# Stability of Uric Acid Used as an Indicator of Insect Contamination During Extrusion of Wheat Flour[1]

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## ABSTRACT

To determine the stability of uric acid during extrusion, wheat flours were spiked with uric acid such that the samples had a uric acid content of approximately 25 µg of uric acid per gram of flour. The samples were then extruded on a Brabender single-screw extruder at feed moisture contents of 28, 30, and 32% (db) and temperatures of 120, 140, and 160°C. Uric acid in the extrudates was quantified by reversed-phase high-performance liquid chromatography, using ion-pairing with tetrabutylammonium phosphate and ultraviolet detection. It was found that 62–80% of the uric acid in flour survived extrusion, depending on the extrusion conditions. On average, about 72% of the uric acid initially present was retained in extruded wheat flour. This high recovery level indicates that uric acid is relatively heat stable and could be used as an indicator of insect contamination in extruded products.


Uric acid, excreted by insects as the end product of nitrogen metabolism, has previously been proposed as an alternative to the insect fragment count as an indicator of insect contamination in grains and cereal products (Pachla and Kissinger 1977, Roy and Kvenberg 1981, Galacci 1983, Wehling and Wetzel 1983, Lamkin et al 1991). Good correlations between uric acid content and levels of infestation have been found in whole grain (Wehling et al 1984). However, little is known about the fate of uric acid during thermal processing. In order to use uric acid as a marker to indicate whether extruded products have been produced from insect-contaminated ingredients, it is necessary to examine the stability of uric acid during the extrusion process. It is also important to know the influence of different processing conditions on the retention of uric acid in the final product. Food extrusion is an important processing technique that can utilize a wide variety of raw materials and processing conditions. Food ingredients are subjected to high temperature, pressure, and shearing stress and therefore undergo physical and chemical changes that affect the quality factors of the extruded products. The retention of uric acid in extrudates may be affected by extruder variables and variables related to the raw material.

Food extrusion is becoming more popular as a processing technique that can be applied to many raw materials (Altschul and Wilcke 1985), and a number of extrusion applications have been reported by Cheftel (1986). Complete descriptions and advantages of extrusion processing have been written (Harper 1979, Linko et al 1981, Mercier et al 1989). Reviews of the effects of extrusion cooking on nutritional properties have been given by Cheftel (1986) and Björck and Asp (1983). They have reported that extrusion affects food product quality factors such as enzyme activity, as well as vitamin and amino acid availability in foods, depending on processing conditions.

Important processing parameters in extrusion that affect product quality include moisture content of the feed material, temperature, shear, residence time, screw speed and configuration, and die geometry. Many researchers have investigated the effects of extrusion variables on the physical properties of extrudates made from different materials (Fletcher et al 1985, Lawton et al 1985, Stanley 1986, Phillips and Falcone 1988). According to many published literature reports (Beetner et al 1976, Seiler et al 1980, Schlude 1987), the moisture content of the feed materials and the barrel temperature are the most important processing variables that affect the final characteristics of the extrudates. There is, however, little literature information on the chemical reactions occurring during extrusion processing and the quality implications related to these changes (Camire et al 1990).

Limited information is available about the thermal stability of uric acid. The *Handbook of Chemistry and Physics* (Anonymous 1991) reports that uric acid decomposes upon heating; however, it does not specify the temperature at which decomposition takes place. The 1-methyl derivative of uric acid, a closely related organic compound, has a melting point of 400°C. This high stability of a uric acid derivative to heat indicates that uric acid may, to a degree, survive cooking temperatures. The objective of this work was to evaluate the survival of uric acid during extrusion using conditions commonly encountered in processing operations. Barrel temperature and feed moisture content were varied within typical ranges. This provided information on the survival of uric acid during thermal processing and indicated whether uric acid survived to a sufficient degree to be useful as a marker of insect contamination in processed foods.

## MATERIALS AND METHODS

### Materials

Three packages of all purpose wheat flour, each 4.53 kg and bearing different lot numbers, were purchased from local markets and used for sample preparation. Each individual package was used as a replicate.

### Sample Preparation

Uric acid (0.045 g, Sigma Chemical Co., St. Louis, MO) was accurately weighed and added to 1,800 g of flour, such that the sample contained about 25 µg of uric acid per gram of flour. This amount of flour provided enough sample to split and prepare feed materials at different moisture contents. The flour was then placed into a rotating V-shaped blender (Patterson-Kelly Co., East Stroudsburg, PA) for 48 hr to ensure uniform distribution of uric acid in the flour. Three replicated samples were made using this procedure. To check the uniformity of uric acid in the flours, two random samples of each replicate were analyzed for moisture and uric acid content. The actual uric acid levels on a dry basis were 22.98 ± 0.11 µg of uric acid per gram of flour for the first replicate, 24.70 ± 0.04 µg/g for the second replicate, and 22.22 ± 0.28 µg/g for the third replicate.

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### Extrusion

The moisture contents of flours were determined in duplicate by AOAC procedure 14.004 (AOAC 1984). Flours were then adjusted to 28, 30, and 32% moisture (db) by spraying with calculated amounts (g) of distilled water in a fine mist. The flours were blended with the water using a Hobart mixer (model C-100, Hobart Corp., Troy, OH). Samples were then extruded with a Brabender (model 2003 GR-8) single-screw laboratory extruder (C.W. Brabender Instruments, Inc., Hackensack, NJ). The screw had a 381-mm length and 19-mm diameter (20:1 length-to-diameter ratio), with only one mixing zone. The die was a rod type that produced bread stick-type products. The screw speed was 120 rpm, and the extruder was fed manually. The experiment was conducted at barrel temperatures of 120, 140, and 160°C for each moisture content. All other extrusion parameters were held constant throughout the experiment, which was replicated three times.

### Extrudate Analysis

Randomly chosen pieces of extrudates from each run were air dried overnight and ground to fine particle sizes using a Janke and Kunkel grinder (model A-10, Tekmar Co., Cincinnati, OH), before high-performance liquid chromatography (HPLC) analysis. Uric acid was extracted from both flours and extrudates using a previously described technique (Wehling and Wetzel 1983). Chromatographic separation of uric acid was achieved by the ion-pair HPLC method of Wehling and Wetzel (1983), as modified by Ghaedian and Wehling (1996). The mobile phase used a 95:5 water-methanol ratio. The moisture contents of the ground extrudates were determined in duplicate, using AOAC method 14.004 (AOAC 1984).

### Statistical Design

The experiment was conducted with a strip split-plot for a 3×3 treatment design. Treatments were barrel temperature at levels of 120, 140, and 160°C and feed moisture content at levels of 28, 30, and 32%. The subunits of the split plot were in strips. Moisture content was controlled by mixing wheat flour with calculated amounts of distilled water, which accounted for one strip. The other strip involved the barrel temperature of the extruder. The experiment was repeated three times. Significant differences at the 5% level of probability were tested by analysis of variance computed by a Statistical Analysis System (SAS 1986) computer program with the general linear model procedure. Comparisons of means ($P < 0.05$) were made using Fisher's protected least significant difference test (Steele and Torrie 1980).

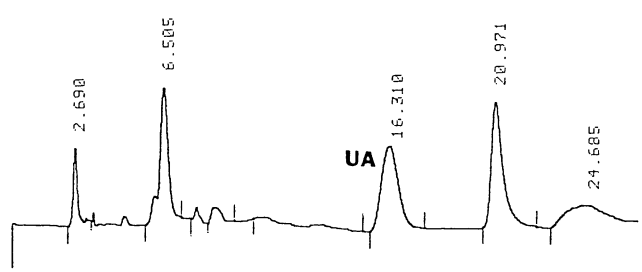


**Fig. 1.** Chromatogram of extract obtained from extruded wheat flour contaminated with uric acid (UA).

**TABLE I**
**Mean Percent Uric Acid Retention and Standard Deviation in Extrudates Produced at Different Barrel Temperatures (°C) and Feed Moisture Contents**

| Temp. (°C) | MC % | Retention (%) | Standard Deviation |
|---|---|---|---|
| 120 | 28 | 65.1 | 2.2 |
| | 30 | 71.3 | 5.6 |
| | 32 | 68.8 | 3.7 |
| 140 | 28 | 69.1 | 4.6 |
| | 30 | 72.5 | 5.4 |
| | 32 | 74.1 | 1.9 |
| 160 | 28 | 72.1 | 7.3 |
| | 30 | 76.6 | 2.1 |
| | 32 | 73.9 | 3.2 |

## RESULTS AND DISCUSSION

### Preliminary Work

To establish a working range of moisture contents for wheat flour and a range of barrel temperatures for the extruder, several extrusion trials were performed using wheat flour with moisture levels ranging from 16 to 32% (db) and a temperature range of 120–160°C. Extrusion processing using 16–26% feed moisture resulted in extrudates that were dark in color, tough in texture, and not typical of most extruded products. As the water content of the wheat flour was increased, the color of the extrudates became brighter and the hardness decreased. Moisture levels of 28, 30, and 32% were selected as the optimum moisture contents of wheat flour for the experiment.

Initially, batches of wheat flour with a uric acid content of about 25 μg/g of sample, with moisture levels of 28 and 32% (db), were extruded at 120°C. The extrudates were then analyzed for uric acid. This method was used to determine whether uric acid is stable under minimal extrusion temperatures. After finding that about 70% of uric acid survived a temperature of 120°C, the effect of higher temperatures (140 and 160°C) on the stability of uric acid was also investigated.

### Stability of Uric Acid

The nine different extrudates from each replicate were analyzed for uric acid using the previously described HPLC technique. Two extracts from each extrudate were subjected to HPLC analysis, with duplicate injection of extracts onto the chromatographic column. A representative chromatogram of an extrudate is presented in Figure 1. The peak areas from the integrator were converted to micrograms of uric acid per gram of sample by comparison with standards. The uric acid content of each extrudate was expressed on a dry basis, and this value was divided by the initial uric acid concentration of the flour to calculate the percent uric acid retained after processing.

Table I shows the mean percent uric acid retention and standard deviation resulting from each set of processing conditions. The

**TABLE II**
**Analysis of Variance for Percent Uric Acid Retention in Contaminated Wheat Flour Extrudates**

| Variance Sources[a] | Degrees of Freedom | Mean Square | *P* Values |
|---|---|---|---|
| Rep | 2 | 51.48 | |
| Temp | 2 | 76.23 | 0.029*[b] |
| Temp × Rep (error a) | 4 | 7.90 | |
| MC | 2 | 53.24 | 0.266ns |
| MC × Rep (error b) | 4 | 28.40 | |
| Temp × MC | 4 | 5.22 | 0.952ns |
| Error c | 8 | 32.53 | |

[a] Rep = replicate, Temp = temperature, MC = moisture content.

[b] * = Significant at the 0.05 level of probability, ns = not significant at the 0.05 level of probability.

**TABLE III**
**Mean Percent Uric Acid Retention of Contaminated Wheat Flour Extrudates as Affected by Barrel Temperature**

| Temperature, °C | Retention (%) |
|---|---|
| 120 | 68.4 a[a] |
| 140 | 71.9 ab |
| 160 | 74.2 b |

[a] Means not followed by the same letter differ ($P < 0.05$).

uric acid retention ranged from 65.1 to 76.6%. Table II presents the results of the analysis of variance for percent uric acid retention in the extrudates as affected by the extrusion variables. Analysis of variance revealed that temperature was the only variable that affected ($P < 0.05$) the retention of uric acid in the extrudates. Feed moisture did not significantly affect ($P > 0.05$) retention of uric acid within the range tested. Uric acid is relatively insoluble in water (Lehninger 1979); therefore a small increase in the water content of wheat flour did not change the retention of uric acid in extrudates. No interaction was found ($P < 0.05$) between temperature and moisture content.

The mean percent uric acid retention of the extrudates as affected by the heat treatment is shown in Table III. A difference ($P < 0.05$) was found in the percent uric acid retained in the extrudates processed at 120°C when compared with the uric acid retained in extrudates processed at 160°C. The highest retention of uric acid (74.2%) was at 160°C, whereas the lowest retention (68.4%) was found at 120°C. This appeared at first to be illogical. However, the reason for the higher retention of uric acid at a higher temperature was attributed to differences in the residence time of the material at different temperatures. Chen et al (1991) reported that when corn meal was extruded (at temperatures of 100–200°C and moisture contents of 20–30%), a lower temperature at any given moisture content caused the feed to become more viscous and increased the residence time. Therefore, longer exposure to heat at a lower temperature must have been more effective in degrading uric acid than higher temperature with shorter residence time.

The results of this study show that uric acid in contaminated wheat flour can survive heat to a relatively high degree. On average, about 72% of uric acid was retained in extruded wheat flour. This high retention level indicates that uric acid is relatively stable at temperatures commonly encountered in processing operations and could be used as an indicator of insect contamination in extruded products. The degradation of uric acid in heat-processed food would depend on the level of temperature and the extent of heating of the contaminated food. The results presented here provide information that can be used as a basis for future investigations of extrusion and other thermal processes. Studies involving a wider range of temperature, as well as close measurement of the residence time of materials, are suggested.

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