>13.22 b</td>
<td>5.71 d</td>
</tr>
</tbody>
</table>

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

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

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.

<table>
<thead>
<tr>
<th>Heat treatment</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw germ</td>
<td>80.57 cd</td>
<td>-2.76 c</td>
<td>26.28 a</td>
</tr>
<tr>
<td>120°C for 30 min</td>
<td>82.75 a</td>
<td>-2.93 d</td>
<td>24.40 de</td>
</tr>
<tr>
<td>140°C for 10 min</td>
<td>82.40 ab</td>
<td>-2.81 cd</td>
<td>24.02 e</td>
</tr>
<tr>
<td>140°C for 20 min</td>
<td>81.01 bc</td>
<td>-1.95 b</td>
<td>24.98 cd</td>
</tr>
<tr>
<td>140°C for 30 min</td>
<td>80.28 d</td>
<td>-1.43 ab</td>
<td>25.30 bc</td>
</tr>
<tr>
<td>160°C for 10 min</td>
<td>79.27 d</td>
<td>-0.56 a</td>
<td>26.07 ab</td>
</tr>
</tbody>
</table>

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.