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ORIGINAL ARTICLE

Optimization and evaluation of microencapsulated artificial diet for mass rearing the predatory ladybird *Propylea japonica* (Coleoptera: Coccinellidae)

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Abstract Artificial diet optimization is a key aspect in mass rearing of natural enemies since it influences the quality and feeding effectiveness, and thus the success of the biological control program. Here, we introduced the microencapsulation method to package liquid artificial diet for feeding of the ladybird *Propylea japonica*. An orthogonal test of the quality of microencapsulated artificial diets (ADMs) was performed on key variables in production; Ca-alginate concentration, chitosan concentration and weight ratio of wall material to inner diet. We compared the development and reproduction of *P. japonica* fed on the ADMs under different cold-stored periods with those fed on fresh aphids and liquid artificial diets, in addition to a comparison of respiration, locomotion and predation. Our results indicated that chitosan concentration and ratio of shell to core significantly influence the quality of ADMs. The optimal recipe is 1.0% Ca-alginate, 1.6% chitosan and shell : core = 1 : 2. Insects reared on fresh optimized ADMs were similar to those fed on fresh prey in all developmental and reproductive characteristics except for survival ratio and female fertility. ADMs appeared more beneficial than using a liquid artificial diet, although this may decrease with the prolonging of the cold-storage period. *P. japonica* fed either on fresh ADMs or fresh prey showed improved respiration and predation abilities compared to where liquid artificial diet was used. Our study indicates advantages of microencapsulation in the production of artificial diet for predatory ladybird rearing. A microencapsulated diet can directly increase the efficiency and stability of mass rearing.

Key words alginate; chitson; development; locomotion; predation; quality evaluation; reproduction; respiration

Introduction

The development of artificial diets has facilitated mass rearing of predatory insects for biological control purposes (Vanderzant, 1974). Over the last century, much intensive research has been carried out on recipe optimization, functional supplementary components and rearing substrate material of artificial diets (Bruzzone *et al*., 1990; Murai *et al*., 2001). The increasing commercial demands of mass biological control agents for agricultural production has intensified the need for effective artificial diets, not only in providing sufficient insect nutrition and rearing efficiency, but also in terms of ease of storage and feeding capabilities (House, 1961; Chambers, 1977).

In addition to the components of the artificial diets, the properties and state (liquid or solid) can strongly affect feeding efficiency. Although most artificial diets have been made in liquid form due to ease of mixture and insect ingestion, the adhesive nature of the material also increases mortality (Zhang *et al*., 2008). Furthermore,

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moisture loss in liquid and solid artificial diets is a problem when exposed during storage and the course of feeding (Vanderzant, 1974; Thompson & Hagen, 1999). Without any protective packaging both liquid and solid artificial diets can be easily contaminated and lose nutritional value during storage. These shortcomings have hindered development of artificial diets in scalable biological control insect mass rearing and prospects of effective practical application in the long term.

To address some of these negative features of liquid artificial diets, the microencapsulation technique was introduced for improving physical properties (Thompson, 1999). Although this technique has been widely utilized in packaging of microbial agents and chemical pesticides in pest management, and in the production of medicines (Jones *et al*., 1974; Yazlovetsky, 1989; Gharsallaoui *et al*., 2007; Tan *et al*., 2013), few studies have been made on the application of microencapsulation for packaging artificial diet. Our previous work indicated a significantly higher consistency in characteristics of microencapsulated artificial diets, which were sufficient for development and reproduction of the flower bug *Orius sauteri* (Tan *et al*., 2010). Further, we developed a series of suitable artificial diet recipes in the form of microcapsules (Tan *et al*., 2013). Based on these results, a commercial massive rearing line of *O. sauteri* fed on artificial diet microcapsules were established. After three generations fed on artificial diet microcapsules, the mortality of the flower bug decreased by almost 50% and the cost of mass rearing was reduced approximately 35% compared to liquid diet, and did not show significant differences when fed on natural aphid prey *Myzus persicae* (S. Wang, X.L. Tan, F. Zhang, unpublished data).

The crop field and greenhouse release of the euryphagous predatory ladybird beetle, *Propylea japonica* (Coleoptera: Coccinellidae) as an efficacious biological control agent to suppress the outbreak of various arthropod herbivores, has been widely reported (Obrycki & Kring, 1998; Ragsdale *et al*., 2001; Pang *et al*., 2004). Due to its performance in pest management, many studies have been performed on aspects of biology, physiology, behavior and ecology of *P. japonica* (Obrycki & Kring, 1998; Pan *et al*., 2010; Pervez & Omkar, 2011; Ouyang *et al*., 2012; Tang *et al*., 2013). Moreover, the biological control population needs abundant ladybirds for inundative or inoculative release, which may influence the efficiency of pest management (Obrycki & Kring, 1998). Therefore, we have addressed the need for practical solutions in a system of mass rearing of *P. japonica*, especially in commercial aspects (Yang *et al*., 2014). Many previous efforts have focused on the selection and optimization of artificial diet as alternative foods for successfully rearing *P. japonica*. Most have studied the liquid form artificial diets which contain saccharides, insect tissue and viscera of livestock (as liver) (Shen *et al*., 1992; Li *et al*., 2007; Zhang *et al*., 2007). Practically, the high rate of putrescence (even in cold-storage) and difficulty in feeding by insects with chewing mouth parts, inhibit the efficiency of massive rearing with liquid diets. Based on these results, we introduced a new diet form, the microcapsule, to improve the features of artificial diets and efficiency of *P. japonica* rearing for commercial biological control application.

The purpose of the present study was to confirm the optimal ingredients of microencapsulated artificial diets based on various characters of the microcapsules. Moreover, we evaluated the effects of the artificial diet microcapsules under different cold-storage periods on the development and reproduction of *P. japonica*, and compared the respiration, locomotion and predation of *P. japonica* fed on artificial diet microcapsules with the ladybirds fed on insect prey and liquid artificial diet.

Materials and methods

Ladybird stock

A total of 223 *P. japonica* (ladybird) adults were captured in an alfalfa *Medicago sativa* L. field in the experimental area of the Beijing Academy of Agriculture and Forestry Sciences (BAAFS) during July to August of 2011. The ladybirds were maintained in the insect-rearing chamber of the Institute of Plant and Environment Protection. We reared the ladybird adults in a culturing cage (50.0 × 45.0 × 50.0 cm, constructed from plastic frames and 80 mesh fabric net) with daily supplement of aphid *Myzus persicae* as food. We controlled the environmental factors at 25 °C, 65% relative humidity (RH), photoperiod 16 : 8 L : D and light intensity 900 lux using an automatic environmental regulation system (Sun-Tech®, LP-100, Beijing, China).

Optimization of microencapsulated artificial diet

Microcapsule ingredients and orthogonal test design To optimize the microencapsulated artificial diet, we examined three main factors, the proportion of sodium alga acid (factor I, ALG%, set as 1.0%, 2.0%, 3.0% and 4.0%) and Chitosan (factor II, CHI%, set as 0.4%, 0.8%, 1.2% and 1%), and the ratio of wall material to contents (factor III, set as (shell to core) 2 : 1, 1 : 1, 1 : 2 and 1 : 3). These characters were assessed for two different wall materials. An orthogonal L_{16} test design was used to evaluate the optimal microcapsule recipes (Table 1).

Table 1 The composition of different orthogonal experiment treatments.

Recipes	Composition of microcapsule		
	Ca-ALG proportion (A)	CHI proportion (B)	Shell to core (C)
1	1.0%	0.4%	2 : 1
2	1.0%	0.8%	1 : 1
3	1.0%	1.2%	1 : 2
4	1.0%	1.6%	1 : 3
5	2.0%	0.4%	1 : 1
6	2.0%	0.8%	2 : 1
7	2.0%	1.2%	1 : 3
8	2.0%	1.6%	1 : 2
9	3.0%	0.4%	1 : 2
10	3.0%	0.8%	1 : 3
11	3.0%	1.2%	2 : 1
12	3.0%	1.6%	1 : 1
13	4.0%	0.4%	1 : 3
14	4.0%	0.8%	1 : 2
15	4.0%	1.2%	1 : 1
16	4.0%	1.6%	2 : 1

Ca-ALG, calcium alginate; CHI, chitoson.

Optimization of artificial diet embedded microcapsule A liquid artificial diet which was optimized previously (Zhang *et al*., 2007) was used in the present study. The artificial diet was made up of a variety of ingredients, including fresh pork liver + pure honey + sucrose (regulated weight ratio of 5 : 1 : 1, respectively) and 0.5% (to total weight of diet) olive oil. The fresh pork liver was mashed bya triturator (PT1600E, Kinematica, Luzern, Switzerland) with pure honey, sucrose and olive oil in described proportions. Afterwards, the liquid artificial diet was maintained in a 250 mL glass jar covered by parafilm and stored in a freezer at 4 °C.

The complex coacervation method (Schrooyen *et al*., 2001) was used to make the artificial diet microcapsules (ADMs). Based on the different treatments shown in Table 2, the microcapsules were produced as follows: (i) raw materials preparation; (ii) sterilization (all the raw materials of artificial diets were treated in 85 °C for 10 sec according to the requisition of food ultra-high temperature [UHT] sterilization method); (iii) burdening; (iv) emulsification; (v) granulation; (vi) rinsing; (vii) filtration; and (viii) packaging (Tan *et al*., 2010). The production process was as our previous work on the microencapsulated artificial diet for *O. sauteri* (Tan *et al*., 2013). First, we prepared a 2% mass concentration of calcium chloride solution with distilled water. The frozen liquid artificial diet and the sodium alginate, produced according to the various settings shown in Table 1, were mixed using a magnetic stirrer for 10 min at 3 500 r/m to ensure uniform mixing. A medical micro-injection pump (Top-5300, Top Corporation, Kyoto, Japan) was then filled with the mixed core liquid. As Figure 1 shows, we adjusted the pinhead of the injection pump to 5.0 cm to the surface of the 350 mL 2% calcium chloride in a 500 mL glass beaker. The flow of the injection pump was set at 1.5 mL/min (0.1 mL/drop × 15 drops / min) by regulating the pressure of the compressed air. The rough artificial diet microcapsules were maintained in the calcium chloride solution for at least 10 min and then were moved to the sodium alginate solution according to various settings shown in Table 1, for shaking at 15 min periods. The formed calcium alginate-chitosan-sodium alginate colloid microcapsules were maintained in 0.15% sodium alginate solution for 30 min. After that, the microcapsules were put into a 0.055 mol/L sodium citrate solution for 15 min. Afterwards, we rinsed the microcapsules in physiological saline and distilled water gently, five times. All the ADMs were stored in airproof plastic bags and at 4 °C for special time periods. A total of 16 co-allocated types of ADMs were produced according to the orthogonal setup in Table 1, including over 2 000 microcapsules.

Quality evaluation of ADMs and optimization of microencapsulation recipe To confirm the optimal ingredients of the ADMs, we evaluated the microcapsules according to four criteria: (a) % of productivity rate = (weight of the microcapsule / total weight of wall and core materials) × 100; (b) % of inner artificial diet embedding rate = (total weight of encased artificial diet in microcapsule / total weight of artificial diet) × 100; (c) % of moisture content = (weight of microcapsule − weight of dried microcapsule) / weight of microcapsule × 100; and (d) the scores of sensory evaluation based on the contents shown in Table 2. A total of 50 microcapsules were randomly selected for each orthogonal treatment. We calculated the "quality score" = [% (a) + % (b) + % (c)] × 100 + (d) and confirmed the influences and optimal ingredient of each factor for making ADMs.

Rearing investigation of optimal ADMs with different cold storage periods

Both to evaluate the practical effects of the ADMs optimized by the above procedures for mass rearing of *P. japonica*, and due to possibility of some decay of ADMs during storage, the newly produced ADMs and the 5- and 10- day cold-stored ADMs were offered to newly

Table 2 The grade criteria of sensory evaluations of artificial diet microcapsule.

Contents	Evaluation criterions	Full score
Shape	Perfectly spherical	20
Surface color	Homogeneous white or pale yellow	20
Resilience	High resilience and hard to break	20
Shell tissue	Distinct separation of shell and core materials, compact tissue structure	20
Surface smoothness	Smooth and without embossment or debossment	20

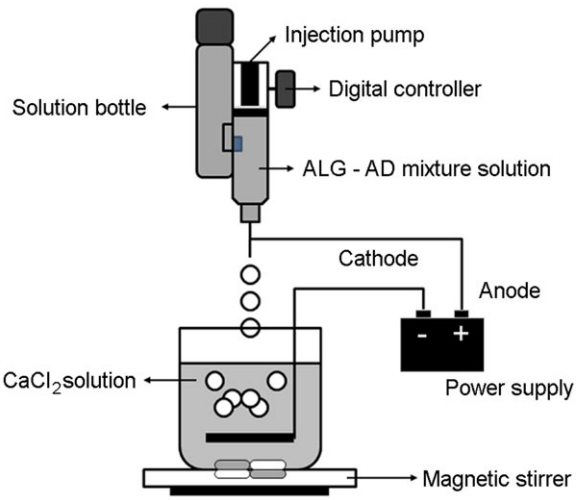

Fig. 1 Schematic diagram of the devices for forming of microencapsulation artificial diet by means of the complex coacervation methods (as shown in Tan *et al*., 2013).

hatched larvae of *P. japonica* under the regulated environmental conditions described above in an artificial environment chamber (MLR-351-H, Sanyo, Osaka, Japan). Newly hatched larvae were placed into a clean plastic Petri dish ($\phi = 9.0$) with five newly produced ADMs supplied daily. The Petri dish was covered with parafilm with several ventilation holes. Then we observed and recorded the total larval development time and adult eclosion ratio (%) once they had pupated to adults, and total life span. Furthermore, a pair of 8-day-old *P. japonica* adults reared by newly produced optimized ADMs was placed into a plastic Petri dish ($\phi = 9.0$) with eight ADMs. After 48 h, we removed the male and reared the mated female continuously until death. The oviposition duration and net female fertility (amount of hatched first instar larvae of offspring) were recorded. We repeated the above procedures with the ADMs which were stored both for 5 and 10 days. *P. japonica* fed on only *M. persicae* and liquid artificial diet (10 mL daily) under the same environmental conditions were tested as different control treatments.

For each experimental treatment, five replications, each with 10 observations, were performed in total.

Respiratory and locomotory activity of P. japonica *reared by optimal ADMs*

We used a respiratory measurement device (Sable System Co., Ltd., Copenhagen, Denmark) to determine the respiratory quotient of the adults of *P. japonica* reared by ADMs, aphid and liquid artificial diet (see the details of the measurement device in Tan *et al*. [2013]). The input airflow was adjusted to 0.2 L/min. Next we activated the CO_2 infrared analyzer and O_2 sensor to determine the initial CO_2 and O_2 concentrations. When they were stable, we placed newly emerged *P. japonica* adults which had been fed consistently on ADMs, into the glass sample chamber for 30 min. We recorded the CO_2 and O_2 concentrations at the end of the 30 min period. All experimental conditions were regulated as above. We calculated the respiratory quotient = (CO_2% expired − CO_2% inspired) / (CO_2% inspired − O_2% expired). Moreover, we used newly emerged *P. japonica* adults fed on *M. persicae* or liquid artificial diet as controls. Each experimental treatment and control group was replicated five times with 10 adults each.

The locomotory capacity of *P. japonica* adults fed on ADMs and control treatment diets (aphid *M. persicae* and liquid artificial diet) were tested by LC-100 Tracksphere (Synthetic, Stuttgart, Germany). After adjustment of focus, a newly emerged *P. japonica* adult was placed on the top of the monitoring sphere. The sphere adjusted the moving speed automatically and kept the ladybird located in a relative rest position. The Tracksphere tracked the locomotory status of the ladybirds over 5 min and recorded the average moving speed. The observation of ADMs and two control groups were replicated five times, with 10 replications.

Predatory ability of P. japonica *fed on ADMs*

The predatory ability of *P. japonica* fed on ADMs was tested on greenhouse tomatoes. We established a 2.0 × 2.0 m area with nine tomato plants (each in 30–35 cm height, with five main leaves) covered by a column cage ($\phi =$ 2.2 m, height = 60 cm, made by 80 mesh fabric net) for the

Table 3 The quality evaluations of various microencapsulated artificial diet.

No.	% productivity rate	% inner artificial diet embedding rate	% moisture content	Sensory evaluation score	Quality score
1	65.0 ± 1.2	75.8 ± 1.9	12.2 ± 1.5	60.8 ± 1.6	213.8 ± 2.9
2	63.8 ± 0.6	70.6 ± 0.8	11.6 ± 1.4	64.8 ± 1.8	210.8 ± 1.8
3	75.6 ± 0.8	71.4 ± 1.2	11.2 ± 0.8	71.4 ± 2.3	229.6 ± 2.1
4	81.0 ± 0.7	79.4 ± 2.7	9.6 ± 1.3	66.4 ± 4.1	236.4 ± 4.1
5	67.6 ± 3.3	76.4 ± 2.3	11.2 ± 0.9	60.0 ± 3.6	215.2 ± 5.8
6	63.6 ± 1.1	66.0 ± 0.9	10.0 ± 1.6	63.0 ± 2.1	202.6 ± 3.5
7	76.8 ± 2.1	74.6 ± 1.5	11.6 ± 0.5	75.4 ± 1.7	238.4 ± 1.5
8	64.8 ± 1.1	74.6 ± 3.1	12.8 ± 0.4	66.4 ± 2.4	218.6 ± 3.8
9	65.6 ± 0.9	70.2 ± 2.6	11.4 ± 1.1	71.2 ± 3.3	218.4 ± 3.9
10	63.0 ± 1.4	69.6 ± 3.8	13.8 ± 0.9	69.0 ± 2.3	214.6 ± 4.2
11	65.2 ± 0.9	67.6 ± 3.7	12.0 ± 0.8	74.6 ± 2.6	219.4 ± 4.5
12	63.6 ± 0.7	74.8 ± 3.1	11.2 ± 0.5	68.2 ± 3.5	217.8 ± 5.1
13	63.4 ± 1.8	67.6 ± 2.2	12.0 ± 1.0	70.4 ± 1.4	213.4 ± 4.1
14	72.4 ± 1.4	75.6 ± 1.7	13.8 ± 1.4	62.6 ± 2.4	224.4 ± 3.5
15	61.6 ± 1.4	72.2 ± 3.2	13.4 ± 0.5	66.0 ± 1.6	213.2 ± 6.1
16	73.2 ± 2.4	78.4 ± 1.4	15.0 ± 1.6	67.8 ± 1.7	234.4 ± 6.6

tests in a glass greenhouse at NOYA® Organic Vegetable Production Station (Pinggu county, Beijing, China). Five newly emerged *P. japonica* adults were placed in a plastic Petri dish and maintained for 12 h without food supply. The starved ladybirds and a total of 800 *M. persicae* aphids were simultaneously released into the cage. From that time, we observed the predation of *P. japonica* and counted the residual amount of the aphids every 12 h for a total of 48 h. We repeated the above procedures with the *P. japonica* adults fed on *M. persicae* and liquid artificial diet as control treatments. All the treatments were replicated 10 times.

Statistical analysis

The statistical analysis was performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The average values of all the sets of observed data were calculated as the mean ± SE. Based on the quality scores which were calculated by observed percentages of productivity rate, inner artificial diet embedding rate and moisture content, and the scores of sensory evaluation of ADMs in orthogonal tests, we confirmed the optimal recipes by variance analysis and the most suitable proportions by range analysis of different influential factors (ALG proportion, CHI proportion and the ratio of shell to core). We also analyzed the differences in the total larval developmental time, adult eclosion ratio, the reproductive characters, respiratory quotient, locomotory capacity and predatory capacity (residual amount of aphids in 24 and 48 h) among the various cold-stored ADM treatments and control groups by one-way analysis of variance (ANOVA) at the $P = 0.05$ level.

Results

Optimization of ADMs

A total of 16 groups of ADMs were made and characterized according to productivity rate, inner artificial diet embedding rate and moisture content, and quality scores were integrated (Table 3). The multiple ANOVA analysis of the quality scores showed that the ingredients, CHI proportion and the ratio of shell to core may each significantly affect the quality score of ADMs. Characters ranked in terms of impact on the quality are as follows: CHI proportion > ratio of shell to core > ALG proportion (Table 4). Intensive range analysis indicated that the optimal ADMs were those produced with 1.0% ALG, 0.8% CHI and weight ratio of shell to core of 1 : 2 (Table 5).

Bioassay of P. japonica *fed on optimal ADMs*

Development and reproduction Our results showed there were significant differences in the total larval developmental time of *P. japonica* when fed on newly made ADMs and *M. persicae* aphids (Table 6, $F = 27.2$, df = 4,243, $P < 0.01$). Conversely, the pure liquid

Table 4 Variance analysis of different influential factors according to the quality scores of the artificial diet microcapsule.

Factors	Type III sum of squares	df	Mean of square	F	P
A	349.8	3	116.6	1.092	0.358
B	3071.2	3	1023.7	9.590	< 0.001
C	2462.1	3	820.7	7.688	< 0.001

Table 5 Range analysis of different influential factors according to the quality scores of the artificial diet microcapsule.

Features	Level	Means of influential factors		
		A	B	C
Quality score	1	222.7	215.2	217.6
	2	218.7	213.6	214.2
	3	217.6	222.3	229.9
	4	221.7	229.6	218.9

artificial diet may significantly inhibit the development of *P. japonica* larvae. Although the developmental time was significantly shorter than the liquid artificial diet, ADMs after 7 days of cold storage also showed a negative influence on the development of ladybird compared to other treatments.

Not all larvae fed on ADMs developed into adults under present conditions. The survival of *P. japonica* decreased with the prolonging of the cold storage period (Table 6, $F = 62.1$, df = 4,20, $P < 0.01$). The liquid artificial diet showed the most negative influence on the survival of *P. japonica. P. japonica* did not show significant differences in life span of males and females when they fed on newly made ADMs or aphids (Table 6, $F_{male} = 3.8$, df = 4,109, $P_{male} = 0.01$; $F_{female} = 13.5$, df = 4,64, $P_{female} < 0.01$). We only observed significant prolonged lifespan, both in males and females, when they were fed on the ADMs after 7 days cold storage.

The pre-oviposition across all diets was longer than in the aphid prey treatment (Table 6, $F = 16.9$, df = 4,244, $P < 0.01$). Fresh ADMs showed the same influence on the pre-oviposition duration as did liquid artificial diet, and regressed with increasing of the cold storage period. The various diets influenced the net fertility of females significantly (Table 6, $F = 38.8$, df = 4,244, $P < 0.01$). The fertility of females significantly decreased with the prolonging of the ADM cold storage period, whereas females produced the most viable offspring when they fed on aphids. Furthermore, the liquid artificial diet showed mostly negative impacts on female fertility.

Respiration, locomotion and predation We compared the respiratory quotients of *P. japonica* fed on newly made ADMs, aphid *M. persicae* and liquid artificial diet. The results showed there were significant differences between ADMs and aphid feeding treatments, but both were significantly higher than the liquid artificial diet feeding group (Fig. 2, $F = 7.462$, df = 2,147, $P < 0.01$). The results of *P. japonica* adult creeping speed variation assessed utilizing the Tracksphere system are shown in Figure 3. The creeping speed of ladybirds fed on aphid *M. persicae* were significantly higher than other treatments ($F = 5.492$, df = 2,146, $P < 0.01$). In contrast, the slowest speed was observed with *P. japonica* fed on liquid artificial diet ($P < 0.01$). We did not find any significant differences in the number of remaining aphid prey among various diet treatments (newly made ADMs, *M. persicae* and liquid artificial diet), both at 24 h and 48 h checkpoints (Fig. 4).

Table 6 Developmental and reproductive characteristics of *Propylea japonica* fed on the different diets.

Diets	Total larval duration (days)	Survival ratio (%)	Lifespan (male, days)	Lifespan (female, days)	Pre-oviposition duration (days)	Female fertility
F-ADMs	6.2 ± 0.3 c	87.2 ± 3.9 b	41.3 ± 2.1 ab	46.8 ± 1.3 a	6.4 ± 0.4 bc	277.4 ± 13.3 b
5-ADMs	6.4 ± 0.3 bc	81.6 ± 6.9 c	39.8 ± 2.7 ab	43.2 ± 0.9 ab	7.5 ± 0.2 b	240.8 ± 18.8 b
10-ADMs	7.4 ± 0.4 b	69.2 ± 9.3 d	34.5 ± 1.1 b	38.6 ± 2.3 bc	8.8 ± 0.4 a	154.1 ± 6.4 c
Liquid AD	9.2 ± 0.5 a	58.7 ± 4.2 e	38.7 ± 1.5 ab	35.3 ± 1.2 c	7.1 ± 0.3 b	183.6 ± 5.8 c
M. persicae	5.5 ± 0.4 c	95.5 ± 2.6 a	44.6 ± 1.9 a	47.2 ± 0.9 a	5.4 ± 0.3 c	344.7 ± 11.4 a

F-ADMs, fresh ADMs; 5-ADMs, 5 days cold-stored ADMs; 10-ADMs, 10 days cold stored ADMs; Liquid AD, liquid artificial diet. Letters following the means in the same column indicate significant differences at the level of $P = 0.05$ by ANOVA.

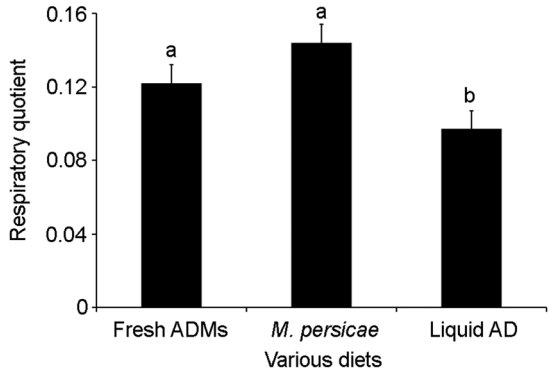

Fig. 2 Respiratory quotient of *Propylea japonica* fed on fresh artificial diet microcapsules, fresh aphid *Myzus persicae* prey and pure liquid artificial diet. The columns and bars represent mean + SE. Letters at the top of the columns indicate significant differences based on the Turkey test ($P < 0.05$). The ADM and AD indicate artificial diet microcapsule and artificial diet, respectively.

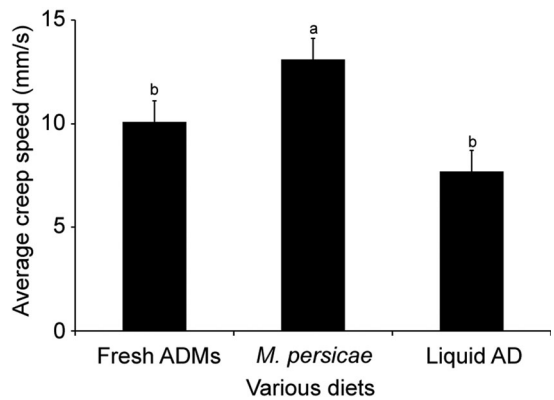

Fig. 3 Locomotory performance of *Propylea japonica* fed on fresh artificial diet microcapsules, fresh aphid *Myzus persicae* prey and pure liquid artificial diet. The columns and bars represent mean + SE. Letters at the top of the columns indicate significant differences based on the Turkey test ($P < 0.05$). The ADM and AD indicate artificial diet microcapsule and artificial diet, respectively.

Discussion

By orthogonal tests, we evaluated the influences of the ALG, CHI proportion and weight ratio of core artificial diets to wall material, on the quality of ADMs, and confirmed the most suitable recipes for production. All three factors showed significant influence on productivity, inner artificial diet embedding rate, moisture content and even sensory evaluation. The concentration of chitosan probably has most impact on the quality of ADMs. As

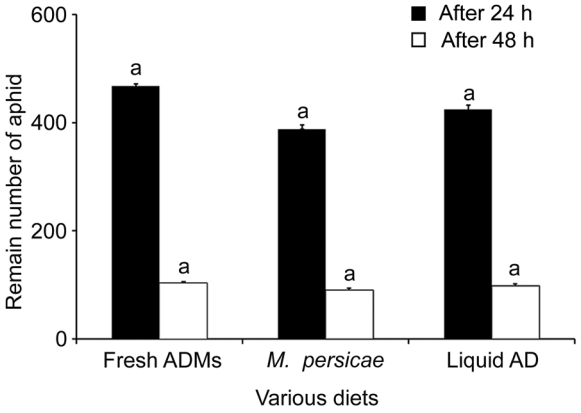

Fig. 4 The number of prey *Myzus persicae* left at 24 h and 48 h of predation by of *Propylea japonica* fed on fresh artificial diet microcapsules, fresh aphid *Myzus persicae* prey and pure liquid artificial diet. The columns and bars represent mean + SE. Letters at the top of the columns in the same color indicate significant differences based on the Turkey test ($P < 0.05$). The ADM and AD indicate artificial diet microcapsule and artificial diet, respectively.

a biocompatible polymer, chitosan has been widely used as a primary wall material in microcapsulation for the packaging of functional proteins for medicine, and as nutritional components of food (Park *et al*., 2005; Peniche *et al*., 2003). Similarly, our results showed in many cases that high concentrations of chitosan in wall materials of microencapsulation enhances the strength stability of the microsphere, helps maintain moisture and increases the encapsulation yield range (Ribeiro *et al*., 1999). In addition, an intermediate level of core to shell ratio in weight showed most influence on the integrity of microcapsules. During the formation of microcapsules, loss of inner liquid diet components could be due to the integrity of wall materials, thus a lower ratio of core to shell may increase the formation speed and decrease the loss of inner liquids (Gouin, 2004). Contrasting with our previous work on the microencapsulated artificial diet for feeding of the flower bug *Orius sauteri*, the calcium alginate concentration did not show significant influence on the quality of the ADMs (Tan *et al*., 2010). Actually, the ratio of Ca-alginate/chitosan plays very important roles in microencapsulation, including the formation speed, rigidity and the standing time (Hari *et al*., 1998). The presence of Ca-alginate results in liquid inner materials forming initially as bead structures, and the chitosan coating may increase the terminal strength of the microcapsules (Murata *et al*., 1993; Krasaekoopt *et al*., 2004). In practice, the size of ADMs may be varied to fit the predatory habits of the insects. Thus, we can form even tiny ADMs by

modulation of the chitosan coating process, which may improve the chemical and mechanical stability of the inner artificial diet bead, and improve the effectiveness of the microencapsulation.

The external status of the artificial diets, including shape, surface color and smoothness, smell and resilience could influence the preferences of the insect consumers, and may cause variation in insect development, and other biological and physiological characters (Hagen, 1987). In the present study, we introduced sensory evaluation, a popular investigative method in food research (Hoppert *et al*., 2012), as one of the key indicators used to optimize the recipes and production conditions of ADMs, although few previous reports are available for comparison. We controlled the size of ADMs to that between 1.5–2.2 mm, which is very close to the body size of aphids. Further, the bright surface color and soft texture may be preferred by the ladybirds during practical mass rearing, compared to ADMs with darker surface color and harder shell texture. Furthermore, a suitable body size and surface structure may aid the ladybirds in holding the ADMs during feeding. Although the components are the most influential factors in artificial diet optimization, the sensory aspects of artificial diet may also affect the feeding efficiency and the terminal productivity of natural enemies, based on our observation. We recommend sensory evaluation of any packaged artificial diets is examined in further studies.

We observed significant advantages in development and reproduction of *P. japonica* fed on ADMs compared to liquid artificial diets. The microcapsule materials (Ca-alginate and chitosan) do not provide any nutrition to the insects directly, thus the differences clearly suggest that it is the microencapsulated packaging that benefits food quality. In contrast to the results herein, the flower bug *O. sauteri* showed a longer developmental period, especially in 1–2 instar nymphs (Tan *et al*., 2010). Despite the influences of variation in artificial diet components, this could be attributed to the differentiation of mouthparts, which are either of the chewing or sucking type. In fact, even though preying on natural arthropods, the predator preferences of insects with various mouthparts are influenced by the specific physiological and morphological adaptations of prey (Coll & Guershon, 2002). Within coccinellidae species, the variation in structure of chewing mouthparts strongly influences the prey type (Samways *et al*., 2010). In the present study, preference of the artificial diet microcapsule in a compact capsule wall structure is higher in the predators with chewing mouthparts. This also points to the requirement of adjusting components and their concentrations to fit the particular morphology and behaviors of different predators.

Furthermore, we found extending of the cold-storage period decreased the effectiveness of ADMs in terms of survival ratio, lifespan of adults and reproductive efficiency of *P. japonica*, although the feeding effectiveness of ADMs with 10 days cold storage was higher than with the liquid artificial diet. The results suggested that the cold-stored microencapsulated artificial diet may meet the nutritional demands for basic development, but does not provide sufficient nutrition for sexual maturation. A few publications have mentioned that environmental temperature influences the quality of microcapsules. Microcapsules of smaller size could withstand the stress from the slurry flow, and the volumetric expansion/contraction of phase change with undulating temperature (Yamagishi *et al*., 1996). Long-term cold storage may induce dissociation of the wall material of microcapsules and loss of inner materials (Chen *et al*., 2009). Intuitively, the addition of antifreeze may prevent negative impacts of freezing, but there are no studies on this. Thus we intend to assess the introduction of food-grade nontoxic coolants in improving the performance of artificial diet microcapsules during cold storage. This could reduce the costs of corruption and contamination of fresh ADMs, increasing the value of the microcapsules in commercial transportation and application by prolonging shelf life. Generally, we must be concerned both with the development and fertility of mass rearing populations if we are to keep consistently high productivity for commercial biocontrol applications. Based on our results, it may be more efficient using cold-stored ADMs to feed young larvae, switching to fresh ADMs for mature larvae and adult ladybirds.

The effectiveness of practical application of reared biological control agents requires evaluation in many characteristics of physiology and behavior (Boller & Chambers, 1977; van Lenteren, 2003). Similar respiratory quotients were observed in fresh ADMs and aphid feeding treatments, and both were significantly higher than the ladybirds fed on liquid artificial diet. Respiration performance should be considered an external signal of internal metabolism and nutrient assimilation (Buck, 1962), and could influence development and sexual maturity, since the higher respiration performance brought by microcapsule packaged artificial diet may increase the metabolic rate of *P. japonica*, as observed with *O. sauteri* (Tan *et al*., 2010; Tan *et al*., 2013). Moreover, high locomotory capacity is indicative of superiority of prey and mate searching ability of insects (Matthews & Matthews, 2010). We observed lower average creeping speed of *P. japonica* fed on ADMs than those fed on fresh aphids, but higher than those using liquid artificial diet. The results indicated some disadvantages of ADMs on the dispersal and field colonization in augmentative biological control

application of *P. japonica*, while not inhibiting mass rearing efficiency. The variation of artificial diet ingredients may influence the locomotory ability and field dispersal of *O. sauteri* (Tan *et al*., 2013). We were only concerned with one artificial diet recipe here, so the intensive optimization of artificial diet ingredients based on locomotory status should be performed in future studies. Furthermore, we did not find significant differences in preys consumption among the ladybirds fed all fresh ADMs, fresh aphids and liquid artificial diet at either 24 h or 48 h. It was most important that the microcapsule packaging did not reduce the pest control ability of the ladybirds produced by mass rearing. Actually, the characters of locomotory propensity and prey consumption are subject to tight quality control for mass rearing (Chambers, 1977). In general, extending the evaluation of feeding effectiveness of the biocontrol agents should relate to most characteristics, in practical biological control applications.

In conclusion, our study introduces a new type of microencapsulated artificial diet for mass rearing of predatory ladybirds. The results of the present research showed more suitability of the microencapsulated artificial diet compared to a liquid form of the diet in feeding predatory ladybirds. The microcapsule packaged artificial diets may benefit *P. japonica* development, reproduction and other biological or physiological performances, in a way similar to fresh aphid prey. Our results promote the further use of microencapsulated artificial diet in mass rearing of predatory ladybirds, and may help in improving the feeding efficiency and conservation quality of artificial diets in commercial practices.

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Disclosure

XLT, SW and FZ all contributed to experimental design; XLT and SW executed the experimental processes and data calculation; FZ did the statistical analysis and composition of figures. XLT and SW composed the manuscript. The authors declare that they have no conflict of interests.

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