J Food Sci Nutr
Vol 12, p 89~94 (2007)

J Food Science and Nutrition

Nutritional Quality of Dried Pig Placenta

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Abstract

Nutrients and hormone levels of dried pig placenta were studied. Placentas were freeze-dried (FD), oven-dried at 60 (OD-60), and 90°C (OD-90) and then crushed by a blender into small pieces. FD and OD-60 pig placenta had a higher moisture content than did OD-90, with no difference between FD and OD-60. There were no large differences in compositions of crude protein, crude fat, and crude ash of dried placenta among the treatments and the contents of K, Fe, and α-tocopherol were highest in FD ($p<0.05$). Glutamine and glycine were the most abundant amino acids in all dried placenta and tyrosine was highly retained in FD placenta, compared with OD ($p<0.05$). Estradiol was the major sex hormone, followed by progesterone and testosterone in all dried placentas. Antibiotics including amoxicillin, sulfamethazine, tylosin, and chlorotetracyclin were not detected from the pig placentas tested. These results demonstrate that placenta is a good biomaterial with high nutritional quality, and that freeze drying is superior to oven drying for processing pig placenta.

Key words: pig placenta, nutritional quality, drying methods

INTRODUCTION

Human placenta has been used as a medicine in Korea and China for over 1,400 years due to its anti-aging and cosmetic properties (1). Human placental extract is a reservoir of a large number of bioactive molecules such as hormones, proteins, lipids, nucleic acids, glycosaminoglycan, amino acids, vitamins, and minerals (2). Also it contains many unknown compounds and is believed to have various bioactivities including inhibition or delay of aging, inflammation, sunburn, mutagenicity, anaphylacticity, and oxidation (1). Moreover the placenta has been known to have no toxic effects (2).

Pig placenta has been also used partially for a source of biomedical material. However, approximately 1,500 tons of pig placentas in Korea are produced and destroyed each year (3). Therefore, it is worthwhile found useful properties for pig placenta, so it can be a value-added product with economic benefits for industry while reducing environmental pollution. One of the problems to be solved before introducing biomaterials to industry is to find ways of preserving nutrients or bioactive components during processing, especially for heat-sensitive materials.

Conventional heat drying is the most commonly used dehydration method in the food and chemical industry (4). It provides quick dehydration of samples economically, but may change nutrient content. Freeze drying is one of the best alternatives, but is also the most sophisticated dehydration method (5). Freeze-dried foods are considered higher quality than any other dehydrated products, mainly because they can be reconstituted with water rapidly to products closely resembling the original food. Another important advantage is that freeze drying is accomplished at relatively low temperatures, thereby protecting heat-sensitive biological compounds. Freeze drying provides dried products of porous structure (6,7) and little or no shrinkage, superior taste retention, and better rehydration properties, compared with the products of other drying processes. However, its advantages are directly offset by its corresponding high cost for processing.

Therefore, the nutritional qualities of pig placenta are critical to evaluating its potential as biomaterial. Little research has been done to determine the influence of drying method on the nutritive value of pig placenta. The aim of the present study was, therefore, to asses the influence of drying methods (i.e. oven drying (OD) and freeze drying (FD)) on the nutritive value of pig placenta and compare it with that of human placenta, which has already been reported.

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MATERIALS AND METHODS

Sample preparation

Pig placentas were collected immediately after parturition from 200 pigs of Landrace×Yolkshire×Duroc cross breeds (approximately 120 kg of body weight) reared in Nonghyup Pig Breeding Institute (Youngkwang, Korea). All dust and blood of the placenta were washed with distilled water and dehydrated with paper towels. This was homogenized with a blender (model KSB5, Kitchenaid Co., St Joseph, Michigan, USA) for 5 min at 20,000 rpm.

Drying

The blended pig placenta was frozen at -50°C and then freeze-dried using a freeze drier (model FD-5508, Ilsin Engineering. Co., Siheung, Korea) for 72 hrs. The drying was conducted under constant vacuum conditions. The placenta was also oven-dried at 60 and 90°C for 12 hrs until a constant weight was obtained. These freeze- and oven-dried samples were homogenized (12,000 rpm for 3 min) and the powder was sterilized with ultraviolet irradiation for 3 min by means of a Sterilaire Series unit (UVP Ultra-violet Products, Cambridge, UK) at an intensity of 250 microW/cm^2 at a distance of 30 cm and kept in a desiccator for 2 hrs at room temperature and stored in aseptic condition at -78°C until used.

Proximate analysis

Each dried sample was analyzed for moisture, crude protein, crude fat, and crude ash an AOAC method (8).

Amino acid composition

The amino acid composition of dried pig placenta was determined after acid hydrolysis with 6 N HCl at 110°C. After hydrolysis the contents were derivatized with phenylisothiocyanate (PITC, Sigma Co., St. Louis, MO, USA) following the method of González-Castro et al. (9) and the amino acid composition was measured using L-8500 analyzer (Hitachi, Tokyo, Japan). Tryptophan assay was carried out with 3 M mercaptoethanesulphonic acid hydrolysis of the placenta at 110°C (10).

Minerals

Dried pig placenta (5~20 g) was ashed at 450~550°C and analyzed for mineral content using the method of Han et al. (11) using an inductively coupled plasma spectrometer (PS1000, Teledyne, Leeman Labs., Inc., Kreuztal, Germany). The spectrum wavelength was 393.36 nm for Ca, 177.49 nm for P, 588.99 nm for Na, 766.49 nm for K, 259.94 nm for Fe, 324.75 nm for Cu, 213.85 nm for Zn, 257.61 nm for Mn, and 280.27 nm for Mg.

α-Tocopherol

α-Tocopherol was analyzed according to the method of Elmastas et al. (12). Methanolic extract of placenta (50 mg) was mixed with 6 mL of pyrogallol (6% in 95% ethanol, v/v) and 4 mL of 60% potassium hydroxide aqueous solution and saponified at 70°C for 20 min. Distilled water (15 mL) was added and extracted with 15 mL of n-hexane. The n-hexane layer was washed with distilled water after vigorous vortexing and evaporated using a rotary evaporator (Laborata 4001, Heidolph WB, Schwabach, Germany). The residue was redissolved in 5 mL of n-hexane and filtered through a 0.45 mm filter paper prior to HP 1100 HPLC injection. The mobile phase was methyl alcohol/acetonitrile (2:8 (v/v)) at a flow rate of 1.0 mL/min and UV detection at 295 nm. The content of each α-tocopherol was calculated on the basis of the calibration curve of commercial α-tocopherol (Sigma Co.).

Sex hormones

Estradiol, progesterone, and testosterone were measured by method of Kwon et al. (13) using ACTIVE total estrogens ^{125}I RIA kit (ICN, Costa Mesa, California, USA), ACTIVE total progesterone (DSL 3900, Webster, TX, USA), ACTIVE testosterone ^{125}I RIA kit (DSL-4000, Webster) and gamma counter (cobra II, Packard instruments Co., Meriden, CT, USA).

Antibiotics residue

Amoxicillin, sulfamethazine, tylosin, and chlorotetracyclin were assayed by the Charm II test (Charm Blue/Yellow test, Charm Science Inc., Lawrence, MA, USA). Placenta samples for antibiotic analysis were prepared by homogenizing the sample at 12,000 rpm with Charm II extraction buffer provided with the test kit. The sample mixture was heated at 80°C for 20 min and placed in an ice water bath for 10 min and centrifuged for 10 min at 3,000×g. The pH of the supernatant was measured and adjusted to 7.5 using 0.1 N HCl. The sediment was homogenized, mixed with the scintillation fluid with subsequent of the scintillation counts according to the method of Charm Science Inc. (Lawrence, MA, USA). Concentrations of antibiotics in placentas were calculated from calibration curves obtained by different concentrations of antibiotic standards added to antibiotic-free tissues (14).

Statistical analysis

All measurements were performed in triplicate and Analysis of variance (ANOVA) was conducted by the General Linear Model procedure using SAS software (15). Duncan's multiple range test was used to identify the differences between mean values for the treatments.

Mean values and standard errors of the mean (SEM) were reported at $p<0.05$ level.

RESULTS AND DISCUSSION

Proximate composition

Proximate composition of raw, freeze-dried (FD), and oven-dried placenta at 60 (OD-60) and 90°C (OD-90) are shown in Table 1. Moisture and crude protein of raw placenta were 90.02 and 5.97%, respectively. After FD, the moisture and fat were higher than those of OD-60 and OD-90. Oven drying, at both 60 and 90°C, resulted in a similar composition of protein, fat, and ash except the moisture which was significantly decreased in OD-90, from 5.11 to 1.76%. From this result, the pig placenta was mostly composed of protein and was low in fat. The protein composition was higher in OD-90 (78.88%) than that of FD (76.50%), yet no significant difference was found. Phuapradit et al. (16) reported that protein composition of human female and male placenta showed 81.62 and 80.06%, respectively, when heat dried at 80~100°C for 24 hrs.

Mineral and α-tocopherol

A range of both macro (Ca and Mg) and micro (Fe, Cu, Mn, and Zn) mineral nutrients are important in human nutrition (17). The mineral and α-tocopherol contents of raw and dried pig placenta are shown in Table 2. Ca, P, K, and Na were the majority of the minerals in raw placenta. FD placenta showed higher contents of most minerals than those of OD placenta. The Fe contents of FD, OD-60, and OD-90 were 1238.72, 330.18, and 319.39 ppm, respectively. Since the Fe is a nutrient that is often deficient in diets (11), it is important to note that FD pig placenta was significantly higher in Fe. Na, Ca, Fe, and K of heat-dried (80~100°C) human placenta were 980.00~10,202.20, 1,040.00~10,418.00, 1,525.50~8,590.90, and 2,287.70~8,367.10 ppm, respectively (16). Dried pig placenta was 5 times higher in Ca content than that of human dried placenta. This suggests that dried placenta can be a good source of Ca for fortifying food products. The intake ratio of Ca and P for humans is recommended at 1:1 considering the absorption and use of Ca and P (11). The ratio of pig placenta with FD, OD-60, and OD-90 was 1.05, 0.99, and 0.90, respectively. Manganese is an enzyme cofactor, participating in phosphorylation and fatty acid synthesis (11,18). α-Tocopherol is effective as an antioxidant agent (19) and it was highly affected by drying method. FD showed the highest content of α-tocopherol, followed by OD-60 and OD-90.

Amino acid composition

Amino acid composition of raw and dried pig placenta

Table 1. Proximate compositions (%) of pig placenta dried by different methods

	Raw placenta	FD[1)]	OD-60[2)]	OD-90[3)]	HDHP[4)]
Moisture	90.02±1.308[5)]	5.96±0.906[a]	5.11±1.729[a]	1.76±0.814[b]	6.79
Crude protein	5.97±0.857	76.50±2.914	76.24±3.279	78.88±2.949	80.84
Crude fat	0.78±3.072	5.30±1.458	4.94±0.432	4.72±0.313	1.59
Crude ash	0.39±0.154	5.94±1.600	6.04±1.475	5.98±1.314	5.75
Drying time (hrs)	-	72	12	12	24

[1)]FD, Freeze drying; [2)]OD-60, oven drying at 60°C; [3)]OD-90, oven drying at 90°C; [4)]HDHP, mean value of heat dried (80~100°C) female and male human placenta (16); [5)]Mean±standard deviation.
[a,b]Means with different letters within the same row, except for raw placenta column, differ significantly ($p<0.05$).

Table 2. Mineral (ppm) and α-tocopherol (IU/kg) contents of pig placenta dried by different methods

	Raw placenta	FD[1)]	OD-60[2)]	OD-90[3)]	HDHP[4)]
Ca	700.00±0.000[5)]	7,800.00±108.563	7,267.70±272.881	7,000.00±1,177.70	1,906.75
P	800.00±0.035	7,400.00±408.248	7,300.00±346.410	7,700.00±115.470	2,803.50
Na	1,086.96±848.555	10,239.98±3,823.050	9,600.62±1,661.99	9,510.77±3,808.21	10,310.00
K	839.96±693.542	11,145.15±3,478.690	10,834.79±1,636.41	11,370.64±1,721.58	8,479.00
Fe	39.53±10.138	1,238.72±178.377[a]	330.18±12.309[b]	319.39±19.420[b]	1,010.00
Mg	62.32±28.637	611.95±12.863	590.87±69.674	599.36±42.551	382.90
Zn	8.87±4.939	66.31±3.078	60.27±4.041	68.36±13.355	47.15
Cu	2.94±2.260	8.11±3.142	9.94±1.107	11.14±2.310	43.80
Mn	2.11±1.039	6.81±1.219[a]	5.22±0.439[b]	5.43±0.324[b]	1.20
α-Tocopherol	1.25±0.070	8.56±0.087[a]	6.54±0.135[b]	6.01±0.174[c]	5.45 (μg/g)

[1)]FD, Freeze drying; [2)]OD-60, oven drying at 60°C; [3)]OD-90, oven drying at 90°C; [4)]HDHP, mean value of heat dried (80~100°C) female and male human placenta (16); [5)]Mean±standard deviation.
[a,b]Means with different letters within the same row, except for the raw placenta column, differ significantly ($p<0.05$).

is shown in Table 3. Among the essential amino acids, leucine, lysine, and arginine comprised 5.48~5.62%, 4.14~4.72, and 4.48~5.09% of the dried placenta. Those amino acids were the major component regardless of drying methods. Marshall et al. (20) and Korhonen et al. (21) reported that the amino acids which are considered to be physiologically beneficial are arginine, glutamine, histidine, lysine, taurine, tyrosine, and tryptophan. Guo et al. (22) determined that autoclaving (120 or 132°C for 60 min) reduced the levels of available lysine and methionine of sodium caseinate by about 5 and 10%, respectively. They assumed that destruction of lysine was probably due to protein-protein interactions involving ε-NH_2 groups with the amide groups of asparagines and glutamine and the loss of methionine may be due to damage to the methyl-thiol group by alkylation or other interactions during heating. In the present study, the lysine and methionine were reduced in proportion to drying temperature, but not significantly. Tyrosine of freeze-dried placenta was significantly higher than that of oven-dried pig placenta ($p<0.05$). Although arginine and aspartic acid were higher in FD pig placenta, no difference was found between FD and OD-90. Also, arginine and aspartic acid were higher in the pig placenta treated with OD-90, compared with those of OD-60. Guo et al. (22) reported that the apparent values for arginine and tryptophan increased after heating.

Total essential amino acid content of FD, OD-60, and OD-90 were 32.44, 29.75, and 30.82%, respectively. Glutamine was the most concentrated non-essential amino acid, followed by glycine, proline, and aspartic acid. This result agreed well with Phuapradit et al. (16). They reported that heat-dried human female and male infant placentas contained amino acids in order of glutamine, aspartic acid, glycine, alanine, proline, and arginine. They also suggested that there was no sexual difference in amino acid composition. Ratios of essential amino acid and non essential amino acid compositions of raw pig placenta, FD, OD-60, and OD-90 were 0.74, 0.75, 0.75, and 0.73, respectively. This result indicated that drying method did not affect the ratio of essential and non-essential amino acid compositions in pig placenta. However, the ratio of heat dried human placenta was 0.87, which was 15% higher than pig placenta. This may be due to species-specificity.

Hormone and antibiotic residues

There is growing evidence that estrogens may be key regulators of bone health in the elderly (23,24). Amin et al. (25) reported that low serum estradiol levels of men from the Framingham cohort were associated with low bone density. The sex hormones including estradiol in dried pig placenta were affected significantly by drying methods (Table 4). Estradiol, progesterone, and tes-

Table 3. Amino acid composition (%) of pig placenta dried by different methods

Amino acid	Raw placenta	FD[1)]	OD-60[2)]	OD-90[3)]	HDHP[4)]
His	0.13±0.012[5)]	2.03±0.222	1.71±0.170	1.80±0.289	3.95
Ile	0.14±0.017	2.15±0.188	2.09±0.085	2.61±0.851	2.18
Leu	0.36±0.061	5.62±0.363	5.50±0.238	5.48±0.254	9.73
Lys	0.30±0.029	4.72±0.552	4.31±0.483	4.14±0.497	7.61
Met	0.10±0.017	2.20±0.230	1.66±0.554	1.73±0.672	0.56
Phe	0.19±0.035	2.84±0.140	2.76±0.143	2.77±0.183	5.19
Thr	0.18±0.035	2.77±0.211	2.60±0.050	2.75±0.165	5.06
Val	0.19±0.081	3.50±0.318	3.49±0.295	3.47±0.235	5.48
Arg	0.33±0.087	5.09±0.152[a]	4.48±0.061[b]	4.83±0.256[a]	5.71
Trp	0.05±0.005	1.52±0.107[a]	1.15±0.115[b]	1.24±0.086[b]	0.94
Asp	0.42±0.078	6.53±0.422[a]	5.58±0.349[b]	6.37±0.551[a]	9.78
Ala	0.34±0.084	5.34±0.498	5.55±0.481	5.46±0.492	7.12
Cys	0.07±0.006	1.81±0.291	1.39±0.401	1.46±0.620	1.26
Glu	0.59±0.107	9.10±0.691	8.47±0.204	9.17±0.567	13.19
Gly	0.57±0.185	8.60±0.161	8.00±0.349	8.35±0.429	7.22
Pro	0.35±0.110	6.85±1.730	6.46±1.675	6.70±1.831	5.78
Ser	0.20±0.035	3.25±0.238	2.91±0.027	3.11±0.263	5.56
Tyr	0.11±0.023	1.89±0.053[a]	1.52±0.056[b]	1.57±0.092[b]	3.67
EAA[6)]	1.97	32.44	29.75	30.82	46.41
NEAA[7)]	2.65	43.37	39.88	42.19	53.59
EAA/NEAA	0.74	0.75	0.75	0.73	0.87

[1)]FD, Freeze drying; [2)]OD-60, oven drying at 60°C; [3)]OD-90, oven drying at 90°C; [4)]HDHP, mean value of heat dried (80~100°C) female and male human placenta which was recalculated as a percentage from data reported as mg% (16); [5)]Mean±standard deviation; [6)]EAA, essential amino acids; [7)]NEAA, non-essential amino acids.

[a,b]Means with different letters within the same row, except for the raw placenta column, differ significantly ($p<0.05$).

Table 4. Sex hormone contents (ng/g) in pig placenta dried by different methods

	Raw placenta	FD[1)]	OD-60[2)]	OD-90[3)]	HDHP[4)]
Estradiol	1,811.37±177.643[5)]	13,127.02±1617.350[a]	8,391.26±605.778[b]	8,474.73±301.426[b]	8.92
Progesterone	311.9±86.809	1,952.71±365.598	1,716.26±230.681	1,436.58±271.893	135.87
Testosterone	3.1±2.085	33.10±9.227	30.11±3.596	19.16±5.412	20.25

[1)]FD, Freeze drying; [2)]OD-60, oven drying at 60°C; [3)]OD-90, oven drying at 90°C; [4)]HDHP, mean value of heat dried (80～100°C) female and male human placenta (16); [5)]Mean±standard deviation.
[a,b]Means with different letters within the same row, except for the raw placenta column, differ significantly ($p<0.05$).

tosterone amounts of pig placenta by FD were higher than those of pig placenta by OD-60 and OD-90, respectively. However, no significant difference was found between oven-dried placentas.

During pregnancy, the fetus metabolizes placental progesterone. This is for the adrenal mineralo- and glucosteroids production. Phuapradit et al. (16) described that the heat-dried placenta from human female and male infants contained 123.47 and 148.27 ng/g of progesterone which are approximately 15-fold higher than those of estradiol. In the present study, estradiol was found to be a major sex hormone in raw and dried pig placenta (Table 4); the amount of estradiol was also much higher than that of human placenta. In addition, in contrast to human placenta, the amount of estradiol was higher than that of progesterone in pig placenta because, in general, estrogen and estradiol increased steadily during pregnancy of pigs until term reached.

Often a β-lactamic derivative is used in veterinary medicine as a broad spectrum antibiotic (26). Also, it has high activity against Gram-positive and Gram-negative bacteria *in vitro*. Therefore possible antibiotic residues including amoxicillin, sulfamethazine, tylosin, and chlorotetracyclin were determined to evaluate the safety of pig placenta used in this study. None of the residues were detected in raw and dried pig placenta (data not shown).

The present study showed that freeze dried pig placenta contained equal, or in certain circumstance, higher nutritive properties than human placenta. Therefore a high value added product with excellent nutritional quality can be produced using pig placenta. When nutritional quality of pig placenta is a major concern, freeze drying is recommended over conventional oven drying.

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(Received May 16, 2007; Accepted June 7, 2007)