Part IV

NUTRITIONAL AND MEDICINAL ATTRIBUTES OF MUSHROOMS

CHAPTER 23

ANALYSIS, DIGESTIBILITY AND THE NUTRITIONAL VALUE OF MUSHROOMS

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1. INTRODUCTION

In many parts of the world people have recently become much more aware of nutrition and its importance. Several years ago, the U.S. Congress passed new nutritional labeling laws requiring that virtually all processed food and much of the fresh produce, including mushrooms, must show their nutritional content on the label. Those laws take effect this year. In Europe, the EEC also has a new nutritional labeling law. Such laws depend solely on chemical analysis.

Chemical analysis tells what is present, but it does not tell how well it is used. Like any experimental data, it is subject to varied interpretations or misinterpretation.

For many years, we have known that the primary structural carbohydrate of mushrooms is chitin. Chitin is poly-N-acetylglucosamine. The nitrogen of the chitin will be included in a Kjeldahl analysis. While the chitin is non-protein, there seem to be no definitive experiments that allow us to conclude anything about the nutritional value of mushroom chitin. All living tissue also contains nucleic acids which will account for some of the nitrogen.

Many people routinely report crude protein of most materials as 6.25 X Kjeldahl nitrogen (A.O.A.C., 1990; FAO, 1970; Kurtzman, 1975; Watts & Merill, 1963). The nucleic acid, chitin and other miscellaneous nitrogen are normally included, so it should be clear that the reported protein will often be too high. Nonprotein structural nitrogen may be relatively uncommon. It does occur in all mushrooms, yeasts and arthropods. Many plants contain nitrogenous glycosides. All living tissue, but especially actively growing tissue will contain nucleic acid nitrogen. Thus, poultry eggs and seeds contain large amounts of storage protein, with few (eggs, one) living cells. Milk is a secretion which has only a few sloughed cells and contaminating microorganisms.

Official Methods of the Association of Official Analytical Chemists (A.O.A.C., 1990) call for several different factors to be used for various commodities. Those factors range from 5.18 for almonds (amygdalin) to 6.38 for dairy products. Most commodities are lumped together and most often 6.25 is recommended. It is surprising that they specifically recommend 6.25 for yeast. Yeast would be more like mushrooms than anything else that is specifically mentioned, but yeast would

usually have a higher nucleic acid content. Watt and Merrill (1963) proposed 6.25 X 2/3 or 4.17 for *Agaricus bisporus*. Doesburg and Meijer (1965) assayed the amino acids of *Agaricus bisporus* and recovered 5.162 grams of amino acid per gram of nitrogen. Assuming that the amino acids weigh 6% more than the protein they are derived from, due to water of hydrolysis, a factor of 4.78 is a minimum conversion factor for *Agaricus bisporus*. Even 5.03 would be reasonable, if Doesburg and Meijer accounted for 95% of the protein nitrogen. Other mushroom species probably are similar.

2. LABELING LAWS

Nutritional labeling laws introduce new problems into our nutritional understanding. It is good to let the public know what nutritional benefits they should expect from fresh produce. Often regulations may require that the analysis be done in such a manner as to assure that the data applies to all packages of the produce. That too sounds good, but it means that old data, considered to be carefully done, is no longer acceptable. The growers are not prepared to do such analysis and must find a contractor to do it for them.

I was privileged to see the complete contractor's report which is the basis for the official U.S. nutritional label for mushrooms (Table 1) (Contractors Report to Produce Marketing Association, 1982). Several of their data appear to be out of the expected range, based on the analysis of others.

They used samples taken from retail supermarkets, shipped to them from all of the 48 states, but they reported moisture contents of 90.2% to 93.5%. In my own experience, I find that mushrooms picked less than one hour before the initial weighting will usually be less than 92% water when dried at 80°C. However, they ranged from 88.6 to 93.2% (Table 1). There will be some apparent caramelization, that is, some of the water lost will be chemically part of the sugar. I also find that after 20 hours storage in a refrigerator, the same mushrooms have lost water and are about 88% water. The values in the literature vary from the mid eighty-percents to the mid ninety-percents.

TABLE 1. Proximate composition of mushrooms, gm per 100 gm fresh weight. Minerals mg per 100 gm fresh weight.

Species and Ref.	Moisture	Ash	Nitrogen	Protein N X 5.0	Fat	Crude Fiber	Na	K	Ca	Fe	P
Agaricus bisporus[a]	89.7	0.82	0.78	3.90	0.20	0.38					
A. bisporus[b]	89.5	1.26	0.61	3.07	0.19	1.09					
A. bisporus[c]	90.4	0.9	0.64	3.24	0.30	0.80	14.98	414.0	6.05	.797	116.0
A. bisporus[d]	88.7	0.9	0.62	3.08	0.90	0.80	11.98	322.0	8.02	.938	103.1
A. bisporus[e] High	93.0	1.40									
A. bisporus[e] low	87.2	0.87									
A. bisporus[f] High	93.5	0.9*	0.64	3.20	0.5*	1.00	5.40	389	5.03	1.20	124.4
A. bisporus[f] Low	90.5	0.6	0.43	2.16	0.2	0.500*	3.90	329	1.48	0.420	92.6
A. bisporus[f] Label				2.23	2.23	0.558	4.46	357			
Pleurotus ostreatus[g]	92.5		0.34	1.72							
P. ostreatus "florida"[h]	91.5	0.79	0.25	1.25	0.14	1.01					
Volvariella volvacea[a]	88.4	1.46	0.80	3.99	0.74	1.38					

* Exact value in three or more composite samples.

References: [a]Chang (1992); [b]Esselen and Fellers(1946); [c]Watts and Merrill(1963); [d]F.A.O.(1972); [e]Kurtzman, unpublished; [f]Contractor'sReport (1982); [g]Kalberer and Kunsch(1974); [h]Bano, *et al.* (1981).

The mushrooms shipped to the contractor from the greatest distance and from the driest climate had the highest water content. That is also the part of the country where the soil is most saline. If we believe the official analysis we must reject experiments that show that increased salt in cultivation yields mushrooms of higher solid content, although those from the hot-dry-saline area also contained the least ash.

There were other interesting variations in the ash, and calcium was 3.4 times greater from one area of the country than from another (Table 1). As far as I know, all U.S. growers use large quantities of gypsum in their compost and limestone in their casing. Thus, it is not reasonable that calcium should vary by locale. The magnesium content of soils does vary from region to region and from one source of limestone to the next, but the report showed a variation of only 9.6 to 12.0 mg magnesium per 100 g mushrooms. Magnesium and calcium are antagonistic in many biochemical reactions and although most growers avoid magnesium, the largest portion of magnesium is in the limestone. So, although some correlation between magnesium and calcium content might be expected, none was apparent.

While others (Crisan & Sands, 1978; FAO, 1970; Kurtzman, 1975; Watts & Merrill, 1963) have found 0.65 to 0.78% nitrogen, the contractor found 0.52%. It is interesting that 0.52% is exactly 2/3 of the 0.78%. Watt and Merrill (1963) used 2/3 in their calculation of protein from nitrogen. While others found 0.2 to 0.3% fat (Crisan & Sands, 1978; Kurtzman, 1975), the official label says 2%.

The sampling protocol was such that it assured that the sampling was not random, but even the questionable sampling would not appear to account for the unexpected results. It seems equally unlikely that it was due to varietal differences.

3. ANTI-NUTRITIONAL FACTORS

In 1972, Presant and Kornfeld (1972) isolated a hemagglutinin from *Agaricus bisporus*. The hemagglutinin was similar to, but distinct from, the hemagglutinin of bean, *Phaseolus vulgaris*. Apparently, that work went unnoticed by people interested in the nutritional value of mushroom until very recently. Ortiz *et al.* (1992), used the purified hemagglutinin and found that it caused intestinal malabsorption in rats. Malabsorption was determined by weight gain, fecal nitrogen and nitrogen digestibility. Histological lesions were found in the small intestine. They found no histological lesions in rats that were fed material that had been heated at 100°C for 1 h. Weight gain, fecal nitrogen and nitrogen digestibility were not determined for the rats fed the heated material.

Hemagglutinins have also been reported in *Pleurotus ostreatus* and *Pleurotus spodoleucus* (Kogure, 1975). Until some mushroom is specifically shown not to contain hemagglutinins, we should expect that all mushrooms have them.

Bean hemagglutinin is well known to cause intestinal malabsorption (Liener, 1975), so the results of Ortiz *et al.* (1992) were not surprising. It appears that a thorough study is needed to determine the minimum degree of cooking or other processing that will be required to inactivate the hemagglutinin. Other mushrooms should be examined for hemagglutinins. As long as we are aware of the problem, it need not decrease the nutritional value of mushrooms, but we need to know more than we have learned so far.

4. VITAMINS

Fungi have long been used as a source of vitamins. Yeast has been sold as a vitamin source. Years ago it was sold, along with an iron supplement as vitamin pills in the U.S.A. In Australia, New Zealand and the United Kingdom, yeast extract is sold as a spread for breakfast toast. The beneficial value of fungi as yeast was appreciated, at least indirectly, by western civilizations long before yeast was clearly understood. Governor William Bradford, the primary civil leader of the American Pilgrims of the Plymouth Colony, wrote that the colonists had suffered greatly from want of food and from pestilence (Bradford, 1648). He indicated that it was surprising to him that things had not been worse because they did not have beer to drink to give them health; the colonists were reduced to drinking water! If you ever think that mushroom or nutritional science is lagging behind other intellectual endeavors, consider the social sciences in the U.S.A., who tell us that Governor Bradford and his associates, who considered beer a health drink, were somehow responsible for our alcohol prohibition 250 years later. A lesson that we should learn from their error is to read older literature. People a decade or a century ago did have good brains. It is important to build on both past errors and past knowledge.

Most vitamin analyses have shown mushrooms to be very similar to yeast, except that thiamine is usually found to be low in mushrooms. Thiamine is required for decarboxylation, a reaction required by all living tissue. Why then is it low in mushrooms? According to Wakita (1976) most mushroom species have thiaminases, enzymes that destroy thiamine. There are two thiaminases: I, base transferase (Wittliff & Airth, 1970a) and II, hydrolase (Wittliff & Airth, 1970b). Apparently, mushrooms have the hydrolase and a few also have the transferase. Both enzymes destroy the vitamin. If thiaminase is the reason for low thiamine analysis, it may be released during normal food preparation, but destroyed by cooking procedures. It is possible that with proper care, the thiamine in mushrooms could be greatly increased.

If mushrooms contain at least two anti-nutritional factors, a few might discard them as worthless food. That would not be wise, since most foods have some negative factors and both factors known in mushrooms occur in other, commonly consumed foods. Beans and other legumes contain hemagglutinins (Liener, 1975), and fresh-water fish have thiamine base transferase (Wittliff & Airth, 1970a).

5. FEEDING EXPERIMENTS

Another appealing answer is to make all determinations by feeding experiments. Feeding experiments are expensive and rats or other species are generally used. Rodent digestive systems are clearly different than the human digestive system. Specific differences can easily become of great importance. Chitin might easily pass down the short gut of a rodent without being digested. That might keep the digestive enzymes from reaching the cell contents of unbroken cells. Both the chitin and all cell contents might be digested in the complex gut of a ruminant, neither experiment would tell us about humans.

Human feeding experiments can tell much about nutritional value. It might seem that human feeding experiments would give conclusive answers, but it would be nearly impossible to feed only mushrooms to people. What influence can we expect from the remaining diet? If the basal diet and the control were adequate in thiamine, there might be no affect from the thiaminase, but it might be great if the basal diet contained any less than the minimum requirement. It should be apparent that the degree of cooking could make a considerable difference.

Table 2. Protein Efficiency Ratio and digestibility of tunnel-dried mushrooms in rats.

Expt.	Dietary Protein	Final Body	Total Feed	PER[5]			% Digestibility[6]		
No.[1]	10% of diet[2]	Wt., gm[3]	Consumed[3]	Actual[4]	Adjusted	5.0/6.25	Diet	N	5.0/6.25
1	Casein (ANRC)	201 ±9	438 ±36	3.35 ± .13	2.50			93	91
1	*A. bisporus*	141 ±7	286 ±19	3.01 ± .10	2.25	2.81	88	65	81
2	Casein (ANRC)	183 ±11	372 ±24	3.42 ± .09	2.50			94	92
2	*A. bisporus*	116 ±4	278 ±12	2.18 ± .05	1.59	1.99	82	59	74

[1]See Table 3 for the diets used in experiment No.1 and No. 2.
[2]The 10% protein is based on N X 6.25.
[3]Mean ± S.E. Duncan's Multiple Range Test. All values within each experiment are significantly different: P<.01.
[4]Mean ± S.E. Duncan's Multiple Range Test. All values within experiments are significantly different: Experiment No. 1 P<.05. Experiment No. 2 P<.01.
[5]PER (Protein Efficiency Ratio) = weight gain/protein intake. "Adjusted" equivalent with casein reduced to standard 2.5. "5.0/6.25" = "Adjusted"/ 5.0/6.25.
[6]Digestibility: Diet = (feed intake - fecal weight)/feed intake X 100. N = (N intake - fecal N)/ N intake X 100. "5.0/6.25" = (N intake - fecal N)/ N intake X 5/6.25 X 100. Pooled data, day 7 through day 14.
Male, Sprague-Dawley rats, initial age 21 days, initial weight 55 grams.

Some of the problems with feeding experiments are more subtile. Years ago I had some Protein Efficiency Ratios (PER) run. While conferring with the pharmacologist who was in charge of running the experiments, he explained that the results would depend largely on the basal diet even though it would be used as the control.

The results of those experiments are shown in Table 2. The experimental rats never ate as much as the controls and more nitrogen was found in their feces. If we assume that the chitin nitrogen should not be counted, and try to recalculate on the basis of 5.0 rather than 6.25 as the nitrogen to protein conversion factor, the PERs look better. In experiment No. 1, the recalculated PER is better than

Table 3. Diets as percent composition, used in Protein Efficiency Ratio experiments (Table 2).

Ingredients	Experiment No. 1		Experiment No. 2	
	Control	*A.bisporus*	Control	*A.bisporus*
Casein (ANRC)	11.17		11.07	
Dry Mushrooms		37.41		39.68
DL-Methionine		0.57		0.57
N from above	1.60	1.60	1.60	1.60
Corn Oil	8.00	7.33	8.00	6.88
H_2O	4.37	3.94	4.33	2.01
Salts	7.80	3.71	6.49	3.00
Cellulose	3.00	0.61	3.30	0.36
Vitamins	2.00	2.00	2.00	2.00
Corn Starch	20.00	20.00	20.00	20.00
Dextrose	43.66	24.43	44.36	25.50

casein. Digestibility increases when we allow for the 20% of the nitrogen that is apparently in the chitin and nucleic acids, but is never as good as casein. The calculations that I used are questionable, but the whole PER procedure is weak. Table 3 shows the composition of the diets. While the ANRC casein standard is nearly pure protein, and an almost chemically defined diet can be made with it, there are major problems in balancing the experimental diet containing the mushrooms. In their attempt, my colleagues were forced to provide diets of lower energy content with the mushrooms. Of the differences between the diets, the most likely explanation would seem to suggest the rats need some minimum quantity of fat in their diet. The differences in the results, the problems with diet balance and the problems with interpretation of the data should make it clear that there is no absolute answer to nutritional value.

6. CONCLUSIONS

It would be very convenient if all we needed was a Kjeldahl or even an amino acid analysis to determine the nutritional value of mushrooms. Unfortunately, even feeding experiments depend on the degree of destruction of antinutritional factors and on other foods in the diet. We need to pursue all methods that will yield information on the nutritional value of mushroom.

The hemagglutinins and thiaminase may not be the only things found in mushrooms that can reduce their nutritional value and not all species or even varieties may contain them. If all anti-nutritional factors are proteins, then proper cooking may be all that is required to increase nutritional value. Studies need to be run to determine the minimum heating required to destroy the known anti-nutritional factors *in vitro* and the minimum heat necessary to obtain maximum nutritional value from whole mushrooms.

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