Formation of 2-Pentyl Pyridine During Processing of Soybean Protein Isolates as Affected by pH

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ABSTRACT

Levels of 2-pentyl pyridine (2-pp) in protein fractions from Stressland soybeans increased to 0.868 ppm (± 0.027) at pH 7 after removing insoluble components from defatted flours. If similar protein fractions were held at pH 4.5 or pH 9, the 2-pp concentration was 0.182 ppm (± 0.003) or 0.199 ppm (± 0.003), respectively. Soybean cultivars KS4694, Edison, and a type null for lipoxygenase 1, 2, and 3 showed similar changes. The increase at pH 7 occurred in less than 1 h. If the defatted flour solution was not subjected to alkaline extraction to remove insoluble components, the increase of 2-pp at neutral pH did not occur. Thus, the pH conditions should be carefully controlled to produce soybean protein isolates with desired flavor properties.

Key Words: soybean protein isolate, 2-pentyl pyridine, pH, off-flavor, processing

INTRODUCTION

VARIATION OF PH AFFECTS YIELD AND COMPOSITION OF SOYbean protein isolates (SPI) during processing (Honig et al., 1987). However the effects of pH on flavor compounds of soybean protein in processing have not been published. The flavor of soy protein products has been the most important consideration in the expanded use of soy proteins in human foods (Kinsella, 1979; Wilson et al., 1990). 2-Pentyl pyridine (2-pp) exhibited a penetrating-grassy aroma when detected by Gas Chromatography/Oflactometry and a throat-catching taste in water when evaluated by sensory panelists (Boatright and Crum, 1997). With the taste threshold of 0.012 ppb in water solution, it has the greatest reported flavor value of any compound found in SPI. The level of 2-pp was reported to increase when adding pro-oxidants ($FeCl_3$, $CuCl_2$) or exposing the protein slurries to ultraviolet light during SPI processing (Boatright et al, 1998). Other factors, such as pH, temperature, or drying methods, may influence the formation of 2-pp. Our objective was to study the effects of different pH conditions applied at various stages of SPI production on the generation of 2-pentyl pyridine.

MATERIALS & METHODS

Preparation of defatted flour from soybeans

Stressland, KS4694, and Edison soybeans were obtained from U.S. Department of Agriculture's Agricultural Research Service soybean breading and genetics laboratory at Purdue University (West Lafayette, Ind., U.S.A.). Soybeans null for Lipoxygenase 1, 2, and 3 were obtained from the University of Kentucky Agronomy Department (Lexington, Ky., U.S.A.). All chemicals were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo. U.S.A.).

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Defatted soybean flour was prepared as described by Boatright et al. (1998). Beans were cracked in a blender and hulls were removed by aspiration. The dehulled bean pieces were then ground in a coffee mill and passed through a 20-mesh screen. One part full fat flour was mixed with 10 parts hexane, agitated 10 min, then centrifuged at 1000 × *g* for 10 min at 20 °C. The supernatant was discarded. The extraction was repeated two more times. The resulting pellet was dried in a fume hood overnight.

Lipid extraction and measurement of 2-pentyl pyridine

Lipid extraction was achieved by a modification of the long known method using chloroform partitioning. A sample (1 g) was extracted twice, each time with 20 mL of chloroform/methanol/water (5:10:4, v/v/v). The deuterium labeled 2-pentyl pyridine (d_5/d_6) was added as internal standard during the first extraction at a level corresponding to 0.202 ppm of the SPI. A Hewlett Packard model G1800A GCD system (Palo Alto, Calif., U.S.A.), equipped with an electron ionization detector, was used to measure 2-pentyl pyridine, described by Boatright and Crum (1997). The quantitation was done by the ratio of m/z 93 ion to the m/z 99 and 98 ions from the internal standard. For each treatment, 2-pp was quantified in quadruplicate by gas chromatography/mass spectrometry.

pH treatments in preparation of soybean protein isolates (SPI)

To process soybean defatted flour into soybean protein isolate (Fig. 1), defatted flour was mixed with water (1:10 w/w) from a Barnstead Nanopure 4-Module System (Fisher Scientific, Pittsburgh, Pa., U.S.A.), the pH was increased with 1 N NaOH to pH 9.0, and the mixture held for 1 h. After centrifugation at 1500 × *g* for 10 min, the supernatant pH was decreased with 1 N HCl to pH 4.5 and held another 1 h. Then a second centrifugation at 1500 × *g* for 10 min was performed. After freezing overnight, samples were subjected to Freeze-Dry System LYPH LOCR 4.5 from Labconco (Kansas City, Mo., U.S.A.) for lyophilized drying under vacuum (0.008 mm Hg) at room temperature for 2 d.

Eight experiments (Table 1) were designed to determine effects of pH on formation of 2-pentyl pyridine. At the end of each experiment samples were frozen and lyophilized.

Statistical analysis

Analysis of variance (ANOVA) was done using Statistical Analysis System software package (SAS Institute Inc., 1995). Least significant differences (LSD) values were computed at $P < 0.05$, and comparison between means was done using the Tukey-Kramer HSD test.

RESULTS & DISCUSSION

TO UNDERSTAND THE EFFECTS OF PH ON THE OCCURRENCE OF 2-PP, a series of samples were taken at three stages during typical SPI preparation (Fig. 1). For Stressland at stage one, two, and three, the mean concentrations of 2-pp were 0.194 ppm, 0.191 ppm, and 0.201 ppm respectively (Exp. I, Table 2). Statistical analysis showed a difference between stages two and three, but no dif-

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ference between stages one and two, nor between stages one and three. Stressland contained the highest level of 2-pp, followed by KS4694 and Edison (Exp. I, Table 2). The cultivar null for lipoxygenase 1, 2, and 3 (LOX null) had the lowest concentration of 2-pp with mean levels of 0.128 ppm, 0.124 ppm, and 0.134 ppm at stage one, two, and three respectively. The results (Exp. I, Table 2) also showed that the concentrations of 2-pp in the various stages for Edison and KS4694 were not different. But for LOX null the levels were different ($p < 0.05$) between stages two and three.

The Experiments II, III, and IV showed the changes of 2-pp when various pH conditions were applied to individual stages. For Stressland soybeans at stage one (Fig. 1), increases occurred only between pH 4.5 and pH 7 (Exp. II, Table 3). If pH variations occurred at stage two, the concentrations of 2-pp increased to 0.868 ppm, 0.796 ppm, 0.713 ppm, and 0.146 ppm for Stressland, KS 4694, Edison, and LOX null, respectively, at pH 7 (Exp. III, Table 2). They increased by 355%, 320%, 306%, and 17%, respectively, compared to those at pH 4.5. Between pH 4.5 and 9, concentrations of 2-pp showed no significant differences for any cultivar. If the same pH variations were applied at stage three (Fig. 1), increases in 2-pp level from pH 4.5 to pH 7 (Exp. IV, Table 3) were not as high as those at stage two, but were still different. Also the concentrations of 2-pp showed no differences between pH 4.5 and pH 9.

There are at least two possible explanations for the formation of 2-pp in soybean protein processing. Buttery et al. (1977) proposed a mechanism for the formation of 2-pp through interaction of 2,4-decadienal with ammonia. Neutral pH conditions may be favorable for such a reaction or for other chemical or enzymatic processes that induce formation of substrates of such reaction. Zhang et al. (1993) found that protein deamidation for SPI was more structurally sensitive at neutral condition due to a succinimide formation mechanism. That may explain the increase of 2-pp under neutral experimental conditions in our study. The amino acids could also react with 2,4-decadienal to form 2-pp (Kim et al., 1996; Kim and Ho, 1998). Another possibility may be that neutral pH optimized the soybean protein configuration by causing amino acids to take the zwitterion form. Such configuration changes may help to release 2-pp that is bound to protein under alkaline or acidic pH conditions.

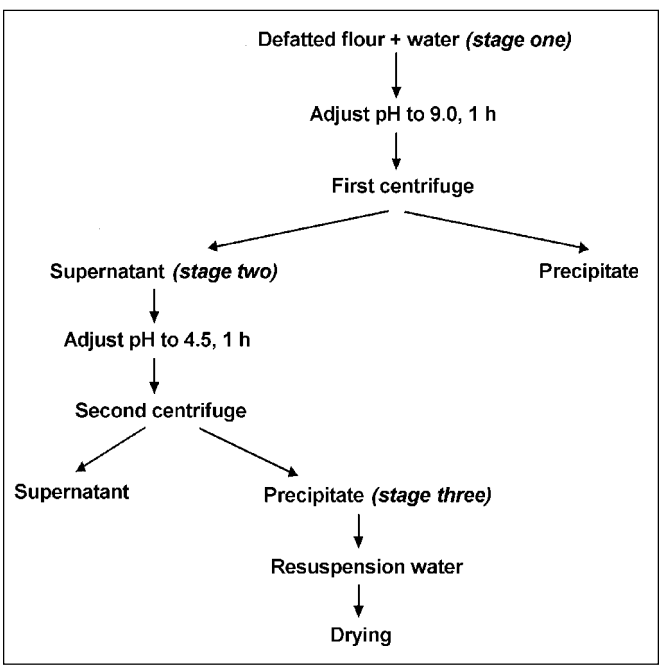

Fig. 1—Scheme for processing defatted flour into soybean protein isolates (SPI).

Table 1—Sample preparation to determine the effects of pH on the formation of 2-pentyl pyridine in SPI processing

Code	Stages during SPI process (Fig. 1)	Treatments at sampling points
Exp. I	Sampling at stage one, two and three	
Exp. II	At stage one	Adjust pH to 4.5, 7.0 or 9.0
Exp. III	At stage two	Adjust pH to 4.5, 7.0 or 9.0
Exp. IV	At stage three	Adjust pH to 4.5, 7.0 or 9.0
Exp. V	At stage two adjust pH to 7.0	Holding for 1 h, 2 h, or 3 h
Exp. VI	At stage two	Adjust pH to 7.0, then to 4.5, then back to 7.0
Exp. VII	After stage one, adjust pH to 7.0, then to stage two	Adjust pH to 4.5, 7.0, or 9.0
Exp. VIII	After first centrifugation	Adjust pH of precipitate to 4.5, 7.0, or 9.0

Exp. = Experiment.

Experiments were designed to search for reasons why 2-pp increased under neutral pH. Experiment V (Table 4) showed the concentrations of 2-pp as related to time at stage two. Results suggested that an increase happened quickly and seemed to reach its maximum level within 1 h. No further increase was observed from 1 to 5 h of holding at pH 7. Considering the fact that the soybeans null for lipoxygenase 1, 2, and 3 showed the least increase of 2-pp under neutral pH (Table 2), it is likely that lipoxygenase was involved in the formation of 2-pp by directly affecting the synthesis of either 2-pp or its precursors. Even though soybean lipoxygenase-1 was optimally active around pH 9 (Whitaker, 1994), lipoxygenase-2 showed a sharp pH maximum around pH 6.5, and lipoxygenase-3 displayed a broad optimum centered around pH 7 (Axelrod et al., 1981). The formation of elevated levels of 2-pp did not occur under either acidic (pH 4.5) or basic (pH 9) conditions (Table 2), possibly due to the decrease of lipoxygenase activities. However, the facts that 2-pp levels at stage one and three did not increase as high as that at stage two implied other mechanism were involved in the generation of 2-pp.

When the pH was held at 7 for the first hour (Exp. VI, Table 4), the concentration of 2-pp reached 0.718 ppm. Then, when the pH had been lowered to 4.5 for the second hour, its level decreased to 0.639 ppm. When the pH had been adjusted back to 7 for the third hour, the concentration increased back to 0.73 ppm. Results indicated that the highest levels of 2-pp corresponded to neutral pH, but not to acidic condition. The most common method (Thanh and Shibasaki, 1976) for preparing 11S soybean proteins is to precipitate and recover them by adjusting an alkaline soy extract to neutral pH, whereas for 7S proteins the pH is adjusted to 4.8. Our results suggest that 2-pp may be bound to 11S proteins rather than to 7S proteins.

If the pH before the first centrifugation (Fig. 1) was adjusted to 7 instead of 9, the level of 2-pp increased only to 0.196 ppm at stage two under neutral pH (Exp. VII, Table 3), lower than the 0.868 ppm in Experiment III. Snyder and Kwon (1987) pointed out that strong alkali would extract more protein but would also cause damage particularly to the sulfur-containing amino acids and destabilize the three-dimensional structure of proteins. This might explain why the neutral pH effect at stage two occurred only when the protein had been formerly subjected to the alkaline condition after stage one. Also, 2-pp might be released due to a change in configuration of proteins under the alkaline conditions.

The final experiment (Exp. VIII, Table 3) was to determine whether changing pH of the precipitate (insoluble components) after the first centrifugation (Fig. 1) would affect its 2-pp levels. Most of the precipitate materials were carbohydrates from cell

Table 2—Levels of 2-pentyl pyridine (ppm) during SPI processing

Soybean cultivar	Experiment[c] I			Experiment III		
	Stage one	Stage two	Stage three	pH 4.5	pH 7	pH 9
Stressland	0.194 ± 0.004[a]	0.191 ± 0.005[a]	0.201 ± 0.005[b]	0.182 ± 0.003[a]	0.868 ± 0.27[b]	0.199 ± 0.003[a]
LOX null[d]	0.128 ± 0.001[a]	0.124 ± 0.001[a]	0.134 ± 0.002[b]	0.126 ± 0.004[a]	0.146 ± 0.005[b]	0.124 ± 0.001[a]
Edison	0.167 ± 0.006[a]	0.174 ± 0.015[a]	0.164 ± 0.004[a]	0.170 ± 0.002[a]	0.713 ± 0.036[b]	0.181 ± 0.004[a]
KS4694	0.184 ± 0.004[a]	0.191 ± 0.002[a]	0.189 ± 0.005[a]	0.183 ± 0.001[a]	0.796 ± 0.015[b]	0.196 ± 0.003[a]

[a-b]Means (± standard deviation) within a row series in each experiment with no common superscripts differ (P < 0.05; n = 4).
[c]Experiment details in Table 1.
[d]Soybean variety null for lipoxygenase 1, 2, and 3.

Table 3—pH Effects on formation[c] of 2-pentyl pyridine (ppm) during SPI processing

	Exp.[d] II	Exp. IV	Exp. VII	Exp. VIII
pH 4.5	0.167 ± 0.0008[a]	0.028 ± 0.010[a]	0.174 ± 0.002[a]	N/D[e]
pH 7.0	0.182 ± 0.004[b]	0.238 ± 0.004[b]	0.196 ± 0.001[b]	0.193 ± 0.001[a]
pH 9.0	0.177 ± 0.002[ab]	0.214 ± 0.006[b]	0.174 ± 0.003[a]	0.210 ± 0.017[a]

[a-b]Means (± standard deviation) within a column series in each experiment with no common superscripts differ (P < 0.05; n = 4).
[c]Soybean cultivar was Stressland.
[d]Experiment details in Table 1.
[e]N/D = not detectable.
Exp. = Experiment.

Table 4—pH and time effects on formation[c] of 2-pentyl pyridine at stage[d] two

Exp. V[e]	Conc., ppm	Exp. VI	Conc., ppm
1 h	0.764 ± 0.010[a]	pH 7.0 first	0.718 ± 0.045[a]
3 h	0.760 ± 0.010[a]	pH 4.5	0.639 ± 0.016[b]
5 h	0.755 ± 0.002[a]	pH 7.0 back	0.7300 ± 037[a]

[a-b]Means (± standard deviation) within a column series in each experiment with no common superscripts differ (P < 0.05; n = 4).
[c]Soybean cultivr was Stressland.
[d]See Fig. 1.
[e]Experiment details in Table 1.
Exp. = Experiment. Conc. = Concentrations.

walls (Waggle et al., 1989). After the first centrifugation, the pH of the precipitate resuspended in water was close to 9, and it contained 0.199 ppm of 2-pp. When the pH dropped to 7, the level of 2-pp was not different from that at pH 9. However if the pH was lowered to 4.5, the concentration of 2-pp was too low to be detected. Since 2-pp is water soluble, its level in the fraction at pH 9 may have been left from stage one. Some inhibitors of lipoxygenases, and the generation of 2-pp, may be present in the carbohydrate fraction in soybeans. It is also possible that there were not enough substrates or precursors for the formation of 2-pp in the precipitate.

CONCLUSION

THE INCREASE OF 2-PENTYL PYRIDINE UNDER NEUTRAL CONDITIONS at stage two was an interesting result. It remains to be determined whether the formation of 2-pp results from chemical processes, from physicochemical characteristics of soybean proteins, or from both. Tracing the stable-isotope tags in the processing of soybean protein isolates may provide more conclusive evidence.

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