Chemical Composition and in vitro Anti-inflammatory Activity of Wheat Germ Oil Depending on the Extraction Procedure

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Abstract: This study aimed to examine the chemical composition of wheat germ oil extracted by three different methods, and to evaluate its inhibitory effect on the cyclooxygenase and proteinase activities. The results showed that the contents of policosanols, tocopherols and phytosterols were affected by the extraction procedure. However, the fatty acid composition of the different oil extracts was nearly the same. Among the tested oils samples, cold pressed oil exhibited the strongest inhibitory activity against proteinase (93.4%, IC50 = 195.7 µg/mL) and cyclooxygenase 1 (80.5%, IC50 = 58.6 µg/mL). Furthermore, the cold pressed oil had the highest content of octacosanol, β-sitosterol and α-linolenic acid, suggesting that those bioactive compounds could be essential for the potent anti-cyclooxygenase activity. The present data revealed that wheat germ oil contained cyclooxygenase and trypsin inhibitors, which are the promising therapeutic target for the treatment of various inflammatory diseases. Thus, wheat germ oil might be used to develop functional foods and pharmaceutic products for the human health.

Key words: wheat germ oil, bioactive lipids, anti-inflammatory activity, effect of solvent

1 Introduction

In rheumatoid arthritis, the destruction of cartilage tissue appears to be due to release of proteolytic enzymes by pro-inflammatory mediators such as cytokines1-2. Therefore, proteinase inhibitors provide substantial protection against tissue damage during inflammation3. Furthermore, the destruction of the phospholipids in the cartilage by phospholipase A2 produces arachidonic acid, which is subsequently converted through cyclooxygenase (COX) to various lipid mediators such as pro-inflammatory prostaglandins. During the inflammatory process, COX-1 activity does not change whereas an important increase in COX-2 levels occurs leading to amplified production of pro-inflammatory lipid mediators. So, the COX-2 activity is widely targeted for the treatment of inflammation4.

Due to the various side effects of the current anti-inflammatory drugs, much attention has been focused recently on the development of new alternative anti-inflammatory agents from natural phyto-resources5. So, different plant extracts were evaluated for their in vitro anti-inflammatory potential using proteinase and cyclooxygenase inhibitory assays1,5,6. According to Salehi et al.7, the mixture of avocado and soybean unsaponifiable lipids (Piasclédine300, France) is characterized by a potent anti-inflammatory effect and is recommended for the treatment of various autoimmune diseases such as arthritis.

Wheat (Triticum aestivum L.) germ is an important by-product of wheat milling and its oil content ranged from 5.2 to 15.5%8-10. Wheat germ oil is a rich source of health promoting compounds such as the essentials fatty acids and unsaponifiable lipids including tocopherols, phytosterols and policosanols. Different studies have shown that wheat germ oil possess various therapeutic proprieties like anti-hyperglycemic and antioxidant effects8,9. In addition, Hussein et al.10 reported that wheat germ oil protects against lipid peroxidation and oxidative stress. The extraction of oil from germ is mostly done by mechanical pressing

Abbreviations: GC-MS, Gas chromatography coupled to mass spectrometry; COX, cyclooxygenase; IC50, The fifty percent inhibitory concentration; SD, Standard deviation
or solvent extraction. The method of extraction and the solvent type affect the yield and quality of oil\(^8\)\(^{11}\). Among the oil extraction techniques, cold-press method does not require an external heat or organic solvent, maintains the proprieties of the bioactive compounds, and preserves the high quality of the extract. Consequently, the cold pressed oils are nutritional\(^{12}\) and are currently in high demand.

Wheat germ oil has been shown to possess various biological proprieties\(^8\)\(^{10},\)\(^{13}\), however much less is known about its inhibitory effect on cyclooxygenase and proteinase activities. The phytochemical profile and biological proprieties of plant extracts were influenced by different factors such as the method of extraction, environmental conditions, and the genotype\(^5\)\(^,\)\(^6\). Therefore, this study aimed to determine and compare the chemical composition, as well as the proteinase and cyclooxygenase inhibitory capacities, of wheat germ oil extracted with different methods including cold pressing.

### 2 Materials and Methods

#### 2.1 Material and reagents

Fresh wheat germ was obtained from National Agriculture Institute of Tunisia (INAT). The wheat germers were ground using a commercial IKA grinder. All solvents were of analytical grade.

#### 2.2 Wheat germ oil extraction

Lipids were extracted from wheat germ using different three methods: cold pressing, Soxhlet and chloroform/methanol methods. Oil samples were extracted by chloroform/methanol according to the method of Folch and co-workers\(^{14}\). The cold pressed oil was obtained by pressing wheat germ using automatic oil presser (304 Stainless Steel, 1500 W). Oil extraction was also conducted with a Soxhlet extractor during 6 h. After Soxhlet extraction with petroleum ether, the used solvent was removed by rotary evaporator (55°C). The experiment was performed in triplicate.

#### 2.3 Determination of total unsaponifiable matter and thin layer chromatography

The unsaponifiable fraction were extracted and fractioned by thin layer chromatography as described previously by Harrabi and co-workers\(^{16}\).

#### 2.4 Analysis of bioactive lipids

The policosanol and sterol composition of the tested oils was determined by GC-MS as described by Harrabi and co-workers\(^{16}\). GC-MS analyses were performed using a capillary HP5MS column (30 m × 0.25 mm I.D., 0.25 μm film thickness; Agilent Technologies) with gas chromatography (Agilent Technologies 7820A) coupled directly to the mass detector (Agilent Technologies 5975 series MSD). Tocopherols were analysed by HPLC according to the method described by Deiana and co-workers\(^{17}\). The separation of tocopherol isomers was accomplished on an Atlantis column (4.6 × 150 mm, 5 μm).

The fatty acid composition of the seed oil was determined by gas chromatography (GC). Fatty acid methyl esters were analysed by GC using a HP 6890 chromatograph equipped with a flame ionisation detector (FID) on a capillary column, CP-Sil 88 (50 m length, 0.25 mm id, 0.2 μm film thickness; Varian).

#### 2.5 Measurement of in vitro anti-inflammatory activity

Determination of proteinase inhibitory activity of the tested extracts was performed according to the method described by Oyedepo and co-workers\(^{17}\). Wheat germ oil extracts and diclofenac sodium (positive control) were tested for trypsin inhibition, at different concentrations (10 to 400 μg/mL). The percent inhibition of proteinase activity was calculated as: % Inhibition =

\[
\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}}\times 100
\]

The anti-cyclooxygenase activity was determined using the COX (ovine/human) inhibitor screening assay kit (Cayman Chemical, N° 760151), as reported previously by Boudjou and co-workers\(^{18}\).

#### 2.6 Statistical analysis

Statistical analysis was performed by using the SPSS statistics (Software version 21). Duncan’s Multiple Range Test was applied.

### 3 Results and Discussion

#### 3.1 Oil extraction yield

According to the extraction method used, the oil yield of the tested wheat germ varied from 6.4 to 11.5% (Table 1). The Soxhlet petroleum ether method led to the highest extraction level of total lipids (11.5%), however the methanol-chloroform method led to the lowest value (6.4%). The cold pressing method was less effective than Soxhlet method in the extraction of oil from germ. This might be because the Soxhlet extraction requires an organic solvents

| Extraction method          | Oil content (% of dry weight) |
|----------------------------|-------------------------------|
| Methanol-chloroform        | 6.4 ± 1.2\(^a\)               |
| Soxhlet Petroleum ether    | 11.5 ± 0.5\(^b\)              |
| Cold pressing              | 7.8 ± 1.3\(^a\)               |

Mean values with different letters are significantly different at \(p < 0.05\).

Mean values are presented as mean ± SD (\(n=3\)).
and external heat, which facilitates the shatter of cell structure and the release of bound lipids. Similarly, Kostić et al. showed that Soxhlet method was more efficient than cold pressing to remove oil from plum kernels. However, cold pressing is a more appropriate method when considering the need to maintain the proprieties of the bioactive lipids and preserve the high quality of the extract. According to Ghafoor et al., the wheat germ oil yield ranged from 5.2 to 15.5% depending on the types of solvent used for the extraction.

3.2 Total unsaponifiable lipid content
Due to their numerous nutritional and health benefits, unsaponifiable compounds are important constituents of vegetable oils. Ham et al. demonstrated the protective effects of unsaponifiable matter from rice bran on oxidative damage. The total unsaponifiable contents of the tested wheat germ oils were affected by the method of oil extraction (Table 2). The oil extracted by Soxhlet petroleum ether method contained more unsaponifiable matter (3.9%) than those extracted by chloroform/methanol (2.7%) and cold pressing (3.1%). Unsaponifiable matter is a mixture of polar and non-polar compounds, as well as of free and bound lipids, therefore extracting conditions (temperature, time, pressure) and solvent polarity could affect the extraction efficiency of those compounds. In general, the unsaponifiable level of wheat germ oil was in the range of 1.5-7.8%.

3.3 Tocopherols content and composition
α-Tocopherol is essential in small quantities to normal metabolism to prevent the human deficiency disease AVED “Ataxia with Vitamin E Deficiency”. As shown in Table 2, the tocopherol content was higher in the oil extracted by Soxhlet method (22.2 mg/g) than in the oils obtained by the other tested methods (12.8-16.9 mg/g). The main tocopherol of the tested wheat germ oil was α-tocopherol (98% of total tocopherol) (Fig. 1), which was in accord with the literature data. Our results showed that cold pressing method cause substantial tocopherol loss. Similarly, Özcan et al. reported that the supercritical CO2 method was more effective in extracting α-tocopherol from wheat germ oil than cold pressing procedure. Furthermore, the tocopherol solubility could be affected by the type of solvent and the temperature. Konopka et al. showed that 90%-94% of total tocopherols present in wheat grain were extractable by petroleum ether.

3.4 Phytosterols content and composition
Phytosterols consumption is known to reduce the risk of cardiovascular diseases by decreasing intestinal cholesterol absorption and blood LDL-cholesterol levels. As shown in Table 2, the phytosterol level of the wheat germ oil was affected by the method of extraction. Total sterols were more represented in the chloroform/methanol oil extract (4.16 mg/g of oil) than in the oils extracted with cold pressing (3.92) and Soxhlet method (3.1 mg/g of oil). This result is probably due to the solvent polarity and the extraction temperature used. Similarly, Kozłowska et al. showed that the total sterol content of anise oil extracted with methanol/chloroform mixture was 1.5 times higher as compared with the Soxhlet hexane method. According to Jafarian Asl et al., the amount of phytosterols decreased significantly at 80°C due to thermal degradation of these compounds at higher temperatures. Neutral lipids are extracted with non-polar solvents, such as hexane, whereas polar lipids, which are membrane-associated lipids, needs polar solvents (ethanol, methanol) or bipolar solvents (water-saturated butanol) to disrupt hydrogen bonds and electrostatic forces.

GC-MS analysis was carried out to identify the different

![Fig. 1 HPLC chromatogram of the tocopherol analysis of wheat germ oil obtained by cold pressing.](image)

**Table 2** Total unsaponifiable matter, policosanol, tocopherol and phytosterol content of wheat germ oil obtained with different extraction methods.

| Extraction method          | Unsaponifiable Matter (%) | Policosanol mg/100 g oil | Tocopherol mg/g oil | Phytosterol mg/g oil |
|----------------------------|----------------------------|--------------------------|---------------------|----------------------|
| Methanol-chloroform        | 2.7 ± 1.1                 | 25.8 ± 2.1               | 12.8 ± 1.2          | 4.16 ± 1.2           |
| Soxhlet Petroleum ether    | 3.9 ± 1.2                 | 20.4 ± 3.1               | 22.2 ± 1.3          | 3.10 ± 1.2           |
| Cold pressing              | 3.1 ± 0.7                 | 35.6 ± 1.5               | 16.9 ± 1.1          | 3.92 ± 1.1           |

Mean values in the same column with different letters are significantly different at p < 0.05. Values are presented as mean ± SD (n=3).
phytosterols present in the wheat germ oil samples. Typical GC-MS chromatogram of the oil obtained by cold pressing is shown in Fig. 2. Six sterols were identified, and the sterol profile of the tested oil samples was nearly the same (Table 2). β-sitosterol was the major compound (60.80-66.47%) followed by campesterol (13.53-18.50%). The sterol composition of wheat germ oil reported in this study is in agreement with the literature data.

3.5 Policosanol content and composition

Various beneficial physiological activities have been attributed to policosanol, such as anti-inflammatory, antioxidant and cholesterol-lowering effects. Policosanol contents of the different wheat germ oil samples are given in Table 2. Cold pressed oil showed the highest total policosanol content (35.6 mg/100 g of oil), indicating that the cold pressing technique was more effective than organic solvent in extracting policosanols. The pressure could have a significant effect on the extraction efficiency of these compounds. Dunford et al. demonstrated that the policosanol content of wheat germ oil varied with the type of solvent and extraction temperature.

GC-MS analysis was carried out to identify the different policosanol compounds present in the tested wheat germ oils (Fig. 2, Table 3). The major compounds in all extracts were tetracosanol (C24), hexacosanol (C26) and octacosanol (C28) (Table 4). The amounts of the various compounds depended on the extraction method. Octacosanol was the most abundant compound (46.25%) in cold pressed oil followed by tetracosanol (27.38%) and hexacosanol (21.94%). However, tetracosanol (67.48%) was the most abundant compound in Soxhlet petroleum ether extract followed by octacosanol (23.45). Cold pressing extraction is suitable for the extraction of octacosanol. Similarly, Dunford et al. demonstrated that policosanol composition was significantly affected by the type of solvent and extraction temperature.

3.6 Fatty acid composition

The fatty acid composition is an important indicator of
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Table 4 Policosanol (%) composition of wheat germ oil extracted with different methods.

|                  | Cold pressing | Methanol-chloroform | Soxhlet Petroleum ether |
|------------------|---------------|---------------------|-------------------------|
| Docosanol C22    | 3.18 ± 0.21<sup>a</sup> | 4.20 ± 0.27<sup>a</sup> | 3.67 ± 0.2<sup>a</sup> |
| Tricosanol C23   | 1.25 ± 0.06<sup>a</sup> | 2.38 ± 0.04<sup>a</sup> | 1.90 ± 0.04<sup>a</sup> |
| Tetracosanol C24 | 27.38 ± 0.5<sup>a</sup> | 54.33 ± 0.5<sup>b</sup> | 67.48 ± 0.3<sup>c</sup> |
| Hexacosanol C26  | 21.94 ± 0.91<sup>a</sup> | 10.65 ± 0.84<sup>a</sup> | 3.50 ± 0.9<sup>a</sup> |
| Octacosanol C28  | 46.25 ± 0.31<sup>a</sup> | 28.44 ± 0.29<sup>a</sup> | 23.45 ± 0.45<sup>a</sup> |

Mean values with different letters within a row are significantly different at p < 0.05.
Values are presented as mean ± SD (n=3).

Table 5 Fatty acid (%) composition of wheat germ oil obtained with different methods.

|                  | Cold pressing | Methanol-chloroform | Soxhlet Petroleum ether |
|------------------|---------------|---------------------|-------------------------|
| C16:0            | 22.93 ± 0.52<sup>a</sup> | 24.19 ± 0.74<sup>a</sup> | 22.28 ± 0.69<sup>a</sup> |
| C18:0            | 1.25 ± 0.04<sup>a</sup> | 1.18 ± 0.04<sup>a</sup> | 0.95 ± 0.02<sup>a</sup> |
| C18:1            | 17.38 ± 1.27<sup>a</sup> | 15.86 ± 1.11<sup>a</sup> | 16.90 ± 1.20<sup>a</sup> |
| C18:2            | 52.19 ± 1.43<sup>a</sup> | 54.33 ± 1.38<sup>a</sup> | 57.48 ± 2.14<sup>b</sup> |
| C18:3            | 6.25 ± 1.20<sup>a</sup> | 4.44 ± 0.80<sup>a</sup> | 2.39 ± 0.91<sup>a</sup> |
| Saturated Fatty acids | 24.18 ± 0.50<sup>a</sup> | 25.37 ± 0.68<sup>a</sup> | 23.23 ± 0.56<sup>a</sup> |
| Unsaturated fatty acids | 75.82 ± 1.25<sup>a</sup> | 74.63 ± 1.12<sup>a</sup> | 76.77 ± 1.86<sup>a</sup> |

Mean values with different letters within a row are significantly different at p < 0.05. Values are presented as mean ± SD.

seed oil quality. High oleic-linoleic oils are of interest because their stability and nutritional propierties<sup>14</sup>. Linoleic and oleic acids are essential fatty acids and their consumption is important for human health. The fatty acid composition of the tested oil samples was nearly the same (Table 5). Linoleic acid was the main fatty acid (52.19-57.48%) followed by palmitic (22.24-24.93%) and oleic (15.86-18.8%) acids. The tested oil extracts were found to be rich in unsaturated fatty acids (73.82-76.77%) and low in saturated fatty acids (24.18-26.18%). Interestingly, linoleic acid level in cold pressed oil (6.25%) was higher than that in Soxhlet extract (2.39%), which may be due to thermal and oxidative degradation of this fatty acid. The fatty acid composition of the tested wheat germ oils is in agreement with those reported in the literature<sup>8</sup>. Numerous studies reported that the extraction method, temperature, and roasting process of germ did not change the fatty acid composition of wheat germ oil<sup>13,35,36</sup>. However, Soltan<sup>6</sup> et al. showed that the fatty acid profile of wheat germ was affected by the type of extraction technique.

3.7 In vitro anti-inflammatory activity

The different obtained extracts were evaluated for their

in vitro anti-inflammatory potential with proteinase and cyclooxygenase inhibitory assays. Figure 3 shows that all the tested extracts exhibited trypsin inhibitory activity in a concentration dependent manner. Among the tested oil samples, cold pressed oil exhibited the highest antitryptsin activity of 93.4 ± 0.6% at the concentration of 300 μg/mL and its IC<sub>50</sub> value (195.7 ± 0.2 μg/mL) was similar to the positive control diclofenac (190.3 ± 0.9 μg/mL). The percent inhibition of trypsin activity correlated strongly
with the total policosanol content ($r = 0.94$) but not with the total tocopherol content ($r = 0.38$). The trypsin inhibitory activity could be attributed to the high level of octacosanol in cold pressed oil. This bioactive compound could form complexes with the substrate in the assay (casein), resulting in trypsin inhibitory activity. Due to its hydroxyl group, octacosanol could also bind to the catalytic cavity of protease ensuing in the inhibitory activity. Similarly, Anyasor et al. found that anti-proteinase activity of *costus afer* leaf extracts was greatly influenced by the solvent type. Furthermore, positive correlation was observed between anti-proteinase activity and the total phenolic content of different methanolic plant extracts.

Cyclooxygenase (COX) inhibitors are the promising therapeutic target for treating various inflammatory related diseases such as arthritis. Results for COX1 inhibitory activities of the tested extracts are shown in Fig. 4. Among the tested oil samples, cold pressed oil exhibited the highest inhibitory effect against COX1 of 80.5% at the concentration of 500 μg/mL. Cold pressed oil displayed COX1 (IC$_{50}$, 58.6 ± 0.21 μg/mL) inhibitory activity similar to that of aspirin (55.4 μg/mL). The Soxhlet extract, which had the tetracosanol as the major policosanol compound and the highest level of tocopherol, did not display greater than 60% inhibition against COX1 at the concentration of 500 μg/mL. COX-1 inhibitory activity was highly correlated with the amount of β-sitosterol ($r = 0.86$) and with the total policosanol content ($r = 0.90$). In contrast, there were no correlation between COX inhibitory activity and the tocopherol content ($r = 0.45$). According to Akinloye et al., hydrophobic interactions favour selective inhibition of COX-1.

Based on its relatively high trypsin and COX1 inhibitory activity, the cold pressed oil was selected for the COX2 assay. The IC$_{50}$ value (92.2 μg/mL) of the cold pressed oil was similar to aspirin (90.7 μg/mL), but lower than the potency of Celebrex, a known COX-2 inhibitor (IC$_{50}$, 50.3 μg/mL). Cold pressed oil was found to have the highest content of β-sitosterol, octacosanol and α-linolenic acid, suggesting that those bioactive compounds could be essential for the potent anti-COX activity. These compounds have in their structure functional groups such as hydroxyl and carboxyl, capable of reacting with the amino or carbonyl groups of the amino acids of the enzyme to form inactive conjugates. These findings are in accord with the mechanism proposed by Akinloye, et al., where selective inhibition of COX-2 by phytosterols was observed and attributed to formation of hydrogen bonds as well to repression the expression of COX-2 mRNA. β-sitosterol and campesterol showed greater binding energies with COX-2 when compared with COX-1. Furthermore, Henry et al. reported that linolenic acid exerted appreciable COX inhibitory activity.

4 Conclusion

In summary, our results showed that the extraction procedure of wheat germ oil affected the content and efficacy of bioactive compounds. The cold pressed oil contained the highest levels of total policosanol and α-linolenic acid compared to the other extracts. However, the tocopherol content was higher in the oil extracted by Soxhlet method than in the oils obtained by cold pressing and methanol/chloroform methods. Furthermore, the cold pressed oil exhibited the highest anti-inflammatory potential which may be due to the synergistic effect of octacosanol, β-sitosterol and α-linolenic acid. The cold-press technique does not require an external heat or organic solvent, maintains the proprieties of the bioactive compounds, and preserves the high quality of the extract.

The results revealed promising anti-inflammatory proprieties of wheat germ oil. Cold pressed oil could be effective for the prevention or treatment of various inflammatory diseases. The present study provides the biochemical foundation for further *in vivo* studies and chemical analysis in order to potentially exploit these natural sources for medicinal health, nutraceuticals applications and functional food.

Acknowledgements

The authors thank Pr. Moncef Harrabi (INAT) for providing the germ wheat. They thank also Mejri Zied for the statistical analysis.

Competing Interests

The authors declare that they have no competing interest.
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