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HIGH VITAMIN B_{12} VEGETARIAN DIETS by MIXED FERMENTATION

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A I D Grant Projet Officer Science Attache,
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EXECUTIVE SUMMARY

HIGH VITAMIN B_{12} VEGETARIAN DIETS BY MIXED FERMENTATION

The project aims to develop the process for producing the novel and vitamin B_{12} rich vegetarian diets from soybeans The products could be used among the group of vegetarians as well as the low-income, low-protein consuming people in order to prevent vitamin B_{12} deficiency

Experiments were conducted in the last phase (March 31, 1998-February 28, 1999) (1) Finished product development (2) Food formulation and (3) Sensory evaluation by Thai group whereas those on Nutritional and Toxicological evaluations were studied by Israel group (Professor Dr Arich Gertler group)

Products derived from selected Process C and Process F(which centaining vitamin B_{12} about 300 ng per 100 g wet basis) were used for finished product development (FPD) It is found out that the FPD could be done in 3 ways (I) the direct uses of fermented soybean product as high vitamin B_{12} soy sandwich or soy-salads (ii) Uses of fresh high vitamin B_{12} soybean masses as soymilk, soy-soup, tofu or soy-kapi (iii) Indirect uses of fermented masses as soy powder by spray drying technique The spray dried powder could serve as soy-soup or soy-milk Food formulation is needed to meet consumer acceptance Sensory evaluation of the main product have been carried out

Kasetsart University through Dr B Yongsmith have given the finished products materials to Professor Dr A Gertler since October 1997 Both groups are communicated regardling project progress and direction Dr B Yongsmith and Dr W Krusong visited the project consultant, Professor Di K H Steinkraus at Cornell University in November 1998 Professor Steinkraus visited Kasetsart University, discussed the experimental results and gave advices for writing up the final report in early 1999

Section I

A) Research Objectives

The overall aim of this project is to develop a process for producing the novel and vitamin B_{12} rich vegetarian diets This project also aims to prevent vitamin B_{12} among vegetarians and the group of low income, low protein consuming people

The experiments conducted from March 31, 1998 to February 28, 1999 were designed as follows

1) Finished product development
2) Food formulations
3) Sensory evaluation
4) Nutritional study
5) Toxicity study

Experiments numbering (1), (2), (3) were conducted by Thai group (led by Dr Busaba Yongsmith) and those of (4) and (5) were conducted by Israel group (led by Professor Dr Arich Gertler)

B) Research Accomplishments

Our plan is to study the feasibility of using mixed cultures of lactic acid bacteria and for propionic acid bacteria to be incorporated in the traditional tempe or thua nao preparation These innovative process are expected to produce an acceptable novel high vitamin B_{12} vegetarian diets that will be suitable for rural poor in term of economical, practical and simple application

The project is divided into major phases as follows

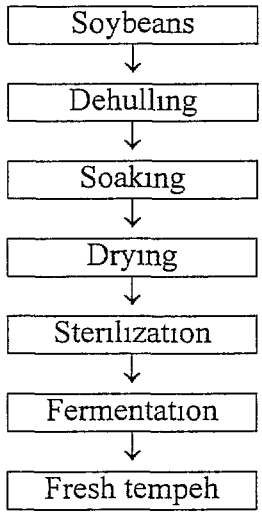
Phase I Culture preparation and selection of process variables applicable to soybean substrate

Phase II Optimization of the process and scaling up

Phase III Product development and evaluation

This annual report is covering the experiments of Phase III Some of these experimental results are shown in Management Report III and this report of Table 1-4 and (Fig 1)

Fig 1 showed the mass balance in the production of product from Process C prepared in the tray with 2 cm thickness of soybean mass and covered with perforated aluminum foil was calculated and showed that the total solids loss was 30 14% during the processing step



	Yeld (%)	Solid loss (5 dry basis)
Soybeans	1,000	-
Dehulling	75 (hulls)	7 62
Soaking	1,770	12 98
Drying	1,485	1 61
Sterilization		
Fermentation	1,448	2 09
Fresh tempeh	691	5 84
	Total	30 14

Fig 1 Mass balances in production of product from Process C at optimum conditions at bench scale after 24 hrs fermentation

Table 1 showed changes in pH, soluble carbohydrate, vitamin B_{12} and total acidity in product from Process C during fermentation Tempe mold alone can increase pH of soybean from 6 3 to 7 6 with ammonia accumulation But the Process C where propionic acid bacteria included, the pH is 6 1-6 6 and deamination of amino acid was reduced The total acidity gradually increased due to the increase in carbohydrate degradation products used as substrate for acid production It was also observed that vitamin B_{12} in the product was produced rapidly in 24 hrs and then slightly increased in 48 hrs This may be due to the rapid growth of propionic acid bacteria after 24 hrs of incubation

Table 2 showed changes in chemical compositions in product of Process C during fermentation These was an increase of protein and fat contents due to the decomposition of the carbolydrate and increase in mold mycelia

Eventhough the soymilk product derived from Process C had inferior characteristics to a commercial soymilk (Table 3) but it contained more soluble protein and vitamin B_{12} than that of commercial soymilk (Table 4)

Table 1 Changes in pH, soluble carbohydrate, vitamin B_{12} and total acidity of product from Process C during fermentation

Fermentation Hour	PH	Soluble Carbohydrate[1] (g/100g)	Vitamin B_{12} (ng/100g)	Total Acidity (%)
0	6 3	15 34	36 0 a	0 78 b
24	6 6	17 53	304 0 a	1 06 b
48	6 2	15 63	308 4 a	1 51 b
72	6 2	14 88	307 6 a	2 44 a
96	6 1	14 71	302 8 a	1 31 b

Table 2 Proximate analysis of product of Process C during fermentation (dry weight basis)

Fermentation	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (% by difference)
0	3 3 bc	46 3 a	20 1 b	2 9 a	27 4 a
24	2 3 d	47 4 a	22 3 b	3 1 a	24 0 a
48	2 8 cd	49 6 a	26 1 a	3 5 a	18 0 b
72	4 2 ab	49 4 a	26 4 a	3 6 a	16 4 b
96	4 7 a	48 3 a	28 4 a	3 7 a	14 9 b

Means of three replications means in the same column followed by the same letters are not significantly different at 5% by DMRT

Table 3 Sensory evaluation of soy milk product derived from Process C

Sample	Characteristics			
	Odor	Color	Texture	Acceptability
A commercial soymilk	6 28 a	5 96	6 60 a	6 56 a
Soymilk from Process C	5 36 b	5 88	5 68 b	5 96 b

Table 4 Nutritional value of soymilk

Sample	Total protein (%)	α aminonitrogen (g/l)	Vitamin B_{12} (ng/100ml)
A commercial soymilk	2 78 b	2 87 b	0 0
Soymilk from Process C	3 47 a	3 50 a	36 1

Means of three replications means in the same column followed by the same letters are not significantly different at 5% by DMRT

C) Scientific Impact on Collaboration

Kasetsart University led by Dr Busaba Yongsmith had handled the product samples of 28 items to Professor Dr Arieh Gertler for his studies on nutritional and toxicity approaches since October1997 Dr Busaba Yongmith and her coinvestigator Dr Warawut Krusong had visited their project consultant Professor Dr K H Steinkraus at Cornell University in November 1998 Professor Dr K H Steinkraus could visit Kasetsart University in March 1999 to further discuss on the experimental results and give advices for the writing up the final report Professor Dr Steinkraus visited Kasetsart University after successful laparoscopy examination of his wife

D) Description of Project Impact

The bacteria namely *Propionibacterium freudenreichii* and *Bacillus megaterium* could be used as potential vitamin B_{12} enhancer to mixed fermentation in soybean substrates More soluble and digestible soybean could be obtained by treating with Tempe mold (*Rhizoups oligosporus*) or Thua-nao bacteria (*Bacillus subtilis*)

Process C (prompt inoculation of Tempe mold and *Propionibacterium freudenreichii* and Process F (prompt inoculation of Tempe mold and *Bacillus* spp were used for Phase III Experiments on Product Development and Sensory Evaluation Results showed that product of Process C could be developed over than those of process F in 3 methods (i) direct used of fermented masses as high vitamin B_{12} soy sandwich or soy salads (ii) Uses of fresh high vitamin B_{12} modified Tempeh as soy soup or soy-milk (iii) Indirect uses of fermented masses as soy powder by spray drying which its modification could be soy soup or soy milk Food formulation is needed to meet consumer acceptance Sensory evaluation of the main product has been carried out

E) Strengthenign of Developing Country Institutions

Many equipments obtained from the project as well as those provided by the Department have given project facilities and resulted successfully Experiences and skills from novel process and novel products of the project would solve the problems of malnutrition among vegetarians and low-income people due to the lack of vitamin and low-protein foods

F) Future work

Project has been accomplished and terminated on February 28, 1999 However, some knowledge should be carried out in the future concerning the basic study on the molecular interaction of the mixed cultures and soybeans sugar (oligosaccharide) utilization in terms of upgrading of soybean substrates

Section II

A) Managerial Issues

Main works of Thai group have been finished Some data are still collected and profiled After the results of Israel group on nutritional and toxicological studies of the finished products have been obtained Results of two groups are thus combined for the final report which can be sent to USAID within a few months from now

B) Budget

Budget allocation for the third period has been delayed for 9 months so this is the reason why the project had been be postponed another year Budget has been spent more to personnel bedget line item By all means, the total budget has not been changed

C) Special Concerns

D) Collaboration, Travel, Training and Publication

Dr Busaba Yongsmith and Dr Warawut Krusong visited the project consultant, Professor Dr H H Steinkraus at Cornell University In return, Professor Dr K H Steinkraus visited Kasetsart University in early 1999 in order to give consultation and advises on writing up the final report and give lecture on his experiences on soybean fermentation to Thai scientists Thai scientists hope to join and present a paper at the International Soy Forum in Chicago, U S A in July, 1999

E) Request for A I D and Bostid Action

F) Travels and meeting Attended

1 A visit of Thai scientists to their consultant in Cornell University U S A in November, 1998
2 A visit of the project consultant to Thailand in early 1999

PROGRAM SCHEDULE OF THE PROPOSED RESEARCH PROJECT PROPOSAL No TA-MOU-C11-194
"HIGH VITAMIN B_{12} VEGETARIAN DIETS BY MIXED FERMENTATION"