Development of a Meat-Like Process Flavoring from Soybean-Based Enzyme-Hydrolyzed Vegetable Protein (E-HVP)


ABSTRACT: Response surface methodology (RSM) was used to develop a meat-like process flavoring agent from enzyme-hydrolyzed vegetable protein (E-HVP). Five factors were evaluated: pH (3.6 to 8.4), temperature (51 to 99°C), heating period (0.3 to 2.7 h), amount of ribose (0 to 1 × 10⁻³ mol) and amount of cysteine (0 to 1x10⁻⁴ mol). Sensory analysis limited to aroma in terms of overall liking and intensity of specific aroma attributes was investigated. The aroma attributes measured included bean-like, potato-like, Brussels sprouts-like, molasses-like, chicken-like, beef-like, egg-like, roasted and apple sauce-like. Based on the fitted surfaces and consumer test data (overall liking), the optimum reaction conditions for production of a meat-like flavoring were pH 6, 99°C reaction temperature, 1.5 h heating time, 5 × 10⁻⁴ mol of ribose and 5 × 10⁻⁴ mol of cysteine.

Key Words: response surface methodology (RSM), ribose, cysteine, process flavoring, enzyme-hydrolyzed vegetable protein (E-HVP)

Introduction

Hydrolyzed vegetable protein (HVP) has been used for more than 100 years to impart meat-like flavor to prepared foods and represents one of the earliest forms of process flavorings (Manley 1994). HVP produced by acid hydrolysis of vegetable protein consists predominantly of amino acids. The use of hydrochloric acid in the production of HVP has been thought to result in the formation of potential carcinogenic compounds such as 3-chloropropane-1,2-diol (Nagawadhana 1992). This in turn has therefore prompted the food industry to reduce the use of HVP in process flavorings. Nevertheless, it is possible to avoid the formation of carcinogens if proteolytic enzymes are employed for the hydrolysis of soybean protein to produce enzyme-hydrolyzed vegetable protein (E-HVP). Another potential advantage with the use of E-HVP is a lower monosodium glutamate (MSG) content in the final product.

Optimal conditions for enzymatic hydrolysis of defatted soybean meal (DSM) into E-HVP using Flavourzyme (Novo) have been established by Baek and Cadwallader (1996). Optimum hydrolysis conditions were pH 6, temperature of 50 to 55°C, 7 h reaction time, 15% (w/v) substrate concentration, and 3.5% (w/w) enzyme/substrate ratio (E/S). The degree of hydrolysis (DH) also was affected by particle size and heat pretreatment of the defatted soybean meal. The three particle sizes studied included >500 µm, 212 to 500 µm and < 63.5 µm of which the smallest particle size (< 63.5 µm) resulted in the highest DH (Bae and Cadwallader 1996). However, preliminary trials in this study using small (< 63.5 µm) and intermediate (< 63.5 to 500 µm) particle sizes did not show differences in DH. As for the heat pretreatment, autoclaving at 121°C for 30 min, was determined to be sufficient to denature the defatted soybean meal and result in higher DH (Baek and Cadwallader 1996).

Process or reaction flavorings are created from precursor materials via the Maillard reaction and other thermal or oxidative reaction occurring during processing and preparation of foods, such as cooking, roasting, baking, frying, and boiling (Giese 1994; Manley and Ahmedi 1995). Precursors play an important role in the generation of process flavorings and include amino acids, reducing sugars, thiamine (vitamin B1), nucleotides and other components generated from the Maillard reaction (for example, dialdehydes) (Swaine 1993). The aroma of a process flavoring is composed of a highly complex mixture of volatile components, most being formed via either sugar and/or sugar-amino acid (or peptide) interactions or by the thermal degradation of specific components. For example, the thermal reaction of cysteine and ribose has been reported to give meat-like aroma (Hofmann and Schieberle 1997). The type and level of reactants, as well as the processing conditions, can be manipulated to obtain process flavorings having distinctive flavor attributes. Extensive work has been conducted on the thermal generation of volatile compounds in model systems containing from just one to several components (Shibamoto and Russell 1976; Shibamoto 1977; Lane and Nursten 1983; Shaw and Ho 1989; Zhang and Ho 1991; Apriyananto and Ames 1993; Meynier and Mottram 1995; Hofmann and Schieberle 1997); however, only limited information has been published on process flavorings from HVP (Aaslyng and others 1997; Baek and others 1997). To our knowledge, no detailed report has been published on the production of a process flavoring by the thermal reaction of E-HVP, ribose, and cysteine.

Use of response surface methodology (RSM) for treatment of data is becoming increasing popular (Henselman and others 1974; Min and Thomas 1980). This technique has been used to optimize enzymatic processes (Baek and Cadwallader 1995) and to evaluate the effects of ingredients on the physical properties of food products (Carballo and others 1995; Penna and others 1997). RSM could be useful in solving complex flavor problems, such as assessing the effects of reaction conditions on Maillard type flavors (Shaw and Ho 1989), or for determining the best combination and concentration of flavor compounds or precursors needed for optimal meat flavor (Bodrero and others 1981). Use of RSM is becoming increasing popular in the sensory evaluation area because it provides a translation medium from sensory evaluation to product developer (Min and Thomas, 1980; Bodrero and others 1981). The objective of the present study was to optimize and evaluate reaction conditions for production of a meat-like process flavoring by the thermal reaction of E-HVP, ribose, and cysteine.
Materials and Methods

Materials
Defatted soybean meal (DSM, protein content 48%) was obtained from Archer Daniels Midland Co. (ADM, Clarksdale, Miss., U.S.A.). Flavourzyme was obtained from Novo Nordisk Biochem North America, Inc. (Franklinton, N.C., U.S.A.). Folin & Ciocalteu’s phenol reagent, D-Glucose, L-cysteine, D(-)-ribose, and amino acid standards were from Sigma (St. Louis, Mo., U.S.A.). All other chemical reagents were purchased from Sigma and Fisher (Pittsburgh, Pa., U.S.A.).

Production of E-HVP
Ground DSM (225 g of particles between 63.5 μm and 500 μm) was suspended in deodorized distilled water to a level of 15% (w/v) and then autoclaved at 121 °C for 30 min. After cooling, the DSM suspension was placed into a glass 2-L jacketed reaction vessel and preincubated at 50 to 52 °C. The pH was adjusted to 6.0 using either aqueous 6 N HCl, 0.1 N HCl, 0.5 N NaOH, or 2 N NaOH. Flavourzyme was added to the solution at an enzyme/substrate (E/S) ratio of 3.5% (7.88 g Flavourzyme), and the mixture was vigorously stirred during reaction using a Labmaster mechanical stirrer (Mixing Equipment Co., Avon, N.Y., U.S.A.). After 7 h reaction time, the solution was pressed through two layers of cheese cloth (Fisher) and the filtrate subsequently heated above 85 °C for 30 min for enzyme inactivation. The solution was clarified by centrifuging at 3,500 × g for 30 min, followed by vacuum filtration of the supernatant (Whatman no. 40 filter paper). The supernatant from this step represented aqueous enzyme-hydrolyzed vegetable protein (E-HVP) (Baek and Cadwallader 1996). Aqueous E-HVP was concentrated under vacuum using a Rotavapor (Büchi, Switzerland) at 50 °C and then freeze-dried (Lyph-Lock 6, Labconco Corp.). E-HVP was stored in a vacuum desiccator. Recovery was about 30 to 35% based on the ratio of freeze-dried E-HVP to DSM.

Reaction Parameters and Effect of Precursors
Reaction parameters such as pH, temperature, reaction time, amount of precursors (mol), and E-HVP content (%) were considered for optimization of reaction conditions. Process flavors were produced using the general procedures outlined by IOFI (Manley 1994). For example, the product temperature and pH during processing shall not exceed 180 °C and 8, respectively. Furthermore, the characteristic cooked meat and savory flavors produced from sulfur-containing heterocyclic compounds may be favored under acid conditions (Mottram and Whitfield 1994). For example, the product temperature and pH during processing shall not exceed 180 °C and 8, respectively. Furthermore, the characteristic cooked meat and savory flavors produced from sulfur-containing heterocyclic compounds may be favored under acid conditions (Mottram and Whitfield 1994). Range of pH, temperature, reaction time, and amount of precursors were selected based on published information, practical considerations, and preliminary experiments. E-HVP content was chosen based on preliminary experiments and later fixed at a level of 10% (w/v). A variety of precursors such as reducing sugars (glucose, xylose, fructose, and ribose), amino acids (cysteine, cystine, proline, lysine, serine, methionine, threonine), thiamine, nucleotides (inosine 5’-monophosphate, guanosine 5’-monophosphate) were evaluated during preliminary experiments. On the basis of the results of preliminary experiments, ribose and cysteine were selected for further study.

Response Surface Methodology
RSM (Box and others 1978; Myers 1971) was applied to determine optimum levels of five factors: pH, temperature, reaction time, ribose amount (mol), and cysteine amount (mol). Each factor was coded at five levels: -2.4, -1, 0, 1, and 2.4 (Table 1). A total of 50 runs, based on a central composite design (CCD) of the second order, was performed for the study of five experimental factors in coded units. Data were analyzed by multiple regression to fit the following second order Eq.:

\[ Y = b_0 + \sum_{i=1}^{5} b_i X_i + \sum_{i=1}^{5} b_{i1} X_i^2 + \sum_{i=1}^{5} \sum_{j=1}^{5} b_{ij} X_i X_j \quad (i < j) \]

where \( b_0 \) is the intercept, \( b_i \) and \( b_{ij} \) are regression coefficients of the model. \( X_i \) and \( X_j \) are coded independent variables and are linearly related to \( X_i \) and \( X_j \).

Compositional and Preparation of Process Flavoring Samples
E-HVP (1.5 g) in about 13.5 mL of deodorized distilled water plus various amounts of ribose and cysteine (depending on treatment) was placed in a 25-mm × 150-mm screw-cap test tube (Pyrex, Corning, N.Y., U.S.A.) and then homogenized using a stir bar. The pH of each experimental sample was adjusted using aqueous 0.1 N HCl or 0.5 N NaOH, and final volume was adjusted to 15 mL to give an E-HVP concentration of 10% (w/v). Tubes were capped with a PTFE-lined screw cap and then placed in a thermostat controlled water bath (model 910, Fisher Scientific) for cooking at the various time-temperature combinations. After cooling in an ice-water bath, samples were stored in a −20 °C freezer until analysis.

Sensory Evaluation
Aroma Acceptability. Aroma quality of the developed process meat-like flavoring was assessed by acceptability testing. Twenty-two male and eight female panelists, consisting of university employees and students aged between 18 and 65, participated in the sensory evaluation. Even though it is generally recommended that at least 50 panelists be used for consumer preference testing (Meilgaard and others 1991), difficulty in the availability of the same panelists for all of the ten sessions (a total of 50 formulations) limited the total number of panelists used in this study to 30. This approach has been previously taken when it was necessary for each panelist to evaluate all products in a large sample set (Pastor and others 1996).

Tests were conducted in partitioned booths in a sensory room. A total of 10 consumer test sessions were conducted from 9:00 a.m. to approximately 12:00 p.m. and 1:30 to approximately 4:30 p.m. during a 5-d period. Experimental samples were prepared the day before the test, held in a freezer, and then warmed to room temperature just before serving to panelists in 3-oz white plastic cups capped with 3-digit random numbers. Each panelist rated the acceptability (overall liking) of the aroma (by sniffing) of each sample using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like or dislike, and 9 = like extremely) (Meilgaard and others 1991). Five samples, evaluated individually, were presented at random in each session (Stone and Sidel 1993).

Descriptive Analysis. Quantitative/qualitative screening of the meat-like process flavoring was performed using descriptive analysis (Meilgaard and others 1991). The objective here was to not only identify and rate meat-like attributes, but also all other aroma at-

Table 1—Levels of independent variables expressed in coded and natural units for production of process flavorings

<table>
<thead>
<tr>
<th>Independent Variables</th>
<th>Code Units</th>
<th>pH (°C)</th>
<th>Time (h)</th>
<th>Ribose (× 10⁻⁴ mol)</th>
<th>Cysteine (× 10⁻⁴ mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code Units</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-2.4</td>
<td>3.6</td>
<td>51</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>-1</td>
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<td>6</td>
<td>79</td>
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<tr>
<td>1</td>
<td>7</td>
<td>85</td>
<td>2.0</td>
<td>7.1</td>
<td>7.1</td>
</tr>
<tr>
<td>+2.4</td>
<td>8.4</td>
<td>99</td>
<td>2.7</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

αpHc = (pH-6)/1; tempc = (temp - 75)/10; timec = (time - 1.5)/0.5; ribosec = (ribose - 5)/2.1; cysteinec = (cysteine - 5)/2.1.
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The objective of this study was to compare the sensory descriptive flavor profiles of a sample made under optimum conditions (data from consumer acceptance and descriptive tests) (R4), an unheated E-HVP sample (UR4), and a heat-treated model system containing ribose and cysteine in 0.1 M phosphate buffer (pH 6.0) (MR4). Each sample was prepared in duplicate as follows: R4 consisted of 10% E-HVP plus 5 × 10⁻⁴ mol of ribose and 5 × 10⁻⁴ mol of cysteine, pH 6, heated at 99 °C for 1.5 h; MR4 consisted of 10% E-HVP plus 5 × 10⁻⁴ mol of ribose and 5 × 10⁻⁴ mol of cysteine, pH 6 and was not heated); and MR4 consisted of 0.1 M phosphate buffer, pH 6 containing 5 × 10⁻⁴ mol of ribose and 5 × 10⁻⁴ mol of cysteine, heated at 99 °C for 1.5 h. Panelists were instructed to evaluate the two sets of samples with a 3-min break between each set. Samples were scored using the same scale and procedures as described under descriptive analysis.

Results and Discussion

Response Surface Plotting

Response surfaces were plotted for two independent variables with the other two independent variables fixed at 1 coded level, except that temperature was fixed at the 2.4 coded level. Direct correlation between temperature and pH with overall liking were observed (Figure 1a). Increasing temperature progressively increased overall liking. Maximum overall liking values were characterized by high levels of temperature and middle levels of pH. The region of optimum response was localized at pH 5.5 to 7.5 and at a temperature above 90 °C.

At optimum pH (pH 5.5-7.5), overall liking increased as the amount of cysteine increased, and remained constant above 4.5 × 10⁻⁴ mol. The response surface obtained when plotting overall liking against pH and reaction time (Figure 1c) showed that overall liking increased with increasing reaction time. Optimum area of reaction time and pH for overall liking was selected from the response surface plot as reaction time > 1 h and pH 5.5 to 7.5.

Benzing-Purdie and others (1985) concluded that in the Maillard reaction, where equimolecular amounts of starting materials are used, an increase in temperature leads to an increase in aromatic character in both high- and low-molecular-weight products. Based on 21 amino acids and 8 sugars treated under different conditions of temperature and time, Lane and Nursten (1983) commented that generally, the aromas become stronger and more unpleasant, aldehydic, and burnt with increased temperature and time, and at 100 °C the pentose mixtures give less burned notes. Increasing the temperature increased the quantity of sugars combining with the same quantity of amino acid, and the incorporation of sulfur into ring systems is favored by prolonged heating periods (El’Ode and others 1966; Galt and MacLeod, 1994). The pH has an important effect on the Maillard reaction. The formation of some aroma volatiles can be favored by higher pH values, but others are formed under acid conditions (Meynier and Mottram 1995). In the present study, reaction under slightly acid or neutral pH (5.5 to 7.5) conditions resulted in process flavorings with most acceptable aromas.

Response surface plots were generated for the recovery of chicken-like aroma intensity model. Temperature values X₃ = -2.4, X₄ = 0, and X₅ = -2.4 were established from this model. Plots for all 3 values were virtually identical. A 3-dimensional response surface plot of recovery of chicken-like aroma as a function of amount of cysteine and pH at X₃ = -2.4 is shown in Figure 2. A cysteine amount of 6.01 × 10⁻⁴ mol at pH 6 was optimum for recovery of chicken-like aroma (Figure 2). An increase in temperature

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tributes that might contribute either desirable or undesirable attributes. Ten panelists (8 men and 2 women), consisting of university employees and students aged between 22 and 65 were selected based on interest and time availability. Training sessions, lasting 1.5 h, to evaluate a wide array of soybean extracts, E-HVP and process flavorings, was provided to the panelists once a week for a total of 12 wk. Initial training sessions of the panel included introduction of the samples and discussion to develop aroma descriptors. The aroma descriptors included bean-like, potato-like, Brussels sprouts-like, molasses-like, chicken-like, beef-like, egg-like, roasted, and apple sauce-like. Familiarity with the 10 aroma descriptors was provided by using commercial products and cooked food items as references. References and samples were rated using a 15-cm universal Spectrum™ line scale with 0 cm representing “none” and 15 cm representing “strong” (Munoz and Civille 1998). Panelists were instructed on the proper use of the scale. That is, panelists were instructed to rate aroma intensity such that product receiving a rating of 10 would have aroma intensity twice as high as a product receiving a rating of 5, independent of aroma characteristic. The panelists discussed individual scores for each of the reference aroma descriptors among themselves and decided on the intensity of the descriptor by mutual consensus. The product obtained by reaction of 10% E-HVP, 5 × 10⁻⁴ mol ribose, and 5 × 10⁻⁴ mol cysteine at pH 6 and 99 °C for 1.5 h, was labeled “Overall Intensity 15.” Similarly, Bush’s Best™ canned pinto beans (Bush Brothers & Co., Knoxville, Tenn., U.S.A.) was labeled “Bean-like 1,” The Allens Sunshine™ canned whole white potatoes (Allen Canning Co., Siloam Springs, Ark., U.S.A.) were labeled “Potato-like 7,” cooked Brussels sprouts (fresh) were labeled “Brussels Sprouts-like 9,” blackstrap molasses (feed grade) (Farm Services Inc., Starkville, Miss., U.S.A.) was labeled “Molasses-like 10,” Swanson premium chunk white chicken in water (98% fat free) (Campbell Soup Co., Camden, N.J., U.S.A.) was labeled “Chicken-like 10,” pot roast (round bottom roast, approximately 250 to 300 g, wrapped with aluminum foil and baked for 1 h at 350 °F) was labeled “Beef-like 8,” hard-boiled egg (white chicken egg boiled for 20 min, peeled, and halved) was labeled “Egg-like 13.5,” ground roast coffee (Maxwell House Coffee Co., Kraft General Foods, Inc., White Plains, N.Y., U.S.A.) was labeled “Roasted 15,” and Mott’s natural style unsweetened apple sauce (Mott’s Inc., Stamford, Conn., U.S.A.) was labeled “Apple Sauce-like 5” (Meilgaard and others 1991).

Labeled references were presented in 3-oz white plastic cups during each session to standardize the panel scores and prevent drifting. Several practice sessions (during end of training period) were conducted to ensure that all panelists understood the task, scorecard, and terminology, and to monitor panel performance for repeatability, consistency, and discriminating ability.

Descriptive sensory evaluations were conducted in a sensory room during the morning (9:00 a.m. to about 12:00 p.m.) and afternoon (1:30 to about 4:30 p.m.), for 5 consecutive days. Experimental samples were prepared 24 h prior to evaluation, held in a freezer, and then warmed to room temperature just before serving to panelists. References were prepared fresh daily: Samples of 4 mL each were placed in 3-oz white plastic cups. Five samples, coded with 3-digit numbers, were served to each panel members in random order during each session (Stone and Sidel 1993). Each panelist evaluated (rated) each sample independently with a 30-s break between samples.

Sensory Descriptive Profiles. The objective of this study was to compare the sensory descriptive flavor profiles of a sample made under optimum conditions (data from consumer acceptance and descriptive tests) (R4), an unheated E-HVP sample (UR4), and a heat-treated model system containing ribose and cysteine in 0.1 M phosphate buffer (pH 6.0) (MR4). Each sample was prepared in duplicate as follows: R4 consisted of 10% E-HVP plus 5 × 10⁻⁴ mol of ribose and 5 × 10⁻⁴ mol of cysteine, pH 6, heated at 99 °C for 1.5 h; R4 contained 10% E-HVP plus 5 × 10⁻⁴ mol of ribose and 5 × 10⁻⁴ mol of cysteine, pH 6 and was not heated); and MR4 consisted of 0.1 M phosphate buffer, pH 6 containing 5 × 10⁻⁴ mol of ribose and 5 × 10⁻⁴ mol of cysteine, heated at 99 °C for 1.5 h. Panelists were instructed to evaluate the two sets of samples with a 3-min break between each set. Samples were scored using the same scale and procedures as described under descriptive analysis.

Statistical and Data Analysis

In the optimization study, data from consumer acceptance testing and sensory descriptive analysis were fitted to procedure of response surfaces, obtained using the RSREG procedure of the Statistical Analysis System (Ver 6.12, SAS Institute, Inc. 1989) program. Significance was defined at p 0.05. Three-dimensional response surface plots and contour plots were drawn using Sigma-Plot (V4.0 for Windows™, SPSS Inc., Chicago, Ill., U.S.A.). In the sensory descriptive profile study, mean values of intensities of each odor note in samples were calculated.
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from 51 °C (X2 = 2.4) (figure not shown) to 99 °C (X2 = 2.4) resulted in a slight decrease in recovery of chicken-like aroma (0.6 to 0.5), indicating little influence of temperature.

Zhang and Ho (1991) found that the formation of sulfur-substituted furans was favored by acidic conditions in a IMP/cysteine model system. These compounds have been shown to possess meat-like aromas (Meynier and Mottram 1995). Gasser and Grosch (1990) reported that 2-methyl-3-furanthiol, 2-furfurylthiol (2-furanmethanethiol), and 2,5-dimethyl-3-furanthiol are important aroma compounds in chicken broth. In addition, 2,4-decadienal also was found to contribute to the aroma of chicken. Carbonyl compounds, formed by the oxidative degradation of unsaturated lipids, change the "meat-like" odor into a "chicken-like" odor. Mottram and Whitfield (1994) showed that the formation of 2-methyl-3-furanthiol in the reaction between hydrogen sulfide and 4-hydroxy-5-methyl-3(2H)-furanone (HMF) appears to be favored by acidic conditions. In a model system containing cysteine/ribose, it was shown that nitrogen containing heterocyclic compounds such as pyrazines were only produced at pH values above 5.5, while furan thiols were favored by lower pH values (Madruga and Mottram 1995; Meynier and Mottram 1995). Whitfield and others (1988) also studied the flavor of a model cysteine/ribose system. They used much higher amounts of both reactants (0.04125 mol of cysteine and 0.02998 mol of ribose) in a phosphate buffer of pH 5.7 and heated the mixture for 1 h at 140 °C. They found relatively large quantities of 2-methyl-3-furanthiol and 2-furfurylthiol. Wasserman (1979) indicated that increasing temperature resulted in lower thiol concentrations. Higher temperatures were reported to favor pyrazine formation and reduce furan production in a serine/glucose model system (Parliment 1989).

High recovery of beef-like aroma results from high pH and low amount of cysteine (Figure 3). Cerny and Grosch (1992) identified 2-acetyl-2-thiazoline, Furaneol, 2-ethyl-3,5-dimethyl-pyrazine, and 2,3-diethyl-5-methylpyrazine as important contributors to the aroma of roast beef. In the study of a glucose-lysine model system and a cooked meat system, it was established that basic pH favors the formation of heterocyclic compounds such as thiazoles and pyrazines in the Maillard reaction (Leahy and Reineccius 1989; Madruga and Mottram 1995). This may be explained by the decreased reactivity of the amino group at lower pH due to its pro-
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Tonsbeek and others (1968) isolated 4-hydroxy-2,5-dimethyl-3(2H)-furanone (Furaneol) and 4-hydroxy-5-methyl-3(2H)-furanone (HMF) from beef broth, and these two compounds were considered to play a role in beef flavor. The formation of HMF, a precursor of several colored Maillard reaction products, and Furaneol, formed by the 2,3-enolisation route, are favored by more basic conditions which allow for the degradation of Amadori compounds in the Maillard reaction (Nursten 1980; Apriyantono and Ames 1993). Lane and Nursten (1983) found that equimolar amounts of cysteine and ribose heated at 100 °C for 30 min exhibited a distinct aroma similar to that of roast beef.

Recovery of egg-like aroma intensity increased with amount of cysteine and pH at 51 °C (Figure 4a). This trend was similar at 75 °C (X2 = 0) (figure not shown), but with a 5% lower recovery of egg-like aroma intensity. Cysteine amount had a greater influence on the recovery of egg-like aroma intensity than did pH. When considering comparable changes in the amount of cysteine and pH, recovery of egg-like aroma intensity decreased more dramatically due to a decrease in the amount of cysteine. At a higher temperature of 99 °C (X2 = 2.4), recovery of egg-like aroma decreased with increasing pH, with the cysteine amount not significant (Figure 4b).

The egg-like aroma was probably due to presence of hydrogen sulfide. Hydrogen sulfide (H2S), which is formed during thermal processing of cysteine, is a precursor of various compounds associated with meat aroma (Shibamoto 1980). However, in the present study, excessive levels of H2S in the process flavorings would be considered undesirable because of its pungent sulfurous and egg-like aroma. The release of H2S increases with time and temperature of cooking (Wasserman 1979), since H2S is a low-boiling-point compound and quickly dissipates. At high temperature, H2S will further react with other compounds to form various volatile compounds with meaty aroma attributes.

Van den Ouweland and Peer (1975) obtained a complex mixture of compounds with an overall odor resembling that of roasted meat by reaction of 4-hydroxy-5-methyl-3(2H)-furanone, which was derived from pentoses in the Maillard reaction, or its thio analog with H2S for 4 h at 95 to 100 °C. Shibamoto and Russell (1976) heated glucose with ammonia (NH3) then bubbled H2S through the solution for 10 min. The mixture was allowed to react at 100 °C for 2 h. It was noted by these researchers that the “resemblance to beef odor was unmistakable.” Shibamoto (1977) also reported that 2-furfurylthiol contributing to the chicken aroma was probably formed by the reaction of H2S with the sugar breakdown product 2-furaldehyde.

Model Fitting Using Results of RSM

Analysis of variance of the regression models for the consumer acceptance (overall liking) and specific descriptive aroma attributes indicated that models for overall liking, recovery of molasses-like, chicken-like, beef-like, and egg-like aromas were significantly (p < 0.05) effective. The models of recovery for all other aroma attributes (bean-like, potato-like, Brussels sprouts-like, roasted, and apple sauce-like) were not significant (p > 0.05). All models showed no lack of fit except recovery of molasses-like and roasted aroma intensity. This probably means that a higher-degree model may be required to explain their recoveries. Concurrently, the

![Figure 3](image-url)

Figure 3—Response surface plot of the effect of amount of cysteine and pH on recovery of beef-like aroma intensity at 99 °C (X2 = 2.4), 2 h reaction time (X3 = 1) and 7.1 × 10–4 mol of ribose (X5 = 1).

![Figure 4](image-url)

Figure 4—Response surface plot of the effect of amount of cysteine and pH on recovery of egg-like aroma intensity at (a) 51 °C (X2 = 2.4) and (b) 99 °C (X2 = 2.4) [2 h reaction time (X3 = 1) and 7.1 × 10–4 mol of ribose (X5 = 1)].
models developed for the overall liking and recovery of chicken-like, beef-like, and egg-like aroma intensity were adequate. The results of sensory assessment of the 50 process flavoring samples for these dependent variables are shown in Table 2. Significance and lack of fit, the effect of independent variables pH, temperature (T), reaction time (t), and amount of cysteine and ribose (C and R) on each dependent variable was divided into first-order (linear), second-order (quadratic), and interactive effects (interaction between pairs of variables) (Table 3). Estimated regression coefficients for each dependent variable were obtained from data shown in Table 2 by multiple linear regression (Table 3).

For descriptive flavor analysis, regression coefficients showed that T and C had linear effects on recovery of chicken-like aroma intensity, and pH had quadratic effects. There was no interaction effect on recovery of chicken-like aroma intensity. The linear effect of pH also needs to be considered because of the quadratic effect of pH on recovery of chicken-like aroma intensity. Amount of cysteine was the most important linear variable influencing recovery of chicken-like aroma intensity and had the largest value of estimated regression coefficient ($b_4 = 0.05$). The quadratic polynomial model equation for recovery of chicken-like aroma intensity ($Y_2$) was as follows:

$$Y_2 = -1.17 + 0.54X_1 - 0.002X_2 - 866.84X_4 - 0.05X_1X_4 - 632902.49X_4^2 + 173.14X_1X_4^2$$

The pH had a linear effect on recovery of beef-like aroma intensity, and C had a quadratic effect. Interaction effect was observed between pH and C. Although a linear effect was not significant on amount of cysteine, the linear effect of C also needs to be considered owing to the existing quadratic and interaction effect of C. The pH ($b_1 = 0.04$) was the most important linear variable affecting recovery of beefy aroma intensity. The fitted model for the recovery of beef-like aroma intensity ($Y_3$) was:

$$Y_3 = -0.49 + 0.13X_1 + 1575.75X_4 - 565283.45X_4^2 + 173.14X_1X_4^2$$

Recovery of egg-like aroma intensity depended on linear effects of pH, T, and C, quadratic effect of T, and interaction of C
The flavor system containing E-HVP, heated to 99 °C for 1.5 h was found to have the highest beef-like and chicken-like aroma in addition to having the highest overall acceptable aroma when compared to a flavor system that contained unheated E-HVP. Heating E-HVP in the presence of ribose and cysteine resulted in a decrease in bean-like, apple sauce-like, and molasses-like aromas. Based on the above evaluations by the panelists, the flavor system containing E-HVP, under the influence of heat, served as a rich source of flavor precursors. Therefore, a flavor system consisting of 10% (w/v) HVP, heated at 99 °C for 1.5 h at pH 6 along with 5 × 10⁻⁴ mol ribose and 5 × 10⁻⁴ mol cysteine does seem essential for the formation of Maillard-type flavor, characterized by an increase in desirable (chicken-like and beef-like) and a decrease in undesirable (bean-like, apple sauce-like, egg-like, and molasses-like) aromas.

**Conclusions**

Generation of meat-like aroma in a process flavoring made from soybean-based E-HVP was highly influenced by reaction time and temperature, pH, and amount of cysteine. Optimum reaction conditions for production of a meat-like process flavoring were pH 6 to 7.5, temperature of 90 to 99 °C, reaction time of 1 to 2 h, and cysteine from 4.5 × 10⁻⁴ mol to 6.01 × 10⁻⁴ mol. Amount of ribose had no effect at the range tested. Chicken-like aroma was favored by reaction at middle pH values, while beef-like aroma were more pronounced in flavorings produced at higher pH values. Bean-like, molasses-like, egg-like, roasted, and apple sauce-like aromas were perceived at low intensity in the meat-like process flavoring produced under optimal conditions.

**References**

Development of a Meat-Like Process Flavoring . . .

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