Nutritional Component and Chemical Characterization of Chinese Highland Barley Bran Oil

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Abstract: The nutritional composition and chemical properties of the Chinese highland barley bran oil were characterized in this study. The barley bran oil extracted with solvent possessed relatively high acid value and peroxide value, indicating that the oil should be further refined before using. The fatty acid composition of the oil showed that the content of unsaturated fatty acids was 80.12 g/100 g, in which the content of polyunsaturated fatty acids was as high as 60.41 g/100 g. The overall triacylglycerol profile showed that the oil contained 27 TAGs including 21 regioisomers. Major TAGs included LLL (21.08 g/100 g), PLL (19.27 g/100 g), LLO (12.24 g/100 g), and LLLn (12.17 g/100 g). The total unsaponifiable matter of the oil reached up to 10.74 g/100 g oil. The total phytosterol content reached 7.90 g/100 g oil, in which β-sitosterol was the most predominant, with the content of 5.69 g/100 g oil. Other important sterols included campesterol (1.32 g/100 g oil), lanosterol (0.70 g/100 g oil) and stigmasterol (0.19 g/100 g oil).

Key words: chemical characterization, highland barley bran oil, nutritional component, sterol, triacylglycerol

1 Introduction

As one of the most ancient cereal crops, hulless highland barley (qingke) is the only crop that can be cultivated in Qinghai-Tibetan Plateau, which has an average elevation of 4000 meters. The extremely cold, hypoxia and extensive UV radiation condition of Qinghai-Tibetan Plateau led to the accumulation of abundant secondary metabolites in hulless highland barley.

The phytosterol level in barley oil from different areas was 1.20-9.60 g/100 g, higher than that of most rice bran oil (1.50-3.50 g/100 g)1,2. Moreau reported that the total tocotrienol content in barley oil reached up to 0.29-0.61 g/100 g oil3, higher than that of the rice bran oil (0.077 g/100 g oil) and the wheat bran oil (0.19 g/100 g oil)4. It has been reported that tocotrienol in barley oil is mainly α-tocotrienol, whose bioavailability is higher than γ-tocotrienol, while the tocotrienol in palm oil is mainly γ-tocotrienol5,6. Barley germ oil has been tested to be rich in tocopherols.

As a byproduct of highland barley processing, highland barley bran is rich in minerals, oil, protein, polyphenols, β-glucan, oryzanol and phytosterols. However, highland barley bran is now mainly used as feed. Nowadays, highland barley bran has been incorporated into bread, cookies, and crackers making. The results showed that barley bran could effectively enhance the health-salutary components and antioxidant capacities of foods7-8. Extraction of protein, dietary fibre, and β-glucan from highland barley bran has been well studied9. But research on barley bran oil is still very limited. Qian investigated physicochemical parameters of the bran oil of hulless highland barley from Tibet10. However, the triacylglycerol (TAG) profile of the highland barley bran oil was still unknown.

In order to promote better utilization and further research of the highland barley bran oil, especially used as a novel functional oil, this study gave an overall characterization of the highland barley bran oil.

2 Materials and Methods

2.1 Materials

Highland barley seed and highland barley bran samples were obtained from Tibet Xinwang Biology & Technology Co. Ltd. in Tibet Province (Northwest of China) in 2019. α-Cholenanol, eicosanol, standards of tocopherols and fatty acid esters were supplied by Sigma-Aldrich, and BSTFA + TMCS (99:1) was purchased from Supelco. n-hexane, isopropanol, acetonitrile and acetone were of HPLC grade. Other solvents were analytical pure.

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2.2 Chemical composition analysis of highland barley bran and highland barley whole seed

Chemical composition of both the highland barley bran and highland barley whole seed were analyzed. The total starch, protein, oil, moisture, ash and fat were analyzed according to the American Oil Chemists’ Society (AOCS) methods [11].

2.3 Oil extraction from the highland barley bran

Briefly, after removing the impurity, highland barley bran was grinded into powder. The highland barley bran powder of 100 g was extracted by 600 mL of n-hexane at 40°C for 4 h. The solvent was collected, evaporated and centrifuged, after which the highland barley bran oil was obtained. The oil was preserved at −18°C before analysis.

2.4 Analysis of the oil

2.4.1 Physicochemical properties

Acid value (AV), peroxide value (PV), saponification value, and unsaponifiable matter content of the highland barley bran oil were determined according to the standard methods of American Oil Chemists’ Society (AOCS Official method Cd 8b-90) [11]. The levels of tocopherol (α-, β-, γ-, and δ-isomers) in crude highland barley bran oil were determined as described by [12] using HPLC method.

2.4.2 Fatty acid composition

Derivatisation reaction of the highland barley bran oil was carried out by using conventional method [10]. The obtained fatty acid methyl esters were analyzed on Agilent 7890B gas chromatograph system by using ISO5508/ISO5509 method. Fatty acids were identified by comparing with the fatty acid esters standards.

2.4.3 TAG profiles analysis

Overall TAG profiles analysis was conducted by using off-line 2D HPLC system consisted of non-aqueous reversed-phase (NARP) and silver ion HPLC (Ag-HPLC) coupled with atmosphere pressure chemical ionisation (APCI)/MS [13]. Each fraction of the NARP eluent was collected following ten injections. Same fractions were combined, condensed by nitrogen dryer, solubilized in hexane, and then injected into Ag-HPLC system.

2.4.4 Unsaponifiable matters analysis

Saponification reaction was performed at present of internal standards (α-cholesterol and eicosanol) according to the literature [13]. The obtained unsaponifiable matters was then boiled with BSTFA + TMCS (99:1) for derivatisation reaction. The derivated unsaponifiable matters analysis was carried out using Agilent Trace 1310 Gas Chromatograph system coupled with ISQ QD Single Quadrupole Mass Spectrometer (Thermo Scientific, USA). Unsaponifiable matters were identified by matching with the mass spectra from the Wiley library.

2.5 Statistical analysis

All experiments were repeated in triplication, and results were expressed as mean ± standard deviation.

3 Results and Discussion

3.1 Nutritional components of highland barley bran and highland barley whole seed

Table 1 shows the nutritional components of the highland barley whole seed and the highland barley bran. The oil content of the highland barley bran was 6.85 g/100 g, which was relatively higher than that of the highland barley whole seed (1.76 g/100 g) and the wheat bran (3.4 g/100 g) [20], but lower than that of the rice bran (18.5 g/100 g). Moreover, that content was extremely lower than that of the rapeseed (30-40 g/100 g), soybean (16-22 g/100 g), olive (15-50 g/100 g), and so on [21]. The protein content of highland barley bran was 16.97 g/100 g, higher than that of the highland barley whole seed (10.32 g/100 g), and comparable with that of the wheat bran (12.18 g/100 g) [10]. Also, the ash and the crude fiber content of the highland barley bran was 5.92 g/100 g and 10.87 g/100 g, respectively, which were higher than that of the highland barley whole seed (1.21 g/100 g, 2.86 g/100 g). But the highland barley whole seed contained a higher level of total starch (56.43 g/100 g) than the highland barley bran (34.39 g/100 g).

3.2 Physicochemical Characteristics of Highland Barley Bran Oil

As shown in Table 2, oil yield of the solvent extracted method was 5.82 g/100 g, with extraction rate of 85.0%. The solvent extracted highland barley bran oil presented brown yellow color with a little turbid. The high acid value (11.85 meq KOH/g oil) and peroxide value (14.50 meq O2/kg oil) were

| Sample     | Total Starch (g/100 g) | Protein (g/100 g) | Oil (g/100 g) | Moisture (g/100 g) | Ash (g/100 g) | Crude Fiber (g/100 g) |
|------------|------------------------|-------------------|---------------|-------------------|--------------|----------------------|
| Whole HB   | 56.43 ± 0.25           | 10.32 ± 0.04      | 1.76 ± 0.02   | 11.27 ± 0.07      | 1.21 ± 0.01  | 2.86 ± 0.01          |
| HB Bran    | 34.39 ± 0.14           | 16.97 ± 0.05      | 6.85 ± 0.03   | 5.52 ± 0.02       | 5.92 ± 0.03  | 10.87 ± 0.08         |

Values are mean ± SD of three seeds, analyzed individually in triplicate.

HB, highland barley seed samples harvested from Xinjiang Province of China.
oil) of the oil suggested that the oil should be further refined before using. High iodine value indicated high unsaturated fatty acids content of the oil. The oil contained an extremely high content of unsaponifiable matters (10.74 g/100 g oil), which was higher than that of the wheat bran oil (2.80 g/100 g oil), olive oil (1.23 g/100 g oil) and peanut oil (0.94 g/100 g oil). Total content of tocopherols in the oil was up to 0.18 g/100 g oil, lower than that of the wheat germ oil (0.27 g/100 g oil) and the wheat bran oil (0.19 g/100 g oil).

3.3 Fatty acid composition

As shown in Table 3, fatty acids of the highland barley bran oil were mainly composed of linoleic acid, followed by palmitic acid and oleic acid. Linolenic acid and gadolenic acid are minor fatty acids. The fatty acid composition of the oil was similar with that of the soybean oil, which contained linoleic acid (49.8-59.0 g/100 g), palmitic acid (8.0-13.5 g/100 g), oleic acid (17.7-28.0 g/100 g) and linolenic acid (5.0-11.0 g/100 g). The linoleic acid content of the oil (54.80 g/100 g) was comparable to that of the sunflower oil (48.3-74.0 g/100 g), corn oil (34.0-65.6 g/100 g) and cottonseed oil (46.7-58.2 g/100 g). The oleic acid content of the oil (18.66 g/100 g) was extremely lower than that of the peanut oil (35.0-67.0 g/100 g) and camellia oil (74.87 g/100 g). The unsaturated fatty acids content (USFA) was 80.12 g/100 g, among which polyunsaturated fatty acids content reached up to 60.41 g/100 g. Therefore, highland barley bran oil was beneficial to human health.

3.4 TAG composition

Depending on the interaction between silver ions and fatty acid double bonds, Ag-HPLC can effectively resolve the separation the positional isomers, which could not be resolved by NARP. Therefore, it is necessary to combine the NARP with the Ag-HPLC separation for overall TAGs profiles analysis. Sixteen TAGs fractions were obtained in NARP dimension, and each fraction was collected and separated in the Ag-HPLC dimension. As the Ag-HPLC-APCI-MS chromatogram shown in Fig. 1, two regioisomers were obtained from fraction 2: sn-Lnn-Ln and sn-LnLnn. As both linolenic acids with three double bonds were located in the external position sn-1/3, sn-LnLnn was more retained in Ag-HPLC dimension. Also resolved was the PLnLn group. As shown in Fig. 3, the

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Table 2  Physicochemical characteristics of the highland barley bran oil.

| Determination                        | EPO-CHI oil |
|--------------------------------------|------------|
| Oil yield (g/100 g)                  | 6.10 ± 0.04|
| Color                                | Brown yellow, a little turbid |
| Acid value (mg KOH/g oil)            | 11.85 ± 0.42|
| Peroxide value (meq O₂/kg oil)       | 14.50 ± 0.28|
| Iodine value (g I₂/100 g oil)        | 119.37 ± 1.72|
| Saponification value (mg KOH/g oil)  | 215.3 ± 5.69|
| Unsaponifiable matter (g/100 g oil)  | 10.74 ± 0.03|
| Tocopherol (mg/kg of oil)            |            |
| α                                    | 1240.1 ± 6.8    |
| β                                    | 52.4 ± 0.4      |
| γ                                    | 471.6 ± 0.8     |
| δ                                    | 78.4 ± 1.2      |

Values are mean ± SD of three seeds, analyzed individually in triplicate.

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Table 3  Fatty acid composition of the highland barley bran oil.

| Fatty acid         | Content (g/100 g) |
|--------------------|-------------------|
| Palmitic acid (C 16:0) | 19.88             |
| Oleic acid (C 18:1)  | 18.66             |
| Linoleic acid (C 18:2) | 54.80             |
| Linolenic acid (C 18:3) | 5.61              |
| Gadolenic acid (C 20:1) | 1.05              |
| SFA                | 19.88             |
| MUFA               | 19.71             |
| PUFA               | 60.41             |
| USFA               | 80.12             |
| PUFA/SFA           | 4.03              |
| SFA/USFA           | 0.25              |

SFA saturated fatty acids, MUFA mono unsaturated fatty acids, PUFA polyunsaturated fatty acids, USFA unsaturated fatty acids.
PLnLn group in fraction 4 was separated into $sn$-PLnLn and $sn$-LnPLn. Similarly, $sn$-LLLn was more retained than $sn$-LLLn (Fig. 2), and $sn$-LnPL was more retained than $sn$-PLnL (Fig. 4) for the same reason.

As shown in Fig. 5, fraction 7 was separated into 2 species in Ag-HPLC: $sn$-LLO and $sn$-LOL. With both linoleic acids with two double bonds located in $sn$-1/3, $sn$-LOL was more retained than $sn$-LLO. $sn$-LPL (Fig. 6) and $sn$-LGL
The TAG profiles results of Chinese highland barley bran oil were listed in Table 4. With content of 21.08 g/100 g, LLL was the dominant TAG of the oil. Principal TAGs included PLL (19.27 g/100 g), LLO (12.24 g/100 g), LLLn (12.17 g/100 g), PLLn (7.68 g/100 g) and OLO (6.17 g/100 g). Other TAGs were minor TAGs, such as LLG (4.28 g/100 g), PLO (4.19 g/100 g) and LLnLn (4.16 g/100 g). Table 5 listed the relative abundance of TAG regioisomers. PLO has three isomers, while other 11 TAGs have two isomers. The total TAG profiles results showed that the oil contained 27 TAGs including 21 regioisomers.

3.5 Unsaponifiable matters composition

Tables 6-8 showed that unsaponifiable matters of the oil were mainly composed of sterols, squalene, tocopherol, alcohols and hydrocarbons. The hydrocarbons composition results showed that the total hydrocarbons content was 0.39 g/100 g oil (Table 6).

The phytosterols results showed that the phytosterols included campesterol, stigmasterol, β-sitosterol, isofucosterol and lanosterol (Table 8). The total phytosterols content of the oil reached up to 7.90 g/100 g oil. According to the literature, the total phytosterols content in barley oils from various hulled and hulless barley varied from 1.20 to 9.60 g/100 g oil. The phytosterols content of the oil was extremely higher than that of the peanut oil (0.21 g/100 g oil), olive oil (0.45 g/100 g oil), rice bran oil (1.27 g/100 g oil) and other vegetables oils.21 The content of β-sitosterol was the highest, up to 5.69 g/100 g oil. Other
important sterols included campesterol (1.32 g/100 g oil), lanosterol (0.70 g/100 g oil) and stigmasterol (0.19 g/100 g oil). It has been reported that phytosterols can help lower blood cholesterol level, thereby decreasing cardiovascular morbidity\(^2^2\). Phytosterols also had important antioxidant, analgesic, and anticancer abilities\(^2^3, 2^4\).

The squalene content of the oil was 0.27 g/100 g oil, lower than that of the rice bran oil (0.32 g/100 g oil)\(^2^2\). The content of \(\alpha\)-Tocopherol and \(\gamma\)-tocopherol was 0.08 g/100 g oil and 0.03 g/100 g oil, respectively. Moreau reported that the total tocopherols in barley oils reached up to 0.05-0.61 g/100 g oil\(^3\). With high content of phytosterols, squalene and tocopherol, highland barley bran oil was considered to be a good source of natural biological active substances.

### 4 Conclusions

The overall characterization results of the highland barley bran oil showed that the solvent extracted barley bran oil possessed relatively high acid value and peroxide value, indicating that the oil should be further refined before using. The content of unsaturated fatty acids was 80.12 g/100 g, in which the content of polyunsaturated fatty acids was as high as 60.41 g/100 g. The overall TAG profiles showed that the oil contained 27 TAGs including 21 regioisomers. Major TAGs included LLL (21.08 g/100 g), PLL (19.27 g/100 g), OLO (12.24 g/100 g), and LLLn (12.17 g/100 g).

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**Table 4** Molecular mass, partition number, fragmentation ions obtained for TAGs identified in the solvent extracted Chinese highland barley bran oil by RPLC-HPLC-APCI-MS.

| Fraction | TAG | Relative abundance (g/100 g) | \(M_r\) | DB | CN | ECN | \([M+NH_4]^+\) | \([DG]^+\) | \([DG]^+\) | \([DG]^+\) |
|----------|-----|-------------------------------|--------|----|----|-----|----------------|----------------|----------------|----------------|
| 1        | LnLnLn | 2.84                          | 872.7  | 9  | 54 | 36  | 890.7          | LnLn 595.5     |                 |                 |
| 2        | LLLn  | 4.16                          | 874.7  | 8  | 54 | 38  | 892.7          | LLLn 597.4     | LnLn 595.5     |                 |
| 3        | LLLn  | 12.17                         | 896.5  | 7  | 54 | 40  | 894.5          | LLLn 597.4     | LL 599.4       |                 |
| 4        | PLLn  | 1.34                          | 850.6  | 6  | 52 | 40  | 868.6          | LnP 573.7      | LnLn 595.5     |                 |
| 5        | LLL   | 21.08                         | 878.6  | 6  | 54 | 42  | 896.6          | LL 599.5       |                 |                 |
| 6        | PLLn  | 7.68                          | 852.7  | 5  | 52 | 42  | 870.7          | LLLn 597.3     | OL 601.4       |                 |
| 7        | PLL   | 12.24                         | 880.6  | 5  | 54 | 44  | 898.6          | LL 599.4       | LO 601.4       |                 |
| 8        | PLL   | 19.27                         | 854.7  | 4  | 52 | 44  | 872.7          | LL 599.4       | LP 575.4       |                 |
| 9        | LLG   | 4.28                          | 908.7  | 5  | 56 | 46  | 926.7          | LG 629.5       | LL 599.2       |                 |
| 10       | OLO   | 6.17                          | 882.7  | 4  | 54 | 46  | 900.7          | OL 601.3       | OO 603.3       |                 |
| 11       | PLO   | 4.19                          | 856.5  | 3  | 52 | 46  | 874.5          | OL 601.4       | OP 577.3       | PL 575.3       |
| 12       | PLL   | 0.66                          | 830.4  | 2  | 50 | 46  | 848.4          | PP 551.4       | PL 575.3       |                 |
| 13       | OLG   | 0.85                          | 910.9  | 4  | 56 | 48  | 928.9          | LO 601.4       | LG 629.5       | GO 631.7       |
| 14       | OOO   | 1.21                          | 884.8  | 3  | 54 | 48  | 885.8          | OO 603.5       |                 |                 |
| 15       | POO   | 0.58                          | 858.8  | 2  | 52 | 48  | 866.8          | PO 603.4       |                 |                 |
| 16       | PHP   | 0.52                          | 832.8  | 1  | 50 | 48  | 850.8          | PO 577.4       |                 |                 |

**Table 5** Relative abundance of TAG isomers in Chinese highland barley bran oil.

| Fraction | TAG | Isomers | Relative abundance (mol % of total) |
|----------|-----|---------|------------------------------------|
| 2        | LLLn| sn-LLnL | 69.27                              |
|          |     | sn-LnLnL | 30.73                              |
| 3        | LLLn| sn-LLLn | 42.17                              |
|          |     | sn-LLL  | 57.8                               |
| 4        | PLLn| sn-LnPn | 98.66                              |
|          |     | sn-PLnL | 1.34                               |
| 6        | PLL  | sn-PnPnL | 97.42                              |
|          |     | sn-PnPnL | 2.58                               |
| 7        | LLO  | sn-OLL  | 3.24                               |
|          |     | sn-LOL  | 96.76                              |
| 8        | PLL  | sn-LPL  | 94.27                              |
|          |     | sn-PLL  | 5.73                               |
| 9        | LLG  | sn-LLG  | 74.31                              |
|          |     | sn-LGL  | 25.69                              |
| 10       | OLO  | sn-OLO  | 46.28                              |
|          |     | sn-LOO  | 53.72                              |
| 11       | PLO  | sn-OLP  | 4.17                               |
|          |     | sn-LPO  | 95.08                              |
| 12       | PLL  | sn-PnP  | 97.65                              |
|          |     | sn-PPL  | 2.35                               |

P, palmitic acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; G, gadolenic acid; TAG, triacylglyceride; \(M_r\), molecular mass; DB, double bond; CN, carbon number; ECN, equivalent carbon number; \([M+H]^+\), pseudomolecular ion; \([DG]^+\), diglyceride ion.
Table 6  Composition of the hydrocarbons from Chinese highland barley bran oil.

| Retention time (min) | Compounds      | Content (mg/kg oil) |
|----------------------|----------------|---------------------|
| 10.6184              | Dodecane       | 3.6                 |
| 12.3481              | Tridecane      | 19.2                |
| 14.0247              | Tetradecane    | 106.9               |
| 15.9728              | Pentadecane    | 358.9               |
| 17.9799              | Hexadecane     | 294.3               |
| 22.012               | Octadecane     | 139.3               |
| 25.861               | Eicosane       | 93.4                |
| 27.7028              | Heneicosane    | 234                 |
| 29.6096              | Docosane       | 121.7               |
| 32.7561              | Tetracosane    | 121.7               |
| 34.35                | Pentacosane    | 412.3               |
| 35.814               | Hexacosane     | 99.7                |
| 37.3253              | Heptacosane    | 532.4               |
| 38.7539              | Octacosane     | 177.4               |
| 40.1235              | Nonacosane     | 910.5               |
| 42.662               | Tetratriacontane | 318.9             |
|                      | Total hydrocarbons | 3944.2          |

Table 7  Composition of the alcohols from Chinese highland barley bran oil.

| Retention time (min) | Compounds                  | Content (mg/kg oil) |
|----------------------|----------------------------|---------------------|
| 12.0116              | 1-Nonanol                  | 5.8                 |
| 12.1297              | Glycerol                   | 170.2               |
| 16.3093              | Butylated Hydroxytoluene   | 3.1                 |
| 17.3719              | 1-Dodecanol                | 36.5                |
| 23.293               | 1-Pentadecanol             | 52.9                |
| 24.8987              | Isophytol                  | 147.6               |
| 25.188               | 1-Hexadecanol              | 107.2               |
| 29.462               | Phytol                     | 4414.4              |
| 30.2649              | Farnesol                   | 1547.5              |
| 31.481               | 1-Eicosanol                | 177                 |
| 33.6239              | 1-Heneicosano              | 68.6                |
| 35.2414              | Docosanol                  | 1193.3              |
| 36.5933              | 1-Tricosanol               | 167.7               |
| 38.0691              | Tetracosanol               | 744.9               |
| 39.362               | 1-Pentacosanol             | 106.4               |
| 39.7221              | 1-Heptacosanol             | 80.1                |
| 40.7847              | 1-Hexacosanol              | 1159.7              |
| 42.172               | 1,6,10,14,18,22-Tetracosahexaen-3-ol | 153.1             |
| 43.2287              | 1-Octacosanol              | 138.1               |
|                      | Total alcohols             | 10474.3             |
The total unsaponifiable matter of the oil reached up to 10.74 g/100 g oil. The total phytosterol content reached 7.90 g/100 g oil, in which β-sitosterol was the most predominant, with the content of 5.69 g/100 g oil. Other important sterols included campesterol (1.32 g/100 g oil), lanosterol (0.70 g/100 g oil) and stigmasterol (0.19 g/100 g oil). The oil was considered to be a good source of biological activity compounds, especially linoleic acid and β-sitosterol.

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Conflict of interest

All the authors declare that there is no conflict of interest.

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