IJIEB Int. J. Indust. Entomol. Biomater. 48(2) 59-66 (2024)
ISSN 3022-7542, http://dx.doi.org/10.7852/ijie.2024.48.2.59

ISSN 3022-7542 (Print)
ISSN 3022-859X (Online)

The Molecular Metabolism of the Key Ingredients in the Steamed and Freeze-Dried Mature Silkworm Powder: Effects and Mechanisms

Min-Ju Kim[1], Seong Ryul Kim[2], Ji Hae Lee[2], Byeongyeob Jeon[3], Seokho Kim[3], Eun Ji Go[3], and Hyunwoo Park[1,*]

[1]*Health Park co.,ltd. Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, 08826 Republic of Korea*
[2]*Department of Agricultural Biology, National Institute of Agricultural Sciences, Rural Development Administration, Wanju, 55365, Republic of Korea*
[3]*QBM co., ltd. 6F 7-25 Gangnam-daero 27-gil, Seocho-gu, Seoul, 06752 Republic of Korea*

Abstract

The mature *Bombyx mori* silkworm is recognized as a rich source of several nutrients. A unique steaming process has been developed to enhance the palatability of *Bombyx mori* silkworm and make it more convenient to consume. Additionally, it has also been freeze-dried into a powder form, which is recognized as a nutritional supplement with many health benefits. Steamed and Freeze-dried Mature Silkworm Powder (SMSP) is said to offer a wide range of benefits, including longevity, improved athletic performance, prevention of alcohol-induced liver fibrosis or tumors, amelioration of fatty liver, prevention of peptic ulcers, regulation of melanin production, and mitigation of Parkinson's and Alzheimer's diseases by improving cognitive function. The nutritional composition of SMSP is particularly high in glycine, alanine and serine. This review aims to summarize the molecular mechanisms underlying the diverse effects induced by these key components of SMSP. Such elucidation will enhance the credibility of future studies on SMSP, which will require more comprehensive analyses. It appears that SMSP represents a natural health supplement that could have a positive impact on global human health while increasing income.

Int. J. Indust. Entomol. Biomater. 48(2), 59-66 (2024)

Received : 23 May 2024
Revised : 5 Jun 2024
Accepted : 25 Jun 2024

Keywords:
Bombyx mori,
Steamed and Freeze-dried Matured Silkworm Powder (SMSP), glycine,
serine,
alanine

Introduction

Currently, greenhouse gas emissions are a significant contributor to global climate change, with 14.5 percent of these emissions stemming from livestock production. Exploring the use of insects as a sustainable food source has emerged as a potential solution to mitigate this issue (Ghosh *et al*., 2021; Kim *et al*., 2022.). Among these insects, the mulberry silkworm (*Bombyx mori*) has a rich history of cultivation spanning 5,000 years, primarily for the production of textiles. The white jade (WJ) cocoon, known for its production of white cocoons, serves not only as a textile material but also as a source for functional foods with diverse health-promoting effects, including the management of conditions such as diabetes, paralysis, and stroke (Kim *et al*., 2022.).

Traditionally, 5th instar 3rd-day dried silkworms have been utilized as functional foods for regulating blood sugar levels (Lee *et al*., 2019; Kim *et al*., 2023a.). However, ensuring the

***Corresponding author.**
Hyunwoo Park
Health Park co.,ltd. Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul, 08826 Republic of Korea
Tel: +82-2-542-7760
E-mail: hwpark75@snu.ac.kr

availability of silkworms precisely at the 5th instar 3rd-day mark poses challenges, as the protein gland in silkworms experiences rapid enlargement at the age of 5th instar 4th-day, diminishing the efficacy of blood sugar regulation and thereby reducing their value. Nonetheless, the development of a method involving the steaming and freeze-drying of silkworms aged 5th instar 4th-day for 130 minutes has rendered them suitable for human consumption, offering promising prospects for increasing the income of sericulture farmers and expanding the industrial base (Ji *et al*., 2015; Ji *et al*., 2017.). Presently, active research is underway to investigate the efficacy of steamed and freeze-dried mature silkworm powder (SMSP) developed through this method.

The reported effects of SMSP include enhanced lifespan and healthspan, boosted mitochondrial activity, upregulation of olfactory genes such as Obp83a and Os-C, and heightened olfactory responses. Moreover, SMSP has shown potential in safeguarding against Parkinson's disease by counteracting rotenone-induced Parkinsonism (Nguyen *et al*., 2016; Mai *et al*., 2022.). In experimental models, SMSP exhibited hepatoprotective effects against diethylnitrosamine(DEM)-induced liver injury and hepatocellular carcinoma, attenuating hepatocyte necrosis, inflammatory responses, and liver enzyme levels (ALP, AST, ALT) (Cho *et al*., 2016.). Additionally, it demonstrated protective effects against alcoholic liver injury, ameliorating hepatic steatosis, fibrosis, cirrhosis, and hepatocellular carcinoma (Cho *et al*., 2016; Lee *et al*., 2017b; Lee *et al*., 2020b). Furthermore, SMSP has demonstrated gastroprotective effects against gastric mucosal bleeding and ulcers (Lee *et al*., 2017a; Yun *et al*., 2017; Lee *et al*., 2023.), as well as inhibitory effects on melanin pigment production, thereby reducing skin darkening (Kim *et al*., 2017.). Notably, SMSP exhibits antioxidant properties (Nguyen *et al*., 2020b) and enhances cognitive functions, including memory enhancement (Nguyen *et al*., 2020a; Kim *et al*., 2023b.).

Despite the extensive research on the multifaceted benefits of SMSP, the specific active ingredients responsible for these effects remain unidentified. However, nutritional analyses have revealed elevated levels of three key proteins—serine, glycine, and alanine—in SMSP compared to other commonly used silkworm products as freeze-dried 3rd day of 5th instar silkworm larval powder (FDSP) (Ji *et al*., 2016.). Our objective is to contribute to future research endeavors on SMSP efficacy by elucidating the molecular mechanisms through which these three proteins impact the diverse known benefits of SMSP.

Effects on liver diseases

The liver undergoes a progressive sequence of events, including hepatitis, hepatic fibrosis and cirrhosis, initiated by fatty liver disease and culminating in liver cancer. In particular, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), the primary enzymes responsible for alcohol metabolism, are predominantly synthesized by hepatocytes to metabolize alcohol, thereby contributing to alcoholic fatty liver disease resulting from chronic alcohol consumption. Several studies have shown that the onset of fatty liver disease can be attenuated by the administration of SMSP. Rats given oral administration of 25% ethanol along with daily SMSP intake for 4 weeks had significantly reduced total liver weight and decreased serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) compared to rats given ethanol alone (Lee *et al*., 2020a). In addition, pretreatment with SMSP for 2 weeks significantly reduced ALT and alkaline phosphatase (ALP) levels in Sprague-Dawley (SD) rats given oral ethanol (Lee *et al*., 2017b). ALT and AST, which are primarily active in the liver, are released into the serum when overactive and their serum activity levels serve as biomarkers of alcohol abuse induced liver injury and fatty liver disease (Sorbi *et al*., 1999.). The presence of alanine in SMSP affects alcohol metabolism (Kreisberg *et al*., 1972; Zhengtao Liu *et al*., 2014.). ADH, which is responsible for the breakdown of alcohol in the liver, requires the coenzyme NAD^+, which is produced by the alanine dehydrogenase-mediated dehydrogenation of NADH (Sattler *et al*., 2012; Klatte *et al*., 2015.). During this process, alanine is converted to pyruvate by alanine transaminase (ALT), thereby enhancing alcohol metabolism (Klatte *et al*., 2015; Häkkinen *et al*., 1975; Chyun *et al*., 2002.).

The degradation of alcohol generates reactive oxygen species (ROS) that affect the sirtuin 1 (SIRT1) signaling pathway, which is central to the pathogenesis of alcoholic liver disease (ALD) (Ren *et al*., 2020.). Ethanol is oxidized by ADH and aldehyde hydrogenase (ALDH) to reduce NAD^+ (Gao *et al*., 2011.). This reduction in NAD^+/NADH levels inhibits SIRT1 activity in the liver, subsequently impeding the activity of sterol regulatory element-binding protein 1c (SREBP-1c), AMP-activated kinase (AMPK) crucial for lipid homeostasis, peroxisome proliferator-activated receptor α (PPAR-α), and mechanistic target of rapamycin (mTOR), mTOR complex 1 (mTORC1), which counteract inflammatory responses, thereby fostering

hepatic lipogenesis and inflammation (You *et al*., 2004; You *et al*., 2008; Purushotham *et al*., 2009; Jiang *et al*., 2015; Hanqing Chen *et al*., 2018.). In this context, serine and glycine have been shown to play a critical role in the prevention of ALD by promoting glutathione (GSH) synthesis to mitigate ROS and increasing the NAD^+/NADH ratio to activate SIRT1 (Ming Yin *et al*., 1998; Senthilkumar *et al*., 2004; Sim *et al*., 2019; Xin Liu *et al*., 2021.). In fact, SMSP significantly increased AMPK phosphorylation and SIRT1 while significantly decreasing the ethanol downregulated adipogenic factor PPAR-gamma (Lee *et al*., 2020a), confirming the beneficial effects of SMSP on lipid metabolism and reduction of malondialdehyde (MDA), an indicator of oxidative stress (Lee *et al*., 2017b). In particular, SMSP from white jade cocoons, which are rich in alanine and serine, showed a greater reduction in ADH, ALDH, AST and ALT levels than SMSP from golden silk and light green cocoons (Lee *et al*., 2017a).

Protect of Gastric injury

The mechanisms underlying gastric mucosal damage resulting from excessive ethanol consumption remain elusive, but it is hypothesized that the production of reactive oxygen species (ROS) during ethanol degradation and an exaggerated inflammatory response contribute. Gastric epithelial cells, rich in mitochondria, play a crucial role in maintaining the normal structure and function of the gastric mucosa by facilitating energy production through oxidative phosphorylation (Duman *et al*., 2002; Shimokawa *et al*., 2003; Yin *et al*., 2003.). Ethanol has been shown to induce gastric injury by generating oxygen free radicals (OFRs) that disrupt and impair mitochondrial structure, thereby precipitating gastric mucosal damage (Alarcon *et al*., 1995; Pan *et al*., 2008.). In addition, lipid peroxidation (LP) induced by ethanol-derived OFRs exacerbates gastric mucosal injury (Yoshikawa *et al*., 1997; Hernández-Muñoz *et al*., 2000.). The reduction in glutathione (GSH) levels induced by ethanol enhances superoxide radical-mediated lipid peroxidation (Yoshikawa *et al*., 1997; Hernández-Muñoz *et al*., 2000.). This highlights the potential of serine and glycine to mitigate gastric injury by ameliorating oxidative stress through restoration of GSH synthesis, as mentioned above (Szabo *et al*., 1992.). Pretreatment with glycine followed by oral ethanol administration attenuated ethanol-induced gastric lesion formation, suggesting a role for glycine in scavenging OFRs (Ligumsky *et al*., 1995.).

Anti-melanogenic activity

Melanin is the skin's pigment and its production is primarily triggered by ageing, hormonal fluctuations and UVB-induced skin irritation. Excessive melanin production leads to skin hyperpigmentation, manifesting as conditions such as melasma, freckles and age spots (Lin *et al*., 2007; Lee, 2015; Fu *et al*., 2020.). The induction of melanogenesis involves three main signaling pathways activated by adrenocorticotropic hormone (ACTH) (G Hunt *et al*., 1994a.), α-melanocyte stimulating hormone (α-MSH) (Hill *et al*., 1989; Gillian Hunt *et al*., 1994b.) and stem cell factor (SCF) (Vanover *et al*., 2009.), which subsequently stimulate the enzymatic actions of tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1) and TRP-2, facilitating the conversion of tyrosine into melanin pigment (Yamaguchi *et al*., 2007.). Glycine has been shown to inhibit melanogenesis by suppressing α-MSH-induced tyrosinase activity (Ishikawa *et al*., 2007.). This is further supported by findings indicating a marked reduction in UVB-induced aberrant pigmentation and inhibition of melanin synthesis in the dorsal skin of mice orally treated with SMSP (Kim *et al*., 2017.).

Effects of memory enhance and resistances to Parkinson's disease

Recent reports have highlighted oxidative stress as a common underlying factor in several central nervous system (CNS) disorders, with increased oxidative stress and decreased antioxidant enzyme activity implicated in age-related cognitive decline and memory impairment (Chen *et al*., 2012; Rehman *et al*., 2017.). Glycine has emerged as a potential cognitive enhancer by modulating oxidative stress, offering promise in the prevention of memory decline and Parkinson's disease (Coyle *et al*., 2004; Castner *et al*., 2014; Tsai *et al*., 2014.). The antioxidant gene Nrf2 serves as a stress-response transcription factor that is activated in response to oxidative stress, particularly in neurodegenerative conditions. Nrf2 in turn triggers the activation of another redox regulator, HO-1 (Li *et al*., 2020.). Treatment of D-galactose-induced ageing mice with glycine significantly upregulated Nrf2 and HO-1 protein expression levels and attenuated D-galactose-induced oxidative stress. This intervention simultaneously attenuated synaptic protein loss and spatial learning/cognitive impairment by suppressing the JNK-mediated apoptotic pathway (Liu *et al*., 2020.). Furthermore, glycine was found to protect against neuronal cell death in the cerebral cortex by phosphorylating transcription factors such as

Table 1. Summary of key ingredients and mechanisms affecting efficacy

Efficacy	Marker compound	Molecular mechanism	Reference
Liver disease	Glycine, Serine	ROS reduction SIRT1 pathway	(Ming Yin *et al*., 1998; Senthilkumar *et al*., 2004; Sim *et al*., 2019; Xin Liu *et al*., 2021.)
	Alanine	Alcohol metabolism	(Kreisberg *et al*., 1972; Zhengtao Liu *et al*., 2014; Klatte *et al*., 2015; Häkkinen *et al*., 1975; Chyun *et al*., 2002.)
Protect of gastric injury	Glycine, Serine	ROS reduction Protection of mitochondria from oxygen free radical	(Szabo *et al*., 1992; Ligumsky *et al*., 1995.)
Anti-melanic activity	Glycine	Down-regulation of tyrosinase	(Ishikawa *et al*., 2007.)
Memory enhancement and Parkinson's disease	Glycine	Modulation of NMDAR by Akt pathway Nrf2/HO-1 pathway JNK-mediated pathway	(Burke, 2007; Manning *et al*., 2007; Hu *et al*., 2016.)
	Serine	TGF-β1 activation	(L. P. Diniz *et al*., 2012; Luan Pereira Diniz *et al*., 2014.)
	Alanine	Energy substrate in mitochondrial metabolism in both astrocytes and neurons ROS reduction	(Suzuki *et al*., 2011; Rabah *et al*., 2023; Ma *et al*., 2021.)

Forkhead/FOXO, NF-kB, mdm2 or Bcl-2, thereby enhancing cell survival through non-ionotropic activation of GluN2AR, a subtype of N-methyl-D-aspartate receptors (NMDARs) central to the regulation of excitatory neurotransmission. This mechanism effectively attenuated glutamate neurotoxicity-induced Akt inactivation (Burke, 2007; Manning *et al*., 2007; Hu *et al*., 2016.). In addition, serine, another amino acid critical for NMDAR function, shows an age-related decline in the hippocampus, affecting the induction of synaptic plasticity (Schell *et al*., 1995; Wolosker *et al*., 1999; Yang *et al*., 2005; Williams *et al*., 2006.). D-serine, required for transforming growth factor β1 (TGF-β1) activation, enhances astrocytic synaptogenic properties and promotes neuronal survival, ultimately increasing the number of cortical excitatory synapses (Diniz *et al*., 2012; Diniz *et al*., 2014.). Alanine, which serves as an energy substrate in mitochondrial metabolism in both astrocytes and neurons, plays a dual role in memory formation and glucose metabolism, the latter being essential for the energy requirements of neurotransmission (Suzuki *et al*., 2011; Rabah *et al*., 2023.). It also modulates the cAMP-PKA pathway, thereby enhancing hippocampus-dependent memory formation by alleviating oxidative stress (Ma *et al*., 2021.).

Taken together, these mechanisms suggest that the actions of glycine, serine and alanine support SMSP's potential to improve cognitive function (Nguyen *et al*., 2020b; Kim *et al*., 2020a) and attenuate the progression of Parkinson's disease (Nguyen *et al*., 2016; Kim *et al*., 2017.).

Conclusion

Bombyx mori is not only a source of textiles, but also a valuable ingredient in the production of functional foods. Specifically, 5th instar 3rd-day dried silkworms are used primarily for their potential to lower blood sugar levels. Numerous studies have investigated the efficacy of Steamed and Freeze-dried Mature Silkworm Powder (SMSP), which has been processed into an easily consumable form using a special technology developed for 5th instar 4rd-day silkworms. These silkworms typically face challenges such as enlarged glands, reduced hypoglycaemic effects and difficulties in harvesting accurately at the ideal age. The benefits associated with SMSP include improved liver and stomach function, inhibition of skin pigmentation, improved cognitive function and potential prevention of Parkinson's disease. However, the precise molecular mechanisms underlying these effects remain largely unexplored. However, it is known that SMSP has a rich nutritional composition, particularly in essential amino acids

such as glycine, serine and alanine. Evidence exists to support the individual effects of these amino acids on SMSP efficacy. This comprehensive review aims to provide insights for future research into the efficacy of SMSP, particularly from a molecular perspective.

Author Contributions

Conceptualization, H.P.; Investigation and resources, S.R.K, J.H.L, B.J, S.K, E.J.G, and H.P., and writing—review and editing, M.J.K and H.P. All authors have read and agreed to the published version of the manuscript.

Sources of support

This work was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. RS2022RD010224)" Rural Development Administration, Republic of Korea.

Conflicts of Interest

The authors declare no conflict of interest.

References

Alarcon J, Quevedo L, Reyes P (1995) Inhibitory action of hydrogen peroxide on a high-resistance epithelium. Pharmacology 50, 111-118. https://doi.org/10.1159/000139272

Burke RE (2007) Inhibition of mitogen-activated protein kinase and stimulation of Akt kinase signaling pathways: Two approaches with therapeutic potential in the treatment of neurodegenerative disease. Pharmacol Therapeut 114, 261-277. https://doi.org/10.1016/j.pharmthera.2007.02.002

Castner S, Murthy N, Ridler K, Herdon H, Roberts B, Weinzimmer D, *et al*. (2014) Relationship between glycine transporter 1 inhibition as measured with positron emission tomography and changes in cognitive performances in nonhuman primates. Neuropsychopharmacology 39, 2742-2749. https://doi.org/10.1038/npp.2014.4

Chen H, Shen F, Sherban A, Nocon A, Li Y, Wang H, *et al*. (2018) DEP domain–containing mTOR–interacting protein suppresses lipogenesis and ameliorates hepatic steatosis and acute-on-chronic liver injury in alcoholic liver disease. Hepatology 68, 496-514. https://doi.org/10.1002/hep.29849

Chen X, Guo C, Kong J (2012) Oxidative stress in neurodegenerative diseases☆. Neural Regen Res 7, 376-385. https://doi.org/10.3969/j.issn.1673-5374.2012.05.009

Cho JM, Kim KY, Ji SD, Kim EH (2016) Protective effect of boiled and freeze-dried mature silkworm larval powder against diethylnitrosamine-induced hepatotoxicity in mice. J Cancer Prev 21, 173. https://doi.org/10.15430/JCP.2016.21.3.173

Chyun J, Yim J, Cha Y (2002) Effects of Alanine and Glutamine Supplementation on Alcohol Metabolism in ICR Mice. Nutr Sci 5, 9-12.

Coyle JT, Tsai G (2004) The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. Psychopharmacology 174, 32-38. https://doi.org/10.1007/s00213-003-1709-2

Diniz LP, Almeida JC, Tortelli V, Vargas Lopes C, Setti-Perdigão P, Stipursky J, *et al*. (2012) Astrocyte-induced synaptogenesis is mediated by transforming growth factor β signaling through modulation of D-serine levels in cerebral cortex neurons. J Biol Chem 287, 41432-45. https://doi.org/10.1074/jbc.M112.380824

Diniz LP, Matias ICP, Garcia MN, Gomes FCA (2014) Astrocytic control of neural circuit formation: highlights on TGF-beta signaling. Neurochem Int 78, 18-27. https://doi.org/10.1016/j.neuint.2014.07.008

Duman JG, Pathak NJ, Ladinsky MS, McDonald KL, Forte JG (2002) Three-dimensional reconstruction of cytoplasmic membrane networks in parietal cells. J Cell Sci 115, 1251-1258. https://doi.org/10.1242/jcs.115.6.1251

Fu C, Chen J, Lu J, Yi L, Tong X, Kang L, *et al*. (2020) Roles of inflammation factors in melanogenesis. Mol Med Rep 21, 1421-1430. https://doi.org/10.3892/mmr.2020.10950

Gao B, Bataller R (2011) Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 141, 1572-1585. https://doi.org/10.1053/j.gastro.2011.09.002

Ghosh S, Gahukar RT, Meyer-Rochow VB, Jung C (2021) Future prospects of insects as a biological resource in India: Potential biological products utilizing insects with reference to the frontier countries. Entomol Res 51, 209-229. https://doi.org/10.1111/1748-5967.12507

Häkkinen HM, Kulonen E (1975) Effect of ethanol on the metabolism

of alanine, glutamic acid, and proline in rat liver. Biochem Pharmacol 24, 199-204. https://doi.org/10.1016/0006-2952(75)90277-4

Hernández-Muñoz R, Montiel-Ruíz C, Vázquez-Martínez O (2000) Gastric mucosal cell proliferation in ethanol-induced chronic mucosal injury is related to oxidative stress and lipid peroxidation in rats. Lab Invest 80, 1161-1169. https://doi.org/10.1038/labinvest.3780124

Hill SE, Buffey J, Thody AJ, Oliver I, Bleehen SS, Mac Neil S (1989) Investigation of the regulation of pigmentation in α-melanocyte-stimulating hormone responsive and unresponsive cultured B16 melanoma cells. Pigment Cell Res 2, 161-166. https://doi.org/10.1111/j.1600-0749.1989.tb00181.x

Hunt G, Todd C, Kyne S, Thody A (1994a) ACTH stimulates melanogenesis in cultured human melanocytes. J Endocrinol 140, R1-R3. https://doi.org/10.1677/joe.0.140R001

Hunt G, Todd C, Cresswell JE, Thody AJ (1994b) α-Melanocyte stimulating hormone and its analogue Nle4DPhe7α-MSH affect morphology, tyrosinase activity and melanogenesis in cultured human melanocytes. J Cell Sci 107, 205-211. https://doi.org/10.1242/jcs.107.1.205

Hu R, Chen J, Lujan B, Lei R, Zhang M, Wang Z, *et al*. (2016) Glycine triggers a non-ionotropic activity of GluN2A-containing NMDA receptors to confer neuroprotection. Sci Rep 6, 34459. https://doi.org/10.1038/srep34459

Ishikawa M, Kawase I, Ishii F (2007) Glycine inhibits melanogenesis *in vitro* and causes hypopigmentation *in vivo*. Biol Pharm Bull 30, 2031-2036. https://doi.org/10.1248/bpb.30.2031

Jiang Z, Zhou J, Zhou D, Zhu Z, Sun L, Nanji AA (2015) The Adiponectin-SIRT 1-AMPK Pathway in Alcoholic Fatty Liver Disease in the Rat. Alcoholism: Clin Exp Res 39, 424-433. https://doi.org/10.1111/acer.12641

Ji SD, Kim NS, Kweon H, Choi BH, Yoon SM, Kim KY, *et al*. (2016) Nutrient compositions of Bombyx mori mature silkworm larval powders suggest their possible health improvement effects in humans. J Asia Pac Entomol 19, 1027-1033. https://doi.org/10.1016/j.aspen.2016.08.004

Ji SD, Kim NS, Lee JY, Kim MJ, Kweon H, Sung G, *et al*. (2015) Development of processing technology for edible mature silkworm. J Seri Entomol Sci 53, 38-43. https://doi.org/10.7852/jses.2015.53.1.38

Ji SD, Son JG, Kim SW, Kim NS, Kim KY, Kweon HY, *et al*. (2017) Production techniques to Improve the Quality of Steamed and Freeze-Dried Mature Silkworm Larval Powder. Int J Indust Entomol 34, 17-22. https://doi.org/10.7852/ijie.2017.34.2.17

Kim AY, Park JW, Kang SK, Jeong CY, Kim NS, Kim KY, *et al*. (2023a) Development of spectrophotometric quality analysis protocols for determining the purity of two silkworm products with different health-promoting effects. J Asia Pac Entomol 26, 102029. https://doi.org/10.1016/j.aspen.2022.102029

Kim HJ, Kim KY, Ji SD, Lee HT (2017) Anti-melanogenic activity of steamed and freeze-dried mature silkworm powder. J Asia Pac Entomol 20, 1001-1006. https://doi.org/10.1016/j.aspen.2017.07.013

Kim KY, Koh YH (2022) The past, present and future of silkworm as a natural health food. Food Sci Indust 55, 154-165. https://doi.org/10.23093/FSI.2022.55.2.154

Kim YH, Nguyen P, Kim SR, Kang SK, Kim KY, Koh YH (2023b) A comparison of nutritional components and memory enhancement effects of HongJam prepared from different silkworm varieties that weave yellow-colored cocoons. J Asia Pac Entomol 26, 102167. https://doi.org/10.1016/j.aspen.2023.102167

Klatte S, Wendisch VF (2015) Role of L-alanine for redox self-sufficient amination of alcohols. Microb Cell Fact 14, 1-10. https://doi.org/10.1186/s12934-014-0189-x

Kreisberg RA, Siegal AM, Owen WC (1972) Alanine and gluconeogenesis in man: effect of ethanol. J Clin Endocrinol Metab 34, 876-883. https://doi.org/10.1210/jcem-34-5-876

Lee AY (2015) Recent progress in melasma pathogenesis. Pigm Cell Melanoma Res 28, 648-660. https://doi.org/10.1111/pcmr.12404

Lee DY, Cho JM, Yun SM, Hong KS, Ji SD, Son JG, *et al*. (2017a) Comparative effect of silkworm powder from 3 Bombyx mori varieties on ethanol-induced gastric injury in rat model. Int J Indust Entomol 35, 14-21. https://doi.org/10.7852/ijie.2017.35.1.14

Lee DY, Hong KS, Song MY, Yun SM, Ji SD, Son JG, *et al*. (2020a) Hepatoprotective effects of steamed and freeze-dried mature silkworm larval powder against ethanol-induced fatty liver disease in rats. Foods 9, 285. https://doi.org/10.3390/foods9030285

Lee DY, Hong KS, Yun SM, Song MY, Ji SD, Son JG, *et al*. (2017b) Mature silkworm powder reduces blood alcohol concentration and liver injury in ethanol-treated rats. Int J Indust Entomol 35, 123-128. https://doi.org/10.7852/ijie.2017.35.2.123

Lee DY, Song MY, Hong KS, Yun SM, Han YM, Kim EH (2023) Low dose administration of mature silkworm powder induces gastric mucosal defense factors in ethanol-induced gastric injury rat model. Food Sci Biotech 32, 1551-1559. https://doi.org/10.1007/s10068-023-01278-1

Lee DY, Yun SM, Song MY, Ji SD, Son JG, Kim EH (2020b) Administration of steamed and freeze-dried mature silkworm larval powder prevents hepatic fibrosis and hepatocellular carcinogenesis by blocking TGF-β/STAT3 signaling cascades in rats. Cells 9, 568. https://doi.org/10.3390/cells9030568

Lee JH, Kim S, Jo YY, Kweon H, Jeon JY, Ju WT, *et al*. (2019) Effect of humidity on the quality characteristics of the 3 rd day of 5 th instar silkworm powder. Int J Indust Entomol 39, 74-81. https://doi.org/10.7852/ijie.2019.39.2.74

Li B, Nasser MI, Masood M, Adlat S, Huang Y, Yang B, *et al*. (2020) Efficiency of Traditional Chinese medicine targeting the Nrf2/HO-1 signaling pathway. Biomed Pharmacother 126, 110074. https://doi.org/10.1016/j.biopha.2020.110074

Ligumsky M, Sestieri M, Okon E, Ginsburg I (1995) Antioxidants inhibit ethanol-induced gastric injury in the rat: role of manganese, glycine, and carotene. Scand J Gastroenterol 30, 854-860. https://doi.org/10.3109/00365529509101591

Lin JY, Fisher DE (2007) Melanocyte biology and skin pigmentation. Nature 445, 843-850. https://doi.org/10.1038/nature05660

Liu X, Liu Y, Liu Z, Lin C, Meng F, Xu L, *et al*. (2021) CircMYH9 drives colorectal cancer growth by regulating serine metabolism and redox homeostasis in a p53-dependent manner. Mol Cancer 20, 1-19. https://doi.org/10.1186/s12943-021-01412-9

Liu Z, Que S, Xu J, Peng T (2014) Alanine aminotransferase-old biomarker and new concept: a review. Int J Med Sci 11, 925. https://doi.org/10.7150/ijms.8951

Liu Z, Yao X, Jiang W, Li W, Zhu S, Liao C, *et al*. (2020) Advanced oxidation protein products induce microglia-mediated neuroinflammation via MAPKs-NF-κB signaling pathway and pyroptosis after secondary spinal cord injury. J Neuroinflammation 17, 1-21. https://doi.org/10.1186/s12974-020-01751-2.

Ma Y L, Yang Y, Thakur K, Cespedes-Acuña CL, Zhang JG, Wei ZJ (2021) Evaluation of spatial memory and anti-fatigue function of long-term supplementation of β-alanine and confirmation through cAMP-PKA and apoptosis pathways in mice. eFood 2, 185-192. https://doi.org/10.53365/efood.k/144395.

Mai LX, Kang SK, Jo YY, Nguyen P, Kim A, Kim KY, *et al*. (2022) An alkaline protease-digestion of silkworm powder enhances its effects over healthspan, autophagy, and mitochondria function in a rotenone-induced Drosophila model. Front Nutr 9, 808295. https://doi.org/10.3389/fnut.2022.808295

Manning BD, Cantley LC (2007) AKT/PKB signaling: navigating downstream. Cell 129, 1261-1274. https://doi.org/10.1016/j.cell.2007.06.009

Nguyen P, Kim KY, Kim AY, Choi BH, Osabutey AF, Park YH, *et al*. (2020a) Mature silkworm powders ameliorated scopolamine-induced amnesia by enhancing mitochondrial functions in the brains of mice. J Funct Foods 67, 103886. https://doi.org/10.1016/j.jff.2020.103886

Nguyen P, Kim KY, Kim AY, Kim NS, Kweon H, Ji SD, *et al*. (2016) Increased healthspan and resistance to Parkinson's disease in Drosophila by boiled and freeze-dried mature silk worm larval powder. J Asia Pac Entomol 19, 551-561. https://doi.org/10.1016/j.aspen.2016.05.003

Nguyen P, Kim SW, Jo YY, Beteta SP, Kang SK, Kim SB, *et al*. (2020b) A comparative study on the phytochemical and anti-oxidant activity differences in HongJam prepared with various silkworm varieties. Int J Indust Entomol 41, 19-27. https://doi.org/10.7852/ijie.2020.41.2.19

Pan JS, He SZ, Xu HZ, Zhan XJ, Yang XN, Xiao HM, *et al*. (2008) Oxidative stress disturbs energy metabolism of mitochondria in ethanol-induced gastric mucosa injury. World J Gastroenterol: WJG 14, 5857. https://doi.org/10.3748/wjg.14.5857

Purushotham A, Schug TT, Xu Q, Surapureddi S, Guo X, Li X (2009) Hepatocyte-specific deletion of SIRT1 alters fatty acid metabolism and results in hepatic steatosis and inflammation. Cell Metabol 9, 327-338. . https://doi.org/10.1016/j.cmet.2009.02.006

Rabah Y, Francés R, Minatchy J, Guédon L, Desnous C, Plaçais P Y, *et al*. (2023) Glycolysis-derived alanine from glia fuels neuronal mitochondria for memory in Drosophila. Nat Metab 5, 2002-2019. https://doi.org/10.1038/s42255-023-00910-y

Rehman SU, Shah SA, Ali T, Chung JI, Kim MO (2017) Anthocyanins reversed D-galactose-induced oxidative stress and neuroinflammation mediated cognitive impairment in adult rats. Mol Neurobiol 54, 255-271. https://doi.org/10.1007/s12035-015-9604-5

Ren R, Wang Z, Wu M, Wang H (2020) Emerging roles of SIRT1 in alcoholic liver disease. Int J Biol Sci 16, 3174. https://doi.org/10.7150/ijbs.49535

Sattler JH, Fuchs M, Tauber K, Mutti FG, Faber K, Pfeffer J, *et al*. (2012) Redox self-sufficient biocatalyst network for the amination of primary alcohols. Angew Chem Int Ed 51, 9156-9159. https://doi.org/10.1002/anie.201204683

Schell MJ, Molliver ME, Snyder SH (1995) D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. Proc Natl Acad Sci 92, 3948-3952. https://doi.org/10.1073/pnas.92.9.3948

Senthilkumar R, Sengottuvelan M, Nalini N (2004) Protective effect of glycine supplementation on the levels of lipid peroxidation and antioxidant enzymes in the erythrocyte of rats with alcohol-induced liver injury. Cell Biochem Funct 22, 123-128. https://doi.org/10.1002/cbf.1062

Shimokawa T, Yamagiwa D, Hondo E, Nishiwaki S, Kiso Y, Makita T (2003) Histological observation of the proper gastric gland in Minke whale, Balaenoptera acutorostrata. J Vet Med Sci 65, 423-426. https://doi.org/10.1292/jvms.65.423

Sim WC, Kim DG, Lee W, Sim H, Choi YJ, Lee BH (2019) Activation of SIRT1 by l-serine increases fatty acid oxidation and reverses insulin resistance in C2C12 myotubes (l-serine activates SIRT1 in C2C12 myotubes). Cell Biol Toxicol 35, 457-470. https://doi.org/10.1007/s10565-019-09463-x

Sorbi D, Boynton J, Lindor KD (1999) The ratio of aspartate aminotransferase to alanine aminotransferase: potential value in differentiating nonalcoholic steatohepatitis from alcoholic liver disease. Am J Gastroenterol 94, 1018-1022.

Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, *et al*. (2011) Astrocyte-neuron lactate transport is required for long-term memory formation. Cell 144, 810-23. 10.1016/j.cell.2011.02.018

Szabo S, Nagy L, Plebani M (1992) Glutathione, protein sulfhydryls and cysteine proteases in gastric mucosal injury and protection. Clinica Chimica Acta 206, 95-105. https://doi.org/10.1016/0009-8981(92)90010-N

Tsai CH, Huang HC, Liu BL, Li CI, Lu MK, Chen X, *et al*. (2014) Activation of N-methyl-D-aspartate receptor glycine site temporally ameliorates neuropsychiatric symptoms of P arkinson's disease with dementia. Psychiat Clin Neuros 68, 692-700. https://doi.org/10.1111/pcn.12175

Vanover JC, Spry ML, Hamilton L, Wakamatsu K, Ito S and D'Orazio JA (2009) Stem cell factor rescues tyrosinase expression and pigmentation in discreet anatomic locations in albino mice. Pigment Cell Melanoma Res 22, 827-838. https://doi.org/10.1111/j.1755-148X.2009.00617.x

Williams SM, Diaz CM, Macnab LT, Sullivan RK, Pow DV (2006) Immunocytochemical analysis of d-serine distribution in the mammalian brain reveals novel anatomical compartmentalizations in glia and neurons. Glia 53, 401-411. https://doi.org/10.1002/glia.20300

Wolosker H, Blackshaw S, Snyder S H (1999) Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. Proc Natl Acad Sci 96, 13409-13414. https://doi.org/10.1073/pnas.96.23.13409

Yamaguchi Y, Brenner M, Hearing V J (2007) The regulation of skin pigmentation. J Biol Chem 282, 27557-27561. https://doi.org/10.1074/jbc.R700026200

Yang S, Qiao H, Wen L, Zhou W, Zhang Y (2005) D-serine enhances impaired long-term potentiation in CA1 subfield of hippocampal slices from aged senescence-accelerated mouse prone/8. Neurosci Lett 379, 7-12. https://doi.org/10.1016/j.neulet.2004.12.033

Yin G-Y, Zhang W-N, Shen X-J, Chen Y, He X-F (2003) Ultrastructure and molecular biological changes of chronic gastritis, gastric cancer and gastric precancerous lesions: a comparative study. World J Gastroenterol: WJG 9, 851. https://doi.org/10.3748/wjg.v9.i4.851

Yin M, Ikejima K, Arteel G E, Seabra V, Bradford B U, Kono H, *et al*. (1998) Glycine accelerates recovery from alcohol-induced liver injury. J Pharmacol Exp Ther 286, 1014-1019.

Yoshikawa T, Minamiyama Y, Ichikawa H, Takahashi S, Naito Y, Kondo M (1997) Role of lipid peroxidation and antioxidants in gastric mucosal injury induced by the hypoxanthine-xanthine oxidase system in rats. Free Radic Biol Med 23, 243-250. https://doi.org/10.1016/S0891-5849(96)00625-9

You M, Matsumoto M, Pacold CM, Cho WK, Crabb DW (2004) The role of AMP-activated protein kinase in the action of ethanol in the liver. Gastroenterology 127, 1798-1808. https://doi.org/10.1053/j.gastro.2004.09.049

You M, Liang X, Ajmo JM, Ness GC (2008) Involvement of mammalian sirtuin 1 in the action of ethanol in the liver. Am J Physiol Gastrointest Liver Physiol 294, G892-G898. https://doi.org/10.1152/ajpgi.00575.2007

Yun SM, Cho JM, Hong KS, Lee DY, Ji SD, Son JG, *et al*. (2017) Gastroprotective effect of mature silkworm, Bombyx mori against ethanol-induced gastric mucosal injuries in rats. J Funct Foods 39, 279-286. https://doi.org/10.1016/j.jff.2017