

Furfural–Cysteine Model Reaction in Food Grade Nonionic Oil/Water Microemulsions for Selective Flavor Formation

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The thermal reaction between cysteine and furfural was investigated at 65 °C in five-component food grade oil/water (O/W) microemulsions of *R*-(+)-limonene/ethanol, EtOH/water/propylene glycol, PG/Tween 60 as apart of a systematic study on the generation of aroma compounds by utilizing structured W/O and O/W fluids. The furfural–cysteine reaction led to the formation of unique aroma compounds such as 2-furfurylthiol (FFT), 2-(2-furanyl)thiazolidine (main reaction product), 2-(2-furanyl)thiazoline, and *N*-(2-mercaptovinyl)-2-(2-furanyl)thiazolidine. These products were determined and characterized by GC-MS. Enhancement in flavor formation is termed “*microemulsion catalysis*”. The chemical reaction occurs preferably at the interfacial film, and therefore a pseudophase model was assumed to explain the enhanced flavor formation. The product internal composition is dictated by process conditions such as temperature, time, pH, and mainly the nature of the interface. Increasing water/PG ratio leads to a dramatic increase in the initial reaction rate (V_0). V_0 increased linearly as a function of the aqueous phase content, which could be due to the increase in the interfacial concentration of furfural. Microemulsions offer a new reaction medium to produce selective aroma compounds and to optimize their formation.

KEYWORDS: Amino acid; aroma; cysteine; furfural; Maillard reaction; O/W food microemulsions; phase diagrams; *R*-(+)-limonene; sulfur-containing flavors

INTRODUCTION

The Maillard reaction between reducing carbohydrates and amino acids is an important route to produce desirable flavor and taste in foods (1, 2). This well-known reaction takes place during thermal processing and storage and affects the flavor, appearance, and nutritive value of food products (1–3). Thus, it is important to control this nonenzymatic browning reaction to produce food products such as coffee, beverages, and bakery with desirable flavor and color and to improve the quality of these processed foods. These types of reactions involve complex reaction pathways leading to a wide range of products (1, 3).

Among the precursors of sulfur-containing flavor compounds, cysteine has been reported to be an important ingredient in the formation of these browning flavor compounds (4–10). Mainly, systems involving ribose and cysteine (10–14) have generated a wide range of sulfur-containing flavors (>200 volatile compounds). Sulfur-containing flavors have been detected in many different thermally processed foods [roasted coffee (15), cooked chicken and beef (9, 16–18), roasted seeds (19), and wheat bread (20)] as well as in model Maillard reaction systems containing ribose and cysteine (9–14, 21–23).

The best-known examples of the sulfur-containing flavors are 2-furfurylthiol (FFT) and 2-methyl-3-furanthiol (MFT). They have recently been identified by applying an aroma extract dilution analysis (AEDA) as the key aroma contributors to several thermally processed foods (10–13). Hofmann and Schieberle (11–13) studied the reaction of cysteine and ribose at pH 5.0 under dry heating and aqueous conditions. They found that the dry-heating process is very effective in the generation of significant amounts of FFT. It was found (7, 8, 14, 24) also that the formation of both flavors increased significantly under acidic conditions (pH 3.0–5.0).

Studies on the cysteine–ribose model reaction have elucidated that furfural (aldehyde) is considered to be involved in the formation of FFT and MFT through its reaction with either hydrogen sulfide or sulfur-containing amino acids (24, 25). Recently quantitative studies, using stable isotope dilution analysis, confirmed that FFT is indeed the major product formed during thermal treatment of furfural in the presence of cysteine (24). Also recently, Vauthey et al. (26) described the application of cubic phases as microreactors for the thermal generation of flavors from cysteine–furfural and cysteine–ribose model systems. In that work, the cubic phases facilitated a significant high rate of flavor enhancement as efficient reaction media.

In our work, we are interested in exploring the use of food grade oil-in-water (O/W) microemulsions as a chemical reaction medium for the generation of sulfur-containing aroma com-

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pounds. To carry out this study, we chose the simple model reaction of cysteine–furfural.

Microemulsions are thermodynamically stable mixtures of two immiscible solvents (one apolar and the other polar) stabilized by amphiphilic molecules (27). In the majority of the studies microemulsion water is the polar liquid. These systems can be classified structurally, as O/W microemulsions or oil-swollen micelles. When oil is the continuous phase, water-swollen micelles are formed, termed also water-in-oil (W/O) microemulsions. When the water and the oil concentrations are comparable, bicontinuous microemulsions are formed for which both water and oil domains are the continuous phase, separated by surfactant sheets (27).

The formulation of food grade O/W microemulsions using suitable nonionic surfactants and addition of polyols and short-chain alcohols has been recently discussed in our previous paper (28). We found that the addition of short-chain alcohols such as ethanol and polyol (glycerol or propylene glycol) induces the formation of water-in-oil (W/O) and oil-in-water (O/W) microemulsions. The phase behavior of our system of *R*-(+)-limonene, ethanol, water/PG (1:1), and polyoxyethylene sorbitan monostearate (Tween 60) was characterized by a single continuous microemulsion region starting from a pseudo-binary solution (surfactant/oil phase) to the microemulsion water/PG (1:1) corner. This means that all of the structural changes occurring in the isotropic phase develop continuously (no phase transition takes place). In our future work we will focus on the investigation of the microstructure of these microemulsions and the role of the components in it by means of the NMR pulsed gradient spin–echo (PGSE) self-diffusion method, conductivity, small-angle X-ray (SAXS) and neutron (SANS) scattering techniques, and dynamic light scattering (DLS).

Microemulsions are attractive systems serving as microdomains for chemical and enzymatic reactions because they are thermodynamically stable, macroscopically homogeneous, and isotropic yet heterogeneous on a molecular scale (29). Microemulsions as microreactors have attracted considerable recent attention, and extensive work by numerous researchers has been reported on many different reactions with different types of surfactants, primarily in the past 10 years (29–35). Microemulsions are appropriate systems for the solubilization of lipophilic and hydrophilic reactants causing a local increase of reagent concentrations in widely different environments (three solubilization regions: the oleic phase, the interface, and the aqueous phase), in accordance with their physicochemical properties, provide a large contact surface between them and are therefore suitable for solubilizing chemical interactions leading to specific and selective products (29–32).

MATERIALS AND METHODS

Materials. The reagents used were L-cysteine (Aldrich Chemical Co., Milwaukee, WI) and furan-2-aldehyde (furfural) (Sigma Chemical Co., St. Louis, MO). Brij 96v [polyoxyethylene (10) oleyl alcohol, (C₁₈:1E₁₀)] was purchased from ICI, Specialty Chemicals (Essen, Germany). Ethoxylated sorbitan esters in use were Tween 60 [polyoxyethylene (20) sorbitan monostearate], Tween 80 [polyoxyethylene (20) sorbitan monooleate], Tween 40 [polyoxyethylene (20) sorbitan monomyristate], and Tween 20 [polyoxyethylene (20) sorbitan monolaurate]. All Tweens were of commercial grade and purchased from Sigma Chemical Co. *R*-(+)-Limonene (98%) was supplied by Sigma Chemical Co. Ethanol (EtOH) was obtained from Frutarom (Haifa, Israel). Propylene glycol (PG; 1,2-propanediol, BDH, Poole, U.K.) was used. All of the ingredients were used without further purification. The water was double-distilled.

Phase Diagrams. The five-component systems were described on a pseudo-ternary phase diagrams. They were constructed as reported

recently at 25 °C (28): stock solution of water and PG at constant weight ratio 1:1 was made. The ethanol/oil weight ratio was held constant at 1:1. Mixtures of surfactant–“oil phase” (ethanol and oil) or mixtures of surfactant–aqueous phase (water and PG) were prepared in culture tubes sealed with viton-lined screw caps at predetermined weight ratios of oil phase to surfactant or of aqueous phase to surfactant and kept in a 25 ± 0.3 °C water bath. Microemulsion areas were determined in phase diagrams by titrating either the oil–surfactant phase or aqueous phase–surfactant mixtures with the aqueous phase or the oil phase, respectively. All samples were vigorously stirred. The samples were allowed to equilibrate for at least 24 h before they were examined. In all samples tested, evaporation loss was negligible. The different phases were determined using ocular and optical (crossed polarizers) methods. All samples, which remained transparent and homogeneous after vigorous vortexing, were considered to belong to a monophasic area in the phase diagram. The accuracy in the location of the phase boundaries is within 4 wt %.

Model Reactions. Reactions were carried out at 65 °C in Tween 60-based O/W microemulsions and were performed as follows: cysteine (6 mmol) was dissolved in the phosphate buffer (10 g, 0.5 M, pH 5.0) while furfural (3 mmol) was dissolved in the oil phase [*R*-(+)-limonene plus ethanol]. The nonionic surfactant was dissolved in propylene glycol (PG) at 37 °C and added to the aqueous phase. The oil phase was added to the aqueous phase, transferred to a 100-mL vial, and mixed to obtain a single-phase O/W microemulsion with the desired compositions. The microemulsion was heated at 65 °C in a water bath stirred with a magnetic stirrer. The reaction was quenched by rapid cooling on ice.

Isolation of Volatile Reaction Products. After cooling, the reaction mixture was extracted with diethyl ether. The extract (organic phase) was dehydrated by anhydrous Na₂SO₄ at 4 °C. Naphthalene was used as an internal standard.

Gas Chromatography. A Hewlett-Packard model 5880 gas chromatograph equipped with a flame ionization detector (FID) and an Rtx-1701 fused silica capillary column (30 m × 0.32 mm × 0.25 mm; Thames Restek U.K. Ltd., Berkshire, U.K.) was used for quantification. The operation conditions were as follows: nitrogen was used as carrier gas (column head pressure = 107 kPa), the injector temperature was 170 °C, and the detector temperature was 250 °C; the oven temperature was programmed from 50 °C (2 min) at 6 °C/min to 240 °C (10 min).

High-Resolution Gas Chromatography—Mass Spectrometry (HRGC-MS). HRGC analyses for the identification of the compounds were performed by an HP 5890 GC-MS equipped with an HP5 capillary column (30 m × 0.32 mm × 0.25 mm). Helium was used as the carrier gas [column head pressure = 103 kPa (15 psi)]. Mass spectra in the electron impact mode were generated at 70 eV and scan mode in the range of 40–250 amu. An aliquot (1 mL) of concentrated samples was injected. The GC oven temperature was initially held at 50 °C and then programmed at 4 °C/min to 240 °C (10 min). The injector temperature was 190 °C. The compounds were tentatively identified by comparing their mass spectra with those contained in the mass spectrometer data system library and in previously published literature (10, 26).

Statistical Analysis. Data from all investigations in each experiment were represented as the mean value of three replicates. Significance of differences among means was defined by Student's *t* test (*p* ≤ 0.05). In some of the figures, standard errors are presented.

RESULTS AND DISCUSSION

Phase Diagrams. The pseudo-ternary phase diagram for the *R*-(+)-limonene/ethanol/water/PG system, based on Tween 60, is shown in **Figure 1**. The phase behavior system is characterized by a monophasic region starting from the pseudo-binary solution (surfactant/oil phase) to the water/PG corner. It is easily seen that the microemulsion can be diluted with the aqueous phase to infinity (see, for example, dilution lines T64 and T73 in **Figure 1**) without passing through a two-phase or mesophase region. The model reaction cysteine–furfural was investigated in the O/W region of the microemulsions along these aqueous phase dilution lines.

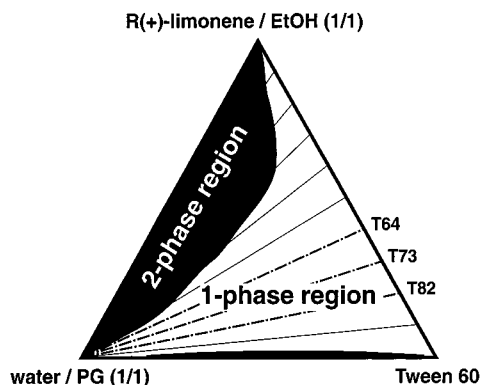


Figure 1. Phase diagram of the water/PG/R-(+)-limonene/EtOH/Tween 60 system at 25 °C with a constant weight ratio of water/PG (1:1) and a constant weight ratio of R-(+)-limonene/EtOH (1:1). The model reaction was carried out in the aqueous phase rich region along dilution line T64.

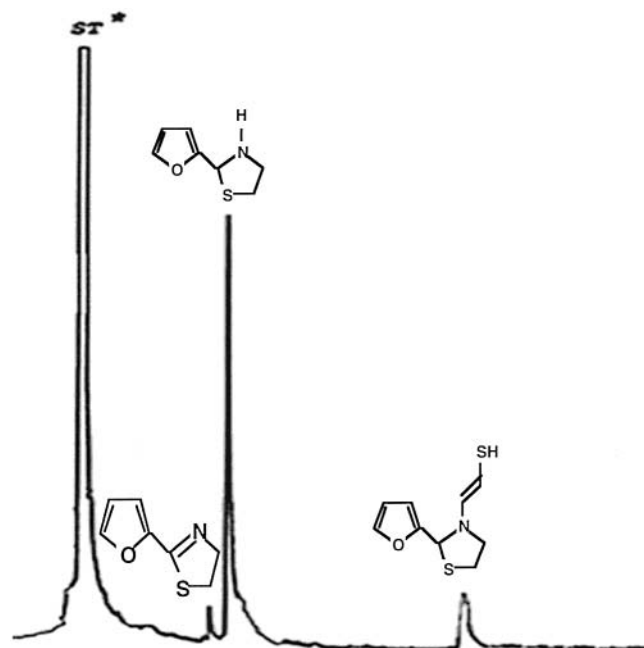


Figure 2. GC-FID chromatogram (Rtx-1701 fused silica capillary column) of volatile flavor compounds generated from the model reaction of furfural–cysteine in a food grade O/W microemulsion based on Tween 60. The internal standard naphthalene is indicated by ST*.

Synthesis of Sulfur-Containing Flavors in Food Grade O/W Microemulsions. The reaction products were monitored by GC-FID (see **Figure 2**) and found to be FFT (roasty, coffee-like), 2-(2-furanyl)thiazolidine, 2-(2-furanyl)thiazole, and *N*-(2-mercaptovinyl)-2-(2-furanyl)thiazolidine as the major flavor compounds. More detailed information about hypothetical pathways for their generation could be obtained from recently reported work (26).

There was a significant increase in the concentration of FFT in the model reaction carried out in the O/W microemulsion system compared to the reaction in water, in which only traces were detected. The formation of FFT is enhanced in the O/W microemulsion with increasing aqueous phase content. Besides FFT (compound **A**), the formation of 2-(2-furanyl)thiazolidine (compound **B**), 2-(2-furanyl)thiazoline (compound **C**), and *N*-(2-mercaptovinyl)-2-(2-furanyl)thiazolidine (compound **D**) was favored in this structured medium. It was reported (26) that compound **B** may react with mercaptoacetaldehyde, released from cysteine upon Strecker degradation, to finally give rise to

Table 1. Products Isolated from the Reaction of Furfural–Cysteine in O/W Microemulsions^a

aqueous phase (wt %)	ratio ^b of A/B		ratio ^b of C/B		ratio ^b of D/B	
	1 h	8 h	1 h	8 h	1 h	8 h
60	0.01	0.03	0.080	0.09	0.08	0.11
75	0.03	0.07	0.09	0.11	0.09	0.14
90	0.05	0.09	0.10	0.12	0.11	0.21

^a The reaction was carried out at 65 °C in a Tween 60-based O/W microemulsion (see **Figure 2**). Reaction products: **A** = furfurylthiol (FFT); **B** = 2-(2-furanyl)thiazolidine; **C** = 2-(2-furanyl)thiazoline; **D** = *N*-(2-mercaptovinyl)-2-(2-furanyl)thiazolidine. ^b Data are the means of triplicate analyses.

compound **D**. As can be seen in **Table 1**, the main product of the model reaction in the O/W structured medium is 2-(2-furanyl)thiazolidine (compound **B**). It can be, therefore, stated that the O/W microemulsion is a selective reaction medium favoring the formation of compound **B**. The formation rate of compounds **A**, **C**, and **D** (slow process) was significantly lower and increased slightly with time. Recently, Fanun et al. (36) described the use of food grade W/O microemulsions, based on sucrose laurate, as microreactors for the thermal generation of flavors of the same cysteine–furfural model reaction. We have found a dramatic difference in the flavor product distribution in our O/W-based in comparison to the W/O-based reactions. The sucrose laurate based W/O microemulsion (36) shows a strong preference for compound **D**, whereas the Tween 60 based O/W microemulsion favors compound **B**.

The effect of the aqueous phase content on formation of the aroma products is illustrated also in **Table 1**. With increasing aqueous phase content, the ratio of **D/B** in the Tween 60 based O/W microemulsion is increased, whereas it was found that by increasing the water content, the ratio of **D/B** in the W/O microemulsion is decreased (36). These results suggest that the microstructure of microemulsions plays an important role in the generation of “selective” aroma compounds. One possible explanation for the remarkable formation rate enhancement and the different flavor products distribution in both structured media is related to the compartmentalization (partitioning) of the reactants at the interface. This effect was found to accelerate bimolecular reactions by acting to locally concentrate the reactants and providing a large contact between them (29–31, 33, 35).

Effect of Aqueous Phase (Water plus PG) Content on Initial Reaction Rate. The influence of the aqueous phase content on the initial decomposition rate of furfural (V_0) in O/W microemulsions was studied in a series of experiments along dilution line T64 in **Figure 1**. The aqueous phase content was varied between 60 and 90 wt %. There is a sharp linear increase (correlation coefficient, r , >0.97) in the initial reaction rate (V_0) as the aqueous phase content increases (see **Figure 3**). To interpret these results, we used a pseudophase model that treats the microemulsion as a three-layer bulk system (the continuous phase, the micellar interface, and the intramicellar pool) and ignores its actual micellar structure (33, 37). Because cysteine, unlike furfural, does not dissolve in the oil phase, its concentration in the oil should be negligible, and the reaction in principle would be limited to two of the three pseudophases, that is, the aqueous phase (in which furfural is insoluble) and the interface. However, the reaction of solid cysteine and furfural in an aqueous phase, at similar reaction conditions, is very sluggish. It is, therefore, not surprising that the reaction in the O/W microemulsion proceeds much more rapidly at the interface than in the aqueous phase. Thus, it is assumed that the interfacial

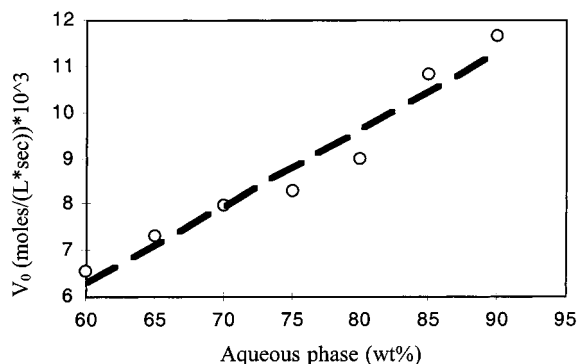


Figure 3. Initial velocity (rate) of the decomposition of furfural (V_0) as a function of aqueous phase content in a microemulsion system containing buffered water (pH 5.0)/PG/*R*(+)-limonene/EtOH/Tween 60 along the dilution line T64 (the aqueous phase contents vary from 60 to 90 wt %). The reaction was carried out at 65 °C.

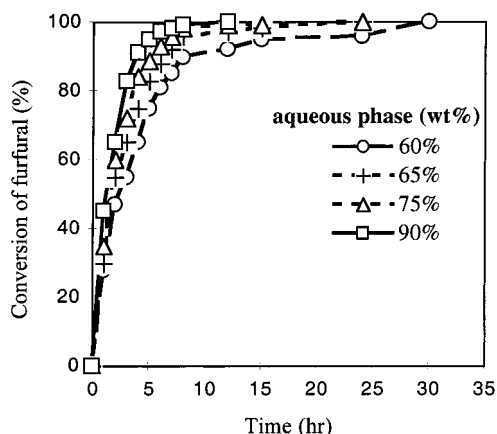


Figure 4. Effect of aqueous phase content in Tween 60 based O/W microemulsions of water/PG/*R*(+)-limonene/EtOH/Tween 60 (see Figure 1) on the conversion of furfural at 65 °C. The reaction was carried out in the aqueous phase rich region along dilution line T64.

film is the main reaction site. The conversion of furfural to sulfur-containing flavors increased with increasing aqueous phase content (see Figure 4). This conversion behavior is typical for cysteine–furfural reactions. As shown in Figure 4, all furfural–cysteine reactions exhibit a plateau in the furfural conversion. Once this plateau is reached, further conversion occurs very slowly; the highest conversion of furfural was observed for O/W microemulsion containing 90 wt % aqueous phase. Increasing the aqueous phase content leads to a decrease in *R*(+)-limonene content (see Figure 5a), and as shown in Figure 5b, it results in an increase in the ratio α [$\alpha = (\text{mass of furfural})/(\text{mass of furfural} + \text{mass of } R(+)\text{-limonene} + \text{mass of ethanol})$]. The enhancement in the initial rate (microemulsion catalysis process) in O/W systems with increasing value of α could be attributed to the increase of the accessibility of furfural to the interface (an increase in the interfacial concentration of furfural). As the value of α increased, more furfural was assumed to be bound to the interface and, thus, the initial reaction rate increased.

Effect of Aqueous Phase Composition on Initial Reaction Rates. The presence of water plays a crucial role in the formation of flavor products (6, 9–13). We examined the water effect on the initial rate of the reaction. Tween 60 based O/W microemulsions solubilize large quantities of oil if part of the water is replaced by PG (28). We examined the effect of the composition of the aqueous phase (water plus PG) on the

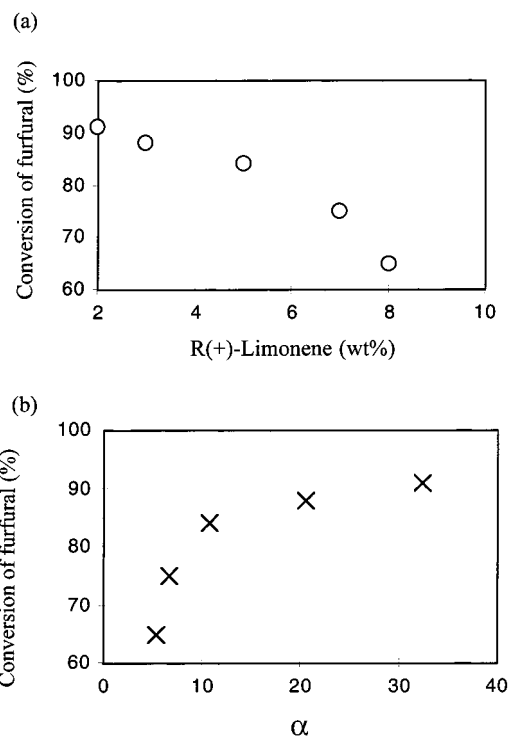


Figure 5. (a) Effect of oil [*R*(+)-limonene] content on the conversion of furfural. (b) Effect of a ratio [(mass of furfural)/(mass of furfural + mass of oil phase)] on the conversion of furfural. The reactions were carried out in Tween 60 based O/W microemulsions along dilution line T64 at 65 °C.

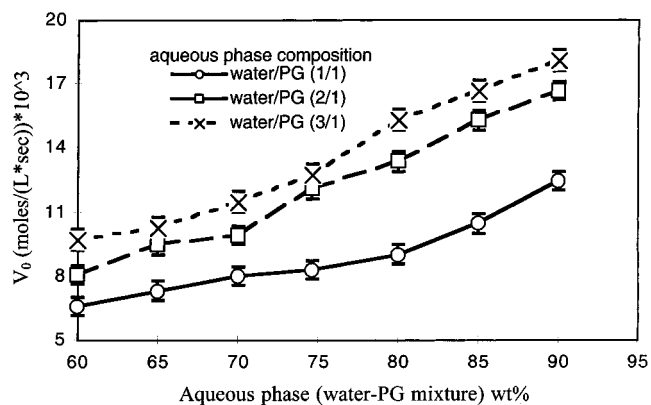


Figure 6. Initial velocity (rate) of the decomposition of furfural (V_0) as a function of aqueous phase content in three microemulsion systems with different aqueous phase composition (water plus PG mixtures) containing buffered water (pH 5.0)/PG/*R*(+)-limonene/EtOH/Tween 60 along dilution line T64 (the aqueous phase contents vary from 60 to 90 wt %). The reactions were carried out at 65 °C.

reactivity of the Maillard reaction. The reactions were carried out in the aqueous phase rich region along three dilution lines containing constant weight ratios of *R*(+)-limonene/ethanol/surfactant 1:1:3 and three different compositions of the aqueous phase (water/PG mixtures). Figure 6 shows the change in the initial reaction rate as a function of the aqueous phase composition along the dilution lines. It can be concluded (as per Figure 6) that increasing the water proportion in the aqueous phase (increasing the weight ratio of water/PG from 1:1 to 3:1) has a significant rate effect on enhancing V_0 . The highest V_0 is observed for the dilution line with water/PG 3:1 and is probably due to the so-called “Strecker degradation” of cysteine (38) that leads to the generation of reactive intermediates such as

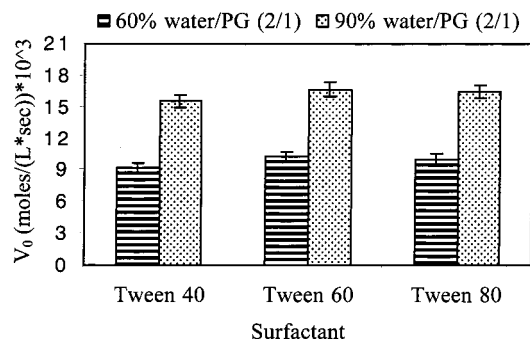


Figure 7. Effect of surfactant nature on the initial velocity (rate) of the decomposition of furfural (V_0) in microemulsion systems containing buffered water (pH 5.0)/PG/R-(+)-limonene/EtOH/ethoxylated sorbitan ester. The reactions were carried out at 65 °C in two O/W microemulsions on dilution line T64 containing 60 and 90 wt % aqueous phase (the water/PG weight ratio was 2:1).

cysteamine, hydrogen sulfide, or mercaptoacetaldehyde (22, 38, 39). It was found that cysteine is thermally unstable when heated in the presence of water, and its degradation leads to the formation of sulfur-containing flavors (22, 38, 39). It was found that generation of an active intermediate such as hydrogen sulfide in heated Maillard systems containing cysteine in the presence of water and its participation in subsequent reactions lead to the formation of sulfur-containing flavors (7, 22–23, 38). In our O/W systems, under acidic conditions, the dramatic increase in V_0 with increasing water/PG ratio could be attributed to an increase in the released amount of the generated reactive intermediates. Therefore, the generation of flavors is based on a possible release of reactive intermediates such as hydrogen sulfide. The reaction of furfural with the “generated reactive intermediates” provides a further increase in the consumption of furfural, leading to high V_0 in comparison to the two microemulsions containing lower weight ratios of water/PG.

Surfactant Nature and Initial Reaction Rates. It is well-known that the “microemulsion catalysis” is influenced by the nature of the surfactant (29, 30, 33, 34). The influence of three different Tweens (ethoxylated sorbitan esters) on the initial rate of the furfural–cysteine reaction was studied in two O/W microemulsions containing 60 and 90 wt % aqueous phase, respectively. In these microemulsions, the surfactant/oil/ethanol weight ratio remained unchanged (3:1:1), and the aqueous phase is water/PG with a constant weight ratio of 2:1. More detailed information on the phase behavior of the Tween systems can be obtained from our recent work (28). **Figure 7** shows the change in the initial rate in two O/W systems as a function of the chain length of the surfactant. Extremely similar V_0 values were obtained when Tween 60 (the most hydrophobic surfactant, HLB = 14.9) was replaced by Tween 40 or Tween 20 (the most hydrophilic surfactant, HLB = 16.7). Although the chain length of Tweens has a significant effect on their phase behavior (28), no differences in the yields of sulfur products and in the initial reaction rates of the Maillard reaction were observed (replacing Tween 60 by Tween 40 or Tween 20). It seems that the surfactant hydrophobic chains do not affect the partition of the reactants among the different microemulsion domains and the microstructure of the reaction media. Further work is required to correlate the nanostructures of microemulsions based on ethoxylated sorbitan esters, shapes of the swollen micelles with the reactivity of the ingredients, and the product internal distribution.

Conclusion. This work describes the reaction between lipophilic (furfural) and hydrophilic (cysteine) reactants to

generate aroma compounds in food grade five-component O/W microemulsions. It was shown that O/W structured systems are “selective microreactors”, imposing a significant influence on the flavor generation. The flavor formation is very efficient if carried out in O/W systems and yields high amounts of key aroma compounds. The reaction initial rate was found to be sensitive to the aqueous phase composition and its content. The rate enhancement in the generation of flavors could be attributed to the nature of the interface and the distribution of the ingredients between the oleic phase, aqueous phase, and interface.

ABBREVIATIONS USED

EtOH, ethanol; FFT, 2-furfurylthiol; FID, flame ionization detector; HLB, hydrophilic–lipophilic balance; GC-MS, gas chromatography–mass spectrometry; PG, propylene glycol; V_0 , initial reaction rate.

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