

# Relationship Between Population Growth of the Red Flour Beetle *Tribolium castaneum* and Protein and Carbohydrate Content in Flour and Starch

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**ABSTRACT** The effects of eight diets (atta flour, wheat flour, self-rising flour, rice flour, custard powder, corn flour, tapioca starch, and potato starch) on the development of the red flour beetle, *Tribolium castaneum* (Herbst), reared at 29–31°C and 66–70% RH were assessed. Five pairs of male and female *T. castaneum* were reared on the respective diets for 28 d before the experimental setup was dismantled and adult counts were recorded. In another experiment, the insects were allowed to mate and oviposit in each flour or starch type over a period of 7 d before being removed. The counting of pupae and adult emergence began on the day of emergence and was continued on a daily basis until day 140. Proximate analysis was performed for chemical composition of each diet, and the numbers of new adults that developed were found to be positively correlated ( $r^2 = 0.97$ ;  $P < 0.05$ ) with the protein content and negatively correlated ( $r^2 = 0.93$ ;  $P < 0.05$ ) with the carbohydrate content. For *T. castaneum*, the suitable diets were ranked as follows: atta flour > wheat flour > self-rising flour > rice flour > custard powder > corn flour > tapioca starch > potato starch. *T. castaneum* larval development to the pupal and adult stages developed significantly faster in atta flour ( $P < 0.05$ ) than in the other diets, and the greatest number of progeny was produced from beetles reared on atta flour. Fewer adults emerged from wheat flour, self-rising flour, and rice flour, and no new emergences were recorded for the remaining diets. Developmental rate was much slower in beetles reared on diets in which a low number in progeny was produced. These data illustrate that different diets can influence the sustainability of these insects and affect their development and growth.

**KEY WORDS** *Tribolium castaneum*, population growth, developmental period, flour, starch

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The red flour beetle *Tribolium castaneum* (Herbst) is a cosmopolitan (Weston and Rattlingourd 2000) pest that infest stored products such as flour and other milled cereal products or broken grains (Cotton 1963, Hill 1990) in food-processing facilities (Mills and Pedersen 1990) and flour mills, warehouses, and retail stores (Campbell and Runnion 2003). This species can colonize small patches of food and sustain itself with small amounts of food (Campbell and Hagstrum 2002). It has often been used as a model for ecological studies because of its ability to colonize a wide range of stored products (Campbell and Runnion 2003). *T. castaneum* can readily fly when the environment is warm (Rees 2004), and it is capable of dispersal in various foods, including grains and flour stored in a storage area (Hagstrum 1973).

Adult females *Tribolium* sp. have a long life span and can lay eggs in a relatively continuous manner throughout their life (Howe 1962, Ziegler 1976) and had been reported to lay up to 1,000 eggs during their lifetime (Rees 2004). The oviposition rate and emer-

gence of new adults depend greatly on the quality and type of the substrate available (Ziegler 1976). In previous studies, preferences for stored food products were evaluated in an attempt to identify diets that are suitable for their growth and development (Verner 1971). Campbell and Runnion (2003) noted that different types of flour contain different nutrients, and a slight difference in quality can affect *Tribolium* sp. development considerably. Sokoloff et al. (1966) showed that the growth of *T. castaneum* was slow when the insects were reared in wheat, brown rice, and rice, but the addition of yeast improved their development. Their study emphasized on the addition of yeast at a ratio of 19:1 (flour: yeast) into the diets but did not examine the level of protein that was suitable for the growth of the *Tribolium* beetles. Other studies had evaluated the dietary requirements of stored product insects such as *Tribolium confusum* Duval, with emphasis on sterols, carbohydrates, amino acids, vitamins, and minerals (House 1961, 1962). According to Sokoloff et al. (1966), most of the previous studies focused on the effects of various components of a diet on developmental rate and mortality of the larvae,

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number of larval instars, or weight of larvae, pupae, or adults. Various minerals and vitamins for the optimal growth of *T. confusum* have been investigated and this includes phosphorus (Chaudary and Lemonde 1962), thiamine, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, and biotin (Fraenkel and Blewett 1947). Nevertheless, studies on the influence of a definite range of nutrients, especially protein and carbohydrates for the optimal growth and development of *T. castaneum* were never attempted.

In granaries or flour mills, *Tribolium* sp. usually is exposed to only one or two grain types. However, mills may also produce and store several types of grain at the same time, thereby providing the opportunity for selection in a spatially heterogeneous environment (Via and Conner 1995). These beetles can disperse over different flour types kept in particular storage areas where the environmental conditions (temperature and humidity) are kept at a constant, but they may or may not thrive. We hypothesize that the development of *T. castaneum* reared on different diets is related to the nutritional content of the diet, particularly the protein and carbohydrate contents. The current study was conducted to investigate the effects of available proteins and carbohydrates in eight different diets on the growth of *T. castaneum* as well as the basic requirement levels of these nutrients. From the data obtained, the developmental time, sex ratio, and number of progeny produced by *T. castaneum* when reared on different diets were evaluated and compared. Earlier, Sokoloff et al. (1966) reported the developmental rates of *T. castaneum*, but their experimental setup was only examined after 42 d of adult introduction and subsequently at every 2 wk intervals, thus the precise determination of larval development was not possible. On the contrary, we determine the daily emergences of adults and pupae reared under different diets over a period of 140 d in our study. This enables more comprehensive determination on the developmental rates of F1 progeny, and also keeping the possibility of cannibalism among the larvae and adult stages at a minimum. The amount of protein and carbohydrate required to sustain their development was investigated.

### Materials and Methods

**Insects.** *T. castaneum* was cultured on wheat flour (Prestasi Flour Mill (M) Sdn. Bhd., Selangor, Malaysia) at 13% moisture content under laboratory conditions ( $30.0 \pm 0.5^\circ\text{C}$ ,  $68 \pm 2\%$  RH, and 12 h photoperiod). The test insects were obtained from laboratory subcultures that had been maintained in the Urban Entomology Laboratory, Vector Control Research Unit, Universiti Sains Malaysia, Minden Campus, Penang, Malaysia since August 2009.

**Insect Growth Assay.** The following six types of flour and two types of starch were evaluated: atta flour (Gemini; Gemini Flour Mills (M) Sdn. Bhd., Kuala Lumpur, Malaysia); enriched wheat flour (Tesco Choice; The Only One Beverage Sdn. Bhd., Bukit Mertajam, Penang, Malaysia); self-rising flour (Nago;

Nago Food Industries Sdn. Bhd., Butterworth, Penang, Malaysia); rice flour (Erawan Brand; Cho Cheng Rice Vermicelli Factory Co. Ltd., Bangkok, Thailand); custard powder (Tesco Choice); corn flour (Tesco Choice); tapioca starch (Cap Kapal ABC; Thye Huat Chan Sdn. Bhd., Bukit Mertajam, Penang, Malaysia); and potato starch (Tesco Choice). The choice of diets was primarily determined by the availability of these flours or starches commercially.

Each type of flour or starch (20 g) was placed into plastic containers measuring 4.5 cm in diameter  $\times$  9 cm in height. The lids of each container were perforated to allow air ventilation, and each flour or starch type was replicated five times. Ten 7-d-old adult beetles (five males and five females) were introduced into each of the containers. The experimental setup was dismantled on day 28, and the numbers of pupae and adults were counted and recorded.

Another experiment was conducted using similar methods, but the beetles were allowed to feed and oviposit for 7 d, after which they were removed. The containers containing the flour or starch were then left undisturbed to observe the emergence of the new generation from any eggs that might have been laid. Once the first pupae developed, the cultures were examined daily, and any new pupae and adults were counted and removed. For each container, pupae numbers and adult emergence were recorded for 140 d beginning from when the pupae were first observed. The pupae that developed were sexed according to Halstead (1963).

**Proximate Analysis.** Proximate analysis was conducted following the standard procedures of the Association of Official Analytical Chemists (AOAC, 1997) to ascertain the chemical composition (ash, lipid, fiber, protein, and carbohydrate) present in each diet type. Before the analyses, the flour and starch samples were stored in a desiccator. Each flour or starch sample was analyzed three times.

Ash content was determined by placing  $\approx 2$  g of flour or starch in a crucible and then ash drying it in a muffle furnace (M15A-1A, Blue M Electric Company, Blue Island, IL) at  $600^\circ\text{C}$  for 5 h. The weight of each flour or starch sample was measured before and after the assay, and the ash content was calculated as the difference between the two measurements. Lipid content was estimated by homogenizing  $\approx 1$  g of the flour or starch in a mixture of analytical grade methanol and chloroform at a ratio of 2:1 and then separating the solution using a separating funnel. The lower phase (solvent and lipid extract) was collected in a beaker and left in an oven (Type UFB 400, Memmert, Germany) at  $105^\circ\text{C}$  for 3 h to evaporate the solvents before weighing the sample. Crude fiber content was estimated by heating  $\approx 2$  g of the flour or starch sample in  $\text{H}_2\text{SO}_4$  (5% vol:vol) and water, then adding NaOH (40% vol:vol) and a few drops of indicator before heating again. The solution then was rinsed with 1% HCl and boiling water through a Buchner funnel equipped with a filter paper (Whatman no. 541). The filter paper was placed in a crucible and left in the muffle furnace at  $600^\circ\text{C}$  for 3 h before

**Table 1. Proximate composition of various flours or starches**

Flour type	% mean composition ± SE				
	Ash	Lipid	Fiber	Protein	Carbohydrate
Atta flour	1.59 ± 0.04	2.65 ± 0.01	2.19 ± 0.29	12.77 ± 0.08	80.79 ± 0.22
Wheat flour	0.57 ± 0.03	1.68 ± 0.17	0.09 ± 0.02	11.71 ± 0.02	85.95 ± 0.19
Self-rising flour	3.08 ± 0.02	1.74 ± 0.06	0.38 ± 0.07	7.34 ± 0.04	87.46 ± 0.10
Rice flour	0.37 ± 0.09	1.46 ± 0.00	0.89 ± 0.02	8.22 ± 0.02	89.05 ± 0.09
Custard powder	0.09 ± 0.02	0.16 ± 0.02	0.53 ± 0.07	0.31 ± 0.02	98.91 ± 0.11
Corn flour	0.03 ± 0.01	0.01 ± 0.00	0.24 ± 0.14	0.17 ± 0.04	99.56 ± 0.17
Tapioca starch	0.09 ± 0.01	0.01 ± 0.00	0.47 ± 0.28	0.14 ± 0.04	99.29 ± 0.24
Potato starch	0.36 ± 0.01	0.04 ± 0.00	0.82 ± 0.30	0.05 ± 0.03	98.74 ± 0.31

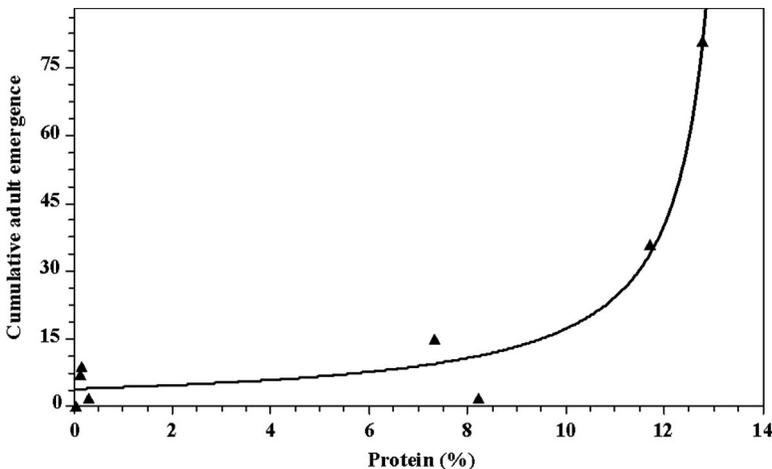
weighing. Each flour or starch sample was also subjected to digestion and analysis using the standard Kjeldahl method and a Rapid Steam Distillation Unit (Vapodest 50s, Gerhardt, Germany) to assess the crude protein content. Crude carbohydrate content was estimated by subtracting the total sum of percent crude protein, crude lipid, crude fiber, and ash from 100%.

**Data Analysis.** The data sets of the protein and carbohydrate content of each substrate were analyzed with the emergence number of pupae and adult using Pearson correlation and regression analysis by applying the curve fitting procedure in Curve Expert Version 1.4 (D. Hyams, Hixson, TN). A paired *t*-test was used to analyze the differences between the number of male and female emergences at *P* < 0.05. Differences in mean development time when reared on different flour or starch types were analyzed using a one-way analysis of variance (ANOVA), and means were separated using the Tukey honestly significant difference (HSD) test. Data (in percentage) of adult and pupae emergences for different flour or starch types were transformed into arcsine values and subjected to one-way ANOVA, and means were separated using the Tukey HSD test. Statistix Version 7.0 (Analytical Software, Tallahassee, FL) was used for data analysis.

**Results and Discussion**

Proximate analysis showed that among the diets tested, atta flour had the highest protein content (Table 1). Atta flour consists of a mixture of refined flour, bran, and shorts (Rani et al. 2001) and is also known as stone ground whole grain flour (Ortiz-Monasterio, 2007). According to Gujral and Pathak (2002), it is an excellent source of nutrition and dietary fiber. Large amounts of enzymes such as amylases, proteases, lipoxigenase, polyphenol oxidase, and peroxidase occur in the bran portion of atta flour. In wheat flour, the bran portion of the wheat, which includes the pericarp, seed coat, and aleurone cells, is discarded during the flour-milling process used to produce refined flour (Rani et al. 2001). The atta flour, wheat flour, self-rising flour, and rice flour tested herein contained >7% crude protein. In contrast, custard powder, corn flour, tapioca starch, and potato starch lacked proteins (<1%). Generally, the ash, lipid, and fiber contents in the diets were low (as low as 0.01% in some of the diets tested).

Over a period of 28 d, the cumulative number of adult emergences significantly increased in substrates with higher protein content (*P* < 0.05; *R*<sup>2</sup> = 0.97; Fig. 1). In *T. castaneum*, protein is essential for egg production of adult females (Pimentel et al. 1965). A



**Fig. 1.** Cumulative adult emergence at day 28 versus different protein content (%) of diets [*r*<sup>2</sup> = 0.97; *y* = (-4.68)/(1-2.48e)<sup>-(6.68)x</sup>].

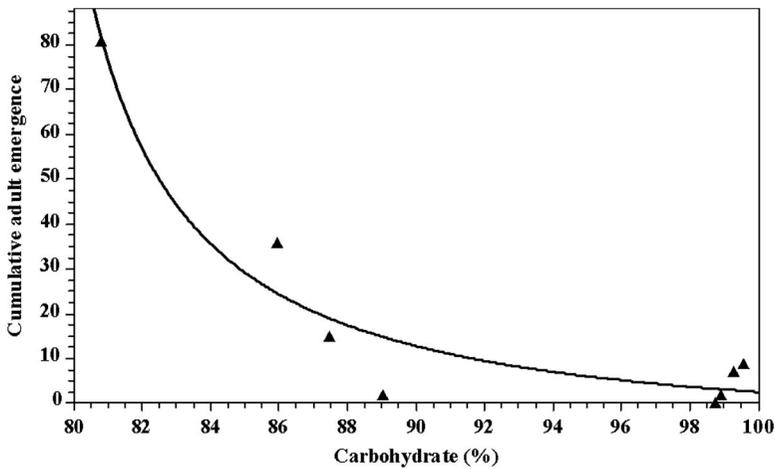
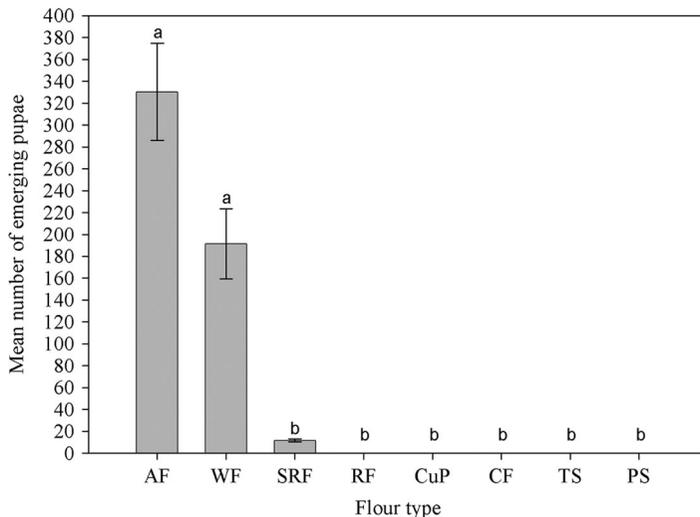


Fig. 2. Cumulative adult emergence at day 28 versus different carbohydrate content (%) of diets [ $r^2 = 0.93$ ;  $y = (2.65 - 2.50x) / (1 - 2.19x + 2.82x^2)$ ].

definite increase in numbers of cumulative adults was recorded in diets with >12–13% protein, and at lower values (<10%) very few new adult beetles emerged. In contrast, the cumulative number of adult emergences decreased in substrates that have higher carbohydrate content (Fig. 2). A significantly lower number of total adult emergences (<10 individuals) ( $P < 0.05$ ;  $R^2 = 0.93$ ) occurred in diets with a high carbohydrate content (>98%). According to Inouye and Lerner (1965), different diets can affect population numbers of the stored product insects. The development of *T. castaneum* in this study was influenced by the availability of protein in the flour, but diets containing high amounts of carbohydrates slowed down their growth and development. Although Sokoloff et

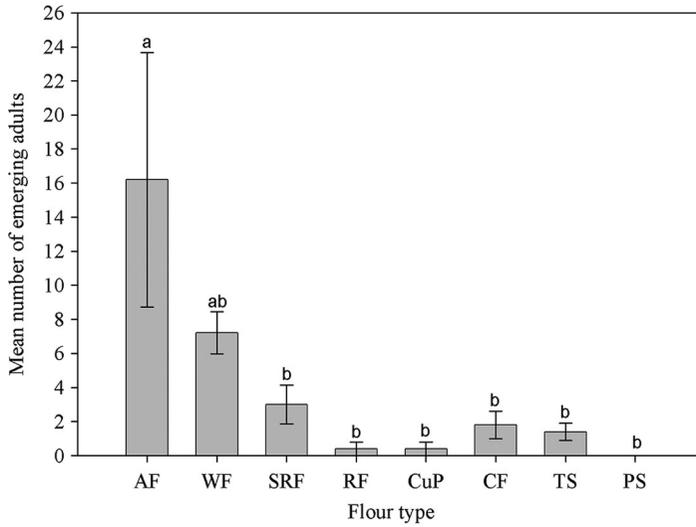
al. (1966) reported that *T. castaneum* can use various flour types, *T. castaneum* is sensitive to insufficient nutrients, and they suggested that a higher amount of leucine, alanine, and aspartic acid is necessary in their diets than compared with the diets for *T. confusum*. Moreover, they reported that *T. castaneum* also requires vitamins, minerals, or both.

Significantly more pupae had emerged from the atta and wheat flours than the other diets tested ( $F = 15.99$ ;  $df = 7$ ;  $P < 0.0001$ ). In fact, no pupae were found in the rice flour, custard powder, corn flour, tapioca starch, and potato starch at day 28 (Fig. 3). According to Mertz and Robertson (1970), it is common for the active stages to eat the inactive stages, with adults cannibalizing the pupae and both larvae and adults



AF= Atta flour; WF= Wheat flour; SRF= Self-raising flour; RF= Rice flour; CuP= Custard powder; CF= Corn flour; TS= Tapioca starch; PS= Potato starch

Fig. 3. Mean ( $\pm$ SE) numbers of emerging pupae reared on different diets at day 28 (Tukey HSD,  $P < 0.05$ ) at  $30.0 \pm 0.5^\circ\text{C}$  and  $68 \pm 2\%$  RH.



AF= Atta flour; WF= Wheat flour; SRF= Self-raising flour; RF= Rice flour; CuP= Custard powder; CF= Corn flour; TS= Tapioca starch; PS= Potato starch

Fig. 4. Mean ( $\pm$ SE) numbers of newly emerging adults reared on different diets at day 28 (Tukey HSD,  $P < 0.05$ ) at  $30.0 \pm 0.5^\circ\text{C}$  and  $68 \pm 2\%$  RH.

feeding on the eggs. Mertz and Cawthon (1973) stated that the cannibalism of eggs is a nutrient acquiring method which can improve the fecundity rate. In  $<28$  d, the larvae of *T. castaneum* in this study may have acquired the necessary nutrients through egg cannibalism to help sustain their growth and development into adults. Several newly emerged adult beetles were recorded from the rice flour, custard powder, corn flour, and tapioca starch. Figure 4 shows that significantly more new adults emerged in atta flour ( $F = 4.06$ ;  $df = 7$ ;  $P = 0.0028$ ) than in self-rising flour, rice flour, custard powder, corn flour, tapioca starch, and potato starch. Flour suitability of *T. castaneum* can be ranked as  $\text{atta flour} > \text{wheat flour} > \text{self-rising flour} > \text{rice flour} > \text{custard powder} > \text{corn flour} > \text{tapioca starch} > \text{potato starch}$ .

In the 140-d experiment, the overall male to female ratio was 1:1.14 in the atta flour, with significantly more females emerging between days 10 and 29 ( $P < 0.05$ ) and significantly more males emerging between days 30 and 39 ( $P < 0.05$ ). No significant differences were detected between the number of males and females that developed when reared in wheat flour, self-rising flour, and rice flour ( $P > 0.05$ ) for any time period (Table 2).

New *T. castaneum* adults failed to emerge from containers containing custard powder, corn flour, tapioca starch, and potato starch at the end of the experimental period (140 d) (Table 3). The numbers of new adults that emerged from the atta flour were significantly higher than for any of the other diets tested ( $F = 7.62$ ;  $df = 11$ ;  $P < 0.0001$ ). In the atta and wheat

Table 2. Mean ( $\pm$ SE) male and female numbers of *T. castaneum* reared on a variety of flours or starches over a period of 140 d at  $30.0 \pm 0.5^\circ\text{C}$  and  $68 \pm 2\%$  RH

Time (d)	Mean no. male and female <i>T. castaneum</i> $\pm$ SE							
	Atta flour		Wheat flour		Self-rising flour		Rice flour	
	Male	Female	Male	Female	Male	Female	Male	Female
10-19	53.33 $\pm$ 2.46a	70.33 $\pm$ 3.21b	37.80 $\pm$ 1.64a	50.80 $\pm$ 1.60a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
20-29	167.60 $\pm$ 3.20a	196.60 $\pm$ 3.20b	94.50 $\pm$ 1.36a	1100.20 $\pm$ 1.96a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
30-39	22.80 $\pm$ 1.29a	15.80 $\pm$ 0.95b	11.60 $\pm$ 0.53a	6.80 $\pm$ 0.42a	0.25 $\pm$ 0.05a	0.25 $\pm$ 0.05a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
40-49	0.80 $\pm$ 0.07a	1.00 $\pm$ 0.10a	1.20 $\pm$ 0.10a	1.40 $\pm$ 0.12a	0.40 $\pm$ 0.06a	0.20 $\pm$ 0.04a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
50-59	1.00 $\pm$ 0.10a	0.40 $\pm$ 0.06a	0.00 $\pm$ 0.00a	0.20 $\pm$ 0.04a	0.60 $\pm$ 0.07a	1.60 $\pm$ 0.17a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
60-69	0.20 $\pm$ 0.04a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	11.60 $\pm$ 0.50a	10.20 $\pm$ 0.57a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
70-79	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	13.00 $\pm$ 0.50a	53.00 $\pm$ 0.37a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
80-89	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	3.00 $\pm$ 0.18a	5.20 $\pm$ 0.32a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
90-99	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	2.00 $\pm$ 0.14a	2.00 $\pm$ 0.15a	0.00 $\pm$ 0.00a	0.75 $\pm$ 0.11a
100-109	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.20 $\pm$ 0.04a	0.40 $\pm$ 0.06a	0.00 $\pm$ 0.00a	0.40 $\pm$ 0.06a
110-119	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.20 $\pm$ 0.04a	0.20 $\pm$ 0.04a	0.20 $\pm$ 0.04a	0.00 $\pm$ 0.00a
120-129	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.20 $\pm$ 0.04a
130-139	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a	0.00 $\pm$ 0.00a
Overall ratio	1.00:1.14		1.00:1.10		1.00:0.98		1.00:6.00	

Means followed by different letters between two columns of the same flour type are significantly different (paired *t*-test;  $P < 0.05$ ).

**Table 3.** Total emergence, mean adult emergence ( $\pm$ SE), and mean developmental time of new *T. castaneum* adults reared on different flour types at  $30.0 \pm 0.5^\circ\text{C}$  and  $68 \pm 2\%$  RH

Flour type	Total no. emerged	Mean no. new adults emerged $\pm$ SE	Length of development (d)	
			Mean	Range
Atta flour	2312	462.4 $\pm$ 16.4a	30.2	20–70
Wheat flour	1474	294.8 $\pm$ 8.5b	38.8	26–62
Self-rising flour	260	52.0 $\pm$ 15.9c	77.9	34–118
Rice flour	4	0.8 $\pm$ 0.4d	114.5	96–130
Custard powder	0	0.0 $\pm$ 0.0d	—	—
Corn flour	0	0.0 $\pm$ 0.0d	—	—
Tapioca starch	0	0.0 $\pm$ 0.0d	—	—
Potato starch	0	0.0 $\pm$ 0.0d	—	—

Means followed by different letters within the same column are significantly different (Tukey HSD;  $P < 0.05$ ).

flours, new beetles began to emerge as early as day 20 and 26, respectively. The new *T. castaneum* adults in the atta flour had a mean developmental time of 30.2 d, whereas those in wheat flour emerged at a mean of 38.8 d. The total number of progeny that emerged from the atta flour was the highest, recording  $\approx 36\%$  more than compared with the number that emerged from the wheat flour. Because *T. castaneum* reared in the atta flour developed within a very short time, many progeny were produced throughout the experimental period. From day 20–39, significantly more emergences were recorded in the atta flour when compared with other time intervals ( $F = 7.87$ ;  $df = 11$ ;  $P < 0.0001$ ). In the wheat flour, significantly more emergences were recorded from day 30–49 ( $F = 15.40$ ;  $df = 11$ ;  $P < 0.0001$ ) (Table 4). In the self-rising flour, new beetles first began to emerge at day 34, and their mean developmental period was 77.9 d, as most of the adults emerged between day 70 and 89. An average of 52 new adults emerged from the self-rising flour. Among the four flour types for which adult emergence occurred, new adult beetles in the rice flour took the longest time to develop into adults (average, 114.5 d). Because only four new adult beetles emerged from containers containing rice flour, the results revealed no significant differences among the different time ranges ( $P > 0.05$ ). Therefore, the development of *T. castaneum* reared on self-rising flour and rice flour took a longer time.

Haines (1991) reported that rearing *T. castaneum* on a poor quality diet can slow their developmental time to  $\geq 45$  d. Ajayi and Rahman (2006) reported that when *T. castaneum* was reared on cassava flour and wheat flour, the developmental period was 54.8 and 27.0 d, respectively. Wheat and millet flour contain high contents of crude protein and other nutrients, including thiamine, riboflavin, and niacin (Rehm and Espig 1991), whereas cassava flour is categorized as a starchy food product (Ajayi and Rahman 2006). In another study, *T. confusum* did not feed when provided with starch; however, they responded well to food containing gluten (Loshiavo 1965), which is a type of protein found in the endosperm of the wheat (Atwell 2001). Lecato and McCray (1973) found that the developmental period for both *T. castaneum* and *T. confusum* was longer when they fed on whole soybeans or cracked black-eyed peas than compared with

a mixture of wheat flour, cornmeal, and brewer's yeast. The larvae deprived of nutrients and appeared to be moribund after feeding on whole soybeans.

The introduced adults in this study were only allowed to mate and oviposit over a 7 d period that limits the number of eggs oviposited. Fewer than two larvae (on average) were present in containers containing custard powder, corn flour, tapioca starch, and potato starch, and these larvae were small and inactive. Lecato and McCray (1973) suggested that the larvae in their study did not develop into adults because of the lack of nutrients in the diets and because of the moribund condition of the larvae. Although cannibalism of eggs by larvae can occur, the number of eggs present in the custard powder, corn flour, tapioca starch, and potato starch may not be sufficient for the surviving larvae to feed on. Therefore, in addition to the lack of nutrients in the diets (custard powder, corn flour, tapioca starch, and potato starch), the development of *T. castaneum* larvae into adults were further inhibited. Howe (1965) suggested that the *T. castaneum* larvae in his study would have been able to develop into adults if the experimental period had been prolonged and the temperature had been increased. Our study was carried out at around  $30^\circ\text{C}$  and according to Rees (2004), this is an ideal temperature for the development of *T. castaneum*. Despite the long experimental period (140 d), the new individuals that emerged remained at the larval stage. Usually, it takes 20–120 d for the larval stage to develop into the pupa stage and another 5 d for the pupae to mature into adult beetles (Rees 2004). Proximate analysis showed that all four of these diets contained large amounts of carbohydrates ( $>98\%$ ). Based on the results, diets with higher amounts of carbohydrates recorded a decrease in the population number. Thus, the high carbohydrate content in these diets is not suitable for the growth of *T. castaneum*.

In conclusion, different types of flour or starch influence the developmental rate of *T. castaneum* in different ways. When the protein content in the diet was high, more adult beetles emerged; in contrast, fewer adults developed in diets with high carbohydrate content. The developmental period of *T. castaneum* was longer when they were reared on diets with high carbohydrate content, and in both of the starches tested, no individuals were recorded at all. Among the

**Table 4.** The mean percentage ( $\pm$ SE) of new *T. castaneum* adults emerging throughout the 140 d of the exp when reared on different diets at 30.0  $\pm$  0.5°C and 68  $\pm$  2% RH

Flour type	n	% mean adult emergence $\pm$ SE												
		20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-99	100-109	110-119	120-129	130-140	
Atta flour	2,312	39.0 $\pm$ 3.3a	58.5 $\pm$ 3.6a	2.0 $\pm$ 0.2b	0.4 $\pm$ 0.0b	0.1 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b
Wheat flour	1,474	1.2 $\pm$ 0.2b	51.2 $\pm$ 1.9a	44.1 $\pm$ 2.6a	3.3 $\pm$ 0.2b	0.3 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b
Self-rising flour	260	0.0 $\pm$ 0.0b	0.4 $\pm$ 0.1b	0.4 $\pm$ 0.1b	3.1 $\pm$ 0.3b	9.2 $\pm$ 1.5b	42.7 $\pm$ 1.0a	31.9 $\pm$ 1.3a	8.8 $\pm$ 0.2b	2.3 $\pm$ 0.3b	0.8 $\pm$ 0.1b	0.4 $\pm$ 0.0b	0.0 $\pm$ 0.0b	0.0 $\pm$ 0.0b
Rice flour	4	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	0.0 $\pm$ 0.0a	25.0 $\pm$ 19.2a	0.0 $\pm$ 0.0a	25.0 $\pm$ 25.0a	25.0 $\pm$ 24.0a	25.0 $\pm$ 26.0a	25.0 $\pm$ 26.0a

Means followed by different letters within the same column are significantly different (Tukey HSD;  $P < 0.05$ ).

tested diets, atta flour was most preferred by *T. castaneum*: A total of 2,312 adult beetles emerged, and their developmental time was also the shortest among the diets tested. Atta flour was followed in preference by wheat flour, self-rising flour, and rice flour. Custard powder, corn flour, tapioca starch, and potato starch were found to be unsuitable for sustaining the growth and development of *T. castaneum*.

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