Effects of L-Cysteine and *N*-Acetyl-L-cysteine on 4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone (Furaneol), 5-(Hydroxymethyl)furfural, and 5-Methylfurfural Formation and Browning in Buffer Solutions Containing either Rhamnose or Glucose and Arginine

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Solutions of L-cysteine (Cys) and *N*-acetyl-L-cysteine (AcCys), containing glucose or rhamnose, with or without arginine, were buffered to pH 3, 5, and 7 and incubated at 70 °C for 48 h. Cys and AcCys inhibited the formation of (hydroxymethyl)furfural (HMF) from glucose and methylfurfural (MF) from rhamnose under acidic conditions. AcCys inhibited the accumulation of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (DMHF, Furaneol) from rhamnose, but Cys, under our experimental conditions, enhanced Furaneol accumulation from rhamnose. Cys and AcCys reacted directly with Furaneol but not with HMF or MF. Both Cys and AcCys inhibited nonenzymatic browning at pH 7. At pH 3, however, Cys reacted with both glucose and rhamnose to produce unidentified compounds that increased the visible absorbency.

INTRODUCTION

Sulfur-containing amino acids such as L-cysteine (Cys) and *N*-acetyl-L-cysteine (AcCys) have antioxidative and antitoxic effects and may inhibit the action of mutagens, carcinogens, and other toxic compounds by direct interaction (Friedman and Molnar-Perl, 1990). In addition, natural thiol compounds can replace sulfite as enzymatic and nonenzymatic browning inhibitors (Friedman and Molnar-Perl, 1990; Richard et al., 1991) in fruits and fruit juices (Montgomery, 1983; Molnar-Perl and Friedman, 1990a,b). Thiols such as Cys, AcCys, and glutathione inhibited browning and ascorbic acid degradation and improved the acceptance of orange juice stored under moderate conditions (Naim et al., 1997). These thiols may inhibit the formation of a variety of compounds related to browning, such as (hydroxymethyl)furfural (HMF) and methylfurfural (MF), as well as the accumulation of citrus juice off-flavor compounds, such as *p*-vinylguaiacol (PVG) and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (DMHF, Furaneol) (Naim et al., 1993, 1997).

Furaneol is an important flavor compound, having a caramel-like burnt-pineapple flavor in various fruits (Rodin et al., 1965; Pickenhagen et al., 1981; Wilson et al., 1988; Pisarnitskii et al., 1992), but it is proposed to be objectionable in citrus juices (Tatum et al., 1975). The presence of low (millimolar) concentrations of Cys reduced Furaneol accumulation in commercial orange juice stored under accelerated conditions (Naim et al., 1993). In addition, heated solutions of Furaneol and

sulfur amino acids can produce meat-flavor aroma compounds, especially at low pH (Shu and Ho, 1988). At higher pH values, Furaneol can be formed through 2,3-enolization of sugars via the Maillard reaction, (Haleva-Toledo et al., 1997). Six-deoxy sugars, such as rhamnose, are particularly relevant precursors for Furaneol formation in a wide range of pH values. The accumulation of Furaneol in citrus juices to above-taste-threshold levels is related, at least in part, to the reportedly small amounts of rhamnose reacting with arginine via the Maillard reaction (Haleva-Toledo et al., 1997).

HMF and MF are degradation products from hexoses and 6-deoxyhexoses, respectively, in the presence or absence of amino acids (Doornbos et al., 1981; Lee and Nagy, 1990). The content of HMF and/or MF is positively correlated with color deterioration in stored citrus juices (Lee and Nagy, 1988). The interaction of sulfur amino acids with sugars can form 2-furfurylthiol and 5-acetyl-2,3-dihydro-1,4-thiazine from glucose and Cys at pH 5, whereas incubation with rhamnose instead of glucose significantly modifies the overall odor note producing Furaneol, 3-hydroxy-6-methyl-2(2*H*)-pyranone, 5-methyl-2-furfurylthiol and additional odorants (Hofmann and Schieberle, 1997).

This study was designed to quantify the effect of Cys and AcCys on browning, Furaneol, HMF, and MF formation in buffer solutions (pH 3, 5, and 7) containing glucose or rhamnose with or without arginine. This may lead to exploration of the manner in which thiols affect browning and Furaneol formation in citrus products.

MATERIALS AND METHODS

Materials. Furaneol, HMF, MF, L-rhamnose, D-glucose, L-arginine, L-cysteine, and *N*-acetyl-L-cysteine were purchased from Sigma Chemical Co. (St. Louis, MO).

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Table 1. Effect of Thiol Fortification on HMF and MF Formation in Incubated Buffer Solutions Containing Glucose or Rhamnose with or without Arginine[a]

pH		no thiols	Cys			AcCys		
			1 mM	5 mM	10 mM	1 mM	5 mM	10 mM
				HMF				
3	Glc	5 ± 1	1.4 ± 0.1*	0.7 ± 0.2*	0.2 ± 0.1*	2.7 ± 0.4*	2 ± 0.5*	0.6 ± 0.2*
	Glc + Arg	92 ± 4	90 ± 5	25 ± 6*	6 ± 1*	118 ± 5	77 ± 7*	13 ± 1*
5	Glc	0.2 ± 0.1	0.1 ± 0.1*	ND	ND	0.1 ± 0.04*	ND	ND
	Glc + Arg	6 ± 1	7 ± 1	3.4 ± 0.6*	ND	6 ± 1	3 ± 1*	2.5 ± 0.3*
				MF				
3	Rhm	3 ± 0.1	1 ± 0.1*	0.4 ± 0.1*	0.3 ± 0.1*	1.4 ± 0.5*	1± 0.1*	0.7 ± 0.1*
	Rhm + Arg	27 ± 3	7 ± 0.4*	1.4 ± 0.2*	1 ± 0.1*	12 ± 1*	7 ± 1*	4.5 ± 0.3*
5	Rhm	2.4 ± 0.6	0.5 ± 0.1*	0.5 ± 0.1*	0.3 ± 0.1*	1.1 ± 0.1*	0.8 ± 0.1*	0.1 ± 0. 01*
	Rhm + Arg	8 ± 1	2.9 ± 0.5*	ND	ND	4.8 ± 0.5*	4.2 ± 0.8*	2.6 ± 0.6*

[a] Values (mg/L) for HMF and MF are the means ± SEM of three samples, each analyzed twice by HPLC. Glc, glucose; Rhm, rhamnose; Arg, arginine; Cys, L-cysteine; AcCys, *N*-acetyl-L-cysteine; ND, not detected. An asterisk indicates significantly ($p \leq 0.01$) lower value than the control incubated without the presence of thiols.

Preparation of Buffer Solutions. Buffer solutions consisted of citrate phosphate (0.1 M, pH 3, 3.5, 5, and 7) modified to contain 55 mM rhamnose or glucose with or without arginine (55 mM) and with either 1, 5, or 10 mM Cys or AcCys. Samples were prepared and immediately stored in sealed 20-mL brown bottles for 48 h at 70 °C. Control samples were stored at 4 °C.

Browning under acidic conditions (pH 3) was monitored following the incubation of buffer solutions containing higher amounts of rhamnose or glucose and arginine (138 mM each) under the same storage conditions, with the addition of 1, 5, 10, 25, or 50 mM Cys or AcCys.

To investigate the possible direct interaction between thiols and furans, buffer solutions (pH 3.5) containing 20 mg/L of either Furaneol, HMF, or MF were incubated under the same conditions with the addition of 0, 0.01, 0.05, 0.15, 0.5, or 2.5 mM Cys or AcCys.

Chemical Analyses. Furaneol, HMF, and MF were extracted from buffer solutions according to the method of Walsh et al. (1997). Each solution (2 mL) was passed through a prewashed (2 mL of methanol and 5 mL of water) C_{18} Sep-Pak cartridge. Each cartridge was then washed with 1.5 mL of water and eluted with 1.5 mL of methanol.

Furaneol, HMF, and MF were analyzed by HPLC equipped with a Lichrospher 100-RP 18 column (5 μm, 250 mm, 4 mm, Merck) with an RP-18 precolumn (25 × 4 mm). Furaneol, MF, or HMF in the incubated sugar-containing buffer solutions was analyzed via a mobile phase consisting of (A) 1.5% (v/v) acetic acid in water or (B) 50% (v/v) methanol/50% (v/v) acetonitrile. The chromatographic conditions were as follows: 0−16 min gradient of 20−40% B, 16−20 min gradient of 40−20% B.

In the analyses designed to measure Furaneol, HMF, and MF stability in the presence of Cys or AcCys (pH 3.5), an isocratic HPLC separation was used. The mobile phase consisted of 80% aqueous acetic acid (1.5%, v/v) and 20% methanol (HMF and Furaneol) or 60% aqueous acetic acid (1.5%, v/v) and 40% methanol (MF). The flow rate was 0.5 mL/min. The injected volume was 20 μL. Peaks were identified and quantified using known standards with a chromoscope UV−visible rapid-scanning detector set at 290 nm (Barspec, Rehovot, Israel).

Browning was determined by measuring absorbance at 420 nm as previously described (Haleva-Toledo et al., 1997). Absorption spectra (200−550 nm) of the extracts were measured in a Hitachi U-300 scanner spectrophotometer (Tokyo, Japan).

Data Analyses. Results of the chemical analyses were tested by *t* test in the JMP statistic computer program (SAS Institute Inc).

RESULTS AND DISCUSSION

Effects of Cys and AcCys on HMF and MF Formation from Sugars and Arginine. Because HMF and MF are formed mainly under acidic condi-

tions, their formation at pH 3 and 5 (conditions relevant to citrus and other acidic fruit juices) was investigated. The formation of HMF from glucose and that of MF from rhamnose in incubated buffer solutions with or without arginine and fortified with either Cys or AcCys are shown in Table 1. In line with previous studies (Lee and Nagy, 1990), the presence of amino acids under acidic conditions increased HMF formation. Cys and AcCys reduced HMF content at both pH values. In fact, at pH 5, 5 and 10 mM Cys and AcCys completely abolished HMF formation when glucose was the only substrate. These thiols are assumed to inhibit HMF formation via the interaction of their SH groups with intermediates during the initial stage of the sugar−amine reaction (Friedman and Molnar-Perl, 1990). As with HMF formation, Cys and AcCys reduced MF content at both pH values, in a concentration-dependent manner (Table 1). Cys and AcCys were slightly more effective at reducing MF than HMF concentrations. For example, at pH 5 no MF was detected when 5 mM Cys was present in the rhamnose and arginine solutions, whereas ~50% of HMF was degraded when glucose was substituted for rhamnose (Table 1). Becuase the hydroxy group at C-2 of rhamnose is axial, whereas it is equatorial in glucose, it is likely that the first stage of the Maillard reaction (nucleophilic attack by an amine and formation of an Amadori compound) is slower with rhamnose than with glucose. Hence, more free rhamnose is available for the attack by a thiol group. The rate of subsequent steps, that is, MF formation from rhamnose and HMF from glucose, should not be different as the intermediate products are of similar configuration.

Effect of Cys and AcCys on Furaneol Formation. Under acidic conditions (pH 3), Furaneol is formed from 6-deoxy sugars (rhamnose), but not from hexoses, in the presence of an amino acid such as arginine (Haleva-Toledo et al., 1997). At pH 3, fortification with AcCys (5 and 10 mM) reduced the Furaneol content significantly, whereas Cys fortification did not significantly affect Furaneol content (Figure 1B). However, at pH 5, Cys increased Furaneol content significantly when rhamnose and arginine were present (Figure 1A). In contrast, AcCys induced some reduction, which became significant only at 10 mM. When rhamnose was present alone, Furaneol was not detected. Nevertheless, in the presence of 5 and 10 mM Cys, small amounts (<0.5 mg/L) of Furaneol were formed. At pH 7, when rhamnose was present without arginine, the addition of 5 and 10 mM Cys significantly increased the Furaneol content
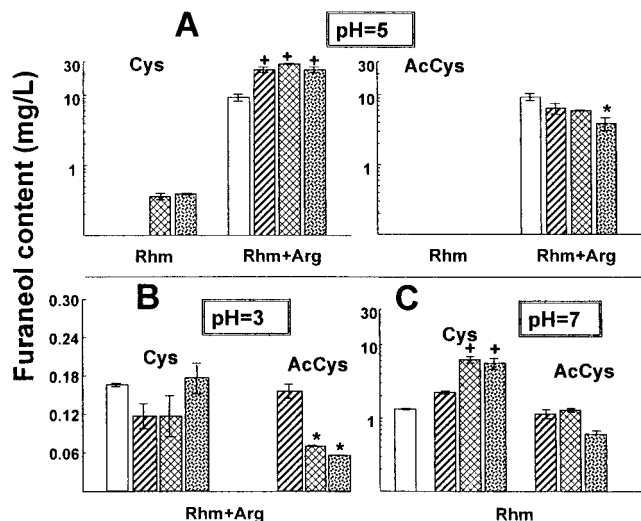
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Figure 1. Effects of Cys and AcCys fortification on Furaneol formation in rhamnose-containing buffer solutions incubated with or without arginine at pH 5 (A), pH 3 (B), and pH 7 (C): (open bars) no fortification; (slashed bars) 1 mM Cys or AcCys; (crosshatched bars) 5 mM Cys or AcCys; (dotted bars) 10 mM Cys or AcCys; (*) significantly lower value ($p \leq 0.01$) than that of the control (without thiols); (+) significantly higher value ($p \leq 0.01$) than that of the control (without thiols). Values for Furaneol are the means ± SEM of three samples, each analyzed twice by HPLC.

(Figure 1C). In the presence of both rhamnose and arginine, Furaneol was formed but its concentration was not affected by the presence of either Cys or AcCys (data not shown). The different effects of Cys and AcCys on Furaneol formation are probably related to the free amino group present in Cys, which can obviously react with sugars. Independent of the thiol group, Cys, as an amino acid, can react with rhamnose to produce Furaneol (Hofmann and Schieberle, 1997). The thiol group is probably responsible for the inhibition effect observed with AcCys, particularly at pH 3, as found in other cases (Friedman and Molnar-Perl, 1990; Molnar-Perl and Friedman, 1990a,b). As mentioned earlier, Furaneol can also be formed from glucose, but only at pH values >6 when Maillard-produced reductones are available (Haleva-Toledo et al., 1997). In the present experiments at pH 7, small amounts (~2 mg/L) of Furaneol were formed from glucose, in the absence and presence of arginine, and neither AcCys nor Cys affected its formation. Apparently, Cys and AcCys have contradictory effects on Furaneol formation from hexoses that are not 6-deoxyhexoses (e.g., glucose). They may provide the reducing power needed for Furaneol formation (Haleva-Toledo et al., 1997), on the one hand, and inhibit Furaneol formation, on the other. Furaneol can react directly with sulfur amino acids during heating at high temperatures to produce typical aroma compounds related to roasted meat and bread crust flavors, especially at low pH (Shu and Ho, 1988). Therefore, the potential interactions between Cys or AcCys and Furaneol were tested in buffers lacking sugars and amino acids but containing Furaneol at pH 3.5, the typical pH of orange juice.

Indeed, Furaneol content was reduced with the addition of Cys and AcCys to buffers fortified by Furaneol, suggesting that at least part of the inhibition of Furaneol formation in citrus juice is related to a direct interaction between Furaneol and these sulfur amino acids (Figure 2). When MF or HMF was incubated under
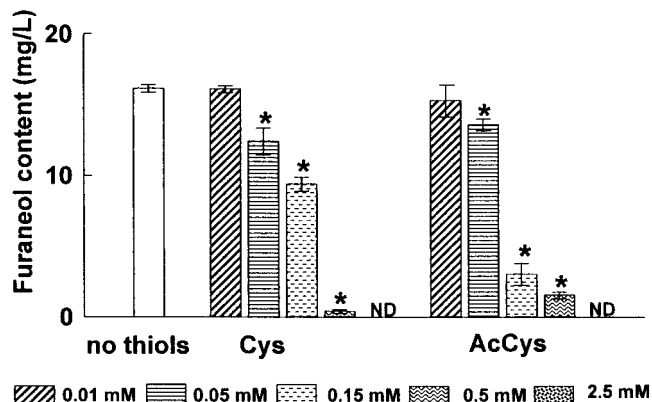

Figure 2. Effects of Cys and AcCys on Furaneol retention in buffer solutions (pH 3.5): ND indicates no detection of Furaneol when 2.5 mM was used; (*) significantly lower value ($p \leq 0.01$) than that of the control (without thiols). Values are the means ± SEM of three samples, each analyzed twice by HPLC.

similar conditions with Cys or AcCys, their contents were not affected. Apparently, the oxygen-ring atom in Furaneol, as in other dihydrofuranones, under acidic conditions, is readily exchanged with the sulfur atom of the sulfide (Zheng et al., 1997). Due to the equilibrium between the open carbonyl form and the furanone ring (Figure 3), the thiol can attack the carbonyl group to create 3(2H)-thiophenone. In the case of furans such a mechanism is not possible as HMF and MF are aromatic with almost no open-chain form. Regardless of thiol fortification, self-degradation of Furaneol, HMF, and MF amounted to 20% during 48 h of incubation at 70 °C.

Effect of Cys and AcCys on Browning. The thiols' ability to compete effectively with amines and inhibit amine−carbonyl interaction is known to contribute to decreased browning (Friedman and Molnar-Perl, 1990). At pH 7, Cys and AcCys reduced the absorbance at 420 nm of the incubated buffer solution containing either glucose or rhamnose with or without arginine (Figure 4). Another possible mechanism for the thiols' inhibitory effect involves suppression of free radical formation, as these may be important in the early stages of browning (Namiki and Hayashi, 1982). Cys may react as a free radical scavenger (Koh et al., 1996). The more intense browning observed with glucose−arginine versus rhamnose−arginine interactions may be related to the fact that rhamnose is known to produce fewer free radicals than glucose (Namiki and Hayashi, 1982).

Differential effects of Cys and AcCys on browning were observed at pH 5 (Figure 4): Cys induced increased browning, whereas AcCys induced a reduced effect. These differential effects are likely to be due to the free amino group present in Cys, but not in AcCys. Because the total absorbance in the visible range under acidic conditions (pH 3) is relatively low when sugars and thiols are tested at the aforementioned concentrations, higher concentrations of rhamnose, glucose, arginine, and thiols were applied (see Materials and Methods) (Figure 5). In contrast to the inhibitory effect observed at pH 7, both Cys and AcCys increased the absorbance under acidic conditions. The OD at 420 of sugar−amino acid solutions incubated with Cys was much higher than that of those containing AcCys. In neither case was the thiol effect concentration-dependent. Low concentrations of thiols produced significant elevations of OD values, which diminished at higher
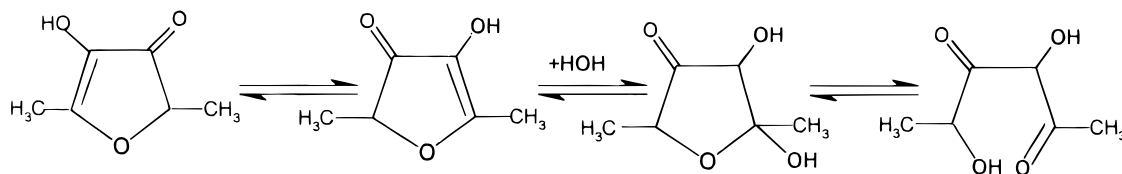
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Figure 3. Proposed equilibrium between the open and ring forms of Furaneol.
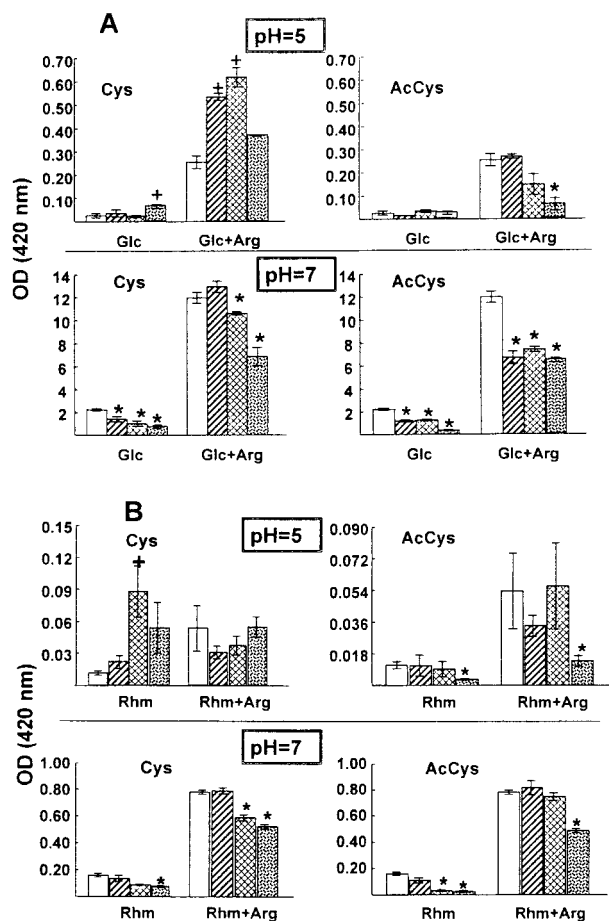

Figure 4. Effects of Cys and AcCys on browning in incubated buffer solutions containing glucose (A) or rhamnose (B) with or without arginine: (open bars) no fortification; (slashed bars) 1 mM of Cys or AcCys; (crosshatched bars) 5 mM Cys or AcCys; (dotted bars) 10 mM Cys or AcCys; (*) significantly lower value ($p \leq 0.01$) than that of the control (without thiols); (+) significantly higher value ($p \leq 0.01$) than that the control (without thiols). Values for optical density (OD) are the means ± SEM of three samples.

Figure 5. Effects of Cys and AcCys on browning in incubated buffer solutions (pH 3) containing glucose (A) or rhamnose (B) with arginine. Values for optical density (OD) are the means and SEM of three samples.

concentrations. Although the reason for this phenomenon is unclear, it does not appear to be related to the presence of oxygen (e.g., pro-oxidation). When argon was bubbled into the incubated bottles to create anaerobic conditions, these browning results were not modified (data not shown). Interestingly, a unique yellow color appeared in the methanol extraction of the incubated solutions, particularly in the presence of Cys.

To further explore this phenomenon, we examined the UV−visible spectra of the methanol extracts of solutions incubated at pH 3 (Figure 6). The formation of HMF from glucose and that of MF from rhamnose produced typical absorbency peaks at 280 (HMF, Figure 6A) or 290 nm (MF, Figure 6B). As expected, and in line with our previous results, the addition of Cys and AcCys reduced the UV absorbency of HMF and MF. As one may expect, when the incubated solution contained glucose alone (Figure 6A, curve 5), there was a lower
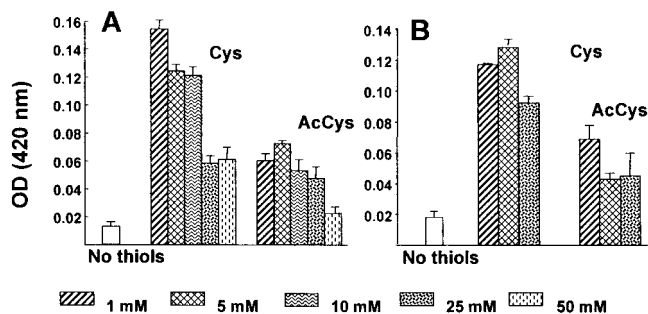
UV absorbance than that of a solution containing glucose and arginine (curve 1). The presence of Cys in solutions containing glucose and arginine (curves 3 and 4) reduced the UV absorbance but increased the visible absorbance, with a typical peak at ∼330 nm. A similar phenomenon occurred when the solutions contained rhamnose and Cys (Figure 6B, curve 3). However, AcCys did not produce such a shift (curve 2). In Figure 6C, the visible spectra of solutions incubated with combinations of rhamnose, arginine, and Cys are shown (AcCys had only a minor effect on the visible absorbency). Cys fortification modified the spectra by adding a typical absorbance spectrum with a peak at ∼390 nm, whereas arginine did not induce any change in the visible absorbance (curve 5 versus curve 4). Selected UV and visible absorbance peaks are summarized in Table 2.

The two absorbency peaks at 320−330 nm (glucose) and 390 nm (rhamnose) are independent of the presence of arginine, and there was no absorbance in this area when Cys alone was present during incubation. Therefore, these peaks are related to the interaction between Cys and the sugars. As mentioned earlier, Cys did not react with the major products of this reaction (HMF or MF). Therefore, these unidentified products were probably formed at an earlier stage of the reaction chain leading to HMF and MF formation. The reaction between glucose or rhamnose and Cys as part of the Maillard reaction at neutral pH was studied by Hofmann and Schieberle (1997). Roasted sesame and cooked rice aromas with strong sulfur notes were observed in a heated glucose−Cys mixture, which resulted mainly in products of the sugar−amino acid interaction (Zhang and Ho, 1991). The fact that Cys has an amine group explains the higher effectiveness of Cys than AcCys on the OD values. To the best of our knowledge, no other data are available on the products formed from glucose− or rhamnose−Cys interactions under acidic conditions. Parts of these products may be similar to those previously identified under similar circumstances at higher pH values. At pH values >5, when the rate of the Maillard reaction is accelerated, these unidentified compounds could not be detected by UV−visible spectra. The low rate of this reaction at pH 3 allows their detection.
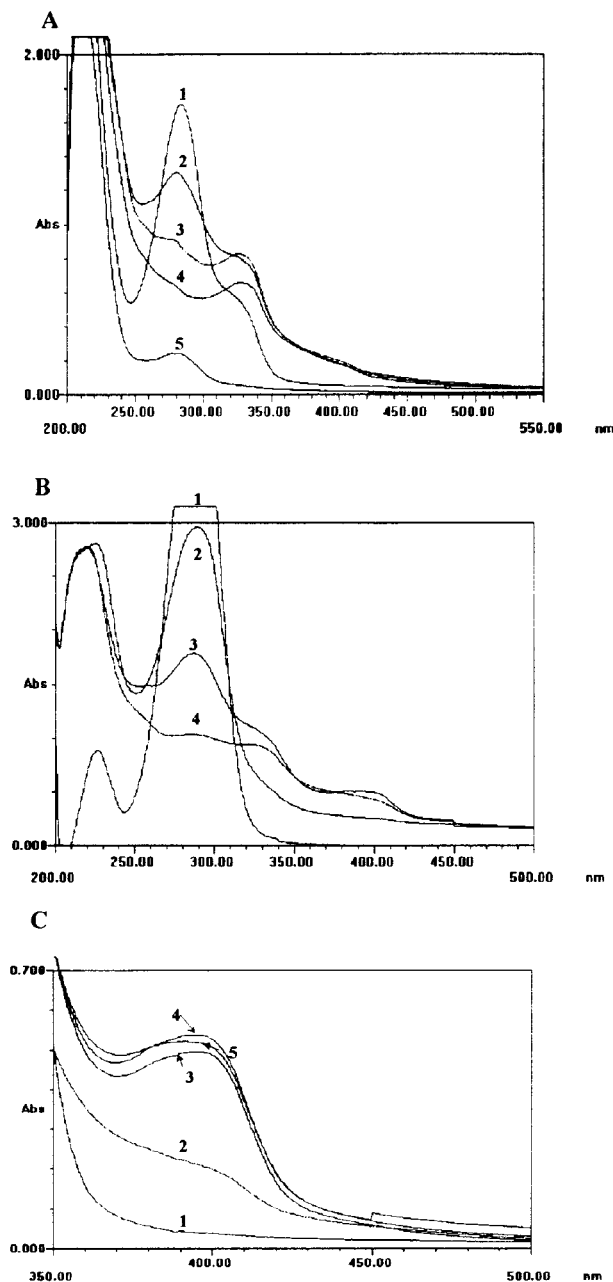
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Figure 6. Absorbance spectra (UV and visible) (200−550 nm) of a methanolic extract of the incubated buffer solutions (pH 3) containing (A) (1) glucose and arginine, (2) glucose and arginine with 1 mM Cys, (3) glucose and arginine with 5 mM Cys, (4) glucose and arginine with 10 mM Cys, or (5) glucose; (B) (1) rhamnose and arginine, (2) rhamnose and arginine with 5 mM AcCys, (3) rhamnose and arginine with 5 mM Cys, or (4) rhamnose and arginine with 25 mM Cys. (C) Visible absorbency (350−500 nm) of (1) rhamnose and arginine, (2) rhamnose and arginine with 1 mM Cys, (3) rhamnose and arginine with 5 mM Cys, (4) rhamnose and arginine with 10 mM Cys, and (5) rhamnose with 10 mM Cys.

In conclusion, the results of this study indicate that Cys and AcCys, depending on the pH, differentially affected the formation of Furaneol, HMF, MF, and browning from the corresponding sugars. In fact, Cys even stimulated Furaneol accumulation. It was further evident that, under the acidic conditions, thiols reacted directly with Furaneol but not with HMF or MF. Factors such as the presence of a deoxyhexose with an axial OH group at C-2 (rhamnose) versus glucose and the presence of an amine group in Cys but not in AcCys were

Table 2. Quantitative Changes (OD) in Selected Visible and UV Absorbencies Due to Thiol Fortification of Buffer Solutions Incubated at pH 3[a]

		320−330 nm	280−290 nm
Glc+Arg	no thiols		1.7
	1 mM Cys		1.3
	5 mM Cys	0.83	
	10 mM Cys	0.65	
	25 mM Cys	0.46	
	50 mM Cys	0.36	
	5 mM AcCys		
Glc	no thiols		0.24
	1 mM Cys	0.8	
	5 mM Cys	0.74	
	10 mM Cys	0.6	
	5 mM AcCys	0.09	

		380−390 nm	280−290 nm
Rhm + Arg	no thiols		2.8
	5 mM Cys	0.5	1.7
	25 mM Cys		1
	10 mM AcCys	0.05	1.5
Rhm	no thiols	0.1	0.6
	10 mM Cys	0.48	

[a] Glc, glucose; Rhm, rhamnose; Arg, arginine; Cys, L-cysteine; AcCys, *N*-acetyl-L-cysteine.

important for the observed results. Furthermore, although both thiols inhibited the nonenzymatic browning at pH 7, under acidic conditions, the reaction between Cys and glucose or rhamnose produced unidentified compounds that increased the visible absorbency. Further investigation is needed to chemically characterize these visible-absorbency-possessing compounds. The phenomenon of elevated absorbency in the visible range due to sugar−Cys and sugar−AcCys interactions under acidic conditions needs to be taken into account when these sulfur amino acids are used to inhibit browning in acidic food products, especially when the products are light in color.

ABBREVIATIONS USED

Furaneol, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone; HMF, (hydroxymethyl) furfural; MF, methylfurfural; Cys, L-cysteine; AcCys, *N*-acetyl-L-cysteine; HPLC, high-performance liquid chromatography.

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