

Research Article

Transformation of Synthetic Allicin: The Influence of Ultrasound, Microwaves, Different Solvents and Temperatures, and the Products Isolation

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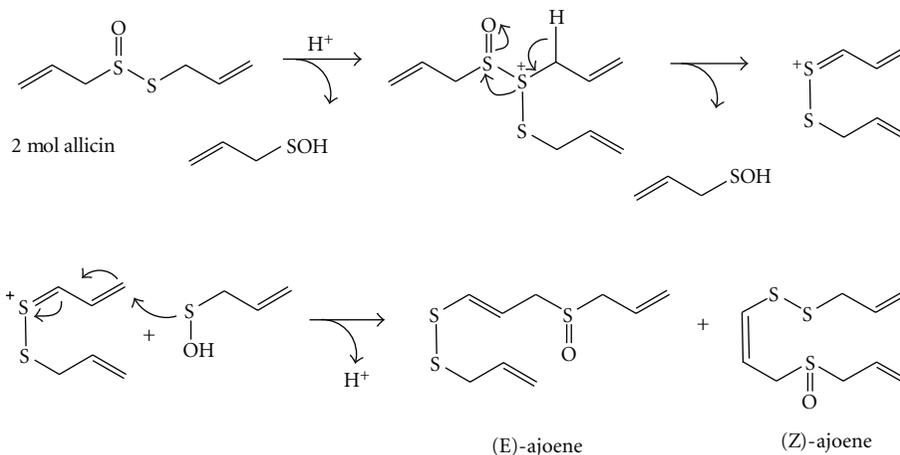
The transformation of the synthesized allicin, using conventional method, the influence of ultrasound and microwaves, in different organic solvents (acetonitrile, acetone, methanol, and chloroform), at various temperatures (room temperature, 45°C, and 55°C) was investigated. Allicin degradation kinetic was monitored by HPLC. Allicin transformation under the effect of microwaves is faster than transformations performed under the influence of ultrasound or by conventional method. Increase of the temperature accelerates allicin transformation. Pharmacologically active compounds of (E)-ajoene, (Z)-ajoene, 3-vinyl-4H-1,2-dithiin, 2-vinyl-4H-1,3-dithiin, and diallyl disulfide were isolated from the mixture of transformation products of allicin under the influence of microwaves in methanol at 55°C, which is according to kinetic parameters (highest values of the order of reaction and the lowest activation energy) the optimal method.

1. Introduction

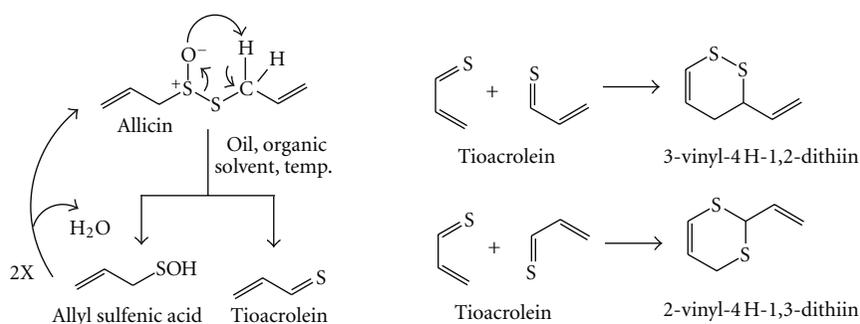
Allicin (3-prop-2-enylsulfanylprop-1-ene) is a thi-oester of sulfenic acid, or allylthiosulfinate. It is an oily, light yellow liquid that has a distinctively pungent smell [1]. Allicin is the most important pharmacologically active substance of the fresh aqueous extract of garlic [2–4] which exhibits antimicrobial [5–10], antiviral [11], antioxidant [7], anticancer [12], antihypertensive [13], and hypolipidemic activity [13]. Isolation of allicin from garlic is a very complex and difficult process because of its instability. The synthesis of allicin is based on the oxidation of allyl disulfide with hydrogen peroxide in acidic media [14–17], the oxidation of allyl disulfide with 3-chloroperoxybenzoic acid in chloroform [18], and the oxidation of diallyl disulfide with magnesium peroxyhydrate in the presence of ammonium butyl sulphate [19]. In our previous paper on the synthesis of allicin [20] we proposed radical mechanism of allyl disulfide oxidation. Due to the significant instability of allicin, synthesis procedures are usually performed at temperatures from 0°C to 25°C.

Allicin was more stable in 20% alcohol than in water, but surprisingly unstable in vegetable oil, with half-life activity of 0.8 hours [22]. In order to increase the stability of allicin, we obtained the inclusion complexes with β -cyclodextrin [8] and carbamide [9], where its pharmacological activity was still preserved. There are data about the transformation of allicin in the polar [21, 23] and nonpolar [1, 3, 4] media, where active products with higher stability than allicin were obtained. In hexane or soybean oil, allicin forms ajoenes and vinyl dithiins [4]. Block and collaborators have proposed degradation mechanisms of allicin to the above-mentioned products (Schemes 1 and 2) [21].

In our previous paper [7] we examined the stability of synthesized allicin by FTIR method, at room temperature. Herein, we present the influence of various factors (ultrasound, microwaves, different solvents, and temperatures) on the kinetics of transformation of allicin. To the best of our knowledge, this is the first report on the influence of ultrasound and microwaves on the allicin transformation. For the optimal transformation process, according to kinetic



SCHEME 1: Mechanism of alliin transformation to ajoenes [21].



SCHEME 2: Transformation of alliin in oil and organic nonpolar solvents [21].

parameters, from the mixture of transformation products by preparative HPLC method, predominant compounds were isolated. Their structures were determined by spectroscopic methods (UV, FTIR, NMR, and MS).

2. Experimental

2.1. Synthesis of Alliin. Alliin was synthesized according to our previous procedure [20]. Synthesis is based on the oxidation of allyl disulfide with the acidic hydrogen peroxide. Alliin was extracted from the crude reaction mixture using multiple liquid-liquid extraction with diethyl ether.

2.2. Transformation of Alliin in the Presence of Solvent at Room Temperature. Transformation of alliin (1 g) in organic solvents (9 cm³ of acetonitrile, acetone, methanol, or chloroform) was obtained using conventional method, the influence of ultrasound and microwaves.

Conventional transformation of alliin has been performed for seven days at room temperature and by heating in a water bath with reflux at 45°C and 55°C.

Transformation under the influence of ultrasound was performed in the ultrasonic bath (Sonic, Nis, Serbia; total nominal power: 3 × 50 W; dimensions of the bath: 30 × 15 × 20 cm) at the frequency of 40 kHz, with reflux at 45°C and 55°C.

Transformation under the effect of microwaves was performed in a “Discover” focus microwave reactor (CEM Corporation, Matthews, NC, USA), at a frequency of 2.45 GHz, with power of 150 W, at 45°C and 55°C.

2.3. Determination of Alliin Content. The content of alliin was analyzed using HPLC-UV method. Chromatographic analysis was performed using Agilent 1100 system equipped with an Agilent 1200 autosampler. Separations were performed on Zorbax Eclipse XDB-C18 (4.6 × 250 mm, 5 μm) column (Agilent, Santa Clara, USA). The mobile phase consisted of acetonitrile and water, 80:20, v/v. The flow rate was 1 mL/min and the injection volume was 20 μL. All separations were performed at 25°C. DAD detection was performed using a surveyor Agilent photodiode array detector at 205 nm.

External calibration was performed in the range of 12.5 μg/mL to 250 μg/mL. Within the range of concentrations injected, the detector response (peak area) was linear. Correlation coefficient for the calibration curve was 0.998.

2.4. Isolation and Structural Determination of Transformation Products. Isolation of transformation products was performed using preparative HPLC chromatography, on the Agilent Technologies 1200 Series device. Separations were performed on a Zorbax XDB-C-18 (6.2 × 150 mm, 5 μm)

column (Agilent, Santa Clara, USA). The mobile phase is consisted of acetonitrile and water, 80:20, v/v. The flow rate was 4 mL/min and the injection volume was 70 μ L. Seventy separations were performed at 25°C. DAD detection was performed using a surveyor Agilent photodiode array detector at 205 nm. For each isolated component an UV spectrum has been recorded.

FTIR spectra were recorded at Bomem MB-100 spectrophotometer (Hartmann & Braun, Baptiste, Canada) using the KBr technique in the range of 4000–400 cm^{-1} .

$^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ measurements were performed by a Bruker AC 250E (Bruker, Germany) spectrometer at the operating frequencies of 250 MHz and 62.5 MHz, respectively, using deuterated chloroform as solvent.

MS analysis of alliin transformation products was performed using a LCQ Fleet Ion Trap LC-MSⁿ system (Thermo Scientific, San Jose, USA). Chromatographic conditions were identical with those of the determination of alliin content. Mass spectra were obtained in positive ionization mode using an extractor voltage of 4.5 kV.

3. Results and Discussion

Figure 1 shows changes in concentrations of alliin during the time, at room temperature, in acetonitrile, acetone, methanol, and chloroform. The most significant change of the alliin concentration occurs in the first five days in all solvents used. The lowest concentration of alliin remaining was detected in acetonitrile, while the highest concentration was detected in chloroform. Transformation of alliin in the above-mentioned solvents, using conventional method at higher temperatures (45°C and 55°C), was significantly faster than the same process at room temperature (Figure 2). The most significant change of the alliin concentration in acetonitrile was achieved in about 75 minutes, while in chloroform it was achieved in about 120 minutes. Also, after the transformation at temperature of 55°C the lower concentrations of alliin remaining were detected in all solvents used compared to the results obtained at room temperature.

Transformation of alliin under the influence of ultrasound (Figure 3) occurs three to four times faster compared to the conventional process of transformation in all solvents used. In these transformations, as well as in conventional ones, the lowest concentration of the alliin remaining was found in acetonitrile.

Microwaves have a bigger impact on the transformation rate, compared to the ultrasound and the conventional process, for the same temperatures and solvents used (Figure 4). The fastest transformation including the complete transformation of alliin was achieved in methanol at 55°C in about 2 minutes.

In order to be able to compare the abilities of different solvents to generate heat from microwave irradiation, their capabilities to absorb microwave energy and to convert the absorbed energy into heat, must be taken into account. Those factors may be considered using the loss angle, δ , which is usually expressed in the form of its tangent

$$\tan \delta = \frac{\epsilon''}{\epsilon'}. \quad (1)$$

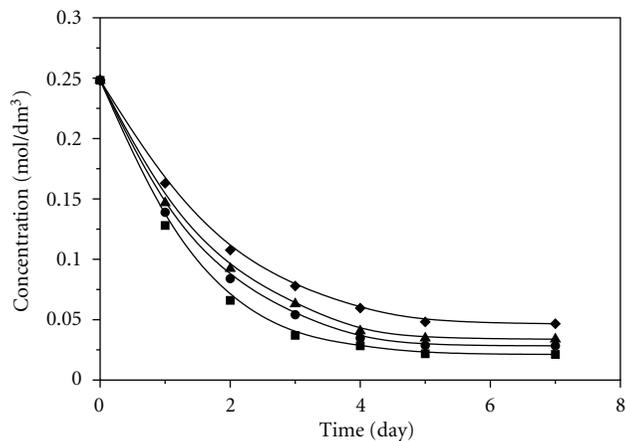


FIGURE 1: Change of alliin concentrations in acetonitrile, acetone, chloroform, and methanol at room temperature (■ Acetonitrile; • Acetone; ▲ Methanol 55°C; ◆ Chloroform).

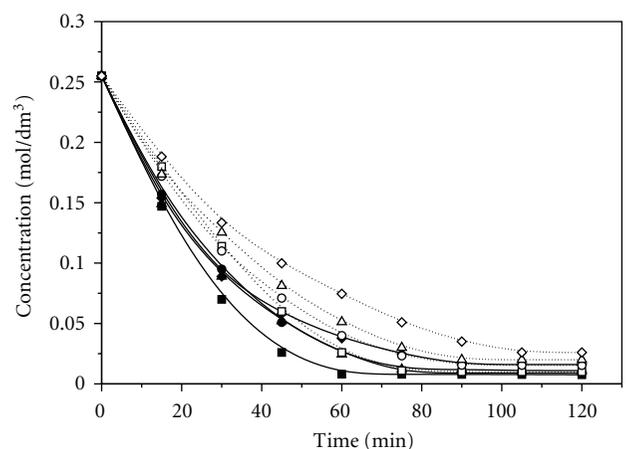


FIGURE 2: Change of the alliin concentration using conventional heating at 45°C and 55°C (■ Acetonitrile 55°C; □ Acetonitrile 45°C; • Acetone 55°C; ○ Acetone 45°C; ▲ Methanol 55°C; △ Methanol 45°C; ◆ Chloroform 55°C; ◇ Chloroform 45°C).

The dielectric constant, ϵ' , represents the ability of dielectric material to store electrical potential energy under the influence of an electric field. The loss factor, ϵ'' , quantifies the efficiency with which the absorbed energy is converted into heat [24]. The transformation of alliin occurs fastest in methanol which can be explained by the fact that this solvent has a much higher value of δ (0.659) compared to the other three solvents, whose δ values range from 0.054 to 0.091 [24].

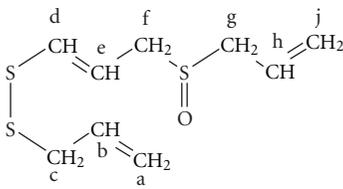
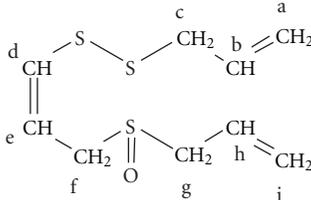
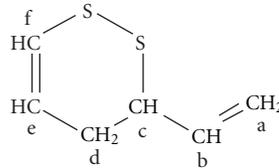
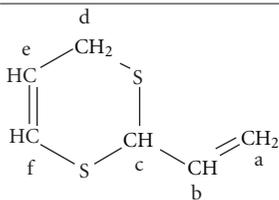
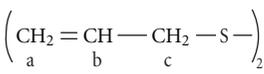
Kinetic parameters of alliin transformations were determined by the reaction rate equation

$$-\frac{dC_A}{dt} = k \cdot C_A^n. \quad (2)$$

Logarithm of this equation is given in (3). By using this equation, kinetic parameters of the reaction of alliin transformation, chemical reaction rate constant (k), and order of reaction (n) can be determined

$$\ln\left(-\frac{dC_A}{dt}\right) = \ln(k) + n \cdot \ln(C_A). \quad (3)$$

TABLE 1: Spectral data for the components isolated from the mixture of alliin transformation products obtained under the effect of microwaves in methanol at 55°C.

Name and structure of compounds	¹ H NMR (δ, ppm)	¹³ C NMR (δ, ppm)	FTIR (cm ⁻¹)	MS (m/z)	UV (nm)
 (E)-Ajoene, 1	6.38 (=CH-S-S, d) 5.9 (=CH-CH ₂ , m) 5.4 (CH ₂ =CH-CH ₂ -SO, m) 5.2 (CH ₂ =CH-CH ₂ -S, m) 3.5 (-CH ₂ -SO-CH ₂ -, m) 3.36 (-S-S-CH ₂ -, d)	41.5 (c) 53 (f) 54.5 (g) 117 (b) 119.5 (e) 123.8 (h) 126 (d) 132.5 (a) 135 (j)	3081 (w, C-H) 3000-2856 (s) 1636 (w) 1563 (w) 1435 (m) 1051 (s, CS(O)C)	234 (10), 145 (24), 111 (11), 103 (91), 73 (39), 68 (32), 45 (100), 41 (91)	244
 (Z)-Ajoene, 2	6.56 (=CH-S-S, d) 5.8 (=CH-CH ₂ , m) 5.4 (CH ₂ =CH-CH ₂ -SO, m) 5.2 (CH ₂ =CH-CH ₂ -S, m) 3.5 (-CH ₂ -SO-CH ₂ -, m) 3.38 (-S-S-CH ₂ -, d)	42.1 (c) 50 (f) 55 (g) 118.2 (b) 119.5 (e) 124 (h) 126 (d) 132.5 (a) 138.5 (j)	3081 (w, C-H) 3000-2856 (s) 1636 (m) 1455 (m) 1402 (m) 1045 (s, CS(O)C) 926 (s)	234 (10), 145 (22), 111 (13), 103 (93), 73 (39), 68 (37), 45 (100), 41 (91)	235
 3-Vinyl-4H-1,2-dithiin, 3	6.31 (S-CH=C, d) 6.01 (-CH=, m) 3.35 (-CH ₂ -, m) 4.51 (-CH-, m) 5.85 (-CH=, m) 5.36 (CH ₂ =, d)	132.5 (a) 121.99 (f) 118 (e) 117.1 (b) 44.5 (d) 39.1 (c)	3081 (m, C-H) 3012 (m, C=CH) 2978 (m, C=CH ₂) 1634 (s, C=C) 982 (s) 917 (s)	144 (49), 111 (75), 103 (31), 97 (48), 72 (25), 71 (60), 45 (100), 39 (20)	227
 2-Vinyl-4H-1,3-dithiin, 4	6.25 (S-CH=C, d) 5.9 (-CH=, m) 5.8 (-CH=, d) 5.37 (CH ₂ =, d) 4.7 (S-CH-S, m) 3.37 (-CH ₂ -S, d)	132.85 (a) 122.1 (f) 117.9 (e) 116.91 (b) 44.98 (d) 41 (c)	3081 (m, C-H) 3012 (m, C=CH) 2978 (m, C=CH ₂) 1634 (s, C=C) 982 (s) 917 (s)	144 (35), 111 (34), 103 (31), 72 (100), 71 (25), 45 (60), 39 (43)	232
 Diallyl disulfide, 5	3.1 (-CH=CH ₂ , d) 5.15 (-CH ₂ -CH=, d) 5.8 (CH ₂ =CH-CH ₂ -, m)	42 (c) 118 (a) 132 (b)	3081 (m, C-H) 3009 (m, C=CH) 2978 (m, C=CH ₂) 2905 (m) 1634 (s, C=C) 1423 (m) 1292 (m) 1214 (m) 721 (m)	146 (41), 113 (46), 105 (40), 81 (55), 79 (33), 45 (78), 41 (100), 39 (50)	200

For all the transformations, dependence $\ln(-dC_A/dt)$ of $\ln(C_A)$ is shown in Figure 5. Activation energy (E_a) was determined from Arrhenius's equation

$$k = A \cdot e^{-E_a/RT}. \quad (4)$$

The changes of E_a and n for the applied solvents and techniques of alliin transformation are shown in Figure 6. Under the effect of microwaves in all solvents used, the highest value

of n (1.5) is achieved. Under the influence of ultrasound n equals 1, while in the conventional process it equals 0.5. The highest values of E_a are required for the conventional method of alliin transformation, while the lowest E_a values are required for the process under the effect of microwaves. According to E_a values, the most suitable alliin transformation process is performed under the influence of microwaves in methanol at 55°C ($E_a = 7902$ J/mol). Also,

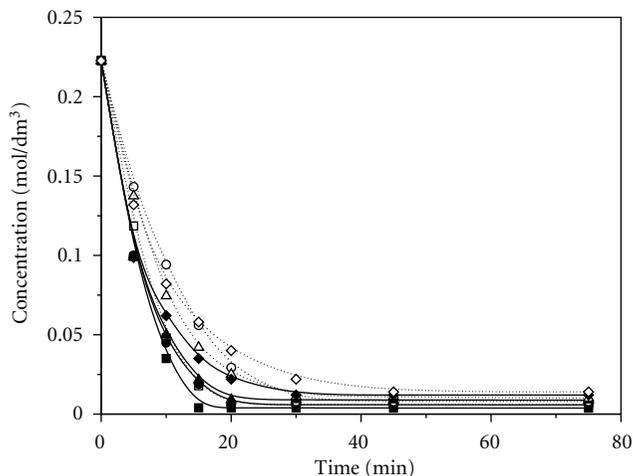


FIGURE 3: Change of the allucin concentration under the influence of ultrasound at 45°C and 55°C (■ Acetonitrile 55°C; □ Acetonitrile 45°C; • Acetone 55°C; ◦ Acetone 45°C; ▲ Methanol 55°C; △ Methanol 45°C; ◆ Chloroform 55°C; ◇ Chloroform 5°C).

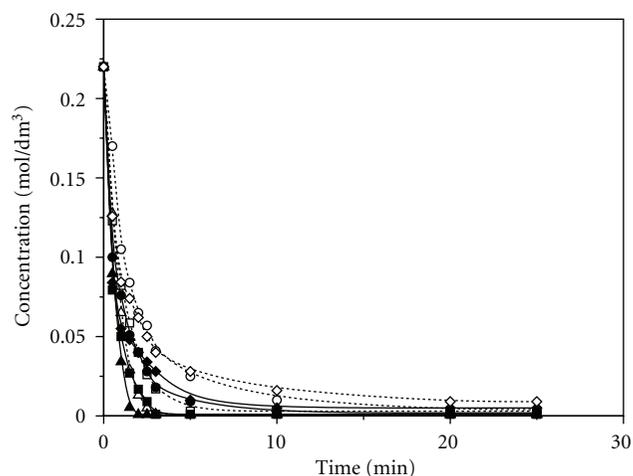
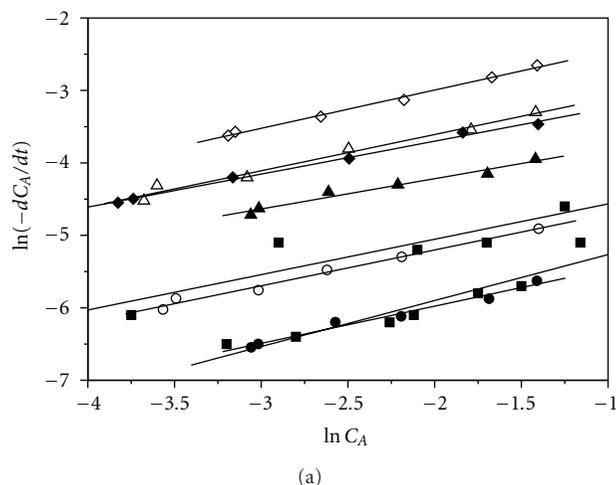


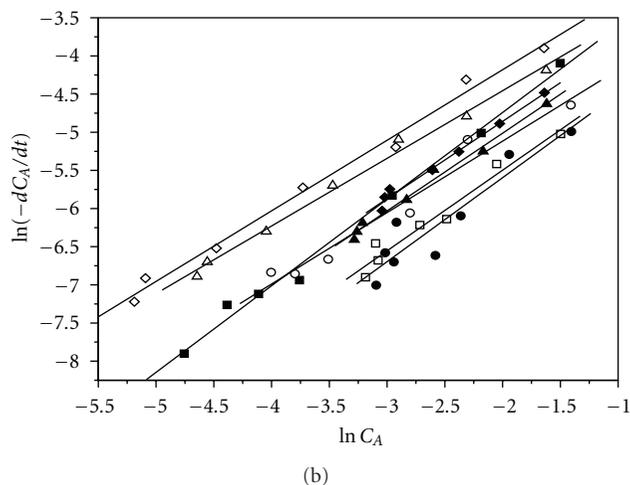
FIGURE 4: Change of allucin concentration under the influence of microwaves at 45°C and 55°C (■ Acetonitrile 55°C; □ Acetonitrile 45°C; • Acetone 55°C; ◦ Acetone 45°C; ▲ Methanol 55°C; △ Methanol 45°C; ◆ Chloroform 55°C; ◇ Chloroform 45°C).

this process occurs within the shortest transformation time (about 2 minutes). The highest E_a value is required for the conventional procedure in chloroform at 45°C ($E_a = 70645 \text{ J/mol}$) and this process requires the longest time of transformation. Therefore, these conditions are least suitable for allucin transformation.

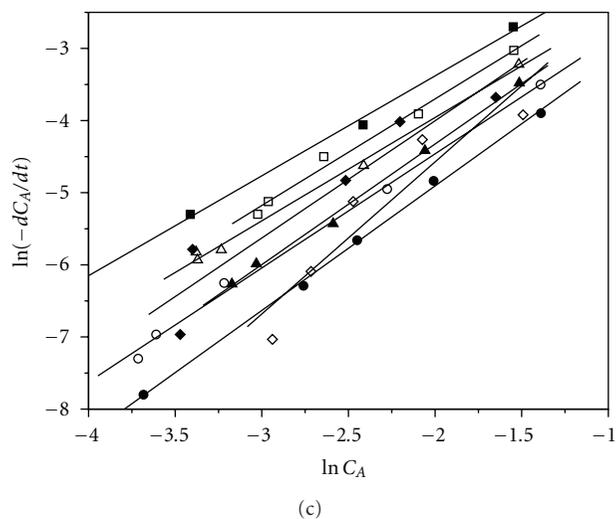
The most common components (see Figure 7) from the mixture of transformation products of allucin under the influence of microwaves in methanol at 55°C were isolated using preparative HPLC chromatography. Their structures were determined by UV, FTIR, NMR, and MS methods and spectroscopic data are given in Table 1. (E)-Ajoene ((E)-1-(prop-2-enylsulfanyl)-3-prop-2-enylsulfanylprop-1-ene;



(a)



(b)



(c)

FIGURE 5: Dependence $\ln(-dC_A/dt)$ of $\ln(C_A)$ for the allucin transformation by conventional method (a), under the influence of ultrasound (b) and microwaves (c). (■ Acetonitrile 55°C; □ Acetonitrile 45°C; • Acetone 55°C; ◦ Acetone 45°C; ▲ Methanol 55°C; △ Methanol 45°C; ◆ Chloroform 55°C; ◇ Chloroform 45°C).

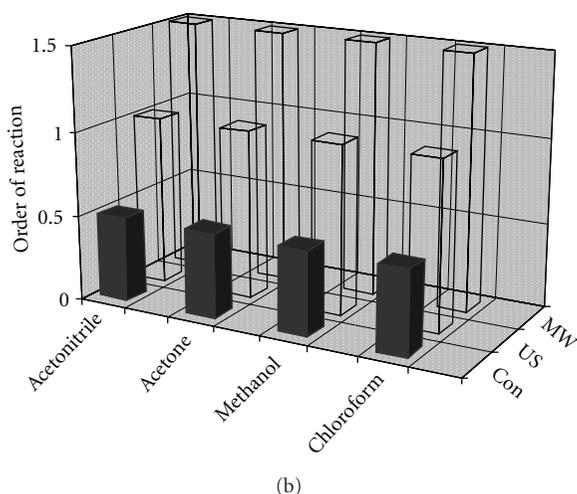
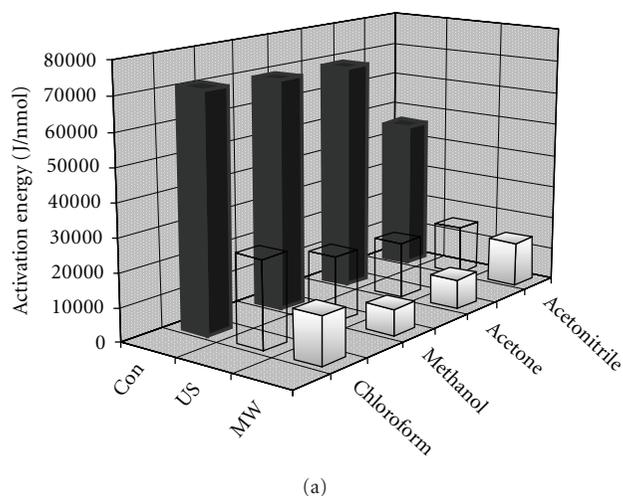


FIGURE 6: Values of E_a (a) and n (b) for the allicin transformation by conventional method (Con), under the influence of ultrasound (US) and microwaves (MW).

(1), (Z)-ajoene ((Z)-1-(prop-2-enylsulfanyl)-3-prop-2-enylsulfanylprop-1-ene; (2), 3-vinyl-4H-1,2-dithiin (3-ethenyl-3,4-dihydrodithiine; (3), 2-vinyl-4H-1,3-dithiin (2-ethenyl-4H-1,3-dithiine; (4), and diallyl disulfide (3-(prop-2-enylsulfanyl)prop-1-ene; (5) were isolated. 1 and 2 exhibit antimicrobial [25, 26] and anticancer effect [27, 28]. 3 and 4 participate in the inhibition of thrombocyte aggregation, cyclooxygenase and 5-lipoxygenase inhibition, and regulation of systolic and diastolic blood pressure [29]. 5 inhibits 1,2-dimethylhydrazine-induced colon and liver cancer in rodents [30]. Presence of these compounds in the transformation mixture is in accordance with the previous findings about the ways of allicin transformation [20].

Due to the complexity of the mixture of transformation products other compounds could not be isolated.

4. Conclusion

The transformation rate of allicin depends on the applied techniques of transformation, temperature, and solvents

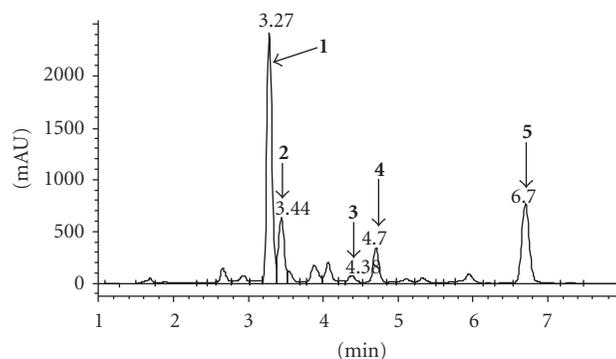


FIGURE 7: HPLC chromatogram of the mixture of allicin transformation products obtained under the effect of microwaves in methanol at 55°C ((E)-ajoene (1); (Z)-ajoene (2); 3-vinyl-4H-1,2-dithiin (3); 2-vinyl-4H-1,3-dithiin (4); diallyldisulfid (5)).

nature. Use of microwaves and increased temperatures accelerate the allicin transformation. Kinetic parameters, n and E_a , depend on the transformation techniques used. The highest n and the lowest E_a values were achieved under the influence of microwaves in methanol at temperature of 55°C.

Acknowledgment

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