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Organosulphur Compounds of Garlic (*Allium Sativum*) Paste Treated with Ascorbic and Citric Acids

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Abstract

The consumption of processed garlic products (e.g. chopped and fried) has considerably increased over the last few years, probably due to its easy of use, when comparing to fresh garlic. To determine the effect of garlic variety and organic acids ((Ascorbic and citric acids) or their blends) on volatile sulphur compounds of garlic paste. Fresh garlic bulbs of two Sudanese varieties (Dongla and Berber) were selected for this study. They were harvested in December 2011. Peeling was done manually; the garlic bulbs were separated into the individual sound cloves and crushed in a blender until a smooth puree was obtained (3 min). In an attempt to maintain the quality of garlic, some chemical additives were added during bulb crushing. (Control (T0), Ascorbic acid 0.5mg/g (T1), Citric acid 2mg/g (T2), Ascordic acid 0.25mg/g and Citric acid 1mg/g (T3), Ascordic acid 0.5mg/g and Citric acid 2mg/g (T4)). Volatile sulphur compounds (VSC) was measured. The result indicated that Berber variety had significant ($P < 0.05$) VSC except Dially disulphide and 2-Vinyl-4(H)-1,3-dithiin. Chemical treatments (T0-T4) had significant ($p < 0.05$) effects on VSC except 3-Vinyl-4(H)1,2-dithiin and 2-Vinyl-4(H)-1,3-dithiin. Garlic paste treated with T4 had higher ($p < 0.05$) VSC except Allyl methyl Disulphide and Diallyl trisulphide. Organic acids (Ascorbic and citric acids) or their blends are recommended to use in garlic processing.

Keywords: *Garlic paste; Preservation; Ascorbic acid; Citric acid; Organic acids*

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Introduction

Garlic is an important crop for culinary purposes, its pungent flavor adding a special taste to food. In some traditional Chinese and Egyptian dishes, garlic is fried in vegetable oil before adding food, which imparts a special taste and smell to the dish (Sun., *et al*. 1995). Garlic is a very important cash crop in Sudan particularly in the Northern states (Dongla and Berber), where there is a long winter season and light soil. It is also grown on a very limited scale in the Gezira, Darfur and Kordofan states (Omar, 1999). The total annual area under garlic in Sudan was estimated to be about 100 hectares and annual production about 16,000 MT with an average yield of 1,778 Kg/ha (FAO, 1994). Cardiovascular disease (CVD) is responsible for 30% of all deaths and 10% of DALYs (disability-adjusted life years) (WHO, 2009). It has a major socio-economic impact on individuals, families and societies in terms of healthcare costs, work absenteeism and national productivity (Opie and Mayosi, 2005). Garlic and garlic extracts are believed to possess beneficial effects for the prevention of cardiovascular diseases (Koscielny., *et al*. 1999; Steiner and Li, 2001). the consumption of processed garlic products (e.g. chopped and fried) has considerably increased over the last few years, probably due to its easy of use, when comparing to fresh garlic (Oliveira., *et al.* 2004). The main objective of the present work was to check the effect of garlic variety and organic acids ((Ascorbic and citric acids) or their blends) on volatile sulphur compounds of garlic paste.

Material and Methods

Preparation of garlic paste: Fresh garlic bulbs of two Sudanese varieties (Dongla and Berber) were harvested in December 2011, collected, packed and transferred to the Department of Food science and Technology Laboratory, Faculty of Agriculture University of Khartoum, Sudan. The garlic bulbs were peeled manually, separated into the individual sound cloves. Prepared garlic cloves were divided into 5 equal portions and crushed by a blender until a smooth puree was obtained after 3 mint. Before crushing, chemical additives were added during bulb crushing, no additive control (T0), (Ascorbic acid 0.5mg/g (T1), 2mg/g Citric acid (T2), 0.25mg/g Ascorbic acid +1mg/g Citric acid (T3), 0.5mg/g Ascorbic acid +2mg/g Citric acid (T4). The garlic paste was packaged in aseptic glass containers and hermetically closed.

Determination of volatile sulphure compounds: Gas chromatography was used a Perkin-Elmer model Sigma 3B. For identification by mass spectrometry a Hewlett Packard model 5980 gas chromatograph equipped with a Hewlett Packard model 5988 A mass spectrometer was used. Operational parameters were as follows: carrier gas, helium; ionisation voltage, 70 eV; ion source temperature 200°C. Dimethyi sulphide (DMS), diethyl ether (Merck), ally1 methyl sulphide (AMS), *p*-cymene (Janssen), diallyl sulphide (DAS), diallyl disulphide (DADS) (Fluka) and dipropyl disulphide (DPDS) (Aldrich), all of analytical grade, were used as standards. Linearity and sensitivity of the detector were calculated from a series of solutions from 2×10^{-2} M to $1 \times l0^{-4}$ M in diethyl ether. The quantitative analyses of sulphur derivatives were performed using p-cymene as the internal standard. To determine the extraction yield from aqueous solutions, 10 ml 5 mol of the pure compounds AMS, DMS, DAS, DPDS and DADS were dissolved in 100 ml distilled water. The solution was extracted three times with 150 ml diethyl ether. The extracts were pooled and concentrated to a volume of 4 ml in a Kuderna-Danish apparatus set in a water bath (40°C), then further concentrated under a nitrogen current to a final volume of 1 ml. To this volume 20 µl of a 5×10^{-3} M solution of *p*-cymene were added, and a 0.2 µl aliquot was injected into the chromatograph under the conditions described above.

Statistical analysis

Data generated were subjected to SAS (SAS, version 2002). Two-factor CRD was performed, where factor A = variety and factor B = temperature for the 1st batch, for the 2nd batch also two factors were assessed where factor A = chemical treatment and factor B = storage period. Duncan multiple range test (DMRT) was used to separate the treatments means.

Results and Discussion

Effect of garlic variety and chemical treatments on volatile sulphur compounds of garlic paste

The interactive effect of variety and organic acids treatment on the ally methyl sulphide (AMS), Dimethyle disulphide (DMDS) and Dially sulphide (DAS) are shown in Table 1. Results indicated that irrespective of chemical treatments variety had led to a significant ($p \leq 0.05$) difference in the amount of AMS. Berber variety had significantly ($p \leq 0.05$) higher (0.419mg/100g) AMS than Dongola variety (0.416mg/100g). The AMS of garlic was significantly ($p \leq 0.05$) affected by the treatments to which garlic paste was subjected. Garlic paste treated with T4 had the highest AMS (0.428mg/100g) compared to the control (0.414mg/100g). The value obtained in this study was higher than those obtained by Martin-Lagos., *et al*. (1995), who reported that garlic contain 0.167 mg AMS /100g. Treatments had led to a significant ($p \leq 0.05$) difference in the amount of DMDS between varieties. Berber variety had significantly ($p \leq 0.05$) higher (0.463mg/100g) DMDS than Dongola variety (0.461mg/100g). The DMDS of garlic was significantly ($p \leq 0.05$) affected by the treatments to which garlic paste was subjected. Garlic paste treated with T4 had the highest DMDS (0.468mg/100g) compared to the control

(0.454mg/100g). The value obtained in this study was higher than those obtained by Martin-Lagos., *et al*. (1995), who reported that garlic contain 0.204 mg DMDS /100g. Treatments had led to a significant ($p \leq 0.05$) difference in the amount of DAS between varieties.

Treatment	Variety		Average trt.
	Dongola	Berber	
AMS Allyl methyl sulphide (mg/100g)			
T_0	0.4130 ± 0.00^h	0.4140 ± 0.00^g	0.4135^E
T_1	0.4160 ± 0.00^e	0.4163 ± 0.00^e	0.4162^C
T_2	0.4130 ± 0.00^h	0.4153 ± 0.00^f	0.4142^D
T_3	0.4170 ± 0.00^d	0.4183 ± 0.000^c	0.4177^B
T_4	0.4227 ± 0.00^b	0.4330 ± 0.02^a	0.4278^A
Average variety	0.416^B	0.419^A	
DMDS Dimethyle disulphide (mg/100g)			
T_0	0.4533 ± 0.00^h	0.4547 ± 0.00^g	0.4540^E
T_1	0.4603 ± 0.00^f	0.4600 ± 0.00^f	0.4602^D
T_2	0.4623 ± 0.00^e	0.4657 ± 0.00^c	0.4640^B
T_3	0.4620 ± 0.00^e	0.4640 ± 0.00^d	0.4630^C
T_4	0.4677 ± 0.00^b	0.4690 ± 0.00^a	0.4683^A
Average variety	0.461^B	0.463^A	
DAS Dially sulphide (mg/100g)			
T_0	0.4143 ± 0.00^i	0.4150 ± 0.00^i	0.4147^E
T_1	0.4223 ± 0.01^h	0.4303 ± 0.00^g	0.4263^D
T_2	0.4317 ± 0.00^f	0.4367 ± 0.01^e	0.4342^C
T_3	0.4460 ± 0.00^c	0.4447 ± 0.00^d	0.4453^B
T_4	0.4467 ± 0.00^b	0.4503 ± 0.00^a	0.4485^A
Average variety	0.432^B	0.435^A	

Values are mean ± SD.

*Means in the same columns and rows bearing same superscript small letters are not significantly different ($P \geq 0.05$).

**Means in the same row with the same superscript capital letters are not significantly different ($P \geq 0.05$).

***Means in the same column bearing different superscript capital letters are significantly different ($P \leq 0.01$).

T_0 = Control

T_1 = Ascorbic acid (0.5mg/g)

T_2 = Citric acid (2mg/g)

T_3 = Ascorbic acid (0.25mg/g) and Citric acid (1mg/g)

T_4 = Ascorbic acid (0.5mg/g) and Citric acid (2mg/g)

Table 1: *Effect of garlic variety and treatments on allyl methyl sulphide, dimethyle disulphide and dially sulphide compounds of garlic paste*

Berber variety had significantly ($p \leq 0.05$) higher (0.435mg/100g) DAS than Dongola variety (0.432mg/100g). The DAS of garlic was significantly ($p \leq 0.05$) affected by the treatments to which garlic paste was subjected. Garlic paste treated with T4 had the highest DAS (0.449mg/100g) compared to the control (0.415mg/100g). The value obtained in this study was higher than those obtained by Martin-Lagos., *et al*. (1995), who reported that garlic contain 0.122 mg/100g DAS. Hedge and Lister (2007) stated that, animal *in vitro* studies have shown that diallyl sulphide can inhibit the activity of particular phase I enzyme families. These enzymes are endogenous one that can transform potential carcinogens into active ones. Also Leelarungrayub., *et al.* (2006) postulated that the antioxidant activity of garlic could be closely associated with DAS content. Accordingly some of the beneficiary health effect could be partially attributed to the DAS content of garlic. The interactive effect of variety and treatment on the allyl methyl Disulphide (AMDS), Dimethyle trisulphide (DMTS) and Dially disulphide (DADS) are shown in Table 2. Results indicated that chemical treatments had led to a significant ($p \leq 0.05$) difference in the amount of AMDS between varieties. Berber variety had significantly ($p \leq 0.05$) higher (2.21mg/100g) AMDS than Dongola variety (2.19mg/100g). The AMDS of garlic was significantly ($p \leq 0.05$) affected by the treatments to which garlic paste was subjected. Garlic paste treated with T1 had the highest AMDS (2.32mg/100g) compared to that of T2 (2.12mg/100g). The value obtained in this study was higher than that obtained by Martin-Lagos., *et al.* (1995), who reported that garlic contain 1.193 mg/100g AMDS. The observed discripancy between the two studies could be attributed to variatal and/or agronomic practices. The two varieties (Berber and Dongola) had similar ($p \geq 0.05$) DMTS contents (0.132mg/100g vis 0.131mg/100g respectively). The DMTS of garlic was significantly ($p \leq 0.05$) affected by the chemical treatments to which garlic paste was subjected.

Treatment	Variety		Average Treatment
	Dongola	Berber	
AMDS Allyl methyl Disulphide (mg/100g)			
T_0	2.10 ± 0.18^{c}	2.25 ± 0.18^{abc}	2.18^{B}
T_1	2.31 ± 0.01^{ab}	2.33 ± 0.02^{a}	2.32^{A}
T_2	2.14 ± 0.02^{abc}	2.10 ± 0.09^{c}	2.12^{B}
T_3	2.12 ± 0.00^{bc}	2.16 ± 0.06^{abc}	2.14^{B}
T_4	2.28 ± 0.06^{abc}	2.21 ± 0.18^{abc}	2.25^{AB}
Average variety	2.19^{B}	2.21^{A}	
DMTS Dimethyle trisulphide (mg/100g)			
T_0	0.1003 ± 0.02^{i}	0.1123 ± 0.02^{i}	0.106^{E}
T_1	0.1240 ± 0.01^{g}	0.1213 ± 0.00^{h}	0.123^{D}
T_2	0.1357 ± 0.00^{f}	0.1390 ± 0.00^{e}	0.137^{C}
T_3	0.1417 ± 0.00^{d}	0.1423 ± 0.00^{c}	0.142^{B}
T_4	0.1523 ± 0.00^{a}	0.1457 ± 0.00^{b}	0.149^{A}
Average variety	0.131^{A}	0.132^{A}	
DADS Dially disulphide (mg/100g)			
T_0	6.71 ± 0.25^{bc}	6.54 ± 0.04^{c}	6.63^{B}
T_1	7.01 ± 0.54^{b}	6.68 ± 0.37^{bc}	6.84^{B}
T_2	7.62 ± 0.01^{a}	7.51 ± 0.18^{a}	7.57^{A}
T_3	7.52 ± 0.01^{a}	7.52 ± 0.01^{a}	7.52^{A}
T_4	7.65 ± 0.00^{a}	7.60 ± 0.09^{a}	7.63^{A}
Average variety	7.30^{A}	7.17^{B}	

Values are mean ± SD.

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**Means in the same row with the same superscript capital letters are not significantly different ($P \leq 0.05$).

***Means in the same column bearing different superscript capital letters are significantly different ($P \leq 0.01$).

T_0 = Control

T_1 = Ascorbic acid (0.5mg/g)

T_2 = Citric acid (2mg/g)

T_3 = Ascorbic acid (0.25mg/g) and Citric acid (1mg/g)

T_4 = Ascorbic acid (0.5mg/g) and Citric acid (2mg/g)

Table 2: *Effect of garlic variety and chemical treatments on allyl methyl disulphide, dimethyle trisulphide and dially disulphide compounds of garlic paste.*

Irrespective of variety garlic paste treated with T4 had the highest DMTS (0.149mg/100g) compared to the control (0.106mg/100g). The value obtained in this study for the control was higher than these obtained by Martin-Lagos., *et al.* (1995), who reported that garlic contain 0.075 mg/100g DMTS. Generally the chemical treatments had led to substantial increases in DMTS contents of garlic paste. Chemical treatments had led to a significant ($p \leq 0.05$) difference in the amount of DADS between varieties. Dongola variety had significantly ($p \leq 0.05$) higher (7.30mg/100g) DADS content than Berber variety (7.17mg/100g). The DADS of garlic was significantly ($p \leq 0.05$) affected by the chemical treatments to which garlic paste was subjected. Garlic paste treated with T4 had the highest DADS (7.630mg/100g) compared to the control (6.63mg/100g). The value obtained in this study was higher than those obtained by Martin-Lagos., *et al*. (1995), who reported that garlic contain 5.297 mg/100g DADS. Again variatal and/or agronomic practices could account for the observed difference between the two studies. The interactive effect of variety and treatment on the allyl methyl trisulphide (AMTS), 3-Vinyl-4(H)-1,2-dithiin (3VHDT), and Diallyl trisulphide (DATS) are shown in Table 3. Results indicated that treatments had led to a significant ($p \leq 0.05$) difference in the amount of AMTS between varieties. Berber variety had significantly ($p \leq 0.05$) higher (1.31mg/100g) AMTS than Dongola variety (1.29mg/100g). The AMTS of garlic was significantly ($p \leq 0.05$) affected by the treatments to which garlic paste was subjected. Garlic paste treated with T_4 had the highest AMTS (1.42mg/100g) compared to the control (1.23mg/100g). The value obtained in this study for the control sample was higher than that obtained by Martin-Lagos., *et al*. (1995), who reported that garlic contain 0.562mg/100g AMTS. Also generally it is to be noted that the chemical treatment had led to substantial increases in AMTS content.

Treatment	Variety		Average Treatment
	Dongola	Berber	
AMTS Allyl methyl trisulphide(mg/100g)			
T_0	1.23 ± 0.03^{c}	1.24 ± 0.01^{c}	1.23^{D}
T_1	1.23 ± 0.01^{c}	1.24 ± 0.01^{c}	1.23^{D}
T_2	1.24 ± 0.00^{c}	1.30 ± 0.10^{b}	1.27^{C}
T_3	1.32 ± 0.02^{b}	1.33 ± 0.01^{b}	1.32^{B}
T_4	1.41 ± 0.01^{a}	1.43 ± 0.01^{a}	1.42^{A}
Average variety	1.29^{B}	1.31^{A}	
3VHDT 3-Vinyl-4(H)-1,2-dithiin (mg/100g)			
T_0	7.79 ± 0.18^{a}	7.62 ± 0.12^{ab}	7.70^{A}
T_1	7.65 ± 0.00^{ab}	7.65 ± 0.00^{ab}	7.65^{A}
T_2	7.65 ± 0.01^{ab}	7.93 ± 0.38^{b}	7.54^{A}
T_3	7.65 ± 0.01^{ab}	7.65 ± 0.01^{ab}	7.65^{A}
T_4	7.60 ± 0.09^{ab}	7.46 ± 0.23^{b}	7.53^{A}
Average variety	7.67^{A}	7.56^{A}	
DATS Diallyl trisulphide (mg/100g)			
T_0	2.04 ± 0.17^{ab}	1.85 ± 0.54^{b}	1.95^{B}

T_1	2.26 ± 0.05^a	2.26 ± 0.05^a	2.26^A
T_2	2.33 ± 0.00^a	2.35 ± 0.04^a	2.34^A
T_3	2.19 ± 0.10^{ab}	2.16 ± 0.04^{ab}	2.18^{AB}
T_4	2.09 ± 0.08^{ab}	2.17 ± 0.05^{ab}	2.13^{AB}
Average variety	2.18^A	2.16^A	

Values are mean ± SD.

*Means in the same columns and rows bearing same superscript small letters are not significantly different (P ≥ 0.05)

**Means in the same row with the same superscript capital letters are not significantly different (P ≤ 0.05).

***Means in the same column bearing different superscript capital letters are significantly different (P ≤ 0.01).

T_0 = Control

T_1 = Ascorbic acid (0.5mg/g)

T_2 = Citric acid (2mg/g)

T_3 = Ascorbic acid (0.25mg/g) and Citric acid (1mg/g)

T_4 = Ascorbic acid (0.5mg/g) and Citric acid (2mg/g)

Table 3: *Effect of garlic variety and treatments on allyl methyl trisulphide, 3-vinyl-4(H)-1,2-dithiin and diallyl trisulphide compounds of garlic paste.*

Apparently the two varieties (Dongola and Berber) had similar (p ≥ 0.05) amount of 3VHDT. Irrespective of the chemical treatments the two varieties (Berber and Dongla) had similar (p ≥ 0.05) 3VHDT contents (7.56 vis 7.67mg/100g) respectively. Similarly irrespective of the variety the chemical treatments had no effect (p ≥ 0.05) on the 3VHDT contents. Untreated garlic paste (control) had a 3VHDT of 7.70mg/100g. The value obtained in this study was higher than that obtained by Martin-Lagos., *et al*. (1995), who reported that garlic contain 4.09mg/100g 3VHDT. The discrepancies between the two studies could be attributed to varietal and/or agronomic practices. Treatments had led an insignificant (p ≥ 0.05) difference in the amount of DATS between varieties. The DATS of garlic was significantly (p ≤ 0.05) affected by the chemical treatments to which garlic paste was subjected. Garlic paste treated with T_2 had the highest DATS (2.34mg/100g) compared to T_0 (1.95mg/100g). The value obtained in this study was higher than that obtained by Martin-Lagos., *et al*. (1995), who reported that garlic contain 1.29mg/100g DATS. The interactive effect of variety and treatment on the 2-vinyl-4(H)-1,3-dithiin (2VHDT) is shown in Table 4. Results indicated that Treatments had led to a significant (p ≤ 0.05) difference in the amount of 2VHDT between the varieties. Dongola variety had significantly (p ≤ 0.05) higher (776.00mg/100g) 2VHDT than Berber variety (734.73mg/100g). The 2VHDT of garlic was significantly (p ≤ 0.05) affected by the treatments to which garlic paste was subjected, as untreated T0 garlic paste had significantly (p ≤ 0.05) less 2VHDT than the samples treated with organic acids (T_1 and T_2) or their blend (T_3 and T_4). Garlic paste treated with T_4 had the highest 2VHDT (790.70mg/100g) compared to T0 (653.70mg/100g). The value obtained in this study was lower than that obtained by Martin-Lagos., *et al*. (1995), who reported that garlic contain 830.07 mg/2VHDT.

Treatment	Variety		Average Treatment
	Dongola	Berber	
2VHDT 2-Vinyl-4(H)-1,3-dithiin (mg/100g)			
T_0	752.70 ± 2.52^a	554.70 ± 345.26^b	653.70^B
T_1	766.70 ± 17.67^a	758.70 ± 3.21^a	762.70^A
T_2	781.70 ± 2.89^a	780.70 ± 4.04^a	781.20^A
T_3	788.30 ± 0.58^a	789.00 ± 1.00^a	788.70^A
T_4	790.90 ± 1.15^a	790.70 ± 1.15^a	790.70^A
Average variety	776.00^A	734.73^B	

Values are mean ± SD.

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***Means in the same column bearing different superscript capital letters are significantly different ($P \leq 0.01$).

T_0 = Control

T_1 = Ascorbic acid (0.5mg/g)

T_2 = Citric acid (2mg/g)

T_3 = Ascorbic acid (0.25mg/g) and Citric acid (1mg/g)

T_4 = Ascorbic acid (0.5mg/g) and Citric acid (2mg/g)

Table 4: *Effect of garlic variety and treatments on 2-vinyl-4(H)-1,3-dithiin compounds of garlic paste.*

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