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Improvement of the Nutritional Quality of Cottonseed Meal by *Bacillus subtilis* and the Addition of Papain

HONG SUN, JIANG-WU TANG†, XIAO-HONG YAO†, YI-FEI WU†, XIN WANG† AND JIE FENG[1]
College of Animal Sciences, Zhejiang University, Hangzhou, 310058, PR China
†Institute of Plant Protection and Microbiology, Zhejiang Academy of Agriculture Sciences, Hangzhou, 310021, PR China
[1]Corresponding author's e-mails: fengj@zju.edu.cn; gearyou@gmail.com

ABSTRACT

To improve the nutritional quality of the cottonseed meal (CSM), it was fermented by *Bacillus subtilis* ($FCSM_B$) or by *B. subtilis* and the addition of papain ($FCSM_{B+A}$). After 48 h of fermentation, both $FCSM_B$ and $FCSM_{B+A}$ increased ($p<0.05$) the contents of crude protein and crude ash; whereas the contents of crude fat, crude fiber and free gossypol were sharply decreased ($p<0.05$) compared with CSM. $FCSM_B$ and $FCSM_{B+A}$ also raised ($p<0.05$) contents of essential and total amino acids. Moreover, both fermented products increased ($p<0.05$) the degree of hydrolysis of cottonseed protein and soluble protein content, especially in $FCSM_{B+A}$. The distribution of soluble protein showed that $FCSM_{B+A}$ improved ($p<0.05$) the amount of small-size protein fractions (<20 kDa), while decreasing ($p<0.05$) medium-size (20–50 kDa) and large-size fractions (>50 kDa) compared with $FCSM_B$ or CSM. Additionally, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and *in vitro* protein digestibility were improved ($p<0.05$) in the $FCSM_B$ and $FCSM_{B+A}$ as compared to CSM. Collectively, CSM fermented by *B. subtilis*, especially with the addition of papain could substantially improve its nutritional value to some extent. © 2012 Friends Science Publishers

Key Words: Antioxidant activity; *Bacillus subtilis*; Cottonseed meal; *In vitro* digestibility; Papain; Solid-state fermentation

INTRODUCTION

Shortage of protein sources of good quality is a major concern in many developing countries due to the prohibitive cost of animal protein sources. Proteins of plants are alternative sources that are more economical to use (Ei-Sayed *et al*., 1999). Cottonseed meal (CSM), a byproduct of extracting the oil from cotton seeds, is recognized as a good protein source in China because of its high protein content (220–560 g kg^{-1}) and wide availability (Nagalakshmi *et al*., 2007). However, the use of CSM in animal feeds is limited due to the detrimental effects of low lysine levels and free gossypol, which is a toxic substance. Free gossypol is associated with depressed growth performance and increased mortality in broilers (Henry *et al*., 2001). Study has shown that free gossypol could bind with amino acids in CSM, mainly lysine, to form non-digestible substances, further reducing the nutritional value of cottonseed protein (Watkins *et al*., 1993). Therefore, methods need to be developed to reduce the harmful properties of CSM.

Solid-state fermentation is one of the most promising techniques to reduce free gossypol of CSM (Weng & Sun, 2006; Zhang *et al*., 2006; 2007). *Bacillus subtilis* is an important starter culture used in many solid-state fermentation processes (Kiers *et al*., 2000; Shafique *et al*., 2004; Zhu *et al*., 2008). It is easy to introduce in dry feed due to the production of spores and is generally recognized as safe recommended by the U.S. Food and Drug Administration (Kramer & Gilbert, 1989). A significant decrease in free gossypol levels after *Bacillus* fermentation has been demonstrated in our previous study (Tang *et al*., 2012). High nutrient availability of soybean meal could also be observed by degradation of proteins during *B. subtilis* fermentation (Omafuvbe *et al*., 2002; Hong *et al*., 2004; Feng *et al*., 2007). But data on protein degradation in fermented CSM is scarce to date.

On the other hand, enzymatic hydrolysis has been widely used to enhance functional and biological properties of plant-based food and feed proteins through breakage of intact proteins (Moure *et al*., 2006; Chabanon *et al*., 2007). Moreover, proteolysis process could increase peptide contents in protein hydrolysates that are absorbed more rapidly from the intestine than free amino acids (Webb, 1990). Thus, we hypothesize that the inclusion of inoculants and proteinase together may further improve the quality of CSM as reported in similar feed ingredients (Lee *et al*., 2008; Xu *et al*., 2011).

In precious studies, papain was identified an excellent cottonseed-protein degrading enzyme (Gao *et al*., 2010). Therefore, the present study fermented CSM using *B. subtilis* alone ($FCSM_B$) or with both incoculants and the

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addition of papain ($FCSM_{B+A}$), to develop CSM into a high quality protein source with functional benefits and extend the use of CSM.

MATERIALS AND METHODS

Microorganism and enzyme: *B. subtilis* BJ-1 was first isolated from the garden soil for free gossypol degradation study and stored in China General Microbiological Culture Collection, Beijing, China (Tang *et al*., 2012). The culture was maintained on potato dextrose agar (PDA) slopes (CM0139, Oxoid, Beijing, China) at 4°C. Before use in an experiment, culture of *B. subtilis* BJ-1 was prepared by transferring a loop of bacteria from the PDA slant into a 250 mL flask containing 50 mL of nutrient broth (BD234000, Difco, Detroit, USA) and incubated at 37°C, 160 r min^{-1} for 18 h. The culture was diluted in sterile distilled water with 1 g L^{-1} peptone (LP0037, Oxoid, Beijing, China) to about 10^8 colony forming units mL^{-1}. The suspension served as the inoculum for CSM fermentation.

Papain (from papaya latex, activity of 0.5–2.0 units per mg protein) was purchased from Sigma (Shanghai, China). Papain is active at acid to neutral pH and cleaves peptide bonds with alkaline amino acids and leucine, glycine. CSM contains more than 15% of these amino acids, which could be effectively hydrolysed by papain (NRC, 1994).

Fermentation of CSM with papain treatment: The CSM was purchased from Tycoon Group Co., Ltd (Xinjiang, China). CSM (100 g) was transferred into 500 mL flasks at a moisture concentration of 60% (w/w) and then inoculated with 2 mL of diluted culture of *B. subtilis* BJ-1. The enzyme preparations (filtration with 0.22 μm filters, Millipore, Beijing, China) were added to reach a rate of 1.0 g kg^{-1} of CSM with no adjustment of pH (Ghazi *et al*., 2010). The homogenates were incubated in a bed-packed incubator at 30°C for 48 h. After fermentation, fresh samples were dried at 50°C in a hot-air oven to about 900 g kg^{-1} dry matter. The dried samples were subsequently milled fitted with 1-mm mesh screen and stored in plastic bags at –20°C for chemical analysis and protein extraction. Triplicate flasks were used for each experimental variation.

Chemical analysis: Dry matter content of samples was determined by drying at 105°C for 5 h; ash content was measured by incineration at 550°C for 8 h. Contents of crude protein, crude fat crude fiber were determined according to the AOCS (2009) methods. Amino acid assay was based on the AOAC (1999) methods. Free gossypol content was determined according to AOCS (2009) based on high performance liquid chromatography technique. The degree of hydrolysis of the cottonseed protein was measured by the method of Teng *et al*. (2012).

Protein extraction: The soluble cottonseed proteins in CSM, $FCSM_B$ and $FCSM_{B+A}$ were extracted according to Chen *et al.* (2010) with minor modifications. For short, 500 mg samples were mixed thoroughly with 1 mL of extraction solution (50 mM phosphate buffered saline, 100 mM NaCl, pH 8.0) and vortexed for 10 min. The homogenate was centrifuged at 10,000×g for 15 min and the supernatant was collected for electrophoresis.

Electrophoresis: Tricine-sodium dodecyl sulphaye-polyacrylamide gel electrophoresis (Trincine-SDS-PAGE) was performed as previously described (Schagger, 2006) using a 5% stacking gel and 16% separating gel. The soluble cottonseed protein was denatured with sampling buffer (50 mM Tris-HCl, 25% (w/v) glycerol, 2% (w/v) SDS and 0.1% (w/v) bromophenol blue) and boiled for 5 min. Protein bands were stained in a Coomassie brilliant blue G-250 solution for 2 h then discolored with acetic acid (methanol: acetic acid: water, 4:0.8:5.2, v/v/v) over night. The protein markers were purchased from Fermentas™ (SM1881, Shenzhen, China).

Free radical-scavenging assay: The antioxidant activity of the protein extracts was determined as hydrogen-donating or radical-scavenging ability by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method according to Shyu and Hwang (2002). Briefly, 1 mL of protein extracts at a concentration of 10 mg mL^{-1} was added to 2 mL of 100 μM methanolic solution of DPPH (Sigma). After immediately shaken, the mixture was kept at room temperature in dark. The decrease in absorbance at 517 nm was measured at 10 min interval until a plateau was detected. The inhibitory percentage of DPPH was calculated according to the following equation: Scavenging effect (%) = $(A_{control} - (A_{sample} - A_{blank}))/A_{control} \times 100$, where $A_{control}$ was the absorbance of DPPH without samples, A_{sample} was the absorbance of samples mixed with DPPH, and A_{blank} was the absorbance of sample without DPPH addition.

Determination of *in vitro* protein digestibility: The procedure of the *in vitro* protein digestibility was according to the method of Chen *et al*. (2010). Ten gram of sample was put in a 250 mL flask with 50 mL pepsin-HCl solution (200 mg of pepsin (250 units mg^{-1}, sigma, Beijing, China) and 0.01 M HCl) and incubated at 39°C for 4 h. After adjusted pH to 7.0 by Na_2CO_3 (50 mg mL^{-1}), 30 mL of artificial pancreatin solution was added and re-incubated at 39°C for 4 h. Digestion was stopped by adding 10 mL of 5% trichloroaceric acid (TCA) into the flask and the digesta solution was centrifuged at 1,000×g for 15 min. The TCA-insoluble residue was oven dried and the crude protein content was analyzed. *In vitro* digestibility (%) = (content of crude protein of sample – content of crude protein of TCA-insoluble residue)/content of crude protein of sample×100.

Statistical analysis: Each determination was performed on three separate samples and analyzed in triplicate and results were then averaged. Data were assessed by the analysis of variance (ANOVA), and significant difference between the treatments was determined with Duncan's multiple range test procedure (SAS, 1999). A significance level of 0.05 was used.

RESULTS AND DISCUSSION

Chemical parameters of treated and untreated CSM: $FCSM_B$ and $FCSM_{B+A}$ changed the chemical characteristics of CSM (Table I). Contents of crude protein and crude ash increased ($p<0.05$) after fermentation. This may be partly due to the use of carbon sources in CSM during the fermentation process, leading to the concentration of other nutrients (Weng & Sun, 2006; Zhang *et al*., 2007; Khalaf & Meleigy, 2008). The increase in *Bacillus* counts (data not shown) may also account for the elevated level of crude protein, for it constitutes about 63% protein of its biomass (Terlabie *et al*., 2006). The contents of crude fiber and crude fat of $FCSM_B$ and $FCSM_{B+A}$ were decreased ($p<0.05$) compared with CSM. Similarly, Tang *et al*. (2012) demonstrated decreased ($p<0.05$) contents of these nutrients in fermented CSM. The reduction may be attributed to the production of lipase (Terlabie *et al*., 2006) and cellulose (Amartey *et al*., 1999) by *Bacillus* strains.

The free gossypol contents in $FCSM_B$ and $FCSM_{B+A}$ were reduced ($p<0.05$) by 61.9% and 54.7%, respectively (Table I). In accordance with our results, free gossypol content was decreased during solid-state fermentation by fungi (Weng & Sun, 2006; Zhang *et al*., 2006). More importantly, free gossypol contents of $FCSM_B$ and $FCSM_{B+A}$ are lower than the levels (400 mg kg^{-1}) which can adversely affect broiler performance (Henry *et al*., 2001). The decrease in free gossypol likely is the result of the binding of proteins, and/or degrading enzymes secreted by microbes (Zhang *et al*., 2007). However, detoxification of

Table I: Nutrient composition and free gossypol content of cottonseed meals fermented with *Bacillus subtilis* ($FCSM_B$) or with *B. subtilis* and papain ($FCSM_{B+A}$)

Items	Unfermented	$FCSM_B$	$FCSM_{B+A}$	SEM	p
Dry matter (g kg^{-1})	903.9	922.0	899.3	5.90	0.273
Crude fat (g kg^{-1})	14.9^a	11.3^b	10.8^b	0.71	<0.001
Crude protein (g kg^{-1})	468.6^b	525.9^a	513.4^a	9.01	<0.001
Crude fiber (g kg^{-1})	101.2^a	93.3^b	92.7^b	1.54	0.003
Crude ash (g kg^{-1})	54.9^b	61.9^a	61.7^a	1.22	<0.001
Free gossypol (mg kg^{-1})	745.67^a	284.00^b	337.66^b	74.93	<0.001

Note: SEM = standard error of the mean; Means obtained from triplication (n=3) not sharing a common superscript in a same row are significantly different at $p<0.05$

Table II: Amino acids (AA) profile of cottonseed meals fermented with *Bacillus subtilis* ($FCSM_B$) or with *B. subtilis* and papain ($FCSM_{B+A}$) (g kg^{-1} dry basis)

Amino acids	Unfermented	$FCSM_B$	$FCSM_{B+A}$	SEM	p
Lysine	20.93^b	22.82^a	22.57^a	0.33	0.029
Methionine	4.90	5.20	5.01	0.14	0.586
Threonine	15.51^b	17.74^a	17.55^a	0.41	0.003
Arginine	51.64^b	56.02^a	58.68^a	1.32	0.070
Leucine	28.94^c	34.26^a	31.88^b	1.81	<0.001
Isoleucine	17.13	19.11	18.20	0.50	0.054
Valine	22.26	22.71	22.93	0.31	0.670
Histidine	16.99^c	20.33^a	18.79^b	0.53	0.001
Phenylalanine	27.72^c	33.05^a	30.71^b	0.80	<0.001
Serine	22.54^b	26.01^a	25.11^a	0.63	<0.001
Proline	20.71	22.89	21.04	0.55	0.149
Glycine	20.85^c	23.82^a	22.63^b	0.50	<0.001
Alanine	20.52^b	23.11^a	22.84^a	0.43	0.011
Tyrosine	11.61^b	14.28^a	13.23^a	0.43	0.008
Aspartic acid	44.20^b	51.14^a	48.90^a	2.12	0.001
Glutamic acid	111.41^b	126.92^a	123.77^a	2.65	0.007
Essential AA	206.02^b	231.24^a	226.32^a	3.61	0.012
Total AA	457.86^b	519.41^a	503.84^a	13.22	<0.001

Note: SEM = standard error of the mean; Means obtained from triplication (n=3) not sharing a common superscript in a same row are significantly different at $p<0.05$; Essential AA: Lys, Met, Thr, Arg, Leu, Iso, Val, His and Phe

Table III: Hydrolysis degree, soluble protein content and protein distribution by size in cottonseed meals fermented with *Bacillus subtilis* ($FCSM_B$) or with *B. subtilis* and papain ($FCSM_{B+A}$)

	Unfermented	$FCSM_B$	$FCSM_{B+A}$	SEM	p
Hydrolysis degree (%)	0^c	5.81^b	20.12^a	2.99	<0.001
Soluble protein (g kg^{-1})	177.15^c	254.83^b	390.45^a	31.11	<0.001
Molecular weight range (%)					
>50 kDa	35.6^a	33.0^a	19.7^b	2.52	<0.001
20 kDa to 50 kDa	36.6^a	36.4^a	32.5^b	0.71	0.001
<20 kDa	27.8^b	30.6^b	47.8^a	3.20	<0.001

Note: SEM = standard error of the mean; Means obtained from triplication (n=3) not sharing a common superscript in a same row are significantly different at $p<0.05$

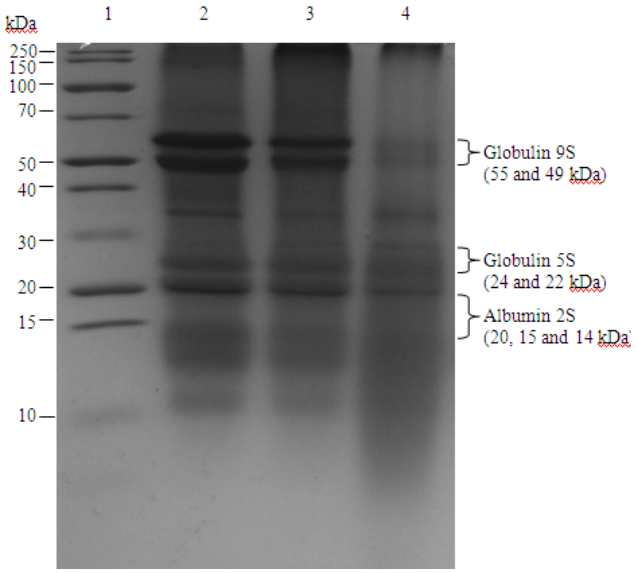
free gossypol is less than those observed by Weng and Sun (2006) and Zhang *et al*. (2006), which probably due to the differences between strains.

The nutritional value of a protein is highly correlated with its amino acid composition, especially essential amino acids required in the diet of animals. The total amino acid content of both $FCSM_B$ and $FCSM_{B+A}$ was increased ($p<0.05$) by 13.4% and 10.0%, respectively (Table II). This is in agreement with the increase of crude protein content after fermentation. Moreover, both $FCSM_B$ and $FCSM_{B+A}$ improved ($p<0.05$) the contents of some essential amino acids, including lysine, threonine, leucine, arginine, histidine and phenylalanine. The content of non-essential amino acid was also increased ($p<0.05$) after fermentation, except proline. Thus, $FCSM_B$ and $FCSM_{B+A}$ potentially improve the nutritional value of CSM from the point view of amino acids. This is partly agrees with Zhang *et al*. (2007) and Khalaf and Meleigy, (2008), who reported a significant increase in total and essential amino acids of CSM fermented with *Candida tropicalis*. The increase in amino acid levels may be ascribed to certain microbial metabolism that occurs during fermentation. Interestingly, $FCSM_{B+A}$ decreased ($p<0.05$) the levels of leucine, histidine, phenylalanine and glycine, compared with $FCSM_B$. Since papain breaks the bonds in proteins rather than affect the total amount of amino acids, the significant drop of amino acids in $FCSM_{B+A}$ may be attributed to the excessive utilization of some amino acids by *B. subtilis*.

Degradation of protein: To access the effects of $FCSM_B$ and $FCSM_{B+A}$ on protein degradation of CSM, protein solubility and degree of hydrolysis were determined. *Bacillus* ferments showed higher ($p<0.05$) protein solubility and hydrolysis degree, especially in $FCSM_{B+A}$, compared with the CSM (Table III). Degree of hydrolysis is one of the determinant parameter for monitoring the protein hydrolysis. The use of protease (papain) as additives is expected to degrade cottonseed protein, contributing to the increase of hydrolysis degree. The increase of protein solubility could result from the breakdown of protein into water-soluble molecules (Chabanon *et al*., 2007). Interestingly, $FCSM_B$ sharply increased the degree of hydrolysis and the soluble protein content, compared with CSM. Teng *et al*. (2012) repoarted similar result in soybean meal fermented with *B. subtilis*. These observations suggest that proteolytic activity has been involved in the fermentation with *B. subtilis* (Omafuvbe *et al*., 2002; Terlabie *et al*., 2006). However, the proteolysis during fermentation in the current study is much less than those reported in other studies (Phromraksa *et al*., 2009; Teng *et al*., 2012). The lack of agreement may be attributed to differences between strains and substrates (soybean meal & CSM, respectively).

The subunit profile of cottonseed protein in $FCSM_B$ and $FCSM_{B+A}$ was assayed by Tricinie-SDS-PAGE (Fig. 1). The unfermented CSM exhibited major protein subunits, including globulin 9S (55 kDa & 49 kDa), globlin 5S (24 kDa & 22 kDa) and albumin 2S (20 kDa, 15 kDa & 14 kDa),

Fig. 1: Tricine-SDS-PAGE analysis of protein fractions of cottonseed meal (CSM) fermented with *Bacillus subtilis* ($FCSM_B$) or with *B. subtilis* and papain ($FCSM_{B+A}$). Lane 1: molecular marker; Lane 2: protein fractions in CSM; Lane 3: protein fractions in $FCSM_B$; Lane 4: protein fractions in $FCSM_{B+A}$



which are in agreement with those of previously reported (Sadeghi & Shawrang, 2007). The subunits of globulin 9S degraded to some extent, after fermentation with *B. subtilis*. More or less complete breakdown of all two subunits (9S & 5S) to low-molecular weight fractions was observed in $FCSM_{B+A}$. These observations are consistent with the protein solubility and the degree of hydrolysis, reflecting the proteolytic activity of *B. subtilis* (Kiers *et al*., 2000; Zhu *et al*., 2008) and papain. The ratio of each band was determined by densitometric analysis with the sizes of peptides grouped as large (>55 kDa), medium (20–55 kDa) and small (<20 kDa) (Table III). The ratio of small-size fraction increased by 10.1% ($p>0.05$) and 71.9% ($p<0.05$) in $FCSM_B$ and $FCSM_{B+A}$, respectively. The large-size protein fraction was decreased by 7.3% in $FCSM_B$ and 44.7% in $FCSM_{B+A}$, respectively. The increased amount of small-size proteins is most likely due to the digestion of large-size proteins in CSM. However, the distribution profile of cottonseed protein has not been significantly affected in $FCSM_B$, which is in conflict with results obtained in fermented soybean meals (Feng *et al*., 2007; Zhu *et al*., 2008). The differences in substrates (CSM versus soybean meal) and bacteria probably account for the discrepancy, suggesting that *B. subtilis* BJ-1 could not degrade some protein subunits in cottonseed protein. On the other hand, Ghazi *et al*. (2010) demonstrated that protease improved the digestion of soybean meal though the improvement of nitrogen usage before to the terminal of the small intestine of broilers. Feng *et al*. (2007) indicated that degradation of soybean protein could improve the digestive

Fig. 2: *In vitro* digestibility of cottonseed meal (CSM) fermented with *Bacillus subtilis* ($FCSM_B$) or with *B. subtilis* and papain ($FCSM_{B+A}$). Values represent the means ± standard error of the mean (SEM) (*n* = 3). Same letter above the bars are not significantly different at p<0.05

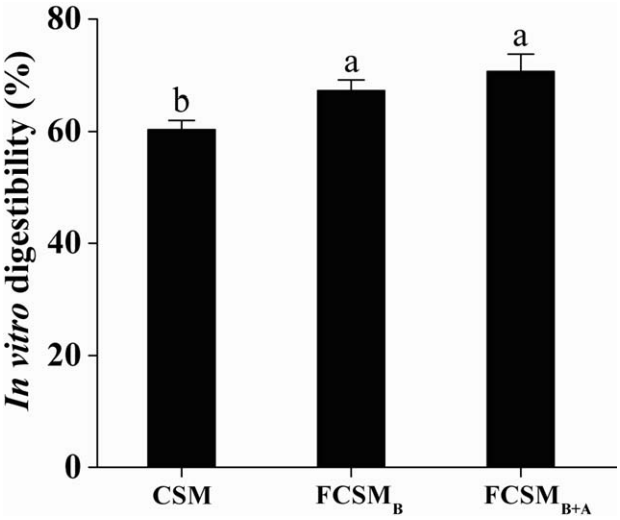
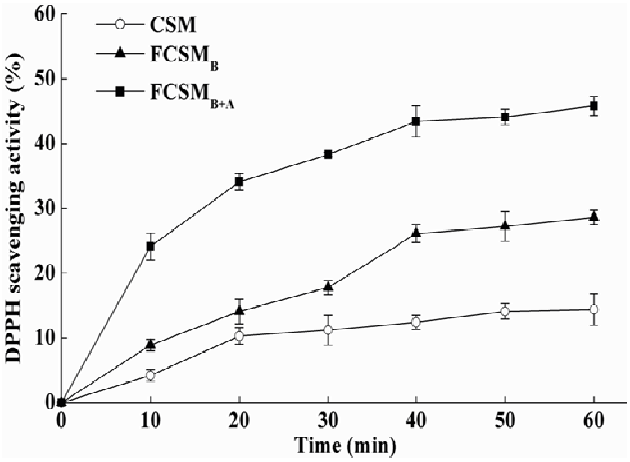


Fig. 3: 2, 2-diphenyl-1-picrylhydrazyl (DPPH)-scavenging activities at 10 min interval of protein extracts of cottonseed meal (CSM) fermented with *Bacillus subtilis* ($FCSM_B$) or with *B. subtilis* and papain ($FCSM_{B+A}$). Values represent the means ± standard error of the mean (SEM) (*n* = 3)



function of piglets. Therefore, the increase in small-molecule proteins of $FCSM_{B+A}$ may improve the digestibility of cottonseed protein. The result also suggests that papain could be use as a suitable enzyme in the fermentation of CSM, which sheds new lights on the strategy for improving the nutritional value of plant-source proteins.

***In vitro* protein digestibility and free radical-scavenging activity:** *In vitro* digestibility of fermented samples was increased (p<0.05), compared with CSM (Fig. 2). Similar improvement was observed in fermented CSM with fungi (Zhang *et al*., 2007). The increase in protein digestibility can be a result of the partial degradation of storage protein into more simple and soluble molecules by enzymatic reactions during the fermentation (Sadeghi & Shawrang, 2007; Zhang *et al*., 2007). However, it would translate to added nutritional value only if it gives rise to better performance in animal feeding trials, which needs to be further investigated.

DPPH is a stable free radical which is used as a substrate to evaluate the *in vitro* antioxidant activities. The scavenging effect increased with the incubation time up to 60 min (Fig. 3). Protein extracts from both $FCSM_B$ and $FCSM_{B+A}$ showed radical-scavenging activity superior (p<0.05) to the CSM during the whole reaction period, with the peak level reached by papain supplement. Similarly, antioxidant activity has been significantly increased in fermented soybean products (Zhu *et al*., 2008; Teng *et al*., 2012). The increased degradation of cottonseed protein can contribute to the increment of antioxidant activity in $FCSM_B$ and $FCSM_{B+A}$ (Zhu *et al*., 2008; Gao *et al*., 2010). This may explain why $FCSM_{B+A}$ depicted a higher antioxidant activity than $FCSM_B$. The improvement of antioxidant activity may be also associated with the increase of amino acids including histidine, alanine, leucine, which play important roles in scavenging free radicals (Kim *et al*., 2001). Other factors cannot be ruled out including the hydrogen-donating ability of microorganisms and other antioxidant components (phenolic compounds, polysaccharides & vitamin) (Yang *et al*., 2000). Since high antioxidant activity is beneficial for inhibiting the oxidation of nutrients in feedstuffs, the fermented CSM may be used as a functional feed ingredient.

CONCLUSION

Fermentation processes with *B. subtilis* BJ-1 could increase the nutritional value, degrade the cottonseed protein which is beneficial for protein utilization and enhance the antioxidant activity of CSM, especially with the supplement of papain. Fermentation with papain may offer a novel strategy to improve the value of CSM.

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REFERENCES

Amartey, S.A., P.C.J. Leung, N.Baghaei-Yazdi, D.J. Leak and B.S. Hartley, 1999. Fermentation of a wheat straw acid hydrolysate by *Bacillus stearothermophilus* T-13 in continuous culture with partial cell recycle. *Process Biochem*., 34: 289–294

AOAC, 1999. *Official Methods of Analysis*, 16th edition. Association of Official Analytical Chemists, Washington, DC, USA

AOCS, 2009. *Official Methods and Recommended Practices of the AOCS*, 6th edition. American Oil Chemists Society, Chicago, Illinois, USA

Chabanon, G., I. Chevalot, X. Framboisier, S. Chenu and I. Marc, 2007. Hydrolysis of rapeseed protein isolates: Kinetics, characterization and functional properties of hydrolysates. *Process Biochem*., 42: 1419–1428

Chen, C.C., Y.C. Shih, P.W.S. Chiou and B. Yu, 2010. Evaluating nutritional quality of single stage- and two stage-fermented soyben meal. *Asian-Australian J. Anim. Sci*., 23: 598–606

Ei-Sayed, A.F.M., 1999. Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. *Aquaculture*, 179: 149–168

Feng, J., X. Liu, Z.R. Xu, Y.P. Lu and Y.Y. Liu, 2007. Effect of fermented soybean meal on intestinal morphology and digestive enzyme activities in weaned piglets. *Digest Dis. Sci*., 52: 1845–1850

Gao, D., Y. Cao and H. Li, 2010. Antioxidant activity of peptide fractions derived from cottonseed protein hydrolysate. *J. Sci. Food Agric*., 90: 1855–1860

Ghazi, S., J.A. Rooke and H. Galbraith, 2010. Improvement of the nutritive value of soybean meal by protease and agalactosidase treatment in broiler cockerels and broiler chicks. *Brit. Poult. Sci*., 44: 3410–3418

Henry, M.H., G.M. Pesti, R. Bakalli, J. Lee, R.T. Toledo, R.R. Eitenmiller and R.D. Phillips, 2001. The performance of broiler chicks fed diets containing extruded cottonseed meal supplemented with lysine. *Poult. Sci*., 80: 762–768

Hong, K.J., C.H. Lee and S.W. Kim, 2004. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Food*, 7: 430–435

Khalaf, M.A. and S.A. Meleigy, 2008. Reduction of free gossypol levels in cottonseed meal by microbial treatment. *Int. J. Agric. Biol*., 10: 185–190

Kiers, J.L., A.E.A. Van laeken, F.M. Rombouts and M.J.R. Nout, 2000. *In vitro* digestibility of *Bacillus* fermented soya bean. *Int. J. Food Microbiol*., 60: 163–169

Kim, S.K., Y.T. Kim, H.G. Byun, K.S. Nam, D.S. Joo and F. Shahidi, 2001. Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. *J. Agric. Food Chem*., 49: 1984–1989

Kramer, J.M. and R.T. Gilbert, 1989. *Bacillus cereus* and other *Bacillus* species. *In*: Doyle, M.P. (ed.), *Food-borne Bacterial Pathogens*, pp: 21–70. Marcel Dekker, New York, USA

Lee, S.M., S.Y. Kim, J. Lee, K.W. Yu, I. Chang and H.J. Suh, 2008. Nonpungent *capsicum* fermentation by *Bacillus subtilis* and the addition of rapidase. *Appl. Microbiol. Biotechnol*., 81: 257–262

Moure, A., J. Sineiro., H. Dominguez and J.C. Parajo, 2006. Functionality of oilseed protein products: A review. *Food Res. Int*., 39: 945–963

Nagalakshmi, D., S.V.R. Rao, A.K. Panda and V.R.B. Sastry, 2007. Cottonseed meal in poultry diets: A review. *J. Poult. Sci*., 44: 119–134

NRC, 1994. *Nutrient Requirements of Poultry*, 9th edition. National Academy Press, Washington, DC, USA

Omafuvbe, B.O., S.H. Abiose and O.O. Shonukan, 2002. Fermentation of soybean (*Glycine max*) for soy-daddawa production by starter cultures of *Bacillus*. *Food Microbiol*., 19: 561–566

Sadeghi, A.A. and P. Shawrang, 2007. Effect of microwave irradiation on rumianl protein degradation and intestinal digestibility of cottonseed meal. *Livest. Sci*., 106: 176–181

SAS, 1999. *Statistical Analyisis System Users' Guide: Statistics*, 8th edition. SAS Institude Inc., Cary, North Carolina, USA

Schagger, H., 2006. Tricine-SDS-PAGE. *Nat. Protoc*., 1: 16–22

Shafique, S., M. Asgher, M.A. Sheikh and M.J. Asad, 2004. Solid state fermentation of banana stalk for exoglucanase production. *Int. J. Agric. Biol*., 6: 488–491

Shyu, Y.S. and L.S. Hwang, 2002. Antioxidative activity of the crude extract of lignan glycosides from unroasted burma black sesame meal. *Food Res. Int*., 35: 357–365

Tang, J.W., H. Sun, X.H. Yao, Y.F. Wu, X. Wang and J. Feng, 2012. Effects of replacement of soybean meal by fermented cottonseed meal on growth performance, serum biochemical parameters and immune function of yellow-feathered broilers. *Asian-Australian J. Anim. Sci*., 25: 393–400

Teng, D., M. Gao, Y. Yang, B. Liu, Z. Tian and J. Wang, 2012. Bio-modification of soybean meal with *Bacillus subtilis* or *Aspergillus oryzae*. *Biocatal. Agric. Biotechnol*., 1: 32–38

Terlabie, N.N., E.S. Dawson and W.K.A. Awua, 2006. The comparative ability of four isolates of *Bacillus subtilis* to ferment soybeans into dawadawa. *Int. J. Food Microbiol*., 106: 145–152

Watkins, S.E., J.T. Skinner, M.H. Adams and P.W. Waldroup, 1993. An evaluation of low-gossypol cottonseed meal in diets for broiler chickens: 1. Effect of cottonseed meal level and lysine supplementation. *J. Appl. Poult. Res*., 2: 221–226

Webb, K.E., 1990. Intestinal absorption of protein hydrolysis products: a review. *J. Anim. Sci*., 68: 3011–3022

Weng, X.Y. and J.Y. Sun, 2006. Biodegradation of free gossypol by a new strain of *Candida tropicalis* under solid state fermentation: Effects of fermentation parameters. *Process Biochem*., 41: 1663–1668

Xu, Ch., H. Wang, F. Yang and Zh. Yu, 2011. Effect of inoculant and enzymes on fermentation quality and nutritive value of erect milkvetch (*Astragalus adsurgens* Pall.) silages. *J. Anim. Feed Sci*., 20: 449–460

Yang, J.H., J.L. Mau, P.T. Ko and L.C. Huang, 2000. Antioxidant properties of fermented soybean broth. *Food Chem*., 71: 249–254

Zhang, W.J., Z.R. Xu, J.Y. Sun and X. Yang, 2006. A study on the reduction of gossypol levels by mixed culture solid substrate fermentation of cottonseed meal. *Asian-Aust. J. Anim. Sci*., 19: 1314–1321

Zhang, W.J., Z.R. Xu, S.H. Zhao, J.Y. Sun and X. Yang, 2007. Development of a microbial fermentation process for detoxification of gossypol in cottonseed meal. *Anim. Feed Sci. Technol*., 135: 176–186

Zhu, Y.P., J.F. Fan, Y.Q. Cheng and L.T. Li, 2008. Improvement of the antioxidant activity of Chinese traditional fermented okara (Meitauza) using *Bacillus subtilis* B2. *Food Cont.*, 19: 654–661

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