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Comparison in nutritional quality between wild and cultured cuttlefish *Sepia pharaonis**

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Abstract In this study, the proximate composition and the amino and fatty acid profiles of shrimp *Litopenaeus vannamei* (prey) and wild and cultured cuttlefish *Sepia pharaonis* (the latter fed the prey) were determined and compared with FAO/WHO recommendations. The resulting scores for isoleucine, phenylalanine+tyrosine, histidine, lysine, threonine, and tryptophan in cultured cuttlefish were ≥150. The ratio of EAA (essential amino acids)/nonessential amino acids in cultured cuttlefish (0.82) was higher than in the wild form (0.80). All EAA amino acid scores for cultured cuttlefish were higher than their wild counterparts, except for histidine and tryptophan. Both groups of cuttlefish possessed similar saturated fatty acid content, with the cultured containing much more total (Σ) monounsaturated fatty acids, Σ *n*-6 polyunsaturated fatty acids (PUFA), and eicosapentaenoic acid (C20:5 *n*-3) but less Σ *n*-3 PUFA, arachidonic acid (C20:4 *n*-6), and docosahexaenoic acid (C22:6 *n*-3) than their wild counterparts. Therefore, the present results suggest that these cultured cuttlefish were better than the wild form for human health. Notably, these results also indicate that the nutritional composition of these cuttlefish might have been significantly affected by diet.

Keyword: cultured *Sepia pharaonis*; wild *Sepia pharaonis*; *Litopenaeus vannamei*; amino acid; fatty acid

1 INTRODUCTION

Cephalopoda, one of the most important marine invertebrate classes, represent an important seafood supply for human consumption worldwide (FAO, 2005a). Japan, China, and South Korea are the leading countries in cephalopod production, with over 1.48 million tons caught annually by the fleets of these countries and representing about 45% of the total world cephalopod catch (FAO, 2005a). Because of their high nutritional and market values, excellent palatability, and increasing demand throughout different regions of the world, cephalopod mariculture has received increased attention in recent years, with recognition of the potential of some species for commercial culture (Almansa et al., 2006; Iglesias et al., 2007; Barord et al., 2010; Domingues et al., 2010; Ferreira et al., 2010; Prato et al., 2010; Biandolino et al., 2010; George-Zamora et al., 2011).

The pharaoh cuttlefish *Sepia pharaonis* is distributed in the Indian and Western Pacific Oceans, including the Red Sea, Arabian Sea south to Zanzibar, Madagascar, Andaman Sea, South China Sea, East China Sea, Japan, and the waters of eastern Indonesia and northern Australia (FAO, 2005a). It is a neritic, demersal species occurring down to 130 m in depth. Around the South China Sea, *S. pharaonis* migrate to

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shallower waters during the mating season, where large numbers of adults congregate at 40–80 m on the continental shelf from November to February. During February and March, they move to the coast for spawning from April to May in water temperatures between 18–24°C. Eggs are laid in clusters and attached to plants, shells, and other hard substrates in ~5–20 m depth (Dong, 1988; FAO, 2005a). *S. pharaonis* is important to commercial cephalopod fisheries, including those of China, Thailand, Australia, Philippines, and Iran. It is the most abundant cuttlefish species and of great commercial importance in Hong Kong because of its flesh being thick, tender, and excellent for human consumption (FAO, 2005a).

Recently, the chemical composition of fish and fishery products has been widely investigated to determine their nutritional quality (Cherif et al., 2008; Akpinar et al., 2009; Telahigue et al., 2010; Wen et al., 2010; Jabeen and Chaudhry, 2011; Prato and Biandolino, 2012). Some studies have reported the nutritional composition of the wild cuttlefish *Sepia officinalis* (Navarro and Villanueva, 2000; Miramand et al, 2006; Ozyurt et al., 2006; Zlatanos et al., 2006; Lacoue-Labarthe et al., 2008; Ozogul et al., 2008; Lourenco et al., 2009; Ayas et al., 2012), and research into cultured cephalopod fatty acid compositions have focused on the cuttlefish *S. officinalis* (Domingues et al., 2004; Almansa et al., 2006; Ferreira et al., 2010) and octopus *Octopus vulgaris* (Navarro and Villanueva, 2000; Biandolino et al., 2010; Prato et al., 2010). Thanonkaew et al., (2006) have reported the fatty acid composition of wild *S. pharaonis* caught in Thailand but, to our knowledge, information on the nutritional quality of cultured *S. pharaonis* is scarce.

The white or whiteleg shrimp *Litopenaeus vannamei* is an important, farmed, penaeid shrimp that has the highest mariculture output of all shrimp, providing ~52% of the world penaeid shrimp output. Since this shrimp's introduction to China, it has become the main, farmed, shrimp species in China, and its farmed yield reaches more than 60% of the total Chinese shrimp yield (FAO, 2005b). Aquaculture is an important industry in Zhanjiang, a coastal city in the western Guangdong Province, China, with a cultured *L. vannamei* farm area of 200 million m^2 and accounting for 15% of the total Chinese white shrimp production (Wu and Yang, 2011).

Therefore, the main objective of the present research was to study the chemical composition and nutritional value of wild and cultured cuttlefish *S. pharaonis* fed with the shrimp *L. vannamei*.

2 MATERIAL AND METHOD

2.1 Sample preparation

Samples of fresh wild *S. pharaonis* (individual wt 1.5–2 kg) were purchased from a local retail market and a supermarket in Zhanjiang, China. Fresh *L. vannamei* and fresh, cultured *S. pharaonis* (individual wt 1.5–2 kg and fed with fresh *L. vannamei*) were provided by Xinhua Shrimp Farm (Zhanjiang Huichang Aquaculture Co. Ltd., Zhanjiang, China).

The cultured *S. pharaonis* used in this experiment were born from eggs collected from Zhanjiang beach (20°55′N, 110°33′E). Cuttlefish hatchlings were cultured in the Xinhua Shrimp Farm and fed live cultured shrimp during the first 20 d of their life cycle. Then, a total of 300 individuals (21-d old; 1.13 ±0.03 g body wt) were cultured in three rectangular, concrete tanks. All tanks had a bottom area of 3.75 m^2 (2.5 m×1.5 m), with maximum water depth of 20 cm and the water depth adjusted according to the animals' size throughout the experimental period. The experiment included three replicates with 100 individuals in each tank. The culture density was 27 individuals/m^2, mean culture temperature at 27±1°C, mean salinity at 29±1, and dissolved oxygen at 7.5±0.1 mg/L. Low light intensity was employed to maintain low stress levels (Koueta and Boucaud-Camou, 2003), and the water in each tank changed every day, filtered through a 53-μm sieve. Several air stones positioned at the bottom of each culture tank provided sufficient aeration and ensured gentle water circulation. The cuttlefish were fed shrimp, as described above, ad libitum twice daily at 9:00 and 17:00. The shrimp were well distributed during feeding to assure all cuttlefish had sufficient food.

The main edible portion of the cuttlefish, the mantle, was cleaned, skinned, eviscerated, and homogenized for subsequent chemical analyses of the fresh material. A sample included 30 individuals, which were divided into three groups, and 100 g of mantle muscle tissue from each group used in the analyses described below.

2.2 Proximate chemical composition

Moisture content was determined by drying a sample in an oven at 105°C until a constant weight was obtained, according to AOAC Method 950.46 (AOAC, 1995). Crude protein content was determined

according AOAC Method 970.42 (AOAC, 1995), and a conversion factor of 6.25 was used to convert total nitrogen to crude protein. Fat was determined according to AOAC Method 960.39 (AOAC, 1995). Ash was determined by incineration in a muffle furnace at 550°C for 24 h, according to AOAC Method 920.153 (AOAC, 1995). All results were presented in wet basis.

2.3 Amino acid analysis

Amino acid was analyzed as per Wen et al. (2010).

2.4 Amino acid score

Essential amino acid scores (EAAS) were also calculated according to FAO/WHO reference amino acid requirements of human adults (FAO/WHO/UNU, 2007) and the FAO/WHO reference amino acid pattern of preschool children (age, 2–5 years; FAO/WHO/UNU, 1985). The amino acid score was calculated using the formula: amino acid score=amount of amino acid per test protein (mg/g)/amount of amino acid per protein in reference pattern (mg/g)×100.

2.5 Fatty acid analysis

Fatty acid was analyzed as per Wen et al. (2010).

2.6 Statistical analysis

All analyses were repeated at least in triplicate. Results were expressed as mean±standard deviation (SD).

3 RESULT AND DISCUSSION

3.1 Proximate compositions of prey, wild and cultured cuttlefish

The proximate composition of the shrimp and the wild and cultured cuttlefish are shown in Table 1. Cultured cuttlefish possessed a slightly higher protein content than their wild counterparts. Generally, these values were in good agreement with those reported for wild *S. pharaonis* (Thanonkaew et al., 2006) and were similar to those reported for wild and cultured octopus *O. vulgaris* (Prato et al., 2010). However, the crude fat content of cultured cuttlefish was significantly lower than reported for the wild cuttlefish *S. officinalis* by Zlatanos et al. (2006) and Ozogul et al. (2008). As the shrimp were found to have significantly higher protein content than the cuttlefish, the cuttlefish protein content might have been affected by diet. Overall, these results indicated that both wild and cultured cuttlefish were excellent protein sources and low in fat content.

Table 1 Proximate composition of prey shrimp *L. vannamei* and wild and cultured cuttlefish *S. pharaonis*

	Moisture	Crude protein	Crude fat	Crude ash
L. vannamei	76.26±0.18*	21.90±0.28	0.36±0.02	1.50±0.05
Wild *S. pharaonis*	83.26±0.23	15.15±0.30	0.41±0.02	1.17±0.03
Cultured *S. pharaonis*	82.86±0.20	15.53±0.33	0.38±0.02	1.23±0.03

* - % of wet wt, mean±SD, n=3.

Table 2 Amino acid profiles (mg/g crude protein, mean ±SD) of prey *L. vannamei* and wild and cultured cuttlefish *S. pharaonis*

Amino acid	*L. vannamei*	Wild *S. pharaonis*	Cultured *S. pharaonis*
Asp	101±1.98*	91.5±1.58	97.5±1.81
Ser	33.8±0.65	37.0±0.81	38.2±0.82
Glu	155±3.18	137±2.38	145±2.58
Gly	93.6±1.87	48.8±0.91	51.1±0.90
Ala	56.6±0.87	49.6±0.88	53.9±0.86
Pro	47.9±0.82	59.8±0.96	47.5±0.81
Arg	102±1.89	72.8±1.36	77.7±1.26
Val#	46.1±0.78	38.1±0.80	41.3±0.68
Met#	24.7±0.48	26.0±0.44	26.4±0.48
Ile#	43.4±0.79	41.9±0.82	45.6±0.84
Leu#	75.8±1.18	74.0±1.16	79.3±1.18
Tyr#	28.8±0.54	32.0±0.66	33.6±0.66
Phe#	41.1±0.69	35.9±0.63	38.1±0.80
His#	15.5±0.28	22.8±0.50	22.5±0.44
Lys#	79.9±1.25	73.8±1.23	78.1±1.25
Thr#	34.7±0.71	40.2±0.56	41.3±0.69
Trp#	8.70±0.18	14.6±0.24	12.8± 0.28
EAA/NEAA	0.68	0.80	0.82
(Arg+Leu+Lys)/EAA	0.51	0.47	0.47

* - % of wet wt, mean±SD. Asp: aspartic acid; Ser: serine; Glu: glutamic acid; Gly: glycine; Ala: alanine; Arg: arginine; Pro: proline; Val: valine; Met: methionine; Ile: isoleucine; Leu: leucine; Tyr: tyrosine; Phe: phenylalanine; His: histidine; Lys: lysine; Thr: threonine; Trp: tryptophan; and EAA/NEAA. Essential amino acids/nonessential amino acids. # Essential amino acids.

3.2 Amino acid profiles of prey shrimp and wild and cultured cuttlefish

The amino acid profiles of the prey shrimp and wild and cultured cuttlefish are presented in Table 2.

Table 3 Essential amino acids scores of wild and cultured cuttlefish *S. pharaonis*

Amino acid	Reference (for adults)	Reference (for children)	Wild *S. pharaonis*			Cultured *S. pharaonis*		
			Content (mg/g crude protein)	Score (for adults)	Score (for children)	Content (mg/g crude protein)	Score (for adults)	Score (for children)
Val	39	35	38.1	98	109	41.3	106	118
Met	22	25	26.0	118	104	26.4	120	106
Ile	30	28	41.9	140	150	45.6	152	163
Leu	59	66	74.0	125	112	79.3	134	120
Phe+Tyr	30	63	67.9	226	108	71.7	239	114
His	15	19	22.8	152	120	22.5	150	118
Lys	45	58	73.8	164	127	78.1	174	135
Thr	23	34	40.2	175	118	41.3	180	121
Trp	6	11	14.6	243	133	12.8	213	116

Note: Abbreviations of amino acid as in Table 2 and n=3.

The most abundant amino acids in both cuttlefishes were glutamic acid, aspartic acid, arginine, leucine, and lysine, constituting half the total amino acids (TAA). Here, arginine, leucine, and lysine represented almost half the essential amino acids (EAA) in both cuttlefishes. This was similar to reports for wild *S. officinalis* (Zlatanos et al., 2006), cultured *S. officinalis* (Domingues et al., 2005), wild squid *Loligo vulgaris* (Villanueva et al., 2004), wild octopus *O. vulgaris* (Villanueva et al., 2004), and cultured *Octopus maya* (Domingues et al., 2007).

In this study, all amino acid contents in cultured cuttlefish were significantly higher than in the wild form, except for proline, methionine, histidine, and tryptophan. When compared with the amino acid profile of the shrimp, the corresponding amino acid contents of shrimp were significantly higher than those of cuttlefish, except for serine, proline, methionine, tryptophan, histidine, threonine, and tryptophan. Thus, these results indicated that the amino acid profile of the cultured cuttlefish was significantly affected by diet, compared with the wild form.

The ratio of EAA/nonessential amino acids (NEAA) in cultured cuttlefish was higher than that in wild cuttlefish; the essential amino acids scores are presented in Table 3. In general terms, when compared with the reference amino acid composition or pattern in adult humans, all amino acid scores were >100, except for valine (98) in wild cuttlefish, which was thus the limiting amino acid. Moreover, the scores for isoleucine, phenylalanine + tyrosine, histidine, lysine, threonine, and tryptophan in cultured cuttlefish were ≥150. When compared with the reference amino acid pattern in preschool children, all amino acid scores were >100. In previous studies, the scores of histidine, leucine, phenylalanine+tyrosine, and valine in wild cuttlefish, leucine in wild *O. vulgaris*, and histidine, leucine, and valine in wild *L. vulgaris* were <100 (Zlatanos et al., 2006), while histidine, leucine, methionine, phenylalanine + tyrosine, valine, and tryptophan in cultured *O. maya* were <100 (Domingues et al., 2007). In comparing cultured and wild cuttlefish, all EAA amino acids scores of cultured cuttlefish were higher than their wild counterparts, except for histidine and tryptophan. Thus, these results suggested that the protein quality of cultured cuttlefish was significantly higher than the wild form.

3.3 Fatty acid profiles of prey shrimp and wild and cultured cuttlefish

The fatty acid profiles and contents of the shrimp and wild and cultured cuttlefish are shown in Table 4. The profiles of both cuttlefish forms were dominated by polyunsaturated fatty acids (PUFA), with docosahexaenoic acid (DHA) the dominant fatty acid. Palmitic acid (C16:0) was the major saturated fatty acid (SFA), while oleic acid (C18:1) was the main monounsaturated fatty acid (MUFA). These values were similar to those reported by Thanonkaew et al., (2006) for wild *S. pharaonis* and cultured *S. officinalis* (Almansa et al., 2006). However, wild *S. officinalis* had higher PUFA content but lower MUFA content (Ozogul et al., 2008) and cultured *S. officinalis* contained higher MUFA (Domingues et al., 2003). Moreover, wild *O. vulgaris* contained higher SFA and lower PUFA, and cultured *O. vulgaris* contained higher MUFA and lower PUFA (Prato et al., 2010), compared with the present wild and cultured cuttlefish.

Eicosapentaenoic acid (EPA) and DHA possess

Table 4 Fatty acid profiles of prey *L. vannamei*, wild and cultured cuttlefish *Sepia pharaonis*

Fatty acid	*L. vannamei*	Wild *S. pharaonis*	Cultured *S. pharaonis*
C14:0	0.48±0.01*	1.65±0.03	1.09±0.02
C15:0	0.40±0.01	0.47±0.01	0.39±0.01
C16:0	16.17±0.42	18.45±0.44	17.62±0.35
C17:0	1.60±0.02	1.65±0.02	1.34±0.02
C18:0	10.89±0.24	11.10±0.30	12.46±0.38
C20:0	0.50±0.01	0.27±0.01	0.35±0.01
C22:0	0.78±0.02	nd	nd
C24:0	0.72±0.01	0.11±0.01	0.12±0.01
C15:1	nd	0.52±0.01	0.98±0.02
C16:1	2.36±0.04	0.80±0.01	0.53±0.01
C17:1	0.17±0.01	2.56±0.05	4.23±0.06
C18:1 *n*-9	20.14±0.42	3.51±0.04	3.96±0.04
C20:1 *n*-9	1.50±0.06	2.20±0.06	2.34±0.05
C22:1 *n*-9	0.44±0.02	0.20±0.01	0.26±0.01
C18:2 *n*-6	12.04±0.33	0.46±0.01	1.37±0.03
C18:3 *n*-3	0.70±0.01	0.11±0.01	0.25±0.01
C20:2 *n*-6	1.76±0.04	0.35±0.01	1.69±0.02
C20:3 *n*-3	0.18±0.01	0.19±0.01	0.44±0.01
C20:4 *n*-6 (AA)	3.59±0.06	7.61±0.20	6.78±0.28
C20:5 *n*-3 (EPA)	13.33±0.32	8.49±0.33	13.01±0.35
C22:4 *n*-6	0.24±0.01	1.36±0.05	1.68±0.05
C22:5 *n*-3	1.07±0.02	1.56±0.04	1.69±0.04
C22:6 *n*-3 (DHA)	9.92±0.28	28.64±0.68	20.62±0.56
Unknown	1.02	7.74	6.80
Σ SFA	31.54	33.70	33.37
Σ MUFA	24.61	9.79	12.30
Σ PUFA	42.83	48.77	47.53
Σ *n*-6	17.63	9.78	11.52
Σ *n*-3	25.20	38.99	36.01
n-6/*n*-3	0.70	0.25	0.32
n-3/*n*-6	1.43	3.99	3.13
DHA/EPA	0.74	3.37	1.58
Σ PUFA/Σ SFA	1.36	1.45	1.42
Σ MUFA/Σ SFA	0.78	0.29	0.37

* - % of total fatty acids, mean±SD. nd: not detected; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; *n*-6, *n*-6 PUFA; *n*-3, *n*-3 PUFA; and *n*=3.

several properties advantageous for human health. In addition to reducing the risk of some cardiovascular diseases (Ness et al., 2002) and cancers (Norat et al., 2005), they can improve various functions in the human body (Berbert et al., 2005). In the present study, DHA was the principal *n*-3 PUFA in these cuttlefish, with the DHA content similar to wild *O. vulgaris* (20.1% of total fatty acids, TFA, Zlatanos et al., 2006; 25.54%–29.57% of TFA, Ozogul et al., 2008), wild *S. officinalis* (23.74% of TFA, Zlatanos et al., 2006; 23.9%–29.5% of TFA, Ozyurt et al., 2006), and cultured *S. officinalis* (22.51%–25.90% of TFA, Almansa et al., 2006). In contrast, this DHA content was lower than in wild *S. officinalis* (30.89%–32.99% of TFA, Ozogul et al., 2008; 27.46%–33.02% of TFA, Ayas et al., 2012) but higher than has been reported for cultured *S. officinalis* (17.02% of TFA, Domingues et al., 2003) and wild and cultured *O. vulgaris* (4.32%–14.33% of TFA, Prato et al., 2010). DHA plays a multifunctional role in cell membrane physiology (Almansa et al., 2003), being involved in formation of the nervous and visual system, reproduction, growth, and embryonic and larval development (Almansa et al., 1999), and might be important for the proper development and survival of fast-growing cephalopods (Navarro and Villanueva, 2000, 2003).

EPA was the second most abundant PUFA in these cuttlefish, accounting for 8.49% and 13.01% of TFA in wild and cultured cuttlefish, respectively. These values were higher than has been reported for wild *S. pharaonis* (7.2% of TFA, Thanonkaew et al., 2006), wild and cultured *O. vulgaris* (3.71%–9.57% of TFA, Prato et al., 2010) but lower than reported for wild *S. officinalis* (13.9%–17.8% of TFA, Ozyurt et al., 2006; Zlatanos et al., 2006; Ozogul et al., 2008; Ayas et al., 2012) and cultured *S. officinalis* (20.06% of TFA, Domingues et al., 2003; 18.54%–19.67% of TFA, Almansa et al., 2006). EPA is one also of the most important eicosanoid precursors, which have been implicated in numerous physiological processes (Sargent et al., 1995).

The principal *n*-6 PUFA in these cuttlefish was arachidonic acid (AA), with AA contents similar to that reported for wild *S. pharaonis* (7.2% of TFA, Thanonkaew et al., 2006) but higher than reported for wild *S. officinalis* (2.78%–4.67% of TFA, Ozyurt et al., 2006; Zlatanos et al., 2006; Ozogul et al., 2008; Ayas et al., 2012), cultured *S. officinalis* (3.16% of TFA, Domingues et al., 2003; 1.99%–3.65% of TFA, Almansa et al., 2006), and wild and cultured *O. vulgaris* (1.08%–2.21% of TFA, Prato et al., 2010). AA is the main precursor of eicosanoids (Gil, 2002) and also plays a role in growth (Mat et al., 1994).

High *n*-3/*n*-6 ratios are a peculiar nutritional

characteristic of seafood (Orban et al., 2004). In the present study, the *n*-3/*n*-6 ratios of wild and cultured cuttlefish were 3.99 and 3.13, respectively. These values were close to those that have been reported for wild *S. pharaonis* (3.1, Thanonkaew et al., 2006) but lower than reported for wild *S. officinalis* (7.69–10, Ozogul et al., 2008; 11.1–14.3, Ayas et al., 2012) and cultured *S. officinalis* (6.46, Domingues et al., 2003; 8.06–11.39, Almansa et al., 2006). These results indicated that, as with fish, differences in fatty acid composition among these cuttlefish were based on their environmental conditions, notably water temperature, which can influence fatty acid composition (Saito et al., 1999). Human dietary intake of food with a high *n*-3/*n*-6 ratio is beneficial. FAO experts have recommended that the *n*-3/*n*-6 ratio in the diet should be higher than 0.2 (FAO/WHO, 2002) and by the UK Department of Health at higher than 0.25 (HMSO, 1994). Thus, these results indicated that both wild and cultured cuttlefish possessed high *n*-3/*n*-6 ratios.

Comparing the fatty acid profiles and contents of wild and cultured cuttlefish showed that both cuttlefishes contained similar SFA contents, but cultured cuttlefish contained significantly higher MUFA and slightly lower PUFA than their wild counterparts. This discrepancy might be attributable to numerous factors, diet being particularly important (Taboada et al., 2003). Comparison with the shrimp fatty acid profile showed the shrimp to have significantly higher MUFA and slightly lower PUFA content than the cuttlefish. Accordingly, in regard to PUFA, cultured cuttlefish contained higher total (Σ) *n*-6 PUFA and EPA, but lower Σ *n*-3 PUFA, AA, and DHA than the wild form. Thus, these results indicated that the fatty acid contents of these cuttlefish might be significantly affected by diet.

4 CONCLUSION

The present study highlights and provides new information on the proximate composition and the amino and fatty acid compositions of commercial cuttlefish *S. pharaonis* fed the commercial shrimp *L. vannamei*, the main, farmed, shrimp species in China. Comparing wild with cultured cuttlefish showed that the cultured contained higher quality protein, with a well-balanced composition of EAA, particularly in valine, methionine, isoleucine, leucine, phenylalanine + tyrosine, lysine, and threonine, and also richer in EPA. Therefore, these results suggested that the meat of these cultured *S. pharaonis* would be beneficial to human health and that the nutritional composition of this cuttlefish might be significantly affected by diet.

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