Life History Parameters of *Ceratitis capitata* (Diptera: Tephritidae) Reared on Liquid Diets

CHIOU LING CHANG,[1] CARLOS CACERES,[2] AND SUNDAY EKESI[3]

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ABSTRACT A liquid diet for rearing *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) was developed. Several yeast-based products were evaluated as diet ingredients, and a combination of whole cell yeast (LBI2240) and hydrolyzed yeast (such as FNILS65) in 1:1–3:1 ratio was selected for use in the study. Larvae reared in a liquid diet with LBI2240:FNILS65 ratios of either 1:1 or 3:1 resulted in the same pupal recovery as to those reared in the conventional mill fee- based control diet. Larvae reared in a liquid diet with whole cell yeast only yielded the lowest pupal production, whereas other parameters such as adult emergence, adult fliers, pupal weight, egg production, and percentage of egg hatch were not affected. In diets fortified with vitamin-fortified yeast (RDA) or glutamine-rich yeast (GSH), there was no enhanced effect on insects, whereas some detrimental effects were observed in some of the parameters measured. A whole cell yeast to hydrolyzed yeast ratio of 3:1 was found to be the most suitable based on quality control parameters measured and cost-effectiveness. This is recommended for use in liquid diets targeting *C. capitata* mass rearing because the cost of whole cell yeast was 5 times cheaper than that of hydrolyzed yeast. This study also demonstrates that it is possible to rear *C. capitata* in a liquid diet at a similar cost to mill feed diet and still maintain the same fly quality as that from the conventional control diet.

KEY WORDS hydrolyzed yeast, whole cell yeast, *Ceratitis capitata*, liquid diet

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most destructive pest of fruits and vegetables worldwide (Vargas et al. 1984, 1994, 1996). It has caused significant losses to crops and marketing opportunities due to plant damages caused by fruit fly infestations and quarantine restrictions imposed by importing countries to prevent its entry and establishment. It has become economically apparent in the horticultural global market economy. A team of United States Department of Agriculture–Agricultural Research Service (USDA–ARS) and University of Hawaii scientists has been implementing an areawide pest management (AWPM) program to suppress economically important tephritid fruit fly populations in Hawaiian islands since 2000, and the technologies developed within the program also have been transferred to many parts of the world that share the same fruit fly problems. At the core of the transfer of technology process of the AWPM is the need to have well-established and cost-effective methodologies to rear high-quality fruit flies and their parasitoids. Currently, fruit fly larvae are reared on a modified Tanaka's conventional solid diet that contains mill feed as a bulking agent in addition to other ingredients, such as yeast-based products, sugar, antimicrobial agents (nipagen, sodium benzoate, and streptomycin), and water (Tanaka et al. 1969). Fruit fly performance from rearing larvae on Tanaka's diet has been satisfactory. However, use of this diet is costly in terms of labor, rearing area, environment control, and spent diet management. It also can be environmentally unfriendly in terms of spent diet disposal. To achieve cost-effective goals and to eliminate these disadvantages, there have been recent advances in the development of alternative methods of rearing. A team in the ARS of the USDA in Hawaii has developed a low-maintenance larval diet for tephritid fruit flies (Chang et al. 2004, 2006). The value of this diet lies in its liquid form and the fact that the mill feed bulking agent can be replaced with an inert sponge cloth. Research into optimization of rearing methodology is a key step toward mass production of the flies. In the optimization process, we are currently exploring the applicability of the liquid diet as an alternative to the traditional solid diet in the mass rearing of *C. capitata.*

Some of the major constraints to the development of liquid artificial diet include development of mold and availability and high cost of yeast-based products. In an earlier phase of developing the liquid diet for rearing fruit flies, mold formation was unavoidable, especially for *C. capitata* rearing, and microbial contamination in the diet is thought to occur through the

[1] Corresponding author: U.S. Pacific Basin Agricultural Research Center, USDA–ARS, 2727 Woodlawn Dr., Honolulu, HI 96822 (e-mail: stella.chang@ars.usda.gov).

[2] Entomology Unit, FAO/IAEA Agriculture and Biotechnology Laboratory, A-24444 Seibersdorf, Austria.

[3] International Center of Insect Physiology and Ecology (ICIPE), P.O. Box 30772–00100 GPO, Nairobi, Kenya.

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Table 1. Composition of the general liquid and mill feed diets for a tray in grams and percentage for rearing larvae of *C. capitata*

Ingredient	Liquid diet			Mill feed diet		
	g	%	Cost (US$)	g	%	Cost (US$)
Sugar	121.80	8.82	0.15834	182.70	12.18	0.23751
Yeast[a]	204.00	14.78	1.22400			0
Torula yeast			0.00000	51.00	3.40	0.14025
Nipagen	2.00	0.14	0.00409	3.00	0.20	0.00614
Sodium benzoate	2.00	0.14	0.00506	3.00	0.20	0.00759
Streptomycin	1.50	0.11	0.08935	2.25	0.15	0.13402
Citric acid	40.00	2.90	0.11894	34.65	2.31	0.10303
Mill feed	-		0.00000	394.50	26.31	0.23276
Water	1000.00	72.45		828.30	55.24	
Sponge cloth (reusable)			0.17231			
Wheat germ oil (specific gravity 0.91)	9.10 (=10 ml)	0.66	0.15470			
Total cost per tray (US$)			1.92679			0.86129

[a] This is the amount of yeast for the generic liquid diet. In total, 25 different diets composed of different protein/yeast sources and combinations were evaluated. For specific liquid diet formulations, see Table 2.

fly's ovipositor or its eggs. A large majority of the larvae often died after reaching the third instar. Lowering the pH of the medium from 3.5 to 3.0 did not solve the mold problem, but improvement of environmental conditions and sanitizing of trays significantly reduced contamination. In addition, it was often difficult to obtain brewer's yeast readily, making it essential to identify locally available substitutes to advance diet-rearing technology. Therefore, in this study, we evaluated various alternative yeast-based products and their combinations using the "Chang liquid diet" formulation (Chang et al. 2004, 2006) in an effort to overcome the constraints of microbial contamination and poor supply of brewer's yeast. We report here on the life history parameters and performance of standard bisexual *C. capitata* strain reared using one or a combination of different yeast-based products in a liquid diet.

Materials and Methods

Insects. One-hour-old eggs of standard bisexual *C. capitata* strain were obtained from the fruit fly rearing laboratory, ARS, Pacific Basin Agricultural Research Center (PBARC) in Honolulu, HI. The flies had been reared on a wheat-based artificial mill feed diet for >360 generations (Table 1) (Vargas et al. 1984, 1994, 1996).

Diet Formulation. The liquid diet was composed of one or several yeast-based products, including whole cell yeast (LBI2240) and hydrolyzed yeast (FNI LS65 and FNI200), vitamin-fortified yeast (RDA), and glutamine-rich yeast (GSH) (Lallemand, Inc., Montreal, QC, Canada), sugar (C & H Sugar Company, Inc., Crockett, CA), sodium benzoate (Noveon Kalama, Inc. Kalama, WA), Nipagen (Sharon Laboratories Ltd. Ashdod, Israel), citric acid (Univar Products Corp., Kirkland, WA), agricultural streptomycin (Nufarm Americas Inc., Burr Ridge, IL), wheat germ oil (WGO) (MP Biomedicals, Aurora, OH), and water (Table 1). The mill feed diet formulation currently used at PBARC served as the control diet (Chang et al. 2004). Initial pH values for both diets were 3.5.

Diet Preparation and Delivery System. The liquid diet and delivery system used in this study was adopted from the diet composition and delivery system for *Bactrocera dorsalis* (Hendel) developed by Chang et al. (2004, 2006). A series of recommended rearing components for *B. dorsalis* are as follows: tray, lid of pupation fiberglass box; seeding egg density, 2.5 ml per tray; diet volume, 1,250 ml; screen, coarse; water quality, distilled water; and additives, 0.66% WGO (Chang et al. 2006). Liquid diet tests were conducted using large fiberglass trays (49.7 by 32 by 2.5 cm^3) (Stack and Nest, LEWI Systems, Watertown, WI) (Chang et al. 2004, 2006). The diet was prepared as described in Chang et al. (2004, 2006) with the following modifications. The diet mixture was formulated by weighing all the dry ingredients and blending (≈2 min) in a 1,000 ml electronic blender (Smoothie plus 600, Back to Basics, Inc., Bluffdale, UT) with 1,000 ml of distilled water until the diet ingredients were fully dissolved and homogeneous. Sponge cloth (Kalle USA Inc., Flemington, NJ) was cut into 25- by 30-cm and 15- by 10-cm rectangles to fit the fiberglass tray, rinsed in cold distilled water, and wrung dry to remove any impregnated germicides or starch. Each side of the sponge cloth had a different surface pattern; one side had a diamond-shaped pattern surface and the other was patterned with grooves. The large sponge cloth, with grooved pattern facing up, served as the primary support matrix for feeding larvae and this was lined on the top by a hardware nylon net (11 by 17 cm; 0.1-cm mesh, Home Depot, Honolulu, HI), and together these lay on the bottom of the tray. Trays were disinfected with 10% Clorox (The Clorox Company, Oakland, CA) and rinsed with distilled water. Diet (1,250 ml) was added to each box containing a 25- by 30-cm sponge and a sheet of screen underneath to increase ventilation area. The sponge was flipped to saturate it with diet whenever necessary. Eggs (2.5 ml; ≈20,000 eggs per ml) were disinfected with 1% Clorox premeasured in a 10-ml cylinder and applied to each 15- by 10-cm sponge by using a plastic pipette. The eggs were sprayed gently with distilled water to spread them evenly across the sponge surface. The sponge was then placed on top of the diet-saturated larger sponge. Each tray was placed inside a plastic underbed box and covered with a screened lid to incubate at ambient temperature (25°C), 65% RH, and a pho-

Table 2. Relative proportion of the different yeast-based protein sources used for each liquid diet tested and cost per recipe

Yeast-based product	LBI2240	FNILS65	FNI200	FNI210	RDA500	GSH	Beer yeast	Brewer's yeast	WGO	% Pupal recovery	Cost ($)[a] per recipe
2240W	100								√		1.31
224065W	50	50							√		2.54
224065RW	50	45			5				√	50.0	2.54
224065GW	50	30				20			√		2.54
224065RGW	50	25			5	20			√		2.54
224065	25	75									3.00
224065W	25	75							√		3.15
224065	50	50									2.38
224065	75	25									1.77
224065W	75	25							√		1.93
200W			100						√	8.8	3.76
210W				100					√	0	3.76
65W		100							√	0	3.76
Korea W							100		√	0	1.31
Brewer's W								100	√	24.6	2.54
200RW			95		5				√		3.64
200GW			80			20			√		3.27
200RGW			75		5	20			√		3.15
210RW				95	5				√		3.64
210GW				80		20			√		3.27
210RGW				75	5	20			√		3.15
65RW		95			5				√		3.64
65GW		80				20			√		3.27
65RGW		75			5	20			√		3.15

[a] Costs posted here are for small quantity purchases. Costs should be cheaper when purchasing in bulk.

toperiod of 12:12 (L:D) h. When the larvae were ready to leave the tray (prepupal stage), 1,000 ml of vermiculite was added into each box for pupation medium. Pupae were collected from the vermiculite for at least five consecutive days after larvae "popped" out of the diet and into the pupation medium. Pupal weights were obtained 1 d after collection.

Experimental Procedures. Larvae of *C. capitata* were reared in the same diet formulation with a variety of yeast-based products as ingredients. In total, 17 yeast-based products and their combinations were tested. These products included brewer's yeast (Brau union Osterreich AG, Seibersdorf, Austria), beer yeast (Beer yeast Korea Inc., Seoul, Korea), and LBI2240, FNI200, FNI210, FNI LS65, FNI200+RDA, FNI200+GSH, FNI200+RDA+GSH, FNI210+RDA, FNI210+GSH, FNI210+RDA+GSH, FNI LS65+RDA, FNI LS65+GSH, FNI LS65+RDA+GSH, LBI2240+FNI LS65+RDA, and LBI2240+FNI LS65 (Lallemand, Inc.). Evaluations of diets were based on the parameters outlined below. RDA and GSH were used at 5 and 20%, respectively, in all the combinations. Combinations of LBI2240 and FNI LS65 were tested in ratios of 1:3, 1:1, and 3:1 (Table 2).

All the physical parameters (such as egg density/diet volume, egg density/tray size), food quality and nutritional parameters (water quality, amount of yeast-based products, and additives), and a biological parameter (generations) were adopted from Chang et al. (2006). After preliminary identification of the best yeast-based products, additional study evaluated the best ratios of each yeast combination among the selected yeast-based products.

Life History Traits. *Pupal Recovery.* For each diet treatment, pupae were collected for at least five consecutive days after larvae popped out of the diet and into the vermiculite pupation medium. The total mass (milligrams) of all puparia collected was obtained, and four sets of 100 puparia each were weighed to obtain a mean weight (milligrams) per 100 puparia. Total pupal yield (number of pupae) was estimated by dividing total pupal weight by the mean weight from the four sets of 100 puparia and then multiplying by 100. Percentage of pupal recovery was calculated as 100 times total pupal yield divided by total eggs times parental percentage of egg hatch. Parental percentage of egg hatch was calculated as follows: after cleansing the eggs using 1% Clorox and rinsing with distilled water, four sets of 100 eggs each were seeded on green blotting paper. The numbers of eggs that did not hatch from each 100 eggs after 4 d were counted and recorded. To calculate mean percentage of egg hatch, the number of eggs that did not hatch was subtracted from 100 and then multiplied by 100.

Larval Duration. Larval duration (days) was estimated by multiplying pupal recovery (in milligrams) from each "pop day" by its developmental age (in days from egg to pop day) and then dividing the sum of the products from each pop day by total pupal production (in milligrams). This measure, adopted from Snedecor and Cochran (1967), yields peak larval developmental duration (days).

Pupal Weight. Pupae from each daily collection from vermiculite were weighed 1 d after pupal collection. Pupal weight (milligrams) from each daily collection was estimated by dividing total pupal weight by the mean weight of four sets of 100 puparia, as described above, and then multiplying by 100.

Adult Fliers. This was tested using a slight modification from Boller et al. (1981) as described below.

Pupae from the largest daily pupal collection were randomly selected and four lots of 100 pupae were taken and each placed in a sauce cup inside a petri dish cover for later use. A tube of black plastic ABS-pipe (20 cm in length and 8.5 cm in diameter, City mill, Honolulu, HI), coated with talc powder (Hawaii Chemical Co., Ltd., Honolulu, HI) was placed over the dish. Pupae were placed inside the sauce cup (1 cm in diameter) inside the tube to allow for emerging adults to expand their wings upon emergence. The tubes and petri dish with pupae were placed inside a lighted flight cage (120 by 120 by 60 cm) without food or water, and they were left until all of the flies had emerged and died, usually 24 d after egg seeding or 4 d after adult emergence. Flies were placed into one of the following five categories: unemerged, partially emerged (part of adult still attached to the puparium), deformed wings (emerged but have deformed wings), nonflying individuals (flies look normal but are not capable of flying), and flies capable of flying. The numbers of flies in each category was recorded. Adult emergence was calculated as 1) the total initial number of puparia (100) minus the average percentage of unemerged puparia or 2) total number of flier, nonflier, and deformed wing. Total flying adults was calculated as the initial number of pupae (100) minus total number of nonflying individuals, partially emerged, unemerged, and deformed wing (Boller et al. 1981).

Egg Production. Twenty grams of pupae were placed in a plastic cup (9 cm in top diameter, 4.5 cm in height, and 3 cm in bottom diameter). Cups were placed individually inside metal egging cages (27 by 25 by 24 cm) with a 3:1 (wt:wt) mixture of sugar to yeast hydrolysate, respectively. A covered plastic container of water with a wick was added inside each egging cage on the day that the first flies emerged. Collection of eggs started at the sixth day after fly emergence. Egg volumes in ml were recorded for each cage for seven consecutive days. Three replicates (i.e., cages) were maintained for each diet.

Egg Hatch. Eggs collected on the first day were used for the percentage of egg hatch assessment. Four sets of 100 eggs from each individual cage were sampled. The numbers of unhatched eggs were recorded 4 d later. The percentage of egg hatch was calculated by subtracting the number of unhatched eggs from 100.

Adult Emergence/Sex Ratio. On the same day that the 20-g lots of pupae were weighed for use in egg production estimates, four lots of 100 pupae each were counted, weighed, and placed into screened plastic containers (8 cm in diameter). Adults were allowed to emerge and die, after which sex ratio and percentage of adult emergence for each lot was determined.

Mating Test. Newly emerged adults were sexed, placed in screened cages, and provided with food and water until ready to be used. Each sex was placed in a separate room. Mating tests were conducted using 6-d-old flies, and the experiments were held both inside the laboratory using acrylic insect cages (30 by 30 by 40 cm) and in field cages outside the laboratory (Rendon et al. 2004). One hundred pairs of flies were released into the clear acrylic cage or field cage at dawn ≈0700 hours. Mating pairs were removed from the cage. Total numbers of mating pairs were tallied and recorded at the end of a test at 0900 hours. Four replicates of each treatment were conducted.

Data and Statistical Analysis. The criteria used for evaluation of the liquid diet and its delivery system were larval duration, pupal recovery (number of pupae produced from number of seeded eggs, expressed as percentage), pupal weight, adult emergence, mating, percentage of fliers, egg production of subsequent generation, and egg hatch as described by Chang et al. (2006) and FAO/IAEA/USDA (2003). Data in Tables 4 and 5 are presented as mean ± SE. Values were obtained from at least four batches of each diet treatment evaluated. Differences among diets were determined by analysis of variance (ANOVA), and means were separated using Tukey's test at $\alpha = 0.05$ (Sigma Stat, version 3.5, Systat Software, Inc. 2006).

Results and Discussion

Of the 25 different diets tested, there was no larval development in diets containing FNI LS65, FNI210, or beer yeast alone. This may be due to the high protein in both FNILS65 (55%) and FNI210 (67.50%). On other diets, overall development was 8.8% from FNI200 series (45.5% protein), 24.60% from brewer's yeast, and 50% from LBI2240+LS65+RDA (75:20:5) (224065R) (47.55% protein) in comparison with individuals reared on the conventional diet. Only diets containing LBI2240 recorded good yields (Table 2). This is probably because diet of 224065R contains more fats (2.98%), calories, and carbohydrates and less protein, vitamins (117.79 mg/100 g), and minerals (1741.38 mg/100 g) than FNI LS65, FNI200, FNI210, and brewer's yeast (Table 3) (Chang and Vargas 2007). Thus, further tests were concentrated on diets using LBI2240 yeast with or without other yeast sources (LBI2240 series).

The yeast-based products of LBI2240 series included LBI2240 (100%), LBI2240+LS65 (50:50), LBI2240+LS65+RDA (50:45:5), LBI2240+LS65+GSH (50:30:20), and LBI2240+ LS65+RDA+GSH (50:25:5:20) with addition of wheat germ oil (W) to each. Abbreviations of each diet are later referred to as 2240W, 224065W, 224065RW, 224065GW, 224065RGW according to the order listed above (also see Table 2). The results from this series of experiments showed that 224065W exhibited the best performance in comparison with the results obtained from the mill feed control diet (Table 4).

Pupal recovery from larvae reared in diets 224065W, 224065RW, and 224065GW showed no significant differences from those reared in the control diet. However, 224065W recovered the most pupae, followed by 224065RW, 224065GW, 224065RGW, and 2240W diets (Table 4). Both RDA and GSH did not seem to have any effect and their combination, 224065RGW diet, had instead a reverse effect on pupal recovery. This may be because of an overdose of B-complex and glutamine, because RDA is an inactivated dried whole cell yeast containing elevated levels of B-complex vi-

Table 3. Total nutritional compositions of yeast of FNI LS65, FNI200, FNI210, LBI2240, RDA, GSH, Brewer's yeast, and 224065R

	FNI LS65	FNI200	FNI210	LBI2240	RDA500	GSH	Brewer's	2240:65 (1:3)	2240:65 (1:1)	2240:65 (3:1)	224065R
Typical data											
Moisture (%)					5.50	5.00					
Protein (%)	55.00	45.50	67.50	45.40	50.00	44.80		52.60	50.20	47.80	47.55
Sodium chloride (%)	<2	<3	<3								
Fat (%)				3.70	4.00	4.50		0.93	1.85	2.78	2.98
Ash (%)				4.70	6.70	7.00		1.18	2.35	3.53	3.86
Carbohydrates, calculated (%)				41.90	31.70	38.80		10.48	20.95	31.43	33.01
Calories (per 100 g)						375.00					
pH	5.2–6.2	4.9–5.3	5.8–6.5	5.9–6.3							
Dietary fiber						23.90					
Soluble						1.90					
Insoluble						22.00					
Amino acids (g/100 g protein)											
Total	56.20	89.30	88.90	100.20	99.50		24.76	67.20	78.20	89.20	91.37
EAA	25.60	53.30	58.00	46.50	45.30		20.76	30.83	36.05	41.28	42.26
NEAA	30.60	36.00	30.90	53.70	54.20		4.00	36.38	42.15	47.93	49.11
Vitamins (mg/100 g)											
Total	200.48	320.80	156.97	65.14	576.80		501.89	166.65	132.81	98.98	117.79
Minerals (mg/100 g product)											
Total	6,735.32	6,285.07	3,934.50	304.60	3,317.30		24.35	5,127.64	3,519.96	1,912.28	1,741.38
Fat (%)						4.5					
Saturated						1.2					
Unsaturated						3.3					
Trans-fatty acids						0.2					

This table is a summary from United States-Canadian Tables of Feed Composition, 3rd revision, National Academies Press, Washington, DC. 1982. EAA, essential amino acids; NEAA, nonessential amino acids.

tamins, and GSH is an enriched yeast with glutamine (Cohen 2004). Therefore, GSH and RDA were deleted from subsequent tests.

Larval durations for larvae reared in the 2240W, 224065W, 224065RW, or 224065GW diets were not significantly different from that of larvae reared in control diet (Table 4). However, larvae reared in 224065RGW recorded the longest developmental time, and this was significantly higher than that for larvae in control diet (Table 4).

There were no significant differences in pupal weight, adult emergence, adult flier, egg production per female per day, and egg hatch among diets in the LBI2240 series and the mill feed (control) diet (Table 4).

From the results, it was concluded that 224065W diet without additives such as GSH or RDA had the best performance. Therefore, different ratios (1:3. 1:1, and 3:1) of LBI2240 and FNI LS65 with or without WGO in the diet were further evaluated to determine the best combination of these two yeast-based products.

Without wheat germ oil in the diet, larvae reared in the diet containing LBI2240 and FNI LS65 at a 3:1 ratio yielded the same pupal production as those from the control diet. The other two ratios (1:3 and 1:1) without wheat germ oil produced significantly fewer puparia than did the control diet. With wheat germ oil in the diet, both 1:1 and 3:1 produced the same pupal recoveries, whereas 1:3 produced significantly fewer puparia than did those reared in the control diet (Table 5). Larvae reared in the 1:3 diet with wheat germ oil produced more pupae than that without wheat germ oil, whereas wheat germ oil did not seem to affect pupal recovery from the 3:1 diet (Table 5).

Larval durations for larvae reared in the diet of 1:3, 1:1, and 3:1 with wheat germ oil were not significantly

Table 4. Comparative performance of *C. capitata* reared in five liquid diets (LBI2240, LBI2240+FNI LS65, LBI2240+FNI LS65+RDA, LBI2240+FNI LS65+GSH, and LBI2240+FNI LS65+RDA+GSH) and a control diet (Mill feed diet)

Parameter[a]	Yeast combinations in liquid diet[b]					Mill feed	ANOVA
	2240W	224065W	224065RW	224065GW	224065RGW		
PR (%)	25.21 ± 8.31c	67.70 ± 4.75a	64.21 ± 2.58a	52.09 ± 7.85ab	40.52 ± 10.37bc	69.60 ± 3.27a	$F = 6.67$; df = 5, 23; $P = 0.0011$
PR/C (%)	37.03 ± 13.34c	98.21 ± 9.36a	92.89 ± 5.84a	74.51 ± 10.09ab	58.05 ± 15.21bc	100.00 ± 0.00a	$F = 6.06$; df = 5, 23; $P = 0.0019$
LD (d)	10.77 ± 0.12ab	10.67 ± 0.30ab	10.83 ± 0.15ab	10.84 ± 0.17ab	11.15 ± 0.16a	10.04 ± 0.59b	$F = 1.52$; df = 5, 23; $P = 0.2317$
PW (mg)	7.74 ± 0.31a	7.30 ± 0.14a	7.20 ± 0.12a	7.70 ± 0.35a	7.65 ± 0.31a	6.90 ± 0.30a	$F = 1.54$; df = 5, 23; $P = 0.2284$
AE (%)	84.44 ± 9.39a	98.75 ± 0.20a	97.31 ± 0.87a	98.50 ± 0.65a	97.31 ± 0.50a	88.25 ± 5.12a	$F = 1.96$; df = 5, 23; $P = 0.1344$
AF (%)	89.71 ± 2.65a	91.70 ± 1.38a	91.45 ± 2.12a	79.59 ± 13.01a	87.07 ± 3.98a	97.42 ± 0.81a	$F = 1.06$; df = 5, 23; $P = 0.4156$
EFD	21.14 ± 4.84a	18.00 ± 4.10a	18.71 ± 2.38a	22.04 ± 6.53a	20.25 ± 4.08a	17.60 ± 5.94a	$F = 0.14$; df = 5, 17; $P = 0.9801$
EH (%)	89.96 ± 1.32a	91.67 ± 1.82a	90.54 ± 1.79a	91.63 ± 1.15a	90.63 ± 1.02a	93.58 ± 0.65a	$F = 0.90$; df = 5, 35; $P = 0.4947$

Within a row, means followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey's honestly significant difference [HSD] for mean separation).

[a] PR, pupal recovery; PR/C, pupal recovery/control; LD, larval duration; PW, pupal weight; AE, adult emergence; AF, adult fliers; EFD, eggs per female per d; EH, egg hatch.

[b] 2240W, LBI2240+WGO; 224065W, LBI2240+FNI LS65+WGO; 24065RW, LBI2240 +FNI LS65+RDA+WGO; 224065GW, LBI2240+FNI LS65+GSH+WGO; 224065RGW, LBI2240+FNI LS65+GSH+RDA+WGO. Mill feed represents control diet.

Table 5. Performance of *C. capitata* reared in six combinations of liquid diets (LBI2240+LS65 in 3:1, 1:1, 1:3, both with and without addition of wheat germ oil) compared with those from control diet (Mill feed diet)

Parameter[a]	Yeast combinations in liquid diet[b]					Mill feed	ANOVA
	224065 (1:3)	224065W (1:3)	224065 (1:1)	224065W (1:1)	224065 (3:1)	224065W (3:1)	
PR (%)	2.47 ± 0.34c	37.23 ± 15.66b	39.08 ± 3.34b	63.67 ± 3.66ab	55.88 ± 7.32ab	59.78 ± 4.45ab	84.56 ± 1.95a	$F = 13.60$; df = 6, 20; $P < 0.0001$
PR/C (%)	2.93 ± 0.46c	43.97 ± 18.78bc	46.17 ± 3.53b	75.49 ± 5.49ab	66.53 ± 10.07ab	71.00 ± 6.79ab	100.00 ± 0.00a	$F = 12.20$; df = 6, 20; $P < 0.0001$
LD (d)	12.51 ± 0.17a	11.09 ± 0.29bc	11.75 ± 0.14ab	10.61 ± 0.20c	11.31 ± 0.04bc	10.79 ± 0.09c	8.88 ± 0.09d	$F = 47.21$; df = 6, 20; $P < 0.0001$
PW (mg)	5.49 ± 1.09b	7.66 ± 0.18a	7.96 ± 0.14a	7.41 ± 0.18a	7.17 ± 0.17a	7.19 ± 0.19a	7.26 ± 0.36a	$F = 3.00$; df = 6, 20; $P = 0.00425$
AE (%)	93.00 ± 1.80a	98.42 ± 0.22a	99.00 ± 0.14a	99.42 ± 0.17a	99.67 ± 0.22a	99.00 ± 0.14a	98.50 ± 1.04a	$F = 8.31$; df = 6, 20; $P = 0.0006$
AF (%)	56.67 ± 14.22bc	72.86 ± 11.23abc	49.21 ± 9.36c	67.78 ± 6.90abc	49.67 ± 10.80c	80.26 ± 6.98a	89.41 ± 6.70a	$F = 2.48$; df = 6, 20; $P = 0.0754$
Mating (%)	NA	76.33 ± 4.63a	70.37 ± 5.28a	71.01 ± 11.05a	73.33 ± 4.33a	59.92 ± 12.28a	74.41 ± 6.78a	$F = 0.52$; df = 5, 17; $P = 0.7552$
EFD	NA	24.64 ± 6.50a	14.61 ± 0.94c	26.00 ± 2.35a	16.10 ± 0.10bc	28.52 ± 0.86a	29.25 ± 2.29a	$F = 4.37$; df = 5, 17; $P = 0.0170$
EFDC (%)	NA	74.11 ± 25.59abc	51.27 ± 4.39c	90.82 ± 13.38a	55.71 ± 4.35bc	9.12 ± 10.76a	100.00 ± 0.00a	$F = 2.82$; df = 5, 17; $P = 0.0657$
EH (%)	NA	92.08 ± 0.34a	83.31 ± 2.70b	92.00 ± 0.59a	88.67 ± 1.86a	91.53 ± 0.41a	90.75 ± 0.17a	$F = 6.06$; df = 5, 17; $P = 0.005$

Within a row, means followed by the same letter are not significantly different ($\alpha = 0.05$; Tukey's HSD for mean separation).

[a] PR, pupal recovery; PR/C, pupal recovery/control; LD, larval duration; PW, pupal weight; AE, adult emergence; AF, adult fliers; EFD, eggs per female per d; EFDC, eggs per female per d per control; EH, egg hatch.

[b] 224065, LBI240+LS65; 224065W, LBI2240+LS65+wheat germ oil. 3:1, 1:1, and 1:3 represent the ratio of LBI2240 and LS65 in wt:wt. Mill feed represents control diet. NA, pupal recovery was too low to analyze indicated parameters.

different from each other, but they were significantly slower than those reared in the mill feed control diet (Table 5). Wheat germ oil speeded up development in the 1:1 and 1:3 diet, but not in the 3:1 diet. Larvae reared in 1:3 and 1:1 diets without wheat germ oil developed significantly slower than larvae reared in the corresponding diets with wheat germ oil (Table 5).

Pupal weights from larvae reared in the 1:3, 1:1, or 3:1 diets were the same except for those reared in the 1:3 diet without wheat germ oil, where pupal weight was significantly lower than for other treatments (Table 5). Percentage of adult emergence values were similar among all diet treatments (Table 5).

Adult fliers and percentage of mating were not significantly different among 1:3, 1:1, and 3:1 diets with or without wheat germ oil. There were not enough emerging adults from puparia whose larvae were reared in the diet of 1:3 without wheat germ oil to perform the mating, egg production, and egg hatch test (marked as NA in Table 5).

Egg production from adults that were reared as larvae in the 1:3, 1:1, and 3:1 diets with wheat germ oil did not differ significantly from that of adults resulting form the control diet. However, egg production from adults whose larvae were reared in 1:1 and 3:1 diet without WGO was significantly lower that for adults reared in the same diet with WGO. Too few adults emerged from pupae whose larvae were reared in the 1:3 diet without wheat germ oil to evaluate egg production (Table 5).

Egg hatch from adults that were obtained from larvae reared in the 1:3, 1:1, or 3:1 diets with WGO did not differ significantly from each other or from those reared in the control diet. However, egg hatch from adults whose larvae were reared in the 1:1 diet without WGO was significantly lower than that of individuals reared in the same diet with WGO and the control diet. These findings agree with our previous results with regard to the beneficial effect of WGO in liquid diet (Chang and Vargas 2007). As mentioned, there were too few adults emerging from pupae whose larvae were reared in the 1:3 diet without WGO to evaluate egg hatch (Table 5). Wheat germ oil contains a lot of saturated and unsaturated fatty acid, and we speculate that these fatty acids may play an important role in insect development and thus may cause the discrepancies between diets with and without WGO. Further study is underway using a proteomic approach to search for the enzyme/protein/genome that triggers all of these functions.

Most organisms rely on chemical energy to meet all of their biological needs such as movement, information processing, growth, and reproduction. Protein, carbohydrates, and lipids are the three major sources of chemical energy. Most insect diets contain proteins. Dietary proteins and their component amino acids function as building blocks for insect proteins, as sources of energy, as components of hormonal peptides, and numerous other metabolic functions. Moreover, the functional roles of proteins in both insects and foods include binding fats, providing flavors, and ensuring storage. Proteins also act as emulsifiers and

film formers at interfaces between other diet components. They may give the diets greater elasticity or other texture features that may be either desirable or detrimental, depending on the circumstances. Proteins are also important in increasing diet viscosity and in helping serve as living sites for insects that burrow into their diets. However, as noted by Cohen (2004), insect diets are chemically and physically complex and dynamic. For example, vitamins and mineral chemistry, and factors such as pH and temperature can interact with food components in various ways and potentially upset diet stability. Therefore, the decision of which protein sources to use will depend on a combination of different qualities and on prices of protein sources. Here we have identified diets with better performances than others, and we attribute this to the varying composition of yeasts used and their relative proportions. However, it is not easy to draw a solid conclusion as to why one recipe is better than the other and further research on the optimization of liquid diet is needed to make proper conclusion.

In conclusion, our results demonstrate that larvae reared in diets of 224065W in both 1:1 and 3:1 ratios perform as well as those reared in the control diet with regard to the quality control parameters measured in our study. The 3:1 diet combination ($1.93) seems to be cheaper than the 1:1 diet ($2.38), because the cost of LBI2240 is 5 times less than LS65 (quoted by Lallemand, Inc.; Table 2) most likely because the LBI2240 is made of whole cell yeast, whereas LS65 is hydrolyzed yeast (Table 2). At the time of this investigation, LBI2240 was ≈$3.00/kg and FNILS65 was approximately $15.00/kg in a smaller order. We therefore recommend rearing *C. capitata* in a liquid diet containing LBI2240 and LS65 at a 3:1 ratio. This is a cost-effective strategy both in terms of the protein source used as well as the convenience associated with using a liquid diet as opposed to the traditional solid diet with mill feed (Chang et al. 2006). This study shows that is feasible to rear standard bisexual *C. capitata* strain in liquid diet. However, additional studies are necessary to test this technology for the temperature-sensitive lethal strain that currently is used for sterile insect technique operations worldwide. Females of this strain have different biological behavior (Cáceres 2002). Therefore, different environment conditions, handling, larval density, and other rearing procedures probably will be required.

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