Investigation of Wheat Germ and Oil Characteristics with Regard to Different Stabilization Techniques

Running head: Wheat Germ Stabilization Techniques

Derya Arslan1*, M. Kürşat Demir1, Ayşenur Acar1 and Fatma Nur Arslan2,3

1Department of Food Engineering, Faculty of Engineering and Architecture, Necmettin Erbakan University, Koycegiz Campus, Konya, Turkey
2Department of Chemistry, Faculty of Science, Karamanoğlu Mehmetbey University, Karaman, Turkey
3Van’t Hoff Institute for Molecular Sciences, Analytical Chemistry Group, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, Netherlands

Received: 18 January 2020
Accepted: 27 September 2020

SUMMARY

Research background. Utilization of wheat germ (WG) and wheat germ oil (WGO) is limited due to high enzymatic activity and unsaturated fatty acids and therefore stabilization techniques are needed to overcome this problem.

Experimental approach. In this study, the effects of stabilization methods (dry convective oven heating at 90 and 160 °C and microwave radiation under 180 W and 360 W output power, and steaming by autoclave) on both WG and WGO were evaluated.

Results and conclusions. Steaming caused the most dramatic changes on lipoxygenase, free fatty acids (FFA), DPPH radical scavenging activity, tocopherols and tocotrienols. The lowest peroxide values (PVs) were measured in oils of convectional heating (160 °C) and steaming treatments which were performed at temperatures above 100 °C. However, para-anisidine values (pAVs) of samples treated at higher temperatures were considerably greater than those of stabilized at lower temperatures. Oven heating at 160 °C was also one of the most effective treatments on inactivation of lipoxygenase coming after steaming. Steaming also induced a significant reduction in total tocopherols which was directly associated with the greater lost in

*Corresponding author:
Phone: +903323252024
Fax: +903322210500
E-mail: dears@erbakan.edu.tr
β-tocopherol content. On the contrary γ- and δ-tocopherols and tocotrienol homologs were abundant with higher amounts in steam applied samples. α-Tocopherol and γ-tocotrienol were the most resistant isomers to stabilization processes.  

**Novelty and scientific contribution.** This study shows that the high temperature oven heating method, which is widely used in the industry for thermal stabilization of wheat germ, does not provide an advantage in oxidative stability compared to steaming and microwave applications. Steaming delayed oxidation in germ, while further inhibiting lipoxygenase activity. Moreover, tocotrienols were more conservable. In industrial application, low power in microwave (180 W instead of 360 W), lower temperature in oven heating (90 instead of 160 °C) would be preferable.

**Key words:** wheat germ oil, stabilization, steaming, tocopherols, tocotrienols

**INTRODUCTION**

Wheat germ (WG) is a highly nutritional product, which contains about 10–15 % lipids, 26–35 % proteins, 17 % sugars, 1.5–4.5 % fiber and 4 % minerals (1). Germ contains approximately 11% oil also with considerable amount of bioactive compounds (2). Wheat germ oil (WGO) is the one with the highest tocopherol content among vegetable oils. In addition, the ratio of polyunsaturated fatty acids, mainly linoleic and linolenic acids, is quite high (almost 80% of the oil) (3). Despite its superior health benefits, WG is mostly used as animal feed and is rarely utilized for human nutrition (4,5). Germ is used worldwide in bread, snacks and cereals. In addition, its oil has an important place in the food, medicine and cosmetics industry (6). The mechanical processes applied during wheat milling, cause the cells to break down and the intracellular oil becomes more prone to oxidation. Degradation occurs by the action of oxidative and hydrolytic enzymes such as lipase and lipoxygenase (LOX) on unsaturated fatty acids (1). Therefore, lipase and LOX inactivation is required to prolong the storage stability of the WG. At the same time, due to the high nutrient content, microorganisms cause the WG to agglomerate, mold or ferment.

For limiting the WG enzymatic activity, several stabilization processes have been applied: extrusion (7), microwaving (8), infrared radiation treatment (9), steaming (10), dehydration (11), atmospheric cold plasma (12), as well as chemical preservation for instance addition of antioxidants (13) or alkalis (14). Currently, the most commonly used heat treatment methods to prevent oxidation are dry heating and steaming (15).
In the present study, it was aimed to evaluate the effects of different thermal treatments on the bioactive components and oxidative stability of WG and WGO with special emphasize on the individual tocopherol and tocotrienols isomers. WG samples and their corresponding oil were investigated for the methods including both wet and dry treatments to determine the relationship, if any, between enzymatic activity, oxidative stability, non-saponifiable components and some physical characteristics.

**MATERIALS AND METHODS**

**Materials**

WG was freshly obtained from the variety *Triticum aestivum* L. just after the production from a local flour mill owned by the ONEL Company located in Konya province. An enough quantity of WG was used for oil extraction and the remaining samples were stored in air tight packages at -18 °C in a freezer for enzyme analysis. The remaining reagents and solvents were of analytical grade.

**WG treatment and extraction of oil**

One kilogram of WG was weighed, in duplicate, and treated as follows: dry heat (oven), wet heat (autoclave, steaming) and microwaves (Fig. 1). Oven drying was performed at 90 and 160 °C for 12 and 6 min. respectively, using a laboratory type electric oven equipped with a forced hot-air circulation system (Nüve, KD 200, Ankara, Turkey). The samples were equally spread onto trays (42 x 33 x 3 cm (length x width x thickness)) to approximately a thickness of 1.0 cm. The samples were placed in the oven after the temperature set was reached. The samples were treated for 12 and 5 min. at 180 and 360 W (2450 MHz), respectively; in a commercial microwave oven (LG SolarDOM, Seoul, S. Korea) using its turntable (35 cm diameter, sample thickness of approximately 1.0 cm). Wet heat treatment was carried out on samples weighed in glass heat resistant screw capped jars in an autoclave (WiseClave, WAC-60, Korea) set at 121°C and 15 min. At the end of the treatments samples were cooled to room temperature in a desiccator. Each sample was put in a separate polyethylene plastic bag and stored at -18 °C. An untreated sample was used as control.

Three hundred grams of each stabilized WG samples were extracted with hexane (Merck, Darmstadt, Germany) ratio of 1:10 (m/V) and vibrated on shaking bath water (WiseBath, WSB-30, Seoul, S. Korea) at 30°C for 2 h. The extract was filtered through No:1 filter paper (Whatman International Ltd., Maidstone, Kent, the United Kingdom) in a Buchner funnel. The extraction procedures were repeated twice under the same condition. Solvent was removed
at 40 °C using a rotary evaporator (Heidolph 91, Laborota 4000 Schwabach, Germany). The resulting oil was kept in a glass container at +4 °C until further analysis.

**Analytical determinations on WGO**

Free fatty acidity and peroxide value (PV) were determined according to AOCS Official Methods (16) Ca 5a-40. p-Anisidine values (pAV) was determined by using the AOCS official method (17). Total oxidation (TOTOX) value was calculated with the equation (18);

$$\text{Totox value} = 2\text{PV} + \text{pAV}$$

**Rancimat analysis**

Rancimat values were expressed as the oxidation induction time (h), using the Rancimat apparatus, model 892 (Metrohm Co., Herisau, Switzerland). 3 g of oil sample heated at 120 °C was aerated with a flow of air (20 L/h). The induction time (IT) was expressed in hours. The rancimat measurements were carried out in triplicate (19).

**Determination of tocopherols and tocotrienols**

The stock standard solutions of tocopherol and tocotrienols isomers in hexane (Merck, Darmstadt, Germany) were prepared at a concentration of 1000 µg/mL. The calibration mixtures were diluted with hexane from the stock standard (10-500 µg/mL). One mL of hexane was added on oil samples (1±0.01 g) and tubes were shaken for 2 min. The samples were filtered through 0.45 µm PTFE membrane filters before injection. Analyses of tocopherols and tocotrienols were performed on an Agilent 1260 Infinity II system (Agilent Technologies Inc, Wilmington, DE, USA) equipped with a model G7121A fluorescence detector (FLD). Data were recorded using Agilent’s Chemstation B.03.02-2008 data processor. The separations were performed on a Develosil C30 (250×4.6 mm, 5 µm; Phenomenex Inc., Torrence, USA) stainless-steel column. Mixture of solvent A (methanol: water, 91:9, V/V) and solvent B (methyl tert-butyl ether: methanol: water, 80:18:2, V/V/V) (Sigma-Aldrich, Taufkirchen, Germany) in gradient program, different flow rates and temperatures were used to perform optimum separations. The mobile phase solvents A and B reported by Knecht et al. (20) were used with a modified gradient program. The following gradient program of solvents A and B was applied:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Solvent A (%)</th>
<th>Solvent B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20.5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>20.5–25</td>
<td>0–40</td>
<td>40</td>
</tr>
<tr>
<td>25–36</td>
<td>40–45</td>
<td>55</td>
</tr>
<tr>
<td>36–46</td>
<td>45–55</td>
<td>55</td>
</tr>
<tr>
<td>46–48</td>
<td>55–60</td>
<td>40</td>
</tr>
<tr>
<td>48–51</td>
<td>60–80</td>
<td>20</td>
</tr>
<tr>
<td>51–53</td>
<td>80–0</td>
<td>0</td>
</tr>
<tr>
<td>53–63</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>63–68</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

The flow rate was 0.5 mL.min⁻¹, and temperature was set at 5 °C. 5 µL of the sample was injected into the system. Fluorescence detector was set at wavelength of 296 nm and 330 nm.
Non-enzymatic browning index (NEBI)

The oil samples were diluted with chloroform (Sigma Aldrich, Steinheim, Germany) at the ratio of 1:20 (m/V). The absorbance of the solution was measured spectrophotometrically (Libra S21-S22, Biochrom Ltd, Cambridge, UK) at 420 nm to represent the Browning Index (21).

DPPH radical scavenging activity

The oil samples 0.2 g were mixed with 900 μM DPPH (1,1-diphenyl-2-picrylhydrazyl) (Fluka, Steinheim, Germany) in toluene (ISOLAB, Wertheim, Germany) and the final concentration was made to 750 μM. The mixture was incubated for 20 min. at room temperature (24±1 °C) and the absorbance was measured at 515 nm against blank sample. The inhibitory percentage of DPPH was calculated by using the equation given below (22).

\[ \text{Inhibition}\% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \]

Lipase activity assay

The reaction solution was prepared with a mixture of 100 mM pH=7.0 phosphate buffer, ethanol, and 50 μM para-nitrophenol butyrate (pNPB) (all these chemicals were from Merck, Darmstadt, Germany) in 95:4:1 ratio, respectively. 2.7 ml of the reaction solution was added on to 0.3 g of enzyme source, the reaction continued at 60°C for 15 minutes. The reaction tubes were allowed to stand at -18 °C for 8 minutes and the reaction was stopped. The absorbance at 400 nm was measured (23). One unit (U/mg) of lipase was defined as the amount of enzyme required for the formation of 1 μmol p-nitrophenol per minute at 400 nm, 60 °C and pH=7.0.

\[ \text{Unit/mg enzyme} = \frac{\Delta A \times V_t}{(\varepsilon_{400\text{nm}} \times t \times b \times V_e)} \]

\[ A = \text{Absorbance}, \Delta A = A_{400\text{nm, Example}} - A_{400\text{nm, Blind}} \]

\[ V_t = \text{Total reaction volume (2.7 mL)} \]

\[ \varepsilon_{400\text{nm}} = \text{The millimolar absorption coefficient of p-nitrophenol (5.081 L/mM)} \]

\[ t = \text{reaction time (15 minutes)} \]

\[ b = \text{light path (1 cm)} \]

\[ V_e = \text{enzyme volume in reaction mixture (0.3 g).} \]

Lipoxygenase activity

The substrate solution was prepared by mixing 157.2 μL of pure linoleic acid, 157.2 μL of Tween-20 (Sigma-Aldrich, Taufkirchen, Germany) and 10 mL of deionized distilled water.
The solution was clarified by adding 1 mL of 1 N sodium hydroxide (Merck, Darmstadt, Germany). Then it was diluted to 200 mL with 0.2 M sodium phosphate buffer, pH=7.0; which gives a 2.5 mM final concentration of linoleic acid (Fluka Sigma-Aldrich, St. Louis, MO, USA). The substrate solution was flushed with oxygen gas at least for 2 min to give an initial absorbance at 234 nm of 0.3-0.4, and allowed to equilibrate in a water bath at 25 °C. The total reaction volume was 3 mL, which contained 2.7 mL of substrate solution and 0.3 mL of enzyme solution. One unit of enzyme activity was defined as an increase in absorbance of 0.001 at 234 nm/min under assay conditions (24). The extinction coefficient for the conjugated diene of linoleic acid was 23,000 M⁻¹cm⁻¹ (25).

**Statistical analysis**

Variance analysis was performed with SPSS statistical software (version 17.0, SPSS Inc., Chicago, IL, USA) (26). Duncan’s multiple range test were used to assess significant differences between the treatments (p<0.05). All the determinations were made in triplicates.

**RESULTS AND DISCUSSION**

**Influence of stabilization methods on FFA content of WG oils**

FFA content is a typical indicator of the degree of hydrolytic rancidity extent of lipids. The FFA content of raw germ samples ranged from 2.68% to 5.47% oleic acid (Table 1). The untreated samples had significantly higher FFA content compared to the oils of stabilised samples. There was no significant difference between thermally treated samples in terms of FFA content. Steaming led to a more reduction in FFA (2.72%) than that of the other stabilization methods applied (3.14-3.93%) even though the difference was not statistically significant. Bergonio et al. (28) also reported that microwave and steaming applications were more effective in reducing acidity. This phenomenon was explained by the higher resistance of proteins to denaturation in a dry environment compared to a wet environment (29).
Peroxide value (PV), p-anisidine (pAV) and TOTOX results of WG oils

Germ oil samples showed PV from 5.40 ±0.60 to 15.28 ±0.47 meq O₂/kg oil, which do not exceed the legal limits (15 meq O₂/kg oil) (FAO/WHO (30) for all samples (Table 1). The stabilization processes ended up with lower PV (between 5.4-12.9 meq O₂/kg oil, as raw WG showed PV of 15.24 meq O₂/kg oil. The lowest PVs were measured in oils of convectional heating (160 °C) (5.40 meq O₂/kg oil) and steaming (5.94 meq O₂/kg oil) treatments which were performed at temperatures above 100 °C. These were followed by high power microwave treated samples (10.50 meq O₂/kg oil), but with almost twice the values of dry convectional heating (160 °C) and steaming. The longer contact of WG to humidity inside the chamber could be the reason of higher PV of samples of low temperature microwave treatment. These results show that output power is more effective than treatment time in the case of stabilization process by means of microwave radiation.

In terms of PV, for applications exceeding 100 °C such as steaming and convectional dry heating, the inactivation of the enzymes was much higher and thus peroxide formation was slower. In microwave treatments, lower values were obtained in the high-power process. Therefore, it can be emphasized that the applied output power is more effective than time in microwave radiation applications.

According to the results of Li et al. (31) raw germ showed higher PV development than stabilized samples; however long time and temperature (45 and 60 min) exposure to short wave infrared were not effective. Hu et al. (27) reported that super heated steam process avoided hydroperoxide formation in wheat bran, while hot air stabilization increased PVs. No or less contact with oxygen is shown as the underlying cause for lower peroxide development in steam heated samples (27). The overall preventive effect of thermal stabilization on oxidation was ascribed to the high tocopherols content in WGO as that the lipid radicals generated during the treatments were stabilised by these compounds.

As for the results of the pAV, which gives an idea about the secondary oxidation products of lipids, mainly aldehydes such as 2,4-dienals and 2-alkenals, all of the stabilization processes except microwave (180 W) significantly increased pAVs of germ oil. pAVs of samples treated at higher temperatures were considerably greater than those of stabilized at lower temperatures. The highest values were determined for the convective dry heating (160 °C), which were followed by steaming performed at 125 °C and by convective dry heating (90 °C). Higher output power used for microwave treatment decreased primary oxidation products while increased secondary oxidation products. In both microwave (180 W) and dry heat
treatments (90 °C), the pAVs were found to be much lower as a result of exposure to low temperatures.

Similarly, germ samples treated by short wave infrared at 70 °C for 60 min and 80 °C for 45 min showed higher pAVs than raw WG (31). The oxidation state of the extracted oils was determined using the TOTOX equation. The results obtained are provided in Table 1. The stabilized samples presented lower results of TOTOX as was the case with PVs.

**Non-enzymatic browning index (NEBI) of oils from stabilized WG**

In order to elucidate the passage of Maillard reaction products formed during thermal processing to the oil phase, the changes in non-enzymatic browning index were determined, and the results were shown in Table 1. Oils from raw and stabilized WG exhibited similar browning index values. The stabilization treatments did not significantly affect the browning index.

**DPPH radical scavenging activity of WG and oil**

WGOs from samples stabilized under low power out microwave radiation presented the highest inhibition of DPPH (64.9 %) even higher than the unstabilized WG (58.19%). However, steaming (48.9 %) and microwave (360 W) (54.6%) stabilization treatments significantly decreased the DPPH radical scavenging activity. This revealed us that steaming with the humidity in the environment caused the highest destruction in the compounds with radical scavenging activity. Even though the duration of steaming is 15 minutes, the sample is exposed to heat until the temperature reaches 125 °C and cools down, and this may be the reason for lower activity of germ oils stabilized by steaming. Likewise, Bergonio *et al.* (28) reported that MW treatments led to decrease in the antioxidant activity of brown rice. Radical scavenging activity of convectional oven heating (90 and 160 °C) applied samples did not vary significantly between each other. These treatments both ended up with oils containing closer radical scavenging activities to untreated samples. During convective oven heating processs, antioxidant compounds resulting from maillard reactions may have increased the DPPH results.

**Inactivation of enzymes**

All treated WG samples had significantly lower lipase and lipoxygenase activities compared with untreated sample (Table 2). There was not significant difference between the treatments in terms of lipase activity; however the results of lipoxygenase were significantly different.
among the treatments. The samples which were treated by steaming exhibited the lowest lipoxygenase activity. Convectional oven heating (160 °C) were also one of the most effective treatment on inactivation of lipoxygenase coming after steaming. Microwave radiation with a power of 360 W for 300 s followed these two treatments even with the values very close to untreated, microwave (180 W) and convective heat (90 °C) processes. 

Hu et al. (27) reported that supersteam processing was more efficient than hot air processing in terms of enzyme inactivation. Ling et al. (32) found radio-frequency heating to 90 °C for a few seconds did not completely inactivate lipase activity in WG. Kapranchikov et al. (33) also reported that the lipase in WG is heat-stable with retaining its original activity over 20 % after being kept at 70 °C for up to 1 h.

Treatment time was found as an important factor on the residual lipase activity as well as a linear association between final residual enzymatic activity and air temperature (6). Further more, Xu et al. (34) reported that a combination of higher temperature and longer time was more effective on inactivation of enzymes. Inactivation of lipase by microwaves in WG was attributed to thermal effects rather than non-thermal effect, and was more effective than conventional heating in the study of Chen et al. (35).

The different secondary structure of the lipase enzyme allows the plasma to be more selective in terms of inactivation of this enzyme. The lower rate of inactivation of lipoxygenase compared to lipase inactivation can be explained by this selectivity (12). Lipoxygenase was less inactivated compared to lipase in all different heat treatment methods including direct steaming, drum drying, fluidized bed drying and atmospheric cold plasma treatment (12,15).

**Effects of stabilization methods on tocopherols and tocotrienols**

Total amount of tocopherols in WGO samples ranged between 960.8-980.9 mg.kg⁻¹oil. All the treatments showed insignificant effect on total tocopherols except steaming which was the only treatment that induced a significant reduction in total tocopherols content. Similarly, more than 96.43% of original tocopherol content was reported to be remained in WG samples stabilized by short wave infrared radiation at 90 °C for 20 min (31). The total tocopherol values were reported between 4133-4181 mg/kg for raw WG and between 3395-3899 mg/kg for treated WG by Gili et al. (9). The values determined in the present study are lower than those reported by Gili et al. (9). The authors also announced slight effect of thermal treatments on total tocopherols.
β-Tocopherol is the cause of the significant difference observed at steaming, as WG samples stabilized by steaming lost significant amount of its original content of this homolog. On the contrary γ- and δ-tocopherols were abundant with higher amounts in steam applied samples than that of stabilized and raw samples. α-Tocopherol as the predominant homolog among the tocopherols was not significantly influenced by stabilization processes. However, tocopherol isomers in rice bran were significantly affected by infrared radiation and the most influenced isomer was α-tocopherol as reported in the study of Yılmaz et al. (36).

As shown in Table 3, β-tocotrienol was the predominant component (54.8-77.5 mg.kg⁻¹), followed by α-tocotrienol (7.6-12.5 mg.kg⁻¹), γ-tocotrienol (1.1-1.9 mg.kg⁻¹) and δ-tocotrienol (0.18-0.32 mg.kg⁻¹). Tocotrienol isomers remained with the highest values in steam-treated samples compared to other stabilized and raw samples as was the case with γ- and δ-tocopherols.

The ratios of increase in γ- and δ-tocopherols caused by steaming were 13.7 and 66.1 %, respectively. Besides, tocotrienol contents increased with the ratios 48.4 %, 33.5 %, 41.2 %, 21.7 % for α-, β-, γ-, and δ-tocotrienols, respectively.

α-Tocopherol and γ-tocotrienol were the most resistant isomers to stabilization processes, as there were not significant changes in their amounts after heat treatments. Li et al. (5), reported that WG samples stabilized with flameless catalytic infrared for 6 min lost 23.82 % of its original α-tocopherol.

The amount of tocotrienols increased in steam applied samples, although this increase was observed in tocopherols too (except β-tocopherol), it was clear that there was a greater increase in tocotrienols.

Yoshida et al. (37) concluded that tocopherols and tocotrienols showed similar mobilities within the membranes, but tocotrienols were more easily transferred between the membranes and incorporated into the membranes than tocopherols. Steaming may increase the transfer of tocotrienols with high cell membrane mobility in the cell. Therefore, the increase in the amounts of individual tocotrienols as a result of the steaming process can be some how explained by this phenomenon.

In the study of Yılmaz et al. (36), the lost in tocopherols induced by infrared was ascribed to the high surface temperature generated during radiation. Yoshida et al. (37) reported that only 80 % of tocopherols remained in soybean roasted in microwave oven for 20 min as cited by Li et al. (31).
CONCLUSIONS

It is well known that the oxidative stability of WG is commonly improved by means of heat treatments which reduce the peroxide number and increase the antioxidant activity. However, in this study several methods and conditions were compared in terms of their effect on some properties of WG and oil with special emphasis on tocopherols and tocotrienols. Convective heating, which is currently the most technologically applied high temperature direct (convective) heat treatment does not offer significant advantages in terms of oxidative stability along with no significant reduction in lipase activity compared to other stabilization methods. Because, convective oven heating at 90 °C resulted in the highest PV values and at 160 °C the highest pAVs were obtained. While convective heat treatment can be preferred when the energy consumption is taken into consideration, steaming comes to the fore in terms of product quality. The steaming process exhibited more reasonable results in terms of lipoxygenase activity, peroxide and pAVs. However, steaming resulted in the most destruction of β-tocopherol which can be associated with the lowest radical scavenging activity determined on steam-stabilized samples.

The most effective procedure that caused notable change in tocopherols was steaming. On the other hand, tocotrienol isomers remained with the highest values in steam-treated samples compared to other stabilized and raw samples as was the case with γ- and δ-tocopherols. α-Tocopherol and δ-tocotrienol are the most resistant isomers to stabilization, no application has created a significant change.

Minimal processing conditions (lower temperatures and output power) can be suggested for both microwave and convective heating treatments based on less formation of secondary oxidation products.

ORCID ID
D. Arslan https://orcid.org/0000-0002-6655-9312
M.K. Demir https://orcid.org/0000-0002-4706-4170
A. Acar https://orcid.org/0000-0002-2086-7109
F.N. Arslan https://orcid.org/0000-0002-5748-8268

REFERENCES


Table 1. Some analytical parameters and oxidative stability of wheat germ oil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Untreated</th>
<th>Microwave (360 W)</th>
<th>Microwave (180 W)</th>
<th>Dry heat (90°C) (convective oven heating)</th>
<th>Dry heat (160°C) (convective oven heating)</th>
<th>Wet heat (steaming)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free fatty acids (%w, oleic acid)</td>
<td>(5.38±0.77)±a†</td>
<td>(3.14±0.45)b</td>
<td>(3.93±0.27)b</td>
<td>(3.44±0.46)b</td>
<td>(3.50±0.22)b</td>
<td>(2.72±0.12)b</td>
</tr>
<tr>
<td>Peroxide value (mmol/kg)</td>
<td>(6.77±0.10)a</td>
<td>(4.66±0.41)c</td>
<td>(5.65±0.38)b</td>
<td>(5.72±0.24)b</td>
<td>(2.40±0.48)d</td>
<td>(2.64±0.82)d</td>
</tr>
<tr>
<td>p-anisidine (pAV)</td>
<td>(3.38±0.05)d</td>
<td>(4.16±0.11)c</td>
<td>(1.16±0.02)e</td>
<td>(4.19±0.06)c</td>
<td>(7.26±0.13)a</td>
<td>(5.86±0.07)b</td>
</tr>
<tr>
<td>TOTOX</td>
<td>(33.94±9.42)a</td>
<td>(25.17±4.73)ab</td>
<td>(26.58±11.75)ab</td>
<td>(30.15±11.27)ab</td>
<td>(18.06±0.75)b</td>
<td>(17.70±0.13)b</td>
</tr>
<tr>
<td>Induction time (t induction/h)</td>
<td>(2.54±0.08)a</td>
<td>(1.71±0.32)ab</td>
<td>(1.78±0.15)ab</td>
<td>(2.00±0.86)ab</td>
<td>(1.50±0.48)b</td>
<td>(1.87±0.18)ab</td>
</tr>
<tr>
<td>NEBI †</td>
<td>(0.47 ±0.05)</td>
<td>(0.39 ±0.05)</td>
<td>(0.43 ±0.06)</td>
<td>(0.48 ±0.03)</td>
<td>(0.49 ±0.07)</td>
<td>(0.45 ±0.03)</td>
</tr>
<tr>
<td>DPPH (inhibition%)</td>
<td>(58.19±0.28)b</td>
<td>(54.57±1.83)c</td>
<td>(64.94±1.83)a</td>
<td>(58.30±1.89)b</td>
<td>(57.96±1.50)b</td>
<td>(48.87±1.64)d</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± standard deviation of 3 determinations.
‡Significant differences in the same row are shown by different lower case letters [comparison between stabilized and unstabilised samples] (p <0.05).
†Non-enzymatic browning index.
Table 2. Enzyme activity in wheat germ samples.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Untreated</th>
<th>Microwave (360 W)</th>
<th>Microwave (180 W)</th>
<th>Dry heat (90°C) (convective oven heating)</th>
<th>Dry heat (160°C) (convective oven heating)</th>
<th>Wet heat (steaming)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase (U/g)</td>
<td>(0.076±0.009)±</td>
<td>(0.060±0.004)b</td>
<td>(0.060±0.004)b</td>
<td>(0.057±0.003)b</td>
<td>(0.064±0.006)b</td>
<td>(0.059±0.005)b</td>
</tr>
<tr>
<td>Lipoxygenase (U/g)</td>
<td>(0.904±0.116)a</td>
<td>(0.733±0.151)ab</td>
<td>(0.829±0.155)a</td>
<td>(0.872±0.069)a</td>
<td>(0.536±0.123)b</td>
<td>(0.216±0.041)c</td>
</tr>
</tbody>
</table>

†[units of activity (U) per gram of enzyme (g)]
*Results are expressed as mean ± standard deviation of 3 determinations.
‡Significant differences in the same row are shown by different lower case letters [comparison between stabilized and unstabilised samples] (p <0.05).
Table 3. Tocopherols and tocotrienols contents (mg.kg⁻¹) in cold pressed wheat germ oil samples stabilized with different methods

<table>
<thead>
<tr>
<th>Tocotrienols</th>
<th>Untreated</th>
<th>Microwave (360 W)</th>
<th>Microwave (180 W)</th>
<th>Dry heat (90°C) (convective oven heating)</th>
<th>Dry heat (160°C) (convective oven heating)</th>
<th>Wet heat (steaming)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-TT</td>
<td>(8.45±0.08)ᵇᶜ</td>
<td>(8.38±0.05)ᵇ</td>
<td>(7.64±0.04)ᵇ</td>
<td>(8.49±0.05)ᵇ</td>
<td>(8.36±0.07)ᵇ</td>
<td>(12.54±0.11)ᵃ</td>
</tr>
<tr>
<td>β-TT</td>
<td>(58.03±0.12)ᵇ</td>
<td>(57.80±0.07)ᵇ</td>
<td>(54.82±0.05)ᵇ</td>
<td>(56.63±0.08)ᵇ</td>
<td>(56.82±0.07)ᵇ</td>
<td>(77.45±0.10)ᵃ</td>
</tr>
<tr>
<td>γ-TT</td>
<td>(1.31±0.01)ᵇᶜ</td>
<td>(1.10±0.03)ᵇ</td>
<td>(1.10±0.03)ᵇ</td>
<td>(1.08±0.02)ᵇ</td>
<td>(1.13±0.01)ᵇ</td>
<td>(1.85±0.03)ᵃ</td>
</tr>
<tr>
<td>δ-TT</td>
<td>(0.23±0.02)</td>
<td>(0.32±0.01)</td>
<td>(0.25±0.01)</td>
<td>(0.18±0.03)</td>
<td>(0.24±0.01)</td>
<td>(0.28±0.02)</td>
</tr>
<tr>
<td>α-T</td>
<td>(484.05±1.09)</td>
<td>(486.47±0.98)</td>
<td>(487.75±1.12)</td>
<td>(485.55±1.29)</td>
<td>(486.60±1.08)</td>
<td>(488.75±1.20)</td>
</tr>
<tr>
<td>β-T</td>
<td>(414.50±1.08)ᵃ</td>
<td>(413.05±1.21)ᵃ</td>
<td>(415.37±1.12)ᵃ</td>
<td>(415.05±1.33)ᵃ</td>
<td>(412.65±1.09)ᵃ</td>
<td>(364.54±1.24)ᵇ</td>
</tr>
<tr>
<td>γ-T</td>
<td>(12.70±0.13)ᵇᶜ</td>
<td>(12.19±0.08)ᵇᵈ</td>
<td>(11.83±0.12)ᵈ</td>
<td>(13.20±0.12)ᵇ</td>
<td>(12.77±0.14)ᵇᶜ</td>
<td>(14.44±0.12)ᵃ</td>
</tr>
<tr>
<td>δ-T</td>
<td>(0.59±0.04)ᵇ</td>
<td>(0.64±0.02)ᵇ</td>
<td>(0.65±0.01)ᵇ</td>
<td>(0.69±0.02)ᵇ</td>
<td>(0.63±0.03)ᵇ</td>
<td>(0.98±0.03)ᵃ</td>
</tr>
<tr>
<td>total content</td>
<td>(979.87±1.26)ᵃ</td>
<td>(979.97±1.20)ᵃ</td>
<td>(979.17±1.24)ᵃ</td>
<td>(980.90±1.31)ᵃ</td>
<td>(979.46±1.21)ᵃ</td>
<td>(960.85±1.21)ᵇ</td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± standard deviation of 3 determinations.
‡Significant differences in the same row are shown by different lower case letters [comparison between stabilized and unstabilised sample (p<0.05).
Fig. 1. Schematic presentation of experimental process.