# Technical

# Microwave Inactivation of Thioglucosidase in Intact Crambe Seeds[1]

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## ABSTRACT

Optimum conditions for microwave inactivation of thioglucosidase in whole crambe seeds (*Crambe abyssinica* Hochst.) were investigated. Whole crambe seeds were left at 7% moisture or tempered to between 10 and 20% moisture contents prior to exposure to various microwave power inputs for 38 sec under controlled conditions. Ten percent moisture crambe seeds coupled the maximum percentage of microwave power. The amount of microwave energy required to inactivate thioglucosidase decreased as the moisture content of the seeds increased from 10 to 20%. Thioglucosidase could not be inactivated in 7% moisture seeds without burning the seeds. With 10, 15, and 20% moisture levels crambe seeds required a minimum of 0.70, 0.60, and 0.65 kW, respectively, of initial microwave power input and 18,12, and 11 kJ, respectively, of coupled energy to inactivate thioglucosidase. Optimum crambe seed moisture contents for microwave inactivation of thioglucosidase were determined to be between 14-16%.

## INTRODUCTION

*Crambe abyssinica* Hochst., a member of the Cruciferae, is under investigation as a new oilseed crop. The oil, which makes up roughly one-third of the seed weight, is high in erucic acid (53-59%). The refined oil has been used as a mold lubricant in steel casting, and the isolated erucic acid can be further processed to manufacture various plastic films and nylon fibers (1). Considering this favorable demand for crambe oil, the defatted meal must be thought of as a by-product from the extraction process even though more than 1.25 tons of hull-free meal are produced per ton of oil (2).

Fat-free, dehulled crambe meal contains 49-55% protein (3) with a well-balanced amino acid composition that is favorably high in lysine and the sulfur-containing amino acids (1,4,5). However, crambe seeds contain glucosinolates that when hydrolyzed by an endogenous enzyme, thioglucosidase, produce toxic compounds which limit palatability to animals and render the meal toxic to nonruminants (6). Various processing methods have been developed to detoxify crambe meal by either decomposing the glucosinolates (2,7,8) or by inactivating thioglucosidase and removing the intact glucosinolates by aqueous extraction (9-11). Both dry and moist heat have been used to inactivate thioglucosidase (12). In preliminary trials, microwave inactivation of thioglucosidase was accomplished in individual crambe seeds (13). For these initial studies, samples were manually moved through either a Litton 500 or 850 home-type microwave oven. Since results differed between the two ovens, the need to determine, under more controlled conditions, the effect of microwave heating on thioglucosidase inactivation became apparent. Accordingly, in our study, laboratory experiments were carried out to determine the optimum conditions for microwave inactivation of thioglucosidase in whole crambe seeds.

## EXPERIMENTAL PROCEDURES

### Materials

Equal blends of three crambe seed varieties (Prophet, Meyer, and Indy) grown at the Purdue University Agronomy Farm, West Lafayette, IN, were used in all experimental work. On a dry basis the blended seeds contained 27.0% crude protein (N x 6.25), 28.6% crude fat, 5.2% ash, and 7.0% total glucosinolate.

### Microwave Equipment

The microwave system, as shown in Figure 1, was adapted from one developed by Vetter and co-workers (14) for microwave research. The generator (HI-1200, Holaday Industries, Hopkins, MN) produced 0.2 to 1.5 kW of 2450 MHz continuous wave microwave radiation which was transmitted to the sample chamber by a waveguide (WR-284). A constant fraction of forward power was extracted from the waveguide by a bi-directional coupler (HI-1001-X, Holaday Industries) to regulate the output of the generator. A triple stub tuner (HI-1004, Holaday Industries) minimized reflection and allowed for maximum transfer of power from the generator to the sample chamber. The sample chamber (HI-1020, Holaday Industries) enclosed a rotating platform (approximate speed of 5 rpm) upon which was placed a round Kimax glass dish (4.25 cm high x 8.25 cm in diameter). This sample container was always filled level to the top with ca. 45 g whole crambe seeds. A single arm directional coupler (4452-30, Sperry Microwave Electronics Co., Clearwater, FL), along

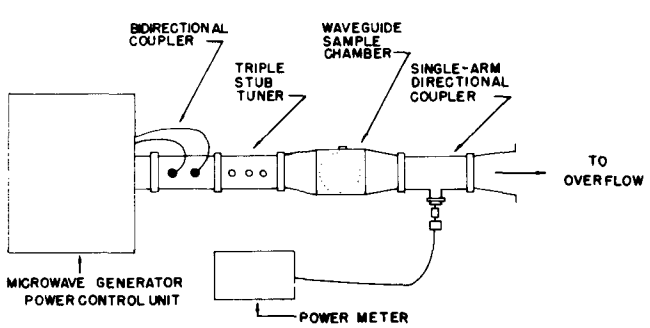


FIG. 1. Schematic diagram of microwave unit.

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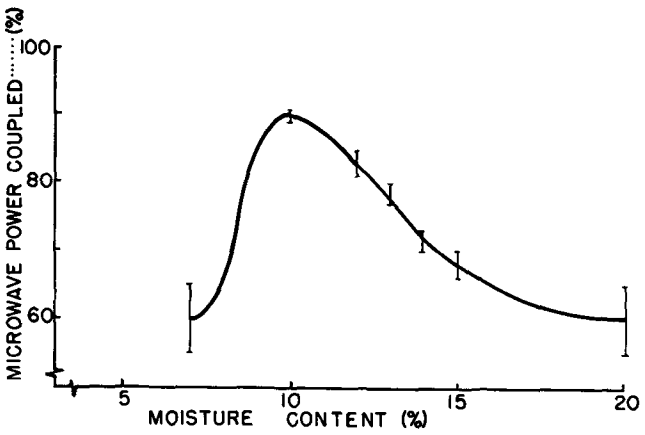


FIG. 2. Percent microwave power coupled (independent of power input and time) by whole crambe seed samples at various moisture contents (I = range of values).

with the power meter (432A, Hewlett Packard), measured the amount of power that bypassed the sample.

*Heating patter of crambe seeds in sample container:* The sample container was filled with whole crambe seeds (15% m.b.) and exposed to microwaves for 5 sec. The heating pattern of the seeds was recorded by laying a liquid crystal sheet on the surface of the seeds and photographing the color pattern displayed by the liquid crystals.

*Measurement of energy coupled by crambe seed samples:* For each sample exposed to microwaves the power transmitted to and past the sample was recorded. The amount of energy actually coupled by the sample was calculated from Equations I and II.

$$\text{Actual microwave power coupled (kW)} = \text{75\% initial microwave power input (kW)} - \text{Microwave power transmitted past sample (kW)} \quad \text{(I)}$$

$$\text{Energy coupled (kJ)} = \text{Actual microwave power coupled (kW)} \times \text{Exposure time (sec)} \quad \text{(II)}$$

In Equation I the 75% initial microwave power input term allows for the approximately 25% of initial microwave power input reflected back to the generator. The amount of reflected power was determined by measuring the incident, reflected, and transmitted power with a Hewlett Packard Model 432A meter when the microwave system was loaded with seeds at 7 to 20% moisture content.

*Temperature measurement of crambe seed samples:* Crambe seed samples were removed from the sample chamber and the temperature was determined within 15 sec with a tubular stainless steel temperature probe (usable to 150 C) placed 1 cm from the circumference of the container.

### Analytical Methods

*Tempering crambe seeds:* Water was added to whole crambe seeds (7% m.b.) ca. 14 hr before microwave treatments. AACC method 44-19 (15) was followed to analyze initial and final moisture contents of the seeds.

*Total glucosinolate content:* Glucosinolate content was determined by modifying the procedure of Van Etten and Co-workers (16). Crambe seeds were ground in a Tekmar micro-mill for 15 sec. To 1 g of this meal in a 50 ml Erlenmeyer flask, 19 ml distilled water at 35 C plus 1 ml aqueous thioglucosidase solution containing 5 mg/ml of the crude enzyme was added. Flasks were held in a 35 C constant temperature water bath shaker and agitated for 50 min. Glucose content of the aqueous extract was measured with a Beckman Glucose Analyzer. Percent total glucosinolate was calculated using Equation III (16):

$$\%\ \text{total glucosinolate} = \frac{\text{glucose weight} \times 2.37}{\text{sample weight}} \times 100 \quad \text{(III)}$$

Crude thioglucosidase was prepared from mustard seed as described by Wrede (17).

*Thioglucosidase activity:* Relative thioglucosidase activity was determined on Tekmar micro-mill ground (15 sec) full fat seeds after microwave exposure. A 5 g sample was measured into a 250 ml beaker and placed in a 35 C constant temperature water bath shaker. One hundred milliliters of distilled water at 35 C was added. This mixture was agitated for 50 min. At 10 min intervals 1 ml of extract was transferred to 2 ml of 0.3 N NaOH in a test tube to be deproteinized according to Somogyi (18). After the precipitate settled the supernatant was analyzed for glucose with a Beckman Glucose Analyzer. Zero time values were otbained by mixing 50 mg of meal directly with the 2 ml of 0.3 N NaOH then adding 1 ml distilled water and following the same procedure as above. If a plot of glucose formed versus time gave a horizontal line, the enzyme was termed inactive.

## RESULTS AND DISCUSSION

### Selection of Optimum Microwave Parameters

To determine optimum conditions for inactivation of

TABLE 1

Thioglucosidase Inactivation Profiles for Whole Crambe Seeds at Various Moisture Levels

| Power input[a] (kW) | Moisture content (%) 7 E.C. | 7 Enz | 10 E.C. | 10 Enz | 12 E.C. | 12 Enz | 14 E.C. | 14 Enz | 15 E.C. | 15 Enz | 20 E.C. | 20 Enz[6] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.50 | 9 | + | 13 | + | 12 | + | 10 | + | 10 | + | 9 | + |
| 0.55 | 9 | + | 14 | + | 13 | + | 10 | + | 11 | + | 9 | + |
| 0.60 | 10 | + | 15 | + | 14 | + | 11 | + | 12 | - | 10 | + |
| 0.65 | 11 | + | 17 | + | 15 | + | 12 | - | 13 | - | 11 | - |
| 0.70 | 12 | + | 18 | - | 17 | - | 14 | - | 14 | - | 12 | - |
| 0.75 | 13 | + | 19 | -[c] | 18 | -[c] | 15 | - | 15 | - | 13 | - |
| 0.80 | 14 | + | | | | | 16 | -[c] | 16 | -[c] | 14 | - |
| 0.85 | 15 | + | | | | | | | | | 15 | - |
| 0.90 | 15 | -[c] | | | | | | | | | | |

[a]Exposure time 38 sec.

[b]E.C. = energy coupled (kJ); Enz = Enzyme activity.

[c]Seeds were burned.

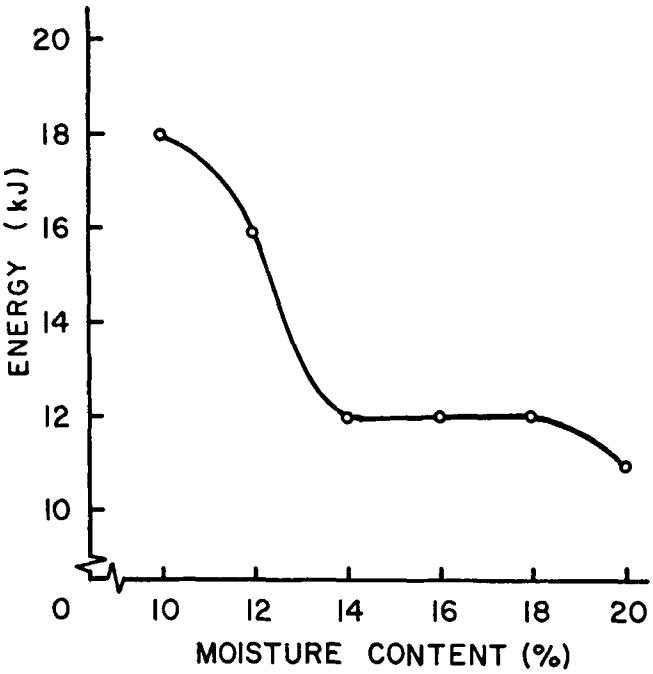


FIG. 3. Minimum amount of energy that must be coupled by whole crambe seed samples at various moisture contents to inactivate thioglucosidase.

thioglucosidase with microwave power certain parameters were studied: (a) microwave exposure time; (b) total microwave power input; and (c) moisture levels to which whole crambe seed samples were tempered prior to microwave exposure.

*Heating pattern of crambe seeds exposed to microwaves:* When crambe seed samples were exposed to microwaves for one complete revolution of the sample container, symmetrical heating of the seeds resulted. The temperature was 32 C in the central portion of the container with a 1.25 cm peripheral zone of narrow even rings gradually decreasing to 29 C around the periphery. Thus, uniform and reproducible heating conditions were achieved in contrast to the uneven heating observed in a conventional home-type microwave oven. The importance of maintaining sample movement to obtain even heating with microwaves has been emphasized (19,20).

*Power coupled by crambe seeds:* Figure 2 shows the summary of percent microwave power coupled by whole crambe seeds at different moisture levels regardless of initial power inputs. The percentage of microwave power coupled by the seeds was dependent on their moisture content. Maximum microwave power (90%) was coupled when seeds contained 10% moisture.

*Energy coupled by crambe seeds:* Preliminary microwave runs using 15% moisture content seeds in which exposure time and initial microwave power input varied indicated that thioglucosidase could be inactivated with either 25 or 38 sec of microwave exposure when 12 kJ or more energy coupled with the seeds. With a 25 sec exposure time, maximum power outputs of the microwave unit had to be employed to provide the required minimum of 12 kJ coupled energy. Whereas, with a 38 sec exposure time a wider range of power input levels resulted in the 12 kJ or more of energy required. Thus, 38 sec was the exposure time used in all remaining experiments.

*Effects of crambe seed moisture content on thioglucosidase inactivation:* First, an attempt was made to duplicate the successful microwave inactivation of thioglucosidase reported by Lessman and Kirleis (13). Crambe seeds (7% m.b.) with no added moisture were exposed to increasingly higher microwave power levels up to 0.90 kW for 38 sec, but the seeds burned before thioglucosidase was inactivated (Table I). At this low moisture content the microwave energy required to inactivate thioglucosidase was absolutely critical and too difficult to reproduce.

TABLE II

Whole Crambe Seed Temperatures and Moisture Contents after Exposure to Various Microwave Power Inputs for 38 sec

| Initial microwave power input (kW) | Seed moisture (%) Initial | Final | Seed temperature attained (C) | Enzyme activity |
|---|---|---|---|---|
| 0.60 | 7 | 5.0 | 81 | + |
| 0.70 | | 4.6 | 112 | + |
| 0.80 | | 3.7 | 121 | +[a] |
| 0.65 | 10 | 7.8 | 100 | + |
| 0.70 | | 5.9 | 110 | - |
| 0.75 | | 5.5 | 120 | -[a] |
| 0.55 | 15 | 12.9 | 90 | + |
| 0.60 | | 12.6 | 95 | - |
| 0.65 | | 12.5 | 96 | - |
| 0.70 | | 10.0 | 97 | - |
| 0.55 | 20 | 16.5 | 89 | + |
| 0.60 | | 16.0 | 90 | + |
| 0.65 | | 14.9 | 95 | - |
| 0.70 | | 14.6 | 96 | - |

[a]Seeds were burned.

To determine what seed moisture would be optimum for thioglucosidase inactivation by microwaves, enzyme inactivation profiles were obtained for seeds ranging from 10 to 20% moisture levels (Table I). The minimum amount of energy absorbed by the seeds to inactivate the enzyme as related to moisture content is shown in Figure 3. For moisture levels below 14%, up to 18 kJ was required to inactivate thioglucosidase. Between 14 and 18% moisture 12 kJ of energy was necessary to inactivate the enzyme, while at moistures greater than 18% only 11 kJ were required. All this indicates that thioglucosidase inactivation with microwaves is a function of seed moisture content as well as the amount of power input.

As seen in Table I, moisture content of the crambe seeds also influenced the working range of microwave power input levels that could be used for thioglucosidase inactivation. For seeds with 10 or 12% moisture, only one power level (0.70 kW) would inactivate the enzyme; that is, one power level above resulted in burned seeds and one level below failed to inactivate the enzyme. However, as the moisture content of the seeds was increased to 15% and 20%, the range of microwave power that would inactivate widened to 0.60 to 0.75 kW and 0.60 to 0.85 kW, respectively.

These findings suggest that 14 to 16% moisture content seeds are optimum for microwave inactivation of thioglucosidase because: (a) a wide range of microwave power inputs can result in enzyme inactivation; (b) in comparison to most other seed moistures, the 14 to 16% range requires relatively lower energy inputs; and (c) the seeds store well without further drying.

*Type of heat necessary for thioglucosidase inactivation:* Table II shows temperatures attained within crambe seed samples after microwave exposure. For moisture levels of 15% or greater, thioglucosidase seemed to be inactivated when a temperature of 95 C was reached. Since natural crambe seeds (7% m.b.) with no added moisture did reach temperatures greater than 100 C when exposed to microwaves, moist heat (e.g., tempered seeds greater than 14% moisture + microwave-generated heat) seemed to be necessary for enzyme inactivation. This explains the failure of Eapen et al. (12) to inactivate thioglucosidase in rapeseed with dry heat.

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