Silkworm Pupae (Bombyx mori) Are New Sources of High Quality Protein and Lipid

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Summary This study was performed to evaluate the nutritional value of silkworm pupae (Bombyx mori) and the content of α-glucosidase inhibitor. The percentages of total protein and lipid contents by dry weight were 55.6 and 32.2%, respectively. Silkworm pupae protein had high levels of essential amino acids such as valine, methionine and phenylalanine. The contents of essential amino acids in silkworm pupae protein satisfied the FAO/WHO/UNU suggested requirements (2007). In addition, they also possessed n-3 fatty acids, especially α-linolenic acid (36.3%), as a major component. The 50% ethanol extract of silkworm pupae contained 1-deoxynojirimycin (DNJ), which is a potent α-glucosidase inhibitor. These results suggest that silkworm pupae are a new source of high quality protein, lipid, and α-glucosidase inhibitor.

Key Words silkworm pupae, amino acid composition, fatty acid composition, α-glucosidase inhibitor, 1-deoxynojirimycin

Materials and Methods

1. Samples. To determine the nutritional value, edible silkworm pupae (Bombyx mori), which are by-products of the silk industry, were purchased from Nishiki Food, Ltd. (Nagano, Japan). The silkworm pupae were lyophilized, followed by grinding into a powder.

2. Analytical methods. Total nitrogen and lipid contents were determined according to the methods of AOAC (8). For amino acid analysis, hydrolysis of the samples was performed according to the method described in our previous study (9). Fatty acid methyl esters were prepared by the method of Hyun et al. (10) and analyzed by a gas-liquid chromatograph according to the method described in our previous study (11).

3. Extraction and quantification of DNJ. DNJ was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and used as a reference standard. Five hundred milligrams of the silkworm pupae powder was extracted two times with 5 mL of 50% aqueous ethanol for 30 min at 60°C. The extract was evaporated and dissolved in 1 mL of a mixture of acetonitrile and water (50 : 50); containing 10 μM ammonium hydrogencarbonate, then filtered (0.5 μm) and a 10 μL aliquot was applied to the HPLC (Jasco 880, Japan Spectroscopic, Tokyo, Japan) using a Unison UK-Amino column (250 mm × 4.6 mm) (Intakt, Kyoto, Japan) with monitoring on a RI detector (Jasco RID-300). The system was developed at a flow rate of 0.8 mL/min with a mobile phase of acetonitrile and water (90 : 10, v/v), containing 10 μM ammonium hydrogencarbonate.

Results

1. Amino acid composition
The percentage of total protein content by dry weight was 55.6%. The moisture content of silkworm pupae...
was 51.9%. The amino acid content (mg/g of protein) of silkworm pupae protein is shown in Table 1. Noteworthy in the amino acid profile are high amounts of aspartic acid, glutamic acid, and proline (10.7, 11.1, and 8.2%, respectively). The essential amino acids were compared with the WHO/FAO/UNU (2007) ideal amino acid (12). The essential amino acids in silkworm pupae protein satisfied the WHO/FAO/UNU suggested requirements.

2. Fatty acid composition

The percentage of total lipid content by dry weight was 32.2%. The fatty acid composition of silkworm pupae oil is shown in Table 2. The silkworm pupae oil is composed of 28.8% saturated fatty acids, 27.7% monounsaturated fatty acids and 43.6% polyunsaturated fatty acids. Among them, the predominant one was linolenic acid (C\textsubscript{18:3}) which makes up 36.3% of the total fatty acid. Oleic acid (C\textsubscript{18:1}) was the second largest fatty acid (26.0%). The polyunsaturated fatty acid (PUFA): monounsaturated fatty acid (MUFA): saturated fatty acid (SFA) ratio of silkworm pupae oil was 1.6:1:1.

3. DNJ content

When standard DNJ was subjected to HPLC, a peak ascribed to DNJ was detected at a suitable retention time (15 min). The DNJ content of silkworm pupae by dry weight was 5.9 mg/100 g (0.006%) (2.84 mg/100 g (0.003%) of raw weight).

Discussion

Silkworm pupae are produced as by-products in the silk industry. In the present study, we determined the nutritional value of Japanese strains of silkworm pupae. We demonstrated that silkworm pupae were good sources of protein because of their high protein content. In addition, silkworm pupae protein had high levels of essential amino acids. In comparison to the essential amino acid content of soy protein isolate (13), the silkworm pupae protein had similar or higher levels of all amino acids except isoleucine and leucine. There are few reports concerning the amino acid limitations of silkworm pupae protein for human consumption. Consuming 100 g (dry weight) of silkworm pupae would equal to ingesting approximately 56 g of protein, which satisfies the recommended dietary allowance of protein for adults (12). Insects, such as bees, beetles, crickets, locusts and termites, have been used as food in tropical and subtropical countries to provide significant nutritional benefits (14). With the increasing human population, global food demand is growing rapidly and therefore silkworm pupae may become a valuable new source of protein.

There has been increased focus on the role of specific dietary fatty acids and their effect on health and disease. Polyunsaturated fatty acids are very important in human diet and physiology (15). Silkworm pupae could be an excellent lipid source for humans, because they possess n-3 fatty acids such as linolenic acid (36.3%). n-3 Polyunsaturated fatty acids have been shown to have potential beneficial effects on chronic diseases (16, 17). Both excessive amounts of n-6 polyunsaturated fatty acids and a very high n-6/n-3 ratio promote the risk of many diseases, including cardiovascular disease, cancer and inflammatory disease, whereas increased levels of n-3 exert suppressive effects on those diseases (15). With the current emphasis on increasing intake of n-3 polyunsaturated fatty acids, the use of silkworm pupae oil in food processing may be acceptable.

Japanese mulberry sericulture was once among the biggest in the world, but in these days it has been in decline. Recently, mulberry leaves have been considered as a functional or medical food for the control of blood glucose. Mulberry DNJ is a potent α-glucosidase inhibitor (2, 3). Kimura et al. reported that the concentration of DNJ in most commercial products of mulberry leaves is about 0.2% (18). Asano et al. reported that DNJ was concentrated 2.7-fold in the midgut of silkworms allowed to feed on mulberry leaves (6). In the present study, we prepared a silkworm pupae extract to investi-
gate whether it has DNJ, a potent α-glucosidase inhibitor.

To determine DNJ by HPLC, we extracted DNJ from silkworm pupae powder with 50% aqueous ethanol according to the method of Asano et al. (6). In this study, the extract was concentrated 10-fold, because the sensitivity of the RI detector was low. It is noteworthy that the DNJ content of silkworm pupae was much lower than that of mulberry leaves (18) or silkworms (6). One of the reasons for this difference may be due to the reeling treatment in the bath. The edible silk worm pupae were by-products of silk industry. Thus, a part of DNJ may be lost in the reeling bath.

Hot water extract of silkworm pupae strongly inhibited porcine pancreatic α-glucosidase, although the activity was lower than that of mulberry leaves (data not shown). This indicates that the α-glucosidase inhibitory activity of silkworm pupae is due to DNJ, but also partly due to other derivatives.

In conclusion, this study demonstrated that silkworm pupae are good sources of high quality protein and lipid and have an α-glucosidase inhibitor, DNJ, which may retard the absorption of carbohydrates and reduce postprandial hyperglycemia. Because silkworm pupae are produced as by-products in the silk industry, they could become new valuable sources of food.

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