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Chicory seeds: a potential source of nutrition for food and feed

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1 SUMMARY

***Cichorium intybus*, commonly known as chicory root, is used as a coffee substitute and grown as a crop for livestock, but little is known about the nutritional value of chicory seeds. Therefore, the chemical compositions of Puna Chicory and Commander Chicory seeds were investigated in this study. The results revealed that the two chicory seeds contained substantial amounts of crude proteins (over 19 %), crude fat (over 22 %) and carbohydrate (over 31 %), respectively. The protein contents were two times higher than those of corn grains, and the fat contents were markedly higher comparable to alfalfa seeds. Chicory seeds were rich of most essential amino acids, and the total amino acid content of Puna Chicory seeds was higher than that of Commander Chicory seeds or alfalfa seeds. The essential fatty acid, linoleic acid was the predominant fatty acid accounted for over 76 % of the total fatty acids in the two chicory seeds, with lower saturated/unsaturated ratios (about 0.11) making them potentially a superior source of nutritional oil. Compared with alfalfa, mineral analysis showed that chicory seeds possess higher K, Ca, P, Mg, Cu, Zn and Mn elements. Overall, the study suggested that the two forage-type chicory seeds had high levels of nutritionally important components and may be of significant importance in the formulation of diets for human and animals.**

2 INTRODUCTION

Chicory *(Cichorium intybus* L.*)* is a perennial plant with blue or white flowers that is easy to grow and can be used for many purposes. It is perhaps best known for the roasted roots used as the traditional coffee substitute with no caffeine and less well known as grazed forage for ruminants (Barbara *et al*., 2007). Chicory also has a history of medicinal use in India (Ramalakshmi *et al*., 1994) and in China (1999), especially of great value for its tonic effects upon the liver and digestive tract. Chicory was also widely used to treat diabetes mellitus, and one recent research supported the traditional belief that ethanol extract of *Cichorium intybus* could ameliorate a diabetic state by reducing the hepatic glucose-6-phosphatase activity (Pushparaj *et al*., 2007). The aqueous extract of chicory leaves has been shown to have a marked radical scavenging properties and offered significant protection against protein oxidation and DNA damage, which could be attributed to the presence of phenolic

compounds. Therefore, chicory leaves could be considered as a source of natural antioxidants for pharmaceutical or dietary needs (Ilaiyaraja and Farhath Khanum, 2010). For grazing animals, chicory is also well known for its toxicity to internal parasites. Studies indicate that ingestion of chicory by farm animals results in reduction of worm burdens (Heckendorn *et al*., 2007; Athanasiadou *et al*., 2007; Tzamaloukas *et al*., 2006), which has prompted its widespread use as a forage supplement, and much of the breeding for improved forage properties was completed in New Zealand. In the last decades, chicory has been used as a relatively new forage crop in China. The superior properties, such as the long productive season, relatively easy to establish, tolerance of drought and soil acidity, make it highly competitive with other grasses and weeds. Chicory leaves are palatable for the majority of livestock and poultry. The digestibility and the mineral content of chicory leaves are greater relative to perennial ryegrass and to red clover (Barry, 1998). At present, much is known about the roots and leaves, however, little is known about the nutritional value of chicory seeds. It was, therefore, the purpose of this study to examine the chemical composition of the seeds and thereby determine the nutritive, as well as food uses, of this material.

3 MATERIALS AND METHODS

3.1 Morphology and average seed weight: Two varieties of seeds of *Cichorium intybus* L. cv. Puna (Puna Chicory), and *Cichorium intybus* L. cv. Commander (Commander Chicory) were bought from Shanghai Barenbrug Group. Two hundred (200) seeds of each variety were randomly taken and dried in the oven at 40 □ for 1 h. The average seed weight for each species was determined after temperature/humidity controlled drying by weighing lots of seeds on an analytical balance and dividing this weight by the total number of seeds (average seed weight=weight of seeds/number of seeds).

3.2. Proximate composition: The moisture content in the two varieties of chicory seeds was detected by the oven drying method (Zhang, 2003). Ash content was determined by the gravimetric method and fat content was assayed by Soxhlet extraction (Williams *et al*., 1962; Chen, 2002). Nitrogen was determined using the Kjeldahl method, and the quantity of crude protein was calculated as 6.25×N. The content of crude fibre was detected by the acid-base method, and the specific operation was conducted according to previously reported method (Zhang, 2003). The total sugar in the two chicory seeds varieties was determined by phenol-sulfuric acid method at 488 nm with visible spectro-photometry (Sun *et al*., 2008). Analysis of each sample was performed in triplicate.

3.3 Amino acid analysis: The analysis of amino acid in the two varieties of chicory seeds was determined on a Hitachi-835-50 amino acid auto analyzer, according the methods by GB/T 18246-2000 of China national standard (2001). In brief, the types and contents of amino acids were detected by ion exchange chromatography using a post-column ninhydrin reaction after acid hydrolysis. Cysteine and methionine were determined as cysteic acid and methionine sulphone after acid hydrolysis and performic acid oxidation.

3.4 Fatty acid analysis: The dried specimens were mixed with chloroform:

methanol (2:1, v/v) and the solid non-lipid material were removed by filtration. The total extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were then re-dissolved in chloroform: methanol (19:1, v/v) and clarified by centrifugation. Transmethylation was performed using 14 % (w/v) boron trifluoride in methanol. Five micrograms of heptadecanoic acid as the internal standard, about 20 % (w/w) of test sample and a 1 ml aliquot of each sample were transferred to a 15 ml Teflon-lined screw-cap tube. After removal of the solvent by nitrogen gas, the sample was mixed with 5 ml of boron trifluoride reagent, placed in a warm bath at 100 °C for 30 min and then cooled. After the addition of saline solution, the trans-methylated fatty acids were extracted into hexane. A calibration mixture of fatty acid standards was processed in parallel (Glew *et al.*, 2006). Fatty acids were separated and quantified using a Hewlett-Packard gas chromatograph (5890 Series II) with a flame-ionization detector. One or two micro-liter aliquots of the hexane phase were injected in split-mode onto a fused-silica capillary column (Omegawax; 30 m × 0.32 mmI.D., Supleco, Bellefonte, PA) according to GB/T 21514 - 2008/ISO/TS 17764:2002. The injector temperature was set at 200 °C, detector at 230 °C, oven at 120 °C initially, then 120–205 °C at 4 °C per min, 205 °C for 18 min. The carrier gas was helium and the flow rate was approximately 50 cm/sec. Electronic pressure control in the constant flow mode was used. The internal standard (heptadecanoic acid, C17:0) and calibration standards were used for quantitation of fatty acids in the lipid extracts. The analysis of fatty acid was also performed in triplicate.

3.5 Mineral Analysis: The two varieties of chicory seeds were ground into fine powder respectively. For each sample, 0.2 g powder was treated with 5 ml of concentrated nitric acid for overnight to predigest in a Teflon vessel, and then added 1 ml hydrogen peroxide. The sample in the vessel was then subjected to a microwave program: 0.5 MPa for 3 min, 0.8 MPa for 2 min and 1.2 MPa for 2 min at 600 W. The last step was 1.5 MPa for 5 min at 800 W. After digestion, the sample was evaporated to dryness and heated at 50 °C for 1 h, and then made up with de-ionized water to 100 ml in acid washed standard flasks. Inductively coupled plasma mass spectrometry (ICP-MS, model Elan DRC-e, Perkin-Elmer Corporation of USA) was used to analyze the following metals: phosphorus (P), molybdenum (Mo), selenium (Se), strontium (Sr) and cadmium (Cd). Atomic absorption spectrometer (AAS, PE 2100) was used to detect contents of sodium (Na), copper (Cu), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn) and potassium (K). The instrument was calibrated with known standards and samples were analyzed at corresponding wavelengths. The analysis of each mineral was performed in triplicate.

3.6 Statistical analysis: The descriptive statistics (mean ± standard deviation) and one-way analysis of variance (ANOVA) were conducted using SPSS13.0 software. Significance was established at $P < 0.05$.

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4 RESULTS

4.1 Seed morphology and average weight: The morphology of the two varieties of chicory seeds was estimated by their appearance. The two seeds were covered by a thin dark-brown skin as shown in Fig.1, and they had similar size and morphology, such as slender or wedge shape, thin, with short pappus and a length of 3 mm. The weight was approximately 1.5 mg, and was lower than that of alfalfa seed (Table 1). There was no significantly difference between the two chicory seeds.

Figure 1: The photos of Puna chicory seeds (A) and Commander chicory seeds (B).

Table 1: Seed average weight of two varieties of chicory seeds (mg)

Seeds	Puna chicory	Commander chicory	Alfalfa[#]
Average weight	1.49±0.03[a]	1.53±0.02[a]	1.77~2.24

Note: Values are mean of three different determinations ± standard deviation, Means with the same letter (a) was not significantly different (P<0.05). # Christos (2006)

4.2 Proximate composition: The proximate composition is presented in Table 2. The two varieties of chicory seeds had abundant proteins (over 19 % of dry weight), fat (about 21-22 %) and carbohydrate (over 30 %). The moisture, crude protein, fat, fibre and carbohydrate contents between them are not different from each other. However, the crude protein, fat and fibre contents in chicory seeds were all significantly higher than those of corn grains where the crude protein, fat and fibre contents are 9.40, 3.10 and 1.20 %, respectively (Xiong *et al.*, 2009). As for the crude fat content, the two chicory seeds had substantially more than that of alfalfa seed, but the crude protein content is lower than alfalfa seed (Yang, 2004). The moisture in the two chicory seeds was markedly lower than that of alfalfa seeds or corn grains.

Table 2: Chemical composition of two varieties of chicory seeds (g/100 g)

Constituent	Puna chicory	Commander chicory	Alfalfa seed※	Corn grain#
Moisture	6.40±0.16[a]	6.65±0.23[a]	15.58	14.00
Ash	6.91±0.15[a]	6.80±0.20[a]	5.17	1.20
Crude protein	19.57±0.17[a]	19.20±0.13[a]	31.63	9.40
Crude fat	22.89±0.67[a]	22.56±0.23[a]	9.85	3.10
Crude fibre	25.68±0.19[a]	25.68±0.17[a]	N	1.20
Carbohydrate	31.66±0.42[a]	34.72±0.23[a]	N	N

Note: Values with different letters (a–b) in one row were significantly different (P<0.05). ※Yang, 2009; #China Feed No. 4-07-0278, Xiong, *et al*., 2009.

4.3 Amino acid composition: The amino acid (AA) composition of the two chicory seeds is present in Table 3. Except for the tryptophan, the total contents of the other amino acids in two varieties of chicory seeds were all calculated. The data showed that the AA contents in the seeds of Puna Chicory and Commander Chicory were higher in most cases than those of corn grain. The total AA content of Puna Chicory seeds except for the tryptophan is up to 14.91% and higher than that of Commander Chicory seeds or Alfalfa seeds.

Table 3: Contents of the amino acids of two varieties of chicory seeds (%)

Amino acids	Puna chicory	Commander chicory	Alfalfa seed※	Corn grain#
Arginine	1.54	1.04	1.68	0.38
Histidine	0.35	0.53	0.38	0.23
Isoleucine*	0.50	0.35	0.64	0.26
Leucine*	0.99	0.63	0.69	1.03
Lysine*	0.56	0.32	0.94	0.26
Methionine*	0.57	0.57	0.07	0.19
Cysteine	0.18	0.34	0.11	0.22
Phenylalanine*	0.70	0.43	0.61	0.43
Tyrosine	0.55	0.33	0.42	0.34
Threonine*	0.48	0.36	0.32	0.31
Valine*	0.67	0.46	0.53	0.40
Serine	0.48	0.36	1.13	N
Glutamic acid	3.58	2.14	1.73	N
Glycine	0.99	0.79	0.88	N
Alanine	0.65	0.47	0.38	N
Aspartic acid	1.58	1.11	0.73	N
Proline	0.54	0.38	0.52	N
Total	14.91	10.61	12.03	N

Note: * essential amino acid; ※Yang, 2004; #China Feed No. 4-07-0278, according to the methods by GB/T 18246-2000 of China national standard. The types and contents of amino acid in *Cichorium intybus* seeds were determined on a Hitachi-835-50 amino acid auto analyzer.

According to the WHO recommended pattern for an ideal dietary protein (WHO, 1973) the two chicory seeds are good sources of most essential amino acids, especially the Puna Chicory. For example, they all contain an appreciable concentration of lysine and

methionine compared to the conventional feed, corn grains, and the methionine contents are higher than that of alfalfa seed, but the lysine contents are lower than that of alfalfa seed. The acidic amino acids, glutamic and aspartic acid together make up more than one third of the total in Puna Chicory, one fourth of total in Commander Chicory. As is known, the lysine is easily damaged during the machine processing, and is often regarded as the limiting amino acid in cereal seeds. In this experiment, the overall quality of the protein in the chicory seeds may be also compromised by its relatively lower lysine content.

4.4 Fatty acid composition: The fatty acid composition of the crude lipid fraction of the two chicory seeds was relatively simple as shown in Table 4. Fatty acids accounted for 14.98 % of the dry weight of Puna Chicory seeds, 10.9 % of the Commander Chicory, respectively. Four fatty acids account for > 98.65% of the fatty acid total: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1n-9) and the essential fatty acid linoleic acid (18:2n-6). From the data, it could be easily found that the saturated fatty acids: palmitic and stearic acid were a lower ratio (about 9.9 %) of the total fatty acids. However, on the percentage basis, in each case, the essential fatty acid, linoleic acid was the predominant fatty acid, and it accounted for 77.25 % and 76.14 % of the total fatty acids in the seeds of Puna Chicory and Commander Chicory, respectively. Meanwhile, oleic acid (18:1n-9) was the second higher content (over 11.6 %) in the Puna and Commander Chicory. In virtue of the higher content and percentage of unsaturated fatty acids in the chicory seeds, the ratio of saturated to unsaturated fatty acids was very low, and it was only about 0.11 in the seeds of Puna Chicory and Commander Chicory, although the amount of linolenic acid (18:3n-3) was very low (0.18-0.20 mg/g dry weight).

Table 4: Fatty acid composition of two varieties of chicory seeds (mg/g dry weight)

Fatty acid	Puna chicory	Commander chicory
C14:0	0.09(0.01)	0.07(0)
C16:0	9.69(0.37)	7.23(0.04)
C16:1	0.20(0.01)	0.15(0)
C18:0	5.16(0.16)	3.84(0)
t11-C18:1	0.06(0.01)	0.07(0.01)
C18:1n-9	17.40(0.61)	12.78(0.01)
C18:2n-6	115.73(4.54)	83.68(1.68)
C18:3n-3	0.20(0)	0.18(0.01)
C20:0	0.65(0.01)	0.49(0)
C20:1	0.16(0)	0.12(0)
C20:2	0.06(0)	0.04(0.01)
C22:0	0.20(0.02)	0.17(0.01)
C24:0	0.20(0)	0.18(0)
Total	149.80	109.00

Note: Number in parentheses is the difference between the means.

4.5 Minerals: The mineral analysis showed that calcium was the highest element in the two

chicory seeds, followed by phosphorus. The concentrations of most minerals are not different between the two seeds except that Fe, K, Mg and Se contents in Commander Chicory seeds are significantly higher than those of Puna chicory seeds ($P < 0.05$). Compared with alfalfa seed, the two chicory seeds possess higher K, Ca, P, Mg, Cu, Zn, Mn and Mo contents except for Na and Fe (Table 5).

Table 5: Mineral contents in two varieties of chicory seeds were detected by ICP-MS and AAS.

Minerals	Puna chicory	Commander chicory	Alfalfa seed
Phosphorus (mg g^{-1})	9.43(0.50)[a]	9.45(0.15)[a]	1.16#
Potassium (mg g^{-1})	5.92(0.29)[a]	6.49(0.05)[b]	1.81(0.05)*
Calcium (mg g^{-1})	19.52(2.28)[a]	20.09(2.07)[a]	1.64(0.43)*
Magnesium (mg g^{-1})	3.59(0.14)[a]	3.96(0.22)[b]	1.21(0.31)*
Sodium (mg g^{-1})	0.31(0.15)[a]	0.39(0.14)[a]	1.70(0.09)*
Iron (mg g^{-1})	0.33(0.01) [a]	0.50(0.04) [b]	51.16(4.64)*
Copper (μg g^{-1})	23.00(3.12)[a]	22.33(3.33)[a]	6.64(0.33)*
Zinc (μg g^{-1})	60.83(7.51)[a]	55.50 (1.80)[a]	21.48(2.07)*
Manganese (μg g^{-1})	32.83(1.26)[a]	37.66(1.76)[a]	7.09(0.08)*
Molybdenum (μg g^{-1})	0.44(0.07)[a]	0.94(0.35)[a]	0.007#
Selenium (μg g^{-1})	0.14(0.02)[a]	0.25(0.08)[b]	N
Strontium (mg g^{-1})	0.24(0.01)[a]	0.24(0.01)[a]	0.003#
Cadmium (μg g^{-1})	0.071(0.006)[a]	0.073(0.003)[a]	0.04#

Note: Values are mean of three different determinations ± standard deviation; Values with the same letters in the same row (a–b) are not significantly different ($P<0.05$). Number in parentheses represents relative standard deviation (RSD). * Lucia *et al.*, 2003; # Yang, B. L., 2004; N: no detection

5 DISCUSSION

The root of the chicory plant is famous as an excellent substitute for coffee for a long time and it also offers extra health benefits that help cleanse the blood and improve the health of liver as a medicinal herb. Many evidences showed that leaves alcoholic extract (Akram *et al.*, 2006), natural root and root callus extracts (Rasheeduz and Ali, 1998) possessed potent hepatoprotective activity in rats against carbon tetrachloride induced hepatic damage. In line with that, the cichotyboside, a sesquiterpene glycoside from seeds of *Cichorium intybus* (Ahmed *et al.*, 2008) was verified to have antihepatotoxic activity, and so did the methanol fraction and one phenolic compound comparable to the standard drug Silymarin (Ahmed *et al.*, 2003). In China, chicory is mainly grown as animal forage, or for producing inulin (one kind of fructan) from its root.

The results in this experiment showed that the two varieties of chicory seeds had affluent levels of crude proteins, over 19 % of the dry weight, which is similar to that of *Achyranthes bidentata* (AB) seeds and 1.6-2.4 times higher than those of more conventional grains, such as wheat, rice, corn and barley (Massimo *et al.*, 2003). As for the crude fat, the content in chicory seeds is about 21-22 %, two times higher than that of AB seeds, except for the carbohydrate concentration relatively lower than AB seeds (Massimo *et al.*, 2003). However, the two chicory seeds contain over 30 % carbohydrate. In fact, chicory (roots and leaves) are recognized as a source of dietary fiber such as inulin and fructo-oligosaccharides, which have health-promoting properties (Bais and

Ravishankar, 2001). Amino acids analysis showed that chicory seeds are good sources of most essential amino acids according to the WHO recommended pattern for an ideal dietary protein, and the two chicory seeds were rich of various amino acids, except for lysine (0.32 % of Commander Chicory). Therefore, for more efficient use of their nutritional values, they should be mixed with other edible materials containing high levels of lysine in an appropriate ratio during the process for food or feed.

As for the fatty acids, surprisingly, all the ratios of linoleic acid (LA) were higher than 76 % of the total fatty acids in the two chicory seeds in the current study. It is known that LA is an unsaturated omega-6 fatty acid, a colorless liquid at room temperature. In physiological literature, it is called 18:2n-6. LA is one of two essential fatty acids that humans and other animals must ingest for good health because the body requires them for various biological processes, but cannot synthesize them from other food components. It is possible to prevent some diseases, such as obesity, heart disease, diabetes and cancer (Kapoor and Huang, 2006). In this study, we found the LA content accounts for 11.57 %, 8.37 % of the dry weight of Puna chicory seeds and Commander Chicory seeds, respectively. The ratio of saturated fatty acids to unsaturated was only about 0.11 in the two chicory seeds, and lower than 0.22 of AB seed (Massimo, *et al.*, 2003). Oleic acid is a mono-unsaturated omega-9 fatty acid found in various animal and vegetable sources. It may hinder the progression of adreno-leukodystrophy, and may contribute to boost memory (Moster and Borel, 1995). Therefore, this would indicate that chicory seeds are potentially a better source of nutritional edible oil for animal and human health.

Chicory contains relatively high levels of minerals (K, Ca, Mg, S, Zn and Na) that are essential for proper animal nutrition. Data from mineral analysis in this study are in accord with previous report, the results showed that the two chicory seeds have similar mineral levels in most cases detected in this experiment, and higher contents of K, Ca, P, Mg, Cu, Zn and Mn compared with those of alfalfa seeds (Lucia *et al.*, 2003), and these trace elements have been proved to have important pharmacological activity. Unlike most forage crops, it is an herb rather than either a grass or a legume, chicory has various beneficial effects on animal and human body as reported in a large number of studies, for example, grazing on chicory can decrease some internal parasites in livestock (Li and Kemp, 2005), and therefore has potential to reduce the use of antihelmintics. As for the safety of chicory is concerned, one toxicological evaluation verified that chicory root extract had no mutagenic activity in the Ames test (Barbara *et al.*, 2007). Measurements included clinical observations, body weights, food consumption, clinical pathology, gross necropsy and histology in the 28-day rat study, confirmed that there were no treatment-related toxic effects or adverse effects from chicory extract administered orally at 70, 350, or 1000 mg/kg/day, therefore, the NOAEL for the extract is 1000 mg/kg/day administered orally for 28 days. The chicory leaves extract were also confirmed without any toxic effects at acute and sub chronic toxicity levels, and free of any cytotoxicity towards rat's lymphocytes (Ilaiyaraja and Farhath Khanum, 2010). In fact, chicory extract is generally regarded as safe by the FDA and appears on the Everything Added to Food in the United States (EAFUS) list. However, the edibility of the chicory seed

proteins and the possible toxicity has not yet to be ascertained. In summary, chicory seeds contain high levels of nutritive components, such as total carbohydrate, especially the amino acids and higher ratio of essential fatty acids. Combining the history usage of chicory grown and consumed by human or animals, and data from the current study, it could suggest that the whole of the chicory plant including roots, leaves and seeds may be of significant importance in the formulation of various foods for human and animal diets although the seeds are not attracted much attention at present.

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