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Microorganisms in Fermented Bio-extract from Irradiated Silk Waste

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Abstract

Microbiological characteristics of four samples of bio-extract from gamma irradiated (300 kGy) silk waste fermented for 0, 1, 2, 3 and 4 months were investigated. The results revealed that total bacterial counts in all samples of bio-extract were initially found to be 2.32×10^7 – 1.69×10^8 cfu/ml. Four months later, total bacterial counts mainly decreased according to the fermentation time. At the beginning, lactic acid bacteria were found in three samples and ranged from 6.0×10^6–2.2×10^8 cfu/ml. Only in one sample that lactic acid bacteria were not found. Four months later, lactic acid bacteria were still found in two samples and ranged from 1.8×10^2–3.8×10^7 cfu/ml. Initially, total yeasts were found in all samples and ranged from 1.0×10^4–3.2×10^5 cfu/ml which exceeded the standard value of Thai Community Product Standard : Plant Extraction [TCPS 481-2547(2004)]. In the fourth month, total yeasts were not found in three samples. However, they were found in one sample and still exceeded the standard value. Three pathogenic bacteria (Salmonella spp., Staphylococcus aureus and Clostridium perfringens) based on the TCPS 481-2547(2004), were not found in any samples after any fermentation length. At zero month, all samples were contaminated with Escherichia coli densities in the range of 15 – >$1.1x10^3$ MPN/ml and exceeded the standard value of TCPS 481-2547(2004). However, one month later the MPN of E. coli were not found in all samples and therefore the samples were up to the standard level of TCPS 481-2547(2004).

Keywords-*microorganisms; fermented bio-extract; Thai Community Product Standard ; irradiation; silk waste*

I. INTRODUCTION

Bio-extract is the local wisdom of the villages in Asian countries. It is well-known extensively and can be applied in several fields. The fermentation process of bio-extract is simple, low cost and easy to use a variety of materials in each local area. Bio-extract is a liquid or yellowish-brown liguid derived from the fermentation of plant and animal residues. It contains effective microorganisms, vitamins, minerals, hormones, enzymes and organic substances. Effective microorganisms (EM) compose of 80 species of microorganisms, which include lactic acid bacteria, photosynthetic bacteria, actinomycetes, yeasts and fermenting fungi [1]. The microorganisms are drawn from 10 genera belonging to 5 different families. The most outstanding characteristic of EM is that it includes both aerobic and anaerobic species coexisting symbiotically in a most beneficially productive manner [2]. The important roles of bacteria in bio-extract are to decompose organic matters to smaller molecules and inhibit microorganisms in plant pathogens [3]. The application of EM were including agriculture, bioremediation, composting, livestock, gardening, landscaping, cleaning septic, water treatment and household uses [1]. It has been hypothesized that the bio-extract may act as a direct source of beneficial microorganisms or/and substrates to stimulate soil microbial activities in decomposing organic fertilizer and to release nutrients to plants. Many small farmers in Northeast Thailand have been using the bio-extract to reduce or replace chemical fertilizer and pesticide use [4].

There were a lot of dirty cocoons from the former projects of Thailand Institute of Nuclear Technology (Public Organization) left as silk waste. According to the Eleventh National Economic

and Social Development Plan (2012-2016) of Thailand, this research was performed to produce fermented bio-extract from irradiated silk waste for agricultural use. This study was focused on the microbiological quality of the product (fermented bio-extract from irradiated silk waste) according to the Thai Community Product Standard: Plant Extraction [TCPS 481-2547 (2004)].

II. MATERIALS AND METHODS

A. Irradiated silk waste

Dirty cocoons were mainly used as silk waste and irradiated at 300 kGy by gamma ray.

Fig.1 Dirty cocoons

B. Fermented bio-extract

Irradiated dirty cocoons were soaked in water overnight. The extraction of sericin from irradiated dirty cocoons was carried out by using autoclave. The concentration of siricin was adjusted to be 1%. Then 1% of sericin was mixed with 3% brown sugar and fermented for 0, 1, 2, 3 and 4 months.

Fig.2 Fermented bio-extract from irradiated silk wastes

C. Determination of microorganisms in bio-extract from irradiated silk wastes

C.1 Enumeration of total bacterial count was examined by BAM Online 2001, Chapter 3 [5].
C.2 Enumeration of lactic acid bacteria was investigated by ISO 15214 : 1998 [6].
C.3 MPN of *E.coli* was determined by BAM Online 2002, Chapter 4 [7].
C.4 *Clostridium perfringens* was detected by BAM Online 2001, Chapter 16 [5].
C.5 *Staphylococcus aureus* was detected by BAM Online 2001, Chapter 12 [5].
C.6 *Salmonella* spp. was detected by ISO 6579 : 2002 [8].
C.7 Yeasts and molds were enumerated by BAM Online 2001, Chapter 18 [5].

III. RESULTS AND DISCUSSION

As shown in Fig.1, total bacterial counts in all samples of bio-extract were initially found to be 2.32×10^7 – 1.69×10^8 cfu/ml. Four months later, total bacterial counts were reduced to 4.25×10^4 – 1.00×10^6 cfu/ml. This result revealed that total bacterial counts were mainly decreased according to the fermentation time. Department of Agriculture also indicated that total bacterial counts were found to be 10^8 cfu/ml at the beginning of fermentation. After one year, not only the number of total bacteria but also the biodiversity of strains were decreased [3].

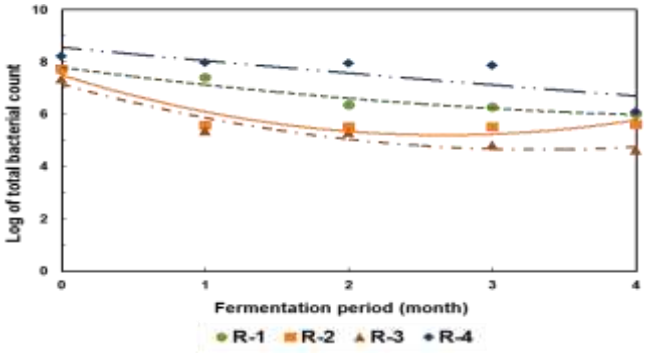

Fig.1 Total bacterial counts in fermented bio-extract from irradiated silk waste at different periods

Total yeasts in fermented bio-extract from irradiated silk waste at different periods is shown in Table 1. At the beginning, total yeasts were found in all samples and ranged from 1.0×10^4–3.2×10^5 cfu/ml which exceeded the standard value of TCPS 481-2547(2004). In the fourth month, total yeasts were not found in three samples. However, they were found in one sample (1.06×10^2cfu/ml) which were slightly higher than the standard value of TCPS 481-2547(2004). Additionally, molds were not found in all samples.

Table 1 Enumeration of total yeasts in fermented bio-extract from silk waste at different periods

Treatment	Total yeasts (cfu/ml)				
	Fermentation period (month)				
	0	1	2	3	4
R-1	1.00×10^4	0	0	0	0
R-2	3.20×10^5	1.00×10^2	5.70 x 10	3.67	0
R-3	2.55×10^4	4.10×10^2	1.22×10^2	8.75 x 10	1.06×10^2
R-4	1.50×10^5	8.16×10^2	2.35x10	0	0

Table 2 shows lactic acid bacteria in fermented bio-extract from irradiated silk waste at different periods. Initially, lactic acid bacteria were found in 3 out of 4 samples and ranged from 6.0×10^6–2.2×10^8 cfu/ml. Four months later, lactic acid bacteria were still found in two samples and ranged from 1.8×10^2–3.8×10^7 cfu/ml. Tripetchkul, et al. reported that after the second week of fermentation, total bacteria and lactic acid bacteria count present in fish and pineapple bioextracts were lower than 10^8 cfu/ml and subsequently, declined continuously. Such decline may be due primarily to a swift depletion of reducing sugar [9]. In addition, lactic acid bacteria in EM suppress harmful microorganism while producing lactic acid which lowers the pH in EM and encourages rapid breakdown of organic substances. Lactic acid bacteria can also suppress the reproduction of a harmful fungus, *Fusarium* spp [10].

Table 2 Enumeration of lactic acid bacteria in fermented bio-extract from irradiated silk waste at different periods

Treatment	Lactic acid bacteria (cfu/ml)				
	Fermentation period (month)				
	0	1	2	3	4
R-1	2.2×10^8	2.3×10^7	9.9×10^7	<10 (not found)	<10 (not found)
R-2	<10 (not found)	<10 (not found)	<10 (not found)	<10 (not found)	<10 (not found)
R-3	6.0×10^6	5.8×10^6	5.3×10^7	6.5×10^7	3.8×10^7
R-4	9.0×10^7	8.9×10^7	1.1×10^6	1.4×10^7	1.8×10^2

MPN of *E. coli* in fermented bio-extract from irradiated silk waste at different periods is shown in Table 3. At zero month, all samples were contaminated with *E. coli* densities in the range of 15 – $>1.1 \times 10^3$ MPN/ml which exceeded the standard value of TCPS 481-2547(2004). However, one month later the MPN of *E. coli* were not found in all samples and were up to the standard level of TCPS 481-2547(2004). For safe utilization from pathogens in bio-extract from irradiated silk waste, it was recommended to use after one month fermentation.

Table 3 MPN of E. coli in fermented bio-extract from irradiated silk waste at different periods

Treatment	*E. coli* (MPN/ml)				
	Fermentation period (month)				
	0	1	2	3	4
R-1	>1100	< 3	< 3	< 3	< 3
R-2	290	< 3	< 3	< 3	< 3
R-3	15	< 3	< 3	< 3	< 3
R-4	>1100	< 3	< 3	< 3	< 3

Three pathogenic bacteria namely *Salmonella* spp., *Staphylococcus aureus* and *Clostridium perfringens* were not found in any samples after any fermentation length, indicating that the samples were up to the standard level of TCPS 481-2547(2004). These results were in accordance with Suksawang indicated that the pathogenic microorganisms, based on the Thai Community Product Standard: Plant Extraction, are not found in Cheng bio-extract [11]. Additionally, Hui et al. also reported that effective microorganisms were environmental friendly, not chemical synthesized, not dangerous and not pathogenic microorganisms [12].

Based on microbiological standard, three samples (75%) of bio-extract from irradiated silk waste which fermented for 4 months were up to the standard value of TCPS 481-2547(2004). On the contrary, only one sample (25%) of bio-extract from irradiated silk waste which fermented for 4 months could not meet the standard value because total yeasts were slightly higher than the standard value of TCPS 481-2547(2004).

IV. CONCLUSION

1. Microbial characteristics of 3 out of 4 samples in bio-extract from irradiated silk waste which fermented for 4 months were up to standard level of TCPS 481-2547(2004).

2. For safe utilization from pathogens in bio-extract from irradiated silk waste, it was recommended to use after one month fermentation

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