Response Surface Methodology in Flavor Research—

Reaction of Rhamnose and Proline

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Flavor chemistry is such a broad and encompassing field because in our daily life we are literally surrounded by aromas. In our search to unlock their secrets our investigations must sometimes be limited to narrow, distinctly defined issues, yet at other times our interest is much broader and far reaching. These two approaches provide us with different perspectives of the ever present aromas.

Food flavors have been made throughout history by the reactions of carbohydrates and proteins with and without the intervention of flavor chemists. Since Maillard's first report some 70 years ago, many studies have been made to understand this prevalent and intricate reaction. The pleasantness of the flavors, their economic importance, and the intrigue they bring to the investigator makes Maillard reaction flavors an exciting and much studied field.

It is frequently desirable and indeed often necessary to optimize a reaction product, enhancing one component at the expense of another by identifying and exploiting synergistic reaction conditions. These might be determined by trial and error but more properly by use of a planned approach using an experimental design.

Rhamnose and proline are precursors of many pleasant aromas which are important to the food industry.[1] Among them are bready, cracker-like and roasted aromas. These aromas are greatly influenced both in quality and quantity by changes in reaction conditions such as temperature, pH and the relative reactant concentration.

RSM and Experimental Design

Response Surface Methodology (RSM), which uses systematic experimental designs, was a tool used to study the reaction of rhamnose and proline, and in particular the effects of temperature, pH and relative concentration on the volatile products. This statistical technique uses quantitative data (chemical assay data, sensory scores, processing parameters etc.) from designed experiments to obtain and solve multivariate equations (mathematical models) as functions of input conditions.

This approach is widely used in the sensory evaluation of foodstuffs with studies reported on systems as diverse as extruded snacks, cured ham, smoked chicken, fruit punches and meat flavor model systems.[2-9] RSM has not, however, been so widely used in studying the chemistry of reaction

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flavors, where investigators typically focus on a severely limited number of variables.

The information obtained from a given investigation can be increased by using a planned experimental design, since the variables in question are studied in a precise, logical fashion, individually and *in concert with each other*. The measured responses can be isolated for each variable and interactions (synergies) between variables, if present, can be identified.

While the experimental designs can be simple in geometry, they are of practical importance because of their efficacy in studying the effects of several variables simultaneously. Perhaps the design's most novel aspect is that the controlled variables are varied simultaneously,[10,11] as opposed to the more conventional approach of studying things one factor at a time.

Simultaneously changing variables, and the experimental efficacy that provides, permit a broader experimental region to be explored. With the larger experimental region, what one may lose in detail is gained in perspective.

The properties of rhamnose and proline were seen not under a handful of conditions but rather under many. With a broader perspective and the understanding it brings, other specific areas of interest become evident. Thus, RSM is a tool that permits us to investigate a wider field.

Table I. Experimental Design

Design Point	Temp °C	pH	Rhamnose Conc (m)	Proline Conc (m)	Ratio Rham/Pro
1	190.0	6.30	0.10	0.10	1.00
2	180.0	6.30	0.10	0.10	1.00
3	152.5	6.30	0.10	0.10	1.00
4	139.0	6.30	0.10	0.10	1.00
5	152.5	2.90	0.10	0.10	1.00
6	152.5	9.70	0.10	0.10	1.00
7	152.5	6.30	0.20	0.00	
8	152.5	6.30	0.03	0.17	0.20
9	170.0	4.60	0.05	0.15	0.33
10	170.0	8.00	0.05	0.15	0.33
11	170.0	8.00	0.15	0.05	3.00
12	170.0	4.60	0.15	0.05	3.00
13	139.0	4.60	0.05	0.15	0.33
14	139.0	8.00	0.05	0.15	0.33
15	139.0	8.00	0.15	0.05	3.00
16	139.0	8.00	0.15	0.05	3.00
17	130.0	8.00	0.15	0.05	3.00

Materials and Methods

Seventeen experimental runs were chosen based on a central composite 3 factor design in temperature (T), pH and rhamnose proline concentration (R/P). This design systematically covered an experimental region bounded by temperature: 130°C-190°C, pH: 2.9-9.7 and concentrations ranging from 0.2 molar (m) rhamnose (without proline) to 0.033 m rhamnose/0.167 m proline. The specific experimental points are listed in Table I.

Flavor volatiles were formed by heating aqueous solutions of rhamnose and proline for 30 minutes in stainless steel reaction cylinders. These solutions were buffered to the reaction pH with mixtures of mono-, di- and tribasic sodium phosphate. Quantification and identification of these volatiles followed standard techniques and have been reported elsewhere.[12]

Reaction Synergies

To determine the prevalence and importance of reaction synergies within this experimental region, mathematical models were prepared for the quantities of 23 volatile compounds. These models took the form of polynomial equations, ie.

$$Y = \beta_0 + \beta_1 T + \beta_2 T^2 + \beta_3 C(r) + \beta_4 C(r)^2 + \beta_5 pH + \beta_6 pH^2 + \beta_7 C(r)T + \beta_8 pHT + \beta_9 C(r)pH$$

Y is the quantity of the individual volatile (ppm) while β's are assumed constants, identified by re-

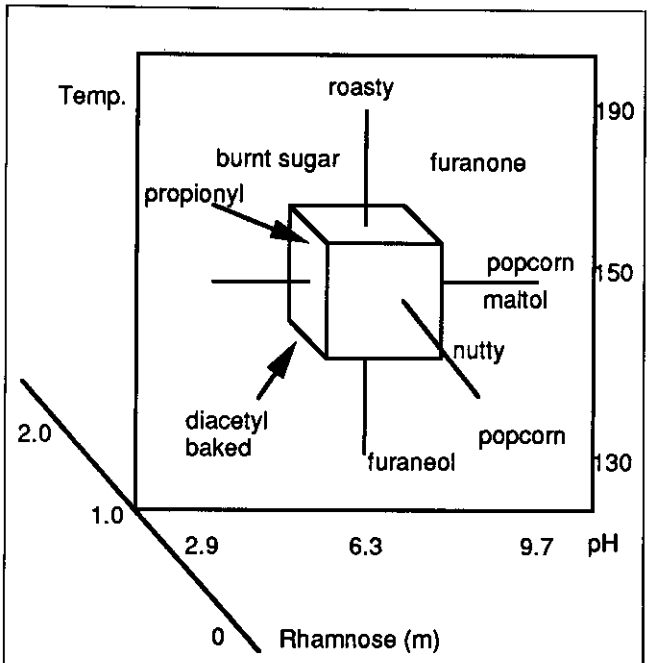

Figure 1. Aromas throughout experimental region.

gression analysis to give the equation which best fits the data. T, T^2, C(r), $C(r)^2$, pH, pH^2, C(r)T, pHT and C(r)pH are derived from the independent variables [temperature (T), rhamnose concentration C(r) and pH].

Thus the models are composed of a constant, 3 linear, 3 quadratic and 3 interaction (synergy) terms. The models use the interaction terms to indicate the prevalence and importance of synergies within the experimental region.

These equations or models describe the quantity of volatiles and are useful for predictive studies. We can use the equations to optimize the quantity of a volatile, individually or relative to another volatile. Qualitative data, however, can also be usefully portrayed over the experimental region. Aroma description is an example of qualitative data, while aroma preference would illustrate quantitative data.

Results and Discussion—Aromas

Figure 1 illustrates some of the key aromas present throughout the experimental region, as described by trained flavorists. These aromas were characterized from perfumers' blotters saturated with room temperature samples. After evaporation of the aqueous solvent, another rating was taken. Detailed information on the aroma, wet and dry, appears in Table II.

Of the 14 groups of aroma descriptions reported by Lane and Nursten,[13] five were found in the present study. These include groups 1, 3, 6, 9 and 14.

Table II. Aroma Descriptions

Design Point	Initial Aroma	Final Aroma
1	smoky, burnt sugar, bready	maltol, smoky
2	smoky, burnt sugar, bready	maltol, smoky
3	smoky, burnt sugar, bready, diacetyl background	strong maltol, not smoky
4	low smoke, high furaneol, bready	furaneol, slight maltol
5	winey, bready, hexanoic, cheesy, sweaty, valeric	high furaneol
6	smoky, sweet, maltol, popcorn	smoky, nutty, maltol, furanone
7	caramel, furfural, fruity, burnt sugar	maltol, caramel
8	low smoky, high diacetyl, bread crust, sweet, furaneol	furaneol, crusty
9	strong furaneol, smoky	sour, burnt caramel, propionyl
10	nutty, popcorn, bread crust, smoky	caramel, slight popcorn
11	smoky, bready, burnt sugar	caramel furanone
12	fruity, furfural, burnt sugar, mild smoke	burnt sugar, cotton candy, furaneol
13	sweet, maltol, baked bread	maltol
14	nutty, popcorn	bready
15	sweet, diacetyl	caramel furanone, burnt molasses
16	maltol, nutty, diacetyl, baked, furaneol	caramel, maltol
17	not recorded	not recorded

Table III. Aroma descriptions from Lane and Nursten

Group	Description(s)
1.	Sweet, boiled sugar, caramel, toffee
2.	Chocolate, cocoa
3.	Bread, crusty, biscuits, cake, toast
4.	Meaty, beefy
5.	Potato, potato skins, potato crisps
6.	Fruity, aromatic ester
7.	Celery, chicory, leeks, brussel sprouts, turnips
8.	Puffed wheat, sugar puffs
9.	Nutty
10.	Floral
11.	Ammonial
12.	Unpleasant, "caused coughing"
13.	Aldehydic
14.	Burnt, charred, scorched, acrid, toast, potato crisps, smoky

The intensity of the aromas varied under different reaction conditions and some aromas types were present only under certain reaction conditions.

The characteristic aroma of furaneol (2,5-dimethyl-4-hydroxy-3(2H)-furanone) was most pronounced in low temperature samples and was apparent at higher temperature if the pH was kept low (i.e. 4.6). At higher pH, where the Maillard reaction proceeds at a faster rate, furaneol was only noted in the 139°C sample.

Table IV. Effects of Changes in Reaction Conditions on the Quantity of Selected Volatiles (ppm)

Volatiles	[i]	[ii]	[iii]	[iv]	[v]	[vi]	[vii]
Temperature							
190.0	42.3	310.3	22.7	381.9	123.3	287.7	101.9
180.0	100.6	267.5	21.0	363.0	119.4	288.9	112.4
152.5	871.4	63.7	87.0	191.8	75.7	374.1	171.0
139.0	718.4	0.0	0.0	27.8	0.0	79.2	29.9
Rhamnose/Proline Content (m)							
0.03/0.17	445.5	40.3	11.7	69.7	35.8	151.8	63.6
0.10/0.10	871.4	63.7	87.0	191.8	75.7	374.1	171.0
0.20/0	1434.5	0.0	318.3	.....caramelization.....			
pH							
9.7	61.6	54.2	52.2	125.5	0.0	122.8	43.4
6.3	871.4	63.7	87.0	191.8	75.7	374.1	171.0
2.9	25.1	0.0	0.0	35.2	154.3	0.0	0.0

Temperature series conducted at pH 6.3, rhamnose and proline 0.10 m each. Ramnose:proline series at 152.5°C and pH 6.3 pH series at 152.5°C and rhamnose and proline 0.10 m each.

[i] furaneol
[ii] sum of 2-hydroxy-3-pentanone and 3-hydroxy-2-pentanone
[iii] 2-acetoxy-3-pentanone
[iv] 5-acetyl-7-methyl-2,3-dihydro-(1H)-pyrrolizine
[v] 5-acetyl-6-methyl-2,3-dihydro-(1H)-pyrrolizine
[vi] 7-formyl-5-methyl-2,3-dihydro-(1H)-pyrrolizine
[vii] 7-acetyl-5-methyl-2,3-dihydro-(1H)-pyrrolizine

This data reflects furaneol stability (it is notoriously unstable) rather than its formation since furaneol is readily formed from rhamnose. The higher temperature samples developed a burnt sugar aroma in place of furaneol.

Bready aromas have been associated with nitrogen heterocycles such as 2,3-dihydro-(1H)-pyrrolizines.[1] So prevalent are these compounds that fully one half of the samples possessed bready aroma. In general the bready aroma is associated with higher temperature samples for which the reaction has gone further to completion.

Popcorn aroma is produced at higher pH irrespective of temperature while maltol aroma tends to be found in lower temperature samples.

Results and Discussion— Quantity of Volatiles

Rhamnose and proline were reacted under a wide range of reaction conditions to produce volatiles of differing type and ratio. Proline as a secondary amine produces different classes of heteroatomic compounds compared to the more common primary amino acids, with ring-fused and multi-cyclic compounds being common.

Near 80% of the compounds identified in this study are heterocyclic compounds, which are typically combination products of the intermediates

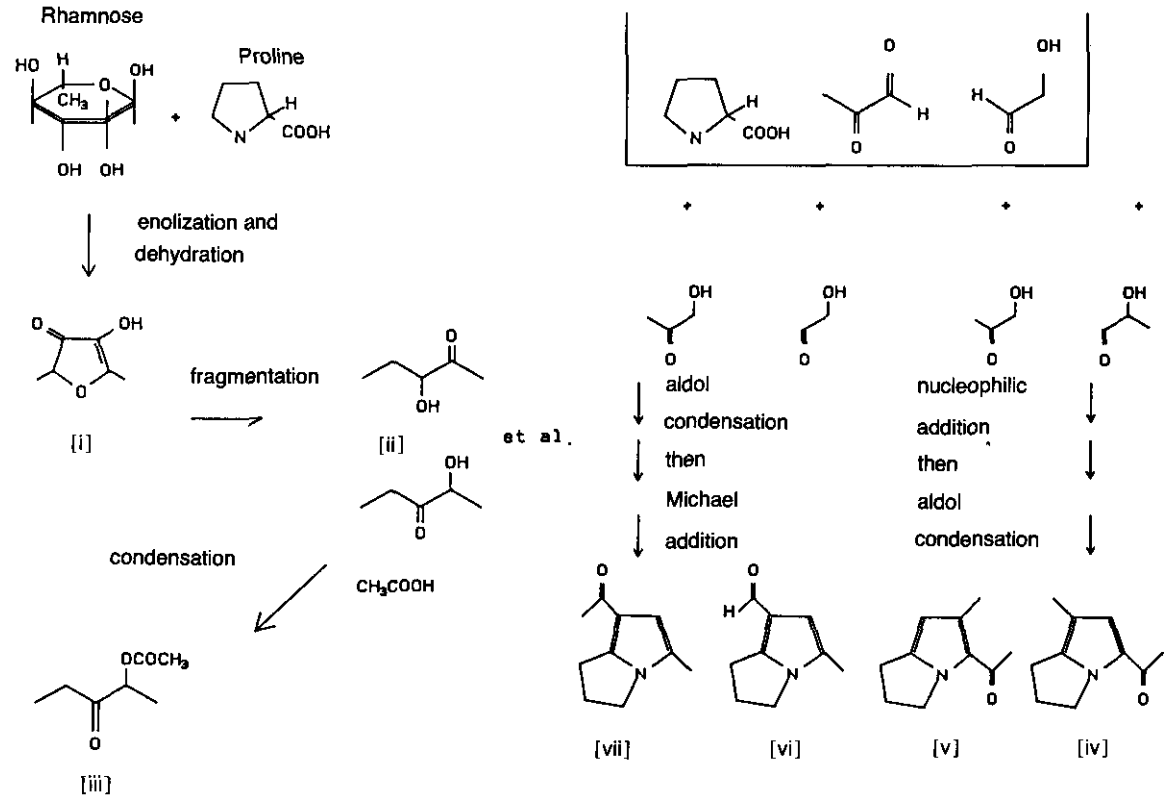

Figure 2. Reaction Overview

formed by Strecker degradation, retroaldolization, dehydration, and enolization. The oxygen heteroatom is provided by the sugar (i.e. rhamnose) while the nitrogen is provided by amino acid.

Forty compounds were identified in this study, and models were prepared for 23, while the aromas and chemistry of 7 were selected for discussion. Table IV shows a portion of the chemical assay data for the 7 volatiles in question. For brevity, only data from the central composite (one factor at a time) portion of the design is shown.

Figure 2 provides an overview of how and when in the reaction volatiles i-vii are formed. It should be evident that furaneol is an initial product whose thermal fragmentation[14,15] accounts for a wide range of smaller molecular weight compounds (hydroxyketones, aldehydes, acids, etc.). These fragmentation products can then combine with proline and, through multiple additions, condensations, eliminations and dehydrations, produce complicated ring structures such as iv-vii.[16-18]

The amount of furaneol measured is strongly temperature dependent, in agreement with Shu, et al.[15] Its content roughly follows the concentration of rhamnose and is found in larger quantity in the lower temperature samples. The furaneol content in this study reflects both its formation and degradation over the 30 minute heating time.

What is surprising is that the hydroxy-pentanone isomers, known furaneol decomposition products, are not found under caramelization. This in part accounts for the unusually high content of 2-acetoxy-3-pentanone during caramelization.

The 2-3-dihydro-(1H)-pyrrolizines have two responses to reaction temperature: increasing with rising temperature or reaching a maxima at 152.5°C. The pyrrolizines are compounds for which proline plays a structural as well as a catalytic role.

The concentration of these compounds does not follow proline content as might be expected because of the presence of the proline ring in these compounds. Rather, the concentrations of rhamnose and the carbohydrate fragments produced from rhamnose determine the pyrrolizine content.

Mathematical Models

Preparing mathematical models to determine the importance of synergies is another step—is it worth it? It is, if more and better information is obtained. One way, though not rigorous, is to compare models containing synergistic interaction terms to models without them. If introduction of interaction terms increases the percentage of total variation explained (the ability to describe the data), then our overall understanding has increased and the effort was worthwhile.

In describing the quantity of furaneol there is no gain (only 0.28 percent) and for the hydroxy-pentanones, 5-acetyl-6-methyl- and 7-acetyl-5-methyl-2,3-dihydro-(1H)-pyrrolizine hardly enough to be worthwhile (5.34, 3.17 and 7.11 percent improvement).

This is not so for 5-acetyl-7-methyl- and 7-formyl-5-acetyl-2,3-dihydro-(1H)-pyrrolizine (17.24 and 10.22 percent improvement) and most notably for 2-acetoxy-3-pentanone, for which there is a 40.77 percent improvement in total data variation explained.

Conclusions

Reaction conditions often influence each other in their effect. These influences may be either large or small, opposite or complimentary, all of which can be confirmed and estimated by the use of mathematical models.

In the present case, where mutual influences were particularly important, up to a 40 percent gain in model precision was achieved by including the interaction terms, a dramatic improvement over the best results of a one variable at a time approach.

These synergies affect the relative quantity of volatile compounds and should be reflected in the organoleptic properties of the reaction flavors.

To be of greatest value, experimental regions can be chosen such that a particular aroma compound is either present or absent, maximized or minimized. Another use of the quantitative descriptive models is to better select reaction conditions that are most appropriate (equipment, cost constraints etc).

Furaneol was exceptional in that variable interaction terms proved to be unimportant. Levels of furaneol were predicted by primary variable effects alone. Furaneol is a thermally unstable compound, both easily formed and readily degraded.

Variable interaction terms are more important in understanding the formation of 2,3-dihydro-1H-pyrrolizines, compounds that are formed through more complicated mechanistic pathways. Where the interaction terms are important, a 17% and 35% improvement in the model's fit to the data is obtained by their inclusion.

In summary, reaction flavors are complex systems and are strongly influenced by changes in reaction conditions. Increasing the number of reaction condition variables increases the possibility of variable interactions (synergies). Without appropriate experimental designs it is not possible to properly assess these interactions along with the contribution of each variable. Response Surface Methodology helps us do this and is another tool for understanding the effects of reaction conditions on Maillard-type flavors.

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