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Hexanal Synthesis in Isolated Soy Proteins

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Abstract Sodium erythrobate (2.3 mM) and dithiothreitol (6.5 mM) caused headspace hexanal from aqueous slurries of commercial isolated soy proteins (ISP) to increase by as much as 13-fold compared with headspace hexanal in the controls. When linoleic acid was also added, headspace hexanal levels from commercial and laboratory-prepared ISP were further increased by as much as 18- and 136-fold, respectively, compared with the levels in the controls. When ^{13}C-labeled linoleic acid was added to aqueous mixtures of laboratory-prepared ISP along with reducing agents, 96% of the resulting hexanal contained the ^{13}C isotope. Commercial ISP samples had about 40% of the isotopically labeled carbon incorporated into hexanal. These large increases in hexanal were almost completely prevented by heating the aqueous ISP to 100 °C or above prior to adding the reducing agent and linoleic acid. Adding cysteine or sodium sulfite reduced headspace hexanal levels from aqueous ISP slurries by more than 88%. Thiol blockers, iodoacetic acid and *N*-ethylmaleimide, did not affect hexanal levels.

Keywords Hexanal · Soy protein · Reducing agents · Linoleic acid · Erythrobate

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Introduction

The taste of soy protein products is a major factor limiting their use in human foods in the Western world [1–3]. Incorporating isolated soy protein (ISP) at a level of 2% [4, 5] or 3% [6] into frankfurters significantly lowered sensory scores. In all three of these investigations of the effects of ISP in frankfurters, sodium erythrobate and/or sodium nitrite were incorporated in the formulations.

The contribution of hexanal to the characteristic odor of soy products has long been acknowledged and has been found to be among the top three major odorants by gas chromatography (GC)/olfactometry methods [7–13]. Hexanal and other lipid-oxidation products in soy products have been thought to result from the action of soybean lipoxygenase [7, 8]. However, food products prepared from soybeans that lack the lipoxygenase (EC 1.13.11.12) isozymes (L-1, L-2 and L-3) had little improvement in the overall flavor [14, 15].

Matoba et al. [16] reported hydroperoxide lyase activity specific for 13-L-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid in the homogenates from both normal and lipoxygenase-deficient varieties of soybeans. In 1996, Matoba et al. [17] noted that soybeans that lack all three lipoxygenase isozymes still produce hexanal, and suggested the presence of a new lipoxygenase in the soluble fraction of soybeans. They found that both the soluble and the membrane fractions from soybeans that contain the three known lipoxygenase isozymes converted linoleic acid to its 13-hydropeoxide and hexanal. These fractions also converted added 13-hydroperoxide to hexanal. Only the soluble fraction from lipoxygenase-deficient soybeans produced linoleic

acid 13-hydroperoxide and hexanal. Both fractions actively converted added 13-hydroperoxide to hexanal.

To our knowledge there is no published work addressing the synthesis of hexanal in ISP; however, in previous research in our laboratory into the effect of various reducing agents on the sulfur-containing odorants in ISP [18], we observed that when 6.5 mM dithiothreitol (DTT) was added to aqueous slurries of commercial ISP there were large increases in headspace hexanal levels compared with the levels in the controls. Monma et al. [19] and Kemal et al. [20] indicated that soybean LOX-1, LOX-2 and/or LOX-3 activities were inhibited by DTT. Zamora et al. [21] indicated that tomato LOX-2 was also inhibited by DTT. The activity of hydroperoxide lyase, another enzyme involved in hexanal synthesis, has been shown to be enhanced with DTT. Rehbock and Berger [22] reported that the activity of hydroperoxide lyase from mung bean was stabilized with DTT. Matsui et al. [23] reported that hydroperoxide lyase from tea leaves was protected from deactivation with DTT. Noordermeer et al. [24] reported that DTT protected fatty acid hydroperoxide lyase in plants from inactivation. Salch et al. [25] reported that hydroperoxide lyase activity from whole soybeans that was lost during isolation procedures reappeared after treatment with DTT.

This current investigation was therefore undertaken to determine if the hexanal in aqueous slurries of ISP is produced only during processing/storage and is released when the protein is hydrated, or if a significant amount is synthesized after the ISP has been hydrated.

Experimental Procedures

Chemicals

Hexanal, DTT, L-cysteine and linoleic acid,were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). ISP samples were provided by Protein Technologies International (PTI; St. Louis, MO, USA) and Archer Daniels Midland (ADM; Decatur, IL, USA). L-Phosphatidyl choline (PC), 95% was obtained from Avanti Polar Lipids (Alabaster, AL, USA). [^{13}C]Linoleic acid was from Spectra Stable Isotopes (Columbia, MD, USA). The hydroperoxide levels of linoleic acid and PC were increased by exposing a portion of each chemical to indirect sunlight for 10 min and then maintaining the temperature at 25 °C for 2 h before storing the portions at –18 °C. Anhydrous sodium sulfite (FCC) and sodium erythrobate (FCC) were obtained from Spectrum Chemical Manufacturing (Gardena, CA, USA).

Soy Products and Preparation of Isolated Soy Proteins

Four samples of ISP were obtained from ADM's processing facility. Three protein isolates were obtained on the same day and from the same plant, but from different processing lines. These samples were designated as ADM-1A, ADM-1B and ADM-1C. ADM-2 was processed by the same method at a later date. An additional sample of ISP was obtained from PTI (St. Louis, MO, USA) and it was designated as PTI Supro 500E ISP. All commercial ISP samples were stored at 4 °C. For production of the laboratory-prepared ISP, defatted (white) flakes were obtained from ADM (Decatur, IL, USA) and stored at 4 °C. The defatted flakes were ground for 15 s in a retail coffee grinder immediately prior to preparing ISP. Laboratory-prepared ISP was made by dispersing the ground hexane defatted soy in water (1 part flour to ten parts water), followed by additions of 1 N NaOH, as needed, until a pH of 8.5 was achieved and maintained for 15 min [18]. After centrifugation at 1,500*g* for 10 min, the supernatant was adjusted to a pH of 4.5 with 1 N HCl to precipitate proteins. Following centrifugation at 1,500*g* for 10 min, the precipitate was washed once with water, and the protein isolate was adjusted to pH 7 with 1 N NaOH and freeze-dried.

Static Headspace Analyses

For static headspace analyses, 0.5 L of 0.6% commercial ISP slurries were placed in a 1-L flask sealed with a septum and a Teflon tube attached to a shutoff valve [18]. For laboratory-prepared ISP samples, 0.1 L of 0.5% slurry was placed in a 0.5-L flask of the same design. After adding the sample, the flask was covered with aluminum foil. After stirring for 30 min at 23 °C, 20 mL of the unconcentrated headspace was withdrawn using a 25-mL gas-tight syringe (preheated to 45 °C) equipped with an inert gas sampling valve as previously described [18]. L-Cysteine and DTT, if added, were placed in the water before the addition of the ISP and the flask was evacuated at 525 mmHg vacuum for 20 min to remove any residual volatile compounds. This vacuum treatment did not significantly change the volume of water. Sodium erythrobate, sodium sulfite and thiol blockers were dispersed in the water before adding ISP without vacuum treatment. Linoleic acid or PC, if added, were suspended in 1 mL chloroform. The mixture was swirled inside the reaction flask with a stream of nitrogen to evaporate the chloroform and leave a thin film of linoleic acid and/or PC inside the flask below the ISP slurry surface.

Gas Chromatography/Mass Spectroscopy

GC/mass spectroscopy (MS) was accomplished using a Hewlett-Packard model 5890 Series II gas chromatograph with a 5971A mass spectrometer, a MS No-vent system (SGE International, Ringwood, Australia) and an indirect liquid-nitrogen trap (SGE International) at the beginning of the column to cryofocus analytes. The injection sequence began by bringing the liquid-nitrogen trap to –60 °C. The purge valve was closed for the first 2 min of the run. The MS No-vent was then turned on and 20 mL of the sample headspace was injected at a rate of 5 mL/min followed by a 2-min wait. The MS No-vent was then turned off, followed by a 0.5-min wait. The flow of nitrogen to the cryotrap was stopped and the GC run was begun. The column was a DB-5MS capillary column (30 m × 0.53-mm inner diameter) of 1.5-μm film thickness (Agilent Technologies, Palo Alto, CA). The helium flow rate through the columns was 3 mL/min, with 2 mL/min emerging from the sniff port (SGE International, Ringwood, Australia). The column temperature was held at 40 °C for 2 min, then increased at 5 °C/min to 165 °C, where it was held for 5 min, then to 220 °C at 20 °C/min, where it was held for 2.75 min. The injection port temperature was maintained at 130 °C. The electron ionization detector was set to detect selected ions; m/z 44 and 56 for hexanal (at the appropriate retention time). Hexanal produced from isotopically labeled linoleic acid was detected initially by a scan of m/z 26–150 and subsequently at m/z 46 and 60 (Fig. 3). Each aqueous ISP preparation was analyzed once after being stirred for 30 and 90 min. Duplicate analyses represent separate analyses performed on the same ISP (or treatment).

Lipid Extraction

Lipid extractions were accomplished by a modification of the method of Bligh and Dyer [26] as previously described [27, 28].

Peroxide Value

The peroxide value (PV) was determined by the procedure of Hick and Gebicki [29]. Values reported as ''nil'' were below the minimum detectable level for this assay of 0.2 nmol hydroperoxide per milligram of lipids.

Thin-Layer Chromatography

One-dimensional thin-layer chromatography (TLC) of the crude lipid extracts from each ISP was accomplished using Adsorbosil Plus-1 20 cm × 20 cm plates coated with 250 μM silica gel G with a preadsorbent strip (Alltech Associates, Deerfield, IL, USA). The mobile phase was hexane/ethyl ether/glacial acetic acid (80:20:1, v/v/v) [30–33]. Linoleic acid (10 μg), 9 ± 0.4 mg of each lipid extract from the ADM ISP samples and 8.2 mg from the PTI ISP sample were applied to the TLC plate. Lipids were visualized by the binding of iodine vapors and by charring with sulfuric acid.

Statistical Analysis

Statistical evaluations of treatments on the level of selected compounds were done using the Statistical Analysis System [34] software package. Analysis of variances was performed by the ANOVA procedure. Least significant difference values were computed at $P < 0.05$ and comparisons between means were done using the Tukey–Kramer honestly significant difference test.

Results and Discussion

When 6.5 mM DTT was added to aqueous slurries of commercial ISP, the level of hexanal in the headspace increased by 1.3- to 7-fold compared with the level in the controls (Fig. 1). While the addition of DTT caused a significant increase in three of the four samples ($P < 0.05$), there was a great deal of variation among ISP samples. From a review of the literature, it seems unlikely that the elevated headspace hexanal owing to the addition of DTT is due to the activity of a lipoxygenase.

Because linoleic acid is a common substrate for the synthesis of hexanal [16, 17], we examined the effect of adding linoleic acid (PV nil) to ISP slurries in combination with DTT (Table 1). With both the laboratory-prepared ISP and the ADM ISP-1A the addition of linoleic acid significantly increased ($P < 0.05$) the level of headspace hexanal. When 95% soybean PC [PV nil or PV 11.6 (0.4)] was added to the ADM ISP slurry along with DTT there was no significant increase ($P < 0.05$) in the hexanal headspace level compared with the level in the ISP-1A/DTT slurry (data not shown). Because the primary fatty acid of soybean PC is linoleic acid [35, 36], soybean PC appears not to be a substrate for hexanal synthesis in ISP with added DTT.

The total lipid contents in the ADM protein isolates were quantified using a modification of the method of Bligh and Dyer [26]. The mean values for the percentage of total lipids in the ADM-1A, ADM-1B and ADM-1C ISPs were 4.87 (0.01), 4.67 (0.02) and 4.50 (0.03), respectively. The PTI Supro 500 E sample

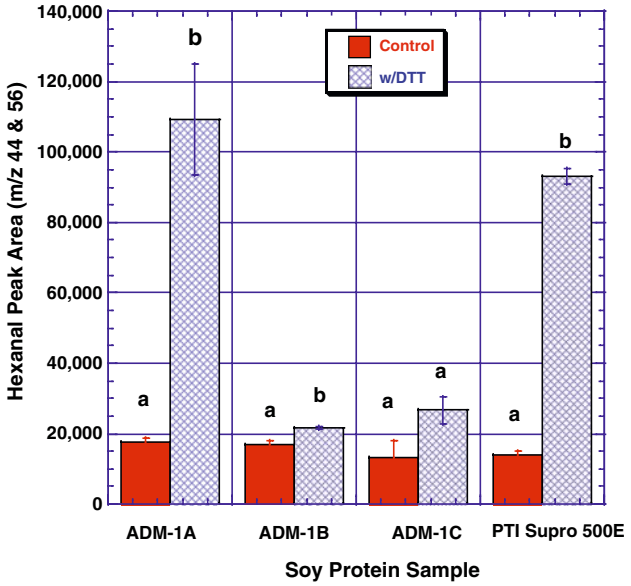

Fig. 1 Levels of hexanal above 0.6% isolated soy protein (*ISP*) slurries (3 g/0.5L) at 90 min as affected by the additions of 6.5 mM dithiothreitol (*DTT*) ($n = 2$)

contained 4.14 (0.004) total lipids. The values in parentheses are standard errors. The types of lipids were approximated using one-dimensional TLC (Fig. 2). This chromatogram indicates that the major classes of lipids are similar to those found in ISP samples by Boatright and Hettiararchchy [37, 38], including phospholipids, triglycerides, sterols and free fatty acids. The level of free fatty acids, as marked by the linoleic acid standard, appears similar in all ISP extracts. The PV of the lipid extracts from the ADM samples were 9.28 (0.34), 10.23 (0.44) and 10.98 (0.26) nmol hydroperoxide per milligram of lipids for ADM-1A, ADM-1B and ADM-1C ISP extracts, respectively. Neither the type, nor the amount, nor the PV of the lipids in the ADM ISP samples provides an explanation for the differences in hexanal produced with added DTT (Fig. 1).

In order to conclusively determine if the elevated levels of hexanal resulting from added DTT are actually due to hexanal synthesis from added linoleic acid, and not somehow due to the release of pre-existing bound hexanal, we added isotopically labeled [^{13}C]linoleic acid where all 18 carbons are ^{13}C. Figure 3 shows the mass spectra of hexanal, and hexanal produced in the aqueous mixture of a laboratory-prepared ISP with added DTT and [^{13}C]linoleic acid. Using a laboratory-prepared ISP with added DTT (6.5 mM) and 20 μL [^{13}C]linoleic acid (PV nil), the hexanal peak area was 4,931,813 (*m*/*z* 44, 46, 56 and 60) with a ratio of isotopically labeled hexanal to nonisotopically labeled hexanal of 20:1 (Table 1). This is a 91-fold increase in hexanal from the the level in the control with no additives (*m*/*z* 44 and 56). Using the same laboratory-prepared ISP with added DTT (6.5 mM) and 20 μL [^{13}C]linoleic acid [PV 17.4 (1.0)], the mean peak area of hexanal was 7,420,874 (*m*/*z* 44, 46, 56 and 60) with a ratio of isotopically labeled hexanal to nonisotopically labeled hexanal of 31:1. The high level of hexanal synthesis with the PV nil substrate indicates that a catalyst other than just hydroperoxide lyase is involved. When [^{13}C]linoleic acid and DTT were added to aqueous slurries of the ADM-1A ISP sample, the

Table 1 Hexanal headspace content and ratio of isotopically labeled carbon in hexanal from aqueous slurries of isolated soy proteins (*ISP*) with various additives

Treatment	Hexanal peak area	Ratio of C to ^{13}C
Laboratory-prepared ISP (0.5%)		
Control	54,430 (7,461)[a]	NA
With 6.5 mM DTT	98,466 (6,637)[b]	NA
With 6.5 mM DTT and [^{13}C]linoleic acid (PV nil)	4,931,813[c]	1:20
With 6.5 mM DTT and [^{13}C]linoleic acid (PV 17)	7,420,874	1:31
ADM ISP-1A (0.6%)		
Control	19,340 (1,847)[a]	NA
With 6.5 mM DTT	111,948 (13,210)[b]	NA
With 6.5 mM DTT and linoleic acid	166,929 (1,848)[c]	NA
With 6.5 mM DTT and [^{13}C]linoleic acid (PV nil)	168,777	2.3:1
With 6.5 mM DTT and [^{13}C]linoleic acid (PV 17)	137,493	1.5:1
With 2.3 mM sodium erythrobate	238,929 (9,892)[d]	NA
With 2.3 mM sodium erythrobate and [^{13}C]linoleic acid (PV 17)	344,654	1.9:1

Hexanal headspace content was determined from the peak area by gas chromatography (*GC*)/mass spectroscopy (*MS*)–selected ion monitoring (*SIM*) from 20 mL of headspace after stirring for 90 min (*m*/*z* 44, 46, 56 and 60). The ratio of isotopically labeled carbon was determined by GC/MS-SIM (ratio of *m*/*z* 56 to *m*/*z* 60). Values within a column (for each ISP) with different *superscript letters* indicate a significant difference at $P < 0.05$. Values in *parentheses* are standard errors, $n = 2$

DTT dithiothreitol, *NA* not applicable, *PV* peroxide value

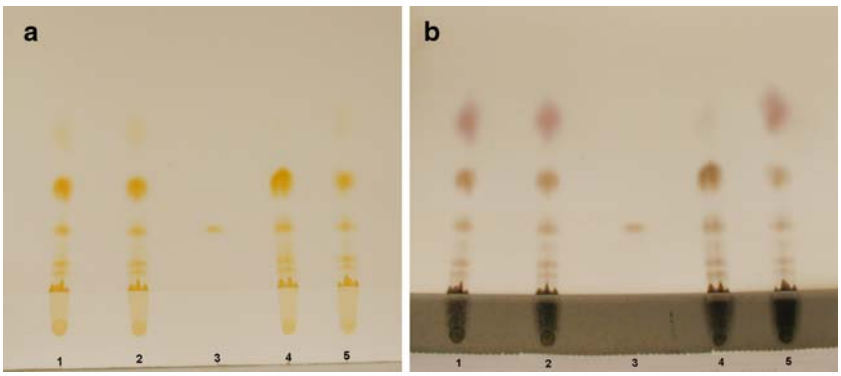

Fig. 2 One-dimensional thin-layer chromatogram of the crude lipid extracts from each ADM ISP (9 ± 0.4 mg each). *Lanes 1*, *2* and *5* correspond to ADM-1A, ADM-1B and ADM-1C ISPs, respectively. *Lane 3* is linoleic acid (10 μg) and *lane 4* is 8.2 mg from The PTI Supro 500E ISP sample. **a** Visualized by the binding of iodine vapors; **b** visualized by charring with sulfuric acid

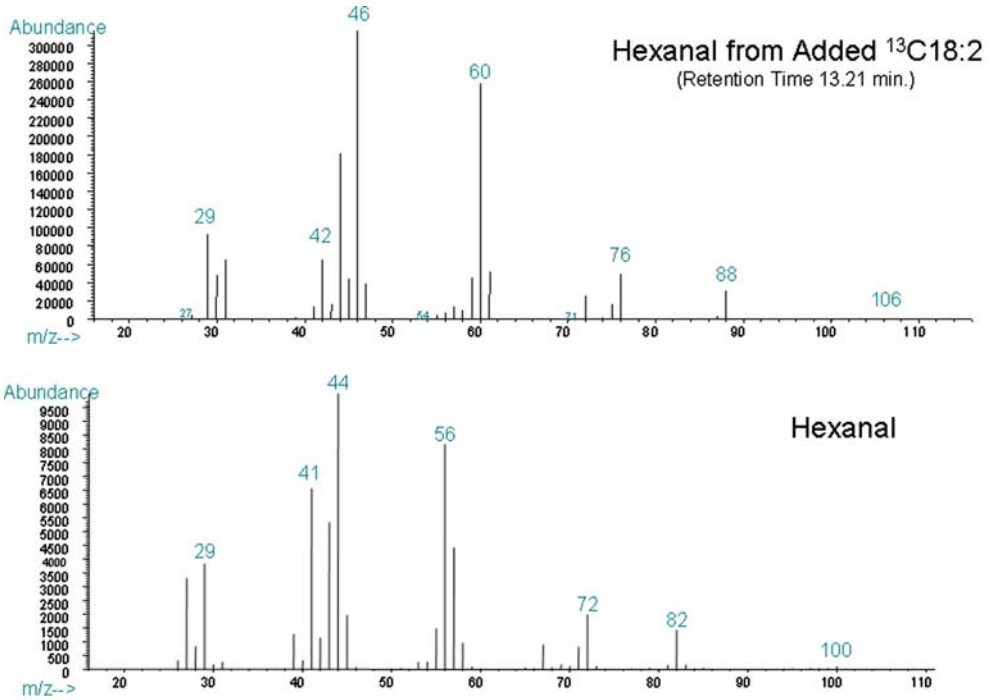

Fig. 3 Mass spectra of hexanal from **a** the headspace of 0.5% ISP slurry with 6.5 mM DTT and 20 L [^{13}C]linolenic acid and **b** NIST mass spectroscopy library. The ratio of *m*/*z* 60 to *m*/*z* 56 (isotopically labeled hexanal to nonisotopically labeled hexanal) in the headspace of the ISP slurry with added [^{13}C]linolenic acid was 31:1

hexanal peak area increased from 14,111 in the control (no additives) to 168,777 and 137,493 with the addition of the PV nil and PV 17.4 [^{13}C]linoleic acid, respectively (Table 1). The ratios of labeled-to-unlabeled hexanal were 1:2.3 and 1:1.5, respectively. Differences between the commercial and the laboratory-prepared ISP are likely due to the thermal processing used in the commercial process and the age of the isolates. At the time these isotopic experiments were performed, the laboratory-prepared ISP was about 1 month old and the commercial ISP samples had been stored at 4 °C for about 2 years.

To determine if the synthesis of hexanal was inhibited by elevated temperatures, the addition of isotopically labeled linoleic acid to the laboratory-prepared ISP was repeated using boiling water to suspend the ISP prior to it being cooled and mixed with the linoleic acid. Suspending the ISP and DTT into boiling water for 5 min prior to adding the mixture to the [^{13}C]linoleic acid resulted in a reduction of the *m*/*z* 60 fragment of the isotopically labeled hexanal from 2,502,021 in the unheated sample to 48,045. Suspending the ISP in boiling water and placing the slurry in a sealed container at 150 °C for 30 min, cooling and then mixing with the DTT and [^{13}C]linoleic reduced the *m*/*z* 60 fragment of the isotopically labeled hexanal from 2,502,021 in the unheated sample to 13,899. These experiments strongly indicate that DTT is acting as an activator of hexanal synthesis from linoleic acid in ISP slurries; possibly by an enzymatic mechanism.

Another reducing agent that was found to elevate headspace hexanal in aqueous ISP slurries was sodium

erythrobate. With ADM ISP-1A, food-grade sodium erythorbate (2.3 mM or 500 ppm) caused a 12-fold increase in hexanal compared with the level in the controls (Table 1). This is about twice as great as the increase observed from the addition of DTT. When [^{13}C]linoleic acid was added to aqueous slurries of commercial ISP and sodium erythrobate, the ratio of labeled-to-unlabeled carbon in the resulting hexanal was 1:1.9; similar to the ratio observed with DTT.

The finding that sodium erythrobate can act as an activator of hexanal synthesis in soy proteins is very important. Sodium erythrobate, alone or in combination with sodium nitrite, is often added to food products (e.g., processed meats) as an antioxidant/reducing agent. In the literature review for this paper we found three studies which reported that adding soy proteins to frankfurters caused off-flavor developments. He and Segranek [4] and Matulis et al. [6] added sodium erythrobate (550 and 485 ppm, respectively) and 156 ppm sodium nitrite to their frankfurter formulations. Lecomte et al. [5] added 156 ppm sodium nitrite as an antioxidant/reducing agent. On the basis of our findings, the off-flavors observed in these frankfurter formulations could have been elevated by the addition of reducing agents added to prevent the formation of lipid-derived off-flavors.

The addition of either sodium sulfite or L-cysteine to aqueous ISP (which caused very large increases in hydrogen sulfide and/or methanethiol [18]) also greatly decreased headspace hexanal contents (Table 2).

Table 2 Effect of various additives on the levels of methanethiol and hexanal from aqueous slurries of commercial ISP

Reaction mixture	Methanethiol	Hexanal
Control ADM ISP-1A (no additive)	765 (161)^{a}	19,340 (1,847)^{a}
With 8.3 mM L-cysteine	10,595 (480)^{b}	2,205(1,255)^{b}
With 7.9 mM sodium sulfite	5,238 (64)^{c}	910 (574)^{b}
With 6.5 mM DTT	14,375 (1,839)^{d}	109,304 (15,855)^{c}
Control ADM ISP-2 (no additive)	2,452 (176)^{a}	11,105 (463)^{a}
With 6.5 mM DTT	34,923 (5,298)^{b}	126,163 (11,182)^{b}
With 10 mM iodoacetic acid	ND	8,698 (1,068)^{a}
With 10 mM *N*-ethylmaleimide	ND	11,262 (2,266)^{a}
Control PTI ISP (no additive)	428 (45)^{a}	14,111(790)^{a}
With 8.3 mM L-cysteine	15,587	ND
With 7.9 mM sodium sulfite	3,673 (294)^{b}	168 (168)^{b}
With 6.5 mM DTT	18,754 (1,202)^{c}	93,091 (2,280)^{c}

The peak area was determined by GC/MS-SIM from 20 mL of headspace after stirring for 90 min, $n \geq 2$. The reaction mixture was in 500 mL water with 3 g ISP contained in a 1-L flask. Values within a column (for each ISP) with different *superscript letters* indicate a significant difference at $P < 0.05$

ND none detected

Unlike reducing agents (e.g., DTT) that can restore essential SH groups near the reaction center of enzymes, sulfites and cysteine can have the opposite effect and react with free sulfhydryl groups to form thiosulfonates [39] and disulfides [23]. While it has been shown that dry ISP already contains some hexanal [28], the inhibition of hexanal synthesis with added sulfite and L-cysteine indicates that a large portion of hexanal occurring in the headspace of rehydrated ISP is the result of ongoing hexanal synthesis. If cysteine and sulfite were inhibiting hexanal synthesis through a thiol-blocking mechanism, then common thiol blockers like iodoacetic acid and *N*-ethylmaleimide (NEM) should have a similar effect. Because the previous ISP samples were considered too old at this point (more than 3 years), the effect of these thiol blockers was examined on a new ISP sample from ADM (ADM ISP-2). The effect of DTT and sulfite was similar as with the other ISP samples. Neither iodoacetic acid nor NEM had a significant effect on hexanal levels (Table 2).

The effects observed for DTT, erythrobate, cysteine, sulfite and iodoacetic acid indicate that there may be more than one activation/inhibition mechanism involved. The activation by reducing agents such as DTT and erythrobate may involve a mechanism similar to the cytochrome P450 cycling where the ferric intermediate is reduced by DTT or erythrobate [40, 41]. Inhibition of hexanal synthesis by sulfite and cysteine, but not by thiol blockers, indicates that thiol modification is not the mechanism. Reducing hexanal levels with added cysteine or sulfite could be used to improve the odor of soy protein products if it were not for the large amounts of hydrogen sulfide and methanethiol resulting from these additives [18].

These results demonstrate that the catalytic activity responsible for converting linoleic acid and/or linoleic acid hydroperoxides into hexanal was most active in the laboratory-prepared ISP. The lower activity in the commercial ISP sample was likely the result of the heat treatment employed during commercial ISP processing. Typical temperatures in ISP processing may reach 70 °C for several hours, which would be anticipated to deactivate most enzymes. However, our findings indicate that there is still some catalytic activity in the ISP samples, which is reactivated with selected reducing agents. While these findings are unexpected, they are not without precedence. Anthon and Barrett [42] reported that several enzymes (including peroxidase and lipoxygenase) from carrots and potatoes are heat-resistant. They reported that heating carrots at 70 °C for 15 min had minimal effect on the activity of peroxidase. Salazar et al. [43] reported on a laboratory

variant of cytochrome P450 peroxygenase with greater heat stability than the corresponding wild type. Machado and Saraiva [44] demonstrated that horseradish peroxidase maintained a significant portion of its activity even after 24 h at 70 °C. Heat inactivation was partially reversed after storage at 4 °C. Lee et al. [45] reported on the thermostability and reactivation of a peroxidase from soybean sprouts.

Using DTT and sodium erythrobate, we have discovered a means to ''strongly'' activate one or more catalysts found in ISP that are involved in the conversion of linoleic acid and/or linoleic acid hydroperoxides into hexanal. Activation in the presence of reducing agents is indicative of a hydroperoxide lyase. However, a novel lipoxygenase enzyme or a chemical mechanism cannot be ruled out at present. The cytosols of most cells possess strong reducing conditions that make disulfide formation both kinetically and thermodynamically disfavorable [46]. The cytosol of eukaryotes normally has a reducing potential of $E_0' = -0.23$ V. A quantity of 10 mM DTT approximately corresponds to the reducing potential of the cytosol. Once cells are ruptured and cytosolic enzymes are exposed to oxidizing conditions, ''misfolding'' can occur owing to the formation of intramolecular and intermolecular disulfide bonds. This may explain why, when soybean cells are ruptured, there is an initially rapid increase in lipid oxidation products such as hexanal. Lipid oxidation is initially so rapid that heating water extracts of soybean immediately after grinding has limited effectiveness at minimizing off-flavors [47].

Fatty acid hydroperoxide lyase (CYP74B) is a cytochrome P450 enzyme [48] and cleaves the carbon–carbon bond adjacent to the hydroperoxy functional group of an unsaturated fatty acid, producing a shorter chain aldehyde and a ω-oxo acid [49]. Hydroperoxide lyases, like other P450 enzymes, are a heme thiolate enzyme with a cysteine thiolate group as the fifth ligand of the heme iron. Unlike most other P450 enzymes, hydroperoxide lyase does not always require oxygen or NADPH as cofactors. DTT and linoleic acid 13-hydroxide protected tea leaf hydroperoxide lyases from being inactivated, which indicates that inactivation may be caused by modification of an essential SH group near the reaction center [23]. Itoh and Vick [50] reported that hydroperoxide lyase from sunflower seed was inhibited by nordihydroguaiaretic acid, a sulfhydryl group reagent.

While there has been no previous report of hexanal synthesis in ISP, there are several investigations of hydroperoxide lyase activity in soybean seeds. Matoba et al. [16] reported hydroperoxide lyase activity specific for 13-L-hydroperoxy-*cis*-9,*trans*-11-octadecadienoic acid in the homogenates from both normal and lipoxygenase-deficient varieties of soybeans. Olias et al. [51] reported on the partial purification of a fatty acid hydroperoxide lyase from Williams variety soybeans that produced hexanal from 13-hydroperoxides, but did not act on 9-hydroperoxides. Hydroperoxide lyase was stabilized with DTT during the extraction and subsequent chromatographic separations. Kondo et al. [52] reported on hydroperoxide lyase activity (also stabilized with DTT) in the enzyme extracts from soybean cotyledons. Other researchers have reported on the activity of hydroperoxide lyase and lipoxygenase enzymes in the seeds and leaves of soybean plants at various stages of growth [53–55].

Having a mechanism to elevate hexanal synthesis to such a large extent provides us with a powerful tool to investigate the catalyst(s) responsible for hexanal synthesis in soy products. Of particular interest is the finding that the same reducing agents (e.g., sodium erythrobate) that accelerate the synthesis of hexanal also accelerate the synthesis of methanethiol. If these two major contributors to the characteristic odor of purified soybean proteins are activated by a similar mechanism, it may be possible to minimize the occurrence of both of these odorants with a single treatment.

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