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Characterization of *E*- and *Z*-ajoene obtained from different varieties of garlics

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Abstract

Synthesised *E*- and *Z*-ajoene were used to determine their amounts in food oils containing various fresh garlics. The best yield of *E*-ajoene (172.0 μg/g of garlic) and *Z*-ajoene (476.0 μg/g of garlic) was obtained from freshly prepared Japanese garlic with rice oil which was heated at 80 °C. Determination of *E*- and *Z*-ajoene from soybean oil containing 15% Japanese garlic samples prepared at 80 °C for 0.5 h gave the amount of *E*-ajoene (170.0 μg/g of garlic) and *Z*-ajoene (127.0 μg/g of garlic). After 9-month storage, 54.0% *E*- and 11.0% *Z*-ajoene remained in Japanese garlic with rice oil. Ajoene (0.1 mM) in ethyl acetate was incubated under UV-light (253.7 nm) for 3 days, 81.7% *E*- and 56.9% *Z*-ajoene remained. 4.3% and 0.5% *E*- and *Z*-ajoene remained when ajoene (0.1 mM in ethyl acetate) was incubated at 100 °C.

Keywords: Garlic, *E*-ajoene; *Z*-ajoene; Incubation temperature; UV-light stability; Temperature stability; Storage stability

1. Introduction

Garlic has historically been one of the most common vegetables served as a spice and a medical herb in many countries (Itakura et al., 2001). The use of garlic as a means of preventing diseases and treating common ailments is one of the earliest documented examples of a plant being used as a medicinal agent (Rivlin, 2001) and its consumption has become a widely accepted general dietary course for promoting overall human health (Rybak, Calvey, & Harnly, 2004). Many of the health benefits associated with garlic consumption have been attributed to the thiosulfinates, the most abundant class of organosulfur compounds, found in freshly chopped or crushed garlic (Lawson & Wang, 2001). Oil-macerated garlic product is common as a health food in Europe but rare in the United States and Japan (Lawson, Wang, & Hughes, 1991). The consumption of oil-macerated garlic products or ether-extracted garlic oil, which is nearly identical in quantitative composition to the oil macerates, decreased serum cholesterol in humans (Bordia, 1981; Bordia, Sharma, Parmar, & Verma, 1982). Oil-macerated heated garlic products contained mainly vinyldithiins, ajoene (Block & Ahmad, 1984; Block, Ahmad, Catalfamo, Jain, & Apitz-Castro, 1986) and small amount of sulfides (Lawson et al., 1991).

Condensation of two molecules of allylsulfenic acid produced one molecule allicin, a major sulfur-containing intermediate, which was isolated and identified as an antibacterial substance (Cavallito, Buck, & Suter, 1944).

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Ajoene (Fig. 1a) is considered as a major natural compound derived from garlic through the conversion of alliin into allicin, by an alliinase-induced cleavage (Lawson et al., 1991). This enzyme, stored in vacuole of mesophyll cells, is liberated during tissue injuries. Allicin is a labile compound, easily transformed to a number of stable lipid-soluble allylsulfides such as ajoene (Block et al., 1986). Ajoene was recognised for its ability to improve health conditions, as reported by many researchers (Apitz-Castro, Badimon, & Badimon, 1994; Apitz-Castro et al., 1988; Rendu et al., 1989; Srivastava & Tyagi, 1993). Ajoene can modify cell adhesion and consequently display effects on cell growth inhibition in several cancer cell lines and antitumor activities (Li et al., 2002; Scharfenberg, Ryll, Wagner, & Wagner, 1994; Scharfenberg, Wagner, & Wagner, 1990; Zhang et al., 1998).

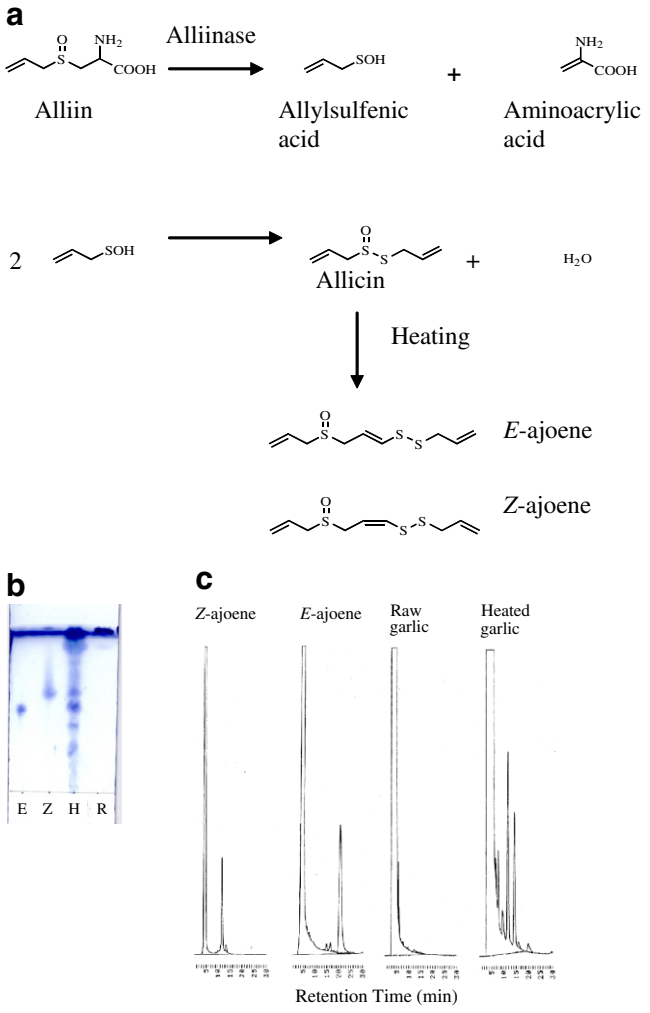

Fig. 1. (a) Formation of ajoene from allicin. (b) TLC and (c) Si-HPLC analyses of *E*- and *Z*-ajoene in garlic. *TLC conditions.* Plate: TLC silica gel 60 (Merck); developing solvent: ethyl acetate; colouring reagent: molybdo phosphoric acid/ phosphoric acid/ sulfuric acid (2.4:1.5:5, v/v/v); R_f value: *E*-ajoene = 0.45, *Z*-ajoene = 0.52. E, *E*-ajoene; Z, *Z*-ajoene; H, heated garlic; R, raw garlic. *HPLC conditions.* Column: Lichrospher Si 60 (250 × 4.0 mm), *n*-hexane/2-propanol (85:15, v/v), 240 nm, 1.0 ml/min. Peak 1 indicate *Z*-ajoene and peak 2 for *E*-ajoene.

Purification of ajoene is rather difficult since the compound is generally obtained as a mixture of *Z*- and *E*-ajoene, with a ratio around 2.2 (Scharfenberg et al., 1994). Purification of (*E*, *Z*) ajoene requires laborious extraction, separation and purification procedures.

Until now, no experiment has been undertaken to compare the characteristics of two ajoene isomers. The objectives of this study are to determine the amount and characteristics of *E*- and *Z*-ajoene in garlic product. The ideal incubation temperature for maximum recovery of ajoene is examined and its temperature, UV-light and storage stability will be investigated.

2. Materials and methods

2.1. Materials

Fresh Japanese garlic (*Allium sativum* L.) and Chinese garlic (*Allium sativum* L.) were purchased at a Japanese market and Bangladeshi garlic (*Allium sativum* L.) was purchased at Bangladeshi markets. Frozen clove, frozen paste, fried garlic, soybean oil, rice oil, olive oil, heated leek oil and 25 heated oil-macerated garlic oils were obtained from Fine Foods Co. (Osaka, Japan). Silica gel (60, spherical) for column chromatography and precoated kieselgel 60 on aluminum sheet, for thin-layer chromatography (TLC), were purchased from Merck (Germany). The solvents used for high performance liquid chromatography (HPLC) were of HPLC grade and all other chemicals were of analytical grade and purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Synthesis of standard ajoene

E- and *Z*-Ajoene were synthesised according to the method of Small, Balley, and Cavallito (1947) with a slight modification. In brief, 0.1 M diallyldisulfide dissolved in 150 ml chloroform (95%) was slowly added to 0.1 M of *m*-perchlorobenzoic acid in chloroform under N_2 bubbling.

The reaction mixture was allowed to stand for 30 min at 25 °C and extracted with 5% aqueous sodium hydrogen carbonate. After standing overnight at 25 °C, the *E*- and *Z*-ajoene were separated using silica gel column.

2.3. Preparation of garlic sample including E- and Z-ajoene

Fresh garlic: Japanese, Chinese and Bangladeshi garlic cloves were cut into 3–4 mm thick slices, and then ground to mix oil using SMT High-Flex disperser apparatus (SMT Co., Tokyo, Japan) mechanically driven at 700 rpm by a drill press. To minimise frictional heating of the sample during the grinding process, the tissue grinder was chilled prior to and during the grinding process with an ice bath. Garlic samples (Japanese, Chinese, Bangladeshi, frozen clove, frozen paste and fried garlic) were mixed with oil (0.25 kg/L) directly in the disperser apparatus. Then the mixture was stored at 40, 60, 80 and 100 °C for 4 h to allow complete ajoene formation. Ten ml samples were extracted

with ethyl acetate and analysed by HPLC. *E*- and *Z*-ajoene were separated using silica gel column chromatography. After washing the column with 200 ml of 40% ethyl acetate in *n*-hexane, the *Z*-ajoene was eluted with 100 ml of 50% ethyl acetate/*n*-hexane (1:1, v/v), whereas *E*-ajoene was eluted with 150 ml of 65% ethyl acetate and found to be over 95% pure. Commercial garlic oils were extracted with ethyl acetate and analysed by ^{1}H NMR spectroscopy, HPLC and TLC.

Tested garlic oil and leek oil: Heated 25 oil-macerated garlic oils (A to Q and R1 to X) and leek oil (R) were extracted with ethyl acetate and analysed by HPLC.

2.4. ^{1}H NMR spectral measurement

^{1}H NMR spectra were measured using a JEOL JNX-270 FT-NMR apparatus (Tokyo, Japan). Chemical shifts were recorded as ppm (δ) values using tetramethylsilane (TMS) as an internal standard in $CDCl_3$.

2.5. HPLC analysis

Extracted compounds were analysed by Si-HPLC using a LiChrospher Si 60 column (250 mm × 4.0 mm, Kanto Chemical Co., Inc., Tokyo). The eluent was *n*-hexane/2-propanol (85:15, v/v) at a flow rate of 1.0 ml/min and the eluate was monitored at 240 nm. The HPLC system consisted of a L-6200 intelligent pump, a L-4200 UV–vis detector and a D-2500 chromato-integrator (Hitachi Co., Ltd., Japan).

2.6. TLC analysis

TLC was developed with ethyl acetate as a solvent and each spot on the plate was detected by spraying colouring reagent (molybdo phosphoric acid/85% phosphoric acid/concentrated sulfuric acid/water, 2.4:1.5:5, v/v/v) and heated at 70 °C for 20 min.

2.7. Storage stability

Heated (80 °C) Japanese garlic with soybean oil was stored at −20 °C to determine its storage stability and ajoene concentration was analysed by HPLC after 3, 6 and 9 months period. The data were expressed as an average of three replications.

2.8. UV-light stability

Ajoene (0.1 mM) in ethyl acetate was incubated under UV-light (Distance of 50 cm; intensity: 253.7 nm, Toshiba GL15, Toshiba, Electronics Co., Tokyo, Japan) at room temperature for 3 days to determine its UV-light stability. Control samples (0.1 mM ajoene in ethyl acetate) were incubated in the dark at room temperature. The concentration of ajoene in samples was analysed by HPLC. Data were the means of three determinations.

2.9. Temperature stability

Ajoene (0.1 mM) in ethyl acetate were heated at 30, 40, 60, 80 and 100 °C for 3 days to check its temperature stability. The concentration of ajoene in samples was analysed by HPLC. Data were the means of three determinations.

2.10. Statistical analysis

The statistical analysis of the measured parameters was made using analysis of variance (ANOVA). Significant differences among samples were evaluated by Duncan's multiple-range test ($P < 0.05$) using SPSS software (v. 11.0, SPSS, Chicago, IL).

3. Results and discussion

3.1. ^{1}H NMR spectroscopy, TLC and HPLC analysis of ajoene

To identify the ajoene, structural elucidation was carried out by ^{1}H NMR spectroscopy. ^{1}H NMR ($CDCl_3$) δ 6.38 (dt, $j = 14.8$, 1H, 1 Hz, SSCH=$CHCH_2$), 5.98 (m, 3H), 5.4 (m, 2H), 5.2 (m, 2H), 3.5 (m, 4H), 3.36 (d, 2H, $J = 7.2$ Hz). These data were consistent with the structure (*E*)-4,5,9-trithiadodeca-1,6,11-triene 9-oxide.

^{1}H NMR ($CDCl_3$) δ 6.55 (dt, $j = 9$, 1H, 1 Hz, SSCH=CH), 5.8 (m, 3H), 5.4 (m, 2H), 5.2 (m, 2H), 3.5 (m, 4H), 3.38 (D, 2H, $J = 7.2$ Hz). This data was identified as *Z*-ajoene. This result agreed with the previous study (Block & Ahmad, 1984; Block et al., 1986).

The sample of heated garlic yielded two spots corresponding to *E*- and *Z*-ajoene compared with raw garlic which showed no spots (Fig. 1b). The spot at $R_f = 0.52$ of heated garlic corresponded to *Z*-ajoene and the other spot, at $R_f = 0.45$ corresponded to *E*-ajoene. This result agreed with the previous study (Lawson et al., 1991).

The samples, with or without heating at 80 °C, were applied on a HPLC column, as shown in Fig. 1c. Before incubation, the raw garlic sample did not contain any ajoene. Whereas, in the sample incubated at 80 °C for 4 h, *E*- and *Z*-ajene peaks were formed. Peak 1 with the elution time of about 11 min was found to correspond to *Z*-ajoene, whereas peak 2 with the elution time of 20 min corresponded to *E*-ajoene.

3.2. Effect of temperature on maximum ajoene recovery

Raw garlic did not contain ajoene but heated garlic contained ajoene. Incubation temperature is a very important factor for ajoene formation. Effect of the temperature (40, 60, 80, 100 °C) on ajoene formation was investigated on a garlic homogenate, to determine the real conditions for maximum ajoene recovery. The effect of incubation temperature on ajoene concentration is shown in Fig. 2a and b. When incubation temperature was at 40, 60 and 80 °C, the amount of ajoene concentration also increased

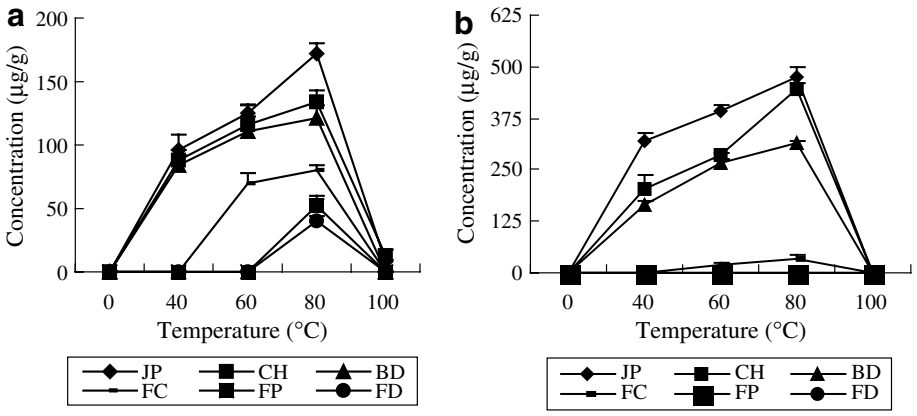

Fig. 2. Effect of temperature on ajoene formation and maximum recovery. (a) *E*-ajoene, (b) *Z*-ajoene in garlic homogenate with rice oil after 4 h incubation. JP, Japanese garlic; CH, Chinese garlic; BD, Bangladeshi garlic; FC, frozen clove; FP, frozen paste; FD, fried garlic. Error bars indicate standard deviations; where no error bars are visible, the standard deviation is less than the size of the symbol. Data were the means of three determinations.

gradually, with the highest amount of *E*- (172.0 μg/g of garlic) and *Z*- (476.3 μg/g of garlic) ajoene found in Japanese garlic with rice oil at 80 °C, indicating its optimal temperature. On the other hand, when the temperature was kept at 100 °C, the amount of ajoene decreased. Very small amounts of *E*-ajoenes (8.0 and 12.6 μg/g of garlic) were found from Japanese and Chinese garlic respectively at 100 °C, but no *Z*-ajoene. Higher temperature decreased the ajoene recovery. However, *Z*-ajoene was degraded at 100 °C, indicating that *Z*-isomer is more unstable to heat than the *E*-isomer. These results agreed with the previously reported study (Pesek & Warthesen, 1988). Ajoene is the degradation product of allicin. Allicin is a moderately unstable compound that can be readily transformed through self-reactions into a variety of oil-soluble thioallyl compounds when crushed garlic is processed (Lawson et al., 1991). Types of compounds formed depend on the temperature and polarity of the medium. Diallyl trisulfide and diallyl disulfide were the main products when crushed garlic was steam-distilled, while ring-structured vinyldithiins and oxygenated ajoene were the main products formed when it was incubated in a vegetable oil, at ambient temperature (Lawson & Wang, 2005).

3.3. Effect of different garlic samples on ajoene production

The ajoene concentration of Japanese garlic is different from Chinese, Bangladeshi, frozen clove, frozen paste and fried garlic as shown in Fig. 3a. This result showed that the amounts of *E*- and *Z*-ajoene in Japanese garlic were higher than Chinese, Bangladeshi, frozen clove, frozen paste and fried garlic with rice oil incubated at 80 °C for 4 h. The homogenate of fried garlic with rice oil showed the lowest amount of *E*-ajoene (40.0 μg/g of fried garlic) and no detectable amount of *Z*-ajoene. The effectiveness of alliinase enzyme was inhibited in fried garlic. These results agreed with the previous study (Lawson & Wang, 2005). Ajoene is derived from garlic through the conversion of alliin into allicin by an alliinase-induced reaction (Block et al., 1986). The lowest amount of *Z*-ajoene was found from frozen paste garlic. Perhaps long time storage in a freezer decreased the effectiveness of alliinase. Japanese garlic showed a higher ajoene concentration than Chinese and Bangladeshi garlic. Therefore, the amount of ajoene may depend on several factors, such as cultivars, soil, climate and clove maturation.

3.4. Storage stability

Fig. 3b shows the concentration of *E*- and *Z*-ajoene in heated (80 °C) Japanese garlic with soybean oil, stored at −20 °C for 9 months. The degradation of *E*- and *Z*-ajoene was dependent on the storage time. The amount of *Z*-ajoene (466.0 μg/g of garlic) was higher than *E*-ajoene (158.6 μg/g of garlic) in freshly prepared garlic oil. *Z*-ajoene concentration decreased rapidly during 9-month storage whereas *E*-ajoene concentration decreased more slowly than *Z*-ajoene. After 6-month storage, the amount of *E*-ajoene (94.3 μg/g of garlic) was higher than *Z*-ajoene (64.2 μg/g of garlic). This phenomena suggests that isomerisation of *Z*-ajoene to trans *E*-ajoene and some degradation occurred during the storage period.

3.5. UV-light and temperature stability of E- and Z-ajoene

The total concentrations of ajoene in all samples decreased after 3 days of incubation under UV-light as shown in Fig. 3c. The concentration of *Z*-ajoene decreased quickly to the remaining amount of 56.8% after 3 days. *E*-ajoene was fairly stable and amount remaining was 81.6%.

Furthermore, the degradation of *E*- and *Z*-ajoene depended on the temperature of incubation, when compared with those at 30 and 45 °C (Table 1). After incubation at 100 °C for 3 days, the concentration of *Z*-ajoene was about 0.5% whereas those at 30 and 40 °C were 41.2% and 30.1%, respectively. Therefore, during storage

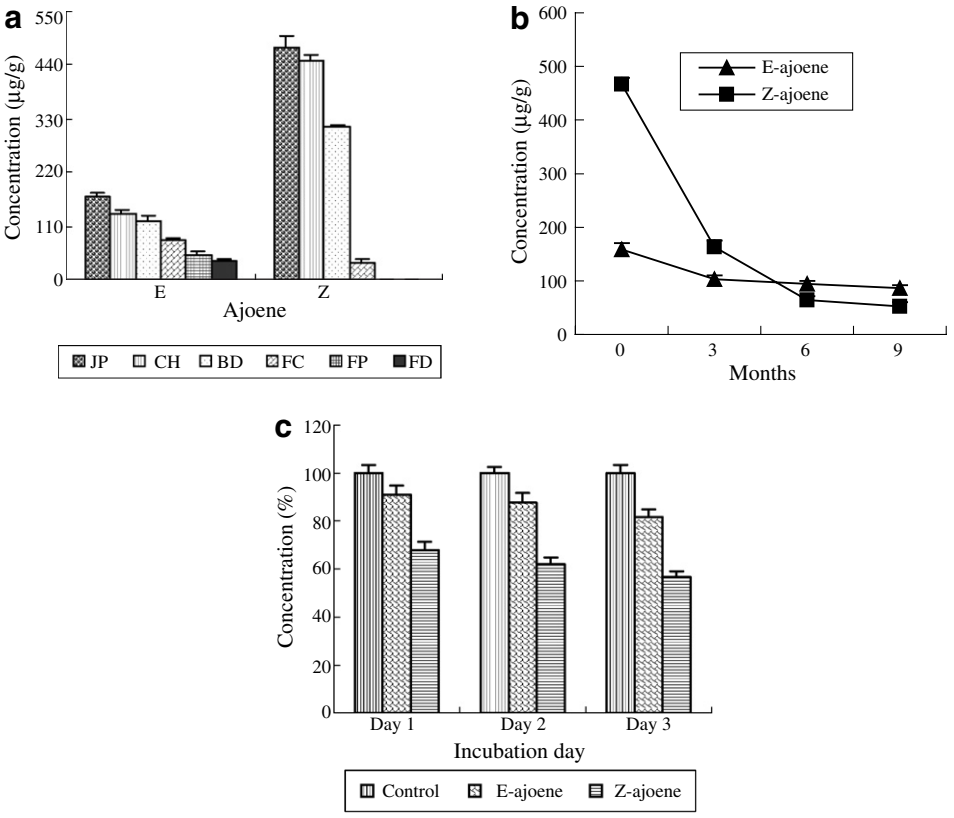

Fig. 3. (a) Effect of different varieties on ajoene concentration. Garlic homogenate with rice oil and heated at 80 °C for 4 h. JP, Japanese garlic; CH, Chinese garlic; BD, Bangladeshi garlic; FC, frozen clove; FP, frozen paste; FD, fried garlic. (b) Effect of storage time on ajoene concentration. Heated (80 °C) Japanese garlic oil (soybean) stored at −20 °C. Error bars indicate standard deviations. (c) Stability of *E*- and *Z*-ajoene toward UV-light. *E*- and *Z*-ajoene (0.1 M) in ethyl acetate were shaken under UV-light at room temperature for 3 days. Control samples were shaken in the dark at room temperature. Error bars indicate standard deviations. Data were the means of three determinations.

Table 1
Stability of *E*- and *Z*-ajoene toward different temperatures

Name	Temperature (°C)	Concentration (%) of ajoene after heating		
		1 day	2 days	3 days
E-ajoene	30	81.3e	76.5g	61.8g
	40	77.9d, e	71.5f, g	56.7g
	60	75.4c–e	67.5e, f	47.2f
	80	33.1b	17.3b	8.1b
	100	18.6a	10.0a	4.3a, b
Z-ajoene	30	73.4c, d	64.2d, e	41.2e
	40	71.4c	59.6d	30.1d
	60	70.2c	53.3c	19.9c
	80	29.3b	12.4a, b	5.2a, b
	100	15.1a	6.1a	0.5a

Ajoene isomers in ethyl acetate (0.1 mM/ml) were heated at 30, 40, 60, 80 and 100 °C for 3 days. Values followed by the same letters on the same column are not significantly different ($P < 0.05$) according to Duncan's multiple-range test. Data were the means of three determinations.

at higher temperature, the *E*- and *Z*-isomer isomerised easily, and then they gradually degraded to other components. These results agreed with the conclusions of a previous study (Tsukida & Saiki, 1983). Therefore, these phenomena should be taken into consideration when using *E*- and *Z*-ajoene as additives in food, or when storing them for a long period.

3.6. Effect of different oil solvents on ajoen production

Recovery of *E*- and *Z*-ajoene depending on different oil solvents are shown in Table 2. The amount of *E*- and *Z*-ajoene in rice oil was not significantly higher than soybean oil in Japanese, Chinese and Bangladeshi garlic. But rice oil was significantly higher than olive oil in Japanese garlic. The amount of *E*- and *Z*-ajoene in frozen clove extracts prepared using rice, soybean and olive oil were not significantly different. No detectable amount of *E*-ajoene was found in frozen paste and fried garlic homogenate with olive oil. *Z*-ajoene was not detected from frozen paste and fried garlic homogenate with rice, soybean and olive oil.

3.7. Amount of ajoene of various brands of garlic products

Table 3 shows the amount of *E*- and *Z*-ajoene in different kinds of garlic oils. Japanese garlic oil (fresh 15%, 80 °C, 3 h and fresh Japanese paste and cut 15%, 55 °C, 0.5 h) contained larger amount of *E*- and *Z*-ajoene than Chinese garlic oils (Chinese fresh Kongo 15%, 55 °C; Chinese fresh Souzan 15%, 55 °C, 0.5 h; frozen and defrosted Chinese 15%, 55 °C, 0.5 h; frozen Chinese 15%, 60 °C,

Table 2
The amount of *E*- and *Z*-ajoene depend on different oil solvents

Oil name	Garlic name	*E*-ajoene Concentration (μg/g garlic)	*Z*-ajoene Concentration (μg/g garlic)
Soybean	Japanese	158e	466g
Rice		172e	476g
Olive		119c, d	441f
Soybean	Chinese	129d	433f
Rice		134d	448f
Olive		116c, d	345d
Soybean	Bangladeshi	117c, d	304c
Rice		121c, d	313c
Olive		101b, c	225b
Soybean	Frozen clove	88.0b	40.3a
Rice		88.4b	36.3a
Olive		80.0b	32.0a
Soybean	Frozen paste	48.0a	nd
Rice		52.0a	nd
Olive		nd	nd
Soybean	Fried garlic	nd	nd
Rice		40.0a	nd
Olive		nd	nd

Garlic homogenate with oil was heated at 80 °C for 4 h. Values followed by the same letters on the same column are not significantly different ($P < 0.05$) according to Duncan's multiple-range test. Data were the means of three determinations. nd = not detected.

0.5 h and frozen Chinese 30%, 55 °C and 60 °C, 0.5 h). However, the amounts of *E*- and *Z*-ajoene varied greatly among the different heating temperatures, and concentrations. Chinese garlic oils (fresh 1% and frozen 9%, 0.5 h; fresh 15%, 0.5 h; fresh 15%, 5 h and fresh 15%, 15 h) heated at 95 °C did not contain any ajoene, perhaps due to thermal degradation. On the other hand, odourless frozen garlic oil (15%, at 55 °C, 0.5 h and 3 h), frozen Chinese garlic oil (15%, at 55 °C, 0.5 h) and fresh leek oil (15%, at 55 °C, 0.5 h) also did not contain any *E*- and *Z*-ajoene. Sulfur-containing compounds are typical components of garlic, so they are found in almost no other *Allium* vegetable (Itakura et al., 2001). From this reason, ajoene was not found in fresh leek. The highest amount of *E*-ajoene (170 μg/g of garlic) was obtained from Japanese garlic oil (fresh 15%, at 80 °C, 0.5 h), but this was not significantly different to Japanese garlic oil (fresh Japanese paste 30%, 55 °C, 3 h; fresh and not dry, 15%, at 55 °C, 3 h; fresh 15%, at 55 °C, 3 h; fresh 15%, at 80 °C, 3 h and fresh 15%, at 55 °C, 15 h), fresh Indian garlic oil (15%, at 55 °C, 0.5 h), fresh Japanese (15%, at 55 °C, 0.5 h) garlic oil, Chinese fresh Kongo (15%, at 55 °C, 0.5 h) garlic oil, Chinese fresh Souzan (15%, at 55 °C, 0.5 h) garlic oil, fresh Japanese pasete and cut (15%, at 55 °C, 0.5 h) garlic oil and frozen and defrosted chinese (15%, at 55 °C, 0.5 h) garlic oil. Whereas, *Z*-ajoene (127.0 μg/g of garlic) in Japanese garlic oil (fresh 15%, at 80 °C, 0.5 h) was present in a significantly higher amount than other kinds of oil, except fresh Indian garlic oil (125.0 μg/g of garlic).

Table 3
The amount of *E*- and *Z*-ajoene in different brands marketed garlic oils[a]

Sample number	Garlics name	*E*-ajoene	*Z*-ajoene
		Conc. (μg/g garlic or leek)	Conc. (μg/g garlic or leek)
Chinese garlic oil			
A	Fresh 1% and freezed 9% (95°C, 0.5 h)	nd	nd
B	Fresh 15% (95 °C, 0.5 h)	nd	nd
C	Fresh 15% (95 °C, 5 h)	nd	nd
D	Fresh 15% (95 °C, 15 h)	nd	nd
Japanese garlic oil			
E	Fresh and not dry 15% (55 °C, 3 h)	154 c–f	99.4g
F	Fresh 15% (55 °C, 3 h)	157d–f	89.8f, g
G	Fresh 15% (80 °C, 3 h)	153c–f	39.4b
H	Fresh 15% (80 °C, 0.5 h)	170f	127h
I	Fresh 15% (55 °C, 15 h)	157d–f	58.3c, d
Garlic oil			
J	Odorless freezing garlic 15% (55 °C, 0.5 h)	nd	nd
K	Odorless freezing garlic 15% (55 °C, 3 h)	nd	nd
L	Fresh Indian garlic 15% (55 °C, 0.5 h)	150c–f	125h
M	Freezing Chinese garlic 15% (55 °C, 0.5 h)	nd	nd
N	Fresh Japanese 15% (55 °C, 0.5 h)	162d–f	19.7a
O	Fresh Japanese with chili 15% (55 °C, 0.5 h)	137c, d	12.7a
P	Chinese fresh Kongo 15% (55 °C, 0.5 h)	152c–f	13.6a
Q	Chinese fresh Souzan 15% (55 °C, 0.5 h)	162d–f	72.3d, e
R	Fresh leek oil (55 °C, 0.5 h)	nd	nd
R1	Fresh Japanese 30% (55 °C, 0.5 h)	131c	82.1e, f
S	Fresh Japanese paste 30% (55 °C, 3 h)	168f	51.9b, c
S1	Fresh Japanese paste and cut 15% (55 °C, 0.5 h)	147c–f	102g
T	Freezing defrosting Chinese 15% (55 °C, 0.5 h)	164e, f	53.0b, c
U	Freezing Chinese 15% (60 °C, 0.5 h)	142c–e	67.5c–e
V	Freezing Chinese 30% (60 °C, 0.5 h)	141c–e	82.0e, f
W	Japanese purified oil (15%) (60 °C, 3 h)	57.6a	37.6b
X	Japanese purifed oil (15%) (55 °C, 3 h)	82.2b	88.8f, g

nd = not detected; oil samples were extracted with ethyl acetate and analysed by HPLC.

[a] Values followed by the same letters on the same column are not significantly different ($P < 0.05$) according to Duncan's multiple-range test. Data were the means of three determinations.

One interesting result of the analysis of various garlic oils was the finding that the supplied garlic oil contained 127.0 μg/g of *Z*-ajoene but freshly prepared Japanese garlic with rice oil contained *Z*-ajoene 476.0 μg/g of garlic. These results indicate that, the *Z* (*Cis*)-isomer is more unstable than the *E* (*trans*)-isomer (Pesek & Warthesen, 1988). Some

degradation might occur during processing and storage time.

Fresh preparation of garlic oil from garlic (Japanese, Chinese and Bangladeshi) contained higher amount of *E*-ajoene than *Z*-ajoene. But processed garlic, like frozen clove and various garlic oils, contained a higher amount of *E*-ajoene than *Z*-ajoene. *E*-ajoene was always found to be dominant over *Z*-ajoene, usually about twice the amount. Fresh preparations of oil-macerated garlic yielded exclusively *Z*-isomer but it gradually isomerised to give the *E*-isomer (Lawson et al., 1991).

4. Conclusion

Maximum recovery of ajoene was obtained from incubating at 80 °C for 4 h. Japanese garlic with rice oil was obtained a higher amount of ajoene than other varieties. The remaining amounts of *E*- and *Z*-ajoene were 54.2% and 11.2%, respectively, after 9-month storage. Ajoene was more stable against UV-light than temperature, while *E*-ajoene was more stable than *Z*-ajoene against temperature, UV-light and storage conditions. These characteristics indicate that *E*-ajoene was considered to have more advantages than *Z*-ajoene, when they are used as food additives. The present study immensely helps when ajoene is used in a wide range of food.

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