The nutritional ingredients and antioxidant activity of donkey milk and donkey milk powder

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Abstract The aim of this work was to shed light on the nutritional ingredients and antioxidant activity of donkey milk and powder. It indicated that basic components of donkey milk were closed to human milk, especially lactose. The fat content of donkey milk was lower than that of cow milk and human milk. The essential amino acids, fatty acids and taurine in donkey milk and powder were richer. The whey protein content of donkey milk was 49.08 g/100 g. The ratio of casein to whey protein in donkey milk and powder was 70.3:100. The cholesterol of donkey milk was 8.6 mg/100 g. Therefore, donkey milk and powder are typical low-fat and low-cholesterol foods. The precious values of donkey milk and powder are suitable calcium and phosphorus proportion, and strongly resistant to oxidation. In conclusion, donkey milk and powder are good supplementary of cow milk and powder, would be better for infant dietary.

Keywords Donkey milk · Donkey milk powder · Nutritional ingredients · Hydroxyl radicals scavenging ability · Superoxide anion radicals scavenging ability · Total antioxidant capacity · DPPH radicals scavenging ability

Introduction

In recent years, donkey milk attracts more attentions because of its similarities to human milk [1, 2], and unique advantage to be a supplementary of human milk. For example whey protein of donkey milk is high. Moreover Donkey milk has a lot of unsaturated fatty acids specially linoleic acid, and low content of fat and cholesterol, is rich in calcium and selenium [3–6]. Donkey milk also has strong antioxidant activity, retarding aging process, and is rich in kinds of immune-boosting substances to human immune system [7]. The medicinal value of donkey milk was recorded on both Valuable Prescriptions for Emergencies by Sun Simiao in the Tang Dynasty and Compendium of Materia Medica by Li Shizhen in the Ming Dynasty. In Peru, donkey milk is used to treat diseases like asthma, bronchitis, diabetes, anabrosis, gastritis, and even ease climacteric discomfort [8]. There are also many people treating tuberculosis and gastric ulcer by drinking donkey milk in Xinjiang, China [9, 10]. In some countries in Europe and America, donkey milk is not only the component of many biological products, a kind of health care products, accepted by more and more people. To summary, donkey milk has high nutrition value and wide medicinal value.

As an insoluble protein casein is hard to be digested and can form larger and harder clots in baby's stomach. However whey protein, belonging to soluble protein, only forms small and soft clots in infant's stomach, being easy to be digested and absorbed. Furthermore whey protein contains a variety of biological activity proteins, enzymes, peptides, immune factors and growth factors, which plays an important role in growth and mental development of human. The nutritional value and biological potency of whey protein are very high, therefore it is praised as "the king of protein". The proportion of whey protein and casein

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in milk relates to the nutrition and absorption for body. In the body, lactose can be converted to glucose which provides energy and participates in the composition of tissues and organs, and galactose which can synthesize glycolipid, one of basic materials for neural tissue, and has a vital role in the development of infant nervous systems. In addition, lactose can promote the proliferation of intestinal probiotics, inhibit the growth of spoilage bacteria, improve the absorption of calcium, phosphorus and other mineral elements, and boost the quality and strength of skeleton.

The yield of donkey milk is relatively low. A donkey only produces 100–150 kg in one lactation period for processing and usage. But donkey milk has special value in scientific researches and breeding efficiency [11]. In China, donkey mainly locates in the northwest region which geographical location is relatively remote and economical development is relatively lagging. Because of the difficulties of milk acquisition, preservation, transportation and ethnic customs, donkey milk's development has not been taken seriously. The nutritional values of donkey milk have not been well studied, and systemic researches are still blank. Nowadays, there is no acceptance criteria of donkey milk, therefore appropriate standards are required to be set for standardizing the donkey milk market, strengthening the donkey milk product quality inspection and market regulation, maintaining rights of consumer and businesses.

Therefore, the development and utilization of donkey milk has great economic value and social value. However the nutrients and functions of donkey milk are not well studied and basic data need to be collected, which will restrict the development of donkey milk industry. Thus, the donkey milk powder was obtained in the study. The composition of conventional nutrients, amino acids, fatty acids, and antioxidant activity of donkey milk and powder were studied. It would shed light on the development of donkey milk industry, improvement of the comprehensive utilization ratio of donkey milk resources, arousing the enthusiasm of raising donkey, increasing the income of farmers and herdsmen, and the promotion of economic development.

Materials and methods

The sources and collections of sample

The donkey milk came from 20 heads and 38 ± 4 month old donkeys in Tianjin LongTian breeding company. Firstly, the milking system was cleaned in 40 °C water. Then, the nipple was cleaned gently and quickly, and stimulated in the order of elution, medicated bath, dry and massage. At last milking was proceeded. The milking was divided into pre-milking and milking. The operation of pre-

milking was to observe and touch the breast whether there were the symptoms of red, swelling, fever, pain, trauma and so on before milking. Milking was used the “S” type cups method. The cups were set on nipples and the action was quick and accurate to avoid air entry to the milking system. It was ensured that 200 mL milk was kept in breast after milking. The milk was processed as donkey milk powder by the Zhongke Yun Health Co., Ltd. The indexes of donkey milk and powder were determined as following.

Measurement of nutritional ingredients

The nutritional ingredients were basic components, essential amino acids, casein, whey protein, fatty acids, mineral elements, taurine, and so on. The detection criteria were the National Standards of the People's Republic of China.

Determination of antioxidant capacity

Hydroxyl radicals scavenging ability

It was operated according to the methods of Shi et al. [12], and the instructions of hydroxyl radical determination kit (Nanjing Jiancheng Company). Each sample was performed three biological repetitions.

Superoxide anion radicals scavenging ability

It was operated according to the instructions of resistance to superoxide anion radicals determination kit (Nanjing Jiancheng Company). Each sample was performed three biological repetitions.

Total antioxidant capacity

It was operated according to the instructions of total antioxidant capacity determination kit (Nanjing Jiancheng Company). Each sample was performed three biological repetitions.

DPPH radicals scavenging ability

The determination method was according to Shi et al. [12], Qu et al. [13] and Ene-Obong et al. [14].

Results and discussion

Donkey milk has become more and more attractive nowadays as a commercial product because of its nutritional values and functional properties [15]. Studies have shown that donkey milk is a kind of effective adjuvant for allergy of milk protein and a variety of food intolerant patients [16].

Donkey milk can be used as a supplementary of human milk, and is the lucky star for infants who are a lack of human milk, especially for those who have allergic reaction of protein when drinking cow milk [16, 17]. So the development of donkey milk as a basic material for infant milk powder or food will inevitably become a trend. We have successfully gained the high quality donkey milk powder.

The basic components of donkey milk and donkey milk powder

The basic chemistry components of donkey milk and donkey milk powder include protein, fat, lactose, moisture and ash. As shown in Table 1, the moisture content of donkey milk and milk powder were 92.5 and 3.63 g/100 g respectively. The content of protein and ash in donkey milk were 2.14 and 0.42 g/100 g. Compared with cow milk, the protein and ash content of donkey milk were closer to human milk. But the average fat content of donkey milk was 1.2 g/100 g, which was 34.3% of human milk or 37.5% of cow milk. Similarly, the fat content of donkey milk powder was also lower than cow milk powder.

Lactose is disaccharide and primary energy resource. Donkey milk contained lactose 5.9 g/100 g, approaching human milk. The lactose content of donkey milk powder was more than that in cow milk powder. Thus donkey milk and donkey milk powder were fitter eating for infants. The G1, blood glucose index, for short raw sugar index, is an effective index for postprandial blood sugar reaction. The G1 of food less than 55 is called low-G1 food. The G1 of lactose was 46, which was 43.8% of maltose or 46% of glucose. Lactose was low-G1 food. Therefore the diabetic can drink donkey milk and milk powder properly.

Biological value of protein of donkey milk and donkey milk powder

The kind of essential amino acids

Donkey milk contained nine kinds of essential amino acids and their proportions were close to human milk, except

isoleucine and lysine (Table 2). In donkey milk, the essential amino acids accounted for 44.3% of total amino acids. In human milk and cow milk, its content was 43.0 and 41.4%. It was shown that donkey milk had a good amino acids proportion and appropriate content, and fitted human body necessarily. In donkey milk powder, nine kinds of essential amino acids content were more than cow milk powder.

The content of taurine in donkey milk and donkey milk powder

Taurine is a kind of sulfur-containing and non-protein amino acids. Taurine is free in vivo and do not synthesize proteins. The activity of cysteine sulfuric acid carboxylase synthesizing in body is low. So taurine which satisfy to the needs of body, mainly depend on food. Taurine can promote infant brain development, nerve conduction and visual perfection. In addition, taurine is conductive to the use of calcium, the development of cardiac muscle, and fat digestion. It is the essential nutrient in infant growing. Besides, it can promote pituitary hormone secretion and pancreas excitation, and then improve the action of endocrinium system, regulate body metabolism, enhance body immunity and help fight fatigue.

The system that generates the enzyme in infant is developmental immaturity and can't synthesize taurine, so if taurine satisfy the needs of body, it must be attracted from food. If taurine intake is not enough, it will result in problems such as poor growth and mental retardation. The content of taurine in donkey milk was 0.87 mg/100 g, lower than that in human milk (6.117 mg/100 g) (Table 2). However, the content in donkey milk was nine times higher than that in cow milk (0.097 mg/100 g). The content of taurine in donkey milk powder was 10.87 mg/100 g, lower than that in cow milk powder. Clearly, donkey milk is more suitable to be the supplementary of human milk.

Table 1 The content of basic component

	Donkey milk	Donkey milk powder	Cow milk ^a	Cow milk powder ^b	Human milk ^a
Moisture	92.5	3.63	87–88	5	87.8
Ash	0.42	4.2	0.7	6	0.25
Protein	2.14	20.2	3.0	34	1.2
Fat	1.2	6.34	3.2	26	3.5–4.0
Lactose	5.9	62.7	4.6	38	6.3–7.0

The unit of basic component content is g/100 g

^aThe data of cow milk and human milk come from Malacarne et al. [25]

^bThe data of cow milk powder come from Zhang [26]

Table 2 The composition and content of essential amino acids, taurine, and protein

	Donkey milk	Donkey milk powder	Cow milk	Cow milk powder	Human milk
Leucine	10.1	1.79	8.7	1.17	10.1
Isoleucine	3.9	0.78	4.2	0.76	5.8
Valine	5.0	1.01	4.8	0.75	6.0
Lysine	7.3	1.42	8.1	0.90	6.2
Methionine	3.4	0.40	1.8	0.27	1.8
Tryptophan	1.7	0.16	1.5	0.162	1.8
Threonine	4.5	0.79	4.5	0.65	4.6
Phenylalanine	5.0	0.99	4.8	0.49	4.4
Histidine	3.4	0.54	3.0	0.23	2.3
Total essential amino acids	44.3	7.88	41.4	5.382	43
Taurine	0.87	10.87	0.097	30	6.117
Casein	41.3	41.3	2.8	Not found	0.5
Whey protein	49.08	46.46	0.7	Not found	0.7
The ratio of casein to whey protein	70.3	70.3	400	Not found	71.4

The above data is the ratio of essential amino acids to total amino acids (%). The unit of taurine content is mg/100 g. The unit of casein, whey protein and the ratio of casein to whey protein is g/100 g. The essential amino acids ratio of cow milk and human milk come from Malacarne et al. [25]. The essential amino acids ratios of cow milk powder come from Zhang [26]. The taurine content of cow milk and human milk come from Pipes [27]. The taurine content of cow milk powder comes from Nestle milk powder in supermarket. The protein content of cow milk and human milk come from Fox et al. [28]

The rate of whey protein

The functional protein focuses on whey protein. The normal way for evaluating the protein nutrition's value is to compare biological value (BV). The BV of egg protein is 100. Compared with BV of egg protein, whey protein's BV is 104, casein's BV is 77. According to Table 2, the ratio of casein to whey protein in donkey milk was 70.3:100, which was close to human milk (71.4:100). In cow milk, the ratio of casein to whey protein is 400:100. The whey protein accounted for 49.08% of total protein in donkey milk. In cow milk, the proportion of whey protein in total protein was 0.7%. Therefore, donkey milk belongs to whey protein dairy products. One of the advantages is fitted for infants. However, we have not found the data of cow milk powder, so there was no discussion.

The content of fatty acids and cholesterol in donkey milk and donkey milk powder

Monounsaturated fatty acids are significant for reducing cardiovascular disease. For example, oleic acid can boost the fat globule's mobility and its metabolic capability. Polyunsaturated fatty acids have all kinds of biological functions. The acids are the primary elements for cytomembrane, and it can prevent cardiovascular disease and promote body development. Donkey milk is low in fat, but the proportion of monounsaturated fatty acids and

polyunsaturated fatty acids was high (Table 3). The percentages of unsaturated fatty acids of total fatty acids in donkey milk and milk powder were 49.1 and 40.5% respectively, exceeding human milk (45.2%) which was significantly higher than cow milk (32.0%).

Linoleic acid (C18:2) and linolenic acid (C18:3) are necessary for human body, but they can not synthesize in body and must be offered by food, so they are called essential fatty acid (EFA). EFA is the significant constituent part for phospholipid and participate in turnover and metabolism in body. EFA can reduce the blood cholesterol content, soften the blood vessel, prevent the atherosclerosis and so on [10]. If human body lacks EFA, it will affect body's metabolism and cause growth retardation, dysgenesis, skin injury, and diseases on blood vessel, viscera, nervous system and vision. The linoleic acid accounted for 19.3% of total fatty acids (TFA) in donkey milk, 6.4% in human milk, 2.9% in cow milk. The content of linolenic acid was 4.3% of TFA in donkey milk, 1.7% in human milk and 1.1% in cow milk. The content of cholesterol was 33.8 mg/100 g in donkey milk powder. The content of cholesterol in donkey milk was 8.6 mg/100 g, 78.2% of human milk (11 mg/100 g) and 57.3% of cow milk (15 mg/100 g). So donkey milk is a typical low-fat, low-cholesterol and high-quality food, which is suitable to drink for those who have cardiovascular and cerebrovascular diseases, and people with obesity.

Table 3 The content of unsaturated fatty acids and cholesterol

	Donkey milk	Donkey milk powder	Cow milk ^a	Cow milk powder ^b	Human milk ^a
Oleic acid	135	872	26.3	246	33.6
Linoleate	121	736	2.9	519	6.4
Linolenic acid	27.2	159	1.1	78	1.7
Palmitoleic acid	15.9	110	1.7	119	3.5
The proportion of unsaturated fatty acids to fatty acids	49.1%	40.5%	32.0%	Not found	45.2%
Linoleate: Linolenic acid	4.45:1	4.63:1	2.63:1	6.65:1	3.73:1
Cholesterol	8.6	33.8	15.0	Not found	11.0

The unit of the above content is mg/100 g

^aThe data of cow milk and human milk come from Malacarne et al. [25]

^bThe data of cow milk powder come from Zhang [26]

Table 4 The mineral and vitamin content

	Donkey milk	Donkey milk powder	Cow milk ^a	Cow milk powder ^b	Human milk ^a
Ca, mg/100 g	68.9	866	122	1000	33
P, mg/100 g	41	577	93	600	14
Mg, mg/100 g	8.89	67.7	12	40	4
K, mg/100 g	65.7	615	152	864	51
Na, mg/100 g	37	237	58	350	15
I, µg/100 g	9.6	35.2	21	60	7
Fe, mg/100 g	0.15	0.40	0.08	5.6	0.20
Zn, mg/100 g	0.14	1.92	0.53	4.8	0.38
Vitamin A, µg/100 g	160	15.9	126	350	190
Vitamin C, mg/100 g	Not detected	30.2	1	32.4	5
Vitamin B ₁ , µg/100 g	12	35	45	320	17
Vitamin B ₂ , µg/100 g	26	176	160	400	36
Vitamin D	30 µg/100 g	Not detected	2.0 IU/100 g	5.8 µg/100 g	1.4 IU/100 g

^aThe data of cow milk and human milk come from Roginski et al. [29]

^bThe data of cow milk powder come from Nestle milk powder in supermarket

The mineral and vitamin content of donkey milk and donkey milk powder

If body ingests too much calcium, it can cause anorexia, constipation, blood calcium symptoms. When the intake of phosphorus is too much, it will form calcium phosphate excreting in vitro and result in loss of calcium. So the appropriate ratio of calcium and phosphorus helps calcium absorption and enhances physical fitness. Donkey milk contained a variety of mineral elements, and the content of calcium (Ca) and phosphorus (P) was 68.9 and 41 mg/100 g respectively, lower than that in cow milk (122 and 93 mg/100 g), but higher than that in human milk (33 and 14 mg/100 g) (Table 4). The optimum ratio of calcium and phosphorus in infant food is 1.5–2.0:1. The calcium phosphate ratio of donkey milk was 1.68:1, and the ratios in human milk and cow milk were 2.36:1 and 1.31:1

respectively. Therefore, the calcium phosphorus ratio of donkey milk was better than that of cow milk. The calcium phosphorus ratio of donkey milk powder was 1.50:1. Hence, calcium phosphorus ratio in donkey milk powder was also appropriate. In addition, donkey milk and donkey milk powder also contain V_A, V_{B1}, V_{B2} and other vitamins.

From the perspective of nutrition needs of infant, the ratio of whey protein in total protein and lactose content were higher (Tables 1, 2), which were the two characteristics and bright spots of donkey milk and donkey milk powder. It will meet the nutritional requirements of certain consumer groups with lactose or cow milk protein intolerance. Donkey milk was remarkable resemblance to human milk in essential amino acids and fatty acids (Table 2, 3). It was in accordance with the results of Polidori and Vincenzetti [18], and Medhammar et al. [19]. The content of proteins and fat in donkey milk was lower

Table 5 The antioxidant ability of donkey milk and cow milk

	1	2	3	4	5	6	7	8	Cow milk
Superoxide anion radicals scavenging ability (U/L)	55.397 ± 1.221	50.423 ± 0.816	58.035 ± 0.904	58.075 ± 0.585	57.101 ± 0.922	50.223 ± 0.651	57.054 ± 0.216	57.307 ± 0.313	556.507 ± 24.276
Hydroxyl radicals scavenging ability (U/mL)	1487.835 ± 1.458	1430.499 ± 5.891	1480.061 ± 5.519	1471.800 ± 1.458	1476.659 ± 6.889	1488.321 ± 3.034	1477.145 ± 0.842	1425.154 ± 1.458	1054.980 ± 16.044
Total antioxidant capacity (U/mL)	14.500 ± 0.384	15.267 ± 0.315	16.542 ± 0.364	16.368 ± 0.125	16.578 ± 0.291	13.956 ± 0.038	13.933 ± 1.387	14.467 ± 0.385	Not detected
DPPH radicals scavenging rate (%)	20.636 ± 0.239	22.724 ± 0.179	23.567 ± 0.197	22.417 ± 0.139	19.533 ± 0.412	19.582 ± 0.296	19.576 ± 0.348	23.390 ± 0.502	63.10 ± 0.99

The sampling time of the eight donkey milk samples was December 19, 2016. The ear number of 1–8 donkeys is respectively 08316 black, 06236 black, 13344 black, 08544 black, 05162 black, 07418 black, 05002 black and 04148 black

than cow milk, and that in donkey milk powder was also inferior to cow milk powder (Table 1). It seems that the lower nutrient concentrations appear to be a defect, but in fact, the lower content is just suitable for infant, especially when kidney function has not yet developed, which can effectively reduce the burden to internal organs, so that nutrients can be easier digested and absorbed. Donkey milk can promote bone growth of infant [20]. It makes more contributions to the growth and development of infant. So donkey milk and donkey milk powder are more suitable for baby than cow milk and powder. Donkey milk is a sort of precious dairy resource with low fat, cholesterol, suitable calcium and phosphorus proportion (Tables 1, 3, 4). The ingestion of donkey milk versus cow milk helps to maintain a normal weight and normal levels of cholesterol and triglycerides in mice [21]. It was revealed that donkey milk can treat hardening of the arteries, and promote the rehabilitation of patients with cardiovascular diseases [20].

The antioxidant ability of donkey milk and donkey milk powder

The OH, one of the free radicals produced by body metabolism, is the most active reactive oxygen species in body, and has very strong oxidization capacities. The ability to remove the OH is an important index for the detection of free radicals antioxidant activity in vitro. The superoxide anion radical is also one of the important radicals in body and has strong oxidization, so the ability to scavenge superoxide anion radicals is often detected to evaluate antioxidant activity [22, 23]. Therefore, the scavenging ability of hydroxyl radicals and superoxide anion radicals, total antioxidant capacity and DPPH radicals scavenging rate were chosen to measure the antioxidant ability of donkey milk and donkey milk powder.

The range of superoxide anion radicals scavenging ability in donkey milk was from 50.223 ± 0.651 to 58.075 ± 0.585 U/L (Table 5). The maximum of hydroxyl radicals scavenging ability was 1488.321 ± 3.034 U/mL, the minimum of it was 1425.154 ± 1.458 U/mL. The minimum value of total antioxidant capacity of donkey milk was 13.933 ± 1.387 U/mL, and the maximum value of it was 16.578 ± 0.291 U/mL. The minimum value of DPPH radicals scavenging rate was $19.533 \pm 0.412\%$, and the maximum value of it was $23.567 \pm 0.197\%$. In cow milk, superoxide anion radicals scavenging ability was 556.507 ± 24.276 U/L, hydroxyl radicals scavenging ability was 1054.980 ± 16.044 U/mL, the total antioxidant capacity was not measured, and the DPPH radicals scavenging rate was $63.10 \pm 0.99\%$.

In Table 6, it was clearly shown that superoxide anion radicals scavenging ability of donkey milk powder was 23.088 ± 0.596 U/g, but the index of XinJiang donkey

Table 6 The antioxidant ability of donkey milk powder

	Donkey milk powder	Xinjiang donkey milk powder
Superoxide anion radicals scavenging ability (U/g)	23.088 ± 0.596	Not detected
Hydroxyl radicals scavenging ability (U/mg)	113.025 ± 0.375	46.257 ± 4.078
Total antioxidant capacity (U/mg)	0.258 ± 0.020	0.055 ± 0.006
DPPH radicals scavenging rate (%)	14.353 ± 0.308	81.207 ± 4.533

The donkey milk powder was as control buy from Xinjiang Barry Kun Hua Lin Dairy Co., Ltd

milk powder was not detected. The hydroxyl radicals scavenging ability was 113.025 ± 0.375 U/mg in donkey milk powder, while the index of XinJiang donkey milk powder was only 46.257 ± 4.078 U/mg. The total antioxidant capacity of donkey milk powder and XinJiang donkey milk powder was 0.258 ± 0.020 and 0.055 ± 0.006 U/mg respectively. According to these indexes, the antioxidant ability of donkey milk powder was higher than XinJiang donkey milk powder. In addition, the DPPH radicals scavenging rate of donkey milk powder was $14.353 \pm 0.308\%$, while that of XinJiang donkey milk powder was $81.207 \pm 4.533\%$. It may be related to the additions of wild sour dates and other plants in XinJiang. To conclusion, the antioxidant capacity in donkey milk was higher than that in cow milk, and the antioxidant activity of donkey milk powder was better than that of Xinjiang donkey milk powder (Tables 5, 6). The results were consistent with asinine whey exhibited radicals scavenging activity [24].

In a word, the lactose content of donkey milk is high and it can promote the beneficial microbial proliferation of intestinal tract. Because of high ratio of whey protein, donkey milk's protein in infant stomach can form small and soft clot, which is easy to digest and absorb. The fat and cholesterol content of donkey milk and donkey milk powder is low and the essential fatty acids content of human body is high, which is suitable for cardiovascular disease, gallstone disease and obesity patients, and the elderly. In addition, the calcium phosphorus ratio of donkey milk is suitable. After drinking, the burden to kidney is lighter, which is suitable for the imperfection of renal function in infant. In general, the difference between donkey milk and human milk is small. The nutritive value and digestibility of donkey milk powder are better than cow milk powder. The antioxidant capacity of donkey milk and powder is high. For these reasons, so far donkey milk and powder are considered as the best mammalian milk for infant especially in case of cow milk allergy, and diet for cerebrovascular diseases and diabetes mellitus patient.

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