

Effect of Moisture Content on Flavor Formation in a Propylene Glycol-based Model System in a Microwave Oven

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Over the past few decades the use of microwave ovens has increased dramatically.¹ It has been indicated that as many as 90% of U.S. homes presently own at least one microwave oven.² This explosion has resulted in a greater demand for new food products that can be microwave processed.

However, foods prepared by using a microwave oven usually generate less desirable flavors and browning than a conventional oven.³ In comparing heating methods used to bake a basic white cake, Whorton and Reineccius⁴ found that microwave-processed cakes had lower levels of isopentanal and furfural than cakes baked conventionally. MacLeod and Coppock⁵ reported that when the volatiles from boiled beef were compared on the basis of doneness, the microwaved beef contained only one third of the amount of volatiles in the conventionally processed beef. The short cooking time and low temperatures common to microwave processing usually do not promote the Maillard reaction, which is responsible for the production of many flavor compounds.⁶

One of the factors being considered in the development of new microwaveable food products is the use of flavor compounds and browning agents.⁷ A possible vehicle for flavor/browning

agent is propylene glycol.⁸ Preliminary work has shown good browning to occur using various amino acids, such as proline, with xylose in a propylene glycol medium.⁹

Xylose was chosen since the industry would prefer to use pentoses due to their higher reactivity in the Maillard reaction.¹⁰ Proline is important as a precursor for the flavor in baked goods. According to its secondary amine structure, during heating with monosaccharides proline forms specific Maillard products which are not observed with primary α -amino acids.¹¹ This is attributed to the blocked transamination during Strecker degradation that prevents the formation of pyrazines and pyrroles but instead forms the reactive Strecker products such as pyrrolidine and 1-pyrroline.¹² These products give a bread/cereal aroma and bitter taste to boiled, baked and roasted foods as characterized in proline/monosaccharide model experiments.¹³⁻¹⁸

We wanted to investigate the potential of propylene glycol to act as part of a microwaveable flavor system. Since water is a component in all food systems and affects the Maillard reaction, water was chosen to be the independent variable. So the specific objective of this study was to investigate the effect of moisture content on the flavor profile as generated by a microwave oven

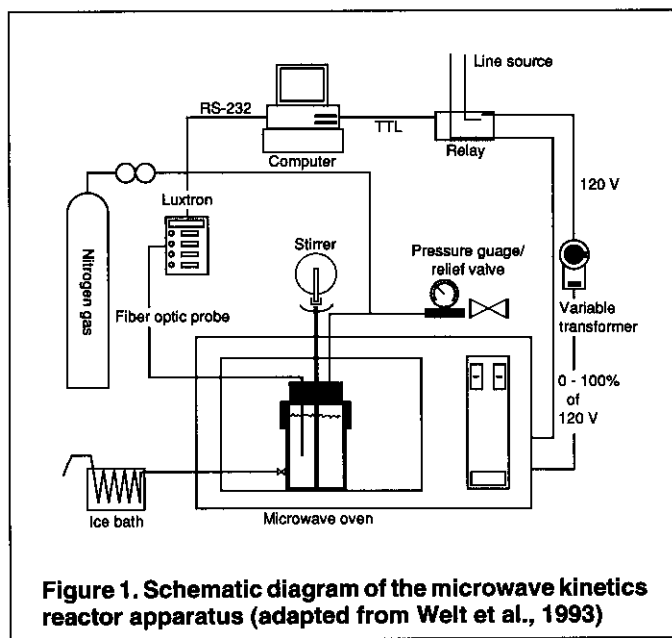


Figure 1. Schematic diagram of the microwave kinetics reactor apparatus (adapted from Welt et al., 1993)

using a model system consisting of proline and xylose as reactants and propylene glycol as the reaction medium.

Materials and Methods

Material: *l*-Proline, propylene glycol and nonadecane were purchased from the Sigma Chemical Co. (St. Louis, Missouri). *d*-Xylose was purchased from Aldrich Chemical Co. (Milwaukee, Wisconsin). Methylene chloride was HPLC grade from Fischer Scientific Co. (Springfield, New Jersey).

Apparatus: The apparatus consists of two major components, a modified microwave oven with a feedback temperature control system, and a microwave kinetics reactor (MWKR). A schematic of the apparatus is shown in Figure 1. The temperature feedback control system was described by Tong et al.,¹⁹ however, a larger capacity oven rated at 900 watts (Model MQS 1403W, Quasar Co., Elk Grove, Illinois) was modified and used in this study. The operation of the feedback control mechanism was demonstrated by Welt et al.²⁰ and showed that the feedback temperature control system was capable of maintaining a desired set-point temperature to within $\pm 0.5^\circ\text{C}$.

A detailed description of the design and operating characteristics of the MWKR was provided by Welt et al.²¹ Briefly, the MWKR is a completely sealed microwaveable

pressure vessel made from ULTEM 1000 (GE Plastics, Pittsfield, Massachusetts). The vessel was designed with penetrations equipped with Teflon® compression fittings to provide access for a fiber optic probe, pressure gauge/over-pressure safety relief valve, and a specially designed sampling port. For this experiment, one large anchor-type impeller was used for mixing such that it would scrape the floor and the walls of the vessel.

A Stedfast laboratory stirrer (Model 14-505-1, Fisher Scientific, Springfield, New Jersey) was mounted above the microwave oven and connected to the drive shaft through a hole drilled through the roof of the oven.

The sampling port consisted of a Teflon "T"-type fitting and a custom ULTEM 1000 screw-on plunger. The plunger was designed so that as it unscrewed from the Teflon fitting, the plunger would be drawn beyond the 90° side (outlet) of the "T", allowing the contents of the vessel to flow through a Teflon tube connected to the outlet side of the sample port "T". This tube ran through a hole drilled in the wall of the microwave oven and was connected to a 6-ft. coiled stainless tube by a Teflon compression fitting. The coil was placed in an ice water bath. An Erlenmeyer flask collected the sample from the coil and also sat in an ice water bath. The top of the flask was covered with two layers of parafilm to act as a septum through which the end of the coil projected. To facilitate the rapid removal of sample, a Teflon "T" was introduced to the connection to the pressure gauge to which a 0.003175m (1/8 in.) O.D. Teflon tube was connected coming from a pressurized tank of nitrogen.

Sample preparation: *l*-Proline was analytically weighed and dissolved in propylene glycol in a volumetric flask by mechanical stirring for 24 h to give a 0.02 M solution. Samples with 0, 2.5, and 5% moisture content were prepared by replacing portions of the propylene glycol with different amounts of deionized distilled water. *d*-Xylose solutions were prepared to a concentration of 0.02 M in the same manner. An internal standard of nonadecane was prepared by dissolving 0.0383 g in 100 ml methylene chloride.

The initial temperature of the reactions was maintained at room temperature. Before each experiment, the optical fiber temperature measurement system was calibrated at 100°C with boiling distilled water. On an analytical balance, 200 g each of proline and xylose solution were weighed directly into the vessel to give a final concentration of 0.01 M for each reactant. The probe and all the tubes were attached to the vessel and the mechanical stirrer was adjusted to give a rotation rate of 240 rpm. The temperature for all reactions was 130°C and trials were performed at 0%, 2.5% and 5% moisture contents. The reaction proceeded for a duration of 10 min. after the set point temperature was attained.

Upon termination of reaction, the valve to the nitrogen tank was opened and the sample port was unscrewed to allow rapid purging of the sample into the recovery flask.

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The cooling system permits the lowering of sample temperature to approximately 35-40°C. The sample was then transferred to a Qorpak bottle (from Fischer Scientific Co.) equipped with a Teflon-lined cap and stored at -20°C until volatile extraction.

To a 100 g portion of the sample, 1 ml of internal standard (0.383 mg nonadecane) was added along with 1000 ml of saturated salt solution. This mixture was then adjusted to pH 8 with 2N NaOH to enhance the extraction efficiency of nitrogen-containing heterocyclic compounds.²³ It was then extracted three times with methylene chloride and the extract was then washed three times with saturated salt solution and then dried over anhydrous sodium sulfate for 12 h.

The extract was concentrated to approximately 6-8 ml by Koderna-Danish concentrator with a 50°C water bath. The extract was further concentrated to a suitable amount and stored at -20°C in a vial fitted with a Teflon-lined cap.

GC/Mass spectrometry: The volatiles were separated using a Varian 3400 gas chromatograph equipped with a non-polar fused silica capillary column (DB-1, 60 m, 0.32 mm i.d., 0.25 µm film thickness, J&W Scientific, Folsom, California). The GC was operated with an injection temperature of 250°C, a helium carrier gas flow rate of 1.0 ml/min, and a split ratio of 10:1. A volume of 1.0 µl was injected with a septum purge. The column was temperature programmed from 40°C (5 min. hold time) to 280°C at a rate of 2°C/min. and held for 55 min. at the upper limit.

The separated volatiles were then detected and identified with a Finnigan Mat 8230 high-resolution double focusing mass spectrophotometer using a GC/MS interface line temperature of 280°C. The ionization (EI mode) was set at 70 eV and the source temperature was 250°C with a filament emission current of 1 mA. All mass spectra were background subtracted and identified by comparison to previously published spectra on proline-specific compounds.^{11,18,21,24}

Gas chromatography: The GC analyses were accomplished using a Varian 3400 gas chromatograph equipped with a non-polar fused silica capillary column (DB-1, 60 m, 0.32 mm i.d., 1.0 µm film thickness, J&W Scientific, Folsom, California). The injector and detector temperatures were 270°C and 300°C, respectively, with a split ratio of 50:1. The program for volatile separation was as follows: initial temperature of 40°C with a 5 min. hold and then a temperature increase of 2°C/min. to a final temperature of 280°C with a 55 min. hold. Helium was used as gas carrier with a flow rate of 1.0 ml/min.

The GC experiments utilized a flame ionization detector (FID) and chromatograms were recorded and processed using a Varian 4270 integrator. The volatiles were quantified from GC analyses by comparing the GC profile with that from the GC/MS and then matching the identified peaks.

Results and Discussion

The flavor profile of a proline/xylose mixture was studied by microwave heating in the MWKR vessel of a 400 g solution of 0.01 M proline and 0.01 M xylose in propylene glycol at moisture contents of 1, 2.5 and 5%. Due to the hygroscopic nature of propylene glycol, the actual moisture content of the solutions may be higher than the amount of water added. The reaction was initiated at room temperature and heated to 130°C where it was then held for 10 minutes. Overall, the aroma of the 0% sample was described as baked cracker, baked taro, nutty and burnt. The 2.5 and 5% samples were the same but progressively weaker. The colors of the samples were reddish-brown, very pale golden and clear for 0, 2.5 and 5% moisture

Table I. Gas chromatographic data for identified compounds generated by the microwave heating of 0.01 M proline and 0.01 M xylose in propylene glycol (10 min. at 130°C)

	Moisture content (% H ₂ O)					
	0		2.5		5	
	Retention time (min.)	Area count	Retention time (min.)	Area count	Retention time (min.)	Area count
(1) 1-formyl pyrrolidine	52.56	4359	52.44	706	52.48	348
(2) 5-formyl-2,3-dihydro-1H-pyrrolizine	74.71	9024	74.17	6731	74.20	2053
(3) 5-acetyl-2,3-dihydro-1H-pyrrolizine	79.53	66811	79.22	7902	79.31	6226
(4) 5-formyl-6-methyl-2,3-dihydro-1H-pyrrolizine	85.46	8944	85.15	2287	85.27	1085
(5) 2-methyl-5,6,7,8-tetrahydroindolizine-8-one	89.28	4605	88.26	1024	88.92	626
(6) 5-acetyl-6 or 7-methyl-2,3-dihydro-1H-pyrrolizine	89.62	3294	89.30	1118	89.34	2044
(7) 7-formyl-5-methyl-2,3-dihydro-1H-pyrrolizine	91.38	2091	91.09	620	91.20	1154
(8) 3-ethyl-2-(1-pyrrol-dinyl)-2-cyclopenten-1-one	100.74	121728	100.38	1059	100.49	1720
(9) Nonadecane (Std)	113.46	83398	113.16	284744	113.38	558293

contents, respectively. Using the same solvent extraction procedure for each sample, the extracts were concentrated to the appropriate amount for GC/MS identification and GC semi-quantification.

Identification of proline-specific compounds: From the analysis of the mass spectrum generated for each sample,

eight proline-specific compounds were identified and are listed in Table I and their structures are shown in Table II. All compounds were identified by comparing their spectra in this study with those previously found in studies on the reaction of proline with reducing sugars. Compound 1 was identified as 1-formyl pyrrolidine by comparison of its spectrum with that found by Tressl et al.²⁴ Mills and Hodge¹³

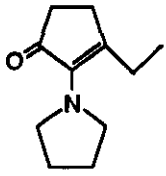
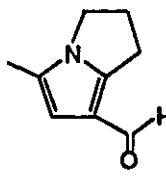
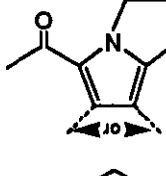
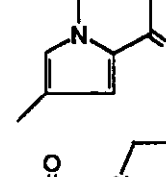
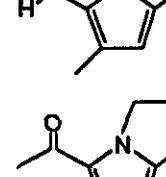
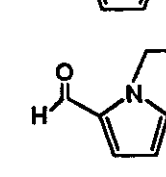
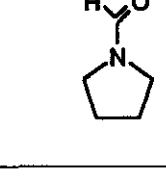
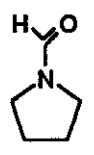
had also identified this compound in the vacuum thermolysis of 1-deoxy-1-*l*-proline-*d*-fructose and described its aroma as minty. Compounds 2, 5-formyl-2,3-dihydro-1H-pyrroli-zone, 3, 5-acetyl-2,3-dihydro-1H-pyrrolizine, 4, 5-formyl-6-methyl-2,3-dihydro-1H-pyrrolizine, and 7, 7-formyl-5-methyl-2,3-dihydro-1H-pyrrolizine, were identified previously by Tressl et al.¹⁸ Compound 6, 5-acetyl-6 or 7-methyl-2,3-dihydro-1H-pyrrolizine, was also identified by comparison with spectra from Tressl et al.¹⁸ but the position of the methyl group could not be distinguished from the mass spectra alone. The spectrum of Shigematsu et al.²⁵ was used to identify compound 5 as 2-methyl-5,6,7,8-tetrahydroindolizine-8-one. Compound 8 was identified to be 3-ethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one by comparison with the spectrum from Tressl et al.²⁴

Shigematsu et al.²⁵ had characterized the aroma of several of these compounds and found compound 3 to be smoky, sweet, weak amine-like; compound 4 was smoky, sweet, cinnamic; compound 5 was mild smoky, weak amine-like; and compound 6 was smoky, bitter, medicine-like. Compound 8 is considered to impart a bitter taste to roasted food-stuffs.²⁵

Quantification of identified compounds: The quantification of the GC peaks at each moisture content and their retention times are shown in Table I. The peak areas were analyzed to give the yield in ppb for each identified compound and the results are shown in Table II.

The compound giving the highest yield for all moisture contents was compound 3 which, like the other seven compounds, gives a dramatic decrease in yield with the addition of a small amount of water. However, analysis of Table II reveals that while the trend in flavor profile change follows the same general pattern

Table II. Yield (in ppb) of compounds identified in a proline/xylose/propylene glycol model system heated in a microwave oven for 10 min. at 130°C

Compound	Moisture content (%H ₂ O)		
	0	2.5	5
 (1) 1-formyl pyrrolidine	200	9.5	2.4
 (2) 5-formyl-2,3-dihydro-1H-pyrrolizine	414	90	14
 (3) 5-acetyl-2,3-dihydro-1H-pyrrolizine	3068	106	43
 (4) 5-formyl-6-methyl-2,3-dihydro-1H-pyrrolizine	411	31	7.4
 (5) 2-methyl-5,6,7,8-tetrahydroindolizine-8-one	211	14	4.3
 (6) 5-acetyl-6 or 7-methyl-2,3-dihydro-1H-pyrrolizine	151	15	14
 (7) 7-formyl-5-methyl-2,3-dihydro-1H-pyrrolizine	96	8.3	7.9
 (8) 3-ethyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one	584	14	11

with increasing moisture content, it decreases at a different rate for each compound. That is, compound **3** decreases at a rate of 30 between 0 and 2.5% moisture, but compound **2** falls off at a rate of 4.5. This implies that the formation pathway of each compound is affected differently by the presence of water.

Conclusions

The basis of this study was that propylene glycol could be used in microwave ovens as a browning/flavor agent. Possible considerations could be to baste a turkey with a propylene glycol solution in the final minutes of microwave cooking or to use it in a similar manner in the baking of bread. It was shown that using proline and xylose as the model system for the Maillard reaction, very good browning and flavor were attained at 130°C for 10 minutes when no water was introduced to the system. Of course, in a real food system, moisture will be present and that is why it was important to study the effect of water on the reaction in the microwave.

The proline/xylose reaction at 0% moisture content gave an aroma characteristic of baked and roasted foods. This aroma was also observed at 2.5% moisture but was less intense and became even less so at 5%. The exciting realization from this is that although there was little brown color at 2.5% moisture and none at 5%, flavors nevertheless developed as revealed by the GC/MS analysis. Whether or not this represents a high enough threshold for practical applications cannot be determined at this point.

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