

Appendices

Appendix A
Calibration curve

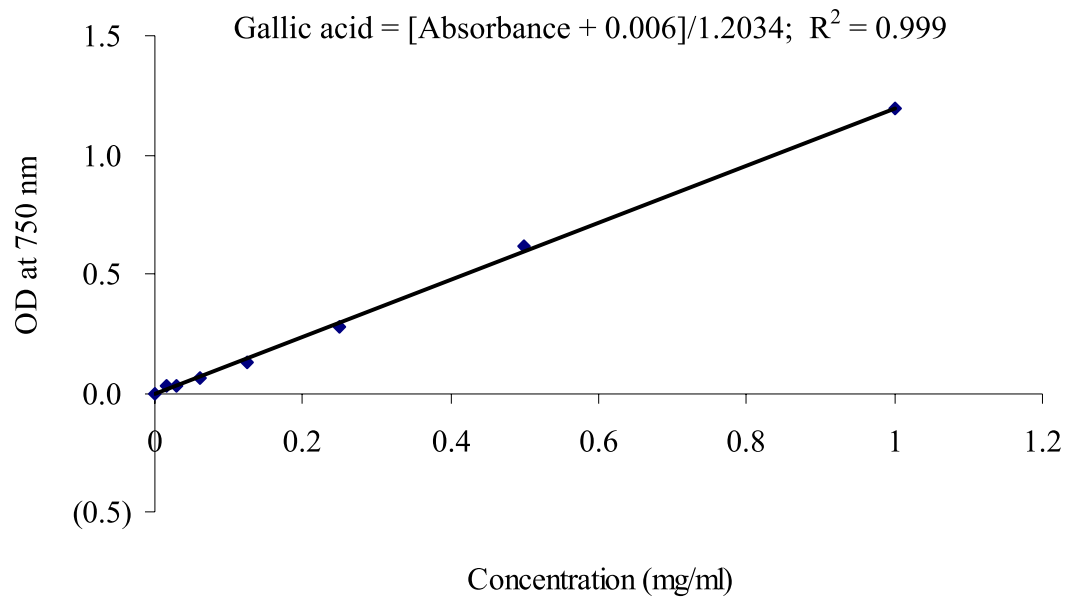


Figure A-1 Standard curve of gallic acid used to quantify total phenolic contents.

Appendix B
Questionnaire for sensory evaluation

Questionnaire for measuring the preference of improved fermented soybeans

Product: Fermented soybeans

Name **Date** **No.**.....

Please evaluate fermented soybean samples and check ✓ on the space that best reflects your feeling about the sample by using the following scores:

- 1 = Dislike very much 2 = Dislike moderately 3 = Dislike
 4 = Neither like nor dislike 5 = Like 6 = Like moderately
 7 = Like very much

Product attributes	Sample code						
	1	2	3	4	5	6	7
Overall preference							
Colour							
Odour							
Texture							
Flavour							

Suggestion:

Thank you for your participation

Appendix C

Bacillus sp. TN51 16S ribosomal RNA, partial sequence

Features Sequence
LOCUS GU451310 500 bp DNA linear BCT 09-FEB-2010
DEFINITION *Bacillus* sp. TN51 16S ribosomal RNA, partial sequence.
ACCESSION GU451310
VERSION GU451310.1 GI:288187224
SOURCE *Bacillus* sp. TN51
 ORGANISM [Bacillus sp. TN51](#)
 Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus.
REFERENCE 1 (bases 1 to 500)
 AUTHORS Chukeatirote, E., Dajanta, K., Apichartsrangkoon, A. and
 Boonkumklao, P.
 TITLE Pure culture for making thua nao
 JOURNAL Unpublished
REFERENCE 2 (bases 1 to 500)
 AUTHORS Chukeatirote, E., Dajanta, K., Apichartsrangkoon, A. and
 Boonkumklao, P.
 TITLE Direct Submission
 JOURNAL Submitted (13-JAN-2010) Biotechnology, Mae Fah Luang
 University, University Avenue, Muang, Chiang Rai 57100,
 Thailand
FEATURES Location/Qualifiers
 source 1..500
 /organism="Bacillus sp. TN51"
 /mol_type="genomic DNA"
 /strain="TN51"
 /isolation_source="fermented soybean"
 /db_xref="taxon:[716595](#)"
 /country="Thailand"
 /PCR_primers="fwd_seq: agagtttgatcctggctcag, rev_seq:
 aaggaggtgatccarccgca"
[rRNA](#) <1..>500
 /product="16S ribosomal RNA"

ORIGIN

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181 cacttacag atggaccgcg ggcgcattag ctagtgtgtg aggtaacggc tcaccaaggc
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361 cgccgcgtga gtgatgaagg ttttcggatc gtaaagctct gttgttaggg aagaacaagt
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481 gccagcagcc gcggtataac
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Appendix D

Presentations and publications

D-1 Posters presentation

D-1.1 To be presented at Food Innovation Asia Conference 2009, The International Food Conference “Value Creation through Innovation in Food Technology” at BITEC, Bangkok, Thailand, June 18-19, 2009.

Title: Composition and quantities of free amino acids in *thua nao* (a Thai fermented soybean)

Authors: Katekan Dajanta, Arunee Apichartsrangkoon and Ekachai Chukeatirote

Abstract: A Thai traditional fermented soybean, *thua nao*, is extensively consumed in the Northern part of Thailand. This study was to quantify free amino acid contents of commercial *thua nao* collected from six local markets in Chiangmai province, Thailand namely, Mae Wang (MW), Mae Hia (MH), Mae Taeng (MT), Jom Thong (JT), San Patong (SP) and San Sai (SS) by HPLC technique. The quantities of total free amino acids were found in the range of 11.03– 61.23 g kg⁻¹, as dry basis. The most predominant free amino acids were Trp, Glu, Cys, Lys and Leu and nearly all essential amino acid (EAA) with proportion from 59.10 to 65.97%. Hence, Thai traditional *thua nao* could be a rich source of bioavailability free amino acids that expected to promote health benefits.

D-1.2 To be presented at RGJ—Ph.D. Congress X: April 3-5, 2009 at Jomtien Palm Beach Resort, Chonburi, Thailand, April 3-5, 2009.

Title: Comparison of Isoflavone Contents in *Bacillus*-Fermented Soybeans

Authors: Katekan Dajanta, Arunee Apichartsrangkoon, Ekachai Chukeatirote and Richard A. Frazier

Abstract: This investigation reflected the effects of using pure culture on the quality and quantity of isoflavones in fermented soybeans with protein-rich variety TG145. Three bacterial starter cultures (10⁴ CFU/g) namely *Bacillus subtilis* BEST195, *B. subtilis* Asaichiban and *B. subtilis* TN51 were inoculated in sterilised (121°C for 40 min) soybeans and subsequently fermented at 42°C for 24 hr (*natto*-style) or 72 hr (*thua nao*-style). The quantities of six major isoflavones; daidzin, genistin, glycitin, daidzein, genistein, and glycitein were determined using HPLC technique. The content of total isoflavones in the fermented products prepared by *Bacillus* starter cultures extensively increased ranging from 43 – 99% in comparison with unfermented sterilised soybeans. In particularly, aglycones found in *Bacillus* sp. strain TN51 *Thua Nao* increased more than 400%. This study suggested a beneficial utilisation of pure *Bacillus* culture in improving isoflavones for future novel functional food.

D-1.3 To be presented at Congress on Science and Technology of Thailand (STT 33) at Walailak University, Nakhon Si Thammarat, Thailand, October 18-20, 2007.

Title: Comparative analysis of protease activity of *Bacillus* species isolated from *thua nao*

Authors: Dajanta, K., Baophoeng, P., Thirach, P., Santithum, P., Chukeatirote, E., and Apichartsrangkoon, A.

Abstract: Various types of fermented soybeans have been produced in Asian countries, i.e., Indian *kinema*, Japanese *natto* and Thai *thua nao*. Presently, *natto*—one of the best-studied fermented soybeans—has been developed using a pure starter culture of *Bacillus subtilis* natto strain. In addition, the production line has been controlled starting from the soybean selection (cultivar and size), systemic fermentation to product quality evaluation. On the contrary, *thua nao* production still remains in a traditional manner and this often leads to inconsistent yield. In this study, several *Bacillus* strains previously isolated from *thua nao* were screened for their proteolytic activity using casein agar. Of 171 strains, two potential isolates namely TN51 and TN69 exhibited highest protease activity (with clear zones of 2.73 and 2.65cm). Further investigation was then performed using three different protein-based media: casein agar, skim milk agar and soy protein hydrolysate agar. Two *B. subtilis* natto strains were also used as reference strains. In addition, cell culture supernatant prepared from overnight incubation was also used to further confirm such activities. Our results showed that both TN51 and its supernatant exhibited highest proteolytic activity (observing from the greatest clear zone diameters). As a result, the bacterium TN51 is expected to be used as a potential starter culture for improvement of *thua nao* production process.

D-2 Papers

D-2.1 Dajanta, K., Chukeatirote, E., and Apichartsrangkoon, A. (2010). Analysis and characterisation of amino acid contents of *thua nao*, a traditionally fermented soybean food of Northern Thailand. *Songklanakarin Journal of Science and Technology*: (in press).

D-2.2 Dajanta, K., Chukeatirote, E., Apichartsrangkoon, A. and Frazier, R.A. (2009). Enhanced aglycone production of fermented soybean products by *Bacillus* species. *Acta Biologica Szegediensis*, 52(2): (in press).

D-2.3 Dajanta K., Wongkham, S., Thirach, P., Baophoeng, P., Apichartsrangkoon, A., Santithum, P., and Chukeatirote, E. (2009). Comparative study of proteolytic activity of protease-producing bacteria isolated from *thua nao*. *Maejo International Journal of Science and Technology*, 3, 269-276.

CURRICULUM VITAE

Name	Katekan Dajanta
Date of birth	April 16, 1971
Education	2003 MSc. (Food Science and Technology) Chiang Mai University, Chiang Mai, Thailand. 1994 BSc. (Agriculture) Chiang Mai University, Chiang Mai, Thailand.
Scholarship	The Royal Golden Jubilee Ph.D. Program, Thailand Research Fund.
Work experience	2008-present Lecturer, Pibulsongkram Rajabhat University, Phitsanulok, Thailand. 1996-2008 Scientist, Department of Food Science and Technology, Faculty of Agro-Industry, Chiang Mai University, Thailand. 1994-1996 Researcher, Department of Plant Pathology Faculty of Agriculture, Chiang Mai University, Thailand.

Presentations

- Dajanta, K., Apichartsrangkoon, A., and Chukeatirote, E. (2009). Composition and quantities of free amino acids in *thua nao* (a Thai fermented soybean). Food Innovation Asia Conference 2009, BITEC Bangna, Bangkok, Thailand, June 18-19, 2009.
- Dajanta, K., Apichartsrangkoon, A., Chukeatirote, E., and Frazier, R. A. (2009). Comparison of isoflavone contents in *Bacillus*-fermented soybeans. RGJ-Ph.D. Congress X, Jomtien Palm Beach Resort, Chonburi, Thailand, April 3-5, 2009.
- Dajanta, K., Baophoeng, P., Thirach, P., Santithum, P., Chukeatirote, E., and Apichartsrangkoon, A. (2007). Comparative analysis of protease activity of *Bacillus* species isolated from *thua nao*. The 33rd Congress on Science and

Technology of Thailand (STT 33), Walailak University, Nakhon Si Thammarat, Thailand. October 18-20, 2007.

Publications

- Dajanta, K., Chukeatirote, E., and Apichartsrangkoon, A. (2010). Analysis and characterisation of amino acid contents of thua nao, a traditionally fermented soybean food of Northern Thailand. *Songklanakarin Journal of Science and Technology* (in press).
- Dajanta, K., Apichartsrangkoon, A., Chukeatirote, E., and Frazier R.A. (2009). Enhanced aglycone production of fermented soybean products by *Bacillus* species. *Acta Biologica Szegediensis* (in press).
- Dajanta K., Wongkham, S, Thirach, P., Baophoeng, P., Apichartsrangkoon, A., Santithum, P., and Chukeatirote, E. (2009). Comparative study of proteolytic activity of protease-producing bacteria isolated from *thua nao*. *Maejo International Journal of Science and Technology*, 3, 269-276.