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Inhibitory Activities of Some Vitamins on the Formation of Cholesterol Oxidation Products in Beef Patties

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ABSTRACT: The capacities of 15 vitamins to inhibit the formation of 7α-hydroxycholesterol, 7β-hydroxycholesterol, and 7-ketocholesterol were examined in beef patties. Their inhibitory activities were tested at a concentration of 0.4 mmol in 30 g of beef. Among them, L-ascorbic acid, retinoic acid, and α-(±)-tocopherol were found to exert a potent inhibitory effect (30−50%) on 7-ketocholesterol formation and (~20%) on 7α-hydroxycholesterol and 7β-hydroxycholesterol formations. Pyridoxamine inhibited 7-ketocholesterol formation by 60% with a statistically significant difference ($p < 0.05$) from that achieved in the control setup. To further elucidate the possible inhibitory mechanism of pyridoxamine against cholesterol oxidation, a chemical model with pyridoxamine added in the cholesterol oxidation system (heated at 140 °C for 240 min in dimethyl sulfoxide) was employed. It was demonstrated that pyridoxamine could directly react with 7-ketocholesterol via the addition reaction. The reaction involved a nucleophilic attack of the free amine group of pyridoxamine on 7-ketocholesterol (an α,β-unsaturated carbonyl compound). This type of reaction was also found to occur in beef patties by chromatographic and spectral analyses.

KEYWORDS: *7-Ketocholesterol, 7-hydroxycholesterol, vitamins, cholesterol oxidation products, beef patties*

INTRODUCTION

Cholesterol oxidation products (COPs) are a family of oxidative products generated in high-temperature processing and/or prolonged storage of foods. They are a group of sterols with an additional functional group(s), such as hydroxyl, ketone, or an epoxide substitution in the ring (cholesterol nucleus) and/or on the side chain of cholesterol.[1−3] Long-term exposure of COPs in animals have been found to show a variety of potentially atherogenic effects. Several studies have suggested that the consumption of excess COPs led to an elevation of the plasma COP level and increased the risk of vascular diseases. Other detrimental health effects of COPs include cytotoxicity, induction of apoptosis, and immunosuppression.[1,2] Some earlier studies on COPs concerned their generation from cholesterol by autoxidation. The formation mechanism and kinetics of a large number of COPs under oxidation were reported by some researchers, offering potential ways for lowering their formation in foods by controlling auto-oxidation.[2]

Some antioxidants have been proven to be effective inhibitors against the formation of COPs.[4,5] As an example, some flavonoids found in fruits, flowers, and herbs were shown to inhibit the formation of COPs in food models.[5,6] Scavenging of free radicals by these effective phytochemicals has been proposed to be their main mechanism to lower the formation of COPs.[1,4,7] In addition to phytochemicals, vitamins have also been tested on their efficacy against the formation of COPs and other foodborne toxicants in food systems, and they were also assumed to act as antioxidants.[4,8] However, recently, the roles and reactivity of vitamins in food systems have been re-investigated, and some vitamins were discovered to play multiple roles. As an example, pyridoxamine (vitamin B6) has been proven as an active reactant in the Maillard reaction and could direct the reaction to different pathways with the formation of some new adducts.[9,10] Thus, it is reasonable to hypothesize that different vitamins may inhibit/promote the COP formation in foods with different effects and through different mechanisms.

In this study, a series of experiments were designed to investigate the effects of vitamins against the formation of COPs in beef patties. With the assistance of chemical model systems and advanced instrumental analysis [liquid chromatography−mass spectrometry (LC−MS) and nuclear magnetic resonance (NMR)], we also tried to tackle the inhibitory mechanisms of some effective inhibitors.

MATERIALS AND METHODS

Materials. Cholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol (7-keto-chol), retinoic acid (vitamin A, VA), thiamin hydrochloride (vitamin B1, VB1), riboflavin (vitamin B2, VB2), nicotinic acid (vitamin B3, VB3), D-pantothenic acid hemicalcium salt (vitamin B5, VB5), pyridoxine monohydrochloride (vitamin B6, PN), pyridoxal hydrochloride (vitamin B6, PL), pyridoxamine dihydrochloride (vitamin B6, PM), biotin (vitamin B7, VB7), folic acid (vitamin B9, VB9), vitamin B12 (VB12), L-ascorbic acid (vitamin C, VC), ergocalciferol (vitamin D, VD), α-(±)-tocopherol (vitamin E, VE), phylloquinone (vitamin K, VK), and Amberlite XAD-16 were purchased from Sigma-Aldrich Co. (St. Louis, MO). 2,2′-Azobis-isobutyronitrile (AIBN) was obtained from Merck Chemicals (Germany). Sephadex LH-20 was from GE Healthcare Bioscience (Sweden). All solvents used were of analytical grade and obtained from BDH Laboratory Supplies (Poole, U.K.). Fresh ground beef was purchased from a local beef vender in Hong Kong. Propylsulfonic acid (PRS) Bond-Elut cartridges (500 mg), Bond-Elut reservoir, and packing materials (diatomaceous earth) were from Varian, Inc. (Harbor City, CA). Screw-cap Tuf-Bond Teflon-fitted glass reaction vials (40 mL capacity) and the Reacti-Therm III

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heating module (model 18840) were purchased from Pierce (Rockford, IL).

Evaluation of the Effects of Vitamins on the Formation of COPs in Beef Patties. The beef samples were prepared following a method published by Cheng et al.,[11] with slight modification. An accurately weighed amount (30 ± 0.2 g) of ground beef was shaped as a disk with the aid of a glass Petri dish (6.2 × 1.2 cm). Powders of each selected vitamins (0.4 mmol) were thoroughly mixed into batches of ground beef before forming into patties and incubated at 25 °C for 1 h. The above procedure was repeated to prepare the samples of control sets without the addition of any vitamin. The patties were fried on a Teflon-coated frying pan at a surface temperature of 200 °C for 6 min (3 min for each side). Each of the control and vitamin-treated groups had three samples, and the experiment was repeated 3 times.[11]

The three beef patties for each treatment were combined and homogenized in 100 mL of 1 M NaOH for 2 min to a dense paste. A total of six portions were weighed into separated beakers, and each of them was equivalent to 5 g of beef. A total of 18 mL of 1 M NaOH was added to each beaker, and the content was stirred to form a suspension. The sample was left for alkaline saponification overnight at 4 °C according to Ubhayasekera et al.[12] The samples were then thoroughly mixed with 45 g of diatomaceous earth and packed into separate reservoirs. The subsequent steps were performed with solid-phase extraction (SPE).

The SPE procedures were adopted from Chen et al.,[13] with slight modification. All together, 10 mL of hexane/diethylether (95:5), 25 mL of hexane/diethylether (90:10), and finally 15 mL of hexane/diethylether (80:20) were used for each sample. The eluate from the reservoirs was combined before eluting through a PRS cartridge, which was previously conditioned with 5 mL of hexane. They were then eluted with 5 mL of acetone, and COP fractions were collected. The eluate was dried under nitrogen gas, and the residue was dissolved in 1 mL of methanol and subjected to filtration with 0.25 μm filter paper for high-performance liquid chromatography (HPLC) analysis. All of the above procedures were repeated 3 times for both control and vitamin sets, and triplicate analysis was performed.

HPLC Analysis of COPs in Beef Patties. The analysis method for COPs was adopted from Chen et al.,[13] with slight modification. The analysis was performed using a Shimadzu HPLC system with a LC-20AT separation module, a SIL-20A autosampler, a DGU-20A3 degasser, a SPD-M20A photodiode array detector, and a YMC-Pack Pro C-18 column (5 μm, 150 × 4.6 mm inner diameter, Waters Corporation, Milford, MA). The mobile phase was a methanol/acetonitrile gradient with a flow rate of 1 mL/min. The initial ratio of methanol/acetonitrile was 95:5 and then gradually changed to 83:17 in 10 min. The acetonitrile concentration continued to increase linearly to 75:25 for the next 10 min, then gradually increased to 55:45 in 10 min, and finally increased to 20:80 in 5 min. The total running time was 35 min, and the post-running time was 15 min for equilibration of the column. 7-Ketocholesterol was monitored at a wavelength of 254 nm, while 7α-hydroxycholesterol and 7β-hydroxycholesterol were monitored at 200 nm. In our analysis, before commencing each analytical run, the column was first conditioned with the initial mobile phase composition for 30 min and the LC-PDA system was tested for system stability using blank and standard solutions. Peak identification was accomplished by comparing the retention times and ultraviolet (UV) spectral characteristics of the HPLC peaks to those obtained from standard solutions analyzed under the same conditions. Spiking with COP standard solutions was also applied to confirm the identity of the target peak and avoid false-positive situations.

Examination of New Chemical Compounds Formed in the Chemical Model System and Beef Patty with the Addition of Pyridoxamine. The role of pyridoxamine in the formation of 7-ketocholesterol was investigated in a chemical model system. Two reaction sets were prepared, with one as the control (no vitamin treatment) and the other with the addition of 0.4 mmol of pyridoxamine. All experiments were repeated 3 times for triplicate investigation.

The method of chemical model systems was adapted from Palozza et al.,[14] with slight modification. Approximately 50 mg of cholesterol was dissolved in 5 mL of dimethyl sulfoxide (DMSO) in a screw-cap Tuf-Bond Teflon-fitted glass reaction vial (40 mL capacity) and heated in a Reacti-Therm III heating module at 140 °C for 4 h. A total of 1.0 mL of AIBN (120 mM in chlorobenzene) was added before the heating process as a radical initiator to initiate cholesterol oxidation. After heating for 4 h, the vial was cooled in ice to terminate the reaction. A total of 5 mL of hexane was added to extract the remaining cholesterol in a separating funnel. The polar layer (DMSO) was collected and further evaporated using a rotary evaporator. A total of 5 mL of methanol was added to dissolve the remaining white crystals. The solution was collected and filtered through a 0.25 μm membrane filter for HPLC and LC−MS analyses. The same procedure was repeated with a chemical model system added with an extra 0.4 mmol of pyridoxamine.

For beef patty preparation, the cooking methodologies were the same as what we used in the Evaluation of the Effects of Vitamins on the Formation of COPs in Beef Patties section. The prepared beef with the addition of pyridoxamine was cooking and then homogenized in 250 mL of hexane. The organic layer was extracted with 250 mL of a 50:50 methanol/water mixture in a separation funnel, and only the lower methanol−water layer was collected. The organic layer was further extracted with 250 mL of 50:50 methanol/water for 2 more times. Then, the methanol/water extract was dried, loaded onto an Amberlite XAD-16 column (40 × 4 cm), and washed with 500 mL of distilled water. The final elution was performed with 500 mL of methanol. The methanol elution was then concentrated using a rotary evaporator. The concentrated extract was collected and loaded on a Sephadex LH-20 column (40 × 4 cm). The elution was performed with 70:30 methanol/water, and the eluted fractions were collected using an automatic fraction collector. The eluate was collected in 10 mL fractions, whose profiles were checked by HPLC−diode array detector (DAD) on a Shimadzu HPLC system. Similar fractions were combined. The samples were further analyzed by LC−MS for their total ion chromatograms.

HPLC Analysis of New Chemical Compounds Formed in Chemical Systems and Beef Patties. The analysis method was adopted from previous studies, with slight modification.[15] Analysis was carried out on a YMC-Pack Pro C-18 column (5 μm, 150 × 4.6 mm, Waters Corporation, Milford, MA). The mobile phase was methanol/acetonitrile gradient with a flow rate of 1 mL/min. The initial ratio of methanol/acetonitrile was 95:5, which was changed to 83:17 during the first 10 min. The acetonitrile ratio continued to linearly increase up to 75:25 for the next 10 min, then linearly increase to 55:45 in the following 10 min, and finally increase to 20:80 in 5 min. The total running time was 35 min, and the post-running time was 15 min for equilibration of the column. The analytes were monitored at wavelengths with a full-scan range of 200−800 nm.

LC−MS Identification of New Chemical Products Formed in Both the Chemical Model System and Beef Patties Extracts. The LC−MS method was referred to a previous publication by our lab.[15] For the identification of new chemical products, the sample was subjected to LC−MS analysis after a single syringe-driven filtering step without further sample processing. All filtrates obtained from the sample preparation were analyzed on a LC−MS instrument equipped with an electrospray ionization (ESI) source interfaced to a (Qtrap 3200 Applied Biosystems) mass spectrometer. Liquid chromatography was run on an Agillent HPLC system with a degasser, a quaternary pump, a thermostatted autosampler, and a DAD. Analysis was carried out with a YMC-Pack Pro C-18 column (5 μm, 150 × 2.0 mm). The mobile phase was composed of 0.1% formic acid aqueous solution (solvent A) and acetonitrile (B) of the following gradients at the flow rate of 200 μL/min: 0 min, 5% B/95% A; 35 min, 80% B/20% A; 37 min, 5% B/95% A; and 50 min, 5% B/95% A. Effluent from the UV detector was split 4:1 directed to the MS for spectrometric analysis and the remaining to waste. The MS conditions were as follows: positive-ion mode; spray voltage, 3.5 kV; scan range, 120−800 Da; and capillary temperature, 300 °C. Enhanced product identification (EPI) was selected as the mode of MS analysis.

Isolation, Purification, and Structural Elucidation of the 7-Ketocholesterol−Pyridoxamine Adduct Formed in a Chemical

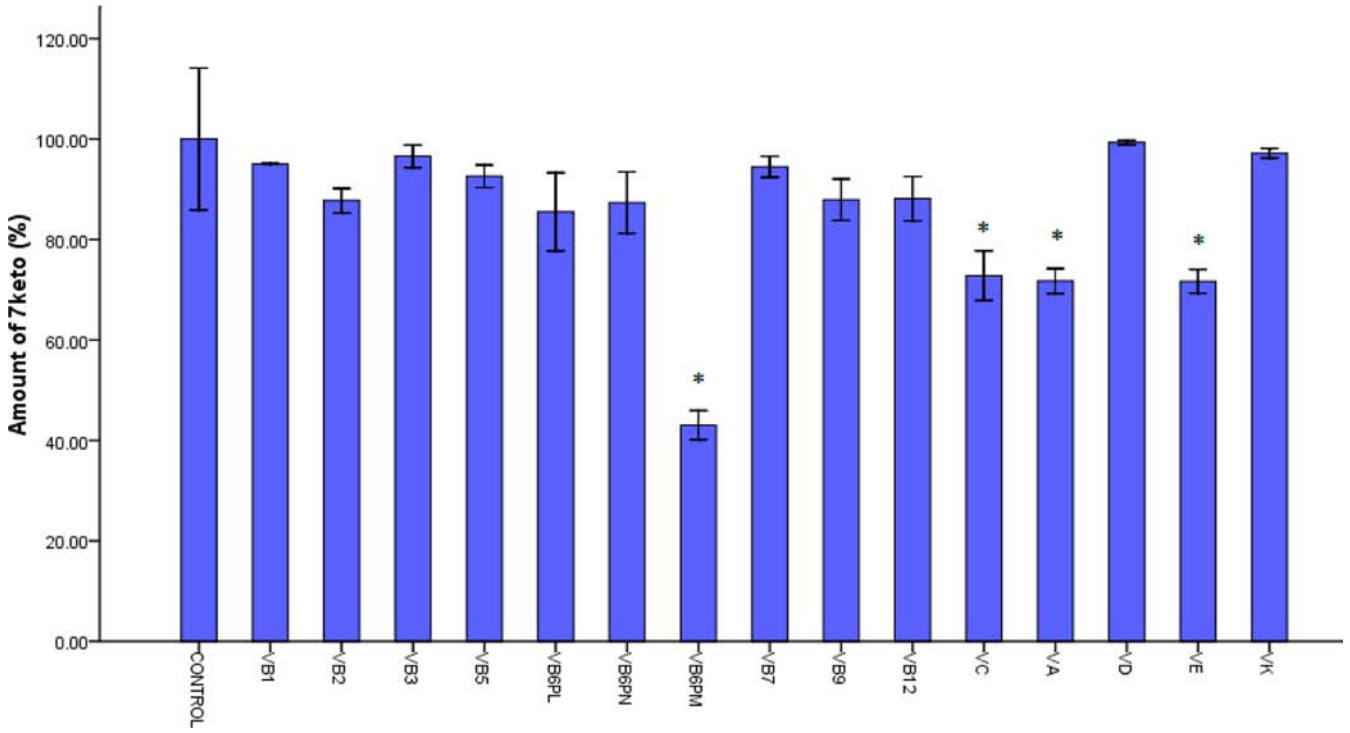

Figure 1. Inhibitory rates of vitamins against 7-ketocholesterol formation in beef patties. Data values are means, and vertical error bars are standard deviations of three independent experiments. $p < 0.05$ was selected as the level of decision for significant differences. Bars with an asterisk indicate significant difference from the control. The control set is set to have 0% inhibition.

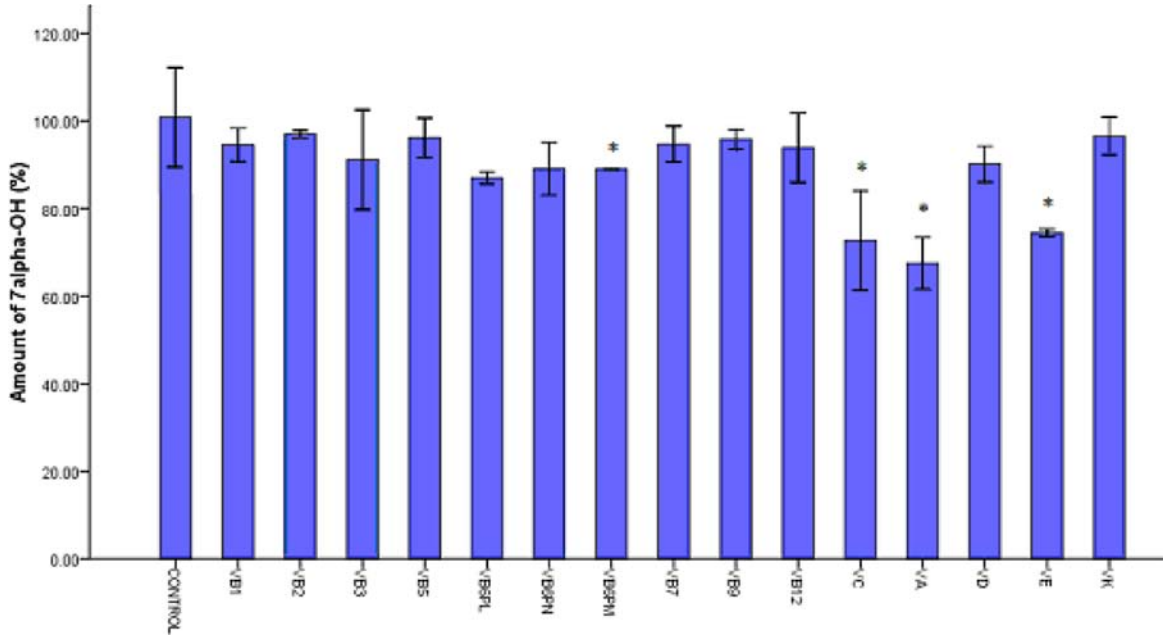

Figure 2. Inhibitory rates of vitamins against 7α-hydroxycholesterol formation in beef patties. Data values are means, and vertical error bars are standard deviations of three independent experiments. $p < 0.05$ was selected as the level of decision for significant differences. Bars with an asterisk indicate significant difference from the control. The control set is set to have 0% inhibition.

Model System. The direct reaction employing pyridoxamine and cholesterol was carried out at 140 °C following the same procedure as discussed above. Prior to isolation, the DMSO solvent was evaporated to dryness using a rotary evaporator and the sample was further dissolved in methanol. The methanol solution was loaded on a Sephadex LH-20 column (40 × 4 cm) and eluted with 70:30 methanol/water. All fractions were collected by an automatic fraction collector. The profile of the all fractions was checked following the same procedures as mentioned above. This open-column chromatographic process eventually led to a new chemical compound with a HPLC retention time of 34 min. The purified compound was further dissolved into CD_3OD for NMR analysis. Its structure was determined by elucidation of 1D NMR spectra obtained from a NMR spectrometer (Bruker, AVANCE 300).

Spectral Data of the 7-Ketocholesterol–Pyridoxamine Adduct. ^{1}H NMR (300 MHz) δ: 8.23 (s, 1H, H-31), 5.66 (pent, 1H, H-3), 4.60

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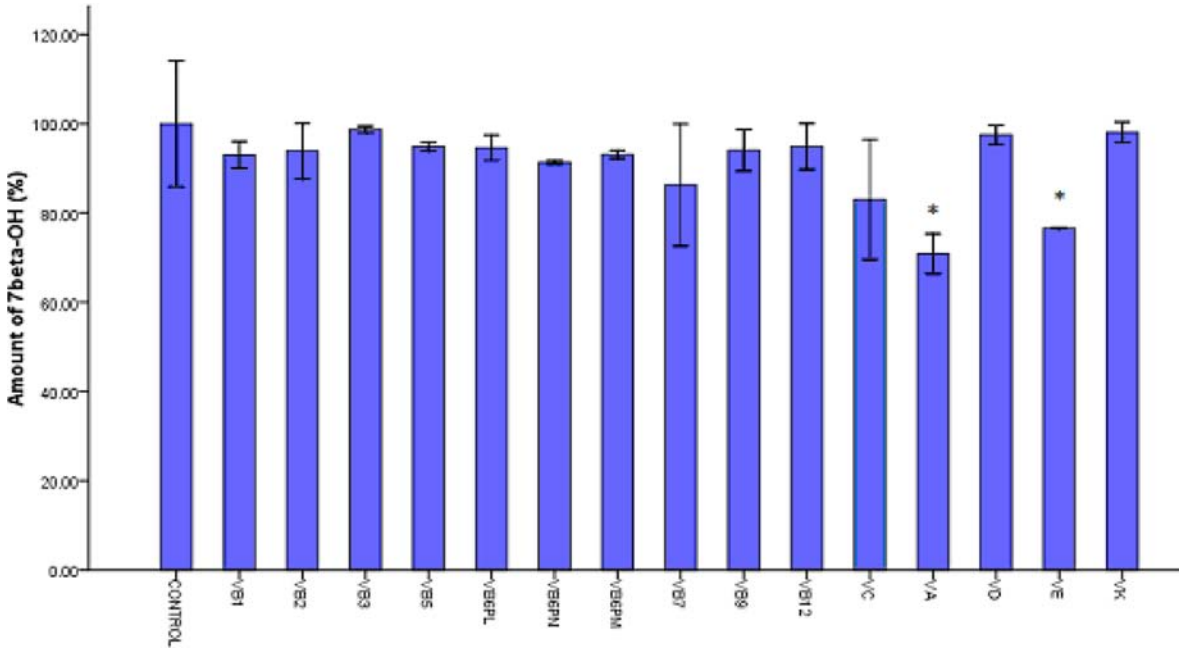

Figure 3. Inhibitory rates of vitamins against 7β-hydroxycholesterol formation in beef patties. Data values are means, and vertical error bars are standard deviations of three independent experiments. $p < 0.05$ was selected as the level of decision for significant differences. Bars with an asterisk indicate significant difference from the control. The control set is set to have 0% inhibition.

(t, 1H, H-8), 3.56 (t, 2H, H-4), 3.35 (s, 2H, H-34), 3.32 (s, 2H, H-28), 3.31 (tetra, 1H, H-9), 3.30 (s, 2H, H-6), 2.37 (tetra, 2H, H-15), 2.35 (tetra, 2H, H-16), 2.06 (tetra, 2H, H-2), 2.01 (tetra, 2H, H-1), 1.89 (tetra, 2H, H-11 and H-12), 1.63 (hept, 1H, H-25), 1.54 (tetra, 1H, H-14), 1.52 (tetra, 1H, H-17), 1.39 (hept, 1H, H-20), 1.24 (s, 3H, H-18 and H-19), 1.23 (tetra, 2H, H-22), 1.18 (pent, 2H, H-23), 1.13 (tetra, 2H, H-24), 0.97 (d, 3H, H-21), 0.89 (d, 3H, H-26 and H-27), 0.73 (s, 3H, H-35).

^{13}C NMR (75 MHz) δ: 204.78 (C-7), 143.68 (C-32), 141.10 (C-31), 138.03 (C-33), 132.34 (C-30), 126.32 (C-29), 71.27 (C-5), 60.21 (C-3 and C-34), 56.29 (C-8), 51.54 (C-4), 49.58 (C-17), 49.41 (C-13), 49.24 (C-14), 49.07 (C-9), 48.90 (C-10), 48.73 (C-6), 48.56 (C-20), 42.79 (C-22), 40.72 (C-12), 40.13 (C-23), 37.63 (C-2), 37.44 (C-1), 37.06 (C-24), 35.60 (C-16), 29.60 (C-25), 27.46 (C-26 and C-27), 24.99 (C-15), 22.98 (C-35), 22.32 (C-11), 19.42 (C-21), 17.75 (C-19), 15.92 (C-18).

Statistical Analysis. Statistical analyses were performed using the SPSS statistical package (SPSS, Inc., Chicago, IL). a paired sample t test was applied to show whether a particular treatment of the sample resulted in significant difference compared to the control set. The value of $p < 0.05$ was selected to determine whether mean values were significantly different with each other.

RESULTS AND DISCUSSION

Effects of Vitamins on the Formation of COPs in Beef Patties. Beef patties are known to contain a high level of COPs among various food products; thus, in this study, we selected beef patties as the model food. In our analysis of the effects of vitamins on COP formation, we only analyzed three COPs, namely, 7-ketocholesterol, 7α-hydroxycholesterol, and 7β-hydroxycholesterol, because they are the most abundant COPs in meat products.[4] Taking into consideration that COPs were produced in a complex food matrix and lots of interfering compounds might have resulted from concurrent chemical reactions in meat samples, we applied SPE using PRS cartridges to purify our meat samples. Similar to what were reported in the literature,[13] the extraction time was shortened substantially with less interference achieved.

Among the 11 water-soluble vitamins in our test, only vitamin C and pyridoxamine showed significant inhibitory effects against the formation of COPs in beef patties. Vitamin C was found to reduce the formation of 7-ketocholesterol and 7α-hydroxycholesterol in beef patties by around 30−35% (Figures 1−3). The inhibitory effect of vitamin C on COP formation was comparable to what was reported in the literature.[6] In Lee's study, vitamin C was applied to egg and pork to test its effect on COP formation.[16] The best inhibitory rate of vitamin C was reported as around 65% in the egg model, while no inhibition was achieved in the pork model. Different from vitamin C, pyridoxamine inhibited 7-ketocholesterol formation by 60% with a statistically significant difference ($p < 0.05$) from that achieved in the control setup, while it did not show significant inhibition on the formation of both 7α- hydroxycholesterol and 7β-hydroxycholesterol. Among the four fat-soluble vitamins, only vitamin A (retinoic acid) and vitamin E (α-tocopherol) showed an inhibitory effect against the formation of COPs; they showed 30 and 35% inhibitory rates, respectively. Tocopherol was well-studied by others for its effect against the formation of COPs in different food systems.[4,5,17] The best inhibitory result of tocopherol was obtained by Kim et al.,[18] which showed up to 90% inhibition of 7-ketocholesterol formation in an aqueous model system.

Dose-Dependent Effect of Pyridoxamine on 7-Ketochoelsterol Formation. The dose-dependent inhibitory activity of pyridoxamine was further studied in beef patties. Pyridoxamine was found to significantly reduce the level of 7-keto-chol at a level as low as 0.05 mmol. The dose-dependent trend could be observed over the concentration range examined (0.05, 0.1, 0.2, 0.3, and 0.4 mmol per each beef patty), and this dependent trend was shown in Figure 4.

Identification of Adducts Formed between Pyridoxamine and 7-Ketocholesterol. In our study, a cholesterol oxidation chemical model was used to investigate the effect of 0.4 mmol of pyridoxamine on 7-ketocholesterol formation.

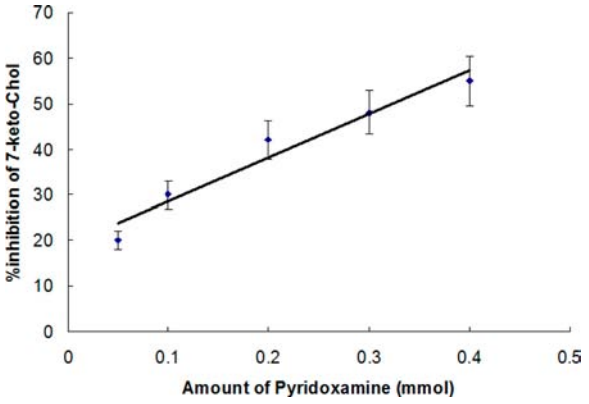

Figure 4. Effects of different concentrations of pyridoxamine on the formation of 7-ketocholesterol. Each data point represents the mean from three replicates, and the vertical bars represent the standard errors of three replicates.

Another cholesterol chemical model proceeded at the same time without the addition of pyridoxamine as a control.[14] Their chemical profiles were compared to elucidate any unknown compound formed with the addition of pyridoxamine.

With LC–ESI–MS analysis, it was found that the addition of 0.4 mmol of pyridoxamine would induce the formation of more chemicals. These chemicals may be some decomposed products of pyridoxamine and some unknown products. The pyridoxamine decomposed products can be easily identified by comparison of their MS spectra to our previous results.[19] Close examination and comparison of the LC–MS total ion chromatograms (Figure 5) revealed the presence of only one newly formed species in the chemical model reaction with pyridoxamine. This newly formed species showed a dominant molecular ion peak at m/z 569.5 $[M + H]^+$ (Figure 5), which is suggested to be a direct combination product of 7-ketocholesterol and pyridoxamine. The two fragment ion peaks at m/z 401.5 and 169.3 further suggest that this new species is the 7-ketocholesterol–pyridoxamine adduct. We further apply LC–MS analysis to check the possible formation of the 7-ketocholesterol–pyridoxamine adduct (m/z 569.5) in beef patty samples. With the application of several steps of purification by column chromatography, we were able to confirm the formation of this 7-ketocholesterol–pyridoxamine adduct in beef patties.

Characterization of the Structure of the 7-Ketocholesterol–Pyridoxamine Adduct. To facilitate a better understanding of the proposed reaction pathway, we tried to isolate and purify this novel chemical from the model system mentioned above. After the performance of subsequent solvent chromatographic purification, the final adduct corresponding to the peak with a retention time of 34.0 min in the HPLC chromatogram was successfully purified. Its detailed structure was then characterized by 1D NMR spectroscopy. All of the NMR data are displaced in details in the Materials and Methods.

The molecular formula of this newly generated compound was estimated to be $C_{35}H_{52}O_4N_2$ based on MS analysis. Its ^1H NMR spectrum exhibited a total of 52 proton signals, and its ^{13}C NMR spectrum exhibited a total of 35 carbon signals. Careful examination of its NMR spectra suggests that its structure may consist of two major parts: the first part was from the pyridoxamine, and the second part was from 7-ketochoelsterol. As for the pyridoxamine partial structure, in the ^1H NMR spectrum, the singlet signals for H-31 (δ 8.23 ppm) and H-35 (δ 0.73 ppm) were assigned to the hydrogen and methyl group attached to the pyridine ring of the PM substructure. Moreover, the two singlet signals at δ 3.32 and 3.35 ppm are assignable to H-28 and H-34, respectively. For the ^{13}C NMR signals, the five carbons of the pyridine ring contributed to signals at δ 155.28 (C-32), 143.68 (C-31), 141.10 (C-33), 138.03 (C-30), and 133.93 (C-29). The methyl, the hydroxymethyl, and the aminomethyl groups attached to the pyridine ring likely exhibited the signals at 22.98 (C-35), 60.21 (C-34), and 48.56 (C-28), respectively. All of the signals are in good agreement with a previous study.[20,21] The remaining signals match very well with the substructure of 7-ketocholesterol. With the proposed addition pathway, the original 7-ketocholesterol loses its double bond at C-5–C-6 and forms an adduct with pyridoxamine. The nitrogen atom of pyridoxamine connected to C-5 as a result of the S_N2 mechanism. Indeed, in ^1H and ^{13}C NMR spectra, we found some related signals. The proton attached at the new C-5–C-6 single bond displaced signal at 3.31 (s, 2H, H-6). The carbon signals displaced at 48.73 (C-6) and 71.27 (C-5), respectively.

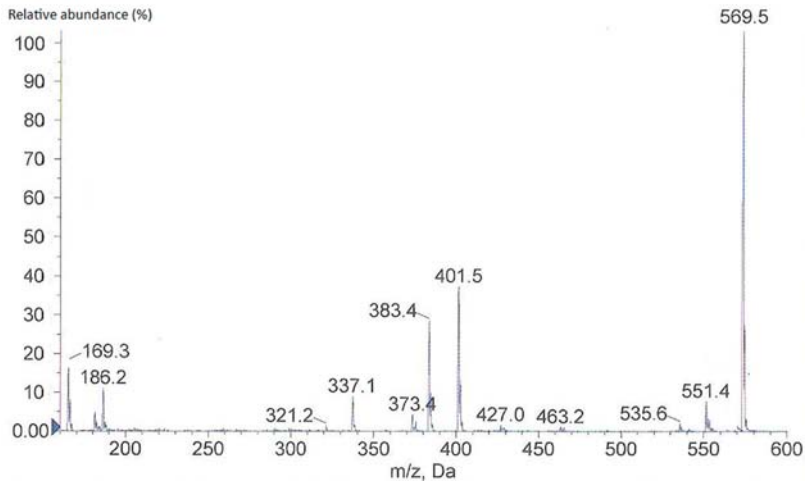

Figure 5. Full (ESI positive) MS spectrum for the 7-ketocholesterol–pyridoxamine adduct.

All other signals are in good agreement with what was reported in the literature.[19,21] For example, the highest signals at δ 204.78 (C-7) is the characteristic signal generated from the ketone functional group. The characteristic peaks at two methyl groups of the large ring systems were found to show signals at δ 17.75 (C-19), 15.92 (C-18), and 1.24 (s, 3H, H-18 and H-19). Thus, the structure of the new chemical is determined as shown in Figure 6.

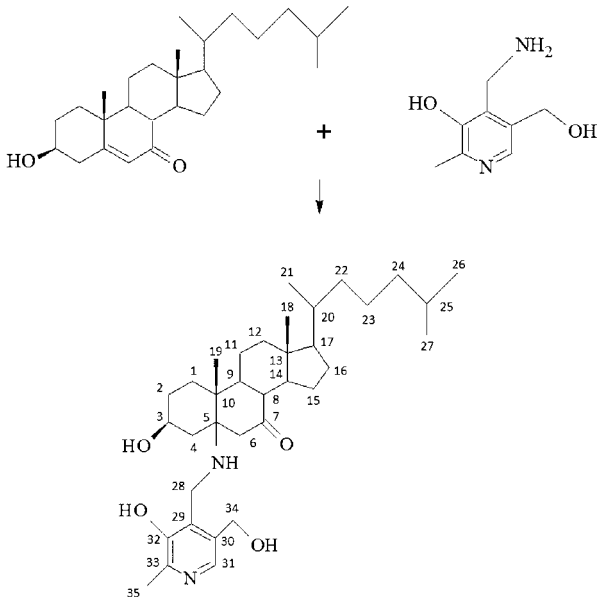

Figure 6. Proposed formation mechanism for the 7-ketocholesterol–pyridoxamine adduct.

In this study, the effect of vitamins on inhibiting the formation of three major cholesterol oxidation products was systematically investigated in beef patties. Pyridoxamine, retinoic acid, α-tocopherol, and L-asorbic acid were effective against the formation of COPs. The effectiveness of these inhibitors suggests their great potential for further development in the food industry. Moreover, the mechanistic study of PM demonstrated that its inhibitory effect comes from its direct participation into the addition reaction with 7-ketocholesterol. Our current study provides extra insight of the mechanism to explain how weak antioxidants act as eliminating agents of cholesterol oxidation products. However, the complicated relationship between inhibitors and cholesterol oxidation may require further investigation.

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Notes

The authors declare no competing financial interest.

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