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Comparison on Antioxidative Capacity of Various Silkworm Strains

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To increase utilities as functional materials, 173 strains of silkworm genetic resources in the form of silkworm powder were evaluated for antioxidative capacity, with minilum L-100 device and ARAW-KIT (anti-radical ability of water soluble substance). Silkworm powder was prepared with freezing method from silkworms at 5th instar 3rd day larvae. All strains of silkworm powder were prepared with 80% methanol extraction. The data of pupation rate, longevity of silkmoth with origin and voltinism were used for data base of silkworm genetic resources. The weight of a silkworm larva with freezing method at 5th instar 3rd day was measured. The average of antioxidative capacity of 173 silkworm strains was 429.68 nmol. The analysis of correlation among variables was significant, showing negative correlation of the antioxidative capacity with longevity of silk moth and weight of 5th instar silkworm larva. The strains from the tropic, Europe and some other origins were comparatively high. In conclusion, short longevity and low weight of 5th instar silkworm larvae showed comparatively effective antioxidative capacity.

Key words: Silkworm, Antioxidative capacity, Longevity

Introduction

Three hundred-forty three silkworm genetic resources are preserved in Korea. These genetic resources are important as the source for the subsequent utilization for a diverse purpose. One such aspect could be utilization of such resources as a health functional food. After the study on the silkworm powder as blood glucose lowering agent was initiated by Rural Development Administration and Kyung Hee University (Chung *et al.,* 1996) a substantial study on the functionality of silkworm powder was underwent in several aspects. For example, the measurement of the active oxygen and the removal effect of liver tissue treated with silkworm powder in SD rat reported about 7.2% reduction (Choi *et al.,* 2000). Nevertheless, still more studies on the functionality of silkworm powder are required to fulfill consumer's demand. One of such demand is about antioxidant effect of silkworm powder.

The biochemical reaction is continuously occurring for the energy supply, which is necessary for the organism. Also, the active oxygen that occurs always in this process meant free radical and hydrogen peroxide, which are created while the triplet oxygen oxidizers were oxidized and deoxidized (Kim, 2005; Hassimotto *et al.,* 2005; Tewari *et al.,* 2006; Chen *et al.,* 2005; Dai *et al.,* 2004a 2004b, 2004c; Andallu and Varadacharyulu, 2008; Lorenz *et al.,* 2003; Chung *et al.,* 2003; Shama *et al.,* 2001; Yen *et al.,* 1996; Yun *et al.,* 1995).

These materials were mostly disappeared by removal mechanism within the organism, which was own defensive organization (Papa and Skulachev, 1997). However, the active oxygen, which surpassed the defensive activity of organization, and creations of free radical damaged the protein, DNA, enzymes and T cells and cause diseases (Fukuzawa and Takaishi, 1990). Especially, the most problematic thing among these was that the active oxygen attacked the unsaturated fatty acid, which was composition ingredient of the cell membrane. The accumulation of the internal peroxide lipid by the peroxide reaction decreased the vital function and recently became the cause of the disease of adult (Halliwell, 1991).

The silkworm powder has been used for blood glucose lowering agent from 1995, but further research is required to meet the demand of the consumers, who wanted new functional effects. Therefore, the new silkworm leading variety, which has the functional effects on antioxidant

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Table 1. The frequency of the origins

Origins	Others	Tropic	Europe	Japan	China	Korea	Total
Frequency	14	9	28	49	69	4	173
%	8.1	5.2	16.2	28.3	39.9	2.3	100

activity, will correspond to this desire well. Comparing anti-oxidative capacity of the silkworm strains in Korea and obtaining fundamental data of silkworm breeding, this research will try to produce silkworm powder, which has high functional effects and creates a new demand on the sericulture products.

Materials and Methods

Experimental silkworm

The silkworms used for the experiment were 173 strains that are successively preserved as the silkworm genetic resource in Korea. These silkworms were reared in spring season at 2008 in National Academy Agricultural Science, and the 5th instar 3rd day larvae were quickly frozen with the liquid nitrogen and grinded into fine powder.

The experimental silkworms used for the experiment are originated throughout Japan, China, Europe, Tropic, Korea, and unknown (Table 1). The cocoon color of these silkworms varied to eight types, including white and yellow (Table 2). The voltinism of these silkworms are monovoltinism, bivoltinism, or multivoltinism (Table 3). The longevity of these silk moths was quoted from the data of Kang *et al.* (1999), and the antioxidative capacity was measured in spring season, 2008. The ANOVA test etc was conducted by using SPSS programs. The frozen and dried silkworm powder was dissolved in 10 ml of 80% MeOH in each 0.1 g of silkworm powder, vortexed for 30 secs., and filtrated with filter paper (No. 6). One ml filtrate was taken and used for sample solution.

Analysis of antioxidative capacity

The antioxidative capacity of the silkworm powder was measured using the equipment such as munilum L-100, ABCD GmbH, and ARAW-KIT (anti-radical ability of water-soluble substance). The ascorbic acid was used as the reference material and made calibration curve using 0, 10, 20, 40 and 50 μl of it. Also, following TIC (according to Thermo-Initiated Chemiluminescence) methods, the reaction was immediately made by adding to antioxidant measuring equipments and calibration curve in 37 degree after injecting 10 μl of mixed sample dilute solution and 1.5 ml of buffer in ARAW-KIT ample. Analysis of the antioxidative capacity of the samples was measured with Oxida-Q program connected to PC. The antioxidative capacity of the sample was marked by the calculation with ascorbic acid consistency (nmol).

Results and Discussions

The shortest longevity among the experimental strains was C108 with 4.30 days, the longest strain was J037 with 15.95 days, and the average was 9.4 days (Fig. 1). The weight of 5th instar 3rd day larva of the Pnd^{re} was lightest with 1.10 g, whereas N59 was heaviest with 4.92 g. The average among strains was 2.91 g. In the case of antioxidative capacity, the Hansaeng 3-Ho was lowest with 42.79 nmol (Fig. 2), PR was highest with 840.06 nmol, and the average was 429.68 nmol (Fig. 3). The longevity of silkmoth, the weight of larva and the antioxidative capacity of silkworm powder were normally distributed with all bilateralism.

After analyzing major characters and the correlation between the weight and antioxidative capacity, the relation between longevity of silk moth and weight of larva revealed a positive correlation about 5% significance level. On the other hand, the relation between antioxidative capacity and weight of larva showed a negative cor-

Table 2. The frequency of the cocoon color

Cocoon color	Light green	Light color	Light yellow	White	Yellow green	Cream	Dull pink	Yellow	Total
Frequency	3	3	4	141	2	1	6	13	173
%	1.7	1.7	2.3	81.5	1.2	0.6	3.5	7.5	100

Table 3. The frequency of the voltinism

Voltinism	Monovoltinism	Bivoltinism	Multivoltinism	Total
Frequency	25	142	6	173
%	14.5	82.1	3.5	100

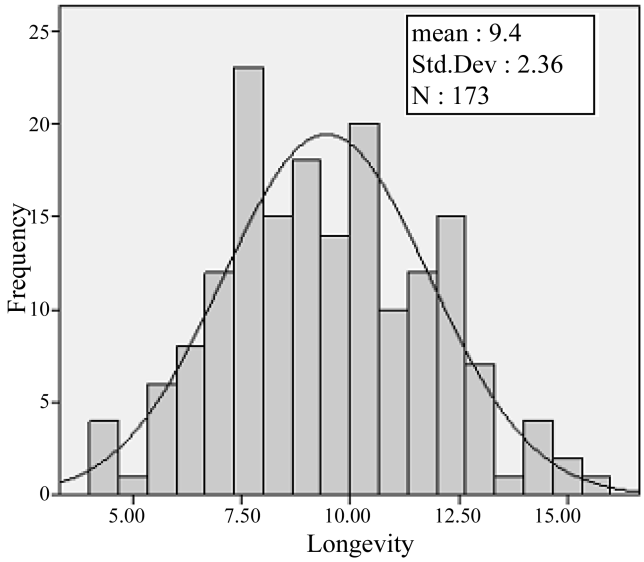

Fig. 1. The frequency of the longevity among silkworm strains. "N" indicates number of strains used in the analysis.

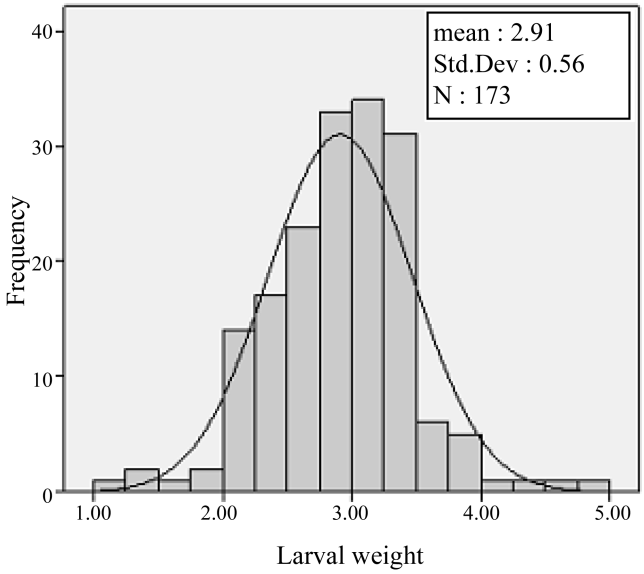

Fig. 2. The frequency of the weight of larvae. "N" indicates number of strains used in the analysis.

relation at 5%. That indicates that the heavier larvae have the longer longevity, whereas the lighter larvae have the higher antioxidative capacity. Particularly, the antioxidative capacity showed the negative correlation with major characters such as pupation ratio, longevity and weight. Thus, it may suggest the difficulty for the breeding of new silkworm variety solely for functional foods (Table 4).

The chi-square analysis of the weight of the larva showed the dependence on the origin of silkworm strains, but had no statistical significance (Table 5). On the other hand, the chi-square analysis of antioxidative capacity

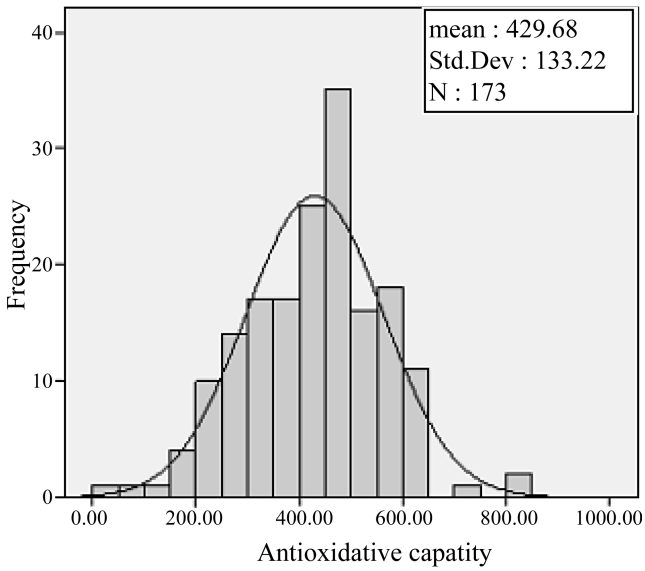

Fig. 3. The frequency of the antioxidative capacity. "N" indicates number of strains used in the analysis.

Table 4. The Pearson correlation coefficients among variables

	Pupation %	Longevity	Weight.	Antioxidative
Pupation %	1			
Longevity	.022	1		
Weight	.035	.164(*)	1	
Antioxidative capacity	-.038	-.040	-.171(*)	1

* Correlation is signification at the 0.05 level.

Table 5. The Chi-square analysis between origins and weight of larvae

Origins \ Larval wt.	4 group (g)				Total
	~2	2~3	3~4	4~	
Others	2	9	3	0	14
Tropic	0	4	5	0	9
Europe	2	19	7	0	28
Japan	0	26	22	1	49
China	1	28	38	2	69
Korea	2	2	0	0	4
Total	7	88	75	3	173

There was no significant between origins and weight of larvae.

showed the correlation of origin of strains with the statistical significance at 5% level (Table 6). Especially, the antioxidative capacity was high among the strains of unknown origin, tropic and Europe (Table 7).

The analysis of variance of the antioxidative capacity and silk moth longevity group showed the significance with F value 2.808 at 5% level (Table 8). And also, the

Table 6. The Chi-square analysis between origins and antioxidative capacity

Origins \ Antioxi.	4 group(nmol)				Total
	~200	200~400	400~600	600~	
Others	0	3	9	2	14
Tropic	0	1	4	4	9
Europe	1	9	15	3	28
Japan	2	16	28	3	49
China	4	28	36	1	69
Korea	0	1	2	1	4
Total	7	58	9475	14	173

X^2=26.03, X^2 is significant at the 0.5 level.

Table 7. The Chi-square analysis between cocoon color and antioxidative capacity

Cocoon color \ Antioxi.	4 group(nmol)				Total
	~200	200~400	400~600	600~	
Light green	0	1	2	0	3
Light color	0	0	3	0	3
Light yellow	0	3	1	0	4
White	7	49	75	10	141
Yellow green	0	0	1	1	2
Cream	0	0	0	1	1
Dull pink	0	2	3	1	6
Yellow	0	3	9	1	13
Total	7	58	94	14	173

There was no significant between cocoon color and antioxidative capacity.

Table 8. The ANOVA test of longevity and antioxidative capacity

Variable	Group	N	Mean	Std. Deviation	F-value
Longevity	~7	23	483.96	138.84	2.808*
	7~10	78	423.23	124.55	
	10~13	62	407.16	137.05	
	13~	10	494.84	126.24	

*$p < 0.05$.

Table 9. The ANOVA test between weight antioxidative capacity

Variable	Group	N	Mean	Std. Deviation	F-value
Weight	~2	7	474.23	165.00	5.923**
	2~3	88	425.07	129.50	
	3~4	75	442.75	123.29	
	4~	3	134.24	86.38	

**$p < 0.01$.

analysis of variance of the antioxidative capacity and weight group of larva had significance with F value 5.923 at 1% level (Table 9). Therefore, the weight of larva is related with the antioxidative capacity more than silk moth longevity. The correlation between antioxidative capacities with female-male longevity of silk moth needs to be analyzed in future study.

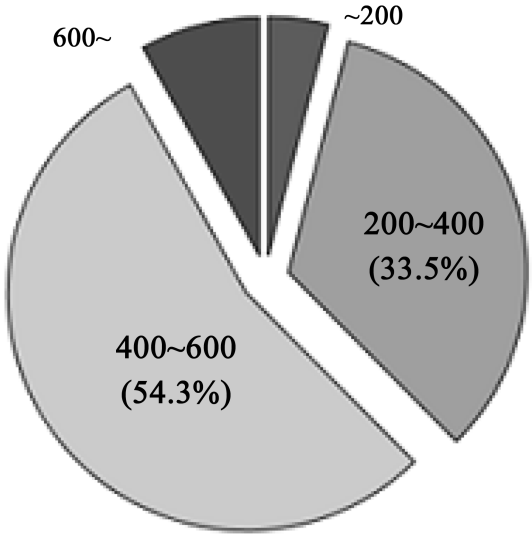

Fig. 4. The distribution of the antioxidative capacity among silkworm strains.

In the distribution of the antioxidative capacity of the silkworm larva, 400~600 nmol occupied a half about 54% and 200~400 nmol was about 33% (Fig. 4). By

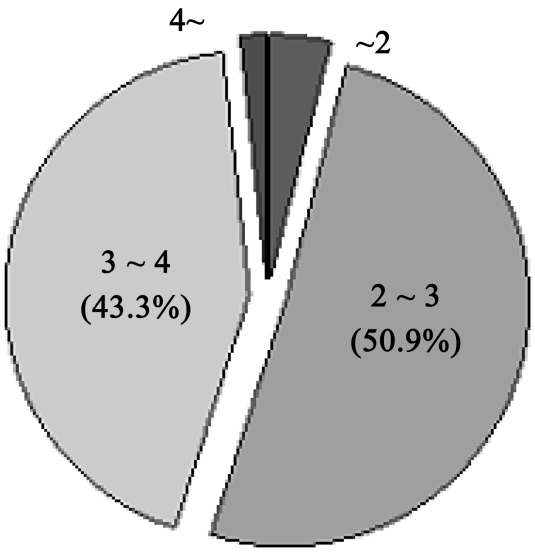

Fig. 5. The distribution of the weight among silkworm strains.

comparison, the weight of the larva 2 ~ 3 g occupies a half and 3 ~ 4 g was about 43% (Fig. 5). The antioxidative capacity of the silkworm powder was quite lower compared to 3,300 nmol antioxidative capacity of the mulberry leaves, according to Kim (2005). Succeeding research is required in order to further enhance current study such as comparative analysis between the raw mulberry leaves and the mulberry leaves within midgut of silkworm larva.

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