

Review Article

Nutritional Composition, Extraction and Utilization of Wheat Germ Oil: A Review[†]**Kashif Ghafoor^{*1}, Mehmet Musa Özcan², Fahad AL-Juhaimi¹, Elfadil E. Babiker¹, Zaidul Islam Sarker³, Isam A. Mohamed Ahmed¹, Mohammed Asif Ahmed¹**¹ Department of Food Science & Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia.² Department of Food Engineering, Faculty of Agriculture, Selcuk University, 42031 Konya, Turkey³ Faculty of Pharmacy, International Islamic University Malaysia (IIUM), Kuantan Campus, Kuantan 25200, Pahang, Malaysia**Running title:** Nutritional components in wheat germ oil**Key words:** Wheat germ oil, extraction, composition, fatty acids, tocopherol, health benefits***Correspondence:** Associate Professor Dr Kashif Ghafoor, PO Box 2460, Department of Food Science & Nutrition, College of Food and Agricultural Sciences, King Saud University, Riyadh 11451, Saudi Arabia**E-mail:** kashif_ft@hotmail.com; kghafoor@ksu.edu.sa**Fax:** +966 11 4618394**Phone:** +966 11 4678403

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Abstract

Wheat germ is a by-product of wheat milling from which wheat germ oil (WGO) can be obtained using different techniques. For a better quality WGO, techniques such as supercritical fluid fractionation, molecular distillation and other innovative methods can be adopted. WGO is composed of nonpolar lipids, glycolipids, phospholipids, alcohols, esters, alkenes, aldehydes, tocopherols, n-alkanols, sterols, 4-methyl sterols, triterpenols, hydrocarbons, pigments and volatile components. The most abundant WGO fatty acid is linoleic acid which composes 42-59% of total triglycerides followed by palmitic (16:0) and oleic acids (18:1). The stearic acid, a saturated fatty acid, is usually less than 2%. WGO is rich in tocopherols particularly vitamin E. It contains α -tocopherol and β -tocopherol which gives various health benefits to it. It is being used in medicine, cosmetic, agricultural and food industry. Some of its applications include production of vitamins and food supplements, animal feed and biological insect control and for treating circulatory/cardiac disorders and weaknesses. More studies are required for producing better quality WGO such as application of more innovative and optimized techniques that can increase its health benefits and hence utilization. More mechanistic approaches for extraction, evaluation and utilization of WGO can help in making this by-product of wheat processing more valuable.

Practical Application

Wheat is a major food crop around the globe and produced and processed in large quantities. Its by-products such as wheat germ can be used to obtain value added products. Oil obtained from wheat germ is found to be a good source of various nutritionally beneficial constituents and carry important health benefits and functional properties. The review will help researchers to carry out further research to improve processing and the quality of oil besides emphasizing on its beneficial aspects. It will also assist in better utilization of this wheat by-product to develop value added products and nutraceuticals after carrying out further studies.

1. Introduction

Wheat is one of the most important food crops which is usually milled to produce flour and other products. Wheat germ (2-3% of grain) can be separated as a by-product during wheat milling. It is considered an important by-product and can be used in different applications such as food, pharmaceutical and other biological purposes [1]. Naturally, there exists a separation line between endosperm and scab of the germ due to which its separation from wheat grain is

considered easy [2, 3]. The embryo consists of two parts, the embryonic axis and the scutellum which is a storage organ [3, 4]. The germ contains high amounts of protein (25%), sugar (18%), oil (16% of the embryonic axis and 32% of the scutellum) and ash (5%). The wheat germ oil (WGO) is rich in phosphorus (1.4 g/kg), contains no starch but high vitamins B contents. Vitamin E can also be as high as 500 ppm [4, 5]. The longitudinal section of a wheat grain is shown in Figure 1 to demonstrate the general structure and location of germ in the grain. The general chemical composition of the commercially available wheat germ is shown in Table 1.

In another study it was stated that germ constitutes about 2.5-3.5% of the kernel and its oil contents range between 10 to 15% [4], of which 2-6% are unsaponifiable matters [6], containing mainly phytosterol and tocopherol [7]. The tocopherol content of WGO is the highest among vegetable oils; can reach up to 2500 mg/kg and α -tocopherol (60%) is predominant [8]. Phytosterols (mainly sitosterol and campesterol) contents of WGO are also generally higher than other commercial oils [7]. It is also contains high levels of unsaturated fatty acids of which 80% is linolenic and linoleic acids. The consumption of these fatty acids is important for human health as they are considered as essential (cannot be synthesized in the organism). They also help in synthesis of a group of hormones called prostaglandins that are important for their role in muscle contractions and for anti-inflammatory properties [8]. WGO, due to its bioactive compounds, is associated with different nutritional and health benefits such as reduction of plasma and liver cholesterol levels, improving physical endurance and delaying aging effects. Some of the positive health effects are also due to polycosanols and especially octacosanol contents of WGO [7]. The higher amount of vitamin E acts as stabilizer for cell membrane by protection of unsaturated fatty acids from peroxidase cleavage [6]. WGO is also used in foods, biological insect control agents and pharmaceutical formulations [9]. The aim of current review is to discuss physico-chemical, compositional and bioactive properties of WGO so as to promote better utilization in formation of different value added products and further research. The review also discuss the current and potential utilization of WGO.

2. The Characteristics, Composition and Bioactive Contents of Wheat Germ Oil

2.1. Physico-Chemical Properties

The values for refractive index, specific gravity, iodine value, saponification value and unsaponifiable matter of WGO are 1.4700-1.4800, 0.9000-0.9300, 120-130, 184-185 and 1.5-7.8, respectively. The free fatty acid content is usually lower than 60 g/kg if oil is stored

without stabilizing. The free fatty acid content of solvent extracted WGO is lower than that obtained by pressing. The free fatty acid content also depends on oil refining process [10, 11]. The lipase enzyme produce free fatty acids by converting triglyceride into diglyceride and monoglyceride [12]. Megahed [12] carried out a study about lipase activity of WGO during periodical storage and observed that the acid value of oil gradually increased and reached up to maximum of 23.46 (mg/g) by comparison with initial value (14.88 mg/g). However, during lipase inhibition at 70°C for 15, 30 and 60 minutes, the acid value decreased from 14.88 mg/g to 12.02 mg/g after 60 minutes. It was determined that the acid value reached to a suitable value by repressing of enzyme activity at 70°C for 30 minutes. Jha et. al [13] studied inactivation of lipase enzyme in WG using γ -irradiation and observed 16.75% lipase inhibition at 12 kGy radiation dose which was increased up to 31.2% at 30 kGy dose. The total lipase inactivation could be achieved at radiation doses above 35 kGy that can enhance the shelf life of WG products. Other treatments have also been studied in relation to improving the shelf life of WG which include treatments using antioxidants, infra- red, epoxy compounds, moisture reduction and microwave [14-17]. However these treatments may negatively affect the nutritive value of WG products. Hence, selection of a suitable method for enzyme inactivation and germ stabilization might be important for commercialization of WGO and other WG products. The constituents isolated from WGO and identified are alcohols, esters, alkene and aldehydes. The volatile compounds present in oil are responsible for the flavor of oil and play their role in consumer preference. The main volatile components are hexanal (15.97%), 2-methyl-2-butene (10.43%), 2,4-heptadienal (8.53%) and limonene (6.83%) [18]. Due to the presence of polyunsaturated fatty acid, mineral and elemental contents in high quantities, WGO is dietetically valuable. The nutritionally important macro and micro elements constitute a substantial part of oil composition [19]. Niu et al. [18] studied the thermal behavior of WGO and found that the values for onset temperature of melting, the peak temperature and ΔH were -29.03°C, -14.61°C and 21.54 J/g, respectively. While the weight loss substantially occurred in the 280-500°C range, the complete weight loss occurred at 516.02°C. The oxidative stability is important for WGO as for all other oils. Niu et al. [18] specified the oxidative induction time of WGO which was 3.81 h. The oils being rich in unsaturated fatty acid are easily soluble, prone to oxidation and can become rancid. WGO melts easily, however it is more stable due to its high amount of tocopherol compared to the oils with high amounts of unsaturated fatty acid. The chemical composition with reference to different individual constituents of WGO is discussed in more details in subsequent sections of this review. Some of these components have significance for establishing utilization of

WGO as quality food product or in the development of nutraceuticals having medicinal importance.

2.2. The Fatty Acid Composition

The free fatty acids cause bitter and soapy flavor in foods [5]. Table 2 shows the average fatty acid composition of WGO. The fatty acid composition of WGO may change depending on wheat variety, germ characteristics (maturity, rancidity, quality etc.), separation method, storage and extraction conditions. WGO is rich in polyunsaturated fatty acids that constitute 80% of triglycerides. The most abundant fatty acid is linoleic acid (18:2) which composes 42-59% of total triglycerides followed by palmitic (16:0) and oleic acid (18:1). The stearic acid, a saturated fatty acid, is usually less than 2%. In general palmitic (13-20%), stearic (2%), oleic (14-23%), linoleic (51-60%) and linolenic (4-12%) compose fatty acid contents of WGO. The different fatty acid profiles and high level of monounsaturated fatty acid concentrations were determined for spelt and einkorn [5, 10, 11]. A study [19] about germ oil extracted from wheat crops cultivated in Turkey showed that the oil contained 56.1 % linoleic acid, 17.4% palmitic acid, 17.1% oleic acid. In a study [20], it was observed that WGO contains 56.5 % linoleic acid, 20.0% palmitic acid and 14.7% oleic acid in WGO. Özcan et al. [8] evaluated quality of WGO obtained by cold pressing and supercritical carbon dioxide extraction and found that the percentages of palmitic, oleic, linoleic and linolenic acids were 15.89%, 15.48%, 54.88% and 7.34% of total fatty acids (cold pressed oil) and 16.50%, 15.05%, 54.79% and 7.29% of fatty acids (supercritical carbon dioxide extracted oil), respectively. The percentages of saturated, mono and polyunsaturated fatty acids were 17.15%, 17.63 % and 62.22% of total fatty acids in the cold-pressed oils, but the proportions of those in oils extracted by supercritical carbon dioxide were 18.14%, 17.58% and 62.08%, respectively. It was observed that the fatty acid profiles of both oils studied were rather similar. The high amount of 18:3 fatty acid makes oil sensitive to oxidative rancidity, however the high level of polyunsaturated fatty acids in WGO is important for human health and can be considered as a valuable property [10] for utilization in food products when the objective is to reduce the presence of saturated fats.

2.3. Lipids

The lipid contents of wheat germ depends on extraction index of the flour and around 25% of those are linked to starch in flour and the rest of lipids (75%) are not linked to starch. These lipids involves nonpolar lipids, glycolipids and phospholipids in different proportions [21]. Much of the reported data for acyl lipid composition of wheat germ are about oils extracted by polar solvents. The total polar lipid content of five commercial oils consisting of almost

completely nonpolar lipids was 0.2-1.8% and the polar lipid content of oil obtained by Soxhlet hexane extraction was 3.6-10.1% [10]. The class of nonpolar lipids involves 4-6% steryl esters, 82-89% triglycerides, 6% free fatty acids, 4-11% diglycerides and 1% monoglycerides [11]. Table 3 shows nonpolar acyl lipid composition of laboratory-extracted WGO. Triglycerides are main components in all oil samples. The differences in the level of hydrolytic rancidity result from variation in the proportion of free fatty acid and that can depend on refining in the commercial oils [10]. The ceramides and glycoceramides being present in wheat can be extracted. The ceramides which are sphingosines N-acylated with a fatty acid are copious in the skin. The ceramides of wheat can be an alternative to animal ceramides [21].

2.4. Unsaponifiable Lipids

WGO has very high nutritional value and contains many bioactive components. It involves 4.16% unsaponifiable matter and tocopherol contents reach up to 319 mg/100 g [19]. The unsaponifiable fraction of WGO composes of mainly 4-dimethyl sterols and a trace of methyl sterols, triterpenols, tocopherols, n-alkanols, carotenoids and hydrocarbons. Niu et al. [18] determined five different unsaponifiable matters in the WGO when the mass spectra analysis results were examined. Those were squalene, cholesterol, campesterol, β -sitosterol and fucosterol, β -sitosterol being the major (64.64%) sterol. Despite being minor components of unsaponifiable fraction, tocopherols and n-alkanols are commercially important [10]. Certain sterol which constitutes a major part of unsaponifiable matter is one of the substantial bioactive components in WGO, and it has an effect on lowering blood cholesterol and reducing the prevalence of cardiovascular disease [13]

2.5. Tocopherols

The cereal germ oils are important source of tocopherols. WGO is rich in vitamin E, which is a type of tocopherol and is composed of a chromanol nucleus and a saturated lateral chain of 16 carbons. There are different forms of tocopherol (α , β , γ , δ) depending on the number and location of the methyl groups in the chromanol nucleus. The antioxidant activity of these tocopherols increases from α to δ , whereas the vitamin activity and the incidence of reaction with peroxide radicals reduce [21]. The amounts of tocopherols in per kilogram of wheat germ of WGO are about 1179, 398, 493 and 118 mg of α , β , γ and δ -tocopherols, respectively [6]. The main tocopherols of WGO are α -tocopherol (α -T) and β -tocopherol (β -T), however α -tocopherol content was much higher in WGO extracted by supercritical carbon dioxide extraction technique (1.27 mg/g) than the one extracted by cold pressing (0.79 mg/g) [10]. Eisenmenger and Dunford [7] found not effects of extraction and refining methods on fatty

acid composition. They however observed that tocopherol content was higher in supercritical carbon dioxide extracted oil than one obtained commercially using hexane. It was reported that while the conventional chemical oil refining technique reduced the tocopherol content in WGO, physical refining such as molecular distillation enhanced its concentration. The total tocopherol content varies in the range of 2000-3000 mg/kg. The obvious variation between values of tocopherol content and percentage composition depends on analytical procedures, extraction methods and the purity and origin of germ [22]. The tocopherols and tocotrienols contents of WGO are shown in Table 4. The WGO also contains, in addition to α -, β -, γ - and δ -tocopherols, smaller quantities of 5,8-dimethyl tocopherol, 7-methyl tocotrienol and 5,7-dimethyl tocotrienol [23]. The presence of good quantities of tocopherols in WGO is an important property as it can not only be beneficial for the prevention of lipid peroxidation it can also be a source of nutraceuticals development as it improves cellular defense against free radicals [23].

2.6. n-Alkanols

The n-Alkanols are present in the unsaponifiable WGO fraction and the major ones are octacosan-1-ol and triacontan-1-ol. It was observed that unrefined, pure, solvent extracted oil contained 80 mg/kg octacosan-1-ol and that this concentration was eight times greater than found in partly-refined oil and pressure-expelled oil [10]. Octacosanol is principal active component of polyicosanol. It is a long carbon chain ($\text{CH}_3(\text{CH}_2)_{26}\text{CH}_2\text{O}_{14}$) and a natural mixture of high molecular weight alcohols. WGO extracts showed beneficial effects on the physical performance of athletes and it was observed that octacosanol was the main WGO constituent producing this health effect [24].

2.7. Sterols, 4-methyl sterols and triterpenols

Sitosterol (60-70% of total sterols) is the main WGO sterol followed by campesterol (20-30% of total sterol). Table 5 shows the composition of sterol, 4-methyl sterol and triterpenol fractions. Gramisterol is present in germ oils of both wheat and maize in high quantity. WGO carries higher proportions of β -amyirin [10].

The unsaponifiable matter in WGO is composed of some minor components in addition to triacylglycerols which include sterols, tocopherols, lipopigments and hydrocarbons. The antioxidant activity of tocopherols has been previously established whereas other such as sterols and pigments may also prevent lipid peroxidation [25].

2.8. Hydrocarbons

The WGO hydrocarbons are squalene, 22-n-alkanes, eight branched and cyclohexylalkanes however, there are some disagreements between published data about the proportion of squalene and composition of alkanes [11]. There are two detailed studies about WGO hydrocarbons one of which [22] reports that squalene constitute 50% of hydrocarbon fraction and the remainder composed of mainly n-C₂₉ and alkanes. In the other study, squalene could not be determined and the carbon number distribution of the alkanes spread in the wide range till maximum n-C₂₄. The hydrocarbons are minor constituents of WGO and the composition can be affected by contamination with mineral oil while processing of the germ and germ oil [10].

2.8. Pigments

Bowden and Moore [26] crystallized the pigment which was responsible for WGO color and determined that the spectrum was incident to xanthophyll. The concentration of that pigment was 60 mg/kg in the oil. Later, xanthophyll, lutein and cryptoxanthin were determined in WGO. It is known that the carotenoids in WGO involves 71-88% xanthophyll, 2-17% xanthophyll esters and 10-12% carotene. In a study about determination of total carotenoid content of WGO by measuring the spectrophotometric absorbance value at 440 nm, carotenoid content of laboratory-extracted oils was found to be higher than that of commercial oil samples. The reason of low carotenoid content of commercial oils was oxidation happening during pressing or refining. Not only carotenoids but also flavonoid glycosides contribute to the yellow color of wheat germ [10, 22].

Plant pigments such as carotenoids are efficient antioxidants capable of eradicating singlet molecular oxygen and peroxy radicals. They can play an important role in antioxidant defense system in human body as they establish synergistic relation with other antioxidants [27].

2.9. Other nutrients

Wheat germ is a good source of other vitamins, mineral and nutrients including A, B1, B3, B5 and B6 vitamins, folic acid, riboflavin, thiamin, magnesium, iron, zinc, potassium, fibre, phosphorous and calcium. Micronutrients such as iron, zinc, potassium, vitamins are deficient in diets of major global populations. Besides the demand for foods that in addition to containing traditional nutrients can also supply other compounds capable of supplying additional benefits for consumer health and well-being is increasing and wheat germ and products containing it can be one such choice [28]. Hence the presence of various nutrients and potential bioactives in WGO makes it a strong candidate for development of natural medicines and nutraceuticals.

3. Extraction of Wheat Germ Oil

WGO is extracted from germ commercially using mechanical pressing or solvent extraction. Although it is possible to avoid organic solvent contamination when using pressure expulsion techniques, however it recovers only half of oil present in the germ. The solvent extraction method is more effective as 99% of oil can be recovered. The solvents used for WGO extraction are hexane, 1, 2-dichloroethane and ethanol. The catabolism of nutritionally important components of WGO can be minimized by avoiding higher extraction temperatures and by using techniques such as cold pressing and supercritical carbon dioxide assisted extraction [8]. These techniques either avoid or reduce the use of extraction temperature that might be detrimental of heat sensitive nutrients [13]. The oil yield is 5.2-15% using petroleum hydrocarbon solvents or diethyl ether during extraction whereas it may increase slightly (7.2-15.5%) with use of more polar solvents. The differences between these values results from oil loss during milling and limitations of some solvent extraction methods. Mechanical pressing, aqueous and enzymatic techniques have also been used for WGO recovery. Mechanical procedures may yield solvent-free oil however yield may be lower than that from hexane extraction. Seed moisture and other operating conditions in respective extraction methods can significantly affect oil yield. Seeds with too low moisture cannot be freed from oil whereas high moisture can result in slippage of the material in the press. The optimum moisture for seeds varies depending on the type of seed and the method used for extraction such that a maximum oil yield (37%) can be obtained at 1.5% moisture content of germ by using a germ oil press [5, 10, 11, 17, 29]. The WGO yield can be 92% during extraction with solvents when innovating approaches such as supercritical carbon dioxide extraction is applied which may also reduce the use of toxic solvent. Hence, taking into account, the effects of various extraction methods and the respective extraction variables is crucial for WGO quality and its industrialization. The oil content is also affected by the bran and endosperm contamination levels, oil contents of these are lower than that of embryonic axis. Losses also occur during peeling [5, 10]. Dunford and Zhang [9] obtained oil by using pressurized solvent extraction and soxhlet method and observed that the yield was higher when ethanol was used in pressurized solvent extraction whereas the oil yields by soxhlet and pressurized solvent extractions were similar while using hexane. The crude WGO, which is usually dark amber-colored clear liquid, has strong odor and flavor depending on oxidative conditions [10, 30].

3.1. Refining Process Loss

Tocopherols and other nutritional components should be protected as the undesirable components must be removed to produce high-quality WGO. Significant amounts of nutritional components are also lost during conventional refining such as deodorization causes appreciable decrease in tocopherol content. The free fatty acids are eliminated from WGO by alkali application, but this application cause substantial loss of oil and tocols. Therefore, alternative ways are proposed for oil refining such as supercritical fluid fractionation and molecular distillation [5, 10]. Wang and Johnson [31] studied the effects of degumming, neutralization, bleaching and deodorization processes on quality of refined WGO. It was determined that the crude oil contained 1.428 ppm phosphorus, 15.7% free fatty acid, 2.682 ppm tocopherol and its peroxide value was 20 meq/kg. The degumming process did not cause significantly phosphorus loss and neutralization process had an impact on eliminating phospholipids. There wasn't considerable alteration in total tocopherol content during degumming, neutralization and bleaching processes. When deodorization was performed at high temperature and for long time, free fatty acid content, peroxide value and red color of oil decreased. There was no significant decrease in tocopherol content during deodorization at 250°C for 9 minutes. However, a considerable decrease occurred during deodorization at 290°C for 30 minutes. Hence, high quality WGO was produced using a modified standard oil refining processes as explained here.

4. The Functional Activities and Utilization Areas of Wheat Germ Oil

In addition to utilization of WGO in medicine, cosmetic industry and vitamin production, it is also being used in foods, feeds, biological insect control and for treating circulatory and cardiac disorders and weaknesses [5, 21]. WGO is sold in bottled or capsule forms currently and some of its preparations also include food supplement for livestock, racehorses and pets. It can be in mixture forms along with lecithin, fish oil and shampoo formulations. Being rich in α -tocopherol it is readily available. Vitamin E activity of tocols is high and vitamin E is regarded as essential nutrient for human health [10].

Cosmetic use of WGO is due the fact that it contains ceramides which prevent or slow down skin ageing. The required enzyme for production of ceramides is sphingomyelinase and the decrease of its activity is associated with skin ageing. Ceramides can protect and moisten skin and as vitamin E also has moisturizing and soothing effects on skin, the moisturizing activity of ceramides can be increased by incorporating WGO being rich in vitamin E [21]. The decrease in level of essential fatty acids causes thickening of the epidermis. Due to deterioration of the epidermal barrier function depending on fatty acids deficiency, the high

level of water loss from stratum corneum occurs. The local applications of these acids help in skin protection and renovation. The soothing the skin and reducing the trans-epidermal water loss effects of local applications of linoleic acid and its polyunsaturated derivatives were proved by different clinical tests [21].

The neutrophil elastase which is a serine protease, plays a crucial role in the inflammatory processes and alteration of the connective tissue components [21]. It can be inhibited by long-chain fatty acids and their derivatives [32]. Ceramide protects extracellular matrix against leukocyte proteinases which cause degradation, by inhibiting this elastase, so it is a good anti-inflammatory agent. The anti-inflammatory activity is result of effect against formation of the lipoperoxide. Its local applications show anti-oedema and anti-erythema effects by preventing the synthesis of the inflammation mediator prostaglandins from arachidonic acid [21]. Also, because of its tocopherol (1300-2700 mg/kg) and carotenoid (56 mg/kg) content, it is a good antioxidant and plays an important role in disease prevention [5]. Ferulic acid esterified with dihydro- γ -sitosterol is another natural antioxidant present in WGO [23]. Megahed et al. [33] deduced that WGO and acylated germ oil was natural antioxidant and a source of nutraceuticals. The antioxidants significantly reduce free radicals which cause cell alterations, photo-induced immunosuppression, and lipid peroxidation increase in skin exposed to UV radiation. It was demonstrated that different derivatives of vitamin E had activity against the production of free radicals, for example 0.5% of vitamin E acetate decreases the production of free radicals at the rate of 23.9%. This activity is protective against both erythema and immunosuppression [21]. Malonyldialdehyde (MDA) which is a reaction product of lipoperoxides, conduces to ageing by putting in collagen fibres of the connective tissue and reducing the elasticity of the skin. In a study, the irradiation with UV-B (290-320 nm) induced the formation of lipoperoxide and free radicals and a raise in the MDA levels of the skin was determined after irradiation. In the later study, mice were exposed to 5% solution of vitamin E acetate before UV-B irradiation and the formation of MDA decreased at the rate of 40-80%. According to these results, it was demonstrated that vitamin E inhibited to the formation of free radicals in the skin [21].

Due to higher vitamin E contents, WGO has an accelerator effect on the venius and arterial microcirculation, helps in the blood flow in vessels and eases the decongestion. Due to the fact that WGO stimulates the microcirculation of the scalp and cures of dystrophic cells in the hair bulb. It plays an important role in hair loss prevention. Improved blood circulation in skin can results in better supply of nutrients to the skin and the removal of catabolites resulting

from skin metabolism is improved by means of protective activity of vitamin E on the membrane lipids at the blood vessels [21].

WGO contains two groups of alcohols causing cholesterol lowering effects: policosanols and phytosterols. The detected policosanols are docosanol (C22), tetracosanol (C24), hexacosanol (C26), octacosanol (C28) and triacontanol (C30). The amount of phytosterols in wheat germ oil is much more than that in other commercial oils and sitosterol (60-70%) and campesterol (20-30%) are also mostly present in WGO [5]. Octacosanol has many beneficial effects on many areas about human health such as especially exercise performance, platelet aggregation and plasma cholesterol levels. Because of its ergogenic properties and cholesterol-lowering effects, there are many studies about it. Octacosanol can be an important drug in future for reducing obesity and cardiac diseases [24].

The extracts prepared from WGO has tendency to reduce 2, 2-diphenyl-1-picrylhydrazyl or DPPH free radicals and this tendency is comparable with those of synthetic antioxidants such as butylated hydroxytoluence (BHT) and butylated hydroxyanisole (BHA) [25]. The antibacterial activity of WGO extracts have also been tested against pathogenic bacteria and it was observed that *L. monocytogenes* and *S. aureus* were the most sensitive strains, followed by *E. coli*, and *S. enterica* [34]. It has been observed that WGO is an excellent source of natural antioxidants, bioactives and antimicrobial compounds. Therefore it also has a strong potential for application in the formulation of nutraceuticals, functional and other food products due to its unique set of various biological activities and health properties. In a study [35] cookies containing WGO were fed to experimental rats to monitor their lipid profile and it was observed to reduce lipid peroxidation which can be a useful tool for reducing heart related health problems. Some other typical applications of WG and WGO include those in germ-enriched bread, cakes, snack foods and supplements to breakfast cereals [36]. In an interesting study [37] an infusion of Calendula flowers was prepared into black seed oil and WGO and studied for *in vitro* cell based experiments (wound healing and radio protective activity) which showed a greater bio-activity and hence WGO can be a potential ingredient in food supplements which have the therapeutic efficiency. The various functional and nutraceuticals attributes associated with WGO and its constituents discussed here are summarized in Table 1.

5. Conclusions

Wheat germ is a by-product of wheat milling and can contain around 10-15% oil which can be separated either by mechanical or chemical means. Conventional refining may reduce

nutritional quality of the oil and hence alternative procedures such as supercritical fluid fractionation and molecular distillation may be adopted. The characteristics and composition of WGO have been discussed in detail in here. It contains low free fatty acids. Alcohols, esters, alkene and aldehydes have been identified in the oil. Hexanal, 2-methyl-2-butene, 2,4-heptadienal and limonene have been identified as volatile components that may be responsible for flavor and hence consumer preference. Despite containing unsaturated fatty acids, WGO is stable due to high amount of tocopherol. The most abundant fatty acid is linoleic acid which composes 42-59% of total triglycerides in WGO followed by palmitic (16:0) and oleic acid (18:1). The stearic acid, a saturated fatty acid, is usually less than 2%. WGO is rich in tocopherols particularly vitamin E. WGO contains α -tocopherol and β -tocopherol, however α -tocopherol content can be increased by supercritical carbon dioxide extraction (1.27 mg/g) instead of using cold pressing (0.79 mg/g). The color of WGO is mainly contributed by carotenoids and some extent by flavonoid glycosides.

WGO is being used in medicine, cosmetic, agricultural and food industry. Some of its applications include production of vitamins and food supplements, animal feed and biological insect control and for treating circulatory/cardiac disorders and weaknesses. More studies are required for producing better quality WGO such as application of more innovative and optimized techniques for increasing its utilization after exploring more health benefits. A mechanistic approach can help in producing a valuable product from a by-product of wheat processing. Some of the functional and therapeutic uses of WGO and its constituent have been discussed and highlighted in this review. More research work is required in order to make maximal benefits from this important by-product for identification and development of potential nutraceuticals and functional foods. Such studies may include isolation and purification of pure bioactives from WGO and systematic *in vitro* and *in vivo* biological studies using such compounds.

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Table 1. The composition of partly-dried commercial wheat germ [10]

Component	Content (g/kg)
Protein	260
Starch	200
Sugar	160
Fat	100
Water	60
Ash	40
Crude fibre	30
Others	150

Table 2. The average fatty acid composition of wheat germ oil (oil content of wheat germ is %8-11 by weight) [8, 21, 38, 39, 40]

Fatty acid	Percent of total fatty acid
Palmitic acid (16:0)	11-17
Stearic acid (18:0)	0.6-3.6
Oleic acid (18:1)	14-25
Linoleic acid (18:2)	49-60
Linolenic acid (18:3)	4-10

Table 3. The nonpolar acyl lipid composition (%age of total) of wheat germ oil [10]

Lipid class	Laboratory-extracted oils	Commercial oils
Steryl esters	5.1-5.8	1.9-5.7
Triglycerides	63.9-88.5	76.3-94.9
Free fatty acids	0.6-22.0	0.7-8.1
Diglycerides	1.8-6.9	2.6-10.9
Monoglycerides	0.3-1.1	0.1-0.7

Table 4. The tocopherols and tocotrienols of wheat germ oil (average composition stated as mg/100 g) [7, 20, 21, 31, 41]

Tocol	mg/100 g
α -T	133.0-256.0
α -T-3	300-700
β -T	60-680
β -T-3	250-360
γ -T	6-700
γ -T-3	-
δ -T	27.1
δ -T-3	-

Table 5. Composition of sterols, 4-methyl sterols and triterpenols in wheat and wheat germ oil (expressed as a percentage) [7, 11, 40, 42, 43]

Lipid ^b	Whole Wheat ^a		Wheat Germ Oil		
	Steryl Esters	Sterols	Unsaponifiable Matter ^c		
Sterols					
β-sitosterol	71.8	65.6	60	67	64
Campesterol	22.8	26.0	19	22	29
Δ ⁵ -avenasterol	2.9	3.6	7	6	2
Δ ⁷ -avenasterol	0.5	0.5	2	2	2
Δ ⁷ -stigmasterol	<0.1	1.0	2	3	1
Stigmasterol	2.0	3.0	4	trace	1
Brassicasterol	trace	<1
Cholesterol	<0.1	0.3	Trace	trace	...
4-methyl sterols					
Gramisterol	38.5	35.2	25	41	41
Citrostadienol	25.8	18.9	30	46	38
Obtusifoliol	11.0	20.0	14	6	7
Cycloeucalenol	6
24-ethyl lophenol	5
Lophenol	2.8	0.4
Others	21.9	25.5	20	7	14
Triterpenols					
24-methyl cycloartanol	...	19.0	33	44	27
Cycloartenol	25	17	17
Cycloartanol	...	9.2	...	3	5
Cyclobranol	1.0	8.2	2	2	6
α-amyrin	32.5	21.0	7	8	13
β-amyrin	66.5	32.1	12	18	22

^a The proportions in steryl esters (like sterol); 85% sterol, 3 4%-methyl sterol and 12% triterpenol.

^b Data for per lipid group in per column are equal about 100%.

^c The amounts in first column are 1.425 for sterols, 59 for 4-methyl-sterols and 59 mg/100 g oil for triterpenols. The proportions in second column are %81 of unsaponifiable matters for sterols, 5% for 4-methyl sterols, 7% for triterpenols and 7% for hydrocarbons. The proportions in third column are 35% of unsaponifiable matters for sterols 17% for 4-methyl sterols, 9% for triterpenols and alkanols, 7% for hydrocarbons and 18% for tocopherols.

Table 6. Summary of some of the functional properties of WGO extracts and its components

Constituent of WGO	Functional uses/Biological properties
Tocopherols/Vitamin E and its derivatives	Lipid peroxidation, Skin moisturizing, free radical scavenging, activity against erythema and immunosuppression, improvement in venous and arterial microcirculation
Octacosanol	Improve physical performance
Carotenoids	Free radical scavenging
Vitamins (A, B1, B2, B3, B5, B6)	Nutritional and health benefits
Micronutrients (iron, zinc, potassium, magnesium)	Nutritional and health benefits
ceramides	Prevent skin ageing, free radical scavenging, anti-inflammatory activity
Linoleic acid and polyunsaturated derivatives	Skin soothing and moisturizing
Ferulic acid esterified with dihydro- γ -sitosterol	Free radical scavenging
WGO or its crude extracts	Antibacterial activity, prevent cell alterations, photo-induced immunosuppression and lipid peroxidation prevention, prevention of hair loss, use in functional food development, radioprotective and wound healing property
Policosanols, octacosanol and phytosterols	cholesterol lowering effects, prevent platelet aggregation, improve physical performance

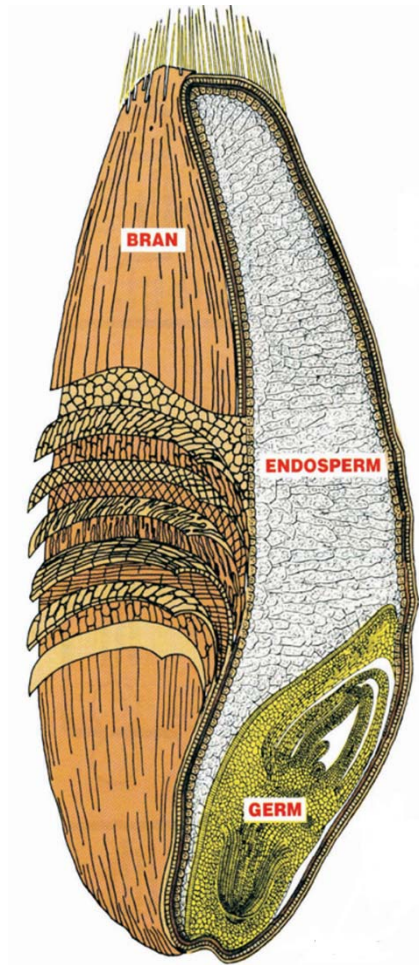


Figure 1. The longitudinal section of wheat grain showing bran (the outermost part of the kernel which contains fibre and is good source of B vitamins), endosperm (contains mostly starch, proteins, minerals and some vitamins) and germ (the embryo or sprouting section of the seed which contains oil and is a rich source of vitamin E and complex B vitamins).