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Production of Meat-Like Flavor

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ABSTRACT Meat-like flavors, prepared by digestion of cooked meats by protease, followed by refluxing the mixture of meat hydrolysates, yeast extract, cysteine or methionine and glucose to obtain meat-like flavors. The sensory evaluation data for beef flavors compared to commercial beef (CB) flavor showed that the prepared flavor BH10YE was the best, among BH-R and BH20YE, and significantly different from CB ($P \leq 0.05$). The prepared pork flavors, PH10YE, PH20YE and PH-R, exhibited higher sensory scores than that of commercial pork flavor (CP) ($P \leq 0.05$) in which PH10YE was found to be more accepted than PH20YE. However, the prepared chicken flavors ,CH10YE and CH20YE, were not significantly different from commercial chicken flavor (CC) ($P > 0.05$).

KEYWORDS: meat flavor, beef flavor, chicken flavor, pork flavor, yeast extract.

INTRODUCTION

The precursors of meat flavor can be divided into two catagories as water soluble components (amino acids, peptides, carbohydrates, nucleotides, thiamines, etc.) and lipid or water insoluble components. The Maillard reaction between amino acids and reducing sugars leads to aroma. The meat flavor precursors obtained by heating meat lead to meat flavor via the Maillard reaction.[1] The flavor precursors have been identified to be free sugars, sugar phosphates, nucleotides, free amino acids, peptides, thiamine and other compounds. The reaction of cysteine and sugar can lead to characteristic meat flavor especially for chicken and pork.[2] Meat flavors can be prepared by the pyrolysis of amino acids and peptides, carameli-zation of carbohydrate, degradation of nucleotides and thiamine or thermal degradation of lipids.[3]

The flavor of beef results from lipid oxidation and/or degradation[4], thermal degradation and inter-reaction of proteins, peptides, amino acid, sugars and nucleotides, and thermal degradation of thiamine. These reactions produce many important beef aroma components.[5] Maarse and Visscher have classified some 880 aroma components from cook beef[6] and found that 25 of them possessed a meaty odor.

The flavors of pork have been found to be hydro-carbons, alcohols, carbonyls, carboxylic acids, esters, lactones, ethers, sulphur containing compounds and heterocyclic compounds.[7] The pork flavor precursors are generally derived from 2-methyl-3-fyranthiol and bis-(2-methyl-3-furyl) disulphide.[8] The major pathway for pork flavor development is from the degradation of lipids [9] and the free fatty acids are released during the process. The lipid degradation products are found to be saturated and unsaturated hydrocarbons, β-keto acids, methyl ketones, lactones and esters.[9] The Maillard reaction between reducing sugars and amino acids, peptides or proteins also leads to pork flavor. The Maillard reaction between cysteine and reducing sugars is especially important in yielding pork like aroma.[10] Other reactions such as the interaction of the Maillard reaction with lipids,[11] and the degradation of thiamine can also generate many sulphur-containing pork flavor compounds.[12]

The flavor of chicken was studied by Gasser and Grosch[13] who identified 2-methyl-3-furanthiol, 2-furfurylthiol, methionol, 2,4,5-trimethyl-thiazole, nonanol, 2-trans-nonenal, and other compounds as important. Volatile compounds generated from the Maillard reaction and lipid oxidation are obviously major sources of chicken flavor.[14] The compound, 2-methyl-3-furanthiol, is the most important compound in chicken flavor. It results from the reaction between ribose and sulphur containing amino acids such as cysteine or cystine.[15]

The flavor industry has developed a range of imitation flavors or meat-like flavors, although the specific flavor profile of a targeted meat item (*eg*, roast beef) has proven much more difficult to achieve. A great majority of patents dealing with the production of meat flavors are based on the Maillard type reactions. Many desirable meat like flavor volatiles are synthesized by heating water-soluble precursors such as amino acids and carbohydrates.[16] It is generally agreed that sulfur compounds, particularly cysteine play a key role in the development of meat like flavors. In 1960, Morton *et al*.[17] received the first patent which claimed that heating cysteine with ribose, glucose or xylose gave a flavor and aroma reminiscent of meat. Many of the patents that followed immediately thereafter chose cysteine as their preferred amino acid for the development of meat flavor. It served as an acceptable substrate for the formation of a large number of sulfur containing flavor chemicals associated with the meaty aroma. Several other patents stress the importance of cysteine/cystine as precursors for the development of meat flavor.[18-23]

Meat hydrolysates or meat extracts have served as condiments which can impart the same flavor as the meat stock from which they were derived. However, it is known that meat extracts do impart poor flavor and odor characteristics and these products are also expensive.

There have been several patents that involve the addition of crude protein hydrolysates. Apart from their role as amino acid precursors, they may also act as a source of reducing sugars or other carbonyl compounds thereby serving as reactants for the Maillard reaction. The best known protein hydrolysates universally accepted for their useful function for flavor are HVP and yeast extract. Several early patents have made use of yeast extracts or yeast hydrolysates as one of the reactants for the thermal generation of meat flavorings.[24-27]

Meat-like flavors are known to make a significant improvement in many savory foods such as soups, gravies, snacks and in a variety of other prepared foods. Almost all these food types can be purchased in a final cooked form so that it requires only heating. It should be noted that, although meat like flavors developed so far are reasonably satisfactory, they are still far from real meat flavors. These products do not yet have the unique characteristic flavor profile, the meat component of beef, chicken or pork has, which in general are clearly distinguishable from each other, organoleptically.

Thailand has to import large quantity of food flavors annually. If these food flavors can be produced in Thailand, it not only reduces the importation of food flavors but it can also be able to export to other countries.

In this study, the formation of beef, pork and chicken flavor and aroma was examined using precursors derived from enzymatic hydrolysis of beef, pork or chicken. The aroma reaction between yeast extract and meat hydrolysates in the presence of cysteine or methionine and glucose is also discussed.

Materials and Methods

Meat of chicken, beef and pork was purchased from the supermarket. Spent Brewer's yeast was obtained from Boonrawd Brewery Co.Ltd, Thailand. Papain was supplied by Sigma Chemical Co, USA. Commercial yeast extract, roasted chicken taste (CC) and roasted beef taste (CB) were obtained from import companies while pork extract flavor was kindly donated by a flavor company in Thailand. L-Methionine was obtained from Fluka, Switzerland. L-Cysteine hydrochloride monohydrate, D-Glucose monohydrate, sodium hydroxide, and hydrochloric acid were supplied by Merck Co Ltd, Germany. IMP and GMP were kindly donated by Ajinomoto Co. Ltd, Thailand.

Proximal analysis of the meat flavors BH10YE, PH10YE and CH10YE

Protein content was determined by the Kjeldahl method. Fat, ash and moisture contents were analyzed according to procedure described in the AOAC manual.

Preparation of yeast extract

Spent brewer's yeast with solid content of about 18-20% (w/w) was adjusted to pH5.0 by addition of either 1M HCl or 1M NaOH. It was then autolyzed at 50°C for 24 h. The autolyzed solution was heated at 85°C for 10 min to deactivate the enzyme, and then it was centrifuged at 4500 rpm for 10 min.[28-30] The clear supernatant was referred as yeast extract with 7-8% (w/w) of solid content, which was then concentrated in rotary evaporator to 30% (w/w) solid content.

Removing of bitterness and beer flavor from yeast extract

The bitter compounds and beer flavor in yeast extract solution prepared from spent brewer's yeast was removed by adding activated carbon (about 3.5g carbon to 100 ml of yeast extract solution) and stirring at 50°C for 1 h. The activated carbon was then removed by centrifugation at 4500 rpm for 10 min.

Preparation of meat hydrolysate

The meat (chicken, pork or beef), which was free of fat, was cut into small pieces and boiled for 10 min. The cooked meat was ground and water was added to obtain 25% (w/w) of solid. Papain was then added to the ground meat slurry at 0.5% (w/w) of the dried meat followed by incubation at 60°C for 12 h. The enzyme was then deactivated at 90°C for 15 min.[31,32] The meat hydrolysate slurry was then clarified by centrifugation at 10500 rpm for 15 min. The clear supernatant was transferred to a separatory funnel and any surface oil removed. The resulting solution was called meat hydrolysate.

Preparation of flavor from refluxed meat hydrolysate

The meat hydrolysate was adjusted to pH 6.0 and refluxed at 90°C on a waterbath. It was then cooled and spray dried using an inlet temperature of 180°C and an outlet temperature of 95°C, to obtain a flavor powder. The reaction time for the refluxed beef hydrolysate (BH-R) and refluxed pork hydrolysate (PH-R) was 2 h, while that for the refluxed chicken hydrolysate (CH-R) was 4 h.[33-35]

Preparation of meat flavors (beef, pork, and chicken flavor) through Maillard reaction containing yeast extract

The mixture formulations and refluxing times used for the Maillard reaction to prepare meat flavors (beef, pork or chicken) are shown in Table 1. Generally, the Maillard reaction mixtures contained meat hydrolysate (beef, pork or chicken hydrolysate), yeast extract, an amino acid and glucose. The reaction mixture was adjusted to pH 6.0 and refluxed at 90°C for the requisite period of time, followed by spray drying to obtain a flavor powder.[33-35]

Sensory evaluation tests

The flavors were evaluated for sensory acceptability by 40 untrained panelists who were staffs, undergraduate and graduate students at Biotechnology Department, Faculty of Science, Mahidol University.

The flavors were served in the form of a soup consisting of 1.5% (w/v) flavor, 0.75% (w/v) salt and 0.25% (w/v) of flavor enhancers. The flavor enhancers were MSG, IMP and GMP in the quantity ratio of 98:1:1, respectively. For evaluation, panelists were asked to smell and taste the odor and flavor of these products. They were asked whether the odor and flavor of the presented soups (from prepared and commercial meat flavors) were similar to those of specific meat products. A 9-point scale was used for evaluating these flavors with 9 = most like the designated meat and 1 = least like the designated meat. An ANOVA-statistical technique was employed for comparison of the sensory evaluations.

RESULTS

The meat flavors that obtained the highest sensory evaluation scores were analyzed for protein, carbohydrate, fat, ash and moisture contents (Table 2). These Maillard reaction flavors included BH10YE (beef hydrolysate, cysteine hydrochloride, yeast extract and glucose), PH10YE (pork hydrolysate, yeast extract, methionine and glucose) and CH10YE (chicken hydrolysate, yeast extract, cysteine hydrochloride and glucose).

The sensory evaluation on odor, flavor and overall acceptability of commercial beef flavor (CB), refluxed beef hydrolysate (BH-R), and those products prepared from Maillard reaction of beef hydrolysate, yeast extract, cysteine hydrochloride and glucose (BH10YE and BH20YE) are presented in Table 3. BH10YE obtained the highest mean evaluation score for oder, flavor and overall acceptability, and this score was significantly different from that for the commercial flavor CB ($P \leq 0.05$).

Table 1. Formulation and reaction time used for preparing meat flavors.

Items (g dry weight)	Beef flavor		Pork flavor		Chicken flavor	
	BH10YE	BH20YE	PH10YE	PH20YE	CH10YE	CH20YE
Beef hydrolysate	90	80	-	-	-	-
Pork hydrolysate	-	-	90	80	-	-
Chicken hydrolysate	-	-	-	-	90	80
Yeast extract	10	20	10	20	10	20
Cysteine hydrochloride	2	2	-	-	4	4
Methionine	-	-	0.4	0.4	-	-
Glucose	1	1	1	1	1	1
Reaction time (h)	2	2	2	2	4	4

Table 2. Proximal analysis of meat flavors.

Samples	% Protein*	% Carbohydrate	% Fat	% Ash	% Moisture
BH10YE	87.10	5.29	0.44	7.17	7.54
PH10YE	89.37	2.55	0.45	7.63	7.24
CH10YE	86.74	3.95	0.43	8.88	6.91

* Nx6.25

Table 3. Acceptance scores[1] of beef flavor samples.

Samples	Score ± S.D.		
	Odor	Flavor	Overall
1. CB	5.68±2.37[a]	6.00±2.13[a]	4.90±2.24[a]
2. BH-R	5.65±1.82[a]	6.68±1.57[a,b]	5.48±1.65[a,b]
3. BH10YE	7.18±0.94[b]	7.59±0.78[b]	6.53±1.46[b]
4. BH20YE	6.68±1.22[a,b]	7.21±1.37[b]	6.07±1.71[a,b]

[a,b]Means with different superscripts in the same column are significantly different at P≤0.05.

[1] Highest score = 9 most similar to beef flavor, Lowest score = 1 = least similar to beef flavor.

Table 4. Acceptance scores[1] of pork flavor samples.

Samples	Score ± S.D.		
	Odor	Flavor	Overall
1. CP	3.47±2.11[a]	4.48±2.23[a]	3.33±1.96[a]
2. PH-R	5.41±1.88[b]	6.78±0.94[b]	5.21±1.24[b]
3. PH10YE	6.38±0.94[b]	7.16±1.14[b]	5.72±1.39 [b]
4. PE20YE	6.31±1.23[b]	6.34±1.43[b]	4.93±1.57[b]

[a,b]Means with different superscripts in the same column are significantly different at P≤0.05.

[1] Highest score = 9 most similar to pork odor or flavor, Lowest score = 1 = least similar to pork odor or flavor.

Table 5. Acceptance scores[1] of chicken flavor samples. No significant differences were found (P≤0.05)

Samples	Score ± S.D.		
	Odor	Flavor	Overall
1. CC	6.50±1.78 [a]	7.17±1.16 [a]	5.57±1.74 [a]
2. CH-R	5.42±1.89 [a]	6.33±1.31 [a]	4.78±1.92 [a]
3. CH10YE	5.69±1.65 [a]	6.61±1.34 [a]	5.37±1.57 [a]
4. CH20YE	6.25±1.42 [a]	6.64±1.44 [a]	5.17±1.69 [a]

[a] Means with different superscripts in the same column are significantly different at P≤0.05

[1] Highest score = 9 most similar to chicken odor or flavor, Lowest score = 1 = least similar to chicken odor or flavor.

The sensory evaluation for odor, flavor and overall acceptability of pork flavors, including commercial pork flavor (CP), refluxed pork hydrolysate (PH-R) and the Maillard reaction of pork hydrolysate, yeast extract, methionine and glucose (PH10YE and PH20YE) are shown in Table 4. The mean sensory scores for odor, flavor and overall acceptability for PH10YE was highest and this was not significantly different from PH20YE and PH-R. All three were significantly different from the commercial flavor CP. Indeed, the mean sensory evaluation scores for CP were very low.

Table 5 shows the sensory evaluation scores for odor, flavor and overall acceptability of commercial chicken flavor (CC), refluxed chicken hydrolysate (CH-R) and the CH10YE and CH20YE Maillard reaction products of chicken hydrolysate, yeast extract, cysteine hydrochloride and glucose. No significant differences were found (P>0.05).

Discussion

The proximal analysis data in Table 2 showed that the spray dried products BH10YE, PH10YE, and CH20YE had moisture contents in the range of 6-7% (w/w) when they were in equilibrium with the humidity in air. Their ash and fat contents were also close. Since too high a fat content can cause rancidity due to the degradation of unsaturated fatty acids by oxidation reactions, some part of the fat had to be removed during the preparation of meat hydrolysates. However, some of the flavor compounds in which characterized the meat flavors was derived from their fat content. The protein content in these flavor was high and at the same level. This protein can act as an encapsulating agent to trap volatile flavor components.[36]

Since the sensory evaluations scores for the beef flavors that contained yeast extract, cysteine and glucose (i.e., BH10YE and BH20YE) were higher than those that did not (BH-R), the results suggested that those compounds were important in helping to enhance beef like flavor. However, the odor of yeast extract is very strong, and if added in too high quantity, it would dominate that of the beef flavor.

Normally, beef hydrolysate possesses the flavor precursors characteristic of beef flavor. In the case of the tested commercial flavor CB, it was not known whether it contained yeast extract in addition to beef hydrolysate.

For the pork flavors, PH10YE gave the best sensory evaluation score, although this was not significantly different from those of PH-R and PH20YE. Thus, any of the three tested methods for these would be suitable for preparing a good flavor. The yeast extract studied was prepared from low cost spent brewery yeast. Thus, the higher the quantity of yeast extract that could be used to replace meat, the lower would be the production cost of the flavor. Thus, in the case of pork flavor, more yeast extract could be used (up to 20% w/w) than that used to prepare beef flavor (*ie*, 10% in BH10YE was best) without obtaining too strong a yeast extract odor. It may be that yeast extract odor is more similar to that of pork than that of beef. Even so, employing more than 20% (w/w) in pork flavor would probably result in a significantly lower evaluation score. The sensory evaluation scores for the commercial CP were too low, since the flavor was not accepted by the panel members who evaluated the odor, flavor and overall acceptability. Thus, the pork flavors prepared in this investigation were better than the commercial pork flavor used for comparison.

The mean sensory evaluation scores on odor, flavor and overall acceptability of chicken flavors (CC, CH-R, CH10YE and CH20YE) were compared. No significant differences were found. Therefore, the chicken flavors prepared in the present investigation were of as good quality as the commercial flavor CC. The high quantity of yeast extract (*eg*, 20% in CH20YE) did not have a negative result on sensory evaluation scores for chicken flavor. Thus, it may be possible to increase the quantity of yeast extract to even higher levels than used in CH20YE. Perhaps the odor of yeast extract is even closer to that of chicken than pork. In this investigation, we demonstrated that chicken flavor could be prepared according to the methods used for preparing CH-R, CH10YE or CH20YE.

The formation of specific meat flavors, i.e., pork, chicken and beef flavors, at high intensity ,can be achieved through the Maillard reactions among amino acids of meat hydrolysate and yeast extract ,cysteine or methionine and glucose. Food flavors obtained from Maillard reaction without amino acids of meat hyrolysate provided the savory flavor, which is not specific to any kind of meat, *ie*, pork, chicken or beef.

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