Production and Stability of (E, Z)-2, 6-Nonadienal, the Major Flavor Volatile of Cucumbers

R.H. BUESCHER AND R.W. BUESCHER

ABSTRACT: Factors affecting the production and stability of (E, Z)-2, 6-nonadienal (NDE) from pickling cucumbers were examined. Production capability was rapidly destroyed in tissues exposed to freezing, fermentation or processing conditions. Lower concentrations were produced by exocarp tissues than mesocarp or endocarp tissues, which probably accounted for an almost linear relationship between fruit size and NDE production. NDE production was reduced by acidification, enhanced by linolenic acid, and unaffected by other unsaturated fatty acids NaCl or $CaCl_2$. E-2-nonenal production was suppressed when NDE production was increased by linolenic acid. NDE was unstable in filtrates of homogenized tissues. Its stability was substantially improved by acidifying to pH 2 and unaffected by $CaNa_2EDTA$. Extraction and concentration by vacuum distillation further enhanced stability. Loss of NDE in distillates averaged 0.3%/d during storage at 5 °C.

Key Words: nonadienal, cucumber flavor volatile, pickling cucumbers

Introduction

(E, Z)-2, 6-NONADIENAL (NDE) IS THE MAIN FLAVOR volatile produced by cucumber fruit (*Cucumis sativus*) when tissues are disrupted (Fleming and others 1968; Hatanaka and others 1975), providing the characteristic, pleasant aroma associated with fresh cucumbers (Forss and others 1962; Schieberle and others 1990). It is enzymatically synthesized from polyunsaturated fatty acids by a sequence of reactions involving lipoxygenases, hydroperoxide lyases, and isomerases (Galliard and others 1976; Grechkin 1998; Grosch and Schwarz 1971; Phillips and Galliard 1978; Wardale and Lambert 1980; Wardale and others 1978). The ability of cucumber tissues to synthesize NDE has been reported to be inactivated by low pH, heating to 60 °C, absence of O_2, or fermentation (Fleming and others 1968; Hatanaka and others 1975; Zhou and McFeeters 1998). Production of NDE by pickled cucumbers is currently unknown, however, since cucumbers are usually exposed to one or more of these conditions during pickle manufacturing, it is unlikely that NDE is capable of being produced by most pickle products.

NDE is an important commercial flavorant used in some food products and is used as a fragrance in cosmetics, perfumes, and detergents (Kula and Sadowska 1993). It has shown potential as a bioactive substance affecting insects, yeast, and certain bacteria (Scriven and Meloan 1984). Due to the lack of an economical source of natural NDE, synthetically manufactured NDE is primarily used for commercial applications (Kula and Sadowska 1993). NDE has been found in various plant materials, such as kiwi, mango, cherries, peas, peppers, rice, tea, and several animal products, however, cucumbers are considered to be the best source. Perhaps cucumbers, rejected for pickle products due to unacceptable size, shape, or other defects, could serve as a source of natural NDE.

The objectives of this study were to determine factors that affect NDE production by pickling cucumbers and to evaluate and enhance its stability. Studies were designed to advance information on NDE production by frozen-thawed, brined, and processed cucumbers and conditions that hinder or stimulate its production and degradation.

Materials and Methods

Source of Materials

Freshly harvested and size-graded pickling cucumbers were donated by Dean Pickle and Specialty Products Co., Div. of Dean Foods Co. and DeGraffenreid and Sons Co. in Springfield, Mo., U.S.A. Decay-free cucumbers were held at 5 °C, sorted, and washed prior to use. Fermentation and processing brines were prepared from ingredients provided by Dean Pickle and Specialty Products Co. in Atkins, Ark., U.S.A. Standards used for gas chromatography and unsaturated fatty acids were purchased from Aldrich Chemical Co. (Milwaukee, Wis., U.S.A.) and Sigma Chemical Co. (St. Louis, Mo., U.S.A.), respectively.

General Production, Extraction, and Assay Methods

Cross-sectional slices (200 g, 2-cm thick) taken at least 3 cm from the ends of cucumbers were homogenized in (200 mL) of deionized water using a Waring blender at high speed for 30 s. The liquid slurry was immediately filtered through Miracloth, 20 mL was placed in glass tubes, and octanol was added to provide an internal standard concentration of 10 mg/L. NDE along with other cucumber volatiles were extracted in capped tubes by 5 mL of n-pentane during mixing on a reciprocal shaker for 30 min. Other methods of disrupting tissues, such as by juice extractors, were observed in preliminary trials and resulted in less NDE production than when homogenized in a Waring blender. The suitability, appropriate amount, and time for complete extraction of NDE by n-pentane were determined from preliminary studies.

One mL of each extract was sealed in an amber vial (1.5 mL) and analyzed by a gas chromatograph equipped with an autosampler, split injector, flame ionization detector, and a 30-m, 0.32-mm dia capillary column coated with 0.25 mm thick 5% phenyl-polydimethylsiloxane. Following injection of

2 μL that was split 1:5, the column oven was raised from 60 °C to 140 °C at a rate of 3 °C/min. NDE was identified based on its retention time compared to that of a known standard and quantified based on the peak area of the internal standard.

Effect of Freezing, Fermentation, and Processing on NDE production

Size 2B (36 ± 2.5 mm dia) cucumbers were frozen to −20 °C and thawed to 5 °C, placed into fermentation brine, or processed as refrigerated or pasteurized fresh pack pickles. The fermentation treatment consisted of 8.5 kg of whole cucumbers submerged in 8.5 L of brine composed of 10% NaCl, 0.7% $CaCl_2$, 0.2% acetic acid, and 0.1% potassium sorbate adjusted to pH 4.0 and held at 25 °C (Buescher and others 1987). Refrigerated (nonpasteurized) and pasteurized pickles were prepared as described previously (Howard and Buescher 1993 but without color or flavor additives.

Effect of Substrates, pH, NaCl, and $CaCl_2$ on NDE production

Cucumber tissues were disrupted in solutions containing 100 ppm linolenic acid and varying amounts of HCl or NaOH, predetermined from titrating homogenates, to provide pH levels ranging from 2.8 to 9.5. Also, tissues were disrupted in solutions containing NaCl to provide final concentrations of 0%, 5%, and 10% and 100 ppm linolenic acid. The effect of 0 and 250 ppm $CaCl_2$ on NDE production was also observed. The influence of different substrates on NDE production was determined by disrupting cucumber tissues in the presence of arachidonic, linoleic, linolenic, or oleic acid that was dissolved in a small amount of ethanol and then uniformly dispersed in water containing cucumber tissues.

Effect of Cucumber Size and Type of Tissue on NDE Production

Six different cucumber sizes, representing commercial size grades designated as1A, 2A, 2B, 3A, 3B, and 4A, that ranged from about 10 to 57 mm in dia, were used to assess the effect of size (maturity) on ability to produce NDE. Another experiment examined NDE production by exocarp, mesocarp, and endocarp tissues dissected from size 2B (36 ± 2.5 mm dia) cucumbers.

Effect of pH and EDTA on NDE Stability

After cucumber slices were disrupted and filtered through Miracloth, juice samples were adjusted to pH 2, 3, 4, or 5 by 1N HCL. The pH-adjusted samples and the control (pH 5.8) were brought to equal volumes with H_2O and assayed for NDE content before and after storage for 24 h at 25 °C. Another experiment examined NDE stability in juice extracts that were adjusted to pH 2 or 3 and contained 0 or 100 ppm $CaNa_2$ EDTA during storage at 5 °C.

Stability of NDE in Distillates

Cucumber slices were disrupted in the presence of 100 ppm linolenic acid, and the filtrate was adjusted to pH 2. The filtrate was heated to 60 °C in a water bath and distilled under reduced pressure (172 kPa) using a rotary evaporator with 2 °C liquid circulated through its condenser coils. About 25 mL of condensate were collected, which was sufficient to eliminate NDE from 200 mL of the juice extract. Samples of the distillate were immediately assayed for NDE and then stored at 5 °C or 25 °C for subsequent analyses.

Table 1—Production of *(E*, Z)-2, 6-nonadienal (NDE) by exocarp, mesocarp, and endocarp cucumber tissues

Tissue	NDE Production (mg/kg)*
Exocarp	1.7c
Mesocarp	10.2b
Endocarp	11.8a

*NDE means were significantly different as determined by LSD (P < 0.05).

Statistics

The data represent the average of 3 randomized replications for each treatment or observation. The data were statistically examined by analysis of variance, and the means were separated (P < 0.05) by Least Significance Difference (SAS Institute, Inc. 1996).

Results and Discussion

Effect of Freezing, Fermentation, and Processing on NDE Production

Fresh size 2B (36 ± 2.5 mm dia) cucumber tissues at 5 °C usually produced 8 to 12 mg/kg of NDE when disrupted in H_20, however, no NDE was produced by tissues that had been frozen to −20 °C and thawed to 5 °C. The ability to produce NDE was also destroyed in cucumbers after being processed by pasteurization. When either exposed to fermentation brine or processed as refrigerated (nonpasteurized) pickles, NDE production capability was lost within 6 d. No NDE was detected in brines of the fermentation, refrigerated, or pasteurized pickle treatments, indicating that NDE was not produced and leached from the tissues.

Lipoxygenase has been reported to be rapidly inactivated in cucumbers when submerged in brines typically used for fermentation (Buescher and others 1987), which probably

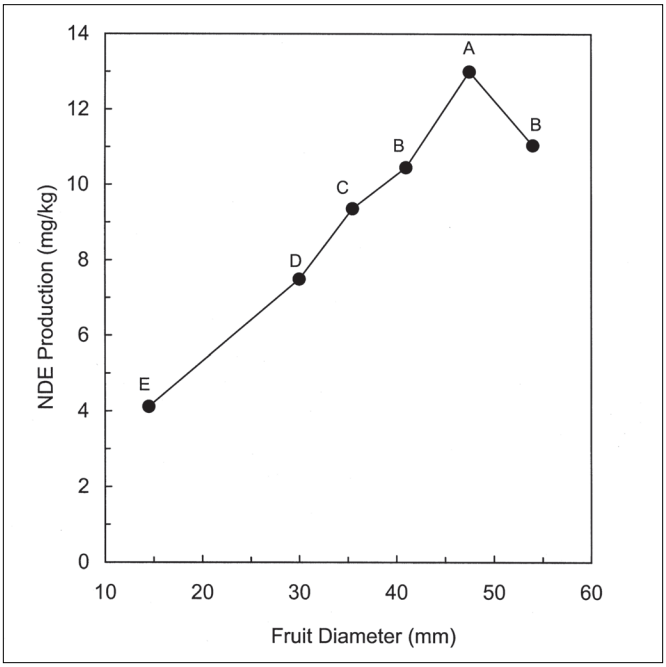

Figure 1—Effect of cucumber fruit size (dia) on production of (E, Z)-2, 6-nonadienal (NDE). Data points represent average fruit dia ± 2.5 mm. NDE means with the same letter are not significantly different (LSD, P < 0.05)

explains the loss in NDE production by cucumbers in the fermentation and refrigerated treatments. Although pasteurization to 70 °C was expected to inactivate NDE production (Fleming and others 1968; Hatanaka and others 1975), the effect of membrane disruption that undoubtedly occurs during the early stage of pasteurization on NDE production by internal tissues was unknown. Apparently, internal disruptions caused by freezing and thawing, osmotic shock from NaCl, or heat compared to disruptions caused by the blender were unfavorable to the NDE production system.

Effect of Cucumber Size and Type of Tissue on NDE Production

Pickling cucumbers are commercially graded into categories based on their dia, which usually corresponds to maturity. NDE production was greatly affected by cucumber size, incrementally increasing from size 1A (15 ± 4 mm dia) to size 3B (47 ± 2.5 mm dia) (Fig. 1). Production of NDE by 4A (54 ± 2.5 mm dia) fruit tissues, the largest size that was tested, was similar to 3A and less than 3B fruit tissues. The effect of fruit size on NDE production may be partially explained by differences observed in NDE production between exocarp, mesocarp, and endocarp tissues (Table 1). Very small amounts of NDE were obtained from exocarp tissues compared to mesocarp and endocarp tissues. Therefore, it would be reasonable to assume that less NDE would be produced by cross-sectional slices of small dia fruit than larger sizes because the proportion of exocarp tissues in relation to mesocarp and endocarp tissues is greater. Although the unexpected low levels of NDE obtained from disrupted exocarp tissues may have been caused by limited substrate or enzyme inhibitors, production with concomitant degradation may have occurred. In fact, production of NDE by exocarp tissues was expected to be greater than by the other tissues because exocarp tissues have higher activities of both lipoxygenase and hydroperoxide lyase (Wardale and others 1978; Wardale and Lambert 1980) and have higher levels of unsaturated fatty acids (Pederson and others 1964).

Effect of Substrates, pH, NaCl, and $CaCl_2$ on NDE production

NDE production was greatly enhanced when cucumber tissues were disrupted in the presence of linolenic acid (Fig. 2).

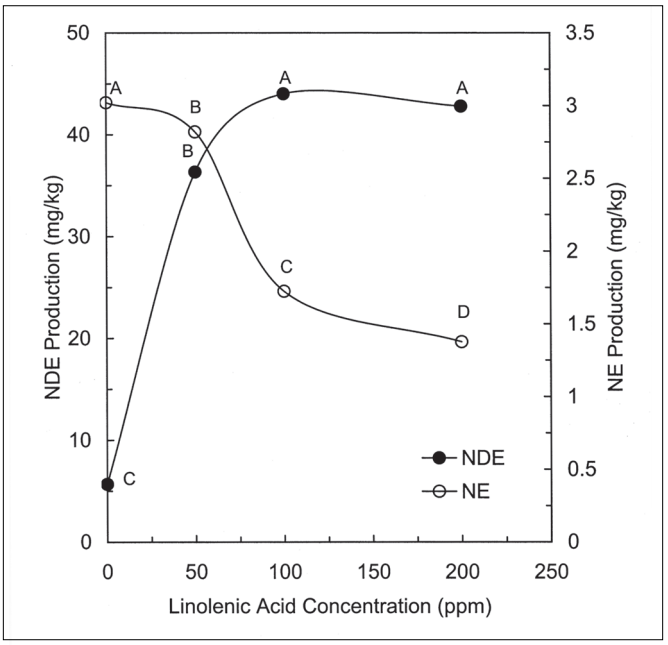

Fig. 3—Effect of linolenic acid concentration on (E, Z)-2, 6-nonadienal (NDE) and E-2-nonenal (NE) production by cucumber tissues. Data points with the same letter within a line are not significantly different as determined by LSD (P < 0.05)

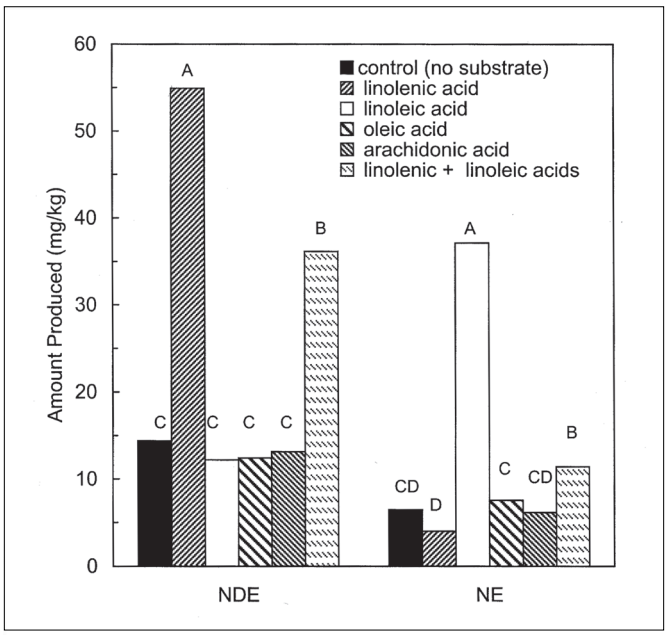

Figure 2—Influence of unsaturated fatty acids (100 mg/L) on (E, Z)-2, 6-nonadienal (NDE) and E-2-nonenal (NE) production by cucumber tissues. Means with the same letter are not significantly different as determined by LSD (P < 0.05)

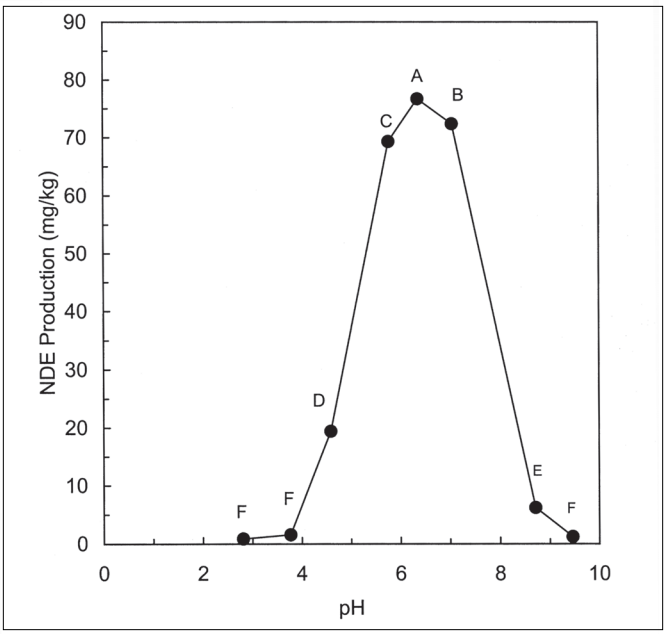

Figure 4—Effect of pH on (E, Z)-2, 6-nonadienal (NDE) production by cucumber tissues supplemented with linolenic acid (100 mg/kg). Means with the same letter are not significantly different as determined by LSD (P < 0.05)

The other unsaturated fatty acids, arachidonic, linoleic and oleic, did not significantly affect NDE production, although linoleic acid stimulated production of E-2-nonenal (NE). The combination of linolenic and linoleic acids increased production of both NDE and NE, but less was produced than when each substrate was added alone. These results confirm that linolenic acid and linoleic acid are precursors of NDE and NE production by cucumbers, respectively (Grosch and Schwarz 1971; Hatanaka and others 1975). Furthermore, it appeared that competitive inhibition may have been involved between the two precursors or their products when they were combined. In another experiment, NDE production was enhanced by 50, 100, and 200 ppm linolenic acid with maximum production achieved by 100 ppm (Fig. 3). The increased production of NDE by linolenic acid significantly suppressed the already low amount of NE production, especially when linolenic acid was increased from 50 to 100 ppm. This suppression of NE production would be favorable for cucumber volatile production since the flavor of NE has been described as being unpleasant and astringent (Forss and others 1962). Although 100 ppm (0.36 moles/L) linolenic acid resulted in the greatest increase in NDE (0.30 mmoles/kg), more efficient production relative to the amount of substrate added occurred from 50 ppm (0.18mmoles/L), which increased NDE by about 0.22 mmoles/kg.

In addition to linolenic-acid supplementation, pH had a major influence on NDE production. Below pH 4 or above pH 8.5, very little NDE was produced (Fig. 4). In contrast, production was very high from pH 5.7 to 7.0, with pH 5.7 being the natural pH of the cucumber-juice filtrates. The pH range for NDE production was similar to the pH requirements reported for lipoxygenase (Wardale and Lambert 1980) and hydroperoxide lyase (Galliard and others 1976).

Relatively minor effects of NaCl and $CaCl_2$ were observed. NDE production was only reduced by about 10% by either 5% or 10% NaCl (data not shown). Although Ca^{++} is known to be an activator of cucumber lipoxygenase (Avdiushko and others 1994), the addition of $CaCl_2$ did not significantly affect NDE production.

Effect of pH and EDTA on NDE Stability

Initial observations clearly demonstrated that NDE was unstable in cucumber juice, necessitating immediate extraction by n-pentane to obtain stable samples for analyses. Several attempts were made to use solid phase microextraction (SPME) fibers for extracting NDE from aqueous samples loaded in a GC autosampler, however, levels were observed to decline with increasing time of holding before analysis, causing inconsistent results. Although NDE was stable in n-pentane extracts, other methods of stabilizing NDE were explored to facilitate post-production handling and potential applications.

Acidification of filtrates substantially reduced NDE degradation. Samples acidified to pH 2, 3, 4, or 5 and the control at pH 5.8 lost 10%, 28%, 40%, 48%, and 55% of their NDE content, respectively, during storage at 25 °C for 24 h. Based on these results, a study was conducted to determine the stability of NDE in samples acidified to pH 2 and 3 with 0 and 100 ppm $CaNa_2$ EDTA during storage at 5 °C.

While NDE in control samples declined about 25%, 65%, and 95% during storage at 5 °C for 1, 6, and 14 d, respectively, NDE in filtrates adjusted to pH 2 declined only about 5%, 15%, and 35% (Fig. 5). Stability at pH 3 was greater than in the control but less than at pH 2. $CaNa_2$EDTA did not affect NDE stability in any of the treatments. Therefore, acidification to pH 2 combined with low temperature greatly improved stability, however, additional improvements would be necessary to preserve NDE during storage for several d.

Stability of NDE in Distillates

Vacuum distillation of acidified-aqueous filtrates of homogenized cucumber tissues further improved NDE stabili-

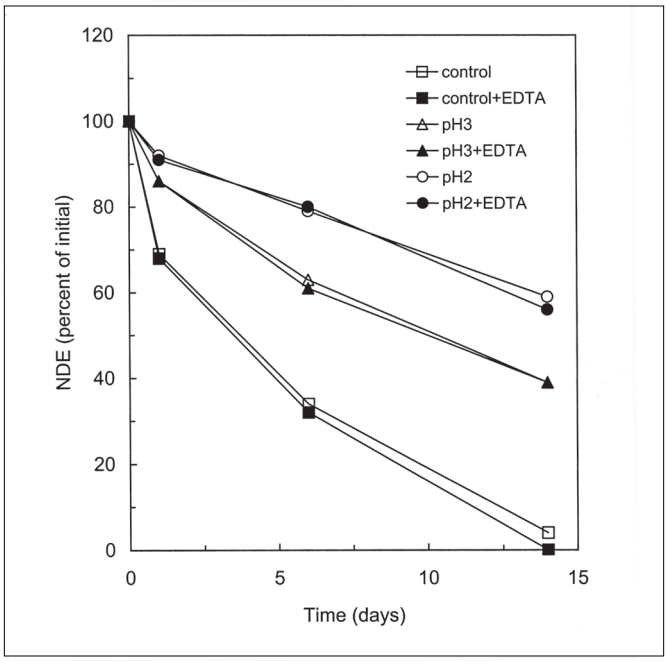

Fig. 5—Stability of (E, Z)-2, 6-nonadienal (NDE) in aqueous filtrates of disrupted cucumber tissues as affected by pH and $CaNa_2$EDTA

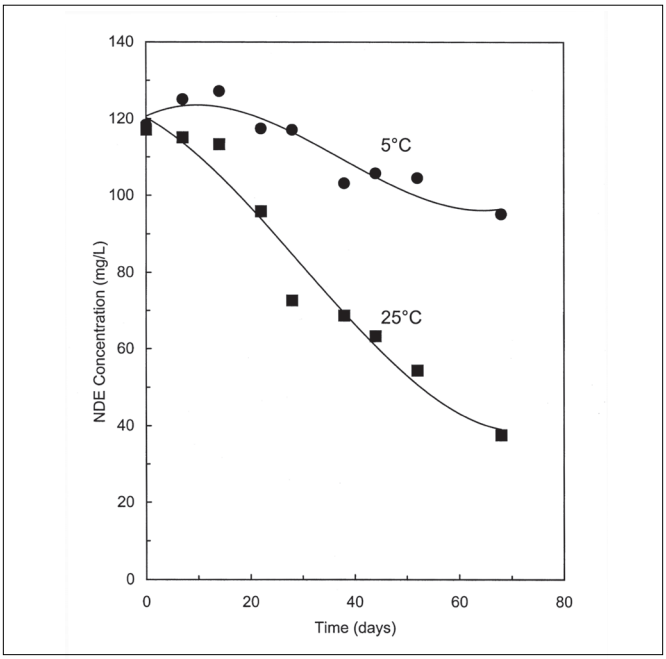

Fig. 6—Stability of (E, Z)-2, 6-nonadienal (NDE) in distillates from cucumber homogenates during stroage at 5 and 25 °C

ty (Fig. 6). After several d in storage, degradation appeared to be almost linear with the rate of loss dependent on temperature. When stored at 25 °C, NDE content declined about 1% per d, while at 5 °C, losses averaged less than 0.25% per d. Because the distillates would not have contained enzymes or other nonvolatile catalysts, it was assumed that other factors were responsible for the gradual degradation of NDE during storage. Using model systems, Josephson and Lindsey (1987) demonstrated that degradation of NDE in aqueous solutions was caused by hydration forming 3-hydroxy-Z-6-nonenal followed by a retro-aldol condensation reaction producing Z-4-heptenal and ethanal. While this reaction sequence provides an attractive explanation for the loss of NDE that we observed, it remains unconfirmed, since heptenal or other degradation products were not detected in any of our samples. However, it is possible that only the hydroxylated product of NDE was formed, which may not have been extracted or detected by the methods we used.

Conclusions

IN SUMMARY, NDE WAS PRODUCED ONLY BY FRESH CUCUMBERS during the time of tissue disruption. Tissues that had been frozen and thawed, exposed to fermentation brine, or processed as refrigerated or pasteurized pickles rapidly lost their capability to produce NDE. Production greatly declined when pH was reduced below pH 5 or elevated above pH 7. In contrast to pH, NaCl had only a minor effect on NDE production. Surprisingly, small cucumbers produced much less NDE than larger sizes. The differences in NDE production between cucumber sizes appeared to be associated to the amount of exocarp tissue relative to the amount of mesocarp and endocarp tissues, since exocarp tissues produced much less NDE than mesocarp or endocarp tissues. Addition of linolenic acid to samples prior to tissue disruption greatly enhanced NDE production. Linoleic acid or other unsaturated fatty acids did not increase NDE production.

NDE was unstable in cucumber-juice filtrates. By acidifying the filtrates to pH 2, NDE stability was greatly improved. Stability was further improved in extracts obtained by vacuum distillation, especially when subsequently stored at 5 °C. Hydration was suspected to be involved in the degradation of NDE in aqueous extracts, although other factors also may be involved because degradation of NDE in aqueous filtrates was considerably faster than degradation of NDE in aqueous distillates. Although additional research may be needed to further improve NDE stability in aqueous extracts, pickling cucumbers appear to have the potential of providing a source of natural NDE.

References

Avdiushko SA, Ye XS, Kuc J, Hildebrand DF. 1994. Lipoxygenase is an abundant protein in cucumber exudates. Planta 193:349-357.

Buescher RW, McGuire C, Skulman B. 1987. Catalase, lipoxygenase, and peroxidase activities in cucumber pickles as affected by fermentation, processing, and calcium chloride. J Food Sci 52:228-229.

Fleming HP, Cobb WY, Etchells JL, Bell TA. 1968. The formation of carbonyl compounds in cucumbers. J Food Sci 33:5 72-576.

Forss DA, Dunstone EA, Ramshaw EH, Stark W. 1962. The flavor of cucumbers. J Food Sci 27:90-93.

Galliard T, Phillips DR, Reynolds J 1976. The formation of cis-3-nonenal, trans-2-nonenal, and hexanal from linoleic acid hydroperoxide isomers by a hydroperoxide cleavage enzyme system in cucumber fruits. Biochim Biophys Acta 441:181-192.

Grechkin A. 1998. Recent developments in biochemistry of the plant lipoxygenase pathway. Prog Lipid Res 37:317-352.

Grosch W, Schwarz JM. 1971. Linoleic and linolenic acid as precursors of the cucumber flavor. Lipids 6:351-352.

Hatanaka A, Kajiwara T, Harada T. 1975. Biosynthetic pathway of cucumber alcohol: trans-2,cis-6-nonadienol via cis-3, cis-6-nonadienal. Phytochem 14:589-2592.

Howard LR, Buescher RW. 1993. Effect of redox agents on visual cure in refrigerated pickles. J Food Biochem 17:125-134.

Josephson DB, Lindsay RC. 1987. Retro-aldol degradations of unsaturated aldehydes: Role in the formation of c-4-heptenal from t-2, c-6-nonadienal in fish, oyster and other flavors. JAOCS 64:132-138.

Kula J, Sadowska H. 1993. Unsaturated aliphatic C9-aldehyde as natural flavorants. Perfumer Flavorist 18:23-25.

Pederson CS, Mattick LR, Lee FA, Butts RM. 1964. Lipid alterations during the fermentation of dill pickles. Appl Microbiol 12:513-516.

Phillips DA, Galliard T. 1978. Flavour biosynthesis. Partial purification and properties of a fatty acid hydroperoxide cleaving enzyme from fruits of cucumber. Phytochem 17:355-358.

SAS Institute Inc. 1996. Statistical Analysis System. Version 6.12 Cary, N.C.: SAS Institute Inc.

Schieberle P, Ofner S, Grosch W. 1990. Evaluation of potent odorants in cucumbers (*Cucumis sativus*) and muskmelons (*Cucumis melo*) by aroma extract dilution analysis. J Food Sci 55:193-195.

Scriven R, Meloan CE. 1984. (E, Z)-2,6-nonadien-1-al and (E)-2-nonen-1-al present in crushed cucumbers are natural repellents for the American cockroach (*Periplaneta americana)*. Ohio J Sci 84(3):82-85.

Wardale DA, Lambert EA, Galliard T. 1978. Localization of fatty acid hydroperoxide cleavage activity in membranes of cucumber fruit. Phytochem 17:205-212.

Wardale DA, Lambert EA. 1980. Lipoxygenase from cucumber fruit: localization and properties. Phytochem 19:1013-1016.

Zhou A, McFeeters RF. 1998. Volatile compounds in cucumbers fermented in low-salt conditions. J Agric Food Chem 46:2117-2122.

MS 20000247

This research was supported and approved for publication by the Arkansas Agricultural Experiment Station, Division of Agriculture in Fayetteville, Ark. The authors gratefully acknowledge contributions of supplies provided by Dean Pickle & Specialty Products Co. in Atkins, Ark., and J. L. DeGraffenreid & Sons in Springfield, Mo., and financial support provided by Pickle Packers International, Inc. in St. Charles, Ill..

Authors are with the Dept. of Food Science, Univ. of Arkansas, 2650 N. Young Ave., Fayetteville, AR 72704. Direct correspondence to author Ron Buescher (E-mail: buescher@uark.edu)