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SHORT COMMUNICATION

Extraction of chitin and chitosan from larval exuvium and whole body of edible mealworm, *Tenebrio molitor*

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Abstract

The purpose of this study was to investigate the production of chitin and chitosan from both the exuvium and whole body of mealworm (*Tenebrio molitor*) larvae. Chitin from the exuvium and whole body of *T. molitor* larvae was chemically extracted with acid and alkali solutions to achieve demineralization (DM) and deproteinization (DP), respectively. The average DM (%) and DP (%) on a dry weight (DW) basis was 32.56 and 73.16% from larval exuvium, and 41.68 and 91.53% from whole body, respectively. To obtain chitosan, chitin particles from the exuvium and whole body of *T. molitor* larva were heated at various temperatures in different concentrations of NaOH. Average chitin yields were 18.01% and 4.92% of DW from the exuvium and whole body, respectively. The relative average yield of chitosan from whole body was 3.65% of DW. On average, over 90% of chitosan derived from whole body was deacetylated. The viscosity of chitosan from whole body was ranged from 48.0 cP to 54.0 cP. The chitin content of dry and wet byproducts from whole body were 17.32% and 16.94% respectively, compared to dry weight. The chitosan contents of byproducts on a DW basis were 14.48% in dry and 13.07% in wet byproduct. These results indicate that the exuvium and whole body of *T. molitor* larva may serve as a source of chitin and chitosan for use in domestic animal feed.

Key words: chitin, chitosan, exuvium, mealworm, *Tenebrio molitor*.

Introduction

Edible insects such as mealworms, crickets, and grasshoppers are consumed as food globally. The number of species appropriate for human consumption is reported to be approximately two thousand species worldwide (Jongema 2013), from which the chemical composition of 236 species has been published (Rumpold & Schlüter 2013). Common mealworm, *Tenebrio molitor* L.(Coleoptera: Tenebrionidae), and giant mealworm, *Zophobas morio* L. (Coleoptera: Tenebrionidae) are commercially produced as edible insect products. Common mealworm is among the most economical species for the production of protein-rich food and animal feed. The nutritional composition of edible mealworm larvae is protein (47–49%), fat (38–43%), fiber (6–7%), nitrogen-free extract (0.26%), and ash (2–3%) based on dry matter (Ramos-Elorduy Blasquez *et al*. 2012; Ramos-Elorduy *et al*. 2007). Protein content in larvae (Rumpold & Schlüter 2013) and chitin content in larvae and pupa (Kramer *et al*. 1995; Adámková *et al*. 2017) have been studied in the common

mealworm. Among other edible insect species, chitin and chitosan content have been investigated in the exoskeletons of two-spotted field crickets (Kim *et al*. 2017a). The chitin content of the Moroccan locust, *Dociostaurus maroccanus,* was reported for adults and nymphs (Erdogen & Kaya 2016). A special function among edible insects, including *T. molitor* larva, is the production of chitinase in the midgut for digestion of chitin from the exoskeleton cuticle (Genta *et al*. 2006). Two-spotted field crickets secrete chitinase and cellulase in the digestive tract (Weidlich *et al*. 2013).

Whole body and exuvium of common mealworm are experimentally manufactured from Korea Beneficial Insects Lab. Co., Ltd. and KEIL Co., Ltd. They are a beneficial resource for insect-based treats for domestic dogs and cats. Chitin composition has been well studied in *T. molitor* larva, pupa, and adult (Adámková *et al*. 2017). However, the extraction of chitosan from *T. molitor* larva whole body and exuvium has not been extensively studied. In this study, chitin and chitosan were extracted from the exuvium and whole body of *T. molitor* larva using a chemical manufacturing process. The objective of this study was to estimate the production level of chitin and chitosan from the exuvium and whole body to improve yield and functionality of insect-based domestic animal treats.

Materials and methods

Preparation of chitin and chitosan

Whole body and exuvium of mealworm (*Tenebrio molitor*) larvae were supplied by Korea Beneficial Insects Lab. (KBIL) Co., Ltd. (Goksung, Jellanamdo, Korea). Byproducts of 80% protein removal from *T. molitor* larval whole body were supplied by KEIL Co., Ltd. (Seoul, Korea). Chitin was extracted from the whole body and exuvium of *T. molitor* larvae using the method of Hackman (1954) with modifications. The exuviae (DW 30 g) were decalcified for 3 h in 1,500 mL 2 N HCl at 20°C. After decalcification, the samples were incubated in 500 mL 5% NaOH at 95°C for 3 h to deproteinize the sample. They were then washed with water until a neutral pH was achieved, followed by drying in an oven at 70°C for 24 h, producing chitin particles. The chitin particles obtained from *T. molitor* were heated in 500 mL of NaOH at various concentrations (50% NaOH (w/v), 50% NaOH (w/w), 55% NaOH (w/w), and 60% NaOH (w/w)) at 95 or 105°C for 3 h or 5 h (Song *et al*. 2014).

The whole bodies (DW 100 g) of *T. molitor* larvae were decalcified for 3 h in 500 mL 2 N HCl at 20°C. After decalcification, the samples were incubated in 500 mL 1.25 N NaOH at 95°C for 3 h to deproteinize the sample. The chitin particles obtained were boiled in 500 mL 40% NaOH (w/w) and 50% NaOH (w/w) solutions at 105°C for 3 h.

The two types of byproduct (dry and wet) (DW 50 g) of 80% protein removal from larval whole body of *T. molitor* were decalcified for 3 h in 500 mL 2 N HCl at 20°C. After decalcification, the samples were incubated in 500 mL 1.25 N NaOH at 95°C for 3 h to deproteinize them. The chitin particles obtained from byproducts were boiled in 500 mL of 50% NaOH (w/v) and 50% NaOH (w/w) solutions at 100°C for 3 h. Finally, chitosan was washed with tap water until a neutral pH was achieved and then dried for 24 h in an oven at 70°C.

Determination of the viscosity of chitosan and the degree of deacetylation

The viscosity of chitosan samples obtained from *T. molitor* larvae was measured in acetic acid solution at 20°C using a Brookfield DV-II+ Pro Viscometer (Brookfield AMETEK, Middleboro, MA, USA) equipped with an LV-3 spindle (model no. 63; rpm = 100). Viscosity was measured in cP units (centipoise = mPa·s).

To determine the degree of deacetylation (DAc) of chitosan, the free amino group content was measured using the method of Terayama (1952) with modifications. One gram of chitosan was suspended in 100 mL water and then mixed with 100 mL 0.4 M sodium acetate buffer. Dissolved chitosan solution (1 g) was added to 30 mL of distilled water containing 2 to 3 drops of indicator. The mixture was titrated with a colloidal solution composed of N/400 poly vinyl sulfate potassium salt (PVSK). The DAc was determined using the method of Kim *et al*. (2016a).

Results

The process of extracting chitin and chitosan from the whole body of *Tenebrio molitor* larva is shown in Fig. 1. The relative yield of chitin and chitosan from the whole body of larva was 4.92% and 3.65% of DW, respectively.

Exuvium of larval *T. molitor* was used to compare the degree of demineralization (DM) and deproteinization (DP) after acid and alkali treatments (Table 1). After acid treatment, the average DWs of the eight test samples was 20.23 g, or 67.45% of initial DW. The average DM (%) and DP (%) of larval exuvium was 32.56 and 73.16%, respectively.

Larval whole body of *T. molitor* was used to compare the degree of demineralization (DM) and deproteinization (DP) after acid and alkali treatments (Table 2). After acid treatment, the DWs of four tests on whole body of *T. molitor* larvae were 55.80, 61.87, 53.72 and 61.90 g. The average yield of the larval whole body from DW was 58.32%. The DMs (%) were 44.20, 38.13, 46.28, and 38.10%, respectively. The DPs (%) were 91.20, 92.16, 90.97, and 91.79%, respectively. The

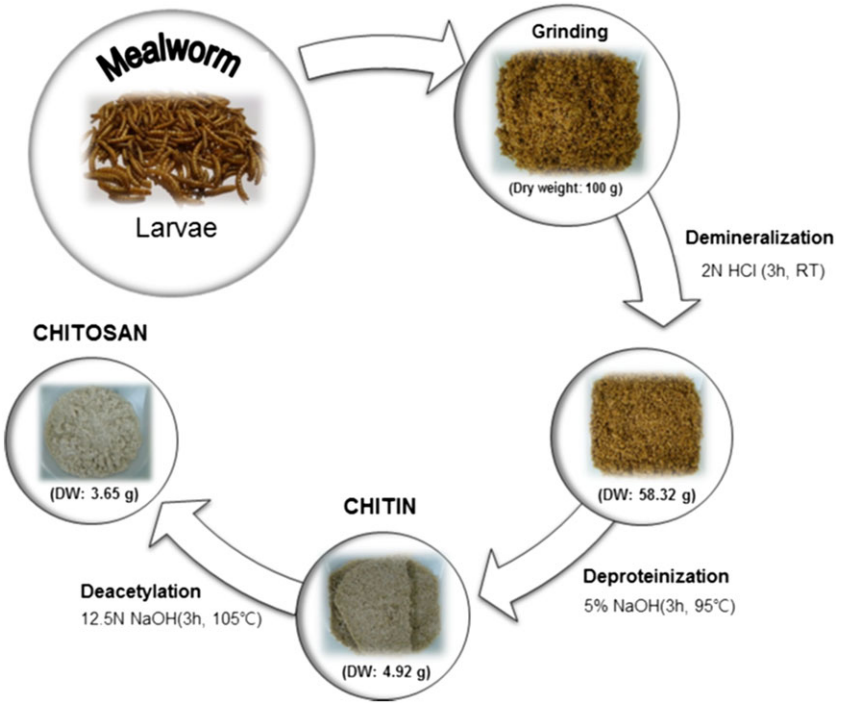

Figure 1 Process for producing chitin and chitosan from mealworm (*Tenebrio molitor*) whole body larvae.

Table 1 Demineralization (DM), deproteinization (DP), and yield after treating exuvium of mealworm larvae (*Tenebrio molitor* L.) with HCl and NaOH

Items	*T. molitor* Whole DW† (g)	Acid treatment DW (g)‡	Yield§ (%)	DM¶ (%)	DP†† (%)
T 1	30	18.38	61.27	38.73	67.25
T 2	30	19.48	64.93	35.07	70.23
T 3	30	20.68	68.93	31.07	73.79
T 4	30	21.51	71.70	28.30	74.06
T 5	30	19.15	63.83	36.17	74.31
T 6	30	20.66	68.87	31.13	75.70
T 7	30	22.45	74.83	25.17	76.61
T 8	30	19.56	65.20	34.80	73.31
Avg.	30	20.23	67.45	32.56	73.16

†DW: Dry weight of *T. molitor* larval exuvium.
‡Dry weight of the *T. molitor* after acid treatment with 2 N HCl at 25°C for 3 h.
§Yield (%) of base dry weight of *T. molitor* larval exuvium after acid treatment.
¶DM (%) = [1 – (DW after 2 N HCl treatment / DW of *T. molitor*)] × 100.
††DP (%) = [1 – (DW after 5% NaOH treatment / DW after 2 N HCl treatment of *T. molitor*)] × 100.

Table 2 Demineralization (DM), deproteinization (DP), and yield after treating whole body of mealworm larvae (*Tenebrio molitor* L.) with HCl and NaOH

Items	*T. molitor* Whole DW† (g)	Acid treatment DW (g)‡	Yield§ (%)	DM¶ (%)	DP†† (%)
Test A	100	55.80	55.80	44.20	91.20
Test B	100	61.87	61.87	38.13	92.16
Test C	100	53.72	53.72	46.28	90.97
Test D	100	61.90	61.90	38.10	91.79
Avg.	100	58.32	58.32	41.68	91.53

†DW: Dry weight of *T. molitor* larval whole body.
‡Dry weight of the *T. molitor* exoskeleton after acid treatment with 2 N HCl at 25°C for 3 h.
§Yield (%) of base dry weight of *T. molitor* larval whole body after acid treatment.
¶DM (%) = [1 – (DW after 2 N HCl treatment / DW of *T. molitor*)] × 100.
††DP (%) = [1 – (DW after 5% NaOH treatment / DW after 2 N HCl treatment of *T. molitor*)] × 100.

average DM (%) and DP (%) of larval whole body from DW was 41.68 and 91.53%, respectively.

Chitin obtained from the whole body of *T. molitor* larvae was used for the manufacture of chitosan (Table 3). For chitin manufacture, the whole body was heated in 1.25 N NaOH at 95°C for 3 h. After alkali treatment in four tests, the chitin yield was 4.91, 4.85, 4.85, and 5.08% of DW. To assess deacetylation, the chitin extracted from the whole body was heated in 40% NaOH (w/w) and 50% NaOH (w/w) solutions at 105°C for 3 h. After 40% NaOH (w/w) and 50% NaOH (w/w) treatment (tests C and D), the yield of chitosan was 3.65% of DW.

Table 3 Manufacturing process of chitin and chitosan from whole body of mealworm larvae (*Tenebrio molitor* L.) after acid and alkali treatments

Items				
Test	Whole body	Manufacturing process & products		Treatment condition for chitosan
A	DW 100 g	Chitin	-	-
		DW 4.91 g	-	
B	DW 100 g	Chitin	-	-
		DW 4.85 g	-	
C	DW 100 g	Chitin	Chitosan	40% NaOH (w/w) at 105°C for 3 h
		DW 4.85 g	DW 3.65 g	
D	DW 100 g	Chitin	Chitosan	50% NaOH (w/w) at 105°C for 3 h
		DW 5.08 g	DW 3.65 g	

During the extraction of chitin and chitosan from larval exuvium of *T. molitor,* the chitin yields were 20.07, 19.33, 18.07, 18.60, 16.40, 16.73, 17.50, and 17.40% of DW (Fig. 2A). The chitosan yields were 10.20, 10.13, 9.53, 8.80, 9.07, 8.50, 7.80, and 9.57% of DW. The average yield of chitin and chitosan was 18.01 and 9.20% of DW, respectively (Fig. 2B).

The free amine content and viscosity of chitosan were measured to determine the DAc and molecular weight (Fig. 3). The deacetylation percentages of chitosan extracted from exuviae were 5.76, 12.41, 32.66, 47.06, 42.97, 40.36, 50.38, and 39.48%.

The relative chitin and chitosan yields from whole body larvae were 4.92 and 3.65% of DW, respectively (Fig. 4A). The free amine content and viscosity of chitosan obtained from whole body larvae were measured to determine the DAc and molecular weight (Fig. 4B and 4C). The deacetylation percentages of chitosan extracted in tests C and D were 91.90 and 96.19%, (Fig. 4B), with viscosity measurements of 54.0 and 48.0 cP, respectively (Fig. 4C).

Two types of byproducts of 80% protein removal from whole body larvae (dry and wet, 57.33% moisture) were compared for chitin and chitosan content (Table 4). After acid treatment, the DWs of dry and wet byproducts were 25.76 and 11.38 g, respectively. After alkali treatment, the DWs of dry and wet byproducts were 8.66 and 3.62 g, respectively. The chitin and chitosan content were 17.32 and 14.48% in dry byproduct, 16.94 and 13.07% in wet byproduct, respectively. The deacetylation percentage of extracted chitosan byproducts was 5.13% (50% NaOH, v/w) in dry byproduct and 86.64% (50% NaOH, w/w) in wet byproduct. The viscosity of chitosan extracted from the wet byproducts was 44.1 cP.

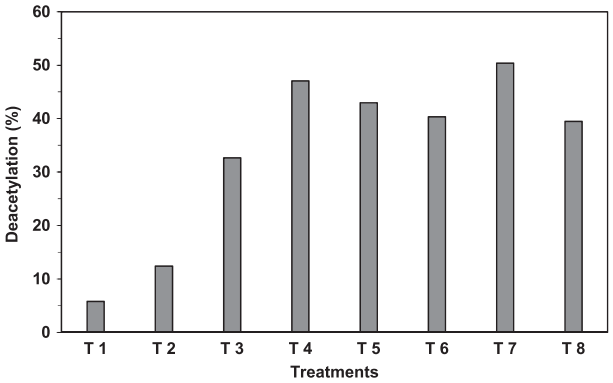

Figure 3 Degree of deacetylation (%) of chitosan from mealworm (*Tenebrio molitor*) larval exuviae. Test 1: 50% NaOH (w/v) at 95°C for 3 h. Test 2: 50% NaOH (w/v) at 105°C for 3 h. Test 3: 50% NaOH (w/w) at 95°C for 3 h. Test 4: 50% NaOH (w/w) at 105°C for 3 h. Test 5: 50% NaOH (w/w) at 105°C for 5 h. Test 6: 50% NaOH (w/w) at 105°C for 5 h. Test 7: 55% NaOH (w/w) at 105°C for 5 h. Test 8: 60% NaOH (w/w) at 105°C for 5 h.

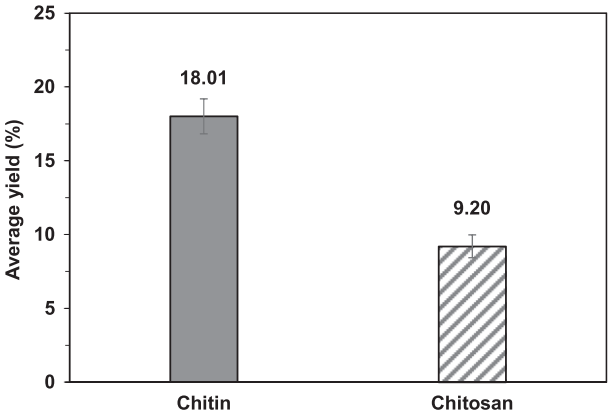

Figure 2 Yield of chitin and chitosan from mealworm (*Tenebrio molitor*) larval exuviae.

Discussion

Breeding of edible insects provides an alternative strategy for providing interesting food and feed for meat products and fishmeal. Mealworm meal is used as a protein source for

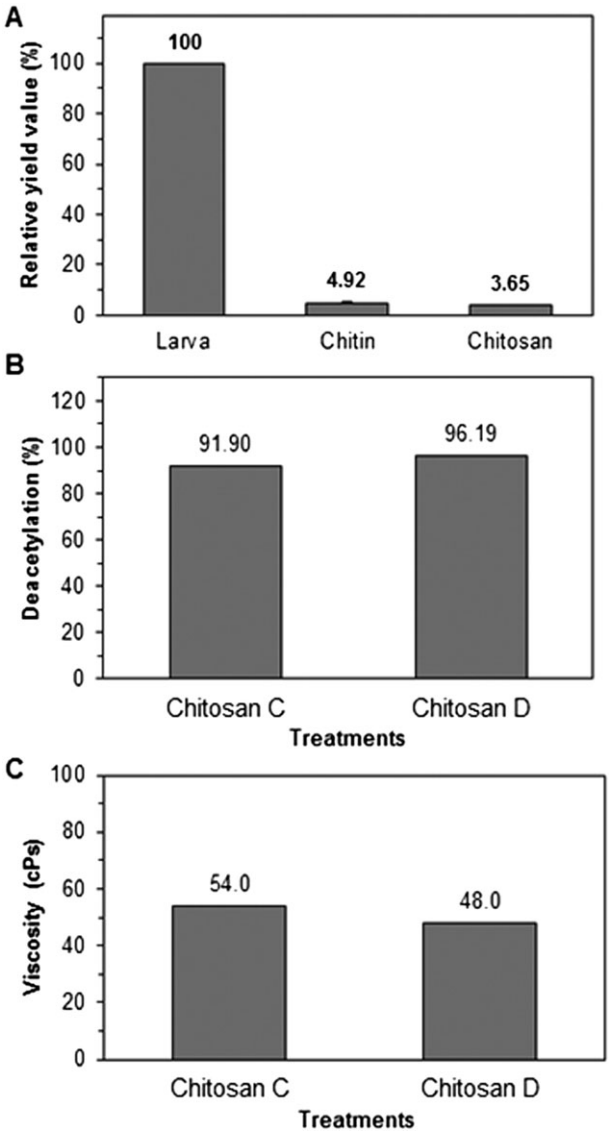

Figure 4 Relative yields of (A) chitin and chitosan, (B) degree of deacetylation (%), and (C) and viscosity of chitosan obtained from mealworm (*Tenebrio molitor*) larval whole body.

farmed Pacific white shrimp *Litopenaeus vannamei* (Panini *et al.* 2017) and for processing of emulsion sausages (Kim *et al.* 2016b). The average DM (%) and DP (%) of larval exuvium and whole body were determined (Table 1 and Table 2). In this study, the average DM (%) and DP (%) of larval whole body were higher than larval exuvium (9.12 and 18.37%), respectively. Adult, larvae, and pupae mealworm are composed of protein ranging from 47% to 65% of dry matter (Rumpold & Schlüter 2013). Protein content of *T. molitor* whole insect larvae was calculated to be 44.74% based on amino acid composition (Janssen *et al.* 2017). *T. molitor* flours contain 60% crude protein (Bußler *et al.* 2016). The average DM (%) and DP (%) of cockroach exoskeleton were 26.65% and 81.25%, respectively (Kim *et al.* 2017b).

The average yield of chitin from the exuviae of larval *T. molitor* was 18.01% of DW (Fig. 2B). The average yield of chitosan from the larval exuvium was 9.20% of DW. The chitin content of edible insects is 6% in giant mealworm larvae, 12% in common mealworm pupa, and 13% in common mealworm larvae (Adámková *et al.* 2017). The chitin and chitosan content from exoskeletons of two-spotted field crickets is 2.42 and 1.65% of DW, respectively (Kim *et al.* 2017a). The chitin content of grasshopper, *Dociostaurus maroccanus* is 14% in adults and 12% in nymphs (Erdogen & Kaya 2016). Chitin content ranges from 5.3% to 8.9% among seven species of Orthoptera (Kaya *et al.* 2015).

The deacetylation of chitosan extracted from the larval exuvium of *T. molitor* was the most complete at 50.38% after heating in 55% NaOH (w/w) at 105°C for 5 h (Fig. 3). Fig. 4 shows the relative yield of chitin and chitosan, and the deacetylation and viscosity of chitosan from whole body *T. molitor* larvae. Deacetylation of chitosan extracted from the exoskeletons of two-spotted field crickets was 95.5% in a reaction with 50% NaOH (w/w) at 105°C for 3 h (Kim *et al.* 2017a). Viscosity of this chitosan was 62.4 cP with a molecular weight of 308.3 kDa. Molecular weight of chitosan in the exoskeleton of a cockroach (*Periplaneta americana* L.) ranges from 210 to 230 kDa (Kim *et al.* 2017b). The molecular weight of chitosan from grasshopper is 7.2 kDa in adult and 5.6 kDa in nymph (Erdogen & Kaya 2016).

As shown in Table 4, the chitin and chitosan yields were calculated for byproducts from production processes using *T. molitor* larva. The DM (%) and DP (%) was 51.52 and 82.68% in dry byproduct, and 60.30 and 87.37% in wet byproduct, respectively. Chitin content of the byproducts was as much as 3.44 fold higher than the whole body of *T. molitor* larva. Chitosan content of byproducts was as much as 3.58 fold higher than the whole body of *T. molitor* larva. The deacetylation percentage of chitosan extracted from the byproducts of larvae was very low at 5.13% after treatment with 50% NaOH (w/v).

In this study, chitin and chitosan content were determined for the exuvium, larvae whole body, and byproducts from whole body of *T. molitor* larvae production processes. The chitin and chitosan yields from the whole body of *T. molitor* L. larvae were 4.92, and 3.65% of DW, respectively. Chitin and chitosan content of the byproducts were 16.94, and 13.07% of DW, respectively.

In conclusion, there is a keen interest in edible insects as an alternative protein source for human food, pet treats, and animal feed. With concern over the availability of protein sources in the future, the industrial production of chitin and chitosan extracted from edible insects could be used

Table 4 Chitin and chitosan content in byproduct obtained from larval whole body of mealworm (*Tenebrio molitor* L.)

Items	By-product of *T. molitor*† (g)	Acid treatment DW (g)‡	Alkali treatment DW (g)§	Chitin (%)	Chitosan (%)	DAc§§ (%)	Viscosity (cPs)
Dry	50	25.76	8.66	17.32	14.48††	5.13	-
Wet¶	50	11.38	3.62	16.94	13.07‡‡	86.64	44.1

†By-product of 80% protein removal from *T. molitor* larval whole body.
‡Dry weight of *T. molitor* byproduct after acid treatment with 2 N HCl at 25°C for 3 h.
§Dry weight of *T. molitor* byproduct after acid treatment with 5% NaOH at 100°C for 3 h.
¶Wet byproduct (including 57.33% moisture) of 80% protein removal from *T. molitor* larval whole body.
††Content of chitosan from *T. molitor* byproduct after alkali treatment with 50% NaOH (w/v) at 95~100°C for 3 h.
‡‡Content of chitosan from *T. molitor* byproduct after alkali treatment with 50% NaOH (w/w) at 95~100°C for 3 h.
§§DAc: Deacetylation of chitin.

as an oligosaccharide source for pet, animal, and human nutrition.

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