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Aliphatic Aldehyde Reductase Activity Related to the Formation of Volatile Alcohols in Vietnamese Coriander Leaves

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Vietnamese coriander (*Persicaria odorata* Lour.) belongs to a group known as cilantro mimics with the 'cilantro' flavor, in which C10 and C12 aldehydes and alcohols have been found as the potent odor compounds. Their composition isolated by different extraction methods varied. The enzyme activity was assayed, and the reductase acting on some aliphatic aldehydes with NADH/NADPH as a coenzyme was found in a crude enzymatic system of fresh leaves. The maximum activity was observed at pH 8.0 in Na-phosphate and at pH 8.5 to 9.0 in a glycine-NaOH buffer, using heptanal as a substrate. The activated reductase that caused the alcohol generation to increase after a time was inhibited by *p*-hydroxymercuribenzoate. Our results, which are the first to be reported on Vietnamese coriander leaves, reveal the presence of aliphatic aldehyde dehydrogenase, which is responsible for acid formation, and elucidate the strong activity of the aliphatic aldehyde reductase.

Key words: Vietnamese coriander; aliphatic aldehyde reductase; *Persicaria odorata*; *Polygonum odoratum*; aliphatic aldehyde dehydrogenase

Vietnamese coriander (*Persicaria odorata* Lour., syn. *Polygonum odoratum* Lour., Polygonaceae) belongs to a group of fresh culinary herbs collectively known as the cilantro mimics with the 'cilantro' flavor consisting of coriander (*Coriandrum sativum* L.) and long coriander (*Eryngium foetidum* L.). This herb has been widely used in the same manner by many cultures at various times. There are many common names for this plant in different countries. English names include Vietnamese cilantro, Vietnamese mint and Vietnamese coriander. In Vietnamese, it is "rau răm," while in Singapore and Malaysia, it is called daun kesom or laksa plant. Vietnamese coriander possesses a strong green, citrus peel, and coriander leaf odor. It is also one of those numerous herbs that give Vietnamese cuisine its unique touch and is often used interchangeably with cilantro herb or mint in Southeast Asian cuisine. It is commonly eaten fresh in salads, meat dishes, some hot soups such as "canh chua," and stews. In Singapore and Malaysia, the shredded leaf is an essential ingredient of *laksa*, a spicy soup.

The aroma profile of Vietnamese coriander and the composition of the volatile compounds have been reported differently, depending on the place of harvest, procedure and analytical techniques used to isolate the volatile constituents.[1–5] Dung *et al.*[1] have studied the composition of this Vietnamese herb oil isolated by steam distillation and identified 28 volatile components, of which β-caryophyllene (36.5%), dodecanal (11.4%) and caryophyllene oxide (8.2%) were the main ones. Hunter *et al.*[2] have also employed steam distillation for the isolation of the Australian herb oil constituents and identified 17 volatile compounds, with dodecanal (44.1%) and decanal (27.7%) as the major components. Additionally, Cadwallader *et al.*[3] used a cold direct solvent extraction method and reported that dodecanal (27.5%) and decanal (23.2%) were in highest abundance, accounting for over 50% of the volatile composition.

Vietnamese coriander leaves are often best used raw, as the flavour vanishes after prolonged cooking, or to add additional flavour to hot soups and curries. For this reason, we studied the volatile compositions of this essential oil isolated by three different methods similar to the ways this herb is eaten or cooked; namely water extraction, hot water extraction, and cold direct solvent extraction. The later method was used to obtain the aroma of Vietnamese coriander similar to that when eaten fresh. The GC analyses show that the sample preparation and extraction method have a dramatic effect on the extract composition and variability of results. The increasing alcohol content seemed to be related to a decrease of the corresponding aldehyde content. The release of an enzymatic system in this herb probably caused this effect.

The formation of volatile alcohols by oxidoreductase activity has been reviewed in several plant species by Schreier.[6] Smallfield *et al.*[7] have reported that the aldehyde content rapidly decreased with an increase in corresponding alcohols for chopped cilantro and they suggested this might have been due to the presence of a nonspecific oxidoreductase. This phenomenon was observed in Vietnamese coriander when isolating the essential oil of this herb by the above mentioned different sample preparation and extraction methods. However, we could not find any references to oxidoreductase, or alcohol/aldehyde dehydrogenase, for this plant. The purpose of this present study is therefore to clarify the aroma composition resulting from different extrac-

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tion methods, and to examine the enzymatic activity in relation to the alcohol formation in Vietnamese coriander. In addition, we also investigated the relationship between the quantity of alcohol formed and the change in enzyme activity with time to clarify the mechanism for the formation of an alcohol group in Vietnamese coriander leaves.

Materials and Methods

Chemicals. Most of the chemicals listed in Table 1 and in the enzyme-related experiments were purchased from Wako Pure Chemical Industries (Osaka, Japan), except for heptanal, heptanol, *p*-hydroxymercurybenzoate, and polyvinylpolypyrrolidone which were from Aldrich Chem. (USA), and ethyl nonanoate from Tokyo Kasei Kogyo (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 certain 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. The Vietnamese coriander leaves used in the experiments for isolation of the aroma concentrate and in the enzyme-related experiments were harvested on a farm and immediately exported from Hanoi, Vietnam in July 2006 and in August 2008, respectively, and botanically authenticated at the National Center for Scientific Research Technology, Vietnam. The herb was stored under cool conditions during transportation, and kept at 4 °C until it was used. The plant was handled within 4 d after being harvested.

Isolation of the aroma concentrate.

Water extraction. Fresh leaves (10 g) were cut into about 1 cm in length and suspended in purified water (with 5% methanol). The suspension was stood overnight (18 h) at 4 °C, and then filtered through a nylon cloth. The filtrate was 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 into 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.

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, methanol was added. The mixture was filtered, and the filtrate 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. The aroma concentrate was obtained in the same way as that just described.

Examination of the contents of aldehydes and alcohols in the homogenized leaves. Fresh leaves (0.5 g) with 2 ml of a 100 mM glycine-NaOH buffer at pH 9.0 or Na-phosphate buffer at pH 5.7 were homogenized for 1 min by a homogenizer. Ten mM NADH or NADPH was added, and the mixture was briefly vortexed and incubated at 25 °C for 30, 60, or 90 min. After incubation, 1 ml of pentane/diethyl ether (8:2) with a certain amount of an internal standard (ethyl nonanoate) was added to the tube, and the mixture was briefly vortexed, and centrifuged to separate the phases. The solvent layer was directly placed into a GC vial for GC and GC-MS analyses.

Preparation of the crude enzyme.

Preparation of acetone powder from fresh leaves. Acetone powder was prepared by following the procedure described by Sekiwa *et al.*[8] All steps were conducted in a cold room at 2–4 °C. Leaves (80 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 6.1 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 Vietnamese 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. The standard assay contained, in a total volume of 1 ml, 400 µl of a 250 mM glycine-NaOH buffer (pH 9.0), 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 (pH 9.0) was incubated at 25 °C for 0.5, 1, 2, 4, 6, or 18 h, and the enzyme reaction was stopped by adding sodium chloride, before putting in an ice bath. 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.

Aliphatic aldehyde dehydrogenase assay. In this experiment, 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, before putting in an ice bath. After acidification, solvent extraction was performed in the same way as that just described for the aliphatic aldehyde reductase assay. A control was prepared by using a boiled enzyme solution.

Aliphatic alcohol dehydrogenase assay. To determine the enzyme activity, 250 µl of the crude enzyme solution was incubated at 25 °C for one hour in 400 µl of a 250 mM Na-phosphate buffer (pH from 5.0 to 7.0) or glycine-NaOH buffer (pH from 8.0 to 10.0), 200 µl of purified water, 100 µl of 10 mM NAD or NADP, and 50 µl of 20 mM heptanol. The formation of heptanal was recorded and quantitatively identified. The enzyme reaction was stopped by adding sodium chloride, before putting in an ice bath. Solvent extraction was performed in the same way as that just described for the aliphatic aldehyde reductase assay. A control was prepared by using a boiled enzyme solution.

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 the rate 2 °C/min. The oven temperature for the enzyme experiments was programmed from 80 °C to 220 °C at a rate 4 °C/min. The injector and detector temperatures were set at 200 °C and 220 °C, respectively. GC-MS analyses were performed with an Agilent-MSD-5973 mass selective detector in the full scan mode. GC conditions were the same as those used for the GC analysis. Each compound was identified by the agreement of its Kovats' GC retention index (KI) and mass spectrum with that of the authentic compound.

GC-olfactometry (GC-O). The flavor dilution (FD) factors of the potent odor compounds in the aroma concentrate were determined by

an aroma extract dilution analysis (AEDA) as described by Guth and Grosch.[9] The original aroma concentrate was diluted stepwise 4-fold by volume with diethyl ether and then subjected to GC-olfactometry. The dilution was continued until the sniffers could not detect any significant odor in a run. Analyses were performed by two trained assessors in duplicate. The highest dilution at which an individual component could be detected is defined as the flavor dilution (FD) factor for that odorant. The GC-O conditions were the same as those described for the GC and GC-MS analyses, with the exception of the column size (60 m × 0.53 mm i.d., and 1 μm film thickness) and the flow rate of 8.3 ml/min of helium carrier gas. The effluent was split into equal parts at the end of the column, each part being respectively conveyed to an FID and sniffing port.

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

Results and Discussion

Profile of the aroma concentrate

Each aroma concentrate isolated by the three extraction methods reproduced the characteristic odor of Vietnamese coriander well. Agreement of the mass spectra and KI retention indices with those of authentic compounds enabled 61 compounds to be identified as the volatiles in the aroma concentrate of Vietnamese coriander. The concentration of each compound and its peak area % are expressed in Table 1.

Qualitatively, the Vietnamese coriander samples varied with different proportions of odor compounds in each aroma group. By employing solvent extraction and hot water extraction, aliphatic aldehydes were the most abundant compounds of this fresh herb (86.71% and 44.43%, respectively), where decanal and dodecanal accounted for the major proportions. These findings are in agreement with those of some studies in which aliphatic straight-chain aldehydes were the predominant essential oil components of this fresh herb.[1–5] However, the content of dodecanal isolated by water extraction and hot water extraction was relatively low compared to that prepared by solvent extraction. This was probably due to the solubility of dodecanal. Aliphatic alcohols were the second major group of these two samples, accounting for 9.18% and 28.71%, followed by acids at 1.51% and 16.52%, respectively. However, the water extract was markedly different from the others, with aliphatic alcohols (38.55%) being the most abundant compounds, with decanol as the major component (16.38% of total area), followed by the acidic group (23.43%), aliphatic aldehyde group (3.57%), and ester group (3.15%). It was obvious that there were some differences in the contents of aldehyde, alcohol and acid when these extraction methods were applied; for instance, the aldehyde content seemed to be replaced by the corresponding alcohols or acids in the water extract. The flavor dilution (FD) factors of potent odor compounds in the aroma concentrate were determined by an aroma extract dilution analysis (AEDA). Despite giving the lowest content of the aldehyde group among the three extracts, the AEDA results obtained from the water extract showed that such saturated aldehydes as decanal and dodecanal were most likely the principal contributors to Vietnamese coriander's characteristic aroma with the highest FD factor (256), followed by undecanal and the aliphatic C_6 aldehydes (64). Additionally, the alcohol group represented by (*Z*)-3-hexenol, decanol, and dodecanol also contributed to the overall aroma of this herb with a relatively high FD factor of 16. These compounds may contribute to the characteristic odor of Vietnamese coriander: the sweet, floral and citrus odor present in the green odor. On the other hand, in spite of the high abundance in the aroma concentrate isolated by water extraction, acids were detected by AEDA with low FD factors ($\leqq$4), so these make a minor contribution to the overall aroma of this herb compared to that of the aliphatical aldehyde and alcohol groups. For these reasons, the differences and formation of aldehydes, alcohols, and acids in these aroma concentrates played an important role in determining the flavor quality of this herb. Elucidation of this point is one of the critical objectives in our study. These differences were probably due to the release of an enzyme which changed the aldehydes to the corresponding alcohols or acids. We assume that aldehyde reductase or other enzymes exist in this plant. To check this assumption, the differences in the contents of decanal-related compounds in the aroma concentrate were measured by using homogenized leaves.

Differences in the contents of aldehydes and alcohols in the homogenized sample

The fresh herb was homogenized in several buffers with the addition of NADH or NADPH and incubated at 25 °C in order to investigate the differences in the contents of aldehydes and alcohols. The amounts of each compound were calculated by the ratio of the peak area and the response factor compared to those of the internal standard and by the calibration curves of authentic samples by the GC analysis. The differences in content of C10 and C12 aldehydes and alcohols after incubating for different times are shown in Fig. 1A and B. These differences were not clearly detectable with the Na-phosphate buffer at pH 5.7 for short times (data not shown). However, in the glycine-NaOH buffer at pH 9.0, these differences were significant with the addition of either NADH or NADPH. In this experiment, the decanal and dodecanal levels dropped rapidly, especially within 30 min after the enzymatic reaction had started, accompanied by a substantial increase of the corresponding alcohols. Additionally, these differences could be more clearly observed by the addition of NADH as a coenzyme than by NADPH. These results strongly suggest the presence of an enzyme system related to the change of the aldehydes to the corresponding alcohols in this plant. To confirm this observation, enzyme assays were conducted with the crude enzyme isolated from Vietnamese coriander leaves.

Enzyme activity

In this experiment, the enzyme activities were investigated at various pH values in the crude enzyme solution isolated from acetone powder prepared from these fresh leaves. The use of aldehydes, which occurred in the crude enzyme extracted directly from leaves such

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

KI[a]	Compound	Solvent extraction		Water extraction		Hot water extraction	
		Area[b] %	Content[c] (μg)	Area[b] %	Content[c] (μg)	Area[b] %	Content[c] (μg)
	Aliphatic alcohols						
1163	1-penten-3-ol[d*]			0.14	4.38	0.06	4.11
1209	3-methylbutanol[d*]			0.13	4.57		
1322	(*Z*)-2-pentenol[d*]			0.16	4.67		
1383	(*E*)-3-hexenol[d*]			0.03	3.33		
1386	(*Z*)-3-hexenol[*]			7.59	96.30	0.46	8.78
1393	(*E*)-2-hexenol[d*]			0.11	5.03	0.10	5.87
1408	(*Z*)-2-hexenol[d†1]			0.32	7.55		
1453	1-octen-3-ol[d*]			0.35	5.87		
1492	2-ethylhexanol[d*]	0.41	6.10	0.48	7.53		
1502	2-octanol[d†2]			0.18	5.55		
1357	hexanol[d*]			0.47	8.95		
1561	octanol[d*]			0.11	4.83	0.08	5.31
1663	nonanol[d*]			0.06	4.45	0.20	5.59
1765	decanol[*]	3.73	31.14	16.38	139.15	20.77	179.17
1883	undecanol[*]	1.56	15.50	1.26	15.50	0.31	6.73
1969	dodecanol[*]	3.48	32.71	3.31	37.99	4.38	50.57
2169	tetradecanol[d*]			0.74	58.02	0.38	7.83
1466	6-methyl-5-hepten-2-ol[d†3]			6.73	49.09	1.97	16.77
	total	**9.18**		**38.55**		**28.71**	
	Aliphatic aldehydes						
1083	hexanal[*]	0.25	12.48	0.05	10.30	0.13	11.39
1497	decanal[*]	27.00	288.01	1.58	32.78	38.60	512.58
1602	undecanal[*]	1.83	21.28	0.30	4.72	0.91	13.54
1708	dodecanal[*]	57.55	602.00	0.52	22.74	4.14	69.58
1910	tetradecanal[*]	0.08	20.42	0.15	24.60	0.46	43.05
1143	(*Z*)-3-hexenal[*]			0.24	21.62	0.08	13.92
1220	(*E*)-2-hexenal[*]			0.73	20.61	0.11	12.38
	total	**86.71**		**3.57**		**44.43**	
	Acids						
1975	(*Z*)-2-hexenoic acid[d†4]			0.17	12.38	0.49	15.25
1849	hexanoic acid[d*]			0.92	12.75		
2061	octanoic acid[d*]			2.22	26.81		
2272	decanoic acid[d*]	0.32	9.47	15.07	129.29	7.26	66.73
2379	undecanoic acid[d*]			0.68	11.61	0.30	8.16
2486	dodecanoic acid[d*]	1.19	28.04	4.37	57.78	8.47	108.80
	total	**1.51**		**23.43**		**16.52**	
	Esters						
816	ethyl acetate[d*]			0.18	4.39	0.76	4.19
886	methyl butanoate[d*]	0.18	4.19	0.19	4.59		
932	methyl 2-methylbutanoate[d*]	0.05	10.30	0.09	11.02	0.48	21.20
1187	methyl hexanoate[d†5]			0.28	16.25		
1390	methyl octanoate[d†5]			0.54	19.68		
1594	methyl decanoate[d†5]			0.57	19.99		
1800	methyl dodecanoate[d†5]			0.41	18.12		
2230	methyl hexadecanoate[d†5]			0.46	27.17	0.47	27.48
1619	methyl benzoate[d*]			0.15	3.55		
2067	methyl cinnamate[d*]			0.28	4.63		
	total	**0.23**		**3.15**		**1.71**	
	Terpenoids						
1597	β-caryophyllene[*]	0.35	4.92	0.09	3.05	1.57	12.75
1673	α-humulene[†6]	0.27	3.98	0.26	4.14		
1985	β-bisabolene[d*]	0.14	10.35	1.34	54.15	0.38	19.74
2242	γ-elemene[*]			0.22	27.09	0.22	50.05
1623	carbitol[d*]	0.10	4.10	0.52	10.92	0.59	10.17
1673	nerol[d*]			0.13	3.62	0.19	4.19
1796	geraniol[d*]			1.08	9.84	0.29	4.84
2208	(*E*)-nerolidol[d*]	0.17	4.73				
	total	**1.03**		**3.64**		**3.24**	
	Others						
1096	undecane[*]	0.95	9.36	0.28	4.93	0.10	9.78
1339	6-methyl-5-hepten-2-one[*]			0.50	6.26	0.12	4.21
1673	4-hexanolide[d*]			0.42	7.30	0.14	5.24
1868	benzyl alcohol[*]			1.70	15.02	0.29	3.73
1896	2-phenylethyl alcohol[*]			0.41	5.26	0.29	7.43
1980	caryophyllene oxide[†6]	0.37	5.07	4.73	32.38		
2132	4-decanolide[d*]			4.65	33.79	0.10	3.15
2162	eugenol[*]			0.19	3.86		
2341	chavicol[d*]			0.80	10.90		
2373	4-vinylphenol[d*]					0.77	88.92
2386	indole[d*]					0.91	9.53
2555	vanillin[d*]			0.29	6.85	0.17	14.47
	total	**1.32**		**13.97**		**2.89**	

[a]KI, Kovats index on DB-WAX.

[b]Peak area % on GC.

[c]Contents in 100 g of fresh herb.

[d]Newly identified compound in the Vietnamese coriander herb aroma composition by agreement of the mass spectrum and KI index with those of the authentic compound.

[*]Contents were calculated by using calibration curves for authentic samples.

[†]Contents were calculated by using calibration curves for the following authentic sample: [†1], (*E*)-2-hexenol; [†2], octanol; [†3], 6-methyl-5-hepten-2-one; [†4], (*E*)-2-hexenal; [†5], ethyl nonanoate; [†6], β-caryophyllene.

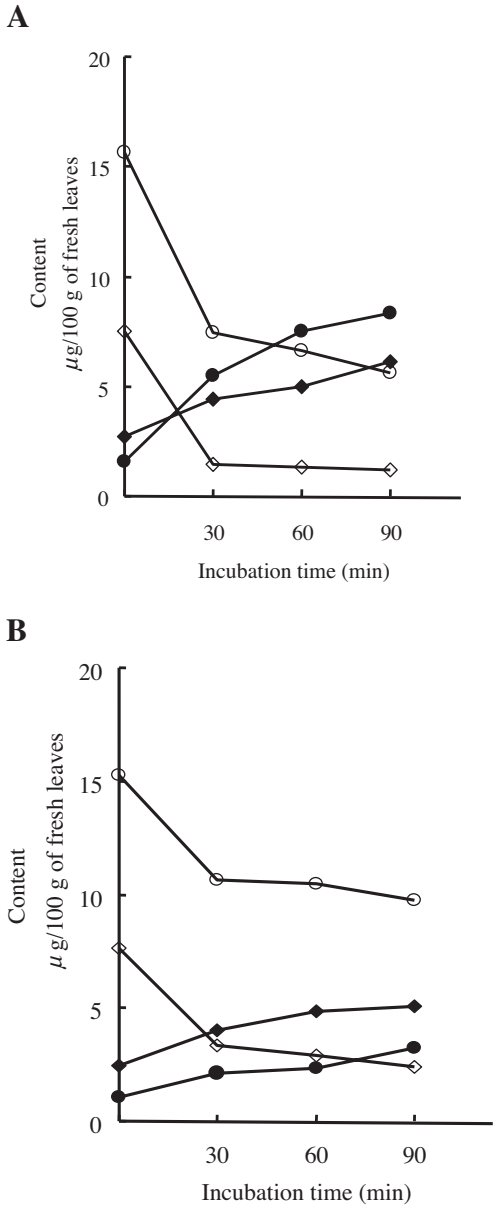

Fig. 1. Changes in the Contents of C10 and C12 Aldehydes and Alcohols in Homogenized Leaves with NADH (A) and NADPH (B).
The reactions were conducted in a glycine-NaOH buffer (pH 9.0) at 25 °C. Each value is shown as the mean, n = 2. Symbols: ◇, decanal; ◆, decanol; ○, dodecanal; ●, dodecanol.

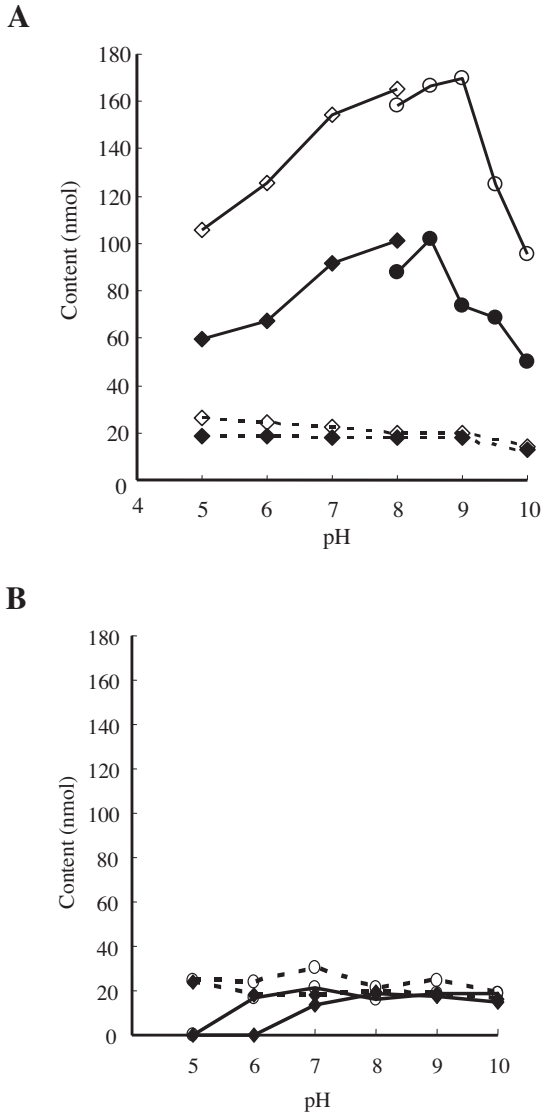

Fig. 2. Effect of pH on the Changes of Heptanol and Heptanoic Acid Contents in the Crude Enzyme Solution.
A, With the addition of NADH/NADPH. The enzyme assay used heptanal as a substrate. The assay mixture (1 ml) contained a 250 mM Na-phosphate or glycine-NaOH buffer (pH range from 5.0 to 10.0), purified water, 10 mM NADH/NADPH, the crude enzyme, and 20 mM heptanal to start the reaction. Each value is shown as the mean, n = 2. —, heptanol; ----, heptanoic acid; Symbols: ◇, Na-phosphate (NADH); ○, glycine-NaOH (NADH); ◆, Na-phosphate (NADPH); ●, glycine-NaOH (NADPH). B, With the addition of 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. Each value is shown as the mean, n = 2. —, heptanol; ----, heptanoic acid; Symbols: ○, NAD; ◆, NADP.

as 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. Furthermore, it was difficult to use a long-chain aldehyde as a substrate because it was not completely soluble in DMSO. For these reasons, heptanal, which was absent from the volatile components in the aroma concentrate of Vietnamese 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 effects of pH on the reduction of heptanal in the two different buffer systems of Na-phosphate (pH range from 5.0 to 8.0) and glycine-NaOH (pH range from 8.0 to 10.0) are expressed in Fig. 2A. The optimum pH range depended on the buffer and the coenzyme used in the assay. Maximum activity was observed at pH 8.0 in the Na-phosphate buffer with either NADH or NADPH, and at pH 8.5–9.0 or 8.5 in the glycine-NaOH buffer with NADH or NADPH, respectively. In this experiment, small amounts of heptanoic acid was also detected and quantitatively identified by the GC analysis. This was probably due to the presence of aldehyde dehydrogenase in the crude enzyme solution.

To examine this effect, an aliphatic aldehyde dehydrogenase assay was conducted by using heptanal as a substrate and NAD or NADP as a coenzyme, and the

Table 2. Effect of Inhibitors on Vietnamese Coriander Aliphatic Aldehyde Reductase.

The reaction mixture contained, in 1 ml: 250 mM glycine-NaOH (pH 9.0), purified water, 10 mM NADH or NADPH, the crude enzyme, inhibitor, and 20 mM heptanal to start the reaction.

Inhibitor	Concentration (mM)	Relative activity (%)
None		100
1,10-phenanthroline (NADH)	5	84
1,10-phenanthroline (NADH)	10	32
1,10-phenanthroline (NADPH)	5	75
1,10-phenanthroline (NADPH)	10	37
p-hydroxymercuribenzoate (NADH)	10	0
p-hydroxymercuribenzoate (NADPH)	10	5
p-hydroxymercuribenzoate (NADPH)	15	0

results are shown in Fig. 2B. The formation of heptanoic acid was apparent in the wide range of pH. This explains why the large amount of the acidic group was present in the aroma concentrate of Vietnamese coriander isolated by water extraction, in which the plant was immersed in water with 5% soluble methanol at 4 °C for 18 h (pH 5.9). However, in the foregoing experiment on the homogenized herb, the formation of the acidic group could not be significantly detected by the GC analysis. This was probably because the aliphatic aldehyde dehydrogenase was not completely activated in a homogenized mixture during the reaction time. Moreover, this enzyme was found to have weak activity when compared to that of reductase. Besides the formation of a low level of heptanoic acid, the appearance of heptanol was also observed in the reaction mixtures. This suggests interaction between the products and coenzyme in this enzymatic reaction. Further work is in progress to elucidate this observation.

Effect of incubation time

In order 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 pH 9.0. 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, and 18 h of incubating the reaction solution at pH 9.0 are shown in Fig. 3A and B. The formation of heptanol was observed when either NADH or NADPH was added, and its quantities increased with increasing incubation time. The total amount generated after 18 h of incubation was about 4 times that after 30 min of incubation, and the final content of heptanol formed with the addition of NADH as the coenzyme was much more than twice that with NADPH. These results suggest that Vietnamese coriander leaves contained aliphatic aldehyde reductase which plays an important role in the formation and difference of alcohols and aldehydes in the volatile composition of this herb.

Following these experiments, an aliphatic alcohol dehydrogenase assay was conducted in Na-phosphate or glycine-NaOH over a range of pH 5.0 to 10.0 with 20 mM heptanol as the substrate. The result shows that no significant activity could be detected under this experimental condition when either NAD or NADP was

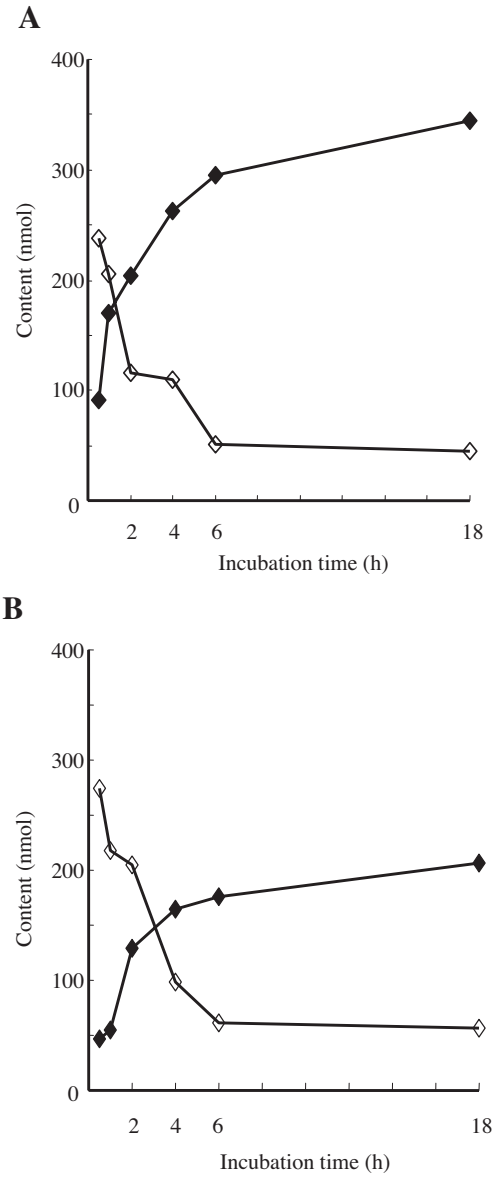

Fig. 3. Changes in Heptanal and Heptanol Contents with Incubation Time in the Crude Enzyme Solution with NADH (A) and NADPH (B).

Symbols: ◇, heptanal; ◆, heptanol. Each value is shown as the mean, n = 2. The enzyme assay used heptanal as a substrate. The assay mixture (1 ml) contained a 250 mM glycine-NaOH buffer (pH 9.0), purified water, 10 mM NADH/NADPH, the crude enzyme, and 20 mM heptanal to start the reaction.

added. This suggests that the differences in aldehydes/alcohols might have been due to aliphatic aldehyde reductase activity. Some other studies have found that alcohol dehydrogenase was activated in alkaline conditions at pH 8.0 to 10.0.[10–14] On the other hand, some reductases have been reported to be activated in a low pH range from 5.0 to 7.0 in many studies.[14–20] However, in our study, the enzyme of Vietnamese coriander leaves was activated in a higher pH range with the maximum activity at pH from 8.0 to 9.0. A similar response to the pH of the assay mixture has been reported for aldehyde reductase from *Euonymus japonica* leaves with the cofactor of NADPH.[21] Additionally, we found that the Vietnamese coriander enzyme seemed to be more strongly activated with the addition of NADH than with NADPH when heptanal was used as the substrate. This

suggestion is supported by the strong activity of this enzyme in either the homogenized mixture (Fig. 1A and B) or in the crude enzyme solution (Fig. 2A), in which the content of heptanol formed with the addition of NADH as the coenzyme was much more than twice that of NADPH (Fig. 3A and B).

Effect of inhibitors

The crude enzyme from Vietnamese coriander leaves and NADH or NADPH were incubated for 2 min prior to adding the substrate to test their inhibitory effect with 1,10-phenanthroline, a potent inhibitor of alcohol dehydrogenase, and *p*-hydroxymercuribenzoate (Table 2). Assays were conducted at 25 °C.

The results show that 1,10-phenanthroline with NADH or NADPH at 5 mM caused 16% or 25% inhibition, respectively. On the other hand, the enzyme activity with the addition of either NADH or NADPH was inhibited completely by *p*-hydroxymercuribenzoate. Our findings agree well with those of Negm, F.B.,[21] who have reported *p*-hydroxymercuribenzoate was a strong inhibitor of aldehyde reductase from *Euonymus* leaves. These results also distinguish the Vietnamese coriander enzyme from aliphatic alcohol dehydrogenase and help to confirm the presence of aliphatic aldehyde reductase in this plant. This is the first report of aliphatic aldehyde reductase activity being detected in Vietnamese coriander leaves.

It is concluded from these results that the aliphatic aldehyde reductase activity would have influenced the formation of alcohols related to corresponding aldehydes in Vietnamese coriander. Aliphatic aldehyde dehydrogenase, which is responsible for the acidic form, was also found in this herb with weaker activity than that of reductase. This implies that the expression and strength of the aliphatic aldehyde reductase activity are strongly correlated with the flavor quality of this herb.

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