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Physical, chemical, and sensory characteristics of dehydrated shiitake mushrooms

David D. Wilson

To the Graduate Council:

I am submitting herewith a thesis written by David D. Wilson entitled "Physical, chemical, and sensory characteristics of dehydrated shiitake mushrooms." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

John R. Mount, Major Professor

We have read this thesis and recommend its acceptance:

Sharon Melton,Marjorie P. Penfield

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Associate Vice Chancellor
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Date April 14, 1992

PHYSICAL, CHEMICAL, AND SENSORY CHARACTERISTICS OF DEHYDRATED SHIITAKE MUSHROOMS

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

David D. Wilson
May 1992

DEDICATION

This thesis is dedicated to the authors mother, Jean Marie Wilson, for all the love and support she provided for David and his sisters Elaine, Erica, and Debra, throughout their childhood and college years.

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The author would also like to recognize two individuals that are primarily responsible for spurring his studies in food science, and they are executive chefs Herman Schwaiger of The Grand Hotel (Mackinac Island, MI) and Dan Huntsbarger of The Gandy Dancer (Ann Arbor, MI).

ABSTRACT

Shiitake mushrooms grown in Claiborne County, Tennessee were cryogenically frozen, held at -31°C until processing and then dehydrated in a Armfield forced-air, tray drier. The mushrooms were initially dried at 50°C for 3 hr and then finished at 40, 50, or 60°C for 6, 5 or 4 hr, respectively.

The shiitake mushrooms were then evaluated for free glutamate (MSG), water soluble carbohydrates (WSC) and ninhydrin reactive materials (NRM), proximate composition, color, and flavor strength. The flavor strength was evaluated by a sensory panel of 8 members from Asian countries selected on the basis of their familiarity with shiitake flavor and sensory methodology.

MSG and NRM were not significantly affected by dehydration conditions but increased under lower temperatures and longer times for dehydration and decreased slightly at higher temperatures and shorter times of dehydration. MSG increased from 3.11 mg/g in partially dehydrated shiitake to 3.12 mg/g in shiitake dried at 40°C for 6 hr, and decreased to 2.40 mg/g in the shiitake dried at 60°C for 4 hr. NRM increased from 29.2 mg/g to 32.8 mg/g and decreased to 25.1 mg/g under the same conditions. WSC was significantly affected by dehydration conditions. The WSC decreased from 3.63 mg/g in the partially dried shiitake to 2.47 mg/g in the shiitake dried at 60°C. The mushrooms

dried at 50 and 40°C were significantly lower than the other treatments at 1.56 mg/g and 1.11 mg/g, respectively.

The shiitake mushrooms are mostly water, 88.2%, and the solid content is 76% carbohydrates of which 9% is dietary fiber. The dehydration temperature and time did not affect the proximate composition of the mushroom caps and stems. However, the mushroom caps contained more moisture, protein and ash while the stems contained more dietary fiber.

The mushrooms decreased in lightness from a Hunter L value of 66.2 in the partially dehydrated caps to 42.4-43.7 in the completely dried caps.

Mushrooms that had second phase drying temperatures of 40 and 50°C had significantly stronger flavor than either freeze-dried mushrooms or mushrooms with a second phase drying temperature of 60°C. Mushrooms finished at 40 and 50°C were equal in flavor strength to two commercial shiitake samples and had a significantly stronger flavor than one commercial sample. Dehydrated stems were found to be similar in flavor to the dried caps.

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CHAPTER I

INTRODUCTION

Exotic mushrooms are gradually gaining acceptance in the American diet and forecasts for the next few years are for increased demand (USDA, 1989a,b). The current market value of exotic mushrooms is approximately 22 million dollars, 75% of which is from sales of shiitake (USDA, 1989c).

The shiitake mushroom is a native cultivar of Japan, where it has been enjoyed for over 2000 years (Mori, 1986). Shiitake (Lentinus edodes, Sing.) means "tree mushroom" in Japanese, and is also known as "black", "forest", or "oak" mushrooms (Jones, 1988).

Late in the 1970s, the first American grown shiitake mushrooms were sold in up-scale restaurants and groceries (San Antonio, 1981). The mushrooms were marketed for their exotic name, unique appearance-dark brown cap with white gills, and healthy nutrient value (Backas, 1988; Duggleby, 1989; Mook, 1989). The shiitake market solidified in the mid-1980s because of several developments: the formation of growers associations/co-ops and extension research (Anthes, 1987; Turner, 1988), widespread approval of the forest industry (Anonymous, 1987; Cook, 1989), the presence of Japanese interests and technology in America (Miller and

Jong, 1987), and a steady retail price of approximately $5/lb. (USDA, 1989a,c).

Shiitake mushrooms are a wood decay fungi that can utilize a wide variety of low-cost/waste substrates for growth nutrients, including: small, 5-10 cm diameter hardwood logs, bran, corn husks, grains, or sawdust (Diehle and Royse, 1986; Ito, 1978; Leatham, 1983; Leatham and Leonard, 1989; Pettipher, 1988; Wuest, 1989). Since shiitake utilized low- value readily available substrates, provided a high retail price, and required minimal start-up revenue, the first shiitake mushrooms were grown by farmers who wanted to supplement their incomes with small outdoor mushroom operations (San Antonio, 1981). Commercial success can be attributed to a steady, year-round supply, which was made available through indoor cultivation in large, environmentally controlled mushroom growing houses (Przybylowicz and Donoghue, 1988).

Shiitake mushrooms are now grown in more than 20 states in America (Anonymous, 1989; USDA, 1989c) and Canada (Patrick et al., 1983). Recently, shiitake were successfully cultivated outdoors in Claiborne County, Tennessee, but due to an erratic market and poor refrigerated shelf-life characteristics (Kikuchi et al., 1984; Minamida et al., 1980), potential revenue was lost from unsold product (Heiskell, 1990). This is not an isolated problem, however, since in 1989 over 189,000 lb of

fresh shiitake went unsold (USDA, 1989c). Thus, an alternate preservation method for shiitake mushrooms is needed domestically to capture revenues during market shortfalls. Drying shiitake mushrooms is a potential answer to this problem.

Dried shiitake products are not currently produced domestically, however, imported shiitake products are marketed at a premium price and a variety of food applications exist in soups, sauces, and processed foods (Anonymous, 1991). Additionally, drying greatly intensifies the flavor of shiitake (Anonymous, 1991; Tanaka et al., 1974), the technology is readily available (Cho et al., 1981), and start-up expenditures are minimal.

The objectives of this study were: to determine physical and chemical changes that occur in pre-frozen shiitake caps and stems during optimal dehydration conditions, to relate these changes to dried shiitake flavor strength, and to compare the flavor of experimental treatments with that of commercially dried shiitake products.

CHAPTER II

REVIEW OF LITERATURE

Similarity between Allium spp. and Shiitake

The Allium family of vegetables includes onion, garlic, scallions, shallots, leeks, chives, and ramps (Carson, 1987; Nock and Mazelis, 1986). The fungi Lentinus edodes (shiitake) has several similarities with the members of the Allium family, including:

i. almost identical enzymatic pathways for derivatization of characteristic sulfur volatiles (Heath and Reineccius, 1986; Iwami and Yasumoto, 1980; Lindsay, 1985),

ii. high sugar levels (very hygroscopic) when rehydrated (Carson, 1987; Przybylowicz and Donoghue, 1988), and

iii. reported beneficial physiological effects on health, which are supported by scientific evidence on hypocholesterolemic, antiviral, and antitumor properties (Breene, 1989; Carson, 1987; Przybylowicz and Donoghue, 1988; Wood, 1989).

The remainder of this section will focus on the similarities and differences in the flavor development of allium spp. and shiitake.

Tissue Disruption

The development of the sulfurous aromas in allium and shiitake begins with the disruption of cellular tissue by bruising, cutting, or crushing. In allium spp., the aromatic precursors are physically separated or compartmentalized from the hydrolytic enzymes by cell walls. Cysteine-sulfoxides (CS) are stored in the cytoplasm, while the enzyme CS lyase is found in the vacuoles (Lancaster and Collin, 1981). After tissue disruption has occurred, sulfur volatiles are produced from classic enzyme-substrate interactions (deMan, 1980; Lindsay, 1985; Richardson and Hyslop, 1985; Zubay, 1988).

Compartmentalization of shiitake enzymes and substrates is currently unexplored, however, freezing is known to disrupt tissue in mushrooms and cause increased enzymatic browning (Fuster et al., 1984). Since shiitakes' dried flavor is developed through tissue disruption, it seems likely that freezing would enhance flavor development during dehydration, however, this theory is also unexplored.

Enzymatic Pathways

The enzymatic pathways in allium spp. and shiitake generate the same primary products: ammonia, pyruvic acid, formaldehyde, and sulfur aromatics. The pathways for onion, garlic, and shiitake are shown in Fig. 1, 2, and 3, respectively. Sulfur aromatics are very unstable, however, and decompose rapidly into smaller sulfur volatiles even at

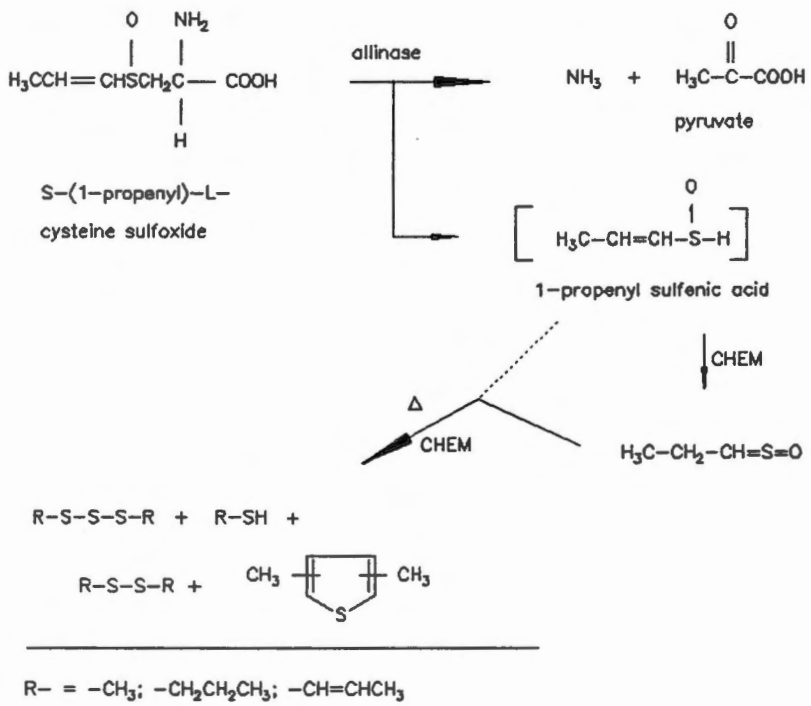

Fig. 1-Reactions involved in the formation of onion aroma.

Adapted from:

Carson, J.F. 1987. Chemistry and biological properties of onions and garlic. Food Rev. Int. 3(1/2): 71.

Lindsay, R.C. 1985. Flavors. In Food Chemistry, O.R. Fennema (Ed.), p. 601. Marcel Dekker, Inc., New York.

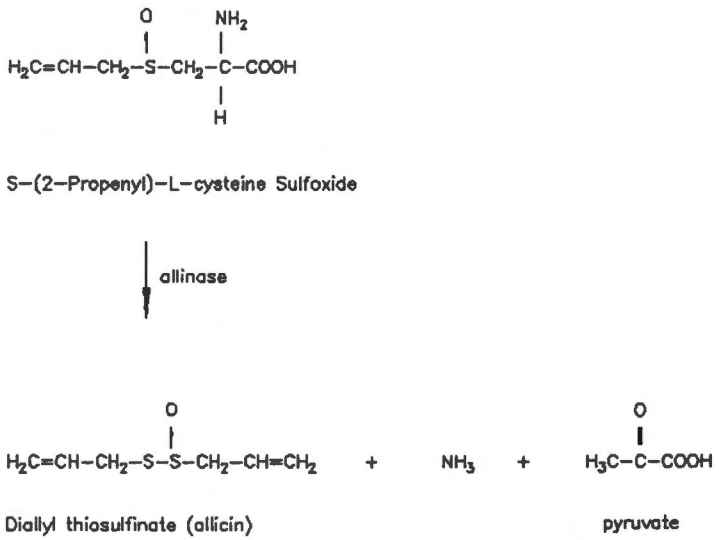

Fig. 2-Formation of the principal aroma compound of fresh garlic.

Adapted from:

Carson, J.F. 1987. Chemistry and biological properties of onions and garlic. Food Rev. Int. 3(1/2): 71.

Lindsay, R.C. 1985. Flavors. In Food Chemistry, O.R. Fennema (Ed.), p. 601. Marcel Dekker, Inc., New York.

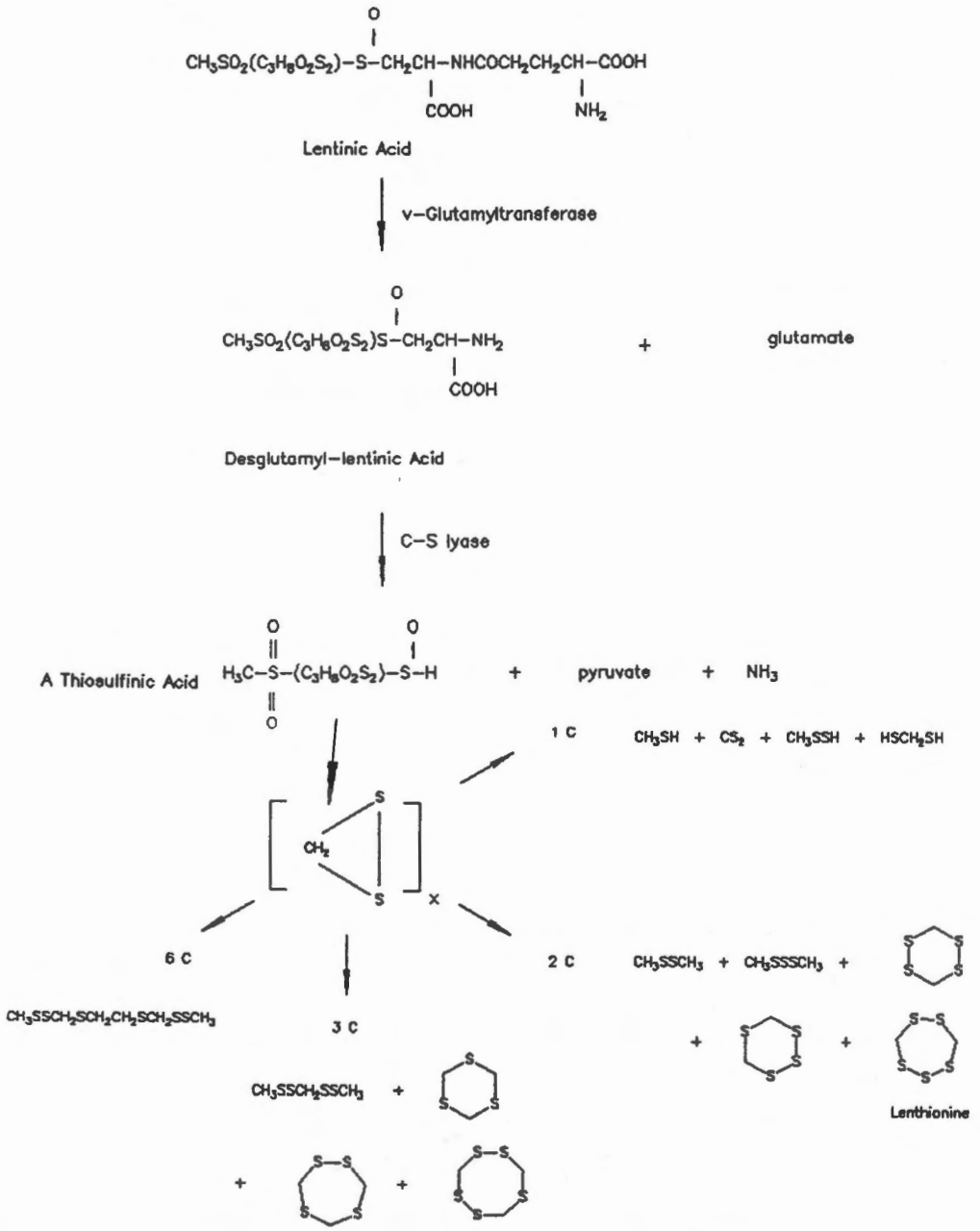

Fig. 3-Formation of sulfur volatiles in Shiitake mushrooms.

Adapted from:

Chen, C.C., Liu, S.E., Wu, C.M., and Ho, C.T. 1986. Enzymatic formation of volatile compounds in shiitake mushroom (Lentinus edodes Sing.). In Biogeneration of Aromas, T.H. Parliment and R. Croteau (Ed.), p. 176, Amer. Chem. Soc., Washington, DC.

optimal conditions (Chen and Ho, 1986a; Shankaranarayana et al., 1982). Since isolation and analysis are especially difficult for these compounds, several researchers have indirectly estimated sulfur aroma by using pathway by-products such as pyruvic acid or formaldehyde. Pyruvic acid is produced on an equi-molar basis to total sulfur volatiles and has thus been used as a crude, but accurate, measure of fresh, canned, and dehydrated onion aroma intensity (Chua et al., 1968; Peleg et al., 1970; Schwimmer and Guadagni, 1962; Schwimmer and Weston, 1961). Although the relationship between pyruvic acid and onion aroma intensity is well documented, an analogous relationship between shiitake aroma by-products and aroma intensity has not been examined by sensory evaluation techniques. The levels of pyruvic acid, formaldehyde, and lenthionine (the main aroma compound), however, all increase with drying (Fujimoto et al., 1974; Yamazaki et al., 1980; Yasumoto et al., 1971b).

Sulfur Aromatic Precursors

The substrates for sulfur aromatic compounds in allium spp. and shiitake are oxidized amino acid (cysteine) compounds cumulatively known as cysteine sulfoxides. CS are acted upon by the enzyme CS lyase (alliinase), which produces unstable thiosulfinates, ammonia, and pyruvic acid (Fig. 1 and 3). The thiosulfinates decompose into di- and trisulfides, and can polymerize into heterocyclics such as trithiolanes and thiophenes (Carson, 1987; Schwimmer and

Mazelis, 1963; Yasumoto et al., 1974). The aromatic differences between allium spp. and shiitake are primarily attributed to the quantity and nature of the CS precursors. For example, onion , garlic, and shallots have significantly higher CS levels than chives, leeks, and scallions. The specific CS are also slightly different between garlic and onion, although they are primarily the same for onion, leek, and scallion. In addition, the lyase enzyme (EC 4.4.1.4) for each allium species exhibits slight differences in substrate specificity and optimal activity based upon the level and concentrations of the CS (Carson, 1987; Ho et al., 1989; Iwami et al., 1975a). Finally, CS lyase activity is inhibited by thiamin in both allium spp. and shiitake (Carson, 1987; Iwami et al., 1982).

While allium spp. have several CS precursors, shiitake mushrooms have only one known flavor precursor-lentinic acid (Iwami et al., 1975c). Lentinic acid forms shiitakes' characteristic aroma (Fig. 3) by the transfer of a gamma-glutamyl group, and decomposition of its CS and thiolsulfinates. Shiitakes' CS residues are known to form lenthionine and other heterocyclic sulfur compounds by polymerization of a disulfide intermediate (Fig. 2) (Chen et al., 1984; Chen and Ho, 1986a; Morita and Kobayshi, 1966). The resulting heterocyclic sulfur compounds are very unstable, and rapidly fragment into smaller molecular weight sulfur compounds including several sulfides (di, tri, and

tetra).

The enzyme gamma-glutamyl transferase (GGT) (EC 2.3.2.2) exhibits a very high enzymatic activity in fresh shiitake mushrooms and is similar to GGT's from other plant and animal sources (Goore and Thompson, 1967; Iwami and Yasumoto, 1982a,b; Iwami et al., 1975a,b,c; Leibach and Binkley, 1968). GGT is pH dependent: below pH 5.5 the enzyme is inactive, between pH 5.5 and 7 the enzyme hydrolyses free glutamate, and above pH 7 the glutamate is transferred to suitable acceptor molecules (Aoyagi et al., 1980a, 1982a; Iwami et al., 1975a). Aoyagi and coworkers (1980a,b; 1982b) have shown that free amino acids act as acceptors for the glutamyl transfer, and that cysteine, serine, methionine, phenylalanine, and leucine are among the best amino acid acceptors (Yasumoto et al., 1971b).

Non-Protein Nitrogen Compounds

Allium spp. contain high concentrations of non-protein sulfur amino acids (1-5% DWB). Gamma-glutamyl (GG) peptides are recognized as necessary intermediates in the production of sulfurous volatile flavor components in onion and shiitake (Aoyagi et al., 1980b, 1982b; Lancaster and Shaw, 1989). In fact, sulfur volatiles are generated in this fashion for many vegetables, thus it's not surprising that shiitake and allium have several GG peptides in common (Aoyagi et al., 1980b; Kasai and Larson, 1980; Nock and Mazelis, 1986; Yasumoto et al., 1971a).

In onion, GG peptides are synthesized in the chloroplasts, transported to the cytoplasm, and are acted upon by GGT, which produces sulfoxides/flavor precursors as products (Lancaster et al., 1989; McCallion and Lancaster, 1984). GG peptides can also be found in other locations besides the chloroplasts (roots, tubers, etc.), but are synthesized less efficiently in these locations (Lancaster et al., 1989).

Extrinsic Factors Affecting Shiitake Flavor

Shiitake is unique among mushroom species because it is preferred in the dried state everywhere in the world (Przybylowicz and Donoghue, 1988), except the United States where fresh shiitake are predominant. Many extrinsic factors affect the flavor of dried shiitake, including: natural flavor components, growing conditions, and mushroom anatomy (cap versus stem).

Natural Flavor Components

Mushrooms in general are characterized by several common flavoring compounds (Cronin and Ward, 1971; Maga, 1981; Pyysalo and Suihko, 1976). For example the main volatile compound of many fresh and canned mushroom species is 1-octen-3-ol, which is commonly called "mushroom alcohol" by flavorists (Picardi and Issenberg, 1973; Wu and Chen, 1986). In general, mushrooms contain high levels of flavor

potentiators including glutamic acid and a variety of 5′-nucleotides (Maga, 1987; Nakajima et al., 1961). Dijkstra (1976) indicates that glutamic acid is present in fresh, canned, and dried mushrooms at levels sufficient to have an important influence on flavor.

Inositine-5′-monophosphate (IMP), guanosine-5′-monophosphate (GMP), and monosodium glutamate (MSG) are found in high levels in dried shiitake mushroom (Anonymous, 1991). The chemical structures of GMP and IMP are similar, with GMP containing an amino group at the 2 position of the purine base, while IMP has only a hydrogen at that position (Yamaguchi et al., 1968; Yamaguchi and Kimizuka, 1979).

Nucleotides and MSG have taste potentiator effects on a wide variety of foods, and are collectively known as "umami" compounds. Several MSG-related substances also have savory tastes, but are generally less intense than MSG. Multidimensional scaling and animal taste receptor mechanism studies indicate that the umami taste is independent of the four basic tastes of sweet, sour, bitter and salty (Kawamura and Kare, 1987; Sugita, 1986). This fifth taste has been described as having a brothy or MSG-like flavor. Umami compounds are potent flavorants (very low flavor thresholds) because of synergistic effects with one another, and tertiary synergism with free amino acids (Mabrouk, 1976; Sugita, 1986). Several extensive reviews of umami research are available for further information (Filer et al., 1979;

Kawamura and Kare, 1987; Maga, 1987; Sugita, 1986).

Growing Conditions

Mushrooms are directly affected by growing conditions, thus, proximal constituents and flavorant levels are somewhat variable (Bano and Rajarathnam, 1988; Crisan and Sands, 1978; Haney, 1989; Leatham and Leonard, 1989; Przybylowicz and Donoghue, 1988; Wuest, 1989). Moisture, carbon dioxide levels, lighting, temperature, substrate, and strain/species can all affect composition. Other minor variations can occur due to mushroom maturity and post-harvest treatments (time since picking, storage atmosphere).

Mushroom Anatomy

Notable differences occur between cap and stem and can affect dried shiitake flavor, including: flavor substrate concentrations, enzyme activities, and rehydration characteristics. Lignin and other cellulose materials have less favorable rehydration characteristics and are predominant components of shiitake stems (Aoyagi and Sugahara, 1986). Secondly, caps have twice the concentration of proteinaceous materials responsible for flavor derivation, versus those in the stem (Hong et al., 1989; Sugahara et al., 1975). Thirdly, enzyme levels are substantially higher in mushroom caps (Burton, 1986). Thus, it appears that differences exist between cap and stem that could cause systematic sampling error (Ott, 1989) in analyses and sensory evaluation (Larmond, 1977; O'Mahony,

1982).

Intrinsic Factors Affecting Dried Shiitake Flavor

Enzymatic Flavor Formation

Ribonucleases The characteristic taste of shiitake mushrooms is imparted by 5′-guanosine monophosphate (GMP) (Nakajima et al., 1961). This compound can be found in both fresh and dried fruit bodies, but in much higher levels in the dried product (Shimazono et al., 1979). This fact led to the isolation and identification of several ribonucleases from shiitake mushrooms, including both acid and alkaline acting enzymes.

The nuclease responsible for increased concentrations of 5′-nucleotides (GMP, IMP, AMP), and GMP in particular, is believed to be an alkaline endonuclease (Kurosawa et al., 1983). This alkaline enzyme exhibits optimal production of 5′-nucleotides at pH 8.5-9.5, is inactivated at temperatures in excess of 50°C or pH < 5.0, and is enhanced by 0.3-0.5 mM sodium chloride at pH=8 (Kurosowa et al., 1983).

Most sources of ribonucleases are acid acting enzymes and require a cofactor such as pyridoxal phosphate for activity (Sumner and Somers, 1947; Whitaker, 1972). One acid nuclease from shiitake is enhanced with pyridoxal phosphate buffer, but it is not required for activity (Iwami et al., 1975b). A total of two acid nucleases have been

isolated from fresh shiitake, but their effects on shiitake flavor are not well characterized. Their optimal activities are at pH's of 4.0-4.5 and 7.0-7.7 (Endo et al., 1980).

Sulfurous Compounds Cyclic and straight chain sulfur volatiles are believed to be formed by polymerization of a cyclic methyl disulfide intermediate of CS decomposition (Fig. 3) (Chen and Ho, 1986b). Lenthionine (1,2,3,5,6-pentathiepane) is the major volatile formed and has the character impact aroma of shiitake (Chen and Ho, 1986a; Wada et al.,1967). Lenthionine is physically unstable under mild aqueous conditions, however, it is quite stable when crystallized (Morita and Kobayashi, 1966). Lenthionine is relatively nonpolar and is thus more soluble in oil (2.3%) and nonpolar solvents, than water (0.0017%) at 25°C. The recognition thresholds for lenthionine in water and vegetable oil are 0.27-0.53 ppm and 12.5-25 ppm, respectively (Wada et al., 1967). Thus, although lenthionine is more soluble and stable in oil, oil inhibits the aroma recognition almost 50 fold versus that of water. Three other cyclic sulfur compounds are also formed with lenthionine, including, 1,2,4,5-tetrathiane, 1,2,3,5-tetrathiane, and 1,2,4-trithiolane (Chen et al., 1986). The aroma of 1,2,4-trithiolane has been described as having a garlic note when isolated from egg (Gil and MacLeod, 1981).

All of the above mentioned compounds are also found from chemical syntheses of lenthionine and 1,2,4-

trithiolane. Chen et al.(1986) have proposed that chemical reactions may be the dominant forces in the final stages of shiitake's volatile generation, but this theory is not presently supported by completed research. Chen et al.(1986) believe that dried mushrooms should be void of enzymatic activity. In fact, the enzymes responsible for sulfur volatiles (GG transferase and CS lyase) have not been isolated from dried shiitake. Both enzymes are heat labile, with enzyme temperatures higher than 50°C for 30 min drastically reducing rehydrated flavor development (Chen et al., 1986). Furthermore, typical drying schedules for shiitake exceed 50°C for at least 6 hr (Przybylowicz and Donoghue, 1988).

Lipoxygenase Mushroom alcohol and other 8 carbon carbonyl compounds are very common components of the volatile fraction of many types of fresh fungi (Cronin and Ward, 1971; Maga, 1981; Pyysalo and Suihko, 1976), and it is well known that 8 carbon carbonyl compounds are common end products of unsaturated free fatty acid oxidation (Chen et al., 1986; Heath and Reineccius, 1986; Ho et al., 1989; Leegwater et al., 1962; Tressl et al., 1981, 1982). Therefore, it is not surprising to find a high percentage of unsaturation (about 70% linoleic) in the crude fat of many species (Hashiguchi et al., 1984; Hong et al., 1988; Yoshida et al., 1979).

Although 8 carbon carbonyl compounds are important

flavor impact chemicals for many fresh mushrooms, they are extremely volatile and are generally lost during heat processing (Dijkstra, 1976; Picardi and Issenberg, 1973; Wu and Chen, 1986). For all practical purposes, the level of 8 carbon carbonyl compounds is negligible and does not significantly affect the flavor of dried shiitake. Fresh shiitake mushrooms, however, have been utilized in batch fermentation of free fatty acids (linoleic) to produce mushroom alcohol (Chen et al., 1986).

Polyphenoloxidase Polyphenoloxidase (PPO) is the enzyme responsible for formation of browning pigments called melanins in a variety of fruit and vegetable products (Burton, 1986; deMan, 1980; Richardson and Hyslop, 1985; Schultz, 1960). PPO catalyzes the oxidation of phenolic compounds into quinones, and the subsequent polymerization of quinones into brown compounds. Enzymatic activity of PPO is dependent on the availability of oxygen, substrate, and a copper cofactor. Several methods are used to deter PPO, including inhibition by lowering the pH or preventing quinone oxidation (sulfites, amino acids), and heat inactivation. Control of PPO is important during food processing because tissue is disrupted, and enzymes and substrates are allowed to interact. PPO can be beneficial in products such as tea and coffee, but is usually harmful to food quality attributes.

The activity of PPO in mushrooms is generally very

high, with much of the activity concentrated in the tissue layer covering the cap (Burton , 1986). Frozen mushrooms brown due to PPO on thawing because cell walls are disrupted (Fuster et al., 1984). Shiitake mushrooms, however, are naturally dark brown in color and the effect of further browning due to PPO on quality attributes has not been evaluated.

Drying Conditions

Since shiitake mushrooms contain approximately 90% water, the drying process can be expected to last several hours. Traditional Japanese cabinet drying methods control moisture loss, temperature, and aeration meticulously over a 14-16 hr drying schedule (Fig. 4). The initial drying stage appears to remove the free water from the mushroom, while the remaining periods are said to minimize case hardening and enzyme inactivation (Cho et al., 1981; Przybylowicz and Donoghue, 1988). Although "optimal" processing conditions (relative humidity, air flow rate, temperature) for shiitake mushrooms using the tray-drier are available, Cho et al. (1981) neglected to evaluate the effects of these factors on flavor development.

Rehydration Conditions

Rehydration conditions (pH, temperature, time of rehydration) can drastically affect the flavor quality of rehydrated shiitake. Rehydration conditions can effect the quantity and stability of the volatile and nonvolatile

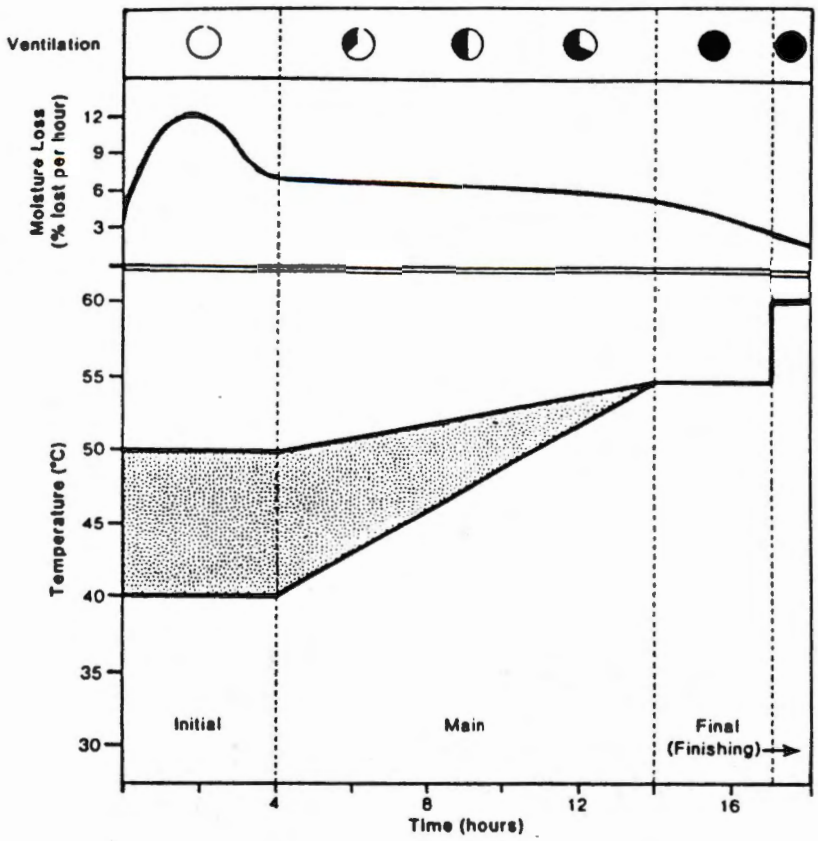

Fig. 4-Traditional Japanese dehydration schedule using a cabinet dehydrator.

Adapted from:

Przybylowicz, P. and Donoghue, J. 1988. Shiitake Growers Handbook. Kendall/Hunt Pub. Co., Dubuque, IA.

flavorants.

Aroma compounds such as lenthionine and the three other cyclic sulfur volatiles are optimal at pH 9 and 50°C, when soaked for 1 hr (Chen and Ho, 1986b; Chen et al., 1986; Ito et al., 1978). Total sulfur volatiles peak at pH=7-7.5 (Chen et al., 1984), possibly as the result of the decomposition of lenthionine and other heterocyclic sulfur volatiles. Higher temperatures and acidic pH drastically reduce aroma intensity, but increase the concentration of small molecular weight sulfur volatiles (Chen et al., 1984).

Nucleotide and free amino acid availability is strongly dependent upon the extraction temperature and degree of cell wall disruption. Twenty times more GMP was detected in boiled samples versus samples held at 40°C for comparable time periods (Aoyagi et al., 1980a). Boiling water extracts of dried shiitake showed twice the level of many 5′-nucleotides (CMP, AMP, UMP, and GMP) versus similar levels in fresh shiitake (Shimazono et al., 1979). These results show a dramatic increase in flavor potentiator availability in dried shiitake because cell wall disruption is increased by enzymatic action and drying (Przybylowicz and Donoghue, 1988). Sasaki et al. (1988) found analogous effects with amino acid availability in dried shiitake.

Although flavor potentiator stability is very good, 5′-nucleotides are susceptible to phosphate cleavage at extreme temperature and acid treatments (Matoba et al.,

1988). For example, the halflife of GMP at 100°C is 8.7 hr. Although nucleotides can lose flavor potentiation activity by several chemical modifications, phosphate removal is the predominant reaction (Maga, 1987; Matoba et al., 1988; Yamaguchi et al., 1968). In general, IMP is somewhat more susceptible to inactivation than GMP at comparable pH, processing times, or temperature abuse. Additionally, free glutamate is more durable than the 5′-nucleotides to heat and pH extremes (Maga, 1987). Although temperature effects influence nucleotide availability much greater than pH, the optimal pH for GMP and glutamate stability is 8 (Matoba et al., 1988; Yamaguchi and Kimizuka, 1979).

Non-Enzymatic Browning

Non-enzymatic browning (NEB) was first discussed by Louis-Camille Maillard in 1912, and has been the subject of intense study in foods since that time (Namiki, 1988). NEB is the reaction of alpha amino compounds (amino acids, peptides, protein residues) with reducing sugars to form brown pigments and flavor compounds. Several factors effect NEB, including: oxygen availability, pH, temperature, metal ions, inhibitors such as phosphates and sulfites, light, sugar type and quantity, and water availability (Cheftel et al., 1985; deMan, 1980; Heath and Reineccius, 1986; Namiki, 1988). NEB is also known to destroy essential amino acids (lysine, arginine) and form toxic compounds including nitrosamines and pyrroles (Namiki, 1988).

NEB is important to the flavor of cooked meats, breads and bakery products, but is undesirable under certain processing conditions such as spray-dried eggs. The effect of NEB during drying of shiitake mushrooms has not been evaluated despite the high occurrence of sugars and sugar related compounds (Hong and Kim, 1988), free amino compounds (Hong et al., 1989), intermediate moisture conditions, and low molecular weight sulfur compounds (Ito et al., 1978; Sakaguchi and Shibamoto, 1978; Shu et al., 1985; Toyoda et al., 1978). Furthermore, since the flavor of shiitake has been described as meaty or brothy, the optimization of NEB flavors might enhance dried shiitake flavor.

Proximate Composition

As previously discussed, compositional differences in fungi are influenced by several factors, yet the proximal constituents of different mushrooms show striking similarities. Consequently, several extensive publications have been collected on fungi spp. of many types (Bano and Rajarathnam, 1988; Crisan and Sands, 1978). In general, mushrooms contain 90% water, and are good sources of fiber and minerals (Haytowitz and Matthews, 1984; Horie et al., 1989; Kurasawa et al., 1982a,b). On the other hand, the mineral content of mushrooms can sometimes contain dangerous levels of heavy metals such as mercury, lead, and cadmium,

because these compounds can be accumulated in mature fruit bodies if present in the growth substrate and/or surrounding air (Ketz, 1982; Ogata et al., 1981). Heavy metals, however, are generally lower in indoor cultivated commercial varieties commonly found in groceries. Certain species of fungi also contain high levels of hydrazines and other natural toxins (Hunter, 1989).

Shiitake mushrooms in particular are a good source of vitamin D (Ono et al., 1974; Yokokawa and Mitsuhashi, 1981; Yoshida et al., 1979), and have all the essential amino acids (Breene, 1989; Chang, 1980).

Several researchers (Bano and Rajarathnam, 1988; Crisan and Sands, 1978; Pecora, 1989) have noted that conventional protein determinations for mushrooms are inaccurate because of high non-protein nitrogen (NPN) levels, which inflate the value for crude protein. Conventional protein calculations assume complete protein digestability and negligible NPN, however, approximations of the digestability of mushroom protein are only 60-70 %, and NPN levels are as high as 20% of the total protein (Aoyagi et al., 1982a). The low digestability of mushroom protein has been attributed to a fibrous component (chitin, a polymer of N-acetylglucosamine) in mushroom cell walls, while the high level of NPN can be attributed to a variety of free peptides, amino acids, and oligopeptides (Bano and Rajarathnam, 1988). Two alternative methods for protein

determination have been proposed for mushrooms, with the former receiving wider support in recent years:

i. an adjusted factor in protein calculations of 4.38, or 6.25 x 70% digestability of protein (Bano and Rajarathnam, 1988), and

ii. a modified Coomassie blue dye-binding method (Pecora, 1989).

Similarities between Savory Flavor and Shiitake

The flavor compounds of dried shiitake have many similarities with the compounds responsible for meaty or savory flavors. For example, the vast majority of flavor compounds described as "meaty" contain sulfur heterocyclics and have very low odor thresholds (MacLeod, 1986). Shiitakes' characteristic aroma is based on heterocyclic sulfur compounds that are degraded into a variety of smaller weight sulfur volatiles. Secondly, 5'-nucleotides are dominant flavor compounds in meat because of the breakdown of ATP to IMP (MacLeod, 1986; Maga, 1987). Shiitake also has high levels of flavor potentiators, including MSG, IMP and GMP. In other words, the "umami" taste is dominant in both shiitake and meat broths such as chicken or beef (Kawamura and Kare, 1987). Thirdly, many of shiitakes' natural and enzymatically formed chemicals are recognized precursors to non-enzymatic browning flavors, many of which

contribute to cooked meat flavors, including: ammonia, sulfur compounds, free amino acids, free sugars, and small peptides (Heath and Reineccius, 1986; MacLeod, 1986; Mabrouk, 1976; Mussinan et al., 1976; Schutte, 1976; Takken et al., 1976). Shiitake powders can be used in much the same way that hydrolyzed proteins, flavor potentiators, and onion powders are currently used. These applications include soups, sauces, and processed foods of all kinds (Anonymous, 1991; Maga, 1987).

CHAPTER III

MATERIALS AND METHODS

Shiitake Source, Preparation, and Storage

Twelve kilograms of fresh shiitake mushrooms were delivered to the McLeod Food Technology and Science Building at the University of Tennessee, Knoxville campus on October 24, 1990. The mushrooms were grown outdoors on natural oak logs in Claiborne County, Tennessee. On October 26, 1990, the whole mushrooms were cryogenically frozen using a liquid carbon dioxide system (Kryospray-model number BF300SD, Cryo-Chem Inc., Gardena, CA) operated on a 25-min cycle at -67°C. The frozen mushrooms were then vacuum packaged using the Multivac system (Cryovac Division, W.R. Grace and Co., Duncan, S.C.) and 25.4 cm x 55.9 cm clear plastic barrier bags (Cryovac, Simpsonville, S.C.). The mushrooms were stored at -29°C until experimental drying treatments were carried out in late June, 1991.

The type of shiitake mushrooms used in the experiment was the Koshin variety, with the mushroom veil open 90-100% (Matsumoto et al., 1979). The mushrooms were fully mature and fairly large, with most of them being 6-13 cm in diameter. Schroeder (1989) has proposed American grading standards for shiitake mushrooms, which are similar to

button grading standards: 50-70% opened caps yield are #1's, and caps expanded more than 70% are #2's. The #2 grade fresh shiitake are considered to be of inferior quality because of poor texture and reduced shelf life.

Drier Systems

A modified Armfield Tray Drier (catalog no. U0P8, Ringwood, Hampshire, England) was used for the tray-dried treatments. A small steel pinwheel fan was added to the drier to disperse the heated air evenly around the drier from top to bottom, and from side to side. Relative humidity (RH) and air flow rates were measured initially and after each hour of drying for each treatment. RH was estimated from wet bulb/dry bulb data and conversion tables (Ockerman, 1978). The air flow rate for all tray-dried treatments was calculated from measurements taken at the "open" end of the dehydration tunnel (exit air velocity). Since a constriction at the end of the tray-drier effectively increases the flow, the air flow rate at the point of drying (POD) was lower than the exit air velocity. The POD air flow rate is equivalent to the ratio of the cross sectional area at the exit to that of the POD cross sectional area times the exit air velocity. The correction factor was determined to be 0.6148. Net weight loss of the

mushrooms was calculated each half hour using a top loading scale, which was interfaced with the drier.

Experimental Design/Drier Operating Conditions

The frozen mushrooms were handled as shown in Fig. 5. The mushrooms were sliced to uniform width (2.54 cm) using a Hobart food processor (Model no. 84142), and sorted into caps and stems. Prior to tray drying, 200-g portions were weighed and two portions (400 g) were used for each treatment. Initial samples of caps and stems were freeze-dried to minimal moisture content, ground to 30 mesh in a Wiley mill, and stored under nitrogen in Whirl-Pak bags. Samples of caps and stems were dried at 50°C for 7 and 4 hr, respectively. The dehydrated cap and stem samples were freeze-dried, ground to 30 mesh and chemical, physical, and sensory analyses were conducted on the final dried powders.

The experimental dehydration scheme was based upon traditional cabinet drying characteristics of shiitake mushrooms (Fig. 4) (Przybylowicz and Donoghue, 1988). Optimal processing/tray-drying conditions that were used as guidelines for the experiment (Table A-1) included a relative humidity of 38-41%, a temperature of 40-55°C, and air flow velocity of 1.0-2.0 m/sec (Cho et al., 1981). The mushroom caps were dehydrated during the first phase at 50°C for 3 hr. This was considered the "half-dried" mushroom

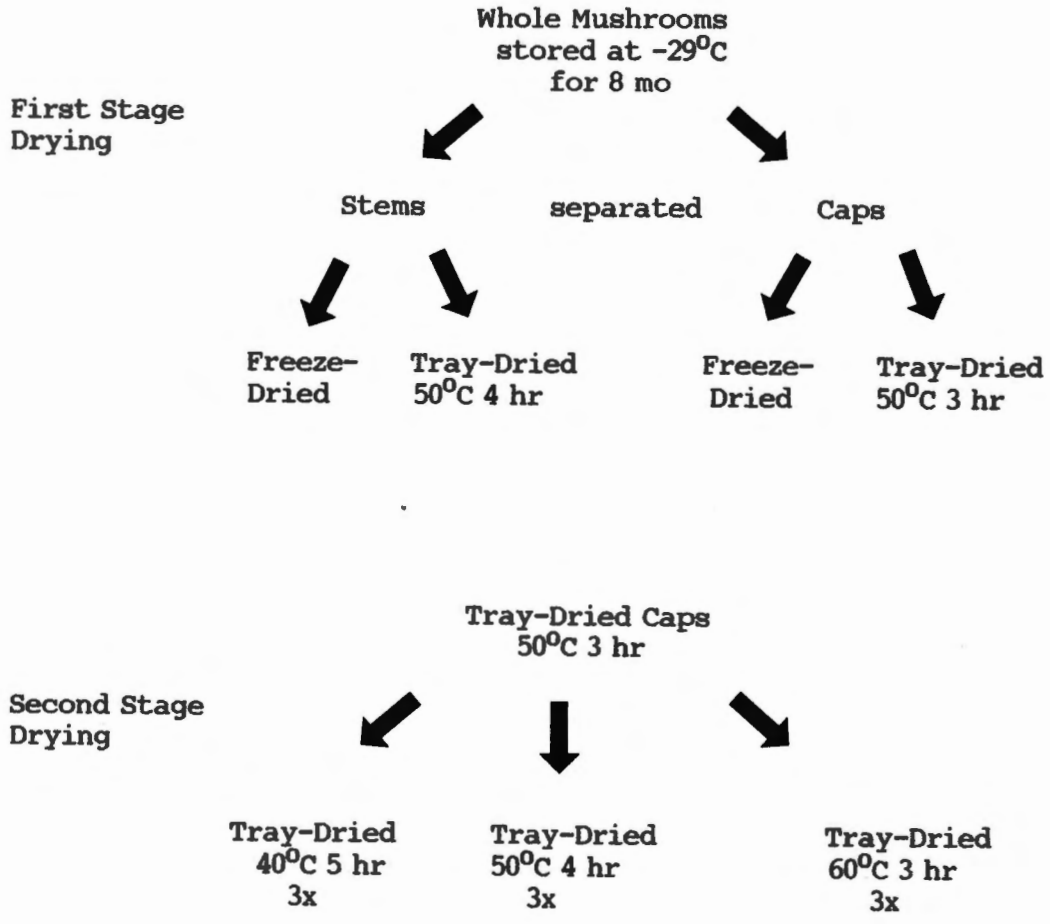

Fig. 5-Experimental design for dehydration of pre-frozen shiitake mushroom caps and stems.

sample and the drying time coincided with the critical moisture content, or the point at which the free water was removed and the dehydration rate began to fall. Mushroom caps that were half-dried were then fully- dried at one of three temperatures: 40, 50, or 60°C. After tray-drying the samples were immediately frozen to -29°C in a blast freezer, freeze-dried to minimal moisture content, ground to 30 mesh, and stored under nitrogen in Whirl-Pak bags. Chemical, physical, and sensory analyses were conducted on the final dried powders. The second stage drying combinations were completed in triplicate at 40, 50, and 60°C, for 5, 4, and 3 hr, respectively.

Proximate Composition

Fat, moisture, ash, and protein were determined by AOAC (1990) methods 920.39, 934.01, 942.05, and 976.06, respectively. Protein was calculated using an adjusted conversion factor for mushrooms (4.38) that accounts for non-protein nitrogen and proteinaceous fiber interferences (Pecora, 1989). Total fiber was determined using a modified method of Prosky et al. (1985).

Physical and Chemical Analyses

Hunter Color

The luminosity (L) of all samples was determined using the Hunter Colorimeter (Hunterlab model D25, Reston, VA). The machine was standardized against a standard white tile (L=91.03, a=-1.3, b=1.6) and contained a 10.1 V lamp.

High Performance Liquid Chromatographic Determination of Monosodium Glutamate

High pressure liquid chromatography (HPLC) was used to separate and quantify free glutamate ion using a modified isocratic method of Waters Associates (Bellinger, 1991; Kenney, 1990). The following system components were used: a Waters Associates pump (Milford, MA), a Waters Associates Lambda-Max Model 480 LC spectrophotometric detector (Milford, MA), a C18 reversed phase column (Alltech Econosil 5 μm, 250 mm x 4.6 mm I.D.), and a Shimadzu Corp. Model-chromatopac C-R2AX integrator (Kyoto, Japan). HPLC grade water (Baxter, Grand Prairie, TX) was used to dissolve samples and standards to aid in ionic separation and minimize interference. The mobile phase was acetonitrile/water (10:90), with UV detection at 214 nm, a flow rate of 1.5 mL/min, and injection volume of 5 μL.

The glutamate ion standard was dissolved in water at a pH of 5.9, which was similar to several preliminary 1-g samples dissolved in HPLC grade water (pH=7). Phosphoric

acid and 0.025 M anhydrous sodium phosphate dibasic buffer were used instead of dicyclohexylamine phosphate at 0.25%. One-gram samples for all treatments were dissolved in 100 mL of HPLC grade water, heated and held at 50°C for 40 min, filtered through 0.45-μm filters, cleaned up with C18 Sep-Pak filters (Waters Associates, Milford, MA), and diluted four-fold, before injection into the HPLC. A standard curve was prepared for the concentration range 10-20 mg/mL.

Water Soluble Carbohydrates

Total water soluble carbohydrates (WSC) were determined from the dried shiitake samples using the phenol-sulfuric acid method of Dubois et al. (1956). Blanks were prepared by substituting distilled water for the deproteinated shiitake water extract, followed by the addition of the 5% phenol solution (1 mL), and concentrated sulfuric acid (5 mL). A standard curve for glucose equivalents was prepared from visible absorbance at 480 nm for the concentration range 0.125-1.025 μg/mL. A Hewlett-Packard (Palo Alto, CA) 8452A Diode Array Spectrophotometer was used for the analyses.

Ninhydrin Reactive Materials

Ninhydrin Reactive Materials (NRM) were extracted from the dried powder using the method of Piotrowski et al. (1970), and quantified using the method of Mickelson et al. (1969). A standard curve for the concentration range 0.02-1.0 mg/mL for glycine equivalents was determined.

Standard Curve Reproducibility

Standard curves for the chemical analyses (MSG, NRM, WSC) were constructed using peak height or absorbance versus concentration, followed by unknown determination using regression analysis (PROC REG) (Schlotzhauer and Littell, 1987). Standard curves were constructed periodically throughout the HPLC analyses, and daily for the WSC and NRM determinations. The reproducibility of each standard curve was evaluated using the standard deviation coefficient of variation (Table A-2). Two points from each curve were chosen randomly, measured 10 times, and analyzed for variation using PROC MEANS in SAS.

Sensory Evaluation

Eight panelists were asked to compare the shiitake flavor strength of 7 samples to the flavor of a reference sample. The panelists indicated the flavor strength of the sample as "weaker", "equivalent", or "greater" than the reference, then the degree of difference was judged as either "none", "slight", "moderate", "much", or "extreme" (a sample score card is shown in Fig. A-1). A sample that was judged as having equal flavor to that of the reference received a score of 5, while samples with stronger flavor than the reference received scores of 6-"slightly stronger", 7-"moderately stronger", 8-"much stronger", and 9-"extremely

stronger". Weaker samples than the reference received scores of 4-"slightly weaker", 3-"moderately weaker", 2-"much weaker", and 1-"extremely weaker". The panelists were screened for their ability to match the reference sample with an identical test sample coded with a 3-digit random number (Day 1), or identify a plain beef broth sample (Day 2) coded in the same manner. The multiple comparison (Larmond, 1977) test was used to detect differences in rehydrated shiitake flavor strength. The 8 untrained panelists were selected based upon their familiarity with sensory testing methods and shiitake flavor. All panel members were international (born and raised in China, Japan, or Malaysia) Food Technology and Science students.

On the first day, the following experimental samples were presented to the panelists: freeze-dried stems, freeze-dried caps, dried stems, half-dried caps (same as reference sample), 40°C-dried caps, 50°C-dried caps, and 60°C-dried caps. On the second day, two experimental samples that were similar to the reference were compared to several commercial samples. The samples that were presented to the panelists on the second day included: 40°C and 50°C-dried caps (experimental samples from Day 1), plain beef broth, Nikken shiitake mushroom powder, ground Donko mushrooms #1 (Kame brand), ground Donko mushrooms #2 (a market sample bought in China), and the NEB sample. The NEB sample was identical to the fully-dried 40°C cap sample, except that it was dried

overnight in a drying oven.

Red lighting was used in the individual evaluation booths to mask color and appearance differences among the samples. Samples were composed of 1-g dry weight basis portions of experimental and commercial shiitake powders dissolved in 100 mL of diluted MBT brand beef broth (one part normal concentration broth with two parts water). The water used in the beef broth and the panelists rinse water was a commercial brand of bottled water (Mountain Valley Spring Water). The samples were held for 30 min at 50°C prior to evaluation to allow rehydration and flavor development. Each panelist was provided 25 mL of each sample and 50 mL of the reference sample. All samples were served in 75-mL screw-top clear glass bottles, with lids tightened. Panelists were provided water to rinse their palates between samples and cups for expectorating the sample if desired. The samples were coded with 3-digit random numbers, and each panelists presentation order for the 7 samples was randomized. The pH of all samples was measured following the sensory sessions.

Statistical Analyses

All data were analyzed using the software package Statistic Analysis System (SAS) (Schlotzhauer and Littell, 1987). Differences among cap treatments (half-dried and 40,

50, and 60°C fully-dried) were analyzed using PROC GLM with independent variables replication and treatment. The dependent variables for the cap treatments were ash, fiber, protein, fat, NFE, WSC, NRM , MSG, and Hunter luminosity (L). Means separation was determined using the Student-Newman-Keuls procedure.

Sensory scores were analyzed using PROC ANOVA with independent variables panelist and sample and dependent variable score. Tukey's method of mean separation of variables was used to determine statistical differences among the sample means based upon judges scoring.

The mushroom sections (caps and stems) were analyzed separately to determine compositional differences. Means and standard deviations were reported for the results from the chemical and color analysis of the caps and stems.

CHAPTER IV

RESULTS AND DISCUSSION

Differences between Cap and Stem

The proximate composition of shiitake mushrooms varied with mushroom part (cap versus stem), but agreed with recent literature values for whole shiitake (Table 1). Similar proximate compositional differences were noted between shiitake caps and stems by Przybylowicz and Donoghue (1988). In this study, the cap portion contains 10% more moisture and twice as much protein and ash as the stems, while the stem portion contains twice the fiber content as the caps (Table 1). The NFE and fat contents in the shiitake caps and stems were fairly similar. Overall the solid content is mostly carbohydrate material, 67%, with the remaining third 16% protein, 9% dietary fiber, 6% ash, and 2% lipid [calculation based on 82.1% of whole mushroom as cap (Sugahara et al., 1975)].

MSG, WSC, and NRM results for shiitake caps (Table 2) are in good agreement with published data (Table 3). The experimental MSG cap content was found to be 3.03 mg/g, while Hong et al.(1989) determined the glutamate content to be 3.24 mg/g in shiitake caps. The experimental NRM content in caps was 26.8 mg/g, which is in good agreement with the

Table 1--Mean proximate compositions for experimental shiitake caps and stems and literature values for whole shiitake

Proximate component	Cap[a]	Stem[a]	Literature values	
			Whole[b]	Whole[c]
Moisture	89.80 ± .23	79.51 ± .45	90.0	----
Protein[dc]	17.00 ± .35	8.85 ± .23	17.5	17.5-20.8
Fat[d]	2.22 ± .16	2.28 ± .06	8.0	2.4-3.2
Ash[d]	6.14 ± .08	3.06 ± .25	7.0	3.7-5.2
Fiber[d]	7.34 ± .08	16.42 ± .28	8.0	5.9-8.8
NFE[d]	67.10 ± .03	67.40 ± .31	59.5	56.9-62.5

[a] n=2.
[b] Bano and Rajarathnam (1988).
[c] Yoshida et al. (1979).
[d] dry weight basis.
[e] protein conversion factor = 4.38.

Source(s):

Bano, Z. and Rajarathnam, S. 1988. Pleurotus mushrooms. Part II. Chemical composition, nutritional value, post-harvest physiology, preservation, and its role as human food. CRC Rev. Food Sci. Nutr. 27(2): 1.

Yoshida, H., Hayashi, J., Aoyagi, Y., and Sugahara, T. 1979. Fatty acid compositions and ergosterol contents of different grades of dried shiitake mushroom (Lentinus edodes Sing.). J. Jap. Soc. Food Sci. Tech. 26: 221.

Table 2--Hunter luminosity (L), water soluble carbohydrates (WSC), free glutamate (MSG), and ninhydrin reactive materials (NRM) values for shiitake caps and stems[a]

Mushroom part	L	WSC[b] (mg/g)	MSG (mg/g)	NRM[c] (mg/g)
Stem	61.8 ±15.0	1.44 ± .19	2.20 ± .20	24.1 ± 1.8
Cap	68.8 ± 3.7	3.70 ± .11	3.03 ± .11	26.8 ± 3.4

[a] n=2.
[b] mg glucose equivalents/g.
[c] mg glycine equivalents/g.

Table 3--Literature values for protein and sugar-related materials in shiitake caps and stems

Constituent	Cap	Stem
Protein nitrogen (%)[a]	1.62	0.88
Non-protein nitrogen (%)[a]	4.62	0.79
Total free amino acids/MSG (mg/g)[b]	21.1/ 3.24	10.0/ 1.47
Free 5′-GMP (mg/g)[b]	1.10	0.32
Free 5′-AMP (mg/g)[b]	0.93	0.33
Free 5′-UMP (mg/g)[b]	1.00	0.30
Total free sugars and sugar alcohols (mg/g)[c]	91.5	117.3
Free glucose (mg/g)[c]	3.8	4.6
Free fructose (mg/g)[c]	7.3	4.3
Free arabitol (mg/g)[c]	2.4	3.2
Free mannitol (mg/g)[c]	62.7	89.0

[a] Yoshida et al. (1979).
[b] Sugahara et al. (1975).
[c] Hong et al. (1989).

Source(s):

Hong, J.S, Kim, Y.H., Kim, M.K., Kim, Y.S., and Sohn, H.S. 1989. Contents of free amino acids and total amino acids in Agaricus bisporus, Pleurotus ostreatus and Lentinus edodes. Korean J. Food Sci. Technol. 21: 58.

Sugahara, T., Arai, S., Aoyagi, Y., and Kunisaki, N. 1975. Contents of 5′-nucleotides and free amino acids in different varieties of dried shiitake mushroom (Lentinus edodes Sing.). J. Jap. Soc. Food Nutr. 28: 477.

Yoshida, H., Hayashi, J., Aoyagi, Y., and Sugahara, T. 1979. Fatty acid compositions and ergosterol contents of different grades of dried shiitake mushroom (Lentinus edodes Sing.). J. Jap. Soc. Food Sci. Tech. 26: 221.

sum of total free amino acids (21.1 mg/g) and free ribonucleotide (3.03 mg/g). WSC content of experimental caps was 3.70 mg/g, while Hong and Kim (1988) have reported a value of 3.8 mg/g. The closeness of experimental and literature WSC contents was unexpected because the method used to quantify WSC is not specific for D-glucose (the standard chosen in experimental work). In fact, D-glucose, D-Mannose, L-arabinose, and D-fructose all have absorbance maxima at or near 480 nm. The alcohols of arabinose and mannose, and fructose are found in significant quantities in shiitake mushrooms (Table 3).

Experimental results for MSG and NRM content of stems are slightly higher than published values, while WSC content was lower that literature data. The experimental stem contents of MSG and NRM were 2.20 mg/g and 24.1 mg/g, respectively. On the other hand, literature values for stem MSG and NRM were 1.47 mg/g and 10.95 mg/g, respectively. Since many factors can affect shiitake composition including growing conditions, strain, and maturity, these experimental values are acceptable.

In general, experimentally dried and ground shiitake stems were darker in color than the caps (Table 2). These differences are most likely explained in terms of the structural differences between caps and stems, since caps are bright white on the interior while stems are not. Color

changes in shiitake due to processing are discussed in the next section.

Differences among Dehydrated Shiitake Cap Treatments

The proximate composition of the cap treatments did not vary significantly (Table 4) at $p=0.05$. Other than the moisture content, the proximate composition would not be expected to change during the dehydration process since the maximum temperature during the most severe treatment was only 60°C. Thus, proximal differences exist between caps and stems (Przybylowicz and Donoghue, 1988), but are not significantly altered by drying treatment.

MSG and NRM levels among the cap treatments were not significantly different at $P \leq 0.05$ (Table 5). The concentration of MSG and NRM in experimental cap samples, however, appears to be inversely related to processing temperature when compared to the freeze-dried cap sample (Table 6). It appears that proteolytic enzymes are most active during initial drying periods and at lower drying temperatures, but as dehydration temperature increases the effect of these enzymes is reduced. This agrees with current theory concerning the thermal instability of shiitake flavor/aroma deriving enzymes (Chen et al., 1986) and of all enzymes in general (Bailey and Ollis, 1986). Additionally, non-enzymatic browning can potentially reduce

Table 4--Analysis of variance (ANOVA) sums of squares for experimental shiitake cap treatments for ash, protein, fat, nitrogen free extract (NFE), and fiber[a]

Source	DF	Ash	Protein	Fat	NFE	Fiber
Treatment	3	0.765^{NS}	0.506^{NS}	0.023^{NS}	1.755^{NS}	0.149^{NS}
Error	6	0.495	3.194	0.198	2.025	0.214
Total	9	1.260	3.700	0.221	3.780	0.363

[a] NS Not Significant at P=0.05.

Table 5--Analysis of variance (ANOVA) sums of squares for experimental shiitake cap treatments for Hunter luminosity (L), water soluble carbohydrates (WSC), free glutamate (MSG), and ninhydrin reactive materials (NRM)[a]

Source	DF	L	WSC[b]	MSG	NRM[c]
Treatment	3	493.68*	6.1617*	91.530NS	93.613NS
Error	6	23.43	0.2246	98.216	295.706
Total	9	517.11	6.3864	198.745	389.320

[a] * P<0.01; NS Not significant at P>0.05.
[b] mg glucose equivalents/g.
[c] mg glycine equivalenys/g.

Table 6--Shiitake cap treatment means for Hunter luminosity (L), water soluble carbohydrates (WSC), ninhydrin reactive materials (NRM), and free glutamate (MSG) based upon a dry weight basis

Sample	No. rep	No. hr dried	L	WSC[a] (mg/g)	NRM[b] (mg/g)	MSG (mg/g)
Half-dried cap	1	3	66.2[c]	3.63[c]	29.2[c]	3.11[c]
40°C cap	3	8	43.7[d]	1.11[e]	32.8[c]	3.12[c]
50°C cap	3	7	42.4[d]	1.56[e]	29.9[c]	2.68[c]
60°C cap	3	6	42.4[d]	2.47[d]	25.1[c]	2.40[c]

[a] mg glucose equivalents/g.
[b] mg glycine equivalents/g.
[cde] Means in the same column with a different superscript are different ($P < 0.05$).

the availability of free amino compounds, such as MSG and NRM, at higher temperatures and intermediate moisture conditions (Health and Reineccius, 1986).

The WSC content of experimental shiitake mushrooms was significantly affected by the cap treatments at p=0.05 (Tables 5 and 6). Significant differences were found between half-dried caps and the 40, 50, and 60°C caps, and between 60°C caps and both the 40 and 50°C caps. The 40 and 50°C caps, however, were not significantly different in WSC content. In general, dehydration time and WSC content were inversely related.

The cap treatments also had significant differences in lightness (Tables 5 and 6), as measured by Hunter luminosity (L). The half-dried caps were significantly lighter in color (p=0.05) than the fully- dried samples, but the fully-dried samples were not significantly different in color from one another. The luminosity value of freeze-dried samples was also measured and found to be 71.5. Since polyphenoloxidase (PPO) is found in high concentrations in mushroom caps, cellular disruption from thawing is known to increase polyphenoloxidase browning in mushrooms (Burton, 1986), and freezing is known to darken shiitake (Tanaka et al., 1974), the experimental conditions were ideal for PPO browning.

Although dried shiitake are known to maintain quality for extended periods of time during normal storage, dried

shiitake have favorable conditions for non-enzymatic browning at elevated temperatures (free sugars and free nitrogen compounds, 10-12% moisture, heat). Typical temperatures for non-enzymatic browning are as low as 100°C (Health and Reineccius, 1986). After moisture determinations (100°C) on the fully-dried powders, all of the treatments (cap and stem treatments) were noticeably darker in color. Informal sensory analysis of the overnight dried samples revealed a bitter coffee-like aroma, and decreased "shiitake" flavor and aroma. One of these samples (a 40°C cap treatment) was saved and evaluated against the other samples by the sensory panel, to determine the effects of non-enzymatic browning on shiitake flavor quality.

Sensory Evaluation

Sensory Panel Screening

On the first day of testing it was determined that one panelist did not score the blindly coded reference sample identical to the reference. This persons scores were omitted from further statistical analyses. Similarly on the second day, it was determined that one panelist could not differentiate between a plain beef broth sample and a sample containing shiitake. Again, this panelist was removed from subsequent statistical analyses. Thus, the total number of panelists used for statistical treatments on each day was 7.

Differences among Experimental Samples

Analysis of variance for the experimental samples indicated significant difference among samples and panelists (Table 7) at $p \leq 0.05$. Significant differences among the panelists scores are common when panelists are untrained. In statistical analyses of sensory data, panelist is a source of variation and should be accounted for in the ANOVA model. This procedure improves the likelihood of finding significant differences among samples.

Significant differences were found among experimental samples. Mean sample scores and the panelist frequency distribution for the experimental samples are shown in Table 8. The 8 samples were divided into two significantly different groups of samples. The first group included 4 samples that were statistically equivalent in shiitake flavor to the reference. The samples that were equivalent to the reference were half-dried caps and stems, 40°C caps, and 50°C caps. The second group was composed of 3 samples which had shiitake flavor strength "moderately weaker" or "much weaker" than the reference. The samples with weaker flavor were the freeze-dried samples (caps and stems) and the 60°C cap treatment, which was rated as having a "much weaker" flavor than the reference.

Several generalizations of the experimental samples can be made. As expected, the freeze-dried samples had significantly weaker flavor than the reference whereas the

Table 7--Analysis of variance (ANOVA) sums of squares for sensory scoring of experimental shiitake samples and panelists[a]

Source	DF	Sum of sqrs	F value	Pr > F
Sample	7	115.64	16.00	0.0001*
Panelist	6	21.21	3.43	0.0080*
Error	42	43.35		
Total	55	180.20		

[a] * $P \leq 0.05$.

Table 8--Mean sensory scores and panelist frequency distribution for experimental shiitake samples[a]

Sample	Mean score	Stronger flavor than reference	Equivalent flavor to reference	Weaker flavor than reference
Half-dried cap	5.8[b]	4	3	0
40°C cap	5.6[b]	2	4	1
Reference	5.0[b]	0	7	0
50°C cap	5.0[b]	1	5	1
Tray-dried stem	4.8[b]	3	0	4
Freeze-dried stem	2.7[c]	0	1	6
Freeze-dried cap	2.4[c]	0	0	7
60°C cap	2.0[c]	0	0	7

[a] Evaluation on a 9-point scale (1=extremely, much, moderate, slightly weaker than reference; 5=equal; 6=slightly, moderate, much, extremely stronger than reference).

[bc] Means in the same column with a different superscript are different ($P \leq 0.05$).

half- or fully-dehydrated samples were equal to the reference. Since the dehydration process ruptures cell walls and initiates enzymatic flavor development, freeze-dried samples were expected to have reduced flavor due to the minimal enzymatic action in flavor development. Unexpectedly, the 60°C cap sample was also evaluated as having "much weaker" shiitake flavor than the reference. It appears that lower dehydration temperatures may produce stronger shiitake flavor, and that temperatures higher than 50°C may reduce flavor intensity. The tray-dried stems were equal in shiitake flavor strength to the reference, therefore the stems (approximately 18% of total cap and stem dried weight [Sugahara et al., 1975]) could possibly be blended along with the caps for flavoring purposes.

Two shiitake experimental samples (40 and 50°C caps) that were statistically equal in shiitake flavor to that of the reference were used in the comparison of commercially available shiitake samples.

Differences among Experimental and Commercial samples

Significant differences were found among the experimental and commercial shiitake samples (Tables 9 and 10), and also among panelists (Table 9) at $p \leq 0.05$. The scores for 40 and 50°C caps and Donko #1 and #2 did not differ significantly from the reference. The scores for these samples ranged from "equal in flavor" to "moderately weaker" in shiitake flavor than the reference. The Nikken

Table 9--Analysis of variance (ANOVA) sums of squares for sensory score of commercial and experimental shiitake samples and panelists[a]

Source	DF	Sum of sqrs	F value	Pr > F
Sample	7	95.5536	11.23	0.0001*
Panelist	6	32.9286	4.51	0.0013*
Error	42	51.0714		
Total	55	179.5536		

[a] * $P \leq 0.05$.

Table 10--Mean sensory scores and panelist frequency distribution for commercial and experimental shiitake samples[a]

Sample	Mean score	Stronger flavor than reference	Equivalent flavor to reference	Weaker flavor than reference
Reference	5.0[b]	0	7	0
40°C cap	4.7[b]	2	2	3
50°C cap	4.4[b]	1	4	2
Donko #1	4.3[b]	1	3	3
Donko #2	3.3[bc]	1	1	5
Nikken	2.3[c]	0	0	7
Beef broth	1.8[c]	0	0	7
NEB	1.4[c]	0	0	7

[a] Evaluation on a 9-point scale (1-4=extremely, much, moderate, slightly weaker than reference; 5=equal; 6-9=slightly, moderate, much, extremely stronger than reference).

[bc] Means in the same column with a different superscript are different ($P \leq 0.05$).

sample, the plain beef broth, and the overnight dried sample (NEB) were statistically alike and differed from the reference score. The scores ranged from "much weaker" to "extremely weaker" in shiitake flavor strength than the reference.

The panel was able to recognize and score appropriately a sample without shiitake, which was the plain beef broth sample. Additionally, several panelists made comments on the "bad" or "very bad" flavor of the NEB sample. The overnight sample (NEB) was subjected to non-enzymatic browning conditions and was rated "extremely weaker" in shiitake flavor strength.

Discussion of Results

Despite being stored frozen for 8 mo, and being of inferior quality based upon grading standards, the experimentally dehydrated shiitake samples were judged to be as flavorful as several commercially available dried shiitake products. Several panelists commented that the commercial samples that were bought whole, then ground into a powder (for sample uniformity) had an off-flavor that tasted "grassy" or "gensing/grassy". None of the experimental samples had this grassy characteristic mentioned by the panelists.

The experimental results were promising, especially since there has been very little work done on sensory evaluation of shiitake products. Matsumato et al. (1978)

found no correlations between sensory score, and either 5′-GMP content or amino nitrogen content. In this study, however, the samples that had flavor strength equivalent to the reference (40 and 50°C fully-dried caps) also had the highest levels of NRM and MSG. Tanaka et al. (1974) evaluated the volatiles of fresh, freeze-dried, frozen, and oven dried shiitake by instrumental methods and their aroma by sensory testing. In that study, the mushrooms were evaluated whole for several characteristics including aroma, texture, color, and cohesiveness. The quantitation of volatiles by instrumental methods showed much higher levels of several unidentified volatiles in the dried samples than for fresh, freeze-dried, or frozen. Sensory testing revealed that the oven-dried and freeze-dried samples had higher aromas than the fresh or frozen samples. The present work contradicts these results slightly because the freeze-dried samples were rated "moderately weaker" in shiitake flavor than dried samples.

The panel was able to identify and evaluate successfully, samples of equivalent (positive control) and much weaker shiitake flavor (negative control) than a reference sample. Although the panelists had significant variation among the way they scored the samples, based upon the frequency distributions (Tables 8, 10) of the entire panel the mean scores are representative.

Surprisingly though, none of the commercial or

experimental samples were evaluated as having stronger flavor than the half-dried reference. Thus, the half-dried cap sample dried at 50°C for 4 hr was "equivalent" in shiitake flavor strength to several commercial samples. Additionally, the stem sample had "equivalent" shiitake flavor strength to several half- and fully-dried experimental cap samples.

CHAPTER V

SUMMARY AND CONCLUSIONS

Flavor development in the shiitake mushroom is dependent on a number of factors. As shown in this research the time and temperature of dehydration are important factors. At a dehydration temperature of 60°C the flavor of the mushroom caps was significantly weaker than the flavor of the caps dehydrated at 40 or 50°C. The flavor of freeze-dried caps and stems of the frozen shiitake was also significantly weaker than the caps dehydrated at 40 or 50°C.

The dehydration temperature and time did not affect the proximate composition of the mushroom caps but did alter the WSC content. The WSC content decreased as the dehydration time increased from 50 to 60°C.

The mushroom caps contained more moisture, protein, and ash while the stems contained more dietary fiber. The caps also contained more WSC and MSG. These differences in composition did not result in any differences in shiitake flavor strength as determined by the sensory panel.

In conclusion, the dehydration process is necessary to develop the flavor of the shiitake mushroom. The process required to develop as strong a flavor as commercial samples was as short as 7 hr at 50°C, which is considerably less than most conventional Japanese processes. Further research

is needed to determine the effect that freezing the fresh mushrooms had on the final dehydrated product. Although freezing allowed the mushrooms to be stored for 8 mo prior to processing, it may have caused increased disruption of cellular structure, and thus could have increased enzyme activity and flavor chemical production.

LIST OF REFERENCES

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AOAC. 1990. Official Methods of Analysis, 13th ed. Association of Official Analytical Chemists Inc. Arlington, VA.

Anonymous. 1987. Farming your woodlands for profit. Successful Farming 85: 21.

Anonymous. 1989. Regional and State Updates. In Shiitake Mushrooms: the Proceedings of a National Symposium and Trade Show, p. 181. Univ. Minnesota, St. Paul.

Anonymous. 1991. Technical bulletin: an introduction to nucleotide seasonings. Ajinomoto Co., Inc., Tokyo, Japan.

Anthes, G.H. 1987. And now: yuppie agriculture. Washington Business J. 5(44): 1.

Aoyagi, Y., Sasaki, H., and Sugahara, T. 1980a. On peptides in dried shiitake Lentinus edodes. J. Agr. Chem. Soc. Japan. 54: 253.

Aoyagi, Y., Sasaki, H., Sugahara, T., Hasegawa, T., and Suzuki, T. 1980b. Sulfur containing peptides in Lentinus edodes. Agr. Biol. Chem. 44: 2667.

Aoyagi, Y and Sugahara, T. 1986. Studies on the rehydration of dried shiitake mushroom. J. Jap. Soc. Food Sci. & Tech. 33: 244.

Aoyagi, Y., Sugahara, T., Hasegawa, T., and Suzuki, T. 1982a. Constituents of a cationic peptide-rich fraction of Lentinus edodes. Agr. Biol. Chem. 46: 987.

Aoyagi, Y., Sugahara, T., Hasegawa, T., and Suzuki, T. 1982b. Gamma-glutamyl derivatives of basic amino acids in Lentinus edodes. Agr. Biol. Chem. 46: 1939.

Backas, N. 1988. Mushrooms add exotic flair to simple dishes. Rest. & Institutions 98: 214.

Bailey, J.E. and Ollis, D.F. 1986. Biochemical Engineering Fundamentals. McGraw-Hill, New York.

Bano, Z. and Rajarathnam, S. 1988. Pleurotus mushrooms. Part II. Chemical composition, nutritional value, post-harvest physiology, preservation, and its role as human food. CRC Rev. Food Sci. Nutr. 27(2): 1.

Bellinger, A. 1991. Unpublished data-Waters Associates Application brief no. 1003. Milford, MA.

Breene, W.M. 1989. Nutritional and medicinal value of exotic mushrooms. In Shiitake Mushrooms: the Proceedings of a National Symposium and Trade Show, p. 87, Univ. Minnesota, St. Paul.

Burton, K.S. 1986. Quality-investigations into mushroom browning. Mush. J. 158: 68.

Carson, J.F. 1987. Chemistry and biological properties of onions and garlic. Food Rev. Int. 3(1/2): 71.

Chang, S.T. 1980. Mushrooms as human food. Bioscience 30: 399.

Cheftel, J.C., Cuq, J.L., and Lorient, D. 1985. Amino acids, peptides, and proteins. In Food Chemistry, O.R. Fennema (Ed.), p. 245. Marcel Dekker, Inc., New York.

Chen, C.C., Chen, S.D., Chen, J.J., and Wu, C.M. 1984. Effects of pH value on the formation of volatiles of shiitake (Lentinus edodes), an edible mushroom. J. Agr. Food Chem. 32: 999.

Chen, C.C. and Ho, C.T. 1986a. Identification of sulfurous compounds of shiitake mushroom (Lentinus edodes Sing.). J. Agr. Food Chem. 34: 830.

Chen, C.C. and Ho, C.T. 1986b. High-performance liquid chromatographic determination of cyclic sulfur compounds of shiitake mushroom (Lentinus edodes Sing.). J. Chromatogr. 356: 455.

Chen, C.C., Liu, S.E., Wu, C.M., and Ho, C.T. 1986. Enzymatic formation of volatile compounds in shiitake mushroom (Lentinus edodes Sing.). In Biogeneration of Aromas, T.H. Parliment and R. Croteau (Ed.), p. 176, Amer. Chem. Soc., Washington, DC.

Cho, D.B., Kim, D.P., and Choi, C.S. 1981. Kinetics of drying shiitake mushroom, Lentinus edodes sanryun No.1. J. Korean Soc. Food Nutr. 10: 53.

Chua, G.K., LaCroix, L.J., Levy, R. and Unrau, A.M. 1968. Relationship between the flavor of canned onions and their chemical components as separated by gas chromatography. Proc. Am. Soc. Hort. Sci. 93: 817.

Crisan, E.V. and Sands, A. 1978. Nutritional value. Ch.6. In The Biology and Cultivation of Edible Mushrooms, S.T. Chang and W.A. Hayes (Ed.), p. 137, Academic Press, Inc., San Francisco.

Cronin, D.A. and Ward, M.K. 1971. The characterisation of some mushroom volatiles. J. Sci. Food Agr. 22: 477.

deMan, J.M. 1980. Principles of Food Chemistry. Van Nostrand Reinhold Co. New York.

Diehle, D.A. and Royse, D.J. 1986. Shiitake cultivation on sawdust: evaluation of selected genotypes for biological efficiency and mushroom size. Mycologia 78: 929.

Dijkstra, F.Y. 1976. Studies on mushroom flavours. 3. Some flavour compounds in fresh, canned and dried edible mushrooms. Z. Lebensm. Unters.-Forsch. 160: 401.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Duggleby, J. 1989. Into the woods to start a food company. Adweeks Marketing Week 30: 26.

Endo, K., Umeyama, Y., Nakajima, J., and Kawai, H. 1980. Purification and some properties of nucleases from Shii-take, Lentinus edodes. Agr. Biol. Chem. 44: 1545.

Filer, L.J.Jr., Garattini, S., Kare, M.R., Reynolds, W.A., and Wurtman, R.J. 1979. Glutamic Acid: Advances in Biochemistry and Physiology. Raven Press, New York.

Fujimoto, K., Tsurumi, T., Watari, M., Akama, K., and Kaneda, T. 1974. The mechanism of formaldehyde formation in shii-ta-ke mushroom. Mush. Sci. 9: 385.

Fuster, C., Prestamo, G., and Espinosa, J. 1984. Frozen mushrooms. II. Changes in whole frozen mushrooms during frozen storage. Alimentaria 132: 33.

Goore, M.Y. and Thompson, J.F. 1967. Gamma-glutamyl transpeptidase from kidney bean fruit. I. Purification and mechanism of action. Biochimica Et Biophysica Acta. 132: 15.

Gil, V. and MacLeod, A.J. 1981. Synthesis and assessment of three compounds suspected as egg aroma volatiles. J. Agr. Food Chem. 29: 484.

Haney, A. 1989. Mushrooming forest profits, shiitake production and woodlot management. In Shiitake Mushrooms: the Proceedings of a National Symposium and Trade Show, p. 57, Univ. Minnesota, St. Paul.

Hashiguchi, M., Itoh, S., and Tsuyuki, H. 1984. Studies on the lipids of shiitake. I. Lipids in cap and stalk of shiitake mushroom. J. Jap. Soc. Food Sci. Tech. 31: 436.

Haytowitz, D.B. and Matthews, R.A. 1984. Composition of Foods: Vegetables and Vegetable Products. Raw, Processed, Prepared. Agr. Handbook No. 8, USDA, Washington, DC.

Heath, H.B. and Reineccius, G. 1986. Flavor Chemistry and Technology. Van Nostrand Reinhold Co. Inc., New York.

Heiskell, M. 1990. Personal communication. State of Tennessee Agricultural Extension, Claiborne County, TN.

Ho, C.T., Zhang, Y., Shi, H., and Tang, J. 1989. Flavor chemistry of chinese foods. Food Reviews Internat. 5: 253.

Hong, J.S., and Kim, T.Y. 1988. Contents of free-sugars and free-sugar alcohols in Pleurotus ostreatus, Lentinus edodes, and Agaricus bisporus. Korean J. Food Sci. Tech. 20: 459.

Hong, J.S, Kim, Y.H., Kim, M.K., Kim, Y.S., and Sohn, H.S. 1989. Contents of free amino acids and total amino acids in Agaricus bisporus, Pleurotus ostreatus and Lentinus edodes. Korean J. Food Sci. Technol. 21: 58.

Hong, J.S., Kim, Y.H., Lee, K.R., Kim, M.K., Cho, C.I., Park, K.H., Choi, Y.H., and Lee, J.B. 1988. Composition of organic and fatty acids in Pleurotus ostreatus, Lentinus edodes and Agaricus bisporus. Korean J. Food Sci. Technol. 20: 100.

Horie, Y., Wahlqvist, M.L., Read, R.S.D., and Jones, G.P. 1989. Dietary fibre content of some traditional Japanese foods. ASEAN Food J. 4: 117.

Hunter, B.T. 1989. Wild mushrooms. Consumers' Res. Magazine. 72: 8.

Ito, T. 1978. Cultivation of Lentinus edodes. Ch. 22. In The Biology and Cultivation of Edible Mushrooms, S.T. Chang and W.A. Hayes (Ed.), p. 461. Academic Press, Inc., New York.

Ito, Y., Toyoda, M., Suzuki, H., and Iwaida, M. 1978. Gas-liquid chromatographic determination of lenthionine in shiitake mushroom (Lentinus edodes) with special reference to the relation between carbon disulfide and lenthionine. J. Food Sci. 43: 1287.

Iwami, K. and Yasumoto, K. 1980. Alliinase-like enzymes in fruiting bodies of Lentinus edodes: their purification and substrate specificity. Agr. Biol. Chem. 44: 3003.

Iwami, K. and Yasumoto, K. 1982a. Chaotropic ions in activation and protection of gamma-glutamyltransferase from fruiting bodies of Lentinus edodes. Agr. Biol. Chem. 46: 761.

Iwami, K. and Yasumoto, K. 1982b. Substrate preference of gamma-glutamyltransferase from caps of fruiting bodies of Lentinus edodes. Agr. Biol. Chem. 46: 2387.

Iwami, K., Yasumoto, K., and Mitsuda, H. 1975a. Enzymatic cleavage of cysteine sulfoxide in Lentinus edodes. Agr. Biol. Chem. 39: 1947.

Iwami, K., Yasumoto, K., Mizusawa, H. 1982. Enzymatic formation of thermostable "antithiamine" factor in fresh fruiting bodies of Lentinus edodes. J. Agr. Chem. Soc. Japan 56: 905.

Iwami, K., Yasumoto, K., Nakamura, K., and Mitsuda, H. 1975b. Properties of gamma-glutamyltransferase from Lentinus edodes. Agr. Biol. Chem. 39: 1933.

Iwami, K., Yasumoto, K., Nakamura, K., and Mitsuda, H. 1975c. Reactivity of Lentinus gamma-glutamyltransferase with lentinic acid as the principal endogenous substrate. Agr. Biol. Chem. 39: 1941.

Jones, R. 1988. The versatile mushroom. Rest. Bus. Magazine 87: 222.

Kasai, T. and Larson, P.O. 1980. In Progress in the Chemistry of Natural Products, W. Hertz, H. Grisebach, and G.W. Kirby (Ed.), p. 173, Springer, Wien. Quoted in Lancaster, J.E., and Shaw, M.L. (1989). Gamma-glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in allium. Phytochem. 28: 455.

Kawamura, Y. and Kare, M.R. 1987. Umami: a Basic Taste. Marcel Dekker, Inc., New York.

Kenney, B.F. 1990. Applications of high-performance liquid chromatography for the flavor research and quality control laboratories in the 1990s. J. Food Sci. 9: 76.

Ketz, H.A. 1982. Nutritional value of edible fungi. Ernahrungsforschung 27: 114.

Kikuchi, M., Yamashita, I., Ishima, T., and Hosoda, H. 1984. Studies on the preservation of shii-take mushroom (Lentinus edodes (Bark) Sing.) at producer farm. Rept. Natl. Food Res. Inst. 48: 9.

Kurasawa, S.I., Sugahara, T., and Hayashi, J. 1982a. Studies on dietary fibre of mushrooms and edible wild plants. Nutr. Rep. Internat. 26: 167.

Kurasawa, S.I., Sugahara, T., and Hayashi, J. 1982b. Proximate and dietary fibre analysis of mushrooms. J. Jap. Soc. Food Sci. Tech. 29: 400.

Kurosawa, S., Higashioka, H., and Uehara, H. 1983. Presence and some properties of alkaline ribonuclease in the fruit body of Lentinus edodes. Agr. Biol. Chem. 47: 1917.

Lancaster, J.E. and Collin, H.A. 1981. Presence of alliinase in isolated vacuoles and of alkyl cysteine sulphoxides in the cytoplasm of bulbs of onion (Allium cepa L.). Plant Sci. Letters 22: 169. Quoted in Lancaster, J.E. and Shaw, M.L. 1989. Gamma-glutamyl peptides in the biosynthesis of S-alk(en)yl-L-cysteine sulphoxides (flavour precursors) in allium. Phytochem. 28: 455.

Lancaster, J.E., Reynolds, P.H.S., Shaw, M.L., Dommisse, E.M., and Munro, J. 1989. Intra-cellular localization of the biosynthetic pathway to flavour precursors in onion. Phytochem. 28: 461.

Lancaster, J.E., and Shaw, M.L. 1989. Gamma-glutamyl peptides in the biosynthesis of S-alk (en) yl-L-cysteine sulphoxides (flavour precursors) in allium. Phytochem. 28: 455.

Larmond, E. 1977. Laboratory methods for sensory evaluation of food. Publ. 1637, Canada Dept. Agr. Ottawa, Ontario.

Leatham, G.F. 1983. A chemically defined medium for the fruiting of Lentinus edodes. Mycologia 75: 905.

Leatham, G.F. and Leonard, T.J. 1989. Biology and physiology of shiitake mushroom cultivation. In Shiitake Mushrooms: the Proceedings of a National Symposium and Trade Show, p. 19, Univ. Minnesota, St. Paul.

Leegwater, D.C., Youngs, C.G., Spencer, J.F.T., and Craig, B.M. 1962. Investigations into the production of lipids by submerged cultures of the mushroom Tricholoma nudum. Can. J. Biochem. Physiology 40: 847.

Leibach, F.H. and Binkley, F. 1968. Gamma-glutamyl transferase of swine kidney. Arch. Biochem. Bioph. 127: 292.

Lindsay, R.C. 1985. Flavors. In Food Chemistry, O.R. Fennema (Ed.), p. 601. Marcel Dekker, Inc., New York.

Mabrouk, A.F. 1976. Nonvolatile nitrogen and sulfur compounds in red meats and their relation to flavor and taste. In Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors, G.Charalambous and I.Katz (Ed.), p. 146. American Chemical Society, Washington, DC.

MacLeod, G. 1986. The scientific and technological basis of meat flavours. In Developments in Food Flavors, G.G. Birch and M.G. Lindley (Ed.), p. 191. Elsevier Applied Science, New York.

Maga, J.A. 1981. Mushroom Flavor. J. Agr. Food Chem. 29: 1.

Maga, J.A. 1987. Flavor potentiators. CRC Reviews in Food Sci. Nutr. 18: 231.

Matoba, T., Kuchiba, M., Kimura, M., and Hasegawa, K. 1988. Thermal degradation of flavor enhancers, inosine 5′-monophosphate, and guanosine 5′-monophosphate in aqueous solution. J. Food Sci. 53: 1156.

Matsumoto, N., Aoyagi, Y., Hirano, and Sugahara, T. 1978. On the preference of dried shiitake mushroom. J. Jap. Soc. Food Sci. & Tech. 25: 129.

Matsumoto, N., Aoyagi, Y., and Sugahara, T. 1979. On the preference of dried shiitake mushroom harvested in different seasons. J. Jap. Soc. Food Sci. & Tech. 26: 193.

McCallion, B.J., and Lancaster, J.E. 1984. Changes in the content and distribution, in different organs, of the flavour precursors, the S-alk(en)yl-L-cysteine sulphoxides, during seedling development of onions(Allium cepa) grown under light and dark regimes. Physiol. Plant. 62: 370.

Miller, M.W. and Jong, S.C. 1987. Commercial cultivation of shiitake in sawdust filled plastic bags. In Cultivating Edible Fungi, P.J. Wuest, D.J. Royse and R.B. Beelman (Ed.), p. 421. Elsevier Scientific Pub. Co., New York.

Minamida, T., Nishikawa, T., and Ogata, K. 1980. Influences of carbon dioxide and oxygen on the keeping freshness of shii-take (Lentinus edodes (Berk) Sing.) after harvest. J. Jap. Soc. Food Sci. Tech. 27: 505.

Mook, R.G. 1989. An overview of the shiitake mushroom industry and marketing implications. In Shiitake Mushrooms: the Proceedings of a National Symposium and Trade Show, p. 1. Univ. Minnesota, St. Paul.

Mori, K. 1986. Cultivated mushrooms in Japan. In Cultivating Edible Fungi, P.J. Wuest, D.J. Royse, and R.B. Beelman (Ed.), p. 455. Elsevier Science Pub., New York.

Morita, K. and Kobayashi, S. 1966. Isolation and synthesis of lenthionine, an odorous substance shiitake, an edible mushroom. Tetrahedron Letters 6: 573.

Mussinan. C.J., Wilson, R.A., Katz, I., Hruza, H., and Vock, M.H. 1976. Identification and flavor properties of some 3-oxazolines and 3-thiazolines isolated from cooked beef. In Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors, G.Charalambous and I.Katz (Ed.), p. 133. American Chemical Society, Washington, DC.

Nakajima, I., Ichikawa, K., Kamada, M., and Fujita, E. 1961. Food chemical studies of 5'-ribonucleotides. J. Agr. Chem. Soc. Japan 35: 797.

Namiki, M. 1988. Chemistry of Maillard reactions: recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. Adv. Food Res. 32: 115.

Nock, L.P. and Mazelis, M. 1986. The C-S lyases of higher plants: preparation and properties of homogenous alliin lyase from garlic (allium sativum). Arch. Bioch. Biophys. 249: 27.

Ockerman, H.W. 1978. Source Book for Food Scientists. The AVI Pub. Co., Westport, CT.

Ogata, M., Kenmotsu, K., Hirota, N., and Naito, M. 1981. Relationship between uptake of mercury vapor by mushrooms and its catalase activity. Bull. Environ. Contam. Tox. 27: 816.

O'Mahony, M. 1982. Some assumptions and difficulties with common statistics for sensory analysis. Food Technol. 36(11): 75.

Ono, T., Arimoto, K., Kano, K., Matsuoka, K., Sugiura, W., Sadone, H., and Mori, K. 1974. Vitamin D2 formation in Lentinus edodes (shii-ta-ke) by irradiation with a fluorescent sunlamp. Mush. Sci. 9: 435.

Ott, L. 1989. An Introduction to Statistical Methods and Data Analysis, 3rd ed. PWS-Kent Pub. Co., Boston.

Patrick, Z.A., Ingratta, F.J., and Macrae, R.M. 1983. Edible mushrooms with commercial potential in Ontario. Highlights Agr. Res. Ont. 6(2): 1.

Pecora, R.P. 1989. Technical note: determination of protein in edible mushroom (Boletus spp.). Int. J. Food Sci. Tech. 24: 207.

Peleg, Y., Mannheim, C.H. and Berk, Z. 1970. Changes in the quality of dehydrated kibbled onions during storage. J. Food Sci. 35: 513.

Pettipher, G.L. 1988. Cultivation of the oyster and shiitake mushrooms on ligno-cellulosic wastes. Mush. J. 183:491.

Picardi, S.M., and Issenberg, P. 1973. Investigation of some volatile constituents of mushrooms (Agaricus bisporus): changes which occur during heating. J. Agr. Food Chem. 21: 959.

Piotrowski, E.G., Zaika, L.L., and Wosserman, A.E. 1970. Studies on aroma of cured ham. J. Food Sci. 35: 321.

Prosky, L., Asp., N.G., Furda, I., DeVries, J.W., Schweizer, T.F., and Harland, B.F. 1985. Determination of total dietary fiber in foods and food products: collaborative study. J. Assoc. Off. Anal. Chem. 68: 677.

Przybylowicz, P. and Donoghue, J. 1988. Shiitake Growers Handbook. Kendall/Hunt Pub. Co., Dubuque, IA.

Pyysalo, H. and Suihko, M. 1976. Odour characterization and threshold values of some volatile compounds in fresh mushrooms. Lebensm.-Wiss. u.-Technol. 9: 371.

Richardson, T. and Hyslop, D.B. 1985. Enzymes. In Food Chemistry, O.R. Fennema (Ed.), p. 371. Marcel Dekker, Inc., New York.

Sakaguchi, M., and Shibamoto, T. 1978. Formation of sulfur-containing compounds from the reaction of D-glucose and hydrogen sulfide. J. Agr. Food Chem. 26: 1260.

San Antonio, J.P. 1981. Cultivation of the shiitake mushroom. Hort. Sci. 16: 151.

Sasaki, H., Nakamura, N., Aoyagi, Y., and Sugahara, T. 1988. The changes of free amino acids during rehydration of dried shiitake mushrooms. J. Jap. Soc. Food Sci. Tech. 35: 90.

Schlotzhauer, S.D. and Littell, R.C. 1987. SAS System for Elementary Statistical Analysis. SAS Institute Inc., Cary, NC.

Schultz, H.W. 1960. Food Enzymes. AVI Pub. Co., Inc. Westport, CT.

Schutte, L. 1976. Flavor precursors in food stuffs. In Phenolic, Sulfur, and Nitrogen Compounds in Food Flavors, G.Charalambous and I.Katz (Ed.), p. 96. American Chemical Society, Washington, DC.

Schwimmer, S. and Guadagni, D.G. 1962. Relation between olfactory threshold concentration and pyruvic acid content of onion juice. J. Food Sci. 27: 94.

Schwimmer, S. and Mazelis, M. 1963. Characterization of alliinase of allium cepa (onion). Arch. Biochem. Bioph. 100: 66.

Schwimmer, S., and Weston, W.J. 1961. Enzymatic development of pyruvic acid in onion as a measure of pungency. Agr. Food Chem. 9: 301.

Shankaranarayana, M.L., Raghaven, B., Abraham, K.O., and Natarajan, C.P. 1982. Sulfur compounds in flavors. Ch.III. In Food Flavours Part A. Introduction, I.D. Morton and A.J. MacLeod (Ed.), p. 121. Elsevier Scientific Pub. Co., New York.

Shimazono, H., Kubo, K., and Hoji, Y. 1979. Studies on nucleic acid related compounds in fresh fruit body of Lentinus edodes (Berk.) Sing (Shiitake). Bull. Faculty of Agr., Miyazaki Univ. 26: 201.

Shu, C.K., Hagedorn, M.L., Mookherjee, B.D., and Ho, C.T. 1985. Volatile components of the thermal degradation of cystine in water. J. Agr. Food Chem. 33: 438.

Sugahara, T., Arai, S., Aoyagi, Y., and Kunisaki, N. 1975. Contents of 5'-nucleotides and free amino acids in different varieties of dried shiitake mushroom (Lentinus edodes Sing.). J. Jap. Soc. Food Nutr. 28: 477.

Sugita, Y.H. 1986. Recent developments in umami research. In Developments in Food Flavours, G.G.Birch and M.G.Lindley (Ed.), p. 63. Elsevier Applied Science, New York.

Sumner, J.B. and Somers, G.F. 1947. Chemistry and Methods of Enzymes. Academic Press Inc., New York.

Takken, H.J., van der Linde, L.M., deValois, P.J., van Dort, H.M., and Boelens, M. 1976. Reaction products of alpha dicarbonyl compounds, aldehydes, hydrogen sulfide, and ammonia. In Phenolic, Sulfur, and Nitrogen Compounds in Food Flavours, G.Charalambous and I.Katz (Ed.), p. 114. American Chemical Society, Washington, DC.

Tanaka, F., Saito, S., and Esashi, T. 1974. On the differences in the quality and composition of shii-take according to different processing methods. Mush. Sci. 9: 521.

Toyoda, M., Suzuki, H., Ito, Y., and Iwaida, M. 1978. Gas-liquid chromatographic determination of carbon disulfide in shiitake mushroom (Lentinus edodes). J. Food Sci. 43: 1290.

Tressl, R., Bahri, D., and Engel, K.H. 1981. Lipid oxidation in fruits and vegetables. ACS Symposium Series 170: 213.

Tressl, R., Bahri, D., and Engel, K.H. 1982. Formation of eight-carbon and ten-carbon components in mushrooms (Agaricus campestris). J. Agr. Food Chem. 30(1): 89.

Turner, S. 1988. The new fungus among us. Ext. Rev. U.S. Dept. Agr. 59(2): 18.

USDA. 1989a. Vegetables and specialities: situation and outlook report. LPS No. 248: 19.

USDA. 1989b. Vegetables and specialities: situation and outlook report. LPS No. 251: 18.

USDA. 1989c. Agricultural Statistics 1989. United States Government Printing Office, Washington, DC.

Wada, S., Nakatani, H., and Morita, K. 1967. A new aroma-bearing substance from shiitake, an edible mushroom. J. Food Sci. 32: 559.

Whitaker, J.R. 1972. Principles of Enzymology for the Food Sciences. Marcel Dekker Inc., New York.

Wood, D. 1989. Mushroom biotechnology. Intern. Ind. Biot. 9: 5.

Wu, C.M., and Chen, C.C. 1986. Chemical changes in the aromas of mushrooms and mushroom extracts during thermal processing, and methods of flavor supplementation. In Role of Chemistry in the Quality of Processed Food, W.H. Chang and C.Y. Lii (Ed.), p. 142. Food and Nutrition Press, Inc., Westport, CT.

Wuest, P.J. 1989. Shiitake growing in sawdust. In Shiitake Mushrooms: the Proceedings of a National Symposium and Trade Show, p. 47, Univ. Minnesota, St. Paul.

Yamaguchi, S. and Kimizuka, A. 1979. Psychometric studies on the taste of monosodium glutamate. In Glutamic Acid: Advances in Biochemistry and Physiology, L.F. Filer, S. Garattini, M.R. Kare, A.R. Reynolds, and R.J. Wurtman (Ed.), p. 35. Raven Press, New York.

Yamaguchi, S., Yoshikawa, T., Ikeda, S., and Ninomiya, T. 1968. Synergistic taste effects of some new ribonucleotide derivatives. Agr. Biol. Chem. 32: 797.

Yamazaki, H., Ogasawara, Y., Sakai, C., Yoshiki, M., Makino, K., Kishi, T., and Kakiuchi, Y. 1980. Studies on formaldehyde in Lentinus edodes. J. Food Hyg. Soc. Japan 21: 165.

Yasumoto, K., Iwami, K., and Mitsuda, H. 1971a. A new sulfur-containing peptide from Lentinus edodes acting as a precursor for lenthionine. Agr. Biol. Chem. 35: 2059.

Yasumoto, K., Iwami, K., and Mitsuda, H. 1971b. Enzyme-catalyzed evolution of lenthionine from lentinic acid. Agr. Biol. Chem. 35: 2070.

Yasumoto, K., Iwami, K., and Mitsuda, H. 1974. Enzymatic formation of shii-ta-ke aroma from non-volatile precursor(s)-lenthionine from lentinic acid. Mush. Sci. 9: 371.

Yokokawa, H. and Mitsuhashi, T. 1981. The sterol composition of mushrooms. Phytochem. 20: 1349.

Yoshida, H., Hayashi, J., Aoyagi, Y., and Sugahara, T. 1979. Fatty acid compositions and ergosterol contents of different grades of dried shiitake mushroom (Lentinus edodes Sing.). J. Jap. Soc. Food Sci. Tech. 26: 221.

Zubay, G. 1988. Biochemistry. Macmillan Pub. Co. New York.

APPENDIX

Table A-1--Experimental dehydration conditions compared with optimal processing conditions for whole shiitake

Source	Temperature range (°C)	Air velocity (m/sec)	% Relative humidity
Experimental	40,50,60	1.10-1.15	35-42
Optimal[a]	40-55	1.0-2.0	38-41

[a] Cho et al. (1981).

Source(s):

Cho, D.B., Kim, D.P., and Choi, C.S. 1981. Kinetics of drying shiitake mushroom, Lentinus edodes sanryun No.1. J. Korean Soc. Food Nutr. 10: 53.

Table A-2--Reproducibility and sensitivity of the chemical methods used in experimental work[a]

Method	MSG	WSC[b]	NRM[c]
Corr. Coeff.	.9666	.9878	.9881
CV 1	± 6.0	± 17.2	± 13.5
Conc.[d]	12.5	0.00005	0.01064
CV 2	± 4.0	± 19.6	± 5.7
Conc.[d]	17.5	0.01	0.1064

[a] n=10.
[b] mg glucose equivalents/g.
[c] mg glycine equivalents/g.
[d] mg/mL.

SHIITAKE/BLACK MUSHROOM SENSORY PANEL

Panelist Number _____

You have been given a reference sample, marked R, to which you are to compare shiitake flavor strength for several coded samples. Taste each sample, determine whether it has stronger, equivalent, or weaker shiitake flavor than the reference. Then mark the degree of difference that exists.

Sample number	____	____	____	____	____	____
Stronger than R	____	____	____	____	____	____
Equal to R	____	____	____	____	____	____
Weaker than R	____	____	____	____	____	____
Degree of Difference						
None	____	____	____	____	____	____
Slight	____	____	____	____	____	____
Moderate	____	____	____	____	____	____
Much	____	____	____	____	____	____
Extreme	____	____	____	____	____	____

Fig. A-1--Sample sensory evaluation scorecard used in sensory evaluation of experimental and commercial shiitake samples.

VITA

David D. Wilson was born on March 22, 1965 in Wyandotte, Michigan to Norman and Jean Marie Wilson. He graduated seventh in his class at Woodhaven High School in May of 1983. The following fall he accepted a partial academic scholarship to begin undergraduate studies at Eastern Michigan University in Ypsilanti, Michigan. In January of 1986, he was accepted as a transfer student into the engineering college of the University of Michigan, Ann Arbor, and for two years studied chemical engineering. During his studies at U of M, the author discovered that his interests in foods were stronger than those in engineering, and eventually decided to pursue those aspirations. The author withdrew from U of M, re-enrolled at Eastern Michigan University in January of 1988, and completed the bachelor of science degree in General Biochemistry in May of 1989. In the summer of 1989, the author accepted a graduate research assistantship in the Food Technology and Science Department at the University of Tennessee, Knoxville. His master's program began the following fall and the requirements for the degree were completed in May of 1992.

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