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Volatile Compounds from Potato-Like Model Systems

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Abstract: The volatile reaction products of aqueous mixtures comprising combinations of methionine, glucose, oleic acid and starch heated in a modified Likens-Nickerson apparatus were extracted and analyzed by gas chromatography-mass spectroscopy (GC/ MS). The majority of volatile compounds were formed from Linoleic acid degradation, hexanal, 2, 4-decadienal and 2-pentylfuran being identified in the greatest amount. Dimethyldisulphide and dimethyltrisulphide were detected in every system containing methionine. 3-(Methylthio) propanal (methional) and other sulphur compounds were detected when methionine was heated with other precursor. No binding of volatiles to starch was observed; rather, starch appeared to act as an additional source of reactive carbohydrate. Almost all the components identified have been identified among the aroma compounds of cooked potato. No pyrazines, pyridines, or thiazoles were identified, may be due to the relatively low temperature/ high moisture conditions.

Key words: Potato volatiles · Glucose · Methionine · Linoleic acid · Maillard reaction · Model systems

INTRODUCTION

Potato is grown in many parts of the world. Egypt where exported large amounts around the world [1]. One reason for its popularity is that the tuber can be processed by boiling, baking and frying. In addition, it is used in the dehydrated form by the food industry and is an important component of many ready to eat. The volatile components of the various forms of cooked potato and also of the raw vegetable, have been the subject of considerable study [1, 2]. More than 25 compounds have identified in boiled or baked potatoes alone [3]. In most cooked foods, including potatoes, the Maillard reaction and lipid degradation are two of the most important reactions leading to the formation of aroma compounds. The compounds that could be found to baked potato aroma include pyrazines, 2, 4-decadienal and methional [4]. Coleman *et al.* [5] also identified thiazoles, furans, acids, alcohols, esters and ketones, while Nursten and Sheen [6]detected aldehydes, alcohols, 2-ethylfuran and 2-pentylfuran in boiled potato aroma and Josephson and Lindsay [7] demonstrated the importance of cis-4-heptanal in potato flavour. Herz and Shallenberger [8] suggested that the reaction involving methionine and glucose heated at 100°C was important in the generation of potato flavour. More recently, methionine/ glucose and methionine sulphoxide/ glucose model systems were reported to produce cooked potato -like aromas on heating for 1h at 180°C and methional, furans and pyrazines were among the major reaction products [9].

Volatile compounds generated from lipid-amino acid-sugar interactions have been reviewed by Whitfield [10]. Most of the published work concerning the effect of lipid on the Maillard reaction has been undertaken by Whitfield *et al.* [11], Farmer *et al.* [12] and Farmer and Mottram [13]. They used a meat model system, i.e., aqueous cysteine-ribose - phospholipids model systems heated at 140°C for 1hour and established that, in the presence of phospholipids, furans, thiophenes and pyridines possessing long-chain alkyl substituted are formed while levels of other heterocyclic compounds are modified. Antioxidants can play a role in the prevention of autoxidation. However, there may be two major problems for the use of conventional antioxidants such as butyl hydroxy toluene (BHT) and butyl hydroxy anisole (BHA). They are approved for use in a limited number of food products and each new synthetic antioxidant must be subjected to a lengthy process of evaluations for proof of safety. Consumers are increasingly concerned about chemical additives used in foods [14]. The antioxidant capacity of MRPs was observed for the first time by Wittayachai *et al.* [15] and some fractions were reported to have strong antioxidant properties comparable to those of commonly used food antioxidants. The heterocyclic compounds, especially those containing sulfur, are very important to generation of potato-like aroma [16].

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Consumers allover the world is becoming more conscious of the nutritional value and safety of their food and its ingredients. At the same time, there is a preference for natural foods ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts. The safety of food flavours has been highly concerned in recent years. Compared with synthetic flavour compounds, natural flavours are more acceptable to consumers. So it has been one of hot topics in flavour chemistry to obtain natural flavours [17]. Egypt is very far away from the field of flavour production and has no share in this concern till now. So the main aim of this study to bring this innovation in Egypt in a relatively less expensive form. My objective is to produce potato-like aroma to replace another synthetic type which has healthy hazards on human health that will be better source of colour flavour and antioxidant activity.

MATERIALS AND METHODS

Materials: The following chemicals were used to conduct research. D-glucose (99%), L-methionine (98%) and potato starch (soluble), β-carotene, linoleic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH°), tert-butyl hydroquinone (TBHQ), polyoxyethylene sorbitan monopalmitate (Tween-80), chloroform (99%) and anhydrous sodium sulphate (Na_2SO_4), were purchased from Merck (Darmstadt, Germany). Sodium hydroxide and HCL, Dichloromethane (DCM) (99.8%) pressurized sealed bottles: with thermal taps were purchased from Aldrich and Sigma Company (Germany). Authentic samples of volatile compounds purchased from laboratory chemical suppliers or were obtained as gifts from flavour companies.

Methods

Preparation of Potato-like Model System: Model systems contained water (400 ml) and combinations of glucose (13.0 g L^{-1})), methionine (20 g L^{-1})), Linoleic acid (0.5 g L^{-1})) and starch (50 g L^{-1}) (Table 1). A blank (water only) was also prepared. Solutions with oleic were mixed before heating to allow better dissolution. Potato starch was added when the solution was boiling to a homogenous mixture in pressurized sealed bottles.

Extraction of Volatiles of Potato-like Flavour: The reaction mixtures obtained from pressurized bottle after reaction complete were subjected to a simultaneous steam distillation (1 L of water) and solvent (dichloromethane, 200 mL) extraction. The dichloromethane extract was dried over anhydrous sodium sulphate.

Gas Chromatography and Gas Chromatography- Mass Spectrometry (GC-MS): The obtained volatile samples were thermally desorbed, using a modified injector port, directly on the front of a (DB5) (*60 m x 0.32 mm i.d*) fused silica capillary column, in the oven of a Perkin-Elmer outosystem XL gas chromatography and temperature increase from 40°C-240°C by the rate 2°C/min. Kovat's indices were determined by co-injection of the sample with a solution containing homologous series of n-hydrocarbons (C_6-C_{26}) under the same conditions as described above. The separated components were identified by matching with NIST mass-spectral library data and by comparison of Kovat's indices with those of authentic components and with published data.

Determination of the Antioxidant Activity of MRPs Scavenging Effect on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) Radical: The effect of model systems on DPPH radical was estimated according to Hatano *et al.* [18]. Aqueous portion of model systems (200 ppm) was added to a methanolic solution (1 mL) of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and left to stand at room temperature for 30 min; the absorbance of the resulting

Table 1: Composition of model systems and odour after heating

Systems	Reactants	Odour of heated model system
G	Glucose	nd[a]
M	Methionine	Sulfury
L	Linoleic acid	nd[a]
S	Starch	nd[a]
M/G	Methionine/ Glucose	Boiled potato
L/G	Linoleic acid/ glucose	nd[a]
L/M	Linoleic acid / Glucose	Sulfury
L/M/G	Linoleic acid / Methionine/ Glucose	Roasted potato, boiled vegetables
L/M/G/S	Linoleic acid / Methionine/ Glucose/ starch	Roasted potato, boiled vegetables

[a]nd. no detectable odour

solution was measured spectrophotometrically in triplicates at 517 nm. In this test, the percentage of DPPH reduction by the model system was compared to that of BHA and TBHQ (200 ppm of each).

β-carotene/Lnoleic Acid Method: Antioxidant activity of the aqueous solution was determined by a β-carotene/Linoleic acid system, as described by Matthaus [19]. Briefly, 2 ml of β-carotene solution (5 mg /1 ml chloroform), 40 mg of linoleic acid were transferred to a round-bottom flask. Chloroform from the sample was evaporated using a stream of nitrogen. Then 100 ml of oxygenated distilled water were added slowly to the residue and vigorously agitated to give a stable emulsion. To an aliquot of 2 ml of this emulsion, different volumes of the samples were added with the same volumes of standard mixture. Absorbance was measured at 470 nm after incubate the reaction mixtures in water bath (50°C) and the readings takes from zero time till 120 min. Antioxidant activity index(AAI) was calculated as: Equation: (2).

$$\text{AoA} = [1\text{-}(A_{S\,(0)\,-}\,A_{S\,(120)})/_{Ab\,(0)\,-}\,A_{b\,(120)}]\,\text{x}100$$

Where:
$A_{S\ (0)}$ is absorbance of sample at 0 min, $A_{S\ (120)}$ is absorbance of sample at time (t).

RESULTS AND DISCUSSION

The chosen levels of glucose, methionine and linoleic acid in the model systems were about the same as levels in raw potato [20]. The odours of the heated model systems are given in Table 1. The presence of both glucose and methionine were required for the enhancement of a potato odour. Roasted potato flavour was detected only in the presence of linoleic acid. Systems M/ G and L/ G/ M were potato yellow colour after heating. The colour darkened in the presence of starch system.

Identification of Volatile Compounds in the Model Systems: Twenty compounds were identified in the model systems were identified by LRI and GC/ MS in Table 2. The detected compounds can be grouped into those generated from the thermal degradation of the individual model system starting materials, i.e., linoleic acid, starch, methionine and glucose. No compounds were detected in G model system, apart from those which were present in the blank. This reflects the stability of glucose when heated alone in aqueous solution at 140°C [2, 21]. Alkanes and alcohols were identified in S model system. Potato starch may contain traces of lipids which can lead to the formation of these compounds.

Compounds Generated from Methionine: Low levels of dimethyldisulphide and dimethyltrisulphide came from the thermal degradation of methionine alone in M model system. Amounts were higher in M/G and L/ M model systems. However the amounts increased greatly in L/ M/ G and in L/ M/ G/ S, compared to M. Dimethyldisulphide and dimethyltrisulphide were the most abundant compounds in L/ M/ G and L/ M/ G/ S may be generated due to lipid degradation products. Mandin *et al.* [20] reported that the addition of glucose to an aqueous methionine solution increased the yield of dimethyldisulphide. They suggested that a portion of methional, formed by reaction of glucose with methionine, may degrade to methanthio, which in turn could yield dimethyldisulphide and dimethyltrisulphide. When oxidized lipids are present, methionine has been shown to be easily oxidized to methionine sulphoxide. These authors have also shown that the formation of dimethyl sulfides was favored over methional in the methionine sulfoxide system compared to the methionine system. Levels of dimethyl disulfide were greater in their methionine sulfoxide-glucose system compared to methionine-glucose and dimethyl trisulfide and dimethyl tetrasulfide were also identified. It appears that, in the current study, the addition of lipid and of lipid plus starch to the sugar-methionine system results in unexpectedly higher levels of dimethylsulfides compared to systems M, M/G and L/M. Glucose facilitates the degradation of methionine while oxidized linoleic acid can facilitate its oxidation to methionine sulfoxide, which in turn yields sulfides more readily than methionine [9]. Starch represents an additional source of reactive carbohydrate. Given the presence of glucose, methionine, lipid and starch in potato, it is surprising that dimethyl disulfide has only been detected among volatile compounds of boiled or baked potato by Gumbman and Burr [22] (Table 2).

Compounds Requiring Linoleic Acid for Their Formation: By far the majority of the compounds identified in this study derive from lipid degradation. They include 10 aldehydes, 3 alcohols and one furan. The most abundant lipid-derived compounds in this study were hexanal, 2, 4-decadienal and 2-pentylfuran. Oxidation of linoleic acid gives a mixture of mainly the 9- and 13-hydroperoxides, which are readily interconvert [23].

Table 2: Volatile compounds identified in aqueous model systems comprising combinations of methionine, glucose, linoleic acid and starch

No.	Structure	K.I[a]	M/G[b]	L/G[c]	L/M[d]	L/M/G[e]	L/M/G/S[f]
1	Dimethyldisulphide	722	3.2	nd	6.40	39.0	12.5
2	Dimethyltrisulphide	990	2.8	nd	12.60	18.00	17.20
3	1-pentanol	765	nd	1.80	0.98	2.00	2.10
4	Hexanol	809	nd	15.20	21.40	21.20	17.20
5	Heptanal	913	nd	2.00	nd	nd	1.20
6	2-heptanal	940	8.0	7.00	8.00	8.01	10.00
7	1-octen-3-ol	986	nd	3.20	nd	nd	nd
8	2-pentylfuran	993	nd	1.02	12.30	18.20	17.00
9	2-ethylhexanal	1069	nd	nd	4.10	nd	nd
10	(E)-2-nonenal	1172	nd	1.20	2.30	1.06	2.08
11	Octanoic acid	1186	nd	4.20	nd	nd	nd
12	Decanal	1204	nd	1.20	1.30	1.09	nd
13	2,4-decadienal	1218	nd	2.60	2.50	2.10	2.30
14	Tridecane	1309	nd	43.00	4.60	7.20	9.60
15	Hexadecanoic acid	1318	nd	6.30	15.20	26.30	37.20
16	Methional	1300	nd	nd	nd	nd	4.20
17	2-methyl-5-(methylthio)furan	1966	nd	nd	nd	nd	0.70
18	Methylmethane-thiosulfonate	909	ns	ns	ns	ns	0.20
19	2-hexylthiophene	1018	nd	nd	nd	0.20	6.90
20	2,4,5-trithiahexane	1083	nd	nd	nd	0.50	nd
21	2-dimethyltetrasulfide	1280	nd	nd	nd	nd	2.90

a: kovate indices, b: linoleic acid / glucose, c: linoleic acid and methionine, d: linoleic acid, methionine and glucose, e: linoleic acid, methionine and glucose and starch

Thus, they give the same volatile decomposition products, but in different amounts. The most abundant compound in systems L and L/G was 2, 4-decadienal. Of all the systems studied, the amount of 2, 4-decadienal was highest in L/G and lowest in L/M. Interactions between sugars and carbonyl compounds, such as those derived from lipid, have not been studied by flavor chemists [10]. However, initiation of the oxidation of either carbohydrates or of lipids in physiological systems stimulates the oxidation of the other [24].

These autoxidation reactions generate reactive oxygen species, which will promote lipid oxidation. The lowest amount of 2, 4-decadienal in L/M may be related to the identification of 2-pentylfuran in that system. Since 2-pentylfuran is formed from 2, 4-decadienal, which is itself formed from 9-linoleate hydroperoxide, the higher production of 2-pentylfuran may indicate more extensive degradation reactions in the presence of methionine. Methionine can degrade on its own under the chosen experimental conditions M to give products capable of reacting with carbonyl compounds formed by the degradation of linoleic acid and thus promoting lipid oxidation. Another explanation for the lower yield of 2, 4-decadienal in L/M is that this compound may react with methionine, giving nonvolatile products.

The highest levels of 2-pentylfuran in L/M/G and L/M/G/S, compared to L/M, was surprising since some Maillard reaction products are reported to act as antioxidants. The formation of free radicals in the early stages of the Maillard reaction may provide the key to the formation of the observed higher levels of 2-pentylfuran. The third abundant lipid-derived compound identified in this study is hexanal. It is a major volatile carbonyl coming from 13-linoleate hydroperoxid. A number of groups have studied the formation of hexanal from linoleic acid and various mechanisms have been proposed. For example, Schieberle and Grosch [24] demonstrated its formation from 13-linoleate hydroperoxide and from compounds having a double bond in the ö-6-position, e.g.,9-linoleate hydroperoxide, 2,4-decadienal and 2-octenal. Josephson and Lindsay [7] have shown that 2, 4-decadienal can undergo retro-aldol condensation to yield 2-octenal (also identified here) which, in turn, may degrade via a similar mechanism to hexanal. Hexanal was present at relatively similar levels in all systems containing linoleic acid, in contrast to 2, 4-decadienal and 2-pentylfuran. Thus, it appears that whatever the pathways leading to hexanal in the current study, its formation is less affected by glucose and methionine than the formation of 2, 4-decadienal and 2-pentylfuran. The formation of the remaining lipid degradation compounds reported in Table

2 has been explained previously [7, 23, 24]. All of the lipid-derived compounds reported here have been identified among the volatile components of cooked potato (Table 2).

Compounds Formed by Methionine/glucose Model System: The only compound detected in M/ G which was not present in M or G was methional, the Strecker aldehyde of methionine. Yu and Ho [9] reported that methional can be generated from methionine alone through the Shigematsu reaction, by decarboxylation and deamination of the amino acid. Methional was the major component of the extracts from M/ G, L/M/G and L/M/G/S. Its absence from M indicates that the Shigematsu reaction did not occur under the experimental conditions used here.

The amount of methional increased in L/M/ G and L/M/G/S, possibly due to the greater amounts of R-dicarbonyl compounds, which are required for the Strecker degradation of methionine. The odor of methional has been described as “cooked potato” (Table 2). In contrast to its presence as a major reaction product in M/G, L/M/G and L/M/G/S, methional has been detected quantitatively as a minor compound in all studies on boiled or baked potato flavour [1, 3]. No pyrazines, furans, thiazoles, oxazoles and thiophenes, which are known products of the Maillard reaction and which are important compounds in the flavor of baked potatoes, were identified. In most cases, their absence may be attributed to the relatively low reaction temperature/ high moisture conditions, while the absence of 5 hydroxymethylfurfural may be attributed to the relatively high pH.

Linoleic Acid/ Methionine/ Glucose Model System: Three volatile sulfur compounds, i.e., 2-methyl-5-(methylthio)furan, methyl methanethiosulfonate ($CH_3SO_2SCH_3$) and 2-hexylthiophene, were identified in L/ M/ G and are attributed to thermal interactions of linoleic acid, methionine and glucose. As previously mentioned, methionine has been found to be easily oxidized to methionine sulfoxide, especially when an oxidizing agent, such as oxidized lipid, is present. Yu and Ho [9] identified 2-methyl-5-(methylthio) furan and methyl methanethiosulfonate in methioninesulfoxide and methionine sulfoxide/ glucose model systems. Lower levels of 2-methyl-5-(methylthio)-furan were identified on heating methionine alone, but neither compound was identified in the glucose-methionine system. No reaction pathways were reported but the results presented here support a route involving oxidation of methionine to its sulfoxide. 2-Hexylthiophene has been identified in mixtures containing cysteine, ribose and phospholipids [13]. The authors proposed a reaction pathway between 2, 4-decadienal and hydrogen sulfide, which can give 2-hexyl-thiophene. The low number and amounts of compounds from the interaction of glucose, methionine and linoleic acid are probably due to two main reasons. First, the temperature of the model systems was low (100-105°C) and second, lipid oxidation products may mask some interaction products on the gas chromatogram. The three compounds which were identified have not been reported among the flavour components of boiled or baked potatoes or of French fries [3].

Linoleic Acid/ Methionine/ Glucose/ Starch Interactions: Two volatile sulfur compounds, i.e., 2,4,5-trithiahexane and dimethyl tetrasulfide were detected in small quantities in M/G/L/S only, but have not been reported among the volatile compounds of boiled or baked potato [25]. When starch was added to the system, the amounts of most of the volatile compounds remained the same or increased slightly, compared to M/G/L. This result was unexpected because starch has the ability to bind certain components, thereby making them difficult to extract and thus quantify [26]. Hau *et al.* [27] studied the binding of aroma volatiles to starch in a closed system at 25°C and found that the presence of starch in solution decreased the percentage of volatiles in the headspace. BeMiller [26] showed that the rate of hydrolysis of starch depends on the pH and the temperature of the system. At slightly acid pH, the hydrolysis rate increases with temperature. The hydrolysis of starch leads to D-glucose and reversion products and greatly increases the number of carbonyl groups. This could account for increases in levels of some compounds in L/M/G/S.

Antioxidant Activity of Potato-aroma Model Systems: Due to the presence of different volatile compounds in the extracts, it is relatively difficult to measure each antioxidant components separately. Therefore, several assay methods have been developed and applied in recent years to screen and evaluate the total antioxidant activity of such samples [28]. In the present work, the DPPH radical-scavenging assay and the ß-carotene bleaching inhibitory assay systems were successfully used for the evaluation on the antioxidant activity of (MRPs). The values of percent DPPH scavenging activities of (MRPs) were summarized in Fig. 1.

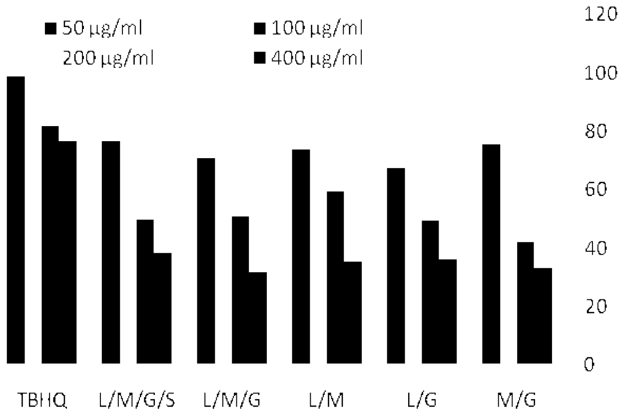

Fig. 1: Free radical of potato model systems by DPPH assay

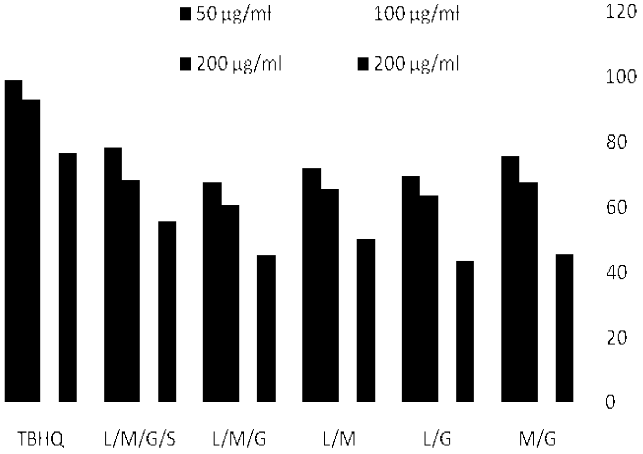

Fig. 2: Free radical of potato model s by β-carotene-linoleate method

These values were compared with those of the well-known antioxidant such as TBHQ. The antioxidant activity of buffalo model systems was found to increase with the increase in concentration of (MRPs). The highly antioxidant activity of (L/M/G/S) model systems are due to dimethyltrisulphide (17.2%), 2-hexylthiophene (6.9%), respectively [29]. Fig. 2 shows the β- carotene assay where model system (L/M/G/S) has highly antioxidant in comparison with other model systems with (78.3%).

CONCLUSION

This study demonstrates the occurrence of important interactions among flavor precursors in a heated model system. By heating each component alone, as well as pairs of reactants, the ability of one component to influence the degradation of the second has been shown. The data emphasize the importance of studying multicomponent model systems and indicate the drawbacks of binary systems. Nevertheless, the composition of the most complex system studied here, i.e., L/M/G/S, is still far removed from that of the target food, i.e. potato.

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