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# Automatic headspace sampler in identifying volatiles released by stored grain insects-a preliminary study

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**Abstract.**

Detecting the presence of insects at low densities can avoid total deterioration of stored grains. *Tribolium castaneum* is one of the major insect pests of the Canadian grain handling industry. Identification of the volatile organic compounds released by insects can be used to detect insects in stored grains. An attempt was made to identify the volatile organic compounds released by *T. castaneum* by headspace analysis, the volatiles in the head space of vials with insects, and insects and wheat, were analyzed using a GC-MS coupled with an automatic headspace sampler. Feasibility of the automatic headspace sampler in headspace analysis was found to be positive. The sampler can do sample conditioning, absorption, trap cleaning and desorption of the volatiles into the GC-MS and speed up the process. The samples conditioned at 250 rpm for 5 min at 30°C were found to be giving detectable amount of volatiles. Wheat with fifteen percent moisture content was used in this study along with two different

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insect numbers. Ethyl-1, 4-benzoquinone, methyl-1, 4-benzoquinone and 1-Pentadecene were the volatiles released in abundance by *T. castaneum*.

**Keywords**: Headspace analysis, Automatic headspace sampler, GC-MS, and Volatile organic compounds.

## INTRODUCTION

The detection of stored-product insect pests is a major issue for the grain handling industry. There are various insect detection methods available for monitoring insects and insect fragments in whole kernels and flour, such as visual inspection, moisture and temperature monitoring, pheromone traps, X-rays and near infrared spectroscopy, however most of these methods are not cost effective and are labor intensive. Analyzing the headspace volatile organic compounds can be a rapid and efficient method for detection of insects. *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), the red flour beetle, is a major insect affecting the quality and quantity of stored grains in Canada resulting in economic losses to producers. Tenebrionids produce quinine-containing secretions from prothoracic and abdominal glands (Ruther et al. 2001). These secretions can contaminate and confer unpleasant organoleptic properties to the food source. A variety of sophisticated and complex methods formerly used to analyze headspace have been summarized by Unruh et al. (1998). Basically their analyses require considerable time and many insects, and it cannot be used as an indicator of incipient spoilage because most of the studies are done without the presence of substrate like cereals or oil seeds.

Based on this back ground information, we initiated this project with the following specific objectives:

1) To determine the feasibility of an automatic headspace sampler for collection and enrichment of volatile organic compounds secreted by insects in a less time, and
2) To find the compounds specific to insects at an incipient level using Gas chromatography and mass spectrometry (GC-MS).

## MATERIALS AND METHODS

### Insects

*T. castaneum* cultures were reared on wheat flour with individuals received from a farm in Landmark, Manitoba in 1998 and The insects used in this study were mixed sex and 40 days old and maintained at 30°C and 70% relative humidity.

### Automatic headspace sampler

A CombiPAL (CTC Analytics, Switzerland) automatic headspace sampler was used in this study for collection and enrichment of volatile organic compounds generated by insects. The system performs all the operations involved in collecting volatile organic compounds such as sample conditioning, absorption, desorption and trap cleaning. The sample conditioning or pre-conditioning was done by heating and agitating a sealed sample at 30°C, 250 rpm for 5 min. The absorption and desorption of volatiles were performed by a microtrap filled with Tenax absorbent material placed between the heated headspace syringe and syringe needle. The headspace syringe was used as a pump to collect a part of the gaseous phase of the pre-conditioned sample vial through the microtrap. This system setup allows rapid, simple and efficient extraction of volatile compounds. The numbers of extraction strokes were 20 at 100µl/s. Desorption was carried out at 230°C and trap cleaning at 270°C.

### Volatiles collection and identification

Fifteen percent moisture content wheat was used along with three and five *T. castaneum* insects per vial *for* the collection of volatiles. The vials were filled with 8 g wheat along with insects and tightly sealed; the volatiles were collected immediately after transferring the insects into the wheat, and at 1 hour. The sealed vials with samples were kept in the automatic headspace sampler for collection of volatiles. The desorbed volatile organic compounds from the automatic headspace sampler were directly injected into the Varian CP-3800 gas-chromatograph (Varian Inc., CA, USA) for separation of compounds and Varian 320 mass Spectrometer (Varian, Inc., CA, USA.) for identification of compounds. A factor four capillary column (30 m length, 0.25 mm I.D., 0.25 µm film thickness) was used with the column oven programming as: 40 °C for 3 min, increased to 80 °C at the rate of 5 °C/min, then to 150 °C at the rate of 20 °C/min and then to 250 °C at the rate of 30 °C/min, with a holding time of 10 min at the final temperature (Villaverde et al. 2007). The mass acquisition was done between 20 and 230 molecular weight. Volatile organic compounds were tentatively identified by interpretation of their mass spectral fragmentation; spectra were also compared to data from MS libraries (NIST/EPA/NIH/NIST 98). Hydrocarbon chain length was confirmed by matching their retention times with those of hydrocarbon standards (Sigma-Aldrich, St.Louis, MO, USA). Vials containing pure wheat without insects were used as control samples.

### Statistical analysis

The difference among the means of the area under the peaks of ethyl-1, 4-benzoquinone, methyl-1, 4-benzoquinone and Pentadecene were compared by analysis of variance (ANOVA), and using Turkey's test to separate treatment means($P<0.05$).

## RESULTS AND DISCUSSION

The automatic headspace sampler reduces the time taken to collect the volatiles to 20 min, within this time the sampler performed absorption, desorption and trap cleaning before collecting the volatiles from the next sample. Following the developed protocol the amount of volatiles obtained was adequate. The sampler is designed and programmed in a way which cannot be operated without agitation and the agitation level used in this study was the minimum level attainable for the programmed sampler. The number of extraction strokes was determined by running the sampler with different strokes, and 20 strokes at 100µl/s were found to be giving detectable amount of volatiles. The other conditions such as syringe temperature maintained at 60°C desorption temperature at 230°C and trap cleaning at 270°C were recommended by the auto sampler manufacturer (CombiPAL, CTC Analytics, Switzerland). The volatiles identified in this study were compared with previous results, and found to be similar, but considerable time was saved compared to other methods.

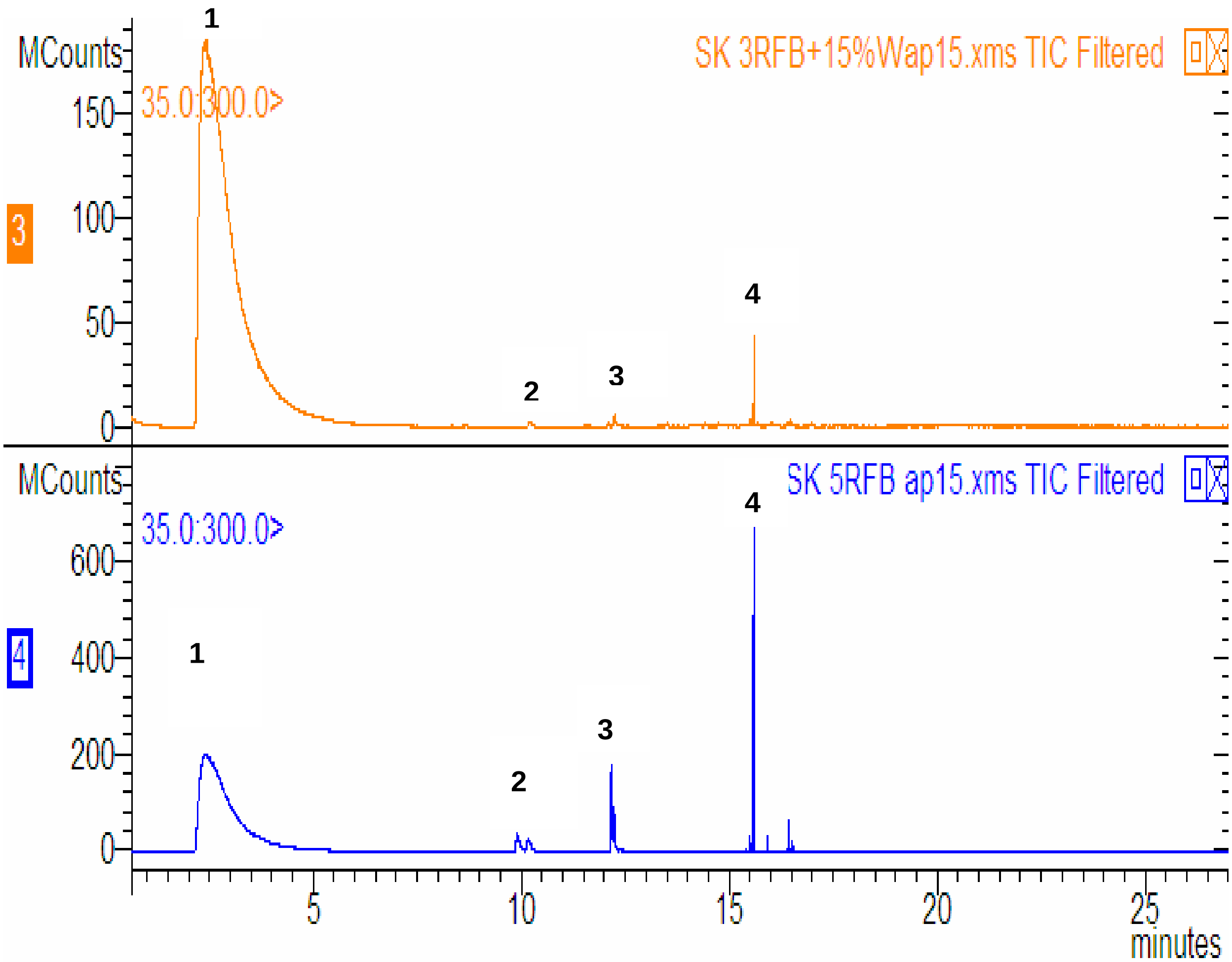


**Figure 1. Ion Chromatograms of the 15% moisture content wheat and three *T. castaneum* adult insects (SK 3RFB) and 15% moisture content wheat and five *T. castaneum* adult insects (SK 5RFB) 1=Carbondioxide, 2=methyl-1, 4-benzoquinone, 3=ethyl-1,4-benzoquinone, 4=Pentadecene.**

The amount of volatiles released by red flour beetles placed in a vial filled with 15 % moisture content wheat increased with an increase in number of insects. From Fig .1 and Table 1.we can see the amount of volatiles released by three red flour beetles was less than the volatiles released by the five red flour beetles. The volatile organic compounds released in abundance by red flour beetle were ethyl-1, 4-benzoquinone, methyl-1, 4-benzoquinone and 1-Pentadecene.The identity of these compounds were confirmed by comparing it with MS libraries (NIST/EPA/NIH/NIST 98). Hydrocarbon chain length was confirmed by matching their retention times with those of hydrocarbon standards (Sigma-Aldrich, St. Louis, MO, USA).

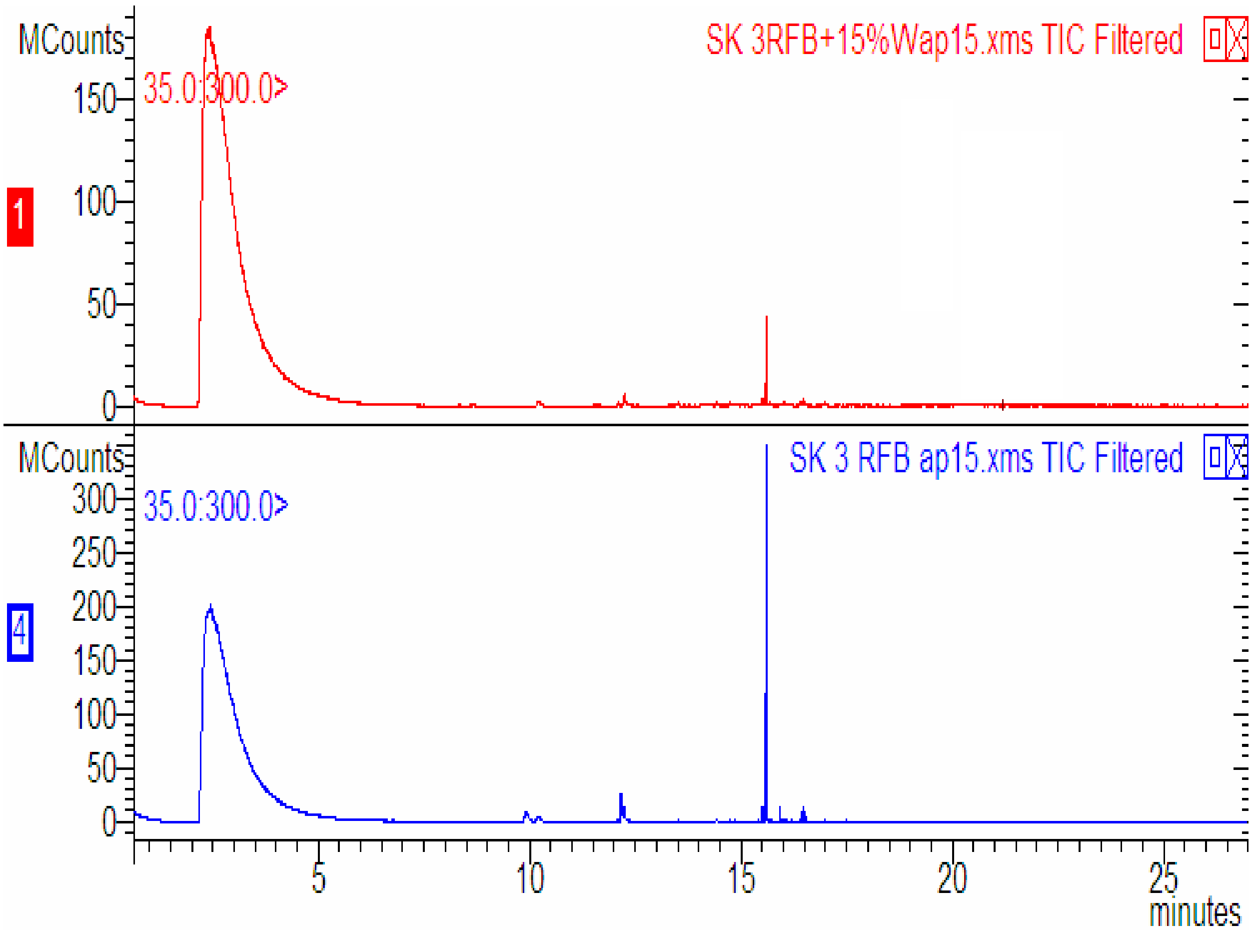


**Figure 2. Ion chromatograms of the 15% moisture content wheat and three *T. castaneum* adult insects immediately after transferring it to vials and after 1 h.**

The amount of volatiles generated by insects increased with the increase in time, three red flour beetles immediately after transferring them to the vials produced less volatiles than the volatiles collected after 1 h (Figure 2). The amount of volatiles increased may be because of the new wheat substratum from the previous wheat flour used for rearing the insects prior to this study and the agitation forced them to produce defensive secretions.

The detection insect infestation at incipient spoilage is still a major issue. A variety of analytical methods for detecting insects can be currently applied, but requires more labor and are often not cost effective. This is the first chemical method performed by automatic headspace sampler for detecting insects causing incipient spoilage.

**Table 1. Amount of volatiles of the three major volatile organic compounds detected by GC-MS in wheat with *T. castaneum* insects (ng)**

| ***T. castaneum*** | **Immediately after adults transferred it to the vial** | | | **After 1 h** | | |
|---|---|---|---|---|---|---|
| | **EBQ** | **MBQ** | **1Pentadecene** | **EBQ** | **MBQ** | **1Pentadecene** |
| Three adult insects | 17.5±2.1[a] | 18.4±6.7[a] | 38.3±5.5[b] | 30±2.5[b] | 34.2±2.2[b] | 52±6.2[c] |
| Five adult insects | 29.6±2.7[b] | 34.2±7.3[b] | 54.2±7.3[c] | 42±3.2[b,c] | 44±3.8[b,c] | 62±2.3[c] |

EBQ= ethyl-1, 4-benzoquinone; MBQ= methyl-1, 4-benzoquinone.

## CONCLUSION

The automatic headspace sampler is found be feasible and more rapid for collection of volatile organic compounds. The time taken to collect the volatiles was 20 min and within 48 min both collection and identification of volatiles was done. The volatiles generated by insects increased with an increase in number of insects and an increase in time. The volatiles specific to *T. castaneum* were found to be ethyl-1, 4-benzoquinone, methyl-1, 4-benzoquinone and 1- Pentadecene. We can conclude that the detection of insects by analyzing the headspace with the automatic sampler coupled with GC-MS could be a promising technique and by identifying the volatiles specific to insects will be helpful in constructing a sensor for detecting the incipient spoilage caused by insects in grain handling industry.

## ACKNOWLEDGEMENTS

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