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EFFECT OF THE ACARID MITE *Acarus siro L*. ON THE COMPONENTS AND NUTRITIVE VALUE OF ROUMYCHEESE (ACARI: ACARIDAE:ACARIDIDA)

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ABSTRACT

The study aimed to investigate the effect of the acarid mite, *Acarus siro* on the components of the Roumy (Turkish) cheese after three months of infestation. The components of cheese ; total protein, total glucose, total lipids and amino acids were evaluated after three months of infestation with (100,200 and300 mite individuals/ 100g.cheese) of *A. siro* whereas, the population increased to 3325.25, 3520.0 and 4615.5 mites respectively. The obtained results denoted that the total protein decreased as well as the number of mites on cheese increased. Also, the study revealed a highly variation in the glucose contents of cheese which decreased with increasing the number of *A. siro* infestation from 200 to 300 mites.The obtained data showed that there were significant differences between the effect of mites number (100, 200 and 300 mites) and the cheese lipids contents. When the number of mites infestation was 100 mites, the differences in the lipids contents was not clearly observed when compared with control (without infestation); but the significant difference in the lipids was observed when the number of mites was 200. However, the study also denoted that when the numbers of *A. siro* infestation on cheese increased from 100–300 mites, the contents of cheese amino acids is increased.

INTRODUCTION

Currently more information is available about the loss or damage caused directly by mites infestation. A number of excellent laboratory studies have been carried in which the potential of mites to damage various products have been examined. Mlodecki (1960) determined some changes in chemical composition of wheat and rye flour infested with mites. The most notable result was a reduction in protein. Zdarkova and Reska (1976) found that a heavy infestation of *Tyrophagus putrescentiae* Shrank caused 44 % loss weight loss in ground nuts compared to a 17 % loss caused by an infestation of *Acarus siro* L. When cheese is ripened with mites, a nutty, fruity flavor and aroma develops. Although no studies have determined how mites induce these flavor changes, it is believed through sensory observations that the mites are responsible for this flavor development, Melnyk *et al*., (2010). The acarid mite *Acarus siro* L. can infest a wide range of foodstuffs, plant and animal material where environmental conditions are suitable, and is considered to be a cosmopolitan stored product pest of significant economic and sanitary importance. The acarid mite is most frequently found in stored food with a relatively high fat and protein content, such as wheat and soy, flour, cheese, rye bread, herring meal, bacon, dried milk and various seeds (Duek *et al*., 2001). The relative humidity of the micro-environment as well as the temperature and food sources are the main ecological factors affecting the growth and development of mites. Mite infestations on cheese can be recognized as a layer of gray dust and cavities on the surface of the cheese. Colonies usually include two or three species growing together. Protein break down during ripening makes old cheese a

better source of nutrients, but old cheese also contains more fatty acids such as butyric acid which inhibits egg-laying by mites, (Sadasivan and Manickam (1991). Many of stored food mites include *Acarus siro*, *Acarus farris*, *Acarus immobilis*, *Tyrophagus putrescentiae*, *Tyrophagus longior*, *Tyrophagus neiswanderi*, *Tyrophagus palmarum*, and *Tyrolichus casei* grow in conditions greater than 4°C and 60% relative humidity, which are common storage conditions for cheese ripening, which makes infestation prevention difficult (Peace, 1983). Attempts were made by Yoshizawa, *et al.,* (1970) to isolate attractants for the cheese mite, *T. putrescentiae*, from Cheddar cheese. Heptan-2-one, octan-2-one, nonan-2-one and 8-nonen-2-one were identified as the active principles. The authors noticed that these compounds did not show significant attractancy to the mite when applied individually, but a mixture of them showed a potent attractancy.The acarid mite, *A. siro* could increase in numbers on cheddar cheese contaminated with the mould *Penicillium verrucosum* and on a sterilized culture of this mould though not as successfully as on uninfected cheddar, Peace (1983). This mite is known to infest cheese and mould and produces a brown dust on the foodstuff it is infesting (Solomon, 1962). The purpose of this study is to investigate the effect of different level infestation of the acarid mite , *A. siro* L. on the components of the Roumy(Turkish) cheese after three months of infestation.

MATERIALS AND METHODS

1. Sample preparation:

To ascertain the effect of mite´s infestation on the constituents of Roumy cheese, the dominant mite, *A. siro* was used. A stock culture of this mite was established from infested Roumy cheese under constant laboratory conditions (25±2 ºC and 70±5 % R.H.). Twelve sliced (freshly prepared) Roumy cheese (5 cm x 5 cm x 0.2 cm) were divided into 4 groups (each group comprise of 3 slices). Each slice was infested with 100 , 200 & 300 of mite, *A.siro* adult (females and males) which represents group 1, 2 and 3, respectively. The non- infested cheese slices represent the control group. Dead mites were removed from each group. After 3 months of infestation, sufficient amount of these cheese (from each group) were used for analyzing their constituents.

2. Extraction of Turkish cheese constituents.

The total protein, lipids were extracted from Roumy cheese (in each group) according to Sadasivam and Manickam (1991).Total carbohydrates were extracted according to Crompton and Birt (1967). For total protein extraction, 500 gm of Roumy cheese from each group mentioned before (in triplicates) were used. Cheese sample was grind well (with pestle and mortar) in 5 ml of 0.01 M phosphate buffer (PH 7) and centrifuge. The pellet was discarded and the supernatant was used for protein estimation. The total lipids were extracted as follows: 100 gm of Roumy cheese from each group, in triplicates, were grinding (using mortar-driven tissue grinder) in 2 x 3 ml portions of chloroform: methanol, 2:1. After centrifugation (2200 rpm for 5 min), the supernatant was evaporated. The fat deposit was resuspended in 3 ml of dist. water. For carbohydrate extraction from Roumy cheese infested with *A. siro* and control one, 100 gm of cheese were used. Sample (in a boiling tube) was hydrolyzed by 100 ml

of 2.5 N HCl for 3 h in a boiling water bathe and then cool at room temperature. Neutrize it with sodium bicarbonate (until effervescences stopped), then collect the supernatant for analysis of carbohydrates. For amino acids extraction from Turkish cheese, the method of protein extraction was used.

Estimation of Turkish cheese constituents

a. Total protein

The extracted protein from Roumy cheese in different groups (as mentioned before) was estimated according to Bradford (1976). Protein reagent was prepared by dissolving 100 mg of comassive Brilliant (G-250) in 50 ml of 95 % ethanol, then add 100 ml of 85 % phosphoric acid. The resulting solution was diluted to a final volume of one liter. Sample solution (50 ml) was pipetted into test tube; the volume was adjusted to 1 ml with phosphate buffer (0.1 M, PH 6.6) and then added 5 ml of protein reagent into test tube. The contents were mixed by inversion and the absorbance was measured at 595 nm after 2 min and before one hour against blank. The blank prepared from 1 ml phosphate buffer and 5 ml of protein reagent.

b. Total lipids:

Total lipids were estimated according to the method of Knight *et al.,* (1972) using phosphor-vanillin reagent. This reagent was prepared by dissolving 0.6 g pure vanillin in 10 ml ethanol and then completed to 100 ml with dist. Water. Add 400 ml of conc. Phosphoric acid. In a test tube add 5 ml of conc. Sulphoric acid to 250 ul of the extracted lipids (Samples). For standard lipid solution (025 ml of olive oil mixed in 199 ml of methanol). The contents were mixed well for 10 sec. and the tubes were placed in a boiling water bath for 1 min. After cooling to room temperature, 6 ml of the color reagent was added to each sample tube and standard tube as well. These tubes were left to stand for45 min (in the dark) and then the intensity of the color was measured at 525 nm. The total lipids (mg/g cheese) were calculated as follows:

$$\text{Total lipids} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard} \times 1/\text{vol. (ml) of sample used}$$

c. Total carbohydrates:

Total carbohydrates (expressed as glucose) were estimated from the acid extract of Roumy cheese (as mentioned before) by the phenol-phosphoric acid reaction. Hundred micro liters of the acid extract were added into tube to 0.5 ml of phenol (20%, w/v), and then 5 ml of concentrated Sulphoric acid was added with shaking. The tubes were allowed to stand for 10 min after that the tubes were shaken and placed in water bath (at 25 ºC) for 20 min before reading. Blank were prepared by substituting distilled water instead of sample containing sugar. Absorbance of the characteristic color (yellow-orange color) is measured at 490 nm against blank. Total carbohydrate is expressed as µg / g cheese.

d. Total amino acids

The estimation method used in the protein was applied also in estimation of amino acids. Double beam ultraviolet/visible spectrophotometer (Spectronic 1201, Milton Roy Co., (USA) used to measure the absorbance of color substances in the present study.

RESULTS AND DISCUSSION

In Nature, the mite infestations on cheese can be recognized as a layer of gray dust and cavities on the surface of the cheese. Mites are able to grow on any cheese of any age. The acarid mite, *A. siro* is very common but usually go unnoticed except in occasions when they become abundant. Four categories of cheese components i.e. total protein, total carbohydrates, total lipids and amino acids were calculated. The number of resulted *A. siro* after 3 months post infestation, (Table 1) was 3325.25, 3520.0 and 4615.5 mites in case of the beginning application of 100, 200 and 300 mites, respectively.

A. Effect of *A. siro* on total protein of Roumy cheese.

Turkish cheese infested with *A. siro* at rate of 200 and 300 mite individuals / 100 gm cheese, obviously decreasing on the total protein content The resulted contents recorded, 19.98 and 18.33 of Protein content (mg/100 gm cheese, respectively compared to control, 23.0 mg (Table 1). On the other hand, there is no any obviously effect on the total protein content of Roumy cheese infested with 100 mite individuals / 100 gm cheese compared to control (Table 1). The obtained results denoted that as well as the number of mites increased, the contents of cheese protein is decreased.

B. Effect of *A. siro* on glucose of Roumy cheese

The tabulated obtained data in Table (2) showed that there was a highly variation in the carbohydrates contents of Roumy cheese and this contents (mg/100 gm cheese) decreased with increasing the number of the acarid mite, *A siro* infestation from 100 mites to 300 mites. Therefore, when the number of mite infestation was 100 mite individuals, the carbohydrate contents decreased to reached (919.40 gm/100 gm cheese) and more decreased in case of 200 mites recorded (865.44 gm/100 gm cheese). However, when the infestation with acarid mite was 300 mite individuals the carbohydrates content of the tested Roumy cheese recorded (730.31 gm/100 gm cheese).The content of carbohydrates in the absence of mite infestation (control) was 1247.0 gm/100 gm of Turkish cheese.

C. Effect of *A. siro* on lipids contents of Roumy cheese:

The tabulated data in Table (3) showed that there were highly differences between the effect of different mites number categories (100, 200 and 300 mites) on the Roumy cheese lipids contents. After 3 months of infestation with 200 and 300 individuals of *A. siro*, the total lipids of Turkish cheese was decreased to recorded 16.10 and 14.30 mg/ 100 g cheese, respectively compared to control (16.26 mg/ 100 g cheese). On the other hand, there is no effect of mite infestation on lipid content when the rate of infestation was 100 individual/ 100 g cheese.

D. Effect of *A.siro* infestation on Roumy cheese amino acids content:

Table (4) denoted that when the number of *A. siro* on Roumy cheese increased from 100 to 300 mites, the total amino acids contents of cheese was obviously increased. The amino acids represented by 4520.22, 6000.22 and 6022.22 (gm/100 gm cheese) when the *A. siro* was applied with 100, 200 and 300 mites, respectively compared with 4111.33 (gm/100 gm cheese) in case of absence of mite infestation (control).The present results are consistent with that of Erban *et al.,* (2009) where studied the adaptation of nine species of

mites that infest stored products for starch utilization was tested by (1) enzymatic analysis using feces and whole mite extracts, (2) biotests, and (3) inhibition experiments. *Acarus siro, Aleuroglyphus ovatus*, and *Tyroborus lini* were associated with the starch-type substrates and maltose, with higher enzymatic activities observed in whole mite extracts. *Lepidoglyphus destructor* was associated with the same substrates but had higher activities in feces. *Dermatophagoides farinae*, *Chortoglyphus arcuatus*, and *Caloglyphus redickorzevi* were associated with sucrose. *T. putrescentiae* and *Carpoglyphus lactis* had low or intermediate enzymatic activity on the tested substrates. The mass loss by feed uptake of the pests is less important than quality losses by contamination caused by secretion of insects (for instance by *Tribolium*, *Tenebrio*, *Trogoderma* and any *Cryzaephilus*), by sensorial and chemical parameters and last not least by containing living pests (mainly mites) and subsequent fungus infestation including the mycotoxin content in forage (Vogel, 1973, Braude *et al.,* 1980, Smith, 1971). Also, the mites *A. siro, A. ovatus, T. lini, L. destructor and T. putrescentiae* showed an accelerated increase in population on a starch-enriched diet (Erban *et al.,* 2009). Pankiewicz *et al*. (1982) evaluated the gustatory attractiveness of *Acarus siro* L. to seven of stored products including wheat germ, dried yeast, oat flaks, powdered milk, fish meal, dried wiled edible mushroom (*Bolitus edilis*), and potato flour, some species of fungi (*Botrytis cinerae*, *Aspergillus flavus* (Link), *Aspergillus rapes* (Corda), and *Penicillium cyclopium* (Westling), and four fractions of wheat germ. The authors found that the stored food products were all suitable for development to this mite. The attractive factor appeared to be a combination of fats and biological active component other than proteins, free amino acids, and carbohydrates.

Table (1): Effect of *A. siro* infestation on Roumy cheese protein content (mg /100 g cheese) after three months of infestation

Variable	Protein content (mg/100 gm cheese)				No. of mites (motile stages) / 5 cm^2
	Mean	S.D. ±	Minimum	Maximum	
100 mites	23.0	1.89	21.43	25.60	3325.25
200 mites	19.98	0.46	19.68	20.64	3520.0
300 mites	18.33	0.93	17.57	19.72	4615.5
Control	23.27	0.89	21.26	23.30	-

Table (2): Effect of *A. siro* infestation on Roumy cheese carbohydrates content (mg/100 g cheese) after three months of infestation

Variable	Carbohydrates content (mg/100 gm cheese				No. of mites (motile stages) / 5 cm^2
	Mean	S.D. ±	Minimum	Maximum	
100 mites	919.40	22.00	890.3	943.32	3325.25
200 mites	865.44	69.33	778	961	3520.0
300 mites	730.31	40.66	689.3	800.3	4615.5
Control	1247.0	166.33	1064.0	1447.0	-

Table (3): Effect of *A. siro* infestation on Roumy cheese lipids content (mg/100 g cheese) after three months of infestation

Variable	Lipids content (mg/100 g cheese)				No. of mites (motile stages) / 5 cm^2
	Mean	S.D. ±	Minimum	Maximum	
100 mites	16.20	0.886	15.24	17.30	3325.25
200 mites	14.30	1.180	13.32	16.05	3520.0
300 mites	13.6	0.298	13.33	14.0	4615.5
Control	16.26	0.763	15.75	17.28	-

Table (4): Effect of *A. siro* infestation on Roumy cheese amino acids content (mg/100 g cheese) after three months of infestation

Variable	Amino acids contents (mg/100 gm cheese)				No. of mites (motile stages) / 5 cm^2
	Mean	S.D. ±	Minimum	Maximum	
100 mites	4520.22	336.64	4141.30	4888.35	3325.25
200 mites	6000.22	658.90	5203.60	6664.32	3520.0
300 mites	6022.22	200.72	5813.60	6200.55	4615.5
Control	4111.33	534.55	3366.0	4557.0	-

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تاثير الحلم الأكاريدى *Acarus siro* L. على المكونات الغذائية للجبن الرومى (Acari: Acaridae:Acaridida)

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تصيب الاكاروسات المواد الغذائية المخزونة وتسبب لها بعض التغيرات فى التركيب الكيماوى لمكوناتها نتيجة للتغذية عليها فضلا عما تسببه من تلوث لها عن طريق فضلاتها وافرازاتها ووجود جلود انسلاخ واجسام ميتة مما تؤثر على هذه المكونات وتأثيرها على صحة الانسان. ولقد اجريت هذه الدراسة بهدف معرفة تأثير الحلم الأكاريدى *A. siro* و يطلق عليه أكاروس الجبن الرومى بأعداد ١٠٠ و ٢٠٠ و ٣٠٠ فرد – ١٠٠جم على مكونات الجبن الرومى من البروتين و الكربوهيدرات والدهون والأحماض الأمينية.انتهت الدراسة بزيادة اعداد الاكاروسات المتحصل عليها بعد ثلاث شهور من التخزين والاصابة بالاكاروس مسجلة اعداد مقدارها ٣٣٢٥.٢٥ و ٣٥٢٠.٠ و ٤٦١٥.٥ اكاروس عند استخدام الاكاروسات ١٠٠ و ٢٠٠ و ٣٠٠ على الترتيب. واتضح من الدراسة ان المعاملة ب ١٠٠ اكاروس ليست لها اى تاثير واضح على البروتين الكلى للجبن الرومى ولكن عندما زادت اعداد الاكاروسات الى ٢٠٠ و ٣٠٠ اكاروس لوحظ ان نسبة البروتين الكلى للجبن قد نقصت بشكل معنوى واضح. وبالنسبة للمواد الكربوهيدراتية و الدهون فقد نقصت نسبة تواجدها جميعها وبشكل معنوى فى الجبن الرومى المختبرة وذلك بزيادة اعداد الاكاروسات الى ٢٠٠ و ٣٠٠ اكاروس. اما بالنسبة للاكاروسات ١٠٠ فقط فلم يكن التاثير واضحا على هذه المكونات. ولقد لوحظ عكس ذلك عند تاثير هذه المجاميع من الاعداد الاكاروسية على مكونات الاحماض الامينية حيث زادت بزيادة الاكاروسات وقلت عندما قل عدد هذه الاكاروسات.