

# Preparation of Peanut Flavor Using Defatted Peanut Hydrolysate

林彥均、游銅錫；張耀南

E-mail: 9019875@mail.dyu.edu.tw

## ABSTRACT

Roasted peanut flavor is preferred by consumers. More than 300 volatile compounds have been identified from roasted peanut. Among the main components in peanut, amino acid, proteins, peptides, sugars and lipids were reported to be the important flavor precursors. The preparation of peanut oil in Western countries and in Taiwan is quite different. In Taiwan peanut oil is prepared from peanut by roasting first, and then cooking and pressing. In foreign, the preparation of peanut oil is without the roasting process. Therefore the residue of defatted peanut is rich in peanut flavor precursors, including sugars and nitrogen-containing compounds (especially proteins). The volatile compounds of peanut are reported to be generated from the Maillard reaction of sugars and amino compounds. In this thesis, proteases were used to break down the peanut protein and to release amino acids or peptides to be used as flavor precursors. In this study, defatted peanut was hydrolyzed using commercial available proteolytic enzymes Protease, Neutrase, Papain, Bromelain, Flavourzyme, and Corolase S-50. The best conditions for the defatted peanut hydrolysate preparation were determined. The defatted peanut hydrolysate with higher hydrolysis degree and less bitterness was then used to react with xylose at different pH and in different time to prepare a roasted peanut flavor. The best addition amounts of xylose, the optimum pH and reaction time were determined using a response surface methodology and the sensory evaluation method. After reaction using a formula consisting of the best amount of defatted hydrolysate, and xylose, the optimum pH and reaction time, volatile compounds in the reaction mixture were analyzed. The best composition of the mixture of defatted peanut hydrolysate and xylose was then used to react with some peanut flavor precursors, i.e., phenylalanine, arginine and histidine, to prepare a peanut flavor. The best addition amounts of these precursors were determined using a response surface methodology and the sensory evaluation method. Volatile compounds in each reaction mixture consisting of defatted peanut hydrolysate, xylose, phenylalanine, arginine and histidine were analyzed using GC-MS. The defatted peanut hydrolysate by Protease treatment was found to have the highest hydrolysis degree and the best total sensory acceptance. The pork hydrolysate by Corolase S-50 or Flavourzyme treatment was found to have the highest bitterness and less sensory acceptance. Two stage enzyme hydrolysate of defatted peanut by Protease-Neutrase (P-N) treatment was found to have higher hydrolysis degree and total sensory acceptance than those of using the individual enzyme. The main volatile compounds found in the heated defatted peanut hydrolysate by P-N two stage treatment were found to be sulfur-containing compounds, pyrazines, pyridines, furans, aldehydes, ketones, alcohols, esters, and acids. By using response surface methodology combined with a sensory evaluation methodology analysis, the optimal or best hydrolysis parameters were found to be: xylose 1.14 g, pH 7.24, hydrolysis time 2.49 hr, phenylalanine 0.88 g, arginine 0.57 g, and histidine 0.94 g, when the amount of P-N defatted peanut hydrolysate was fixed at 200 g. When comparing the volatile compounds in the heated solution using the best addition amount of xylose (X) and the heated solution containing xylose, phenylalanine, arginine and histidine (XPAH), it was found that X have higher amount of acids, aldehydes, alcohols, phenols, esters, furans, pyrans, hydrocarbons, ketones, pyrazines, pyrroles, pyridines, sulfides, thiophenes and thiazoles than those in XPAH. Key words : defatted peanut, protease, hydrolysis, thermal reaction, peanut flavor, volatile compounds, amino acid, Maillard reaction, xylose

Keywords : defatted peanut ; protease ; hydrolysis ; thermal reaction ; peanut flavor ; volatile compounds ; amino acid ; Maillard reaction, xylose

## Table of Contents

封面內頁 簽名頁 大葉大學碩士論文全文授權書 iii 中文摘要 iv 英文摘要 vii 誌謝 x 目錄 xi 圖目錄 xiv 表目錄 xvi 第一章 緒論 1 第二章 文獻回顧 第一節 蛋白質之酵素水解 4 第二節 水解系統的建立、控制及水解液利用 9 第三節 蛋白質水解物之呈味性質 14 第四節 焙炒花生的香氣成分及其前驅物 19 第五節 焙炒花生香氣化合物的形成機構 26 第六節 回應曲面實驗設計法 32 第三章 脫脂花生粉的成分分析及酸水解 摘要 36 第一節 前言 37 第二節 實驗材料與設備 38 第三節 實驗方法 40 第四節 結果與討論 44 第五節 結論 47 第四章 脫脂花生粉的酵素水解 摘要 48 第一節 前言 49 第二節 實驗材料與設備 50 第三節 實驗方法 52 第四節 結果與討論 55 第五節 結論 60 第五章 脫脂花生粉經二階段酵素水解之水解液之密閉加熱 反應所形成的香氣成分 摘要 61 第一節 前言 62 第二節 實驗材料與設備 64 第三節 實驗方法 66 第四節 結果與討論 70 第五節 結論 77 第六章 脫脂花生酵素水解液與木糖之密閉加熱模式反應 摘要 78 第一節 前言 79 第二節 實驗材料與方法 81 第三節 實驗方法 83 第四節 結果與討論 87 第五節 結論 99 第七章 脫脂花生酵素水解液與phenylalanine、arginine、histidine 之密閉加熱模式

反應摘要 100 第一節 前言 101 第二節 實驗材料與方法 103 第三節 實驗方法 105 第四節 結果與討論 108 第五節 結論 123  
第八章 總結論 124 參考文獻 126 圖目錄 圖 2.1 酵素水解蛋白質苦味的產生 18 圖 2.2 pyrazines之生成途徑 27 圖 2.3  
n-alkylbenzenes 之生成機制 28 圖 2.4 2-methylthiazole 之生成機制 29 圖 2.5 furfural 之生成機制 31 圖 2.6 反應曲面法設計  
流程圖 35 圖 4.1 脫脂花生粉經六種蛋白質水解酵素作用後之水解度 57 圖 4.2 脫脂花生粉在不同酵素濃度之Protease  
及Neutralse 作用下之水解度 58 圖 4.3 脫脂花生粉以Protease 和Neutralse在不同溫度作用 下之水解度 59 圖 4.4 脫脂花生粉  
以Protease 和Neutralse在不同pH值作 用下之水解度 59 圖 5.1 混合酵素及二階段酵素水解脫脂花生粉之流程圖 67 圖 5.2 脫脂  
花生粉經不同酵素處理法之水解度 73 圖 6.1 木糖與反應時間對應於模式反應液官能品評值之 回應曲面圖 93 圖 6.2 木糖與  
反應時間對應於模式反應液官能品評值之 等高線圖 93 圖 6.3 木糖與pH對應於模式反應液官能品評值之回應 曲面圖 94 圖  
6.4 木糖與pH對應於模式反應液官能品評值之等 高線圖 94 圖 6.5 pH與反應時間對應於模式反應液官能品評值之回應 曲面  
圖 95 圖 6.6 pH與反應時間對應於模式反應液官能品評值之 等高線圖 95 圖 7.1 Phenylalanine與histidine對應於模式反應液官  
能品 評值之回應曲面圖 113 圖 7.2 Phenylalanine與histidine對應於模式反應液官能品 評值之等高線圖 113 圖 7.3  
Phenylalanine與arginine對應於模式反應液官能品 評值之回應曲面圖 114 圖 7.4 Phenylalanine與histidine對應於模式反應液官  
能品 評值之等高線圖 114 圖 7.5 Phenylalanine與arginine對應於模式反應液官能品評 值之回應曲面圖 115 圖 7.6 Histidine  
與arginine對應於模式反應液官能品評值 之等高線圖 115 表目錄 表 2.1 各種水解法之優缺點的比較 5 表 2.2 苦味胜月太 之  
胺基酸序列 18 表 2.3 對焙炒花生香氣貢獻較大之化合物 20 表 2.4 花生油之一般脂肪組成分 25 表 3.1 本研究所用花生原豆  
及脫脂花生粉之成分分析 45 表 3.2 本研究所用脫脂花生粉之胺基酸組成 45 表 3.3 酸水解處理之脫脂花生粉中之胺基酸組  
成 46 表 4.1 脫脂花生粉蛋白質水解液經加熱後之官能品評結果 58 表 5.1 P-N二階段酵素處理之水解液中之胺基酸組成 72  
表 5.2 經不同酵素處理法處理之脫脂花生水解加熱液之官 能品評之結果 74 表 5.3 P-N二階段酵素水解處理之脫脂花生水解  
物模式反 應液中之揮發性成分 75 表 6.1 中心混成實驗設計變數 85 表 6.2 中心混成實驗設計之組合 85 表 6.3 不同量之木糖  
與脫脂花生粉酵素水解液之熱反應 液之官能品評結果 88 表 6.4 木糖與脫脂花生粉酵素水解液於不同pH值下之 熱反應液之  
官能品評結果 88 表 6.5 木糖與脫脂花生粉酵素水解液於不同反應時間下 之熱反應液之官能品評結果 88 表 6.6 中心混成設  
計及其實驗結果 91 表 6.7 中心混成實驗之迴歸分析表 92 表 6.8 反應曲面模式之變異系數分析(ANOVA) 92 表 6.9 脫脂花生  
水解物與木糖之模式反應液中之揮發性 成分 96 表 7.1 中心混成實驗設計變數 106 表 7.2 中心混成實驗設計之組合 106 表  
7.3 不同量之phenylalanine與木糖、脫脂花生粉酵素 水解液之熱反應液之官能品評結果 109 表 7.4 不同量之argininem與木糖  
、脫脂花生粉酵素水 解液之熱反應液之官能品評結果表 109 表 7.5 不同量之histidine與木糖、脫脂花生粉酵素水 解液之熱  
反應液之官能品評結果 109 表 7.6 中心混成設計及其實驗結果 111 表 7.7 中心混成實驗之迴歸分析表 112 表 7.8 反應曲面模  
式之變異系數分析(ANOVA) 112 表 7.9 由模式反應液中所鑑定到的香氣成份含量比較 116

## REFERENCES

1. 太田靜行 (1990) 天然調味料, *New Food Industry* 32:17.
2. 李秀、賴滋漢 (1976) 食品分析與檢驗. p. 195.
3. 洪哲穎、陳國誠 (1992) 回應曲面實驗設計法在微生物酵素生產上之應用, *化工* 39 (2) :3-18.
4. 徐錫樑、邱義源 (1993) 通氧氣與二氧化碳焙炒花生仁對其化學組成及油脂氧化安定性的影響. *食品科學* 20(1):1-8.
5. 陳怡宏 (1997) 蛋白質酵素水解液之生產技術. *食品工業* 29(11):34-40.
6. 彭秋妹、王家仁 (1991) 食品官能檢查手冊. 食品工業發展研究所. p. 10-33.
7. 鄭靜桂 (1997) 蛋白質之水解與水解液之利用. *食品工業* 29(5):10-17.
8. A.O.A.C. (1984) *Official methods of analysis of the Association of Official Analytical Chemists*. 14th edition, Washington D. C., U. S. A.
9. Adler-Nissen, J. (1982) Limited enzymic degradation of proteins: A new approach in the industrial application of hydrolysates. *J. Chem. Technol. Biotechnol.* 32:138-156.
10. Adler-Nissen, J. (1986) "Enzymic Hydrolysis of Food Proteins," Elsevier Applied Science Publ. Ltd., Barking, Essex. UK. 31:213-254.
11. Arctander, S. (1975) *Perfume and flavor chemicals*. Published by the author. Montclair, N.J. 31:142-158.
12. Basha, S. M. and Pancholy, S. K. (1981) Identification of methionine-rich polypeptides in peanut (*Arachis hypogaea* L.) seed. *J. Agric. Food Chem.* 29:331-335.
13. Box, G. E. P. and Wilson, K. B. (1951) On the experimental attainment optimum conditions. *J. Roy. Statist. Soc.* B13:1-45.
14. Brown, D. F.; Seen, J. V. and Dollear, F. G. (1973) Comparison of carbonyl compounds in raw and roasted runner peanuts. *J. Agric. Food Chem.* 21:463-470.
15. Chiou, R. Y. Y.; Chang, C. Y. and Ho, S. (1991a) Characteristics of peanut kernels roasted under various atmospheric environments. *J. Agric. Food Chem.* 39:1852-1856.
16. Chiou, R. Y. Y.; Chang, Y. S.; Tsai, T. T. and Ho, S. (1991b) Variation of flavor-related characteristics of peanut during roasted as affected by initial moisture contents. *J. Agric. Food Chem.* 39:1155-1158.
17. Frokjaer, S. (1944) Use of hydrolysates for protein supplement -ation. *Food Technol.* 48(10):86-88.
18. Garner, J. (1982) Heterocyclic aroma compounds precursors. In "Chemistry of heterocyclic compounds in flavour and aromas" ed. By Vernin, G., Ellis Horwood Limited, England. p. 23-25.
19. Heath, H. B. (1978) Flavoring materials. Part 6. Imitation flavorings. In "Flavor technology-profiles, products, applications" . AVI Publ. Co., Lnc., Westport Connecticut. p. 352.
20. Heller, S. R. and Milne, G. W. A. (1978) EPA/NIH mass spectral database. Vol. 1., U. S. Government Printing Office, Washington, D. C. U. S. A.
21. Heller, S. R. and Milne, G. W. A. (1980) EPA/NIH mass spectral database. Supplement. 1., U. S. Government Printing Office, Washington, D. C. U. S. A.
22. Heyne, K.; Stute R. and Paulsen H. (1966) Brungsreaktionen and Fragmentierungen von kohlenhydraten. *Carbohydrate Res.* 2:132.
23. Himmelblau, D. M. (1970) *Process analysis by statistical methods*. John Wiley and sons. Inc., New York. p. 230-292.
24. Ho, C. T.; Lee, M. H. and Chang, S. S. (1981) Isolation and identification of volatile components from roasted peanuts. *J. Food Sci.* 47:127 -133.
25. Ho, C. T.; Smagula, M. S. and Chang, S. S. (1978) The synthesis of 2-(1-pentenyl) furan and its relationship to the

reversion flavor of soybean oil. *JAOCS*. 55:233. 26. Johnson, B. R.; Waller, G. R. and Foltz, R. L. (1971a) Volatile components of roasted peanuts:basic fraction. *J. Agric. Food Chem.* 19:1025-1027. 27. Johnson, B. R.; Waller, G. R. and Burlingame, A. L. (1971b) Volatile components of roasted peanuts:neutral fraction. *J. Agric. Food Chem.* 19:1020-1024. 28. Kirmura, J.; Shimizu, A.; Kimizuka, A.; Ninomiya, T. and N. Katsuya. (1969) The contribution of peptides and amino acids to the taste of foodstuffs. *J. Agri. Food Chem.* 19:689. 29. Koehler, P. E.; Mason, M. E. and Newell, J. A. (1969) Formation of pyrazine compounds in sugar-amino acid model system. *J. Agric. Food Chem.* 17:393-396. 30. Koehler, P. E.; and Odell, G. V. (1970) Factors affecting formation of pyrazine compounds in sugar-amine reactions. *J. Agric. Food Chem.* 18:895. 31. Kuninaka, A.; Kibi, M. and Sakaguchi, K. (1964) History and development of flavor nucleotides. *Food Technol.* March:29. 32. Lahl, W. J. and Braun, S. D. (1994) Enzymatic production of protein hydrolysates for food use. *Food Technol.* 48(10):68-71. 33. Lee, M. H., Ho, C. T. and Chang, S. S. (1980) Isolation and identification of volatile components from roasted peanuts. *J. Agric. Food Chem.* 47:127-133. 34. Maga, J. A. (1977) Alkylpyrazine level in various vegetable protein flavor. *Lebensm-Wiss. Technol.* 10(2):100. 35. Mason, M. E.; Newell, J. A.; Johnson, B. R.; Koehler, P.E. and Waller, G. R. (1969) Nonvolatile flavor compounds of peanuts. *J. Agric. Food Chem.* 17:728-732. 36. Mason, M. E.; Johnson, B. R.; Hamming, M. C. (1967) Volatile components of roasted peanuts:the major mono-carbonyls and some noncarbonyl compounds. *J. Agric. Food Chem.* 15:66-72. 37. Mazur, R. H.; Schlatter, J. M.; and A. H. Goldkamp, J. (1969) Structure-taste relationship of some dipeptides. *Amer. Chem. Soc.* 91:2684. 38. Merritt, C. and Robertson, D. H. (1967) The analysis of protein, peptides and amino acids by pyrolysis gas chromatography and mass spectrometry. *J. Gas Chrom.* 5:96. 39. Min, D. B. S.; Ina, K.; Peterson, R. J. and Chang, S. S. (1977) The alkylbenzenes in roast beef. *J. Food Sci.* 42(2):503. 40. Murray, T. K. and Baker, B. E. (1952) Studies on protein hydrolysate. *J. Sci. Food Agric.* 3:470-475. 41. Newell, J. A.; Mason, M. E. and Matlock, R. S. (1967) Precursor of typical roasted peanut flavor. *J. Agric. Food Chem.* 15:767-772. 42. Nonaka, M.; Black, D. R. and Pippen, E. L. (1967) Gas chromatographic and mass spectrometral analysis of cooled chicken meat volatiles. *J. Agric. Food Chem.* 15:713. 43. Oupudissakoon, C. and Young, C. T. (1984) Changes in free amino acid and sugars of peanuts during oil roasting. *Peanut Sci.* 11:6-9. 44. Pattee, H. E.; Singleton, J. A.; Johns, E. B. and Mullin, E. B. (1970) Effects of storage time and conditions on peanut volatiles. *J. Agric. Food Chem.* 18:353-360. 45. Sakaguchi, M. and Shibamoto, T. (1978) Formation of heterocyclic compounds from reaction of cystamine and D-glucose, acetaldehyde or glyoxal. *J. Agric. Food Chem.* 26:1179-1183. 46. Shibamoto T. and Bernhard R. A. (1976) Effect of time, temperature and reactant ratio on pyrazine formation in model system. *J. Agric. Food Chem.* 24:847. 47. Shu, C. K. and Waller, G. R. (1971) Volatile components of roasted peanut:comparative analysis of the basic fraction. *J. Food Sci.* 36:579-583. 48. TNO (1988) Compilation of mass spectra of volatile compounds in food, Central Institute for Nutrition and Food Research-TNO. The Netherlands. 49. Vitzthum, O. G. and Werkhoff, P. (1974) Newly discovered nitrogen-containing heterocycles in coffee aroma. *Z. Lebensm. Unters-Forsch.* 156:300-307. 50. Walradt, J. R.; Pittet, A. O.; Kinlin, T. E.; Murlaidhara, R. and Sanderson, A. O. (1971) Volatile components of roasted peanuts. *J. Agric. Food Chem.*, 19:972. 51. Young, C. T. (1973) Influence of drying temperature at harvest on major volatiles released during roasting of peanuts. *J. Food Sci.* 38:123-130.