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ORIGINAL PAPER

Effect of Wheat Germ Heat Treatment by Fluidised Bed on the Kinetics of Lipase Inactivation

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Abstract

Wheat germ is scrapped during milling due to their unfavourable baking properties and rapid deterioration. Although it is a low-cost by-product with remarkable nutritional features, its widespread utilisation is limited by the action of enzymes. On that basis, the effect of the germ stabilisation process by fluidisation with hot air was studied. Fluidisation, carried out with air between 90 and 150 °C, generated notable lipase inactivation, which reached a minimum residual activity of 15.5% from original. The total tocopherol contents of stabilised samples were not significantly affected. Colour attributes of treated samples showed slight changes compared with raw wheat germ. A mathematical model was fitted to colour data to predict the changes due to heat. A first-order kinetic model was applied to descript the thermal lipase inactivation. The inactivation rate constant, D value, Z value and the activation energy were calculated for this process. The results obtained in this study are expected to contribute to the optimisation of wheat germ stabilisation by fluidisation. This process may enable to obtain a food with good nutritional features for human consumption from a co-product of the wheat milling industry at a short time and high temperature.

Keywords Fluidisation · Wheat germ · Thermal kinetic inactivation · Lipase activity · Tocopherols

Abbreviations

BI	Browning index
BI_0	Browning index before thermal treatment
BI_{MAX}	Maximum browning index reached in the thermal treatment
D	Decimal time, s
E_a	Activation energy (kJ mol^{-1})
FFA	Free fatty acids content, g of oleic acid kg^{-1} of oil
FFA_0	Free fatty acids content before incubation, g of oleic acid kg^{-1} of oil
FFA_{48}	Free fatty acids content after 48 h of incubation, g of oleic acid kg^{-1} of oil
ΔFFA	Change in free fatty acids content, g of oleic acid kg^{-1} of oil
k_{BI}	Browning rate constant, s^{-1}
k	Enzyme inactivation rate constant, s^{-1}
PV	Peroxide value, meq O_2 kg^{-1} oil
R	Universal gas constant, 8.314, kJ kmol^{-1} K^{-1}
t	Time, s
$t_{\frac{1}{2}}$	Time which the half of the maximum BI is reached, s
T	Inlet air temperature, °C
TTC	Total tocopherol content, mg kg^{-1} oil
WI	Whiteness index
Z Z	value, °C

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Introduction

Wheat is one of the most important cereals due to its ability to be ground into flour and semolina that are the main ingredients to bread, other bakery products and pasta (De Vasconcelos et al. 2013). The wheat milling industry is one of most important in the world, annually about 496,800,000 t of wheat are milling generating approximately 15,000,000 t of wheat germ as a main by-product (Bedford et al. 2017). Wheat germ is the embryo of wheat seed and represents about 30 g kg^{-1} of weight of kernel and is removed from the endosperm during the milling due to their rapid deterioration

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despite their excellent nutritional qualities. Wheat germ has a high protein quality, as well as its content of tocopherols, vitamin B, dietary fibre, essential amino acids, functional phytochemicals, such as flavonoids and sterols, and oil content about 150 g kg^{-1} (dry matter) with highly valuables fatty acids such as linoleic acid (594.4 ± 64.2 g kg^{-1}), palmitic acid (187.0 ± 20.2 g kg^{-1}), oleic acid (157.7 ± 17.0 g kg^{-1}) and α-linolenic acid (60.9 ± 6.6 g kg^{-1}) (Gili et al. 2017a). Tocopherols (vitamin E), a major biological antioxidant, are mainly provided by cereal grains and are primarily located in the germ fraction but are also present in the fraction of fine bran. Several factors, as temperature, light, can influence tocopherol stability and leading to reduction in their health benefits (Engelsen and Hansen 2009). Even though the wheat germ particle is a low-cost by-product with valuable nutritional features, the widespread utilisation is limited by the action of lipases and lipoxygenases as well as the high content of unsaturated oil. The hydrolytic and oxidative enzymes bring about a rapid rancidity of wheat germ during storage (less than 10 days) (Gili et al. 2017a). Nowadays, wheat germ is mainly utilised by the cosmetic industry and to animal feeding, and a minor part of the production is used for human consumption (Sjövall et al. 2000). Since lipoxygenases are more sensitive to thermal inactivation (Xu et al. 2016), lipases have been the main focus on germ thermal stabilisation. Several methodologies have been used to reduce the lipase activity in wheat germ: application of heat (thermal processes) (Ferrara et al. 1991; Gili et al. 2017a), dehydration (Rothe 1963), gamma radiation (Jha et al. 2013) or else by chemical preservation, as for instance by adding some chemical compound as antioxidant (H. M. Barnes 1948) or alkalis (Grandel 1959). Thermal processes have demonstrated to be effective in stabilizing wheat germ (Gili et al. 2017a; Kermasha et al. 2007; Murthy et al. 2008; Srivastava et al. 2007). Fluidisation generates a strong exchange between particles and the hot air, which implies a uniform treatment on the material being fluidised and also generates a dehydration of particles contributing with the stabilisation process (Giner and Calvelo 1987).

Thermal treatments generally modify the colour of foods; this change is generally related with some nutritional deterioration. Colour is important for the consumer's acceptance and as an indicator of non-enzymatic browning produced by thermal treatments. Based on this, monitoring the colour changes during the thermal treatments could be an interesting tool to monitoring the processes (Ibanoğlu 2002).

The physical characteristics of wheat germ flakes (low density and non-sticky surface) make them a suitable material to be heat-treated by fluidisation. A spouted fluidised bed (a special type of fluidised bed) was used by Yöndem-Makascioğlu et al. (2005) to stabilise wheat germ. In a previous work, Gili et al. (2017b) studied the minimum fluidisation velocity and drying curves of wheat germ particles in a fluidised bed with hot air.

However, to the best of our knowledge, no studies about the effectiveness of stabilisation process with hot air by fluidised bed of wheat germ nor either their effect on the wheat germ tocopherols were reported in the literature.

On such grounds, the aim of this work was to study the effect of the stabilisation process of wheat germ by thermal fluidisation process with hot air on tocopherol content, oil quality parameters, germ colour and lipase activity.

Materials and Methods

Wheat Germ, Chemicals and Reagents

Wheat germ particles were supplied by a local milling industry (José Minetti y Cia. Ltda. S.A.C.I, Córdoba, Argentina) after grain milling. The ethyl acetate and hexane used for tocopherol quantification were HPLC grade with the tocopherol standards being obtained from ICN Biomedicals, Inc. (Germany). All the other reagents and solvents used in the laboratory determinations were of analytical grade.

Preliminary Operations

Wheat germ was sieved (EJR 2000, Zonytest®) to separate the wheat germ from bran and flour particles (933 g kg^{-1} retained on 20 mesh-size). In order to reduce enzymatic activity and oil degradation until the heat treatment, wheat germ particles were stored at − 18 °C in a three-layer (polyester, aluminium and polyethylene) package with barriers against oxygen and light until further use. The freezing storage process of wheat germ particles does not generate significant effect on wheat germ physical properties (Gili et al. 2017b).

Moisture Content

Moisture content was analysed according to standard method of American Association of Cereal Chemistry (A.A.C.C. 2000).

Oil Extraction

For analytical determinations, germ oil was obtained as follows: Wheat germ was twice extracted with hexane (1:10 *w*/*v*) for 30 min on a mechanical shaker (150 rpm) at room temperature, after which, the hexane portion was centrifuged (5 min, 1000×*g*) and then evaporated in vacuum conditions at 40 ± 1 °C.

Lipase Activity

The activity of wheat germ lipase was measured indirectly through the generation of free fatty acids (FFA) according to Gili et al. (2017a). Briefly, the increase in FFA (ΔFFA) over

time was considered to be proportional to the lipase activity. To obtain ΔFFA, wheat germ samples were wetted to obtain the optimal water concentration of lipase activity: 170 g water kg^{-1} of wet solids. After that, a portion of the wetted sample was utilised to determine the initial oil free fatty acids content (FFA_0), and the remaining sample was maintained at 40 ± 1 °C, in a thermostatic bath, for 48 h to promote the lipase activity or that is the same, to generate free fatty acids as result of lipase activity. After this 48 h, the free fatty acid content of the oil was measured (FFA_{48}). The change in FFA content (ΔFFA) after 48 h in the thermostatic bath was calculated through a simple mathematical operation:

$$\Delta\mathrm{FFA} = FFA_{48} - FFA_0 \tag{1}$$

All determinations of free fatty acids were performed in duplicate according to standard methods of American Oil Chemists' Society (AOCS 2009). The oil utilised in the FFA measurements was extracted with hexane as was detailed before in "Oil Extraction" section.

Total Tocopherol Content

The total tocopherol content (TTC) of wheat germ oil was determined by high-performance liquid chromatography (HPLC) according to Gili et al. (2017a). Briefly, a mobile phase composed of hexane, ethyl acetate with a flow rate of 1.0 mL min^{-1}, was used. Approximately 0.1 g of oil was vortexed in 0.9 mL of hexane for 30 s in darkness condition. HPLC analysis was performed on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan), equipped with a LC-20AT pump, a diode array detector model SPD-M20A and a rheodyne injector fitted with a 20 μL loop, using a Supelco HPLC column (Supelcosil LC-NH2-NP, 250 × 4.6 mm ID × 5 μm). Solutions of tocopherol standards were made in order to perform a calibration curves for the quantification of different isomers. Total tocopherol content was expressed in mg kg^{-1} oil. All determinations of TTC were done in duplicate.

Peroxide Value

The peroxide value (PV) was determined according to the AOCS standard method Cd 8-53 with all determinations being performed in duplicate (AOCS 2009).

Colour Determination

Wheat germ colour was measured according to Martinez et al. (2012). The measurements were made on a 1.5 cm thick layer of germ, which was covered with a low reflectance glass. A spectrophotometer (CM600d, Konica-Minolta®) with a D65 illuminant, 10° angle of observer, andspecular-included component was used. CIELAB parameters (L^*, a^*, b^*) were selected to inform the colour of the wheat germ samples. All colour measurements were made in triplicate.

Whiteness Index

Whiteness index (WI) of wheat germ particles was calculated from L^*, a^* and b^* parameters measured according to Tuncel et al. (2014) using the following equation:

$$WI = 100 - \sqrt{\left(100 - L^*\right)^2 + a^{*2} + b^{*2}} \tag{2}$$

Browning Index

Browning index was calculated according to Sakin-Yilmazer et al. (2013) from L^*, a^* and b^* parameters of CIELAB scale with the following equation:

$$BI = \frac{100(x - 0.31)}{0.17} \tag{3}$$

where

$$x = \frac{(a + 1.75L)}{(5.645L + a - 3.012\,b)} \tag{4}$$

As was reported by Ureta et al. (2014), the evolution of BI could be described by a sigmoid curve, since an induction period, followed by a gradually increase until reaching, asymptotically, the final value. Based on this behaviour, a following equation was fitted to experimental data:

$$BI = BI_0 + \frac{BI_{max} - BI_0}{1 + \exp\left(-k_{BI}\left(t - t_{\frac{1}{2}}\right)\right)} \tag{5}$$

where BI_0 is the initial browning index of wheat germ (at t = 0), BI_{max} is the maximum browning index achieved by heat-treated wheat germ at each temperature of air, t is the treatment time, $t_{\frac{1}{2}}$ is the time (s) when the half browning index is reached and k_{BI} is the browning rate constant (s^{-1}). The results of the fitting were obtained by nonlinear least squares method.

Thermal Inactivation Kinetics of Wheat Germ Lipase

The main goal for thermal treatment of wheat germ particles was lipase inactivation. In general, enzymatic inactivation in foods is often modelled considering a first-order kinetic model (Matsui et al. 2008):

$$Ln\left(\frac{\Delta FFA}{\Delta FFA_0}\right) = -k\,t \tag{6}$$

where ΔFFA_0 and ΔFFA are the lipase activity at time t and zero, k is the first-order inactivation constant (s^{-1}) and t is the

treatment time (s). The k parameter was obtained by a regression of versus $Ln\left({}^{\Delta FFA}/_{\Delta}FFA_0\right)$ versus treatment time (t)

Other parameters used to evaluate the efficacy of the enzyme inactivation by heating are D, Z and activation energy (E_a) values. D value is the time that a 90% reduction of initial enzymatic activity takes under isothermal conditions and can be determined as (Anthon and Barrett 2002):

$$D = \frac{2.303}{k} \tag{7}$$

Z value represents the effect of temperature in D value, and it indicates the temperature increase necessary to reduce D up to one tenth part of initial value (Matsui et al. 2008). Z can be obtained graphically, from the curve $Log(D)$ versus air temperature (T) where the slope is equal to ${}^{-1}/_{Z}$ (Liu et al. 2013).

E_a value was calculated in order to compare the temperature sensitivity of the enzyme inactivation process. The activation energy in chemical reaction is determined by Arrhenius law, which can be expressed in a linearised form as:

$$Ln(k) = -\frac{E_a}{RT} + c \tag{8}$$

where R is the gas constant (8.314 J mol^{-1} K), c is an integration constant, T is the absolute temperature (K) and k is the rate constant of reaction (s^{-1}). Graphically, E_a can be calculated from the slope of the curve $Ln(k)$ versus ${}^{1}/_{T}$ where the slope of this function is equal to ${}^{-E_a}/_{R}$.

Fluidised Bed Drying Setup

The equipment used was a purpose-built fluidised bed dryer (Torrez Irigoyen and Giner 2011). The equipment is made up of a thermally insulated drying chamber, a centrifugal fan, powered with a Siemens electric motor (maximum angular speed, 2800 rev min^{-1}) with a frequency inverter WEG Model CFW-08, Brazil to control the air velocity, a Novus Model N321 temperature controller with two nickel-plated copper resistance U-shaped, 8 mm in diameter each, forms the resistor bank. This resistor bank is capable to heat the air until 325 °C.

Experimental Procedure

The heating treatments of what germ particles were done the purpose-built bed dryer with an automatic control of inlet air temperature and air velocity (Gili et al. 2017b). All treatments were performed at 0.35 m s^{-1} (operational fluidisation velocity of wheat germ determined in a previous work).

Samples were thermally treated at 90, 110, 130 and 150 °C of inlet air temperature for times between 0.5 and 15 min by duplicate. After each period, samples were wrapped in sealed packages and stored under refrigeration.

Statistical Analysis

The means being compared by the LSD (least significant difference)-Fisher test at a significance level of 0.05, and the relationship between the measured parameters evaluated by the Pearson test (significance level $p \leq 0.05$) using the Infostat statistical software. All determinations were performed at least in duplicate.

Results and Discussion

Effect of Processing Conditions

The thermal treatment performed with inlet air temperature at 90 °C did not show a significant lipase inactivation (data not shown). Therefore, this treatment was excluded of the following analysis.

The effect of thermal treatment on the lipase activity is observed in Fig. 1 where ΔFFA values were plotted versus treatment time. Significant inactivation of lipase activity was observed as consequence of thermal treatments between 110 and 150 °C. The final residual activities ($\Delta FFA/\Delta FFA_0$) after 15 min of heat treatment were 15.5, 34.1 and 69.1% for 150, 130 and 110 °C, respectively. These results showed lower residual final lipase activities than the residual activities informed by Vetrimani et al. (1992) for wheat germ stabilised by microwave heating and Yöndem-Makascioğlu et al. (2005) for wheat germ stabilised in a spouted fluidised bed (a special type of fluidised bed) at higher temperatures than those used in this study. These differences could be explained based on the technologies applied; fluidisation generates better heat and mass transfers with a high degree of mixing than the spouted fluidisation, which allowed more effective treatments.

Capitani et al. (2011) informed oil acidity values of 39 and 98 g of oleic acid kg^{-1} of oil for fresh raw and stored (35 days at 27 °C) wheat germ samples, respectively. Our results showed that the final residual lipase activity of the germ which was treated at 150 °C produced 13.6 g of oleic acid kg^{-1} of oil when germ sample was wetted to obtain the optimal conditions of lipase activity for 48 h at 40 °C (to promote the lipase activity). These results confirmed that the heating processes of wheat germ by fluidisation produced the germ stabilisation.

The ΔFFA versus treatment time curves at 130 and 150 °C (Fig. 1) showed a decreasing period followed by a period with small changes in the values, which suggested that after these inflection points (which are very different among treatments), the residual lipase activities tended to constant values. The treatment process at 110 °C showed similar values of ΔFFA up to 300 s, followed by the decreasing period up to ~ 660 s of heating.

Focusing in the decreasing period of the ΔFFA values, the effect of the inlet air temperature could be observed in the

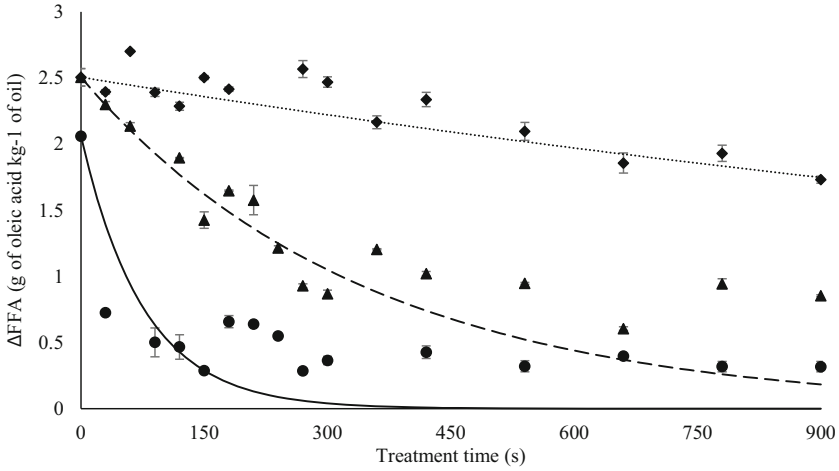


Fig. 1 Lipase activity expressed as ΔFFA in a function of treatment time for the different inlet air temperatures, 110 °C (diamond), 130 °C (triangle) and 150 °C (circle). All determinations were performed in duplicate. Predicted values from the first order kinetic model (Eq. 6) were expressed as a function of treatment time, 110 °C (dotted line, 130 °C (dashed line) and 150 °C (solid line)

negative slope of each curve, and the highest temperature (150 °C) showed the steepest values. As was expected, the 130 °C treatment presented an intermediate behaviour: Its slope was lower than 150 °C slope, but higher than 110 °C slope. These results were in agreement to Kermasha et al. (2007).

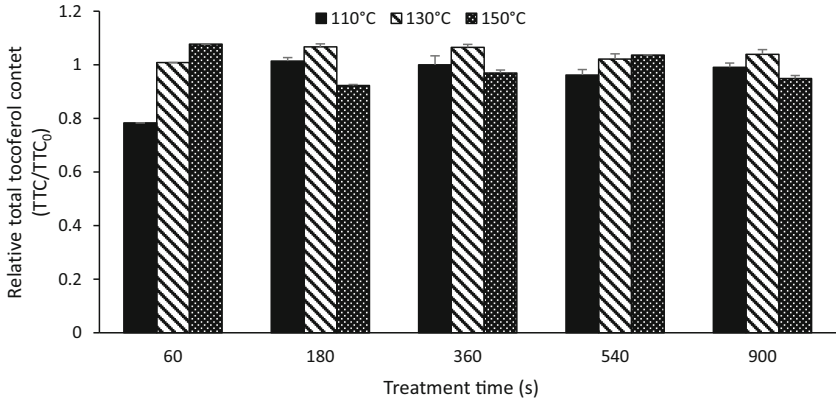
The time to reach the inflection points were ~120, ~270 and ~660 s, and the final residual lipase activities related to these inflection points were ~22.4, ~37.1 and ~74.1% for 150, 130 and 110 °C, respectively.

The observed behaviours can be explained from the energetic exchange developed into the drying chamber, and the higher temperatures increased rapidly the wheat germ temperature, giving the necessary energy to produce the denaturalisation of the enzymes. The great heat exchange, together with the drying generated during the process on the wheat germ particles, contributed to the enzymatic inactivation.

It is important to highlight that thermal treatments, especially at 150 and 130 °C, reached a relative low residual lipase activity in short treatment times. The residual lipase activity at 150 °C decreased 7% (from 20.4 to 15.5% residual lipase activity) during the treatment time period between 120 and 900 s and, at 130 °C the reduction of residual lipase activity between 270 (inflection point) and 900 s was 3% (from 37.1 to 34.1% residual lipase activity). Even though the differences among the residual lipase activities reached at the inflection point times and the final treatment times were statistically significant ($p < 0.05$), the residual lipase activity reduction (generated after the mentioned inflection time point for each temperature) does not justify the resources used to reach the minimum residual lipase activity at each treatment. Therefore, the thermal stabilisation treatment can be applied in a few minutes saving process time and rising the potential daily production. Since there was a linear association between final residual enzymatic activities and air temperature, it is suggested that higher lipase inactivation is only possible if higher temperatures are used.

Total tocopherol content (TTC) of wheat germ samples was analysed to evaluate the effect of thermal treatment on these lipophilic natural antioxidants, with many nutritional and healthy benefits. The TTC of raw wheat germ was 4063.87 ± 137.70 mg kg^{-1} oil, and the relative abundances of the isomers for raw germ were as follows: α-tocopherol (0.616 ± 0.001), β-tocopherol (0.286 ± 0.001) and γ-tocopherol (0.096 ± 0.001) (mg isomer/100 mg total tocopherol), while δ-tocopherol isomer was not detected. These results were in agreement with Gili et al. (2017a), Capitani et al. (2011) and Barnes and Taylor (1980). The effect of thermal treatment on TTC was slight. Figure 2 shows the relative total tocopherol content (TTC/TTC_0) versus treatment time for each air temperature. In general, TTC values were constant in all treatments, only the TTC value of 110 °C at 60 s of treatment showed a reduction; despite this value, the following TTC values observed for the thermal treatments at 110 °C were constant and very close to the initial TTC value. These results indicated that the high TTC of the raw wheat germ remained in the treated samples despite of the intense thermal treatment applied. The reported values of TTC in this study were in agreement with the results published by Li et al. (2016), Gili et al. (2017a) and Magariño et al. (2015) for wheat germ oils. The strong thermal treatments applied may modify the wheat germ structure, and these changes affect the oil extraction yield; this is the reason whereby some relative values shown in Fig. 2 were bigger than one (Özcan et al. 2015).

Peroxide value (PV) is an indicator of the oxidation status of wheat germ oil and a known oxidation initiator of polyunsaturated fatty acids (PUFAs) (Hernández Sánchez et al. 2016). The unsaturated fatty acids contained in the wheat germ oil are susceptible to a rapid oxidation, whether from enzymatic oxidation or by autoxidation. Raw wheat germ showed a peroxide value of 1.76 ± 0.06 meq O_2 kg^{-1} oil, and the treated samples varied between 1.01 ± 0.03 and 1.61 ± 0.01 meq O_2 kg^{-1} oil. It is important to highlight these



TTC: Total tocopherol content
TTC_0: Total tocopherol content of raw wheat germ
n=2

Fig. 2 Relative total tocopherol content of wheat germ samples as a function of treatment time for the different inlet air temperatures. TTC total tocopherol content, TTC_0 total tocopherol content of raw wheat germ. $n = 2$

values because all thermal treated samples showed values well below of legal limits (FAO/WHO 2015) (15 meq O_2 kg^{-1} oil). This fact indicated that the oxidative status of wheat germ oil was not affected by the heat exposition. The low PV determined after the thermal treatments confirmed that oil germ samples did not show thermal oxidation, and it is suggested that the lipid radicals generated during the treatments in the wheat germ oil were stabilised by the high content of tocopherols.

Many authors consider the food colour as an important process parameter in the food processing since it is related to non-enzymatic browning, which may produce a detriment in the food nutritive value (Rahman and Labuza 2007). The pigments generated during the non-enzymatic browning increase as browning and caramelisation reactions progress. Therefore, the control of colour changes seems to be necessary to obtain a good product quality. The raw wheat germ measured had a lightness (L^*) value of 73.93 ± 0.72, a redness–greenness (a^*) of 4.85 ± 0.09, a yellowness–blueness (b^*) of 28.65 ± 0.46, a WI value of 60.96 ± 0.50 and a BI value of 52.72 ± 1.06. The L^* value informed for raw wheat germ was in agreement with the reported values in bibliography (Bansal and Sudha 2011; Gili et al. 2017a), while a^* and b^* values were higher than the that informed by Bansal and Sudha (2011), but in agreement with the published values of Gili et al. (2017a) for infrared stabilisation of wheat germ.

Figure 3a shows the WI versus treatment time for all temperatures analysed, although significant decrease ($p < 0.05$) of WI values only was observed at long treatment times and at high (150 °C) temperature. It is important to highlight that the maximum decrease in WI as consequence of heating was minor to 12%, which was lower than the reductions informed to wheat germ stabilised by infrared radiation (near to 44% from the original value of raw wheat germ) (Gili et al. 2017a). These results indicated that the fluidised bed treatment did not drastically modify the colour of raw germ samples, reducing the undesirable effects of non-enzymatic browning.

Figure 3b presents the evolution of BI values of wheat germ samples treated at 110, 130 and 150 °C as a function of treatment time. To the best of our knowledge, no BI data of wheat germ was published in the literature. As was expected, the heat-treated samples at 150 °C of inlet air temperature showed the highest BI values. Significant differences ($p < 0.05$) of this parameter were found from 300 s of treatment. The ratio of variation regarding to the initial value was minor to 25%. A sigmoid equation (Eq. 5) was fitted to BI experimental data obtained at 150 °C to describe the evolution of this attribute as a function of treatment time. The fitted parameters from Eq. 5 were k_{BI} of 0.012 (0.003) (s^{-1}) and $t_{\frac{1}{2}}$ of 365.24 (26.30) (s), with a coefficient of determination (r^2) of 0.85 (the asymptotic standard error of parameters was included in parentheses).

The sigmoid equation (Eq. 5) did not fit (low r^2 values) the data obtained at 110 and 130 °C treatments since the variation of BI values between raw and heat-treated wheat germ was minor than 8%, which was related to the low air temperature applied in these treatments compared with the temperatures normally used in baking of food products, where BI is usually employed to describe the changes generated during the cooking process of foods.

Considering the thermally treated wheat germ particles as a food ingredient, or supplement, the colour particle is an important property, which defines the acceptability by the consumers (Ureta et al. 2014). The fitted model can be used to determine the total time of the thermal treatment to reach the colour defined as an optimum by the consumers once the inactivation of the lipase activity was achieved.

Thermal Inactivation Kinetics of Wheat Germ Lipase

The thermal fluidisation treatments applied to wheat germ particles produced considerably effects on the wheat germ lipase activity, and it was described by a first-order kinetic

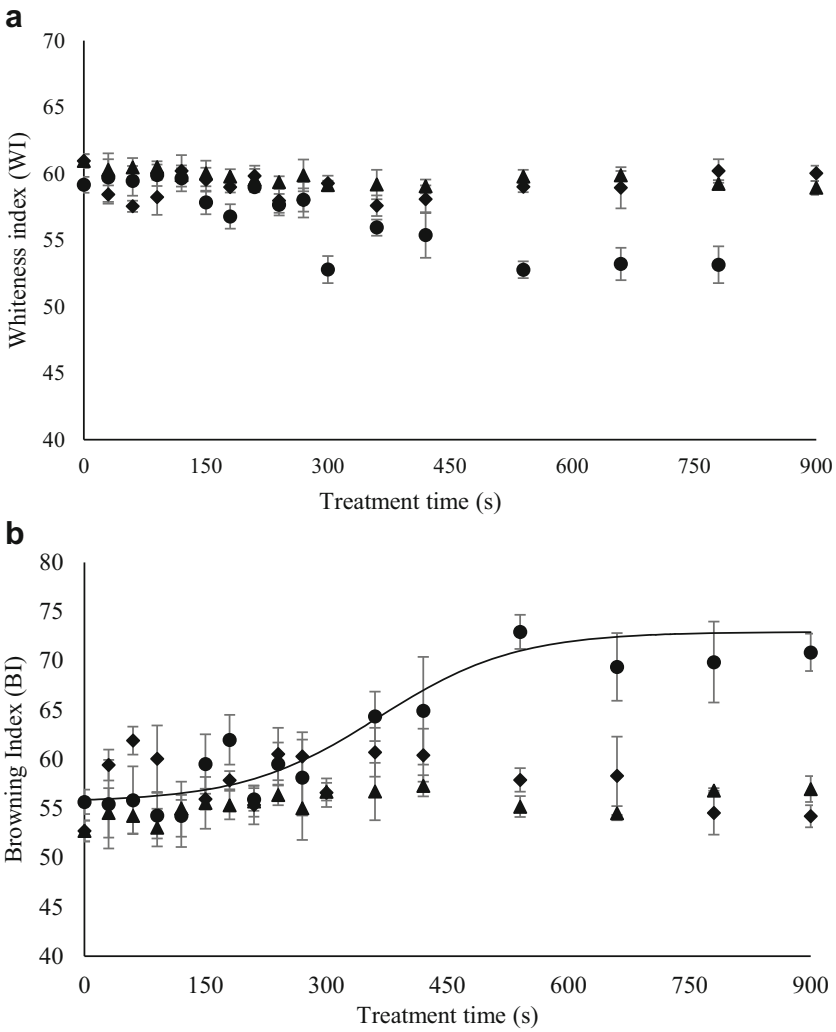


Fig. 3 Whiteness index (WI) and Browning index (BI) expressed as a function of treatment time for the different inlet air temperatures, 110 °C (diamond), 130 °C (triangle), and 150 °C (circle). 150 °C BI predicted values from Eq. (5) were plotted as a function of treatment time. All color parameters were calculated in triplicate

model (Eq. 6). The semi-logarithmic curves of the residual activity of wheat germ lipase versus heating time were lineal to all temperatures analysed with coefficients of determination (r^2) of 0.90, 0.96 and 0.99 at 110, 130 and 150 °C, respectively. The linear behaviour was consistent with a first-order inactivation and related with a rapid inactivation of the enzymes during the first part of the thermal treatment. The obtained parameters from the fitted Eq. 6 are shown in Table 1, and the predicted ΔFFA values for each temperature are shown in Fig. 1. As was expected, the k value decreased continuously from 150 to 110 °C, which confirmed the influence of air temperature on lipase inactivation. These results were according to several published works for enzyme inactivation (Anthon and Barrett 2002; Ganthavorn et al. 1991; Liu et al. 2013). As summarised in Table 1, D values decreased with the temperature indicating that high temperatures needed lower times to reduce the lipase activity up to one tenth of the original ones. This decreasing tendency of D values with the increase of air temperature (Fig. 4) exhibited the heating labile nature of wheat germ lipase (Xu et al. 2016).

The slope of the linear regression applied to $Log(D)$ versus air temperatures (T) experimental data ($r^2 = 0.98$) allowed obtaining the Z value: 25.55 ± 0.01 °C. The calculated Z value was higher than those published by Xu et al. (2016) for wheat

Table 1 Kinetic parameters of lipase thermal inactivation determined by fitting Eqs. 6, 7 and 8 at the analysed temperatures of inlet air

Temperature (°C)	k (s^{-1})	D (s)	Z (°C)	E_a (kJ mol^{-1})
110	0.0004 ± 0.0001	6464.27 ± 693.25		
130	0.0029 ± 0.0002	768.55 ± 85.55	25.55 ± 0.01	121.74 ± 9.24
150	0.0131 ± 0.0007	175.79 ± 13.43		

$n = 2$

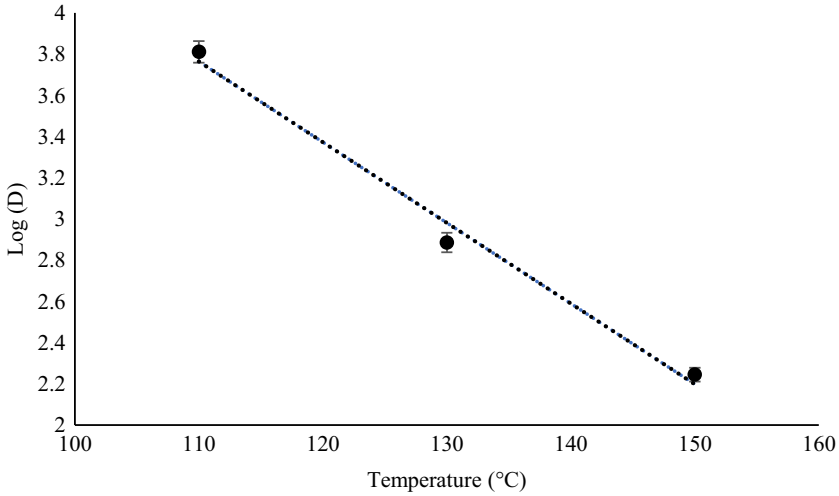


Fig. 4 *Log*(*D*) values (circle) expressed as a function of air temperatures (*T*). Linear regression generated from experimental values (dotted line). *D* values were obtained in duplicate

germ lipase in a buffer solution. On the other hand, the calculated *Z* value was in the order for bacterial and fungus lipase enzymes, analysed in milk (McKellar 1989).

The activation energy of the wheat germ lipase inactivation by fluidised bed process with hot air was calculated from the Arrhenius equation (Eq. 8) by plotting *Ln*(*k*) against the inverse of absolute temperature. The obtained E_a was 121.74 ± 9.24 (kJ mol^{-1}) (Table 1), which was higher than the ones reported in the literature by Xu et al. (2016) and Kermasha et al. (2007). It is important to highlight that both mentioned studies analysed the wheat germ lipase in a buffer solution instead of in the original matrix as was studied in the present work. The higher values obtained for lipase E_a indicated that more energy was necessary to inactivate the enzyme and it may be attributed to a matrix protective effect during the thermal treatment. Meriles et al. (2016) reported a similar lipase activation energy value for wheat germ samples stabilised in a convective oven at high temperatures taking into account the possible matrix effects.

Conclusion

In the present work, the heating processes of wheat germ by fluidisation, carried out at 90, 110, 130 and 150 °C, were performed to analyse the effect of inlet air temperature and treatment time on lipase inactivation, tocopherol content, surface colour and peroxide values.

Air temperatures between 110 and 150 °C produced partial inactivation of wheat germ lipases; the highest degree of inactivation was produced at 150 °C air temperature treatment, which achieved residual lipase activities of 15.5 and 22.4% from the raw germ after 900 and 120 s of treatment, respectively. The residual lipase activity after 120 s of heat treatment at 150 °C produced much lower acidity values of the oil compared to those obtained for raw germ oil showing the effectiveness of the thermal stabilisation by fluidisation.

Total contents and the relative abundances of the tocopherols isomers of wheat germ were not affected by heat treatment. The high concentration of tocopherol in the raw wheat germ was maintained despite the energetic thermal treatment applied. The low peroxide values determined after the thermal treatments confirmed that oil germ samples did not show thermal oxidation. Particle's colour evolution during the thermal treatments showed significant changes only at high temperature (150 °C) and long time treatment.

The thermal lipase inactivation was described by a first-order kinetic model, and the obtained parameters showed an expected behaviour with the treatment temperature. The inactivation rate constant increased and *D* values decreased with the increase of treatment temperature. The *Z* and E_a values of the inactivation process were 25.55 ± 0.01 °C and 121.74 ± 9.24 kJ mol^{-1}, respectively.

The results demonstrated that the heating processes of wheat germ by fluidisation produced the lipase stabilisation in short times, which is the major drawback to the storage and commercialisation of wheat germ. And, at the same time, the heat process did not affect the germ oil quality and tocopherol content and profile.

The data obtained in the present work is expected to contribute in the developing process of wheat germ stabilised by fluidisation. This process considering short time and high temperature conditions may enable to obtain a food ingredient with interesting nutritional features for human consumption from an undervalued co-product of the wheat milling industry.

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