THE EFFECT OF pH ON FLAVOR FORMATION AND ANTIOXIDANT ACTIVITY OF AMINO ACID AND SUGARS INTERACTION PRODUCTS

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ABSTRACT

Background/Aim: Flavor is the crucial part of eating quality. In the preparation of natural identical flavor in different model systems like cysteine- ribose (cys-rib), cysteine-glucose (cys- glu) and cysteine-beef fat (cys-BF) affected at different pH (4.5, 7 and 10) a wide range of flavors was obtained.

Materials and Methods: The Proteins as glutamine, glutamic acid and the sugars as D- ribose and D-glucose, 1,1-diphenyl-2- picrylhydrazyl (DPPH) and β-Carotene and all the Lab grade chemical as Linoleic acid, Polyoxyethylene sorbitan monopalmitate (Tween-80), Chloroform (99%), Anhydrous sodium sulfate, Dichloromethane (99.8%), 0.1 N HCl and 0.1N NaOH, Gallic acid, Sodium carbonate, Folin-Ciocalteu reagent were used to conduct research.

Results: In sugar and amino acid model system, roasted and burnt meat flavor was obvious while in beef fat model system boiled meat flavor was dominated which was strongly supported by sensory evaluation. In rib-cys and glu-cys model systems total phenolic contents (TPC) were highest at pH 7 and pH 4.5, respectively along with browning, leading to strong antioxidant activity. In beef fat–cys model system it was found that as pH increases TPC, browning increases and antioxidant activity becomes maximum at basic pH.

Conclusion: All the results indicated that, there is a positive relationship between the TPC, browning and antioxidant activity of all model systems.

Keywords: Meat flavor, beef fat, meat model systems, pH, antioxidant activity, natural Antioxidants.

INTRODUCTION

Maillard reaction is a non-enzymatic browning reaction, caused by the condensation of an amino group and a carbonyl compounds, resulting complex changes in biological and food system. Maillard reaction occurs when virtually all foods are heated, and also occurs during storage. Heat treatment is vital for generating flavor compounds in a wide variety of food products. Heat induces complex thermal reactions among proteins, carbohydrates, fats, organic acids and vitamins that result in generation of flavor compounds and brown color pigments. The most important reactions contributing to meat flavor generation during the process of meat cooking can be primarily classified as Maillard reaction (interaction of sugar with amino acids or peptides), including Stretcher degradation and the thermal degradation of lipids.

Flavor is a crucial part of the eating quality of meat and an inalienable consideration for consumer acceptance. 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. Meat flavor is determined by compounds contributing to the sense of taste and stimulating the olfactory organs. The flavor of raw fresh meat is bland, metallic and slightly salty and only a blood-like taste whereas desirable meat flavor is apparent only after heating. During cooking, a complex series of thermally induced reactions occur between non-volatile components of lean and fatty tissues resulting in a large number of reaction products. The precursors of meat flavor can be divided into two categories as water soluble components (amino acids, peptides, carbohydrates, nucleotides, thiamines, etc.) and lipid or water insoluble components which on heating lead to meat flavor via Maillard reaction. Due to the great sensory and economic value of beef, enormous amount of researches have been conducted on beef flavor. Almost 1000 volatile compounds have been identified from meat or from model systems consisting of meat ingredients and one might predict that may more thousands of volatiles compounds will be identified from these systems in the future. It is well known that pH greatly affects the nature of Maillard reaction. More colored and polymeric compounds are formed at higher pH. Alkaline conditions tend to facilitate the formation of nitrogen- containing volatiles while other volatiles are more likely formed under acidic environments. The lipids of meat comprise both the neutral triacylglycerols and the structural phospholipids.

Both are capable of undergoing oxidative degradation, leading to both desirable and undesirable aroma volatiles. A number of studies of the effect of phospholipids on the
volatile products of heated aqueous solutions of amino acids and sugars have shown that Maillard reaction products are influenced by the presence of phospholipids, confirming the earlier observations with defatted meat \cite{8-11}. When lipid like phospholipids or triglycerides are removed by polar solvent like chloroform or methanol, the meaty aroma is replaced by a roast biscuit like aroma. Maillard reaction produced from amino-sugar model system is associated with the formation of compounds of strong antioxidant activity \cite{12,13}. During the earlier stages of Maillard reaction, MRP’s of smaller molecular compounds are produced which are pro-oxidant. Their high chemical activity lead to the production of compounds like melanoidins and heterocyclic compounds (furan, pyrazines and pyranone) which are strong antioxidants \cite{14).

Meat flavor is the most important quality of meat and meat products. It has application in liquid form as well as in cubes form that can be used in soups, drinks, snacks and other products to increase its aesthetical value. If we have a glance on laboratory production of meat flavor in the world it will be clear that whole work on the production of flavor is done in developed countries. Asian and Arabic countries are very far away from these innovations and has no share in this concern till now. So, the aim of this work to bring this innovation in a relatively less expensive form meat flavor in Pakistan from economical sources like beef fat to replace it or as alternative of expensive basic component of meat flavor that will be better sources of color, flavor and antioxidant activity.

**MATERIALS AND METHODS**

The following chemicals were used to conduct research. Proteins as glutamine and glutamic acid and the sugars D- ribose and D- glucose, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and β-Carotene were purchased from Merck Std. All the Lab grade chemicals as Linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-80), chloroform (99%), anhydrous sodium sulfate, Dichloromethane (99.8%), 0.1 N HCl and 0.1N NaOH, gallic acid, sodium carbonate, Folin-Ciocalteu reagent were also estimated by Folin-Ciocalteu method (FCM) \cite{16}. An aliquot of MRP sample (200 µL) was treated with 320 µL of distilled water and 2 mL of 0.12 mM DPPH in methanol was added. Then the solution was mixed vigorously and allowed to stand for 6 minutes. The absorbance of mixtures was measured at 517 nm using a UV-1601 spectrophotometer. Gallic was used as a standard.

**Preparation of Maillard Reaction Model Systems**

0.1M (0.5g) cystiene amino acid , 0.07M (0.5g) ribose/glucose and ground beef fat sugar was weighed accurately in 50 ml beaker and mix in distilled water. pH was adjusted to 4.5, 7 and 10 with the help of 0.1N HCl and 0.1N NaOH, volume was made up to mark 50 ml. Then all these system were transferred to flask of Reflux apparatus and heated at boiling temperature for one hour.

**Extraction of Volatiles of Meat Flavor**

Maillard reaction substance was separated by using solvent-solvent producer which also contained water fraction along with volatile of meat flavor dissolved in dichloromethane, then separated by rotary evaporator and kept it at 0°C until further analysis.

**Degree of Browning**

The browning of MRP samples were measured according to the method of Lertittikul, Benjukal and Tanaka \cite{15}. Appropriate dilution was made using distilled water and the absorbance was measured at 420 nm using a UV-1601 spectrophotometer for determining the browning intensity.

**Total phenolic content (TPC) determination**

The total phenolic compounds were estimated by Folin-Ciocalteu method (FCM) \cite{16}. To 125 µL sample was taken in test tube, 500 µL of distilled water was added then 125 µL of Folin-Ciocalteu reagent and allowed to stand for 6 minutes. Then 1.25 mL of 7% sodium carbonate was added in it. Make final volume 3 mL by adding 1 mL of distilled water. Stand the samples for 90 min for completion of reaction and absorbance of the samples were made in triplicate at 760 nm using a UV-vis spectrophotometer. Gallic was used as a standard.

**Determination of Antioxidant Activity**

The antioxidant activity of volatile and non-volatile compounds was determined by two methods; DPPH scavenging activity and β-carotene bleaching assay.

**DPPH Scavenging Activity**

DPPH radical-scavenging activity was determined according to the method of Lertittikul, Benjukal and Tanaka \cite{15}. An aliquot of MRP sample (200 µL) was treated with 320 µL of distilled water and 2 mL of 0.12 mM DPPH in methanol was added. Then the solution was mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of mixtures was measured at 517 nm using a UV-1601 spectrophotometer.

**β-Carotene Bleaching Assay**

Antioxidant activity of the aqueous solution of MRP sample was determined by β-carotene/linoleic acid system, as described by Lertittikul, Benjukal and Tanaka \cite{15}. Accordingly, 1 ml of β-carotene solution (1 mg/ml in chloroform), 40 µL of linoleic acid and 400 µL of Tween 80 were transferred to a round-bottom flask. Chloroform from the sample was evaporated using a stream of nitrogen. Then add 100 ml of distilled water.
slowly to the residue and vigorously agitate to give a stable emulsion. To an aliquot of 4.5 mL of this emulsion, add 500 µL of appropriately diluted samples. To the control reaction mixtures, 500 µL of distilled water is added. Absorbance was measured, immediately at 470 nm.

**Sensory Evaluation of Meat Model Systems**

Sensory evaluation was done according to El-massry, Farouk and El-Ghorab.  

**RESULTS**

**Measurements of degree of browning**

Four different model systems like glucose-cysteine, ribose-cysteine and fat-cysteine at acidic, neutral and basic pH at A 420 nm according to results, it was found that browning is directly proportional to phenolic compounds. The cysteine model system browning degree was highest at pH 7 is 2.060 and the lowest degree of browning was measured at pH 4.5 in fat model system is 0.692, on spectrophotometer analysis. The degree of browning is highest in ribose model system than all others (fig.1).

**Total phenolic contents**

Total phenolics compounds of nine model systems were determined at different pH (fig. 2). The quantity of phenolic compounds were measured in µg/ml by plotting it against the standard curve of gallic acid. The results showed that TPC of cysteine-glucose model system is the highest (409.13 ± 12.96) at pH 4.5 in comparison to other model systems at the same pH. In the cys- glu model system, the phenolic compounds ranges from 4.5 >7 >10. On the other hand TPC in the ribose-cysteine model system is higher at pH 7 than any other pH of its model systems and at the same pH in all model systems. It was found that TPC in different Maillard reaction model systems increases in these order as pH 4.5>10. The phenolic contents in fat-cysteine model system are lower than sugar-amino acid model system. In fat- cysteine model systems phenolic contents ranges from 10>7>4.5. Phenolic contents were the highest in this (cysteine-fat) model system at pH 10 which is 67.47 ± 3.33 and were higher than any other pH in fat model system.

**Determination of the antioxidant activity of Cysteine-Ribose model system at different pH**

The model systems of cysteine-ribose at different pH showed different antioxidant activity patterns. It was found that the highest antioxidant activity at concentration of 500 µg and pH 7 was 52.98 ± 0.38 and the lowest antioxidant activity was observed at pH 4.5.

The inhibition % of cysteine-ribose model system at neutral pH is higher than that at acidic and basic pH because not only it having more phenolic compound than the other two model systems but also have the highest degree of browning.

**Fat-Cysteine model system at pH 4.5,7 and 10**

The results showed the antioxidant activity of Maillard reaction products of cysteine-fat model system at three different acidic, neutral and basic pH at concentrations from 500µl. The percentage of standard BHT antioxidant activity is 91.135 ±0.05 %. It was found that fat-cysteine model system has significant antioxidant activity at pH 10 at concentration of 500 µg in comparison with other model systems at pH 4.5 and 7. These model systems indicated that as pH increases browning degree increases. Phenolic compounds indicates that cysteine-fat model system has more phenolic compounds at basic pH (10) than that at neutral and acidic pH. From these results it was also cleared that basic pH has strong degree of browning that lead to the maximum production of browning pigments, thiazoles, alkylpyrazines and some others.

**Cysteine-Glucose model system at pH 4.5,7 and 10**

The antioxidant activity of Maillard reaction products of cysteine-glucose model system at three different acidic, neutral and basic pH indicates that the highest antioxidant activity was found at concentration of 500 µg and acidic pH 4.5 was 92.62 ± 0.21 and the lowest antioxidant activity was observed at basic pH (10) was 36.76± 0.45%. This indicates a good relationship between inhibition %, phenolic contents and browning degree and binds them in a direct relationship.

**Sensory evaluation of different meat model systems**

Figure (6) demonstrated the sensory profile of the Fat-Cysteine, Ribose-Cysteine and Glucose-Cysteine model systems extracts respectively as well as the intensity of developed flavor and odor acceptability. Pronounced differences were observed in the odor profile. Expected intensities of roasted sulfuric and burnt caramel notes are weak in Fat-Cysteine model system. In fat model system boiled flavor increases with increase in pH and becomes maximum at basic pH (10) (fig. 6). It has strong boiled meat like flavor along with sweaty notes at basic (10) pH as compared to acidic (4.5) and neutral pH (7).
The Effect of Ph on Flavor Formation

Figure 1: The degree of browning of Cysteine-Ribose, Cysteine-Fat and Glucose Cysteine model systems at different pH (4.5, 7 and 10)

Figure 2: The Total phenolic content of Cysteine-Ribose, Cysteine-Fat and Glucose Cysteine model systems at different pH (4.5, 7 and 10)

Figure 3: % inhibition of Cysteine- Ribose model
Figure 4: % inhibition of Cysteine-fat model systems

Figure 5: % inhibition of Cysteine-glucose model systems

Figure 6: Sensory profile of different meat model systems
DISCUSSION

Browning is the most important feature of Maillard reaction products that is responsible for brown color in foods and compounds having browning ability which act as strong antioxidants. Four different model systems like glucose-cysteine, ribose-cysteine and Fat-cysteine at acidic, neutral and basic pH at A 420 nm according to results it was found that browning is directly proportional to phenolic compounds.

In cysteine-fat model system, as pH increases browning also increases and becomes maximum at pH 10. These results were in agreement with Ajandouz and Piigserver (18), who found that the higher browning intensities of MRP of fructose –lysine system heated at 100 °C were observed with increasing pH values. Generally, the ultraviolet (UV) absorbing and colorless compounds formed at intermediate stages contributed to brown pigments formation in Maillard reaction as studied by many authors (12,19, 20).

Cysteine and methionine model systems are yellow orange at acidic pH and tends to brown or red-brown as pH proceeds. Basic pH favors the formation of pyrazines in MR. Pyrazines are not detected at lower pH values (21). Furans derivatives especially 2-methyl-3-furanthiol are mostly detected at acidic pH values between 4.5 -5.5. Nursten (22), found that in lysine model system furfural is major compound for non-enzymatic compound. All results are in agreement with literature (23, 24).

As a result of Maillard reaction, phenolic compounds are produced which are also called polyphenols which are responsible for flavor producing compounds in respective to model systems and also they are in good coordination with antioxidant activity.

Natural antioxidants are believed to intercept the free radical of chain of oxidation and to contribute hydrogen from the phenolic hydroxyl groups themselves by forming stable free radicals which do not initiate or propagate further oxidation of lipids (14).

Lingnert and Eriksson (25), Studied that browning compounds produced in sugar-amino acid interaction have more antioxidant activity in comparison with protein- sugar interaction in Maillard reaction. According to Yen and Hsieh (26), who stated that browning compound produced from ribose and amino acid reaction are free radical inhibitors which can work as primary antioxidants. Browning compounds are also having antioxidant activity.

According to literature, pyrazines start to be produced at neutral pH. At acidic pH like 4.5 no or only traces of pyrazines are noticeable as compare to basic and neural pH. As pH increases, Maillard reaction products with more antioxidant activity are produced that is why pH is important factor for determining antioxidant activity by MPRs (15).

Nitrogen containing compounds that enhance the inhibition percentage at basic pH (27). Similarly, Yen and Lai (28) stated that there is a good correlation between antioxidant compound and total phenolic compounds that have high capacity of scavenging activities. Heterocyclic compounds like 2-methyl pyrrole and pyrrole-2-carboxaldehyde which only produced in fat system inhibit antioxidant activity so acidic model systems have low inhibition. Mottram (29), found that in addition to reducing sugars, carbonyl compounds produced from other sources like lipid peroxidation or derived from lipid are also able to react with amino acid that produced macromolecules pigments which have properties similar to melonoids which in turn have antioxidant activity and able give brown color (30).

At acidic pH most powerful meat flavourous compound like furans its derivatives and most of heterocyclic compound like pyroles, thiazoles pyranones etc. are produces in significant quantity which have antioxidant, anticarcenogenic and antimicrobial efficiency. These compounds are responsible for the development of brown color named as browning which in turn have significant antioxidant activity(27, 31). Osada and Shibamoto (16) reported that Maillard reaction produced from sugar and amino acid interaction possess antioxidant especially heterocyclic compounds are key factors to inhibit lipid oxidation at acidic pH. Murakami et al. (32) stated that pronounced browning are produced in the presence of glucose in study of Maillard reaction with and without sugar while Labuz et al. (33) studied that in glucose model systems high browning rate was observed at acidic pH as compared to basic and neutral pH. Peyrot et al. (34) reported that the degradation of cysteine in acidic condition and in the initial stages of reaction is high that give compounds like thizole are produced more. thiazoline and 2, 5 dimethylpyrazines and tetramethyl pyrazines at acidic pH 4.5 that lead to increase in inhibition activity and degree of browning (35).

The compounds like thiophene, trithiolane, thiazole, thiophene farnamethyl thiol and 2-pentyl 2(H) thiafuran are dominated in Fat-Cysteine model system. These compounds gave boiled like meat flavor rather than roasted and burnt caramel flavor. Sensory evaluation of sugar-amino acid model
system (ribose, glucose and cysteine) showed roasted beef flavor and some what boiled meat flavor. This is due to the presence of compound which is different from that fat model system that is why flavor changed to roasted meat flavor. On the other hand in Glucose-Cysteine model system have roasted meat flavor. On the other hand in Glucose-Cysteine model system that is why flavor changed to roasted meat flavor. This is due to the presence of pyrazine, especially in ribose present in traces. Higher intensity of meat (fig. 2) due to excellent presence of 2-methyl-(pronounced flavor of meat at acidic pH (4.5)


Murakami, M., A. Shigeeda, K. Danjo, Y. Yamaguchi, H. Takamura and T. Matoba. Radical-


الملخص العربي

تأثير درجة الحموضة على تكوين النكهة ونشاط تضاد الأكسدة
وذلك خلال التفاعلات المشتركة بين الأحماض الأمينية والسكريات

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قسم كيمياء مكسبات الطعم والرائحة - المركز القومي للبحوث - الدقي - مصر، المعهد القومي للكيمياء والعلوم الأغذية - الجامعة الزراعية - فصل أباد - باكستان

تعتمد جودة المادة الغذائية اعتمادًا كبيرًا على الكهكلة المميزة لها لما لها من تأثير على مدى قبول المادة الغذائية.

وقد تم عرض نماذج مختلفة للحصول على كهكلة مماثلة للنكهة المتنوعة طبيعياً وذلك محسوب خلال إجراء تفاعلات مختلفة بين كل من (سينسون مع ريبوز) (سينسون مع دهن بقرى) و ذلك تحت ظروف مختلفة محسوب درجات الحموضة [5]. درجة القدمينية والمتعلقة والفعالة والقابلية والتي كانت تشمل الأنس البودروميجي، 5، 4، 2، 0 وتم الحصول على العديد من النكهات المختلفة.

وقد وجد أن في حالة التفاعل بين الجلوكوز أو الريبوز مع الحامض الأميني أدى إلى الحصول على نكهة واضحة من رائحة اللحم المشروى أو المحروق بينما كانت نكهة اللحم المسلح في حالة التفاعل بين الجلوكوز أو الريبوز والدهن البقرى و هي السائدة والتي أكنت بقوة بواسطة التقييم الحي.

ولظهرت نتائج التدفق الكلي لمحتوى الفينول أنه كان أعلى في حالة درجة الحموضة 4، 5 على الترتيب والذي يفق مع الثلومن البني والذي يؤدي بدوره إلى زيادة نشاطية مضادات الأكسدة وقد وجد أن النظام المستروت عن رائحة اللحم الذي ينتج من تفاعل الدهن البقرى مع الحامض الأميني السينسون يعني أعلى درجة تلوين تلك المحتوى الفينولي ونشاطية مضادات الأكسدة وذلك عند الوسط القاعدي.

وأثبتت النتائج السابقة أن يوجد علاج طريقة بين كل من المحتوى الفينولي ونشاطية مضادات الأكسدة، مما سبق بتضخ لـ مـ ـــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــــ~

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