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Insights on traditional and modern oil extractions of wheat germ: Chemical and antimicrobial evaluation

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Abstract : Medicinal plants particularly with higher nutritional values are attracting the attention of both the pharmacological and nutritional affairs. In the present study, we extracted the wheat germ oil with cold press, hexane as well as supercritical carbon dioxide (SC-CO_2). All were chemically and biologically (antimicrobial) evaluated to investigate how far can the differential in fatty acid composition affect the biological properties. The most eminent result was recorded by SC-CO_2 oil. It was the only among the extracted oils that possessed moderate antibacterial and strong antifungal activities.
Keywords : Cold press extraction; Solvent extraction; Supercritical fluid extraction**;** Fatty acid composition; Wheat germ; Antibacterial; Antifungal.

Introduction

Medicinal plants particularly, with higher nutritional values are attracting the attention of both pharmacological and nutritional approaches. No doubts, editing therapeutic benefit to the consumed food resulting in health promotion.[1] In our daily life, we could deal with many nutrients. Of course, if they are accessible in their active forms do a great role of protecting our bodies from several attacker's diseases.

Wheat germ in its flaky form as well as its oil is popular among Egyptians. It is easily purchased from many herbal shops or local markets. It is well known that wheat germ oil is rich in bioactive components that are prone to degradation and oxidation under the conditions used for conventional processing methods.[2] There is a demand for the technological processing techniques that keep its quality and in turn the potential of biological effect. The supercritical fluid technology is complicated and expensive particularly in developing

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countries. However, it is an alternative superior technique to traditional cold press and hexane extractions to get the best benefits from this plant. Several studies have been reported the supercritical carbon dioxide (SC-CO_2) extraction of wheat germ oil.[3] However, as far as we know, the present study is the first that investigated the concerned oil by differential techniques. We extracted wheat germ oil by three techniques; cold press, hexane as well as SC-CO_2. All were subjected to both chemical and biological investigations**.** The obtained results were compared with respect to the chemical and antimicrobial approaches.

Experimental

Plant materials

Wheat germ was provided from the unit of pressing and extraction of natural oils, National Research Centre (NRC), Egypt.

Oil extraction techniques

Cold press, hexane solvent and SC-CO_2 were the methods for the wheat germ oil extraction.

Cold press (Hydraulic Press)

The wheat germ flakes were undergone hydraulic press without heating. We accomplished this type of extraction in the unit of pressing and extraction of natural oils, National Research Centre.

Supercritical fluid using carbon dioxide (SC-CO_2)

The modern technology of extracting oil represented by supercritical fluid using CO_2 (SC-CO_2) was applied in the present study. Oil can be extracted from the wheat germ by means of liquefied gases or supercritical fluids. The oils are recovered by lowering the pressure or increasing the temperature, or both. Supercritical carbon dioxide is ideally suited for food, cosmetics and pharmaceutical industries as it is nontoxic and nonflammable. Moreover, it can be removed easily from the oil as well as the meal.[4] In the present study, wheat germ was extracted under operating conditions of 40 ^{o}C, 300 bar and extraction time 2h.

Solvent extraction by hexane

It is generally accepted that seeds with oil contents of 6–8% is very difficult to press. [5] Normal hexane is the common solvent for oil extraction. Soxhlet n-hexane extraction was carried for 400 gm wheat germ. A rotary evaporator was used for the efficient removal of solvent (n-hexane), then after. The extracts were stored in a glass jars in the deep freeze (−20°C) pending for further analysis.

Gas chromatography (GC)

The fatty acid composition of the three extracted wheat germ oils was determined by gas chromatography (GC). The GC model 7890B from Agilent Technologies was equipped with flame ionization detector at Central Laboratories Network, National Research Centre, Cairo, Egypt. Separation was achieved using a DB-Wax column (60 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were carried out using helium as the carrier gas at a flow rate of 2.1 ml/min at a split-less mode, injection volume of 1 µl and the following temperature program: 50 °C for 1 min; rising at 25 °C /min to 175 °C; rising at 4 °C/min to 235 °C and held for 20 min. The injector and detector were held at 260 °C and 280 °C, respectively.

Antimicrobial investigations

Microorganisms

All extracted oils were screened against 8 microorganisms categorized as two from Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* 6633), two Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028), two yeast-like fungi (*Candida albicans*

ATCC 10231, *Candida tropicalis* ATCC 750) and two fungi (*Aspergillus niger* EM77(KF774181)), *Macrophomina phaseolina* NRRL A62743.

Antimicrobial Assay

The antimicrobial properties of tested oils were determined by the agar diffusion technique.[6] In brief, the sterile nutrient and Czapek's dox agar media were separately, inoculated, with 50µl cell suspension of selected microbes, poured into 10cm diameter petri-dishes. Preliminary experiments were performed to select the optimum concentration for the antimicrobial screen resulted in 10µl for wheat germ oil examination. So, 10µl from each extracted oil was separately, hold on filter paper disc (0.5cm diameter). Prior to incubation, all prepared discs were deposited on to the surface of inoculated agar plates and kept at 4°C for two hours. The latter condition favors diffusion over microbial growth to clearly detect the inhibition zone. 10µg/ml amoxicillin as trihydrate (Amoxicillin, Eipico) and 1mg/ml of clotrimazole (Candistan, ADCO) were used during the antibacterial and antifungal assays, respectively. The plates were finally, incubated at 35 ºC for bacteria and at 30 ºC for both yeast and fungi. The antimicrobial activity was expressed as the diameter of inhibition zone in mm.

Results and Discussion

The obtained yield of the wheat germ oil is of importance at the commercial level. In our study we recorded considerable amount of the oil that represented by 6.3 and 9.3% using the SC-CO_2 and hexane respectively, while the cold press resulted in only 2% collected oil. The obtained yield is comparable to the previous reports[5]. In addition, the main fatty acid compositions of the different extracted wheat germ oils were determined and displayed in Table 1 while the measured chromatograms are indicated in Fig 1, 2, 3. Oleic acid reached the highest amount in the SC-CO_2 oil (41.69%). In the other hand, its percentage in the cold press and hexane was 24.88% and 22.5, respectively. The palmitic quantity was closely in values within all three oils (18-20%). Finally, Linoleic acid displayed 57.80, 53.57, 39.73 with respect to hexane, cold press and SC-CO_2 oil, respectively. Actually, higher contents of monounsaturated fatty acids (MUFA), but lower from both polyunsaturated fatty acid (PUFA) and saturated fatty acids (SFA) is matching the international dietary recommendations of fatty acid ratio. In addition, limiting both PUFA intake and SFA was previously reported for the olive oil. [7] Accordingly, in our results the fatty acid profile represents the SC-CO_2 as the best extracted oil among the rested oils.

Table 1: Fatty acids composition of three different extracted wheat germ oil

Fatty acids	**% Fatty acids &Yield**		
	SC-CO_2	**Hexane**	**Cold press**
Palmitic acid (C16:0)	18.0	19.73	20.37
Oleic acid (C18:1)	41.69	22.47	24.88
11-Eicosenoic acid (C18:1)	ND	ND	1.18
Linoleic acid (C18:2)	39.73	57.80	53.57
Yield (%)	6.3	9.3	2

ND: Not detected

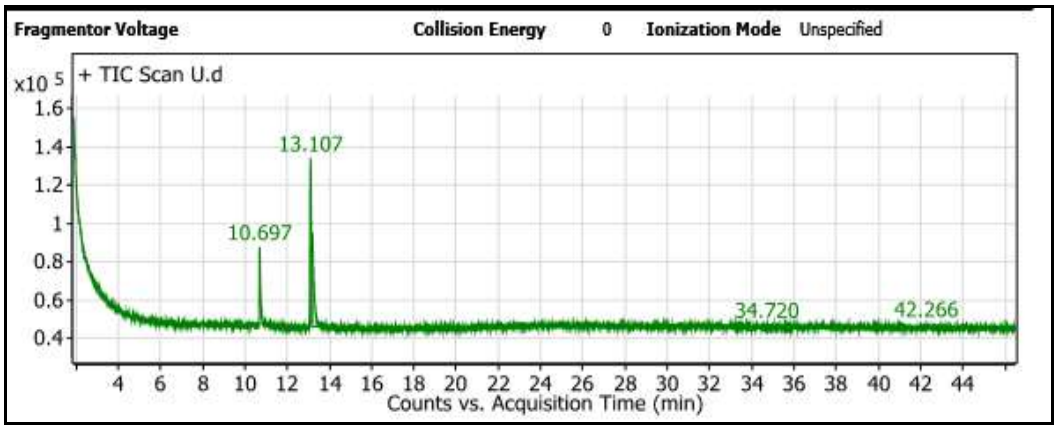

Figure 1: Analysis of fatty acids methyl esters of SC-CO_2 wheat germ extracted oil

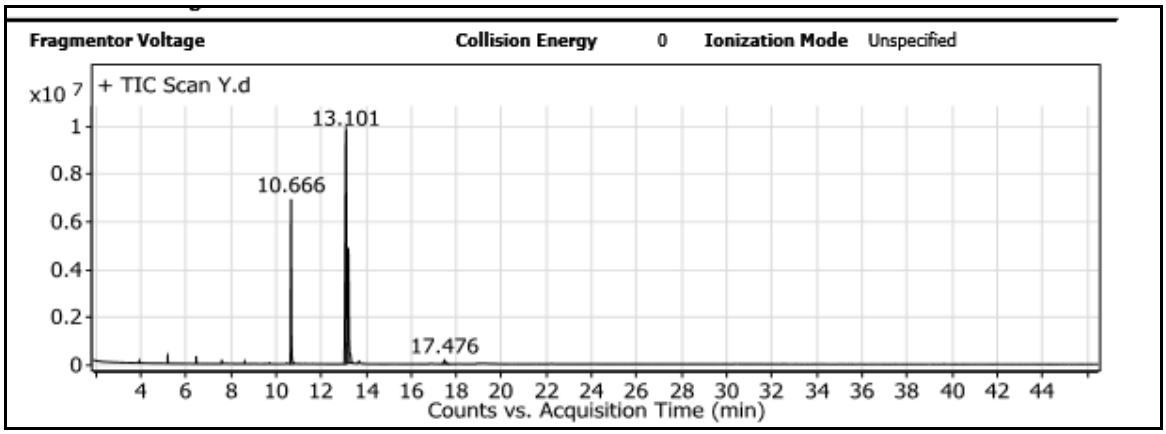

Figure 2: Analysis of fatty acids methyl esters of cold press wheat germ extracted oil

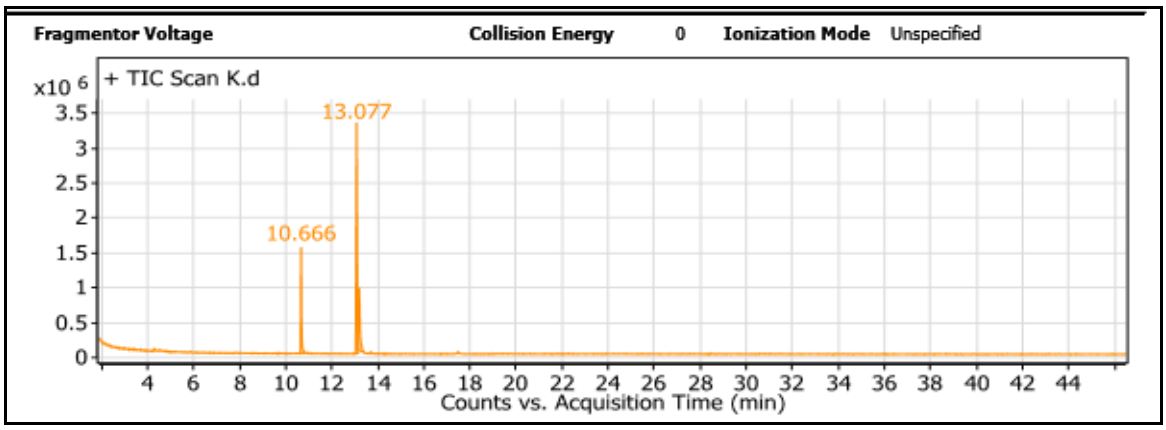

Figure 3: Analysis of fatty acids methyl esters of hexane wheat germ extracted oil

No doubt that the biological activity is the reflex of the oil quality. In the present study, we subjected all extracted wheat germ oils to the antimicrobial assay to evaluate the oil samples against a panel of eight microbes. The results are indicated in Table 2 and introduced SC-CO_2 oil as the only active against all microbes, except the negative bacteria. Together with the chemical profile, the latter technique, SC-CO_2 is considered of importance to obtain a bifunctional; nutrient and therapeutic wheat germ oil.

Table 2: Antimicrobial properties of Wheat germ oil regarding the methods of extraction

Microorganism	Inhibition zone (mm) [Mean ± SEM]			
	SC-CO_2	**Hexane**	**Cold press**	**Reference**
C. Albicans	16 ± 1.0	-	-	22.8 ± 0.7
C. tropicals	16 ± 0.0	-	-	22.2 ± 0.7
E. Coli	-	-	-	16.8 ± 0.4
S. typhimurium	-	-	-	16.2 ± 0.4
B. Subtilis	11 ± 0.5	-	-	16.0 ± 0.4
S. aureus	12 ± 0.0	-	-	19.0 ± 0.8
A. niger	23 ± 2.5	-	-	21.0 ± 1.1
M. phaseolina	23 ± 3.0			17.4 ± 0.9

The values are calculated from at least 2 independent experiments. The obtained results were recorded from 10 µl pure oil. SEM: Standard error of mean; SC-CO_2: Super critical fluid using CO_2; Reference: Antibacterial assay is 10 µg/ml amoxicillin trihydrate while the antifungal assay is 1mg/ml clotrimazole. -:No inhibition zone was recorded

Conclusion

The type of extraction technique is affecting the antimicrobial properties. Extraction by SC-CO_2 is the most important to keep the activity of the wheat germ oil.

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