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[1]

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Alterations of cucumber-, melon-like notes in aromas and flavors caused by retro-aldol degradations of *t*2, *c*6-nonadienal were confirmed using gas chromatographic measurements of volatile compounds in model systems. The data indicated that 3-hydroxy-*c*6-nonenal was formed first by the addition of water to the alpha/beta double bond of *t*2, *c*6-nonadienal, and this was followed by a retro-aldol condensation of 3-hydroxy-*c*6-nonenal to yield *c*4-heptenal and ethanal. Compared to the reaction rate in aqueous systems at neutral pH, formation of *c*4-heptenal was enhanced substantially at alkaline pH, but was greatly diminished at acidic pH values. Heating (to 90 C) of aqueous model systems of *t*2, *c*6-nonadienal held at neutral pH also enhanced the rate of formation of *c*4-heptenal substantially compared to that at ambient temperature (21 C). Rates of formation of *c*4-heptenal in aqueous model systems held under air or nitrogen atmospheres were similar. *c*4-Heptenal was not formed when *t*2, *c*6-nonadienal was held at 21 C for 96 hr under air or nitrogen in nonaqueous commercial corn oil.

During recent studies on the flavor of pickled fish (1), continuing decreases in concentrations of *t*2,*c*6-nonadienal compared to that found in initial unprocessed fish were observed during processing. *t*2,*c*6-Nonadienal, which possesses a distinct cucumber aroma (threshold 0.01 ppb; 2), arises from autoxidative and/or enzymic oxidation of omega-3 polyunsaturated fatty acids following an omega-9-site hydroperoxidation (3,4). In the pickled fish studies which employed smelt, carbonyl-amino reactions appeared to account for some losses of *t*2,*c*6-nonadienal occurring during sodium chloride brining steps that were carried out at neutral pH. However, at lowered pH values existing in the vinegar-pickled fish (pH 3), concentrations of *t*2,*c*6-nonadienal in fish continued to decrease slowly. Because model systems showed that carbonyl-amino reactions were inhibited at pH 3.0, an examination of other processes that could account for the disappearance of this compound in acidic environments was carried out.

The loss of *t*2,*c*6-nonadienal from pickled fish was accompanied with the formation of variable concentrations of *c*4-heptenal which has also been encountered in a wide variety of lipid-containing foods. *c*4-Heptenal exhibits an aldehydic aroma that is somewhat reminiscent of boiled potatoes, and it has a threshold of 0.040 ppb (5). Begemann and Koster (6) first isolated *c*4-heptenal from milkfat, and described a cream-like flavor property for the compound when it was present in fudge candy. These workers believed that the iso-linoleic acids found in milkfat by de Jong and Van der Wel (7) served as the precursor for *c*4-heptenal through classic Farmer-type autoxidation mechanisms (8). Seals and Hammond (9) subsequently reported the isolation of *c*4-heptenal from extensively oxidized soybean and linseed oils, but were unable to assign a precursor compound which could account for its formation though usual autoxidative processes for the fatty acids found in these oils. Carefully controlled experiments by Meijboom et al. (10) using apparently water-free model systems of oxidizing methyl linoleate and oxidizing soybean oils failed to yield *c*4-heptenal, and these workers concluded that the observations of Seals and Hammond (9) must have resulted from the existence of an unknown and possibly spurious precursor in their oils.

*c*4-Heptenal also has been found in frozen butter (11) and frozen cod (5,12), where it has been associated with the cold-store flavors of these foods. Under cold storage conditions the concentrations of *c*4-heptenal sometimes accumulate to the extent that cod becomes unacceptable for consumption (13). In an earlier investigation of volatile compounds in extensively oxidized Great Lakes whitefish, Josephson et al. (14) failed to identify *c*4-heptenal in this fish. However, subsequent studies (unpublished data) have revealed that *c*4-heptenal usually is found in oxidized samples of frozen whitefish.

Because classic Farmer-type autoxidation and photooxidative mechanisms fail to provide an explanation for the formation of *c*4-heptenal through reasonable intermediates, other pathways seem likely to be involved. The acceptance of a retro-aldol condensation mechanism for the conversion of *t*2,*c*6-nonadienal to *c*4-heptenal and ethanal has been reinforced by two very recent reports about similar conversions of mono-unsaturated nine-carbon aldehydes in staling beer (15) and watermelons (16). Thus, in this paper we report the confirmation of the formation of *c*4-heptenal from *t*2,*c*6-nonadienal, and also report the results of experiments which were conducted to characterize the effects of reaction conditions on the rate of formation of *c*4-heptenal in model systems.

MATERIALS AND METHODS

Model systems: Effect of air on the reaction. *t*2,*c*6-Nonadienal (Bedoukian Research Inc., Danbury, Connecticut; 600 ppm), and 1-octen-3-ol (Aldrich Chemical Co., Milwaukee, Wisconsin, internal standard, 80 ppb) were added to degassed (1 mm Hg; 2 hr), distilled water (500 ml, pH 7.5), and this mixture was stirred under nitrogen for 1 hr at room temperature (21 C). To achieve a nitrogen atmosphere, the solution was placed under vacuum (2-3 mm Hg), and then was flooded with

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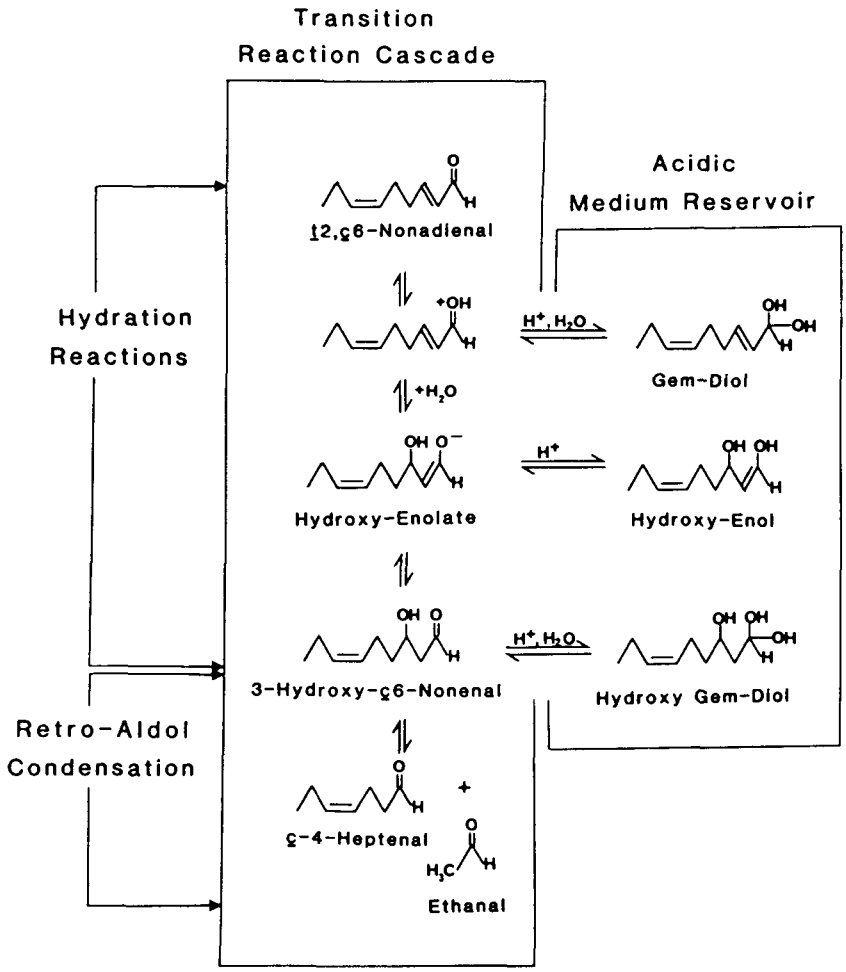


FIG. 1. Proposed mechanism for the formation of *c*4-heptenal from *t*2,*c*6-nonadienal via alpha/beta double bond hydration and retro-aldol condensation.

nitrogen until a slight vacuum remained. After the equilibration period (1 hr) the solution was divided, and 250 ml were retained under nitrogen while the other 250 ml were placed under air. Both systems were then magnetically stirred continuously at room temperature (21 C), and samples (20 ml) were removed for gas chromatographic (GC) analysis from each at times 0, 24, 48, 72 and 96 hr.

Model systems: Effect of pH on the reaction rate. *t*2,*c*6-Nonadienal (600 ppm) and 1-octen-3-ol (internal standard, 80 ppb) were added to distilled water (500 ml, pH 7.4) and stirred for 30 min at room temperature (21 C). The solution was then divided, and 250 ml was left unaltered while the other 250 ml sample was adjusted to pH 2.8 with 85% phosphoric acid. The solutions were then stirred continuously under air at room temperature (21 C), and samples (20 ml) were removed from each for GC analysis at times of 0, 1, 6, 12, 24, 48 and 120 hr.

In a separate series, a similar initial solution of *t*2,*c*6-nonadienal (600 ppm) and 1-octen-3-ol (80 ppb) was added to distilled water (500 ml) which previously had been adjusted to pH 11.1 with 5N NaOH. The solution was stirred continuously, and samples (20 ml) were removed for GC analysis at 10 min and 1, 2 and 4 hr.

Model systems: Effect of heat on the reaction rate. *t*2,*c*6-Nonadienal (600 ppm) and 1-octen-3-ol (internal standard, 80 ppb) were each added to two flasks of distilled water (150 ml, pH 7.4), and stirred for 1 hr at room temperature (21 C). A 20-ml sample was removed from each, and then the remaining portions were rapidly heated to either 60 ± 2 C or 90 ± 2 C in flasks fitted with coldwater condensors. Samples (20 ml) for GC analysis were removed from the heated solutions at 0, 10, 20 and 30 min. These samples were immediately placed into glass stoppered flasks which were then chilled by rotation in a dry ice-methanol bath for 15-20 sec.

Model systems: Effect of nonaqueous oil phase on the reaction. *t*2,*c*6-Nonadienal (600 ppm) and 1-octen-3-ol (80 ppb) each were added to 500 ml of degassed (1 mm Hg; 2 hr) commercial corn oil. This sample was then split, and 250-ml lots were stirred under either nitrogen or air at room temperature (21 C). Samples (25 ml) for GC analysis were removed from each at 0, 24, 72 and 96 hr.

Extraction and analysis of samples. For experiments performed in aqueous environments, each sample (20 ml) was extracted with 2 ml hexane (HPLC grade; EM Science, Cherry Hill, New Jersey), and the extracts

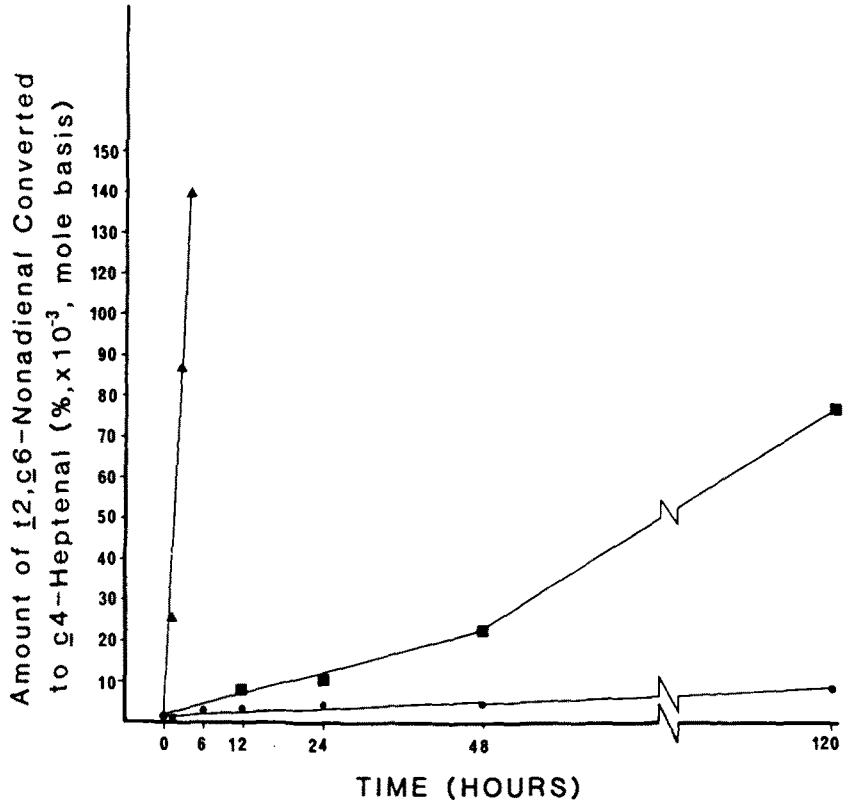


FIG. 2. Effect of pH on the formation of *c*4-heptenal from *t*2,*c*6-nonadienal in aqueous model systems at 21 C (▲, pH 11.1; ■, pH 7.4; ●, pH 2.8).

were concentrated under a slow stream of nitrogen at room temperature (21 C) to approximately 50 μl. For the experiments utilizing a corn oil medium, volatiles from the samples were collected and concentrated by purging each at room temperature (21 C) with a stream of nitrogen (150 ml/min for 3 hr) onto Tenax GC (60-80 mesh, Enka N.V., Holland) as described by Olafsdottir et al. (17). Volatiles subsequently were eluted from the Tenax GC traps with 1 ml of redistilled diethyl ether (Fisher Scientific, Fairlawn, New Jersey) and were concentrated under a slow stream of nitrogen at room temperature (21 C) to approximately 10 μl.

Concentrates of volatile compounds in either hexane or diethyl ether were then analyzed using a Varian 1740 gas chromatograph equipped with an effluent splitter for simultaneous FID measurement and odor assessment of individual peaks. Separations involving odor evaluations were achieved using a 3 m × 2 mm i.d. glass column packed with 7% Carbowax 20M on Chromosorb W AW/DMCS with a temperature programming rate from 50 C to 220 C at 4 C/min.

Additionally, volatile compounds in hexane extracts were analyzed by capillary column gas chromatography in conjunction with mass spectrometry using a Carbowax 20M (60 m × 0.25 mm i.d.) fused silica capillary column (J & W Scientific, Inc., Rancho Cordova California) operated with helium carrier gas. A program rate of 50 C (5 min) to 140 C at 6 C/min followed by a rate of 10 C/min from 140 C to 220 C was used. Identification of *t*2,*c*6-nonadienal and *c*4-heptenal was based on computer matching of mass spectra for this compound (18,19), and coincidence of mass spectral patterns from isolated compounds with those of authentic compounds as well as coincidence for retention indices (I_E; 20). Identifications of 3-hydroxy-*c*6-nonenal and *t*2,*c*6-nonadienoic acid were based on mass spectral fragmentation patterns of compounds which were formed during the experiments.

All quantitative data were obtained with a Spectra-Physics computing integrator (SP 4200). Data are reported as the percent of *t*2,*c*6-nonadienal which was converted to *c*4-heptenal on a mole basis.

RESULTS AND DISCUSSION

The reaction scheme in Figure 1 shows the compounds that can be predicted from classic chemical reactions for aqueous *t*2,*c*6-nonadienal model systems. In addition to *t*2,*c*6-nonadienal [I_E=9.43, Carbowax 20M; 41(100), 70(97), 69(95), 38(70), 67(38), 53(28), 68(24), 55(22), 94(21), 81(14), 109(9), 121(2), 138 M^+(2)], GC-MS analysis confirmed the occurrence of 3-hydroxy-*c*6-nonenal [I_E=13.43, Carbowax 20M; 41(100), 55(66), 68(62), 67(58), 39(44), 84(37), 43(35), 83(28), 42(26), 69(26), 79(20), 138(M-18), 154 M^+ (2)], and *c*4-heptenal [I_E=6.03, Carbowax 20M; 41(100), 68(72), 55(70), 84(50), 39(49), 67(42), 83(37), 56(31), 42(30), 94(22), 112 M^+ (6)]. Compounds with multiple hydroxy groups were not seen in the GC-MS analysis, but this likely reflects either an instability or nonmobility under the conditions of the gas chromatographic analysis. The two gem-diol aldehyde hydrates (Fig. 1) are predicted by

classic mechanisms (21), and their existence would be expected to be favored under acidic conditions. Ethanal also was not measured in the analyses because of its coelution with the solvent peak which was routinely diverted from the analyzer of the mass spectrometer.

The data in Figure 2 dramatically show the effects of pH on the rate of formation of *c*4-heptenal from *t*2,*c*6-nonadienal in aqueous reaction systems. As would be predicted, acidic environments substantially suppressed the rate of formation of *c*4-heptenal when compared to that of a neutral environment. Notably, alkaline conditions greatly accelerated the reaction. These data support a concept where acidic aqueous media create a reservoir of hydrates which compete directly with the reaction cascade, and as a result their existence suppresses the rate of formation of *c*4-heptenal.

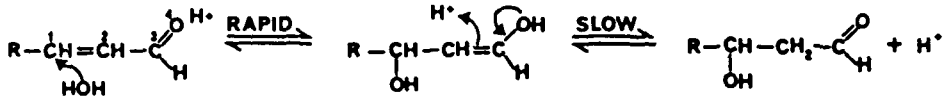
Gracey et al. (15) have proposed that the hydration of the alpha/beta double bond of 2-alkenals involves a rapid 1,4-addition of water followed by a slower acid-catalyzed rearrangement to yield the corresponding 3-hydroxy-derivative (Scheme 1.). However, considering the hydration equilibrium reactions in which *t*2,*c*6-nonadienal is expected to participate (Fig. 1), it follows that conditions promoting the formation of the diol- and triol-hydrates would competitively suppress the formation of *c*4-heptenal. Alternatively, conditions providing suppressed protonations would minimize production of the di- and tri-hydroxy compounds. Thus, under alkaline conditions a rapid production of *c*4-heptenal would be predicted (Fig. 2), because acid-induced aldehyde hydrates of *t*2,*c*6-nonadienal would be essentially nonexistent.

A series of experiments carried out to confirm that the transition of 2-alkenals to their retro-aldol products occurs through a non-oxidative mechanism showed this to be the case. The rates of formation of *c*4-heptenal from *t*2,*c*6-nonadienal in aqueous systems (pH 7.5) held under atmospheres of either air or nitrogen are shown in Figure 3. Initially the hydration and retro-aldol condensation of *t*2,*c*6-nonadienal to *c*4-heptenal does not appear to be affected by the availability of oxygen, but after 96 hr the reaction rate for the sample stored under a nitrogen atmosphere was substantially greater than that found in the sample under air. These results were somewhat unexpected because the atmospheres over the reaction mixtures were not expected to affect the rate of formation of *c*4-heptenal through the hydration and retro-aldol degradation of *t*2,*c*6-nonadienal. However, the apparent slowed rate of formation of *c*4-heptenal in the sample stored under air probably can be explained by degradation reactions involving the oxidation of *c*4-heptenal. In similar experiments performed at pH 3.0, differences in the accumulations of *c*4-heptenal between samples stored in the presence or absence of oxygen were not observed, indicating that spurious secondary oxidations of *c*4-heptenal had occurred in the earlier experiment.

The failure to observe the formation of *c*4-heptenal from *t*2,*c*6-nonadienal when it was dissolved in commercial corn oil (Fig. 3) indicates that insufficient water was present to allow the reaction to proceed. These results, thus, would explain the apparent discrepancy in the findings of Seals and Hammond (9) and Meijboom et al. (10). The extensively oxidized soybean and linseed oils analyzed by Seals and Hammond (9) likely became sufficiently polar to absorb water from the air, and therefore the formation of *c*4-heptenal from *t*2,*c*6-nonadienal was allowed. On the other hand, Meijboom et al. (10) appeared to have excluded moisture from their samples, and hence the retro-aldol process was prevented from occurring. In a related early study, Lillard and Day (22) reported the stoichiometric oxidation of *t*2-nonenal to *t*2-nonenoic acid when it was held under pure oxygen atmpspheres. In the present study, *t*2,*c*6-nonadienoic acid [I_E=16.53, Carbowax 20M; 41(100), 69(83), 86(74), 39(27), 67(15), 68(14), 94(12), 55(10), 79(8), 109(6), 154 M^+ (5)] was produced in the aqueous systems held under air.

Heating an aqueous model system (pH 7.4) containing *t*2,*c*6-nonadienal caused an enhanced formation of *c*4-heptenal (Fig. 4). Further, *c*4-heptenal and 3-hydroxy-*c*6-nonenal were not accumulated in constant proportions in samples, and increased temperatures promoted the conversion of 3-hydroxy-*c*6-nonenal to *c*4-heptenal. Data for a sample heated to 60 C and immediately analyzed showed a ratio of *c*4-heptenal to 3-hydroxy-*c*6-nonenal of 1:9, but when a sample was held at 90 C for 20 min, the ratio was 1:3. When the model system was held at 90 C for 30 min, the ratio became 1:1.6. Although samples held at higher final temperatures immediately provided greater conversions of 3-hydroxy-*c*6-nonenal to *c*4-heptenal in comparison to samples held at lower temperatures (Fig. 4), the large accumulations of 3-hydroxy-*c*6-nonenal resulting at lower heating temperatures would be expected to provide precursors for continuing retro-aldol condensation reactions to yield *c*4-heptenal during and after cooling.

The extreme sensitivity of retro-aldol degradation reactions to elevated temperatures suggests that the process is important in the changes in flavors which occur during even mild cooking of fresh fish and other seafood, such as Pacific oysters which contain substantial concentrations of the nine-carbon aldehydes (23-25). Similarly, raw muscle of fishes that are consumed as sashimi, such as salmon, exhibits subtle, melon-like flavors, and these flavors are substantially diminished upon cooking (unpublished data). Thus, in seafoods containing 9-carbon 2-alkenals, various degrees of heat-induced retro-aldol degradations would result in a variety of flavor modifications. Further, low concentrations of *c*4-heptenal formed in



SCHEME 1

freshly-cooked fish do not appear to contribute unpleasant fishiness as might be concluded from the reorts of McGill et al. (5,12) and Seals and Hammond (9).

Pokorny (27) has reported studies of thermally-induced reactions between oxidized fish oils and fish muscle protein at 100-150 C and concluded that carbonyl-amino reactions resulted in the formation of roasted and baked fish flavors. Because carbonyl-amino reactions progress readily to secondary aroma compounds at elevated temperatures as do retro-aldol degradations (Fig. 4), these reactions combine to provide significant means for the development of flavors during cooking or processing of lipid-containing foods.

In some other recent studies on the mild cooking (broiling and poaching) of Great Lakes whitefish (*Coregonus clupeaformis*), losses of *t*2,*c*6-nonadienal also have been observed (unpublished data). However, other carbonyls including hexanal, heptanal, nonanal, decanal, *c*4-heptenal, 2-octenal, 2,4-heptadienal and 2,4-decadienal were formed in reasonable abundance. With the exception of *c*4-heptenal, oxidative processes yielding these aldehydes could not be distinguished from retro-aldol degradation reactions. Alcohols also survived the mild cooking process in abundant quantities; these included 1-hexanol, 1-penten-3-ol, 1-octen-3-ol, 1,5-octadien-3-ol, 2,5-octadien-1-ol and 3,6-nonadien-1-ol.

Mild cooking of seafoods with pronounced melon-like flavor sometimes leads immediately to only modest suppressions of the fresh melon-like flavors. However, in these cases the melon-like flavor carry-through occurs because sufficient concentrations of relatively stable 9-carbon alcohols, traces of *t*2,*c*6-nonadienal, and *c*3,*c*6-nonadienal survive the heating, and these are adequate to confer the melon-like flavor to the cooked seafood. Although we did not report the identification of *c*3,*c*6-nonadienal in earlier studies of the aromas of a variety of fresh fish (25-26), this compound has been identified subsequently (unpublished data), and it is an important contributor to the melon-like aromas of some species of fish even though it is present usually in sub-ppb concentrations.

The reservoir of the various hydrated species of *t*2,*c*6-nonadienal noted in Figure 1 also likely has a direct bearing on the formation and accumulation of *c*4-heptenal in frozen cod and butter (5,11-12). Cycling of temperatures inducing freeze-thaw conditions is often encountered for frozen foods, and such

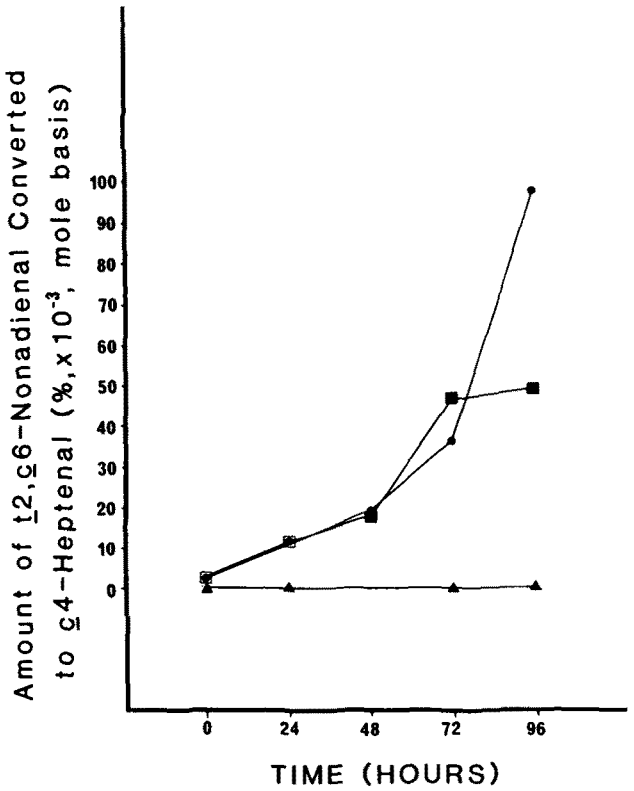


FIG. 3. Effect of solvent (aqueous vs oil) and headspace (air vs nitrogen) on the formation of *c*4-heptenal from *t*2,*c*6-nonadienal at 21 C (●, aqueous under nitrogen; ■, aqueous under air; ▲, corn oil under either air or nitrogen).

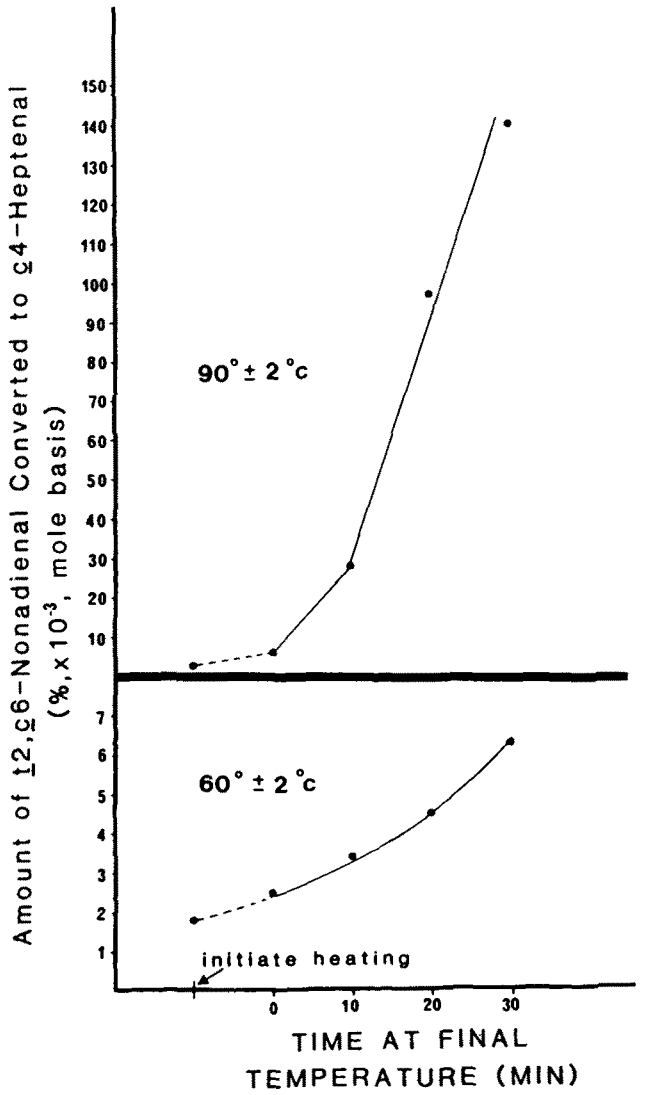


FIG. 4. Effect of temperature on the formation of *c*4-heptenal from *t*2,*c*6-nonadienal in aqueous model systems at pH 7.4 under aerobic conditions.

cycling or storage at high sub-freezing temperatures would be expected to affect the formation and degradation of hydrated *t*2,*c*6-nonadienal species in the non-frozen water phase of foods, including fish. When larger quantities of non-frozen liquid water exist at high sub-freezing temperatures, greater amounts of hydrated 2-alkenals are permitted than at lower temperatures. However, when free water is lost by evaporation or when it is converted to ice by lowered temperatures, the volume of water in which the hydrated species is dissolved diminishes rapidly. The accompanying increase in concentration of hydrated 2-alkenals would force the overall equilibrium toward the formation of *c*4-heptenal in a manner parallel to that noted and described earlier for alkaline reaction conditions. Thus, successive freeze-thaw cycles provide a driving force not only for accelerated traditional autoxidation (3,28), but also for 2-alkenal hydration reactions.

The net effect of retro-aldol degradation of 2-alkenals in fishery products during storage and cooking is the loss of much of their characterizing fresh, green plant-like aromas and flavors which are provided by the 6- and 9-carbon 2-alkenals. However, the overall reaction scheme also provides a means for preserving 2-alkenal flavor compounds when fish are held refrigerated under neutral and acidic conditions. In studies on pickled fish flavors (1), holding of high quality pickled smelt that contained *t*2,*c*6-nonadienal under refrigerated storage in a vinegar brine led to only traces of *c*4-heptenal after 10 wk. In a related study, a commercial vinegar-acidified cucumber juice contained only 9 parts *c*4-heptenal per 1,000 parts *t*2,*c*6-nonadienal after 6 mo refrigerated storage (unpublished data). Thus, retro-aldol degradations of hydrated 2-alkenals occur slowly at low pH, but eventually would be expected to contribute to staling flavors in acidic foods. Beer is a modestly acidic beverage (pH 3.9-4.4), and *t*2-nonenal has been strongly implicated in the development of stale flavors during storage of beers (29,30). Although many mechanisms for the formation of beer staling compounds have been suggested (31-34), Barker et al. (35) and Gracey et al. (15) reported that the hydration of *t*2-nonenal during wort production leads to the formation of 3-hydroxy-nonanal which then appears to provide a dynamic release of low levels of *t*2-nonenal during the storage of beer. Gracey et al. (15) also found modest accumulations of heptanal in staling beers; they attribute its formation to the retro-aldol degradation of 3-hydroxy-nonanal.

Alpha/beta unsaturated aldehydes (2-alkenals) as a class appear to be susceptible to double bond hydrations (15,36,37). Autoxidation of polyunsaturated oils constitutes a major mechanism for the formation of the 2-alkenals encountered in foods (3,38), but 2-alkenals also occur widely as products of enzymic biosynthesis. Examples include cucumbers (2-nonenal, 2,6-nonadienal; 4), mushrooms (2,5-octadienal; 39), soybeans (2-nonenal, 2,6-nonadienal, 2-hexenal; 40) and fish (2-nonenal, 2,6-nonadienal, 2-hexenal; 24). Thus, in view of the potential effects of retro-aldol condensations on expected flavor stabilities of both processed and unprocessed foods, reassessments seem in order for many processing and handling technologies that provide the opportunity for interactions of alpha/beta unsaturated aldehydes with free water.

In summary, this study has documented the susceptibility of *t*2,*c*6-nonadienal in aqueous environments to a degradation to *c*4-heptenal through a double bond hydration/retro-aldol reaction mechanism. In circumstances where this reaction occurs during storage, characteristic fresh-like flavors are subject to replacement with staling-type flavors, such as occur for beer, cold-stored cod and butter. The reaction provides a means for selective removal of 2-alkenals from food flavors during processing or cooking, and it can also act to provide a reservoir of non-flavorful forms of 2-alkenals that can be released upon changes in environmental conditions or through equilibration processes.

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