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Influence of the Isolation Procedure on Coriander Leaf Volatiles with Some Correlation to the Enzymatic Activity

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Coriander leaves (*Coriandrum sativum* L.) have become popular worldwide because of their pleasant and delicate aroma. By a hot water extraction method, in which coriander leaves were cut before suspending in boiling water for 2 min, the contents of the main volatile compounds such as alkanals and 2-alkenals from C10 to C14 decreased, while the levels of corresponding alcohols increased in comparison to those obtained by solvent extraction. To investigate the reasons for this variation, an enzyme activity was assayed. By using aliphatic aldehyde as a substrate and NADPH as a coenzyme, strong activity of an aliphatic aldehyde reductase was found for the first time in this herb in the relatively wide pH range of 5.0−9.0, with the maximum activity at pH 8.5. Additionally, the aliphatic aldehyde dehydrogenase, responsible for acid formation, was also found to have a relatively weak activity compared to that of reductase.

KEYWORDS: Coriander volatile; 2-alkenal; aliphatic aldehyde reductase; *Coriandrum sativum*; aliphatic aldehyde dehydrogenase

INTRODUCTION

The coriander plant (*Coriandrum sativum* L.), a member of the Umbelliferae family, provides a distinctive culinary herb (leaves and root) usually associated with the Latin American and Asian food, and a spice (fruit or seed) used throughout the world in both sweet and savory cooking. Coriander seeds have a bit of a spicy, citrus flavor and are available in the spice aisle of most markets. The leaves, also commonly known as Chinese parsley, are variously referred to as "coriander leaves" in Britain, "cilantro" in the United States, and "rau mui" in Vietnam. Coriander leaves have a very different taste from the seeds; they are similar to parsley but "juicier" and with citrus-like overtones and a definitely strong and pungent aroma.

Previous reports demonstrate the considerable confusion that exits over the volatile composition of coriander herb. Mac-Leod and Islam (*1*) have employed simultaneous steam distillation solvent extraction (SDE) for the isolation of the essential oil of coriander herb that was purchased from a local market in London, identified 17 volatile compounds, most of which were aliphatic aldehydes in the C_8-C_{15} range with 7-dodecenal (21%) as the major component, and did not detect any 2-alkenals. However, 7-dodecenal was not identified by Potter and Fagerson (*2*), who have also studied SDE for the isolation of fresh coriander leaves from Massachusetts (USA) and reported (*E*)-2-decenal (46%) as predominant. On the other hand, Lawrence (*3*) has reported that alkanals and (*E*)-2-alkenals were the major constituents of coriander plants during ontogenesis. Similar results were reported by Mookherjee et al. (*4*). Elsewhere, the detection of 2-alkenals in the $C_{10}-C_{14}$ range was reported in γ-irradiated fresh coriander leaves grown in Pennsylvania (USA) during cold storage by Fan et al. (*5*). They used the solid-phase microextraction (SPME) technique to extract volatile compounds isolated by homogenizing and boiling coriander leaves. They have reported that decanal (51.51%) and (*E*)-2-decenal (31.61%) were the most abundant compounds in this herb. In addition, they have also reported a homologous series of alkanals, related primary alkenols, and alkanols. In other research, Smallfield et al. (*6*) have also studied the composition of coriander oil isolated by steam distillation and solvent extraction. The plants used in their study were immediately subjected after being harvested at Canterbury (New Zealand). A series of alkanals and alkenals were identified, and (*E*)-2-decenal was found as the major component in the essential oil. Similarly, Cadwallader et al. (*7*, *8*) used a cold direct solvent extraction method and reported that decanal (22%), (*E*)-2-decenal (17.4%), (*E*)-2-dodecenal (14.6%), and (*E*)-2-tetradecenal (18.1%) were in the highest abundance in the oil composition of the fresh coriander herb. From these previous reports, although it is clear that alkanals and 2-alkenals are major constituents, the change in the relative levels of these aldehydes due to extraction methods or enzyme activities has not been addressed. In another study, Smallfield et al. (*6*) have reported that the relative levels of aldehydes decreased during storage of chopped coriander, while levels of the corresponding alcohols increased, and suggested it might have been due to the presence of a nonspecific oxidoreductase. However, we could not find any references to oxidoreductase for this plant.

Aldehyde reductases are pyridine nucleotide dependent oxidoreductases, catalyzing the reduction of a variety of aromatic and medium chain aliphatic aldehydes to their corresponding

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alcohols. The occurrence of this enzyme in *Euonymus* leaves (*9*) and in several plant species (*10*) is well documented. We also reported the presence of an aliphatic aldehyde reductase in our previous study (*11*) on Vietnamese coriander leaves (*Persicaria odorata* Lour.), which has influence on the changes of alcohols related to corresponding aldehydes. This herb was used in a comparatively limited area, while coriander has been widely used throughout the world. So it is necessary to investigate the presence of an enzymatic system, if any, in coriander leaves. Although coriander belongs to the same group named "cilantro mimics" as Vietnamese coriander, it differs in the scientific and generic name. It can therefore be presumed that an oxidoreductase exists in coriander herb and its activity has influence on the change of volatile components, which was reported to be different from those of Vietnamese coriander. For all of these reasons, instead of research on the volatile composition, which has been studied in previously, the present study was focused first on investigation of the influence of the aroma extraction procedures, which were similar to the ways coriander herb is eaten or cooked, on coriander leaf volatiles. Second, the enzymatic system in coriander was identified and characterized. These obtained results are also expected to indicate the similarities or differences between coriander and Vietnamese coriander, although these herbs are commonly named "cilantro mimics".

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially from Wako Pure Chemical Industries (Osaka, Japan): (*Z*)-2-hexenol, 2-ethylhexanol, alkanols from C_8 to C_{13}, (*E*)-2-alkenols from C10 to C14, alkanals from C10 to C12, (*E*)-2-decenal, (*E*)-2-undecenal, (*E*)-2-dodecenal, decanoic acid, dodecanoic acid, hydrocarbons from C6 to C26, γ-terpinene, linalool, γ-terpinolene, β-caryophyllene, carbitol, (*E*)-nerolidol, 2-phenylethyl alcohol, eugenol, indole, vanillin, NAD, NADP, NADH, NADPH, all buffers, and DMSO. Heptanal, heptanol, *p*-hydroxymercurybenzoate, and polyvinylpolypyrrolidone were purchased from Aldrich Chemicals (USA), and ethyl nonanoate from Tokyo Kasei Kogyo (Tokyo, Japan). Alkanals and alkenals from C13 to C16 were gifted by T. Hasegawa Co., Ltd. (Tokyo, Japan).

All buffers were adjusted to the desired pH value at room temperature. NADH/NADPH and NAD/NADP were dissolved in purified water at specified concentrations and prepared immediately prior to use. Water-insoluble chemicals were dissolved in absolute dimethyl sulfoxide (DMSO), and dilutions were made in water thereafter. The presence of DMSO in the reaction mixture showed no effect on the enzyme activity.

Plant Material. Coriander leaves grown in Shizuoka Prefecture were purchased in a local market in Tokyo, Japan in September 2008.

Isolation of the Aroma Concentrate. Isolation of aroma concentrates from fresh coriander leaves (10 g) was conducted by solvent extraction and hot water extraction methods according to the procedure described in the previous paper for Vietnamese coriander (*11*) as follows. The procedures were repeated three times.

Solvent Extraction. Fresh leaves (10 g) were immersed in liquid nitrogen. After the frozen contents had been ground into a fine powder with a mortar and pestle, organic compounds were extracted by methanol three times. The obtained extract was adjusted to a 10% methanol aqueous solution with purified water, before being subjected to chromatography in a column packed with 20 g of Porapak Q resin (Supelco, 50/80 mesh). After the water-soluble compounds had been removed with 200 mL of purified water, the absorbed compounds were eluted with 200 mL of a mixture of pentane and diethyl ether (1:1). After desorption, 3 μg of ethyl nonanoate in diethyl ether was added as an internal standard (IS). The eluate was dried over anhydrous sodium sulfate and filtered, and the solvent was evaporated at 39.5 °C at atmospheric pressure to obtain the aroma concentrate. The volatile compounds were then concentrated in a nitrogen stream to 20 μL just before injecting for the GC or GC−MS analysis.

Hot Water Extraction. Fresh leaves (10 g) were cut to about 1 cm in length, suspended in boiling water for 2 min, and filtered. The filtrate was cooled to room temperature and then subjected to chromatography in a column packed with 20 g of Porapak Q resin. The aroma concentrate was obtained in the same way as that just described.

Preparation of Acetone Powder from Fresh Leaves. Acetone powder was prepared by following the procedure described in a previous paper (*11*), in which all steps were conducted in a cold room at 2−4 °C. Coriander leaves (120 g) frozen in liquid nitrogen were milled and dipped in cold acetone (−15 °C). After being homogenized for 1 min by a blender, the acetone layer was removed by filtration. This process was conducted eight times, and the residue was dried under reduced pressure. The yield of resulting acetone powder was 7.62 g, and this was kept at −80 °C until needed.

Preparation of the Crude Enzyme Solution. A crude enzyme solution was prepared from the acetone powder of coriander leaves. Each acetone powder sample (1 g) was homogenized twice for 30 s with 30 mL of a 100 mM Tris-HCl buffer (pH 8.0, 4 °C) containing 10% glycerol, 5 mM dithioerythritol, 2% polyvinylpolypyrrolidone, and 5 mM sodium hydrosulfite. The slurry was centrifuged twice at 13,000 rpm (20 min, 4 °C), and the resulting supernatant was used in the assay for enzyme activity after being appropriately diluted.

Aliphatic Aldehyde Reductase Assay. In this experiment, the aliphatic aldehyde was used as a substrate, and the formation of the corresponding alcohol was recorded and quantitatively identified. The standard assay contained, in a total volume of 1 mL, 400 μL of a 250 mM Na-phosphate or a glycine-NaOH buffer (pH ranges from 5.0 to 10.0), 200 μL of purified water, 100 μL of 10 mM NADH or NADPH, 250 μL of the enzyme solution, and 50 μL of 20 mM heptanal. Unless otherwise specified, the enzyme was incubated with NADH or NADPH for 2 min prior to adding the substrate to start the reaction. This solution was incubated at 37 °C for 1 h, and the enzyme reaction was stopped by adding sodium chloride at a concentration of 15%, followed by putting in an ice bath. The reason for putting salt in the reaction mixture is to easily extract the volatiles from an organic solvent. After ethyl nonanoate had been added as an internal standard, the generated alcohols and remaining aldehydes were extracted with pentane/diethyl ether (6:4, 1 mL), and this extract was subjected to a GC analysis. A control was prepared by using a boiled enzyme solution.

The reverse reaction, i.e., the hydrogenation of alcohols to their aldehydes, was assayed at pH ranges from 5.0 to 10.0 in Na-phosphate or a glycine-NaOH buffer containing NAD/NADP and heptanol as a substrate. The formation of heptanal was recorded and quantitatively identified.

Aliphatic Aldehyde Dehydrogenase Assay. The same enzyme assay as that just described was performed at different pH values but with the addition of NAD or NADP instead of NADH or NADPH. Heptanal was used as a substrate, and the formation of heptanoic acid was recorded and quantitatively identified. The enzyme reaction was stopped by adding sodium chloride, followed by placement in an ice bath. After acidification, solvent extraction was performed in the same way as just described. A control was prepared by using a boiled enzyme solution. The reverse reaction was also assayed.

GC and GC−MS Analyses. GC analyses were performed with an Agilent GC 6890 instrument equipped with a flame-ionization detector (FID). Helium was used as the carrier gas at a flow rate of 1.8 mL/min. The capillary column was 60 m × 0.25 mm i.d. and 0.25 μm film thickness coated with DB-WAX (J&W Scientific, USA). The oven temperature for identifying the volatile composition of the essential oil was held at 60 °C for 4 min and then increased to 220 °C at a rate of 2 °C/min. The oven temperature for the enzyme experiments was programmed from 80 to 220 °C at a rate of 4 °C/min. The injector and detector temperatures were set at 200 and 220 °C, respectively. GC−MS analyses were performed with an Agilent-MSD-5973 mass selective detector and scanned in a m/z range of 30−400 mass units. The GC analytical conditions were the same as those just stated. Each compound was identified by the agreement of its Kovats' GC retention index (KI) and mass spectrum with that of the authentic compound.

Quantitative Analysis. The volatile components in the aroma concentrate of the herb as well as the reactants and the products of the enzymatic reactions were quantitatively analyzed. The absolute concentration of each of these components was determined by the ratio of the

Table 1. Volatile Compounds Identified in the Aroma Concentrate of Coriander Isolated by the Different Extraction Methods[a]

			solvent extraction		hot water extraction	
KI[b]	compound	identification	area[c] %	IS ratio[d]	area[c] %	IS ratio[d]
902	nonane	KI, MS	0.93	0.39	0.97	0.13
1007	decane[e]	KI, MS	0.03	0.01	0.56	0.07
1096	undecane	KI, MS	0.07	0.03	1.20	0.16
1227	dodecane[e]	KI, MS	0.05	0.02	0.12	0.02
1253	γ-terpinene[e]	KI, MS	0.24	0.10		
1329	tridecane[e]	KI, MS	0.07	0.03	0.12	0.02
1386	(*Z*)-3-hexenol[e]	KI, MS			5.66	0.76
1492	2-ethylhexanol	KI, MS	0.27	0.11	0.25	0.03
1497	decanal	KI, MS	3.73	1.56	9.84	1.31
1518	linalool	KI, MS	0.13	0.05	1.17	0.16
1553	γ-terpinolene[e]	KI, MS	0.05	0.02		
1597	β-caryophyllene	KI, MS	0.05	0.02		
1599	phenylacetaldehyde	KI, MS	0.05	0.02	0.18	0.02
1602	undecanal	KI, MS	0.24	0.10	0.57	0.08
1623	carbitol[e]	KI, MS	0.05	0.02	0.17	0.02
1631	(*E*)-2-decenal	KI, MS	3.64	1.52	9.14	1.22
1663	nonanol[e]	KI, MS			0.04	0.01
1708	dodecanal	KI, MS	0.65	0.27	0.55	0.07
1715	(*E*)-2-undecenal	KI, MS	0.80	0.33	1.21	0.16
1765	decanol	KI, MS	0.13	0.05	3.18	0.42
1810	tridecanal	KI, MS	0.27	0.11	0.79	0.11
1820	(*Z*)-2-dodecenal[f]	MS	0.43	0.18	2.68	0.36
1822	(*E*)-2-dodecenal	KI, MS	13.47	5.62	21.85	2.92
1835	(*E*)-2-decenol	KI, MS			0.09	0.01
1883	undecanol	KI, MS	0.11	0.04	0.30	0.04
1896	2-phenylethyl alcohol[e]	KI, MS			0.59	0.08
1904	(*Z*)-2-tridecenal[f]	MS	0.09	0.04	0.23	0.03
1908	(*E*)-2-undecenol	KI, MS			0.17	0.02
1910	tetradecanal	KI, MS	0.25	0.10	0.97	0.13
1920	nonadecane[e]	KI, MS	0.05	0.02	0.13	0.02
1929	(*E*)-2-tridecenal	KI, MS	4.21	1.75	1.37	0.18
1969	dodecanol	KI, MS	0.27	0.11	0.97	0.13
1984	(*E*)-2-dodecenol	KI, MS			0.13	0.02
1987	pentadecanal	KI, MS	0.86	0.36	0.07	0.21
2036	(*E*)-2-tetradecenal	KI, MS	52.60	21.94	26.41	3.53
2088	hexadecanal	KI, MS	0.05	0.02	0.13	0.02
2142	(*E*)-2-pentadecenal	KI, MS	10.04	4.19	2.42	0.32
2162	eugenol[e]	KI, MS			0.56	0.07
2169	tridecanol[e]	KI, MS			0.23	0.03
2208	(*E*)-nerolidol[e]	KI, MS	0.03	0.01	0.30	0.04
2249	(*E*)-2-hexadecenal	KI, MS	3.64	1.52	2.00	0.27
2272	decanoic acid	KI, MS	0.19	0.08	0.56	0.07
2386	indole[e]	KI, MS	0.11	0.05	0.04	0.01
2486	dodecanoic acid	KI, MS	0.42	0.17	1.20	0.16
2555	vanillin[e]	KI, MS	0.34	0.14	0.17	0.02
	total		**98.61**	**41.1**	**99.29**	**13.46**

[a] Identification was performed on the basis of a comparison with reference compounds using the following criteria: retention index and mass spectrum. [b] KI, Kovats index on DB-WAX column. [c] Peak area on GC, average of relative percentage of total peak area, $n = 3$. [d] GC peak area ratio of each component to the internal standard, $n = 3$. [e] Newly identified compound in the coriander herb aroma composition by agreement of the mass spectrum and the KI index with those of the authentic compound. [f] Tentatively identified only by the mass spectrum.

peak area % of the compound to the internal standard (ethyl nonanoate) and by calibration curves of authentic compounds by the GC analysis. The calibration curves were prepared by using a mixture of each authentic sample and the internal standard.

RESULTS AND DISCUSSION

Volatile Profiles Resulting from Different Extraction Methods. The coriander aroma concentrates isolated by solvent extraction and hot water extraction methods were studied by GC analyses. The former method was used to minimize enzyme decomposition of volatiles during tissue disruption and to obtain the aroma of coriander leaves similar to that when eaten fresh. In Asia, coriander leaves are often best used raw or to add additional flavor to hot soups and curries, so the latter method, hot water extraction, was used to obtain the aroma of coriander leaves during cooking by boiling the leaves for 2 min. Agreement of the mass spectra and KI retention indices with those of authentic compounds enabled 45 compounds to be identified as the volatiles in the aroma concentrate of coriander herbs. The peak area % of each compound and its IS ratio are expressed in **Table 1**.

By applying the solvent extraction method, straight-chained aldehydes were the most abundant compounds of this fresh herb (94.97%), where 2-alkenals accounted for the major proportions. In this aroma concentrate, alcohol was the minor group, accounting for 0.78% of total peak area. These findings agree with those of some previous studies (*1*−*8*) in which a series of 2-alkenals and saturated aldehydes were the predominant essential oil components of the fresh herb.

By employing hot water extraction method, the aldehyde group (80.41%) was also found to make up most of the volatile compounds of coriander sample. However, the quantity of this group that is expressed by absolute content was almost one-third that of the one obtained by solvent extraction method (**Table 2**). On the other hand, when hot water extraction was applied, alcohol was the second major group, increasing over 3 times compared to the one detected as applying the other method as shown in **Table 2**.

The above results show that although the different extraction methods were applied, the coriander samples composed mainly of aliphatic aldehydes, where 2-alkenals accounted for the major proportions. It is obvious that the different levels between the content of aldehyde and alcohol group obtained by these two extraction method; for instance, the aldehyde content seemed to be replaced by the corresponding alcohols in the hot water extract. This supported the suggestion of Smallfield et al. (*6*) and our assumption about the presence of an oxidoreductase in this plant, which is responsible for the changes of the aldehyde group to the corresponding alcohols. To confirm this suggestion, enzyme assays were conducted with the crude enzyme isolated from coriander leaves.

Aliphatic Aldehyde Reductase Activity in Coriander Leaves. An aliphatic aldehyde reductase activity was examined by incubating the crude enzyme solution isolated from acetone powder prepared from coriander fresh leaves in different buffers at various values of pH with the addition of NAD(P)H as a coenzyme. The use of aldehydes, which occurred in the crude enzyme extracted directly from leaves, e.g., C8 to C12 saturated aldehydes, as a substrate in the enzymatic reaction probably caused some confusion in calculating the amounts of reactants and reaction products. For this reason, heptanal, which was absent from the volatile components of coriander, was used. The enzyme activity with the addition of NADH or NADPH as a cofactor was assayed by recording the decrease of heptanal and the corresponding increase of heptanol.

The temperature sensitivity of this enzyme activity was preliminarily investigated by incubating the crude enzyme extract at 25 and 37 °C for 1 h in a glycine-NaOH buffer. However, the effect of temperature was not significant under this experimental condition. Therefore, the following assays were conducted at 37 °C in order to obtain the higher activity of this enzyme activity.

Effect of pH. In this experiment, the enzyme activities were investigated at a range of various pH values from 5.0 to 10.0. The effects of pH on the reduction of heptanal in the two different buffer systems of Na-phosphate and a glycine-NaOH are expressed in **Figure 1**. With the addition of NADPH in the reaction

Table 2. Main Odor Characteristic Compounds related to the Changes in Aroma Composition of Coriander Leaf

compound	calibration curve[a]	solvent extraction content[b] (μg)	hot water extraction content[b] (μg)
		Aliphatic alcohols	
nonanol	$y = 1.3137x - 0.1650$ $R^2 = 0.9998$		3.99 ± 0.34
decanol	$y = 1.4999x - 0.3470$ $R^2 = 0.9976$	7.94 ± 0.35	15.34 ± 0.87
undecanol	$y = 1.2998x - 0.1617$ $R^2 = 0.9998$	4.66 ± 0.32	4.66 ± 0.52
dodecanol	$y = 1.1355x - 0.0981$ $R^2 = 0.9923$	5.50 ± 0.43	6.03 ± 0.33
tridecanol	$y = 1.1263x - 0.1851$ $R^2 = 0.9989$		5.73 ± 0.53
(*Z*)-3-hexenol	$y = 0.9842x - 0.0992$ $R^2 = 0.9944$		26.19 ± 2.17
2-ethylhexanol	$y = 1.2618x - 0.1266$ $R^2 = 0.9944$	5.63 ± 0.38	3.72 ± 0.87
(*E*)-2-decenol	$y = 1.3420x - 0.0614$ $R^2 = 0.9981$		1.60 ± 0.01
(*E*)-2-undecenol	$y = 1.4696x - 0.0595$ $R^2 = 0.9979$		1.62 ± 0.03
(*E*)-2-dodecenol	$y = 1.1758x - 0.0505$ $R^2 = 0.9979$		1.80 ± 0.07
total		**23.73**	**70.68**
		Aliphatic aldehydes	
decanal	$y = 0.9615x - 0.4107$ $R^2 = 0.9985$	61.49 ± 2.32	53.69 ± 2.63
undecanal	$y = 0.8698x - 0.0167$ $R^2 = 0.9999$	4.03 ± 0.45	3.34 ± 0.67
dodecanal	$y = 0.9633x - 0.5203$ $R^2 = 0.9962$	24.61 ± 2.67	18.38 ± 3.34
tridecanal	$y = 0.6285x - 0.2130$ $R^2 = 0.9995$	15.42 ± 1.33	15.42 ± 3.32
tetradecanal	$y = 0.2154x - 0.1166$ $R^2 = 0.9998$	30.17 ± 1.76	34.34 ± 3.05
pentadecanal	$y = 0.3701x - 0.1124$ $R^2 = 0.9928$	38.29 ± 2.15	26.13 ± 2.45
hexadecanal	$y = 0.3672x - 0.1063$ $R^2 = 0.9998$	10.32 ± 0.63	10.32 ± 0.97
(*E*)-2-decenal	$y = 0.8627x - 0.2945$ $R^2 = 0.9962$	63.10 ± 3.35	52.67 ± 3.57
(*E*)-2-undecenal	$y = 0.6180x - 0.0787$ $R^2 = 0.9949$	19.84 ± 1.46	11.59 ± 0.78
(*Z*)-2-dodecenal[c]		16.56 ± 0.69[c]	22.44 ± 1.06[c]
(*E*)-2-dodecenal	$y = 0.9191x - 0.3274$ $R^2 = 0.996$	194.13 ± 3.67	106.00 ± 3.73
(*Z*)-2-tridecenal[d]		8.81 ± 0.13[d]	8.51 ± 1.15[d]
(*E*)-2-tridecenal	$y = 1.0071x - 0.2556$ $R^2 = 0.9863$	59.74 ± 3.18	12.98 ± 1.07
(*E*)-2-tetradecenal	$y = 1.3524x - 0.2847$ $R^2 = 0.9904$	493.01 ± 5.67	84.62 ± 2.58
(*E*)-2-pentadecenal	$y = 0.8103x - 0.0908$ $R^2 = 0.9934$	158.49 ± 3.67	15.21 ± 2.78
(*E*)-2-hexadecenal	$y = 0.9202x - 0.2795$ $R^2 = 0.9994$	40.03 ± 3.13	12.30 ± 2.06
total		**1,238.04**	**487.94**

[a] *y*: Peak area ratio of authentic chemical to internal standard detected by FID detector. *x*: Ratio of absolute concentration of authentic chemical to internal standard on DB-WAX column. [b] Content in 100 g of fresh herb, calculated by using calibration curves for authentic samples. [c] Contents were calculated by using calibration curves for an authentic sample of (*E*)-2 dodecenal. [d] Contents were calculated by using calibration curves for an authentic sample of (*E*)-2-tridecenal.

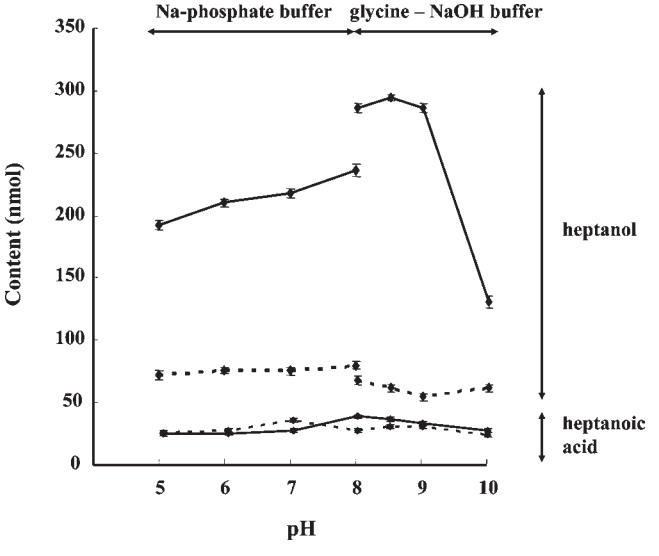

Figure 1. Effect of pH on the changes of heptanol and heptanoic acid contents in the crude enzyme solution with NADH/NADPH. The enzyme assay used heptanal as a substrate. The assay mixture (1 mL) contained a 250 mM Na-phosphate or glycine-NaOH buffer (pH range from 5.0 to 10.0), purified water, 10 mM NADH/NADPH, the crude enzyme, and 20 mM heptanal to start the reaction. The mixture was incubated at 37° C for 1 h. Each value is shown as the mean, $n = 2$. (—) Addition of NADPH; (- - -) addition of NADH.

mixture, the strong activity was observed even in neutral conditions. However, in the glycine-NaOH buffer at pH 8.0 to 9.0, the crude enzyme from coriander leaves was activated the most strongly, and the maximum activity was obtained at pH 8.5. The enzyme activity decreased rapidly at pH 10.0. The content of heptanol formed with the addition of NADPH as the coenzyme was much more than that with NADH, independent of the buffer used in the assay. In this experiment, small amounts of heptanoic acid were also detected and quantitatively identified by the GC analysis. This was probably due to the presence of another enzyme system related to the acid formation in the crude enzyme solution.

Following these experiments, the reverse reaction, related to the hydrogenation of alcohols to their corresponding aldehydes, was conducted according to the aliphatic alcohol dehydrogenase assay. The crude enzyme was incubated in Na-phosphate or a glycine-NaOH over a range of pH 5.0 to 10.0 with 20 mM heptanol as the substrate and 10 mM of NAD/NADP was used as a cofactor. Under this experimental condition, the result of GC analysis showed that no significant activity could be detected when NADP was added, and heptanal formed was only observed in trace with the addition of NAD. This suggests that the aliphatic alcohol dehydrogenase seemed to have an extremely weak activity for this plant, and might be not responsible for the differences in aldehydes/alcohol contents in the aroma concentrates of coriander leaves.

Some other studies have found that alcohol dehydrogenase was activated in alkaline conditions at pH 8.0−10.0 (*12*−*15*). On the

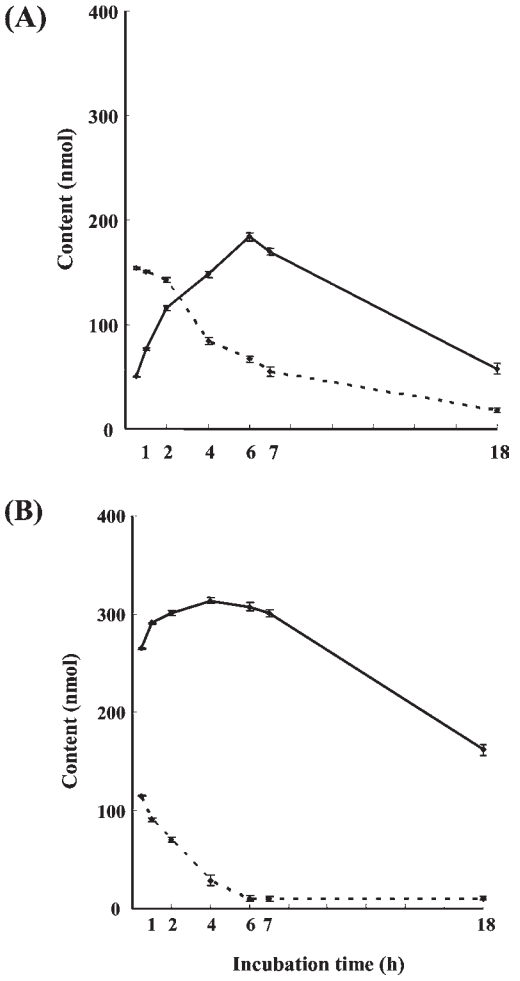

Figure 2. Changes in heptanal and heptanol contents with Incubation time in the crude enzyme solution with NADH (A) and NADPH (B). The enzyme assay was conducted at 37° C using heptanal as a substrate. The assay mixture (1 mL) contained a 250 mM glycine-NaOH buffer (pH 8.5), purified water, 10 mM NADH/NADPH, the crude enzyme, and 20 mM heptanal to start the reaction. Each value is shown as the mean, $n = 2$. (- - -) Heptanal; (—) heptanol.

other hand, some reductases have been reported to be activated in a low pH range from 5.0 to 7.0 in many studies (*16*−*22*). However, in this study, the enzyme of coriander leaves was strongly activated in an alkaline condition with the maximum activity obtained at pH 8.5. A similar response to the pH of the assay mixture has been reported for aldehyde reductase from *Euonymus japonica* leaves (*9*) with the cofactor of NADPH. Additionally, in our previous study reported on the activity of the enzyme system isolated from Vietnamese coriander leaves (*11*), the aliphatic aldehyde reductase from this herb was strongly activated at pH 8.0−9.0 in a glycine-NaOH buffer when NADH was used as a coenzyme, with the highest value of formed heptanol (about 170 nmol) at pH 9.0. However, unlike from Vietnamese coriander enzyme described in this previous study, the enzyme from coriander leaves was found to be more strongly activated with the addition of NADPH than with NADH. The maximum content of heptanol of approximately 300 nmol obtained from coriander was almost twice that from Vietnamese coriander. Even the minimum level of heptanol (198 nmol) isolated from coriander crude enzyme extract at pH 5.0 was higher than the maximum value of that obtained from Vietnamese coriander at its optimum pH. This suggests the tendency of the stronger activity of coriander enzyme.

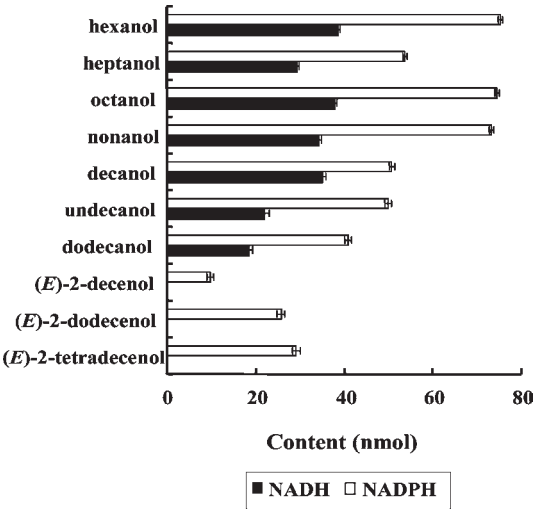

Figure 3. Corresponding alcohols in the enzyme reaction mixtures generated from various aliphatic aldehydes. The assay mixture (1 mL) contained a 250 mM glycine-NaOH buffer (pH 8.5), purified water, 2 mM NADH/NADPH, the crude enzyme, and 2 mM of each aldehyde as a substrate to start the reaction. The mixture was incubated at 37° C for 1 h. Each value is shown as the mean, $n = 2$.

Effect of Incubation Time. To clarify the relationship between the volatile alcohols formed from the corresponding aldehydes during the reaction time, experiments for measuring generated alcohols were carried out with heptanal as a substrate at the optimum pH of 8.5. The changes in heptanal and heptanol contents in the enzyme reaction mixtures with NADH or NADPH as the coenzyme after 0.5, 1, 2, 4, 6, 7, and 18 h of incubation of the reaction solution are shown in **Figure 2A** and **B**. The formation of heptanol was observed when either NADH or NADPH was added, and its quantity was the highest after 4−6 h of incubation. After that time, the heptanol content decreased. It has been also concluded that this enzyme seemed to be more strongly activated with the addition of NADPH than with NADH when heptanal was used as the substrate.

Substrate Specificity. The enzyme activity acting on various aliphatic aldehydes was investigated, and the generated corresponding alcohols were identified and quantitatively analyzed by GC analysis. In these experiments, C6 to C12 saturated and C10, C12, and C14 unsaturated aldehydes, which occurred in the aroma concentrates extracted directly from coriander leaves as the major components, were used as a substrate instead of heptanal. It was difficult to dissolve a long-chain aldehyde at high concentration in DMSO, so the concentration of 2 mM was chosen to investigate the substrate specificity of this enzyme in the reaction mixtures. The crude enzyme with NADH/NADPH was incubated in the glycine-NaOH buffer at 37 °C, pH 8.5 for 1 h, and the result is expressed in **Figure 3**. This enzyme showed broad substrate specificity. The GC analysis results indicated that in almost cases of using these aldehydes as a substrate, their corresponding alcohols were generated when adding NADH or NADPH. It means that aliphatic aldehydes were found to be good substrates for coriander enzyme, but with significantly differing rates of oxidation. Especially, the affinity of this enzyme for the unsaturated aldehydes was rather weak compared to that of saturated aldehydes when either NADH or NADPH was added. However, the enzyme activity with adding NADPH as a coenzyme was remarkably stronger than that with NADH applied to all experimented substrates. The generation of corresponding alcohols was not clearly observed when C10, C12, and C14 unsaturated aldehydes with NADH were used. This might be

Table 3. Effect of Inhibitors on the Activity of Aliphatic Aldehyde Reductases from Coriander Leaves[a]

inhibitor	concentration (mM)	relative activity (%)
none		100
1,10-phenanthroline (NADH)	5	30
1,10-phenanthroline (NADH)	10	25
1,10-phenanthroline (NADPH)	5	17
1,10-phenanthroline (NADPH)	10	10
EDTA (NADH)	10	28
EDTA (NADPH)	10	4
p-hydroxymercuribenzoate (NADH)	5	0
p-hydroxymercuribenzoate (NADPH)	5	0

[a] The reaction mixture contanied, in 1 mL: 250 mM glycine-NaOH, purified water, 10 mM NADH/NADPH, the crude enzyme, inhibitor, and 20 mM heptanal to start the reaction. The mixture was incubated at 37° C for 1 h.

explained by the high hydrophobicity of long-chain aldehydes (> 10 carbons), followed by their low reactivity in the enzyme reaction mixtures, accompanied by weak affinity of the activated reductase for these aldehydes. The obtained results also suggest the strong activity of aliphatic aldehyde reductase in the coriander leaves.

Effect of Inhibitors. The crude enzyme from coriander leaves and NADH or NADPH were incubated for 2 min prior to adding the substrate to test its inhibitory effect with some inhibitors at different concentrations (**Table 3**). Assays were conducted at 37 °C, pH 8.5 for 1 h.

Inhibition of this aliphatic aldehyde reductase was measured in the presence of 1,10-phenanthroline and EDTA, known as inhibitors of alcohol dehydrogenase. 1,10-Phenanthroline inhibited this aldehyde reductase 75% or 90% at a concentration of 10 mM when NADH or NADPH was used as a cofactor, respectively. EDTA on the other hand gave 72% or 96% inhibition at concentrations of 10 mM for the enzyme activity of coriander when NADH or NADPH was added, respectively. Remarkably, this aliphatic aldehyde reductase enzyme was completely inhibited by *p*-hydroxymercuribenzoate at 5 mM when either NADH or NADPH was added. Our findings agree well with those of Negm (*9*), who has reported that *p*-hydroxymercuribenzoate was a strong inhibitor of aldehyde reductase from *Euonymus* leaves. In addition, the results of our previous study (*11*) also confirmed that this inhibitor at a small amount inhibited completely the activity of the aliphatic aldehyde reductase from Vietnamese coriander leaves. These results also distinguish the coriander enzyme from aliphatic alcohol dehydrogenase and help to confirm the presence of aliphatic aldehyde reductase in this plant.

Aliphatic Aldehyde Dehydrogenase Activity in Coriander Leaves. In the above experiments on the aliphatic aldehyde reductase, small amount of heptanoic acid was detected in the product mixtures of the enzyme reaction. This was probably due to the presence of aldehyde dehydrogenase in the crude enzyme solution. To examine this suggestion, an aliphatic aldehyde dehydrogenase assay was conducted by using heptanal as a substrate and NAD or NADP as a coenzyme, and the results are shown in **Figure 4**. The formation of heptanoic acid was apparent in the wide range of pH. However, this enzyme was found to have weak activity when compared to that of reductase. This explained the fact that the difference in acid formation was not apparent when the different extraction methods were applied (**Table 1**). Besides the formation of a low level of heptanoic acid, the formed heptanol was also apparent in the reaction mixtures. This observation implies a formation of NADH/NADPH from NAD/NADP in the products of the original enzymatic reaction. So with its presence as a new coenzyme in the reaction mixture, a part of heptanal was transformed to heptanol by the action of

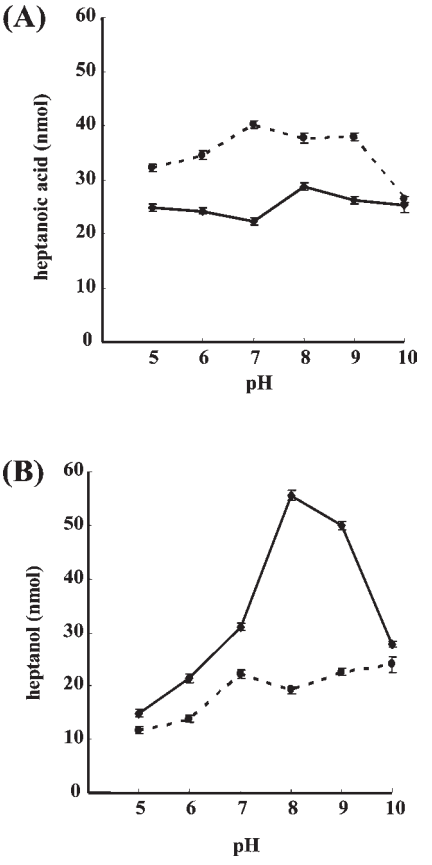

Figure 4. Effect of pH on the changes of heptanoic acid (A) and heptanol contents (B) in the crude enzyme solution with NAD/NADP. The enzyme assay used heptanal as a substrate. The assay mixture (1 mL) contained a 250 mM Na-phosphate or glycine-NaOH buffer (pH from 5.0 to 10.0), purified water, 10 mM NAD/NADP, the crude enzyme, and 20 mM heptanal to start the reaction. The mixture was incubated at 37° C for 1 h. Each value is shown as the mean, $n = 2$. (—) Addition of NADP; (- - -) addition of NAD.

aliphatic aldehyde dehydrogenase that already existed in this coriander crude enzyme.

The reverse reaction, i.e., the dehydrogenation of acids to their corresponding aldehydes, was also assayed in Na-phosphate or a glycine-NaOH over a range of pH 5.0 to 10.0 with 20 mM heptanoic acid as the substrate. No significant activity was detected under this experimental condition when either NADH or NADPH was added.

These above results clearly confirm the presence of aliphatic aldehyde reductase with a relatively strong activity for the first time in coriander leaves. The enzyme was found to be activated in the wide range pH from 5.0 to 9.0 with the maximum activity obtained at pH 8.5. Additionally, the presence of aliphatic aldehyde dehydrogenase, which is responsible for acidic forms from aldehydes, was also found to have weak activity compared to that of reductase. This enzyme, which is also the first to be reported in coriander leaves, was activated even in the neutral condition, an ordinary pH of many dishes. Therefore, as coriander leaves were cut and left free for a long time, these two enzymes were activated, causing the content of the strong odor aldehydes to decrease, accompanied by the increase of levels of weak odor volatiles such as alcohols or acids. This suggests the important influence of food processing on the flavor quality of this herb.

In other research (*6*), it is reported that the relative levels of aldehydes decreased during storage of chopped coriander, whereas levels of the corresponding alcohols increased, and

suggested that it might have been due to the presence of a nonspecific oxidoreductase. In our study, the crude enzyme system being detected in coriander leaves was first clarified and characterized.

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Note Added after ASAP Publication

Due to a production error, there were errors in the text in the version of this paper published ASAP November 16, 2009; the corrected version published ASAP December 18, 2009.

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