J Am Oil Chem Soc (2009) 86:1175–1179
DOI 10.1007/s11746-009-1463-7

ORIGINAL PAPER

Characteristics of Oil from Hulless Barley (*Hordeum vulgare* L.) Bran from Tibet

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Received: 14 June 2009 / Revised: 4 August 2009 / Accepted: 6 August 2009 / Published online: 23 August 2009

Abstract The bran of hulless barley (*Hordeum vulgare* L.) from Tibet was investigated. This paper reports on the physicochemical characteristics, lipid classes and fatty acids of the oil from the bran. The petroleum (60–90 °C) extract of hulless barley bran was found to be 8.1%. The investigated physiochemical parameters included density at 40 °C (0.96 g/cm^3), refractive index at 40 °C (1.41), melting point (30.12 °C), acid value (11.6 mg KOH/g), peroxide value (19.41 μg/g), saponification value (337.62 mg KOH/g), iodine value (113.51 mg iodine/g) and unsaponifiable matter (4.5% of total lipids).The amount of neutral lipids in the crude oil was the highest (94.55% of total lipids), followed by glycolipids (4.20% of the total lipid) and phospholipids (1.25% of the total lipid). Linoleic acid (75.08% of total fatty acids) followed by palmitic acid (20.58% of total fatty acids), were the two major fatty acids in the oil. The results show that the oil from the hulless barley bran could be a good source of valuable essential fatty acids.

Keywords Hulless barley bran · Oil · Physicochemical parameters · Fatty acids composition

Introduction

At present, a large quantity of oils and fats, whether for human consumption or for industry purposes, is derived from plant sources. The characteristics of oils from different sources depend mainly on their fatty acid compositions, and no oil from a single source can be suitable for all purposes. This necessitates the search for new sources of novel oils.

Barley (*Hordeum vulgare* L.), one of the earliest cultivated cereal grains, is grown in large quantities around the world [1]. According to the grain type, barley is classified into hulled barley and hulless barley. At present, most barley varieties are of the hulled form, and they are mainly used for brewing malt and animal feed. By contrast, hulless barley is produced on a small scale and used mainly as human food because of the ease in processing and edibility [2]. In recent years, hulless barley has received increased attention as a health cereal for human consumption, due to its high nutritional value and abundance of dietary fiber [3–6]. Lupton et al. [7] reported that supplements of barley bran flour or barley oil enhanced the cholesterol-lowering effects of the National Cholesterol Education Program step 1 diet in individuals with hypercholesterolemia. Several studies have been reported on the content and composition of barley lipids [8, 9]. The content of total phytosterols and tocotrienols in the oils extracted from both whole kernels and scarified fines of hulless barley have also been studied, and the oils were evaluated as potential sources of functional lipids [10].

In the Tibetan province of China, hulless barley cultivars exist account for more than 97.7% of the total Tibetan barley cultivars and more than 60,000 tonnes of hulless barley is produced each year. Hulless barley plays a very important role in Tibetan life, it is used as an essential food crop, as the main animal feed and is an important fuel [11]. At present, hulless barley has been made into other commercial products such as cake, bread, cookies, and porridge. For the production of most foods, the grains are

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decorticated using mortars and pestles or mechanical dehullers. This treatment removes the outer layers of the grains, i.e., the bran. Barley bran is therefore a by-product of barley-base food manufacturing. A large quantity of barley bran is produced each year in Tibet and used mainly as feed. So if the hulless barley bran is used in some way, valuable byproducts would be produced. To our knowledge, no data has been reported on the properties of the oil obtained from Tibetan hulless barley bran. In this investigation, we analyzed the physicochemical properties, lipid classes and fatty acids of the barley bran oil in order to expedite the utilization of this abundant resource.

Materials and Methods

Materials

Barley bran was collected from a mill after the manufacture of flour from the mixed varieties of ripened hulless barley from Tibet. The standards of fatty acid methyl esters (FAME), phosphatidyl choline (PC), phosphatidyl inositol (PI), phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Silica gel and silica gel plates were purchased from Qingdao Haiyang Chemical Co., Ltd & Special Silica Gel Factory. All other reagents and chemicals used were of analytical grade.

Methods

Extraction of Total Lipids

Bran was finely ground (mesh size = 35) and Soxhlet-extracted with petroleum (60–90 °C) for 8 h. The total lipids (TL) recovered were weighed and stored in chloroform at 4 °C for analysis.

Physicochemical Parameters

Physical and chemical properties including specific gravity, refractive index, melting point, acid value, peroxide value, iodine value and saponification value were determined according to AOAC methods [12].

Column Chromatography Fractionation of the Main Lipid Classes and Fatty Acids Analysis

A glass column (30 cm × 20 mm i.d.) was packed with activated silica gel (100–200 mesh) by applying a slurry of the adsorbent in chloroform (1:5, w/v). Total lipids (30 mg/g of absorbent) were dissolved in 20 ml of chloroform and applied to the column, which was then eluted sequentially with four times the column volume of chloroform (for neutral lipids), with six times the column volume of acetone (for glycolipids), and with five times the column volume of methanol (for phospholipids). The solvent from each fraction was evaporated by rotary evaporation and the percentage of each fraction was determined gravimetrically. The residues were dissolved separately in chloroform and stored in the freezer until required for analysis.

The analyzed lipids were methylated into fatty acid methyl esters and then analyzed using a 6890N gas chromatograph equipped with a HP-5MS fused silica capillary (30 m × 0.25 mm i.d. with 0.32 μm film thickness) and coupled with a gas chromatograph and mass spectrometric detector. For the FAME analysis, a 1-μl sample was injected. The initial column temperature was 70 °C, increased at 4°/min to 90°, then increased at 50 °C/min to 240 °C, and increased at 5 °C/min to 260 °C with a final hold time of 5 min. Column flow rate was 1.0 ml/min using nitrogen as the carrier gas. Mass operating conditions were: 230 °C ion source temperature, 70 eV ionizing voltage, and 33–350 amu mass scan range. Each FAME present in the oil was identified by comparison of its retention time and mass spectrum with those of the standard compounds.

Quantitative Determination of Neutral Lipids and Phospholipids Subclasses

Individual components of neutral lipids were separated by TLC on a silica gel G plate using hexane: diethyl ether:acetic acid (80:20:1, v/v), then individual bands were scraped from the plate and recovered by extraction with 10% methanol in diethyl ether, followed by diethyl ether. Data presented are the averages of three gravimetric determinations. Analytical TLC separation of the phospholipid fraction (from CC) was conducted on silica gel layers, which were activated at 120 °C for 1 h immediately before use. Layers were developed with chloroform/methanol/acetic acid/acetone/water (45:25:7:4:2, v/v). Vaskovsky reagent was used as a general stain [13]. Bands were identified by staining when necessary: Dragendorff's reagent for phosphatidylcholine (PC), ninhydrin for phosphatidylethanolamine (PE) and phosphatidylserine (PS). The R_f values of the phospholipid classes were compared with those of standards which were also included on each plate. For the determination of the PL, the methanol fraction from CC was also separated by TLC in the above given solvent system, and after scraping off of the individual PL subclasses, made to react with the hydrazine sulfate/sodium molybdate reagent at 100 °C for 10 min and photometrically analyzed at 650 nm according to the method of AOCS [14]. From the extinction values obtained, the amount of PL was calculated using a

calibration chart for phosphorus. The individual values were recorded in relation to the PL fraction (methanol fraction from CC) and to the amount of total lipids.

Results and Discussion

In the present investigation, the barley bran was found to contain about 8.1% crude oil. The oil content of hulless barley bran is lower than that of rice bran and is comparable with that of wheat bran and oat bran [15]. Although the hulless barley bran cannot be considered as an oil-bearing resource, its oil content was in the range of other vegetable materials that are used for their health components or their industrial or pharmaceutical applications.

The physicochemical characteristics of the hulless barley bran oil are presented in Table 1. The iodine value, which indicates the unsaturation of fatty acids, was found to be 113.51. A relatively high acid value was found in the oil (11.6 mg KOH/g), this indicates that the oil contains a huge amount of free fatty acids. The high value of the acidity in the oil is perhaps due to the bad conservation of the bran before extraction and analysis.

The proportions of lipid classes and subclasses of neutral lipids and phospholipids of the hulless barley bran oil are shown in Table 2. Among the total lipids, neutral lipids were the predominant lipid class (94.55% of the total lipids), followed by glycolipids (4.20% of the total lipids) and phospholipids (1.25% of the total lipids). Triglycerides (TAG) constituted the major proportion of the neutral lipids, and accounted for 77.12% of the total lipids and 81.56% of the neutral lipids. Monoglycerides (MAG) were at a low level in the oil, it accounted for only 0.52% of the total lipids and 0.55% of the neutral lipids. The presence of diglycerides, monoglycerides and free fatty acids in the oil samples may be due to the partial enzymatic hydrolysis of triglycerides during storage of the bran. Phospholipid subclasses in the oil were separated into four major fractions via TLC. The main component of phospholipids was phosphatidyl choline (0.63% of the total lipids and 50.4% of the phospholipids), followed by phosphatidyl inositol (0.37% of the total lipids and 29.6% of the phospholipids) and phosphatidyl ethanolamine (0.17% of the total lipids and 13.6% of the phospholipids), while phosphatidyl serine was in the lowest level (0.08% of the total lipids and 6.4% of the phospholipids).

Table 1 Physicochemical characteristics of hulless barley bran oil

	Values
Physical characteristics	
Density at 40 °C (g/cm^3)	0.96 ± 0.06
Refractive index at 40 °C	1.41 ± 0.03
Melting point (°C)	30.12 ± 0.63
Chemical characteristics	
Acid value (mg KOH/g)	11.6 ± 0.89
Peroxide value (μg/g)	19.41 ± 1.03
Saponification value (mg KOH/g)	337.62 ± 3.16
Iodine value (mg iodine/g)	113.51 ± 2.39
Unsaponifiable matter (% of total lipids)	4.5 ± 0.26

Data are means ($n = 3$) ± SD ($n = 3$)

Table 2 Lipid classes of hulless barley bran oil

Lipid classes	Values (% of total lipids)
Neutral lipids	94.55 ± 1.63
Triglycerides	77.12 ± 2.13
Diglycerides	8.07 ± 0.97
Monoglycerides	0.52 ± 0.06
Free fatty acids	4.34 ± 0.69
Sterols	3.55 ± 0.24
Hydrocarbons	0.95 ± 0.08
Phospholipids	1.25 ± 0.11
Phosphatidyl choline	0.63 ± 0.06
Phosphatidyl inositol	0.37 ± 0.05
Phosphatidyl ethanolamine	0.17 ± 0.04
Phosphatidyl serine	0.08 ± 0.01
Glycolipids	4.20 ± 0.29

Data are means ($n = 3$) ± SD ($n = 3$)

Fatty acid profiles of the total lipids and lipid classes (neutral lipids, glycolipids and phospholipids) are presented in Table 3. According to the results shown in the table, ten fatty acids were identified in the hulless barley bran oil. The fatty acid composition of the oil was characterized by linoleic and palmitic acids as the major fatty acids. Linoleic acid (C18:2) was the principal unsaturated fatty acid (75.08% of the total fatty acids), eicosenoic acid (C20:1) was also detected and at a relatively low level (1.57% of the total fatty acids). Palmitic acid (C16:0) was the major saturated fatty acids (20.58% of the total fatty acids), and stearic acid (C18:0) was at a relatively low level (1.45% of the total fatty acids). Among them, these four fatty acids represented ca. 98.68% of the total fatty acids present. Six minor fatty acids, myristic (C14:0), hexadecenoic (16:1), heptadecanoic (17:0), eicosanoic (20:0), docosanoic (C22:0) and docosenoic (C22:1), were identified in the bran oil and made up 1.32% of the total fatty acids.

Fatty acids in neutral lipids and polar lipids did not differ significantly from each other. The unsaturated linoleic acid was the most predominant fatty acid in all lipid classes, constituting more than 76% of the total fatty acid content, and linoleic acid in glycolipids is relatively higher than that in neutral lipids and phospholipids. The saturated

Table 3 Fatty acid composition of hulless barley bran lipid classes

Fatty acid	Relative content (%)			
	Total lipids	Neutral lipids	Glycolipids	Phospholipids
Myristic C14:0	0.22 ± 0.01	0.14 ± 0.02	0.52 ± 0.01	0.68 ± 0.03
Palmitic C16:0	20.58 ± 0.35	19.39 ± 0.26	14.43 ± 0.51	13.52 ± 0.14
HexadecenoicC16:1	0.24 ± 0.06	0.30 ± 0.03	0.86 ± 0.07	1.02 ± 0.09
Heptadecanoic C17:0	0.06 ± 0.01	0.06 ± 0.02	0.44 ± 0.01	0.41 ± 0.01
Stearic C18:0	1.45 ± 0.06	1.29 ± 0.10	1.93 ± 0.09	2.28 ± 0.15
Linoleic C18:2	75.08 ± 1.23	76.04 ± 2.10	79.33 ± 1.56	77.70 ± 1.43
Eicosanoic C20:0	0.26 ± 0.03	0.33 ± 0.02	0.32 ± 0.05	0.66 ± 0.08
Eicosenoic C20:1	1.57 ± 0.21	1.83 ± 0.13	1.61 ± 0.11	ND
Docosanoic C22:0	0.16 ± 0.04	0.19 ± 0.03	0.22 ± 0.04	3.44 ± 0.11
Docosenoic C22:1	0.38 ± 0.06	0.43 ± 0.09	0.34 ± 0.05	0.29 ± 0.01
SFA	22.73 ± 1.12	21.4 ± 0.98	17.86 ± 1.16	20.99 ± 1.23
USFA	77.27 ± 3.21	78.6 ± 2.79	82.14 ± 3.94	79.01 ± 2.64
U/S	3.40 ± 0.33	3.67 ± 0.41	4.60 ± 0.32	3.76 ± 0.39

ND not detected, *SFA* saturated fatty acids, *USFA* unsaturated fatty acids, *U/S* unsaturated/saturated fatty acids

Data are means ($n = 3$) ± SD ($n = 3$)

palmitic acid was the major saturated fatty acid in the three fractions. Eicosenoic acid (C20:1) was not detected in phospholipids, however, it was detected in the total lipids (1.57%), neutral lipids (1.83%) and glycolipids (1.61%). Docosanoic (C22:0) was found to be less than 0.22% of the total lipids, neutral lipids and glycolipids, however, it was found with a content of 3.44% in phospholipids. The ratio of unsaturated fatty acids to saturated fatty acids was relatively higher in glycolipids than that of neutral lipids and phospholipids due to the relatively higher percentage of linoleic acid in glycolipids. Price and Parsons studied the composition of fatty acids from hulless barley and their research revealed that linoleic acid was the predominant unsaturated fatty acid and palmitic acid was the major saturated fatty acid of the neutral lipids, phospholipids and glycolipids, which is in agreement with our results [16]. However, they studied the bran and endosperm as a whole, and our research was only concerned with the bran.

Linoleic and alpha-linolenic acids, as the two representative compounds of n-6 and n-3 fatty acid, are essential fatty acids in mammalian nutrition [17]. In our study of the fatty acid composition in the hulless barley bran oil, linolenic acid was not detected. However, the content of linoleic acid is comparable to that of the safflower oil, and is higher than that of cotton, oat, peanut, soybean, rice bran and sunflower oil [17].

The recommended ratio of n-6 to n-3 fatty acids is estimated to be from 3 to 5 [18], so the hulless barley bran oil may not be suitable for being used as an edible oil directly, but the hulless barley bran oil can be used as a good source of linoleic acid. The relatively high linoleic acid content makes the oil nutritionally valuable for its effects on cardiovascular disease and cancer prevention [19].

Based on our study, the hulless barley bran as a by-product seems promising as a new non-conventional resource for the pharmaceutical industries and for edible purposes, such as the production of conjugated linoleic acid and food emulsifiers, and its applications in these fields also needs further research.

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