Accumulation of 1-deoxynojirimycin (DNJ) in Different Species of Silkworm Larvae in Northeast of Thailand

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Abstract

This work aims to investigate 1-deoxynojirimycin (DNJ, a potential antiglucosidase) accumulation in different species of silkworm larvae in northeast of Thailand. Twenty-nine species were cultivated at the Queen Sirikit Department of Sericulture (Sakon Nakhon, Thailand); silkworm specie; SP1, SP2, SP3, N.K.02, N.K.04, N.K.08, N.K.011, SR.5, R.A.05, Chok Amnuai, Non Rue Si, Haujaraud, Kaew Sakon, Sumloung, Vietnam, Vanasavan, Ai Tang, Kuang Wan, Nuangnoi Srisaket, Srisaket1, Tabtim Siam06, Kaki, NuangTui, Nuang Lai, Nuang Kaw, Nuang On Yaung, Naung Saew, Nuang Daeng and Nuang Luang. The silkworm larvae were freely fed with mature mulberry leaves (variety Buriram 60) as common feeding silkworms for sericulture industries. The silkworm's age of 12, 17 and 26 days were investigated DNJ accumulation using HPLC. The results found that silkworms species SR.5 (113±7.5 mg/100g), N.K.04 (97±9.3 mg/100g) and Nuangnoi Srisaket (76±8.2 mg/100g) had high DNJ content at age of 12 days. Kuang wan (111±9.4 mg/100g), Kaki (104±9.3 mg/100g) and Nuangnoi Srisaket (103±6.6 mg/100g) had high DNJ accumulation at 17 days while Nuangnoi Srisaket (96±7.5 mg/100g), Srisaket1 (91±8.1 mg/100g), SP1 (91±9.6 mg/100g) had high DNJ content at 26 days, respectively. As results, DNJ content in silkworms have not relevant on age difference. Considering to DNJ amount, there are several silkworm species had high DNJ (around 100 mg/100g) that was close to DNJ amount in mulberry supplement produced from shoot or young mulberry leaves, and could be applied for supplementary foods.

Keywords: Silkworms larvae, 1-deoxynojirimycin (DNJ), Accumulation

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1. Introduction

At present, diseases concerning modern life style has been healthy problems to people around the world such as hypertension and diabetes. Not only medicine is used to treat patients but plant and herb are also a potential choice for preventing diabetes. For decade, many scientists reported that 1-deoxynojirimycin (DNJ), D-glucose analogue, extracted from mulberry (Moraceae) was an effective anti-glucosidase to suppress glucose absorption. Dietary mulberry DNJ could be suppression of abnormally blood glucose and preventing diabetes in animals and human (Kimura *et al*., 2007; Nakagawa *et al*., 2008). Therefore, several mulberry products are available in supplement food markets.

To control postprandial blood glucose, the amount of DNJ content should be 6.5 mg /meal as effective dose (Kimura *et al*., 2007). It was found that DNJ in mulberry leave was around 0.1% (100 mg/100g) with higher content in shoot, young and mature leave, respectively (Vichasilp *et al*., 2012). However, there was investigation showed that many mulberry products available in the market contained low amount of DNJ that could provide the effective dose (Vichasilp *et al*., 2012). Mulberry products with high DNJ content was highly demanded. There are attempts to find abundant source of DNJ such as DNJ synthesis or DNJ in other sources such as DNJ in silkworms (Nakagawa *et al*., 2010).

A high concentration of DNJ could be found in silkworm that could be used for supplement foods with no toxicity and genotoxicity (Nakagawa *et al*., 2010; Heo *et al*., 2013). The sericulture of Thailand has a various varieties of silkworm species use to produce cocoon and silk. After silk filature, the silkworms have been mortal and cooked for foods. However, there are little studies about the amount of DNJ contents in the silkworms. In this study, DNJ content in silkworm was investigated at different age. This finding could provide the possibility of Thai's silkworm to use as supplement food for suppression of postprandial blood glucose.

2. Materials and methods

2.1 Materials

Twenty-nine species of silkworms larvae varieties that were cultured in Thailand were used in this study following; SP1, SP2, SP3, N.K.02, N.K.04, N.K.08, N.K.011, SR.5, R.A.05, Chok Amnuai, Non Rue Si, Haujaraud, Kaew Sakon, Sumloung, Vietnam, Vanasavan, Ai Tang, Kuang Wan, Nuangnoi Srisaket, Srisaket1, Tabtim Siam06, Kaki, Nuang Tui, Nuang Lai, Nuang Kaw, Nuang On Yaung, Naung Saew, Nuang Daeng and Nuang Luang. The silkworms were fed in the Queen Sirikit Department of Sericulture (Sakon Nakhon, Thailand). Silkworms were cultured in plastic cage in silk house at room temperature (25–30°C). Silkworm were feed freely with mulberry leave varieties Buriram 60 (young leaves;

containing DNJ around 100 mg/100 g DNJ) as normal process for culturing silkworm in sericulture industries. The cage were cleaned and changed every day. The silkworm at different age (12, 17 and 26 days) were slaughtered by dipping into boiled water to de-active proteinase enzyme and then the samples were lyophilized by freeze-drier. Dried silkworm was grinded and sieved with 100-mesh screen (Yin *et al*., 2010) before subjected to DNJ determination by HPLC instruments.

2.2 The analysis of the amount of DNJ accumulation in the silkworm larvae

The dried silkworm powder (sieved with 100-mesh screen) were was extracted with 0.05 mol/L HCl and reacted with 9-fluorenylmethyl chloroformate (FMOC-Cl) for 30 min to form a color 1-DNJ-FMOC complex compound (Kim *et al*., 2003). The obtain solution was filtered through 0.02 µm syringe filter and was injected into HPLC system (Shimadzu, Kyoto, Japan) coupled with photo diode array detector (SPD-M20A, Shimadzu, Kyoto, Japan) at 254 nm with Ultrasphere C18 column (Kim *et al*., 2003). Acetonitrile and 0.1% aqueous acetic acid (4:6, v/v) were used as mobile phase solvent. The DNJ determination in silkworm larvae was done in 3 replication. The results of the analysis were compared with the standard DNJ chart (Figure 1).

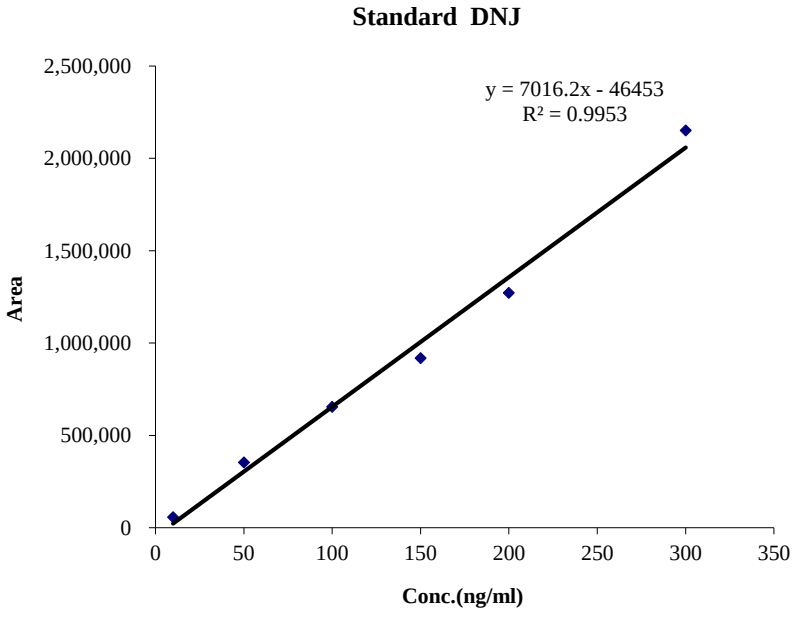

Figure 1 The curve of DNJ standard

3. Results and Discussion

3.1 DNJ determination

As the results of the HPLC-photodiode array detector (254 nm), it took 12.5 min to detect the DNJ-FOMC Chromatogram derived from 9-fluorenylmethyl chloroformate (FMOC-Cl), as shown in Figure 2. For DNJ content in silkworm larvae, it was found that DNJ accumulation at day 12 was 36±2.5 to 97±9.3 mg/100g. Silkworm species SR.5 (113±7.5 mg/100g), N.K.04 (97±9.3 mg/100g) and Nuangnoi Srisaket (76±8.2 mg/100g) showed high DNJ accumulation. For age of 17 days, DNJ content was 44±5.0 to 111±9.4 mg/100g. Silkworm species Kuang wan (111±9.4 mg/100g), Kaki (104±9.3 mg/100g) and Nuangnoi Srisaket (103±6.6) contained high DNJ accumulation. For the age of 26 days, it was found that DNJ accumulation was 40±7.9 to 91±9.6 mg/100g. Silkworm specie Nuangnoi Srisaket (96±7.5), Srisaket1 (91±8.1), SP1 (91±9.6) had high DNJ content, respectively. As results, the amount of DNJ found in some silkworm larvae in this study were close to the amount of DNJ found in mulberry leave that was used for supplement foods for lowering blood glucose levels in the markets (80–20 mg/100g) (Vichasilp *et al*., 2012).

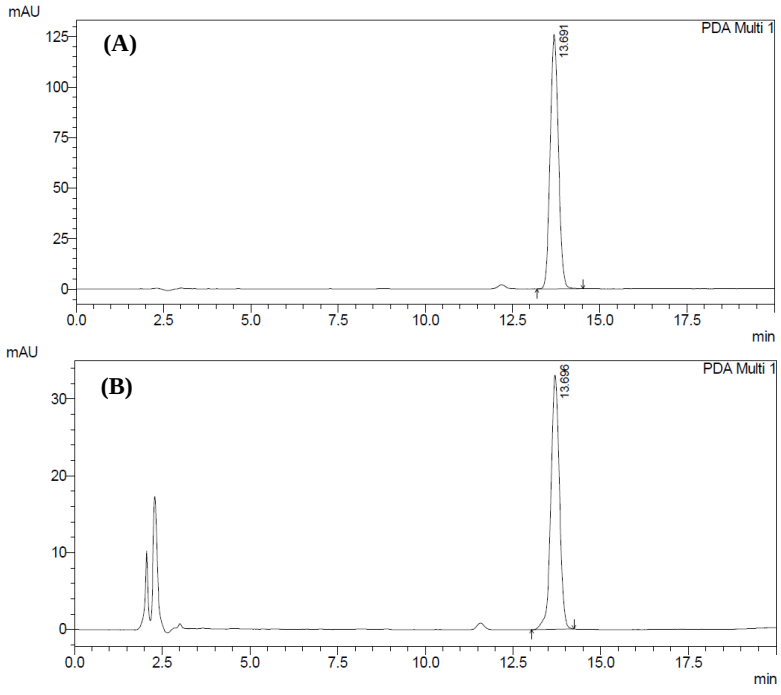

Figure 2 Chromatogram of DNJ standard (A) and DNJ found in silkworms (B)

Table 1 DNJ accumulation in the silkworm larvae at difference of ages

Silkworm larvae species	Age of 12 days (mg/100g)	Age of 17 days (mg/100g)	Age of 26 days (mg/100g)
SP1	70±6.1^{d}	66±2.2^{f}	91±9.6^{b}
SP2	65±5.1^{de}	68±9.9^{f}	74±7.9^{f}
SP3	60±5.6^{e}	72±8.3^{e}	74±7.3^{f}
N.K. 02	72±9.5^{d}	75±8.9^{e}	74±9.4^{f}
N.K. 04	97±9.3^{ab}	87±5.4^{c}	84±9.9^{c}
N.K. 08	81±4.2^{c}	81±7.6^{d}	79±8.8^{d}
N.K. 011	76±4.4^{cd}	90±6.1^{bc}	78±8.1^{ef}
SR. 5	113±7.5^{a}	87±7.7^{c}	82±6.2^{d}
R.A. 5	69±5.7^{d}	79±9.3^{d}	80±7.8^{d}
Chok Amnuai	70±9.6^{d}	91±9.6^{bc}	86±7.4^{c}
Non Rue Si	69±7.5^{d}	90±7.4^{bc}	79±6.0^{d}
Haujaraud	68±9.1^{d}	81±8.5^{d}	86±8.0^{c}
Kaew Sakon	72±7.9^{d}	93±5.4^{b}	87±8.9^{c}
Sumloung	76±6.2^{cd}	92±4.4^{bc}	93±4.2^{b}
Vietnam	80±4.3^{c}	92±4.9^{bc}	92±9.5^{b}
Vanasavan	80±8.8^{c}	94±8.6^{b}	92 ±7.5^{b}
Ai Tang	72±7.8^{d}	97±6.7^{ab}	89±9.9^{bc}
Kuang Wan	38±5.6^{h}	111±9.4^{a}	90±5.3^{b}
Nuangnoi Srisaket	76±8.2^{cd}	103±6.6^{b}	96±7.5^{a}
Srisaket1	88±2.5^{bc}	98±9.5^{ab}	91±8.1^{b}
Tabtim Siam06	43±9.7^{f}	101±9.5^{b}	89±7.4^{bc}
Kaki	82±7.7^{c}	104±9.3^{b}	80±9.1^{d}
NuangTui,	36±2.5^{h}	37±6.2^{i}	40±7.9^{j}
NaungSaew	40±5.2^{g}	64±8.5^{fg}	50±7.3^{i}
Nuang Lai,	49±5.7^{f}	44±5.0^{h}	74±8.1^{f}
NuangKaw	64±7.7^{de}	46±6.0^{h}	66±8.3^{h}
Nuang On Yaung	59±3.3^{f}	61±7.7^{g}	66±8.4^{h}
NuangDaeng	62±7.6^{e}	63±8.4^{fg}	68±9.3^{g}
NuangLuang	63±6.3^{e}	61±4.7^{g}	69±5.5^{g}

Note: Data was shown in average value with standard deviation (n=3). The different letters (a,b,c,..) in the same column indicate significant difference ($p \leq 0.05$).

It was found that DNJ content in silkworms have not relevant on age and species; silkworm specie Nuangnoi Srisaket contained high DNJ in early age and then lower in older age whiles Sumloung contained low DNJ in the younger age and higher DNJ content in older.

For developing supplementary products, the high DNJ content and high yields were considered. The silkworm weight at day 12 was 20–45 g/each, day 17 was 40–60 g/each and day 26 was 50–100 g/each, respectively (data not shown). Considering the yields, silkworm larvae at day 26 was suitable for development of supplement products. As results, silkworm specie Nang Noi, Srisaket, Srisaket, SP1, Samrong, Vietnam, Srisaket1 and Ruby Siam contained high DNJ as shown in Figure 3.

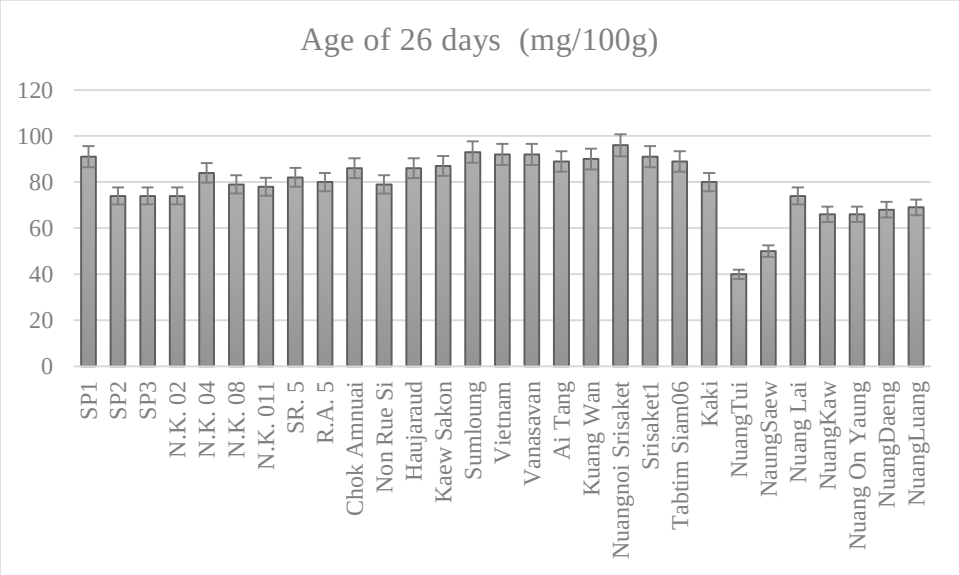

Figure 3 DNJ accumulation of silkworm larvae at day 26

4. Conclusions

The silkworm larvae species have different DNJ accumulation. In this study, DNJ content of 29 species at different age (12, 17 and 26 days) was investigated. DNJ content in silkworms have not relevant on difference of age. DNJ found in several species of silkworm larvae (upper than 80 mg/100g) have high DNJ content suitable for developing a supplement foods as well as functional food from mulberry leaves.

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