THE UNIVERSITY OF ASTON IN BIRMINGHAM

STUDIES ON THE COMPOSITION AND NUTRITIONAL VALUE OF THE CULTIVATED MUSHROOM, AGARICUS BISPORUS (LANGE) SING.

by

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SUMMARY

The effects of various cultural conditions on the composition and nutritional quality of Agaricus bisporus (Lange) Sing. were investigated.

Variation in composition was found between different classes of sample. Sampling techniques were standardised to allow for major variations in the different developmental stages and culture ages.

Fruitbodies were found to be of low calorific value but contained protein of high digestibility and quality, containing all the essential amino acids required by man. Quantitative estimates of the sulphur-containing amino acids indicated that fruitbodies were deficient in methionine and cysteine.

The extent of water application and the supplementation of conventional substrates with various nitrogen-containing substances, influenced yield and composition, establishing the importance of these two factors in the physiology of fruitbodies and cultural management.

Storage conditions influenced composition, high temperatures being deleterious to the nutritional value of fruitbodies.

Submerged culture techniques were used to ivestigate the effects of various nutrients on growth and composition of mushroom mycelium, with special reference to the sulphur-containing amino acids. Yield and composition were greatly affected by the carbon:nitrogen ratio of the medium and by the nitrogen source. Significant increases in mycelial methionine content were observed on the addition of inorganic sulphate, the methionine derivative N-acetyl-L-methionine, and L-methionine. A greater increase in methionine content was obtained when the biomass of a thermophilic bacterium isolated from compost was used as a nitrogen source.

KEY WORDS

COMPOSITION NUTRITION

Agaricus bisporus

DECLARATION

I declare that the work described in this thesis is the result of my own investigations, except where reference is made to published literature and where assistance is acknowledged, and that the work has not been submitted for any other award.

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Candidate

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GENERAL INTRODUCTION

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In recent years, the artificial culture of the cultivated mushroom Agaricus bisporus (Lange) Sing., has advanced from an unsophisticated and unpredictable process to a highly industrialised technology which, in many respects, can be compared to other industrial processes involving micro-organisms, e.g. beer brewing. With these refinements mushroom production has expanded dramatically in Europe and in the U.S.A. and, recently, large-scale industries have been established in many countries in the Orient, notably Japan, The Republic of Free China, Malaysia, India and the Philippines. Mushroom growing is now being introduced into Africa and South America. Production in the U.K. over the last decade has increased by about 10% per annum and, in 1974, the output of the U.K. industry was approx. 60 million Kg, representing a per capita consumption of approximately 1 Kg per annum.

In West Germany, Canada and France, countries in which mushroom consumption is well-established, per capita consumption rates exceed 2.2 Kg per annum (production and consumption data provided by Mushroom Growers' Association, England, 1974).

It is known that mushrooms gathered from the wild have been consumed by Man in small quantities since the earliest of times and even until comparatively recently the cultivated species has not been regarded as a part of the normal diet. However, the improvements to culture techniques and the general availability of the cultivated species is now establishing mushrooms as a regular part of the total diet of Man, especially in Western countries. Attitudes and outlooks are therefore changing on a food product which has been traditionally regarded as a luxury product eaten with other foods to provide flavour and zest. Most of the edible species of mushrooms are members of the genus Agaricus, the type genus of the family Agaricaceae, class Basidiomycetes.

Artificial culture is confined almost entirely to the species Agaricus bisporus (Lange) Sing. which, according to Singer (1961), exists in the wild on soils rich in nitrogen, in greenhouses, near to manure heaps, on roadsides, and in gardens and parks. Its natural geographic area extends over the Northern hemisphere outside the tropics and the arctic.

The basic life cycle is from spore to mycelium to fruitbody, the latter bearing spores again. Mycelium cultured from single spores is usually sterile and does not produce fruitbodies unless a mating occurs by fusion with another compatible mycelium generated from another spore. Multiple spore cultures are usually fertile and are therefore used to establish mycelial cultures from which selections are made for the manufacture of pure culture spawn, the first essential requirement for pure culture methods of production. In this specialised process of spawn manufacture, mycelial cultures are established on grains such as wheat and rye under aseptic conditions.

A second transplant occurs at the stage of spawn inoculation onto the primary food base for culture. The primary food base consists of a compost, traditionally prepared from a fermented mixture of wheat straw and horse manure, involving a series of defined stages, in order to ultimately achieve a nutritionally balanced substrate favouring the growth of A.bisporus at the expense of other possible competitors. Composting is essentially a natural process but conditions are controlled throughout and a pasteurization stage is included which ensures freedom from insects and other undesirable organisms.

Spawn culture and substrate manufacture are technically the most demanding of the entire culture process. The remainder of the process, which is concerned with the establishment of the reproductive or fruitbody stage of the life cycle, which provides the crop for harvest, is

by comparison less demanding.

The reproductive stage is induced by covering the surface of the spawned compost with moist soil (the casing layer) and incubating in a well-aerated atmosphere maintained at 15-18°C. If the correct cultural conditions are maintained the fruitbodies are produced in a series of breaks or flushes at approximately weekly intervals. The yield capacity of the culture diminishes with time and, in industry, it is usual to terminate cropping after five or six weeks. What remains of the culture is disposed of and may be used as a fertiliser, a soil conditioner or as a mulch for allotments and gardens.

With the improvements in the technology of spawn (inoculum) and substrate (compost) manufacture, yields of approximately 20 Kg m^{-2} of cropping area are obtained. In a typical medium sized production unit operating on a weekly input basis of 30 tonnes of substrate, an annual yield of approximately 312 tonnes of mushrooms can be expected. In present-day production units, with compact systems of growing, such a yield is obtained over approximately 1,560 m^2 ground area. Despite high outputs and intensification, harvesting is still manual; however, in recent years progress has been made in the mechanization of harvesting. With this prospect in mind, in addition to the fact that waste agricultural and industrial materials are used as substrates, increasing attention is being given to improve further the efficiency of techniques and systems of growing which may lead to new prospects for the exploitation of this foodstuff.

While, in the past, much attention has been given to the improvement of the pure culture systems, comparatively little attention has been given to aspects concerning the consumption and utilisation of mushrooms, and considerable conflict exists in the literature on the

value of mushrooms as a food. The purpose and objectives of this research is to determine the composition of A.bisporus fruitbodies, to examine possible causes for the conflicting evidence on the composition of the fruitbodies and to elucidate any factors, which are inherent in culture methods, which contribute positively or negatively to the overall value of A.bisporus fruitbodies in human nutrition.

Compared to the traditional art of producing mushroom fruitbodies in compost beds, the submerged culture production of mushroom mycelium is relatively recent. Following the successful production of penicillin and other antibiotics by submerged culture, much attention has been given to the possibility of producing mushroom mycelium by submerged culture.

The technique offers possibility of large scale production of a mushroom-flavoured food material, which may be used for the manufacture of soups and flavourings, and also the possibility of utilizing quantities of low-cost growth media, such as citrus press water or cannery wastes.

The mycelium, like the fruitbody of the mushroom, has been shown to be nutritionally valuable, thus, while it would not serve as a direct substitute for the mushroom sporophore, because it lacks the structure and texture of the fruitbody (which is of significance in a food product), because of its nutritional value, it may be useful as a concentrate of B vitamins and amino acids. The possibility of using mushroom mycelium as a source of protein for the Third World has also been considered, but at present mushroom mycelium is too expensive for use as a food in these areas; it may nevertheless be attractive as a flavouring for other plant proteins.

Section 1

LITERATURE REVIEW

1.1 Nutrition of *Agaricus bisporus*

1.1.1 Compost, casing layer and nutritional requirements

Compost

Since natural materials are used in composting, variation in the composition of compost can be expected which, in turn, may affect the composition of the fruitbodies. In recent years, however, a more complete understanding of the processes involved in composting has been revealed by research. Following early work by Waksman and Nissen (1932) on biological activity in fermenting horse manure, Lambert (1941) identified the major physical and environmental variables which contributed to successful composting and concluded that the degree of aeration was the most important variable which affected microbial activity. This, in turn, is responsible for the necessary heat generation. In practice, attention is given to the shape of the mixture stacks and to frequent turnings during the entire composting time. Lambert also drew attention to the importance of a process known as "peak heating" or pasteurization and this was introduced by Sinden & Hauser (1950 , 1953) in a method of composting known as the "Short" method. During pasteurization, compost temperature is maintained at 60°C for several hours.

Techniques of composting vary in detail but conform to a general schedule involving two stages. Stage 1 is performed out of doors where straw, supplements and water are mixed and stacked to initiate the fermentation. Stacks are regularly turned to maintain aerobic conditions and to ensure thorough mixing of the ingredients. During this stage high temperatures are obtained in the stack as the result of intense microbial activity. This, in turn,

selects progressively for specific groups of thermophilic micro-organisms. Stage 2, which represents the peak heat stage, is performed in specially constructed buildings called heat rooms. Aeration and temperature control are important features of this stage. Sinden & Hauser (1953) recommended for their "Short" method of composting a nitrogen content of the initial mixture of about 1.5% on a dry matter basis, while in Edwards' (1949) formula based on dried blood and wheat straw, the level of nitrogen recommended was 2%. Little attention was given at this time to the detail of ingredient formulation. Pizer (1937) and Pizer and Thompson (1938) attached importance to the dispersion of the various constituents of compost and found that when the colloids were dispersed growth of mushroom mycelium was poor. The addition of calcium sulphate (gypsum) to flocculate the colloids encouraged a strong growth of mycelium. Gypsum addition gave the compost a more granular structure, and its ability to retain water was increased.

During both Stages of composting many changes in the composition and numbers of the natural microflora were shown to occur (Hayes, 1969). Mesophiles flourish at ordinary temperatures but soon give rise to a predominantly thermophilic flora specific to mushroom compost and other heat generating fermentations. Thermophilic bacteria, which utilise simple forms of energy such as sugars and amino acids outnumber the actinomycetes (a group which can utilise more complex forms of energy such as cellulose and hemi-cellulose). The actinomycetes flourish in the later stages of composting, especially during Stage 2. Fungi, also active decomposers of complex sources of energy, remain at a constant

level throughout Stage 1 but their numbers increased slightly throughout Stage 2. These changes are orderly and consistent but variations from the general pattern occur according to the nutrients available for microbial exploitation during composting and the temperature conditions prevailing in the composting mass.

Concurrently with the build-up of vast microbial populations some of the chemical components are transformed into microbial biomass which, together with what remains of the mixture, forms the subsequent substrate for mushroom growth. Much nutriment is lost, however, as gaseous by-products, predominantly carbon dioxide and ammonia, and much energy is released as heat. This results in an overall loss of dry matter during composting, accounted for by breakdown of the simple and most readily available forms of energy, e.g. sugars, and by the removal of lignins, cellulose and hemi-cellulose fractions of straw (Gerrits et al.,1967; Hayes and Randle, 1968). Composting mixtures for mushroom production were seen by Hayes and Randle (1969) as a wasteful but necessary energy-consuming process. While nutrients of value to the mushroom should be conserved, the achievement of this in practice requires a more precise definition of the chemical composition of mixture ingredients and the balance of the primary nutrient, carbon, with that of nitrogen, which previously was the only component adjusted by supplementation. The schedule for composting outlined by Hayes and Randle (1969, 1970) for use in the United Kingdom exploits the use of cheap and plentiful by-products of the sugar industry, e.g. molasses, (to minimise losses of cellulose and hemi-cellulose, nutrients which are important for mushroom growth), sugar beet pulp, leafy hay, apple and grape pumice, or other energy rich materials which are easily broken down by the composting microflora (such as fats and vegetable oils) the latter being materials suggested by Schisler(1967).

Casing layer

The casing soil, which is used to form a layer on the surface of the compost in order to induce fruitbody formation, is prepared from peat made alkaline with calcium carbonate. This is a dilute medium for growth and its function as part of the total substrate has only recently been considered. The investigations of Eger (1961) showed that micro-organisms which naturally colonise the casing layer are implicated in the change from vegetative to reproductive growth. The activities of Pseudomonas putida isolated from casing soils, (Hayes et al., 1969; Arrold, 1972) was linked by Hayes (1972 , 1973) to its ability to solubilize iron, an essential nutrient for the growth and development of primordia (the pre-fruitbody stage) but concluded that iron availability in a casing soil could be influenced by many factors other than those related to microbial activity (such as the formation of acids).

Nutritional requirements

The two substrates (compost and casing layer) required for the commercial culture of mushroom fruitbodies are linked to the activity of other micro-organisms which generally operate by affecting the availability of nutrients. The procedures involved in preparing a compost and casing and the management of a culture to initiate fruitbody formation are concerned in establishing those micro-organisms which contribute to the growth process of a mushroom and are beneficial at the expense of those which are natural competitors or are pathogenic. Because of the difficulties in defining nutritional requirements for growth using the natural media used in commerce, attempts have been made to extrapolate information gained from pure culture studies using standard laboratory techniques.

Treschow (1944) defined the nutritional requirements for the growth of A.bisporus mycelium in liquid culture, but this did not provide any information on the nutritional requirements for the formation of fruitbodies. However, using a Petri plate technique (Hume and Hayes, 1972) and a small tray method, using autoclaved liquid and solid substrates (Smith and Hayes, 1972), a means was provided by which at least broad generalisations as to the minimal nutritional requirements of mushroom fruitbodies could be investigated. Using these studies as a basis, together with what is known about the composting process, Hayes (1972) summarised the nutritional requirements of A.bisporus, in relation to composting, as follows:-

(i) Carbon

This primary energy source is provided largely in the straw cellulose, hemi-cellulose and lignin, these being broken down to simpler carbohydrates by:

(a) the micro-organisms active in composting,
(b) the enzymes secreted by mushroom mycelium, and
(c) the micro-organisms which are active during the growth of the crop.

(ii) Nitrogen

Evidence suggests that there is a specific protein requirement. Only a partially degraded casein supports growth of primordia and fruitbodies in pure culture. In composting, nitrogen provided to activate the fermentation is converted into microbial protein. The biomass of the thermophilic micro-organisms and their by-products probably provides the required protein.

(iii) Minerals

Potassium, phosphorus, magnesium, calcium, iron and trace metals, such as manganese, zinc, copper etc. These are provided in the straw, manure and activators, and when

composting mixtures are correctly formulated they are in adequate supply: they may, however, be deficient in some of the newer synthetic mixtures. Their availability to the mushroom is probably governed by the activity of micro-organisms in composting during the growth of the crop and also by the activities of the mushroom mycelium.

(iv) Vitamins

These growth factors are primarily synthesised by the micro-organisms which are active during the compost fermentation.

(v) Acetate

Biochemically this functions as a building block for many of the essential reactions of cell metabolism. Acetate units are likely to be continually synthesised by latent microbial activity during the growth process and by the probable build-up of fats and oils by the thermophilic micro-organisms.

Although attempts have been made to produced mushrooms by techniques and methods which do not involve composting, e.g. the Till process (Till, 1962), in which a substrate is prepared without animal manure and without composting, for commercial culture compost preparation according to the traditional methods seems necessary to ensure that all food requirements are available for the development of mushroom fruitbodies.

1.1.2 Nitrogen supplementation

Nitrogen, especially in the protein form, was shown to be an important element in mushroom nutrition (Sinden and Schisler, 1962 and Schisler and Sinden, 1962). In recent years, supplementation of composts with nitrogenous materials has become a common practice.

Edwards (1950) and Atkins (1963), found a relationship between

the nitrogen content of finished compost and the production of fruitbodies, conversely Gerrits et al., (1967) reported equal yields from compost with nitrogen contents ranging from 1.6 to 2.7%.

O'Donoghue (1965) suggested that the limitation in yield in compost with a high nitrogen content is associated with the presence of ammonia. During the early stages of composting much of the nitrogen is present as ammonia, in the later stages of composting the ammonia is recombined as microbial protein, which is then itself used by the mushroom (Schisler and Sinden, 1962). Ammonia as such is toxic to the mushroom (Sinden and Hauser, 1953). MacCanna (1969) reported the need of a nitrogen fertilizer which increases compost nitrogen without any corresponding increase in ammonia.

Various feed concentrates (cottonseed meal, wheat flour, dried skim milk and fish meal) were used as nitrogen supplements in pasteurized compost at spawning by Schisler and Sinden (1962). A significant increase in yield resulted from supplementation with cottonseed meal, and dried skim milk, but very high levels of supplement caused no further increase due to mold invasion.

Sinden and Schisler (1962) applied supplements at casing; they reported that protein rich in phenylalanine, leucine, isoleucine and valine were the most stimulating to mushroom production. Stimulation in growth of the commercial mushroom by phenylalanine might be anticipated from its relation to the oxidases, which are an important part of the enzyme systems of the fungus (Hughes and Rhodes, 1959).

Delmas and Laborde (1969) used soya bean by-products as nitrogen supplements at casing, this again resulting in an improved mushroom yield.

MacCanna (1969) observed the effect on yield following the addition of four nitrogen supplements (ammonium sulphate, nitroform (urea-formaldehyde), dried blood and calcium nitrate) to the compost during its preparation; dried blood gave a significant increase in

yield. He suggested that increased compost nitrogen content increases yield if the nitrogen is in a protein form; it is also possible that some factor other than protein may be responsible for the yield increase.

A combination of ammonium sulphate and calcium carbonate has been added, in different concentrations, to the horse manure during compost preparation (Bech and Rasmussen, 1969); the highest concentration of ammonium sulphate and calcium carbonate, when added to fresh manure, resulted in the highest yield, they concluded that decomposed, stored manure requires less ammonium sulphate and calcium carbonate supplementation than fresh manure for the production of maximum yields. They also observed a 25 and 50 per cent reduction in yield from the controls with ammonium sulphate, following the addition of urea and nitroform respectively to the compost, and recommended that urea and nitroform should not be used in the presence of ammonium sulphate and calcium carbonate.

Curto and Favelli (1972) obtained an early picking and increased mycelial density followed by 20-30 per cent increase in total production of fruitbodies (of A.bisporus) after treatment of the culture (in trays) with certain micro-organisms (e.g. bacteria, yeasts and microalgae) when these were added both before and after casing.

The effect of nitrogen supplementation on A.bisporus fruitbody composition (namely ash, urea and amino acids content) has been studied (Maggioni et al., 1968); ammonium sulphate and urea were used, being introduced during composting. Supplementation with urea resulted in an increase in sulfur amino acids, while total nitrogen of fruitbodies was not affected by the type of nitrogen supplement.

Casein hydrolysate and gelatin supplementation have been used at casing (Kissmeyer et al., 1966); supplementation with casein

hydrolysate resulted in an increase in aspartic acid, threonine, serine, proline, leucine, tyrosine, and urea. Upon supplementation with gelatin; alanine, ammonia and arginine decreased while aspartic acid increased (see section 1.2.1).

1.1.3. Water and its relation to mushroom production

Fresh mushrooms contain appreciable quantities of water, and the maintenance of the correct water relationships in the substrates, (compost and casing layer) together with the application of water during cropping, represents one of the most important aspects of husbandry in commercial culture.

Several workers have reported that watering affects yield (Edwards and Flegg, 1953; Reeve et al, 1959; Kindt, 1965; and Flegg, 1965). Generally, increasing the amount of water by routine watering during cropping significantly raised the water content of sporophores by approximately 1%, thus creating sporophores which were individually heavier and more solid in texture. According to Flegg (1965) the increased yield could be accounted for by the increased water content of the sporophores.

In an experimental approach to determine the nature of the water balance in a culture system Flegg (1974) suggested that the optimum watering regime would be one which replaced the water in the compost and casing soil as it was used. His estimates of the water demand showed that there was little demand until sporophore development began about two weeks after applying the casing soil; demand was at a maximum during the first and second "breaks". In the same series of experiments he demonstrated that compost and casing soil contain considerable reserves of water on which the crop can draw. Yields

of up to 70-80% of that normally expected were obtained even when no further water was applied after casing. Consequently Flegg recommended that casing soil should be at approximately 90% of maximum water holding capacity on application and kept wet by frequent and regular waterings.

1.2 Composition and nutritional value of A.bisporus fruitbodies

From the published literature on the composition and the nutritive properties of edible mushrooms, it is clear that there is much confusion, and conflicting views are evident. In some instances figures relate to the chemical composition of mycelium and frequently many different species are reported under the common name - mushroom. According to the limited number of analyses in which it is clearly indicated that the data relate to the fruitbodies of the cultivated mushroom A.bisporus (but sometimes referred to as A.campestris) fruitbodies contain the following components which are of food value: proteins, carbohydrates, fats, mineral elements and vitamins (see Table 1.1).

Quantitative differences are apparent, especially in the water/dry matter content, protein and carbohydrate fractions.

1.2.1 Protein

Mendel (1898) and Koing (1903) stated that the protein content of mushrooms was approximately 5.0% and noted the possible value of this source of protein to man. Since then a number of different values have been reported, as follows:-

	% Protein (fresh weight)
Chatfield and Adams (1940)	0
Fitzpatrick et al. (1946)	2.67
Anderson and Fellers (1942)	3.94
McConnell and Esselen (1946)	3.95
Maggioni et al. (1968)	4.95
Chang (1972)	4.88

Table 1.1 Composition of A.bisporus fruitbodies ($g.100g^{-1}$ fresh weight).

Components	Anderson and Fellers (1942)	McConnell and Esselen (1946)	Jacobs (1951)	McCance and Widdowson (1960)	Chang (1972)
Water	89.50	88.90	91.1	91.5	89.7
Protein (N x 6.25)	3.94	3.95	-	1.8	4.88
Fat (ether extract)	0.19	0.26	0.3	Trace	0.2
Extract matter	4.01	4.75	-	-	-
*Available carbohydrate	-	-	-	0	-
Fiber	1.09	1.00	-	-	0.38
Ash	1.26	1.14	1.14	-	0.82

(*Available carbohydrate = the sum of the starch, dextrins and sugar, all expressed as monosaccharides.)

Considerable variation in the protein digestibility values are evident in the literature. Early work by Saltet (1885) and Morner (1886) indicated approximately 50% of the total nitrogen was in the form of digestible protein. Skinner et al. (1933) determined digestibility, using albino rats, to be 71%. Later work by Fitzpatrick et al. (1946), who performed animal feeding trials according to the paired feeding method of Mitchell and Beadles (1930), concluded that the protein of Agaricus campestris accounted for growth of albino rats equal to one half that produced by soya bean. However, all the essential amino acids required by man were present. It was noted that tryptophan content of the protein was low and the mushroom protein appeared unpalatable to the rats. Earlier work by Anderson and Fellers (1942) found that mushroom protein produced only 30% of the growth of a control set of rats fed on casein. When 20% of the casein was replaced by mushroom protein a growth gain equal to 84% of that of the positive control group was obtained. On this basis mushroom protein is "partially incomplete" according to the definition of Sherman (1941).

Fitzpatrick et al. (1946) qualitatively identified the following essential amino acids in mushroom protein:

phenylalanine, histidine, leucine, lysine, arginine, tryptophan and threonine.

Valine, isoleucine and methionine were not identified chemically but were confirmed to be present by microbiological assay. According to these assays, fresh mushrooms contained ($g.100g^{-1}$):-

Arginine 0.203, Isoleucine 0.458, Leucine 0.242,
Methionine 0.144, Tryptophan 0.005, Valine 0.326.

Block et al. (1953) identified 12 amino acids in mushroom fruitbodies using two dimensional paper chromatography but later studies by Hughes et al. (1958) confirmed the presence of 23 amino acids which included all of the essential amino acids. Hughes et al. also noted that the amino acid tyrosine decreased in amount from first harvest to the fifth harvest, suggesting a change in the amino acid composition according to the age of the culture.

Hughes (1959, 1961) also studied the occurrence of free amino acids in Agaricus bisporus fruitbodies harvested at three successive times during the growth period. Proline and histidine increased, while ornithine, tyrosine and phenylalanine decreased during the growth period. No evidence was found of a change in the total nitrogen content of fruitbodies from successive crops on production units. Variations in the free amino acid pool from the first to the fourth breaks were observed by Kissmeyer et al. (1966). Aspartic acid, threonine, serine, proline, leucine, tyrosine, and to a lesser degree urea, increased, while glutamic acid and arginine decreased. These workers concluded that the observed changes in the nitrogenous compounds contained in the mushrooms may not be the result of nutrient utilisation by the mushrooms but may be indirectly attributable to microbiological metabolism.

Maggioni et al. (1968) showed that the synthesis of sulphur amino acids is stimulated when urea is used as a nitrogen supplement in the compost. Aspartic acid, alanine and valine also increased, while the total free and protein amino acids decreased.

Other nitrogen containing compounds in mushrooms include urea and chitin. Ivanoff (1923) reported that amounts of urea were present in the fresh fruitbodies of A.bisporus varying from a trace amount to as high as 13% of the dry weight, or up to 50% of the total nitrogen

of the fruitbody. No quantitative information on chitin is available in the literature.

1.2.2 Carbohydrates

MacConnell and Esselen (1946) found 24.9% carbohydrate in the dry matter of the cultivated mushroom; this consisted of mannitol, glycogen, hemicellulose and a small quantity of reducing sugar. Similar data was published by Anderson and Fellers (1942).

Using paper chromatography and colorimetric methods, Hughes et al.(1958) qualitatively identified the presence of two pentoses - xylose and ribose, two methyl pentoses - rhamnose and fucose, three hexoses - glucose, galactose and mannose, two amino sugars, glucosamine and N-acetylglucosamine, two sugar alcohols - mannitol and inositol, and two sugar acids - galactouronic and glucuronic acid. The predominant carbohydrate constituents were galactose, mannitol and glucose, followed by rhamnose, xylose, mannose and galactouronic acid.

When comparing the carbohydrate content of a number of different strains of A.bisporus, Dommell (1964) reported values between 3.84% and 4.88% carbohydrate in fresh weight samples. Dommell, and later Lee (1969), followed the changes in carbohydrate content of A.bisporus fruitbodies during the post-harvest period. Proximate analyses of fresh and canned mushrooms showed that carbohydrates were the major constituents lost during canning; soluble solids leached out readily while non-soluble solids were resistant to leaching. Using gas-liquid chromatography, and mass spectrometry, the analysis of carbohydrates in A.bisporus was investigated by Holtz (1971). According to Holtz, 0.48 mg fructose, 2.20 mg of glucose, 11.5 mg of mannitol and 0.52 mg of sucrose per gram fresh weight were contained in mushroom fruitbodies.

Considerable attention has been given to the mannitol and trehalose content of mushrooms, carbohydrates which are generally regarded as being specific to fungi. The polyhydric alcohol mannitol has been shown to exist in a variety of mushrooms. It was first discovered in fungi by Braconnot (1811a, 1811b). In 1934 Inagaki found mannitol present in wild mushrooms (Agaricus campestris) in concentrations of 0.93% of the dry weight of the cap and 0.17% of the stipe. Nickerson and Rettew (1944) isolated and identified mannitol from Agaricus campestris. They reported an average 5.5% of the dry weight of the immature button stage and 9.9% of the dry weight of the mature open stage as mannitol. Esselen and Fellers (1946) reported that 8.6% of the dry weight of Agaricus campestris is composed of mannitol. Hughes et al.(1958) suggested that mannitol is a major constituent of mushroom total carbohydrate. Hughes (1961) isolated and identified mannitol in crystalline form. He reported that the mannitol content of A.bisporus fruitbodies varied from 12-19% of the dry weight, a variation he associated with the time of harvesting. Holtz (1971) qualitatively and quantitatively identified mannitol using gas liquid chromatography and concluded that mannitol was present in amounts in excess of 12% of the dry weight; this is comparable to the findings of Hughes (1961), and Parrish et al. (1976). In addition, Holtz noted variations in mannitol concentration from samples according to the break or flush of the crop cycle. He suggested that, since mushroom tissue is approximately 92% water, mannitol may provide the osmotic potential necessary to maintain a concentration of water in the fruitbody.

The commonly known mushroom sugar or mycose, known chemically as trehalose, (a D-glucosyl D-glycoside) is also known to be present

in mushroom fruitbodies. Hughes et al.(1958), Rast (1965) and Hammond and Nichols (1975, 1976) found trehalose in A.bisporus fruitbodies. Birch (1963 , 1973) has indicated that α,α-trehalose is the only type which occurs in all edible mushrooms.

It is generally believed that both mannitol and trehalose function as reserve carbohydrates in fungi (Cochrane, 1958; Hammond and Nichols, 1976).

Parrish et al. (1976) observed a direct relationship between fluctuation in yield and mannitol content.

1.2.3 Lipid

Anderson and Fellers (1942) reported the fat content of Agaricus campestris to be 0.19%. A more detailed analysis of three varieties of Agaricus campestris was carried out by Hughes (1962). He reported that the ether soluble lipid accounted for approximately 1.3% of the dry weight of the white variety, 1.7% of the golden white variety and 1.8% of the cream variety. According to Hughes, although the total fatty acid contents are similar in all varieties, the unsapponified fatty acids increased from white to golden white to cream. He suggested that, on the basis of fatty acid composition, the golden white variety is closer genetically to the cream variety than to the white variety. Fatty acids present were found to be linoleic, palmitic, stearic, arachidic, oleic and lauric acid. The lipid contains one of the highest proportions of linoleic acid known, and accounted for 63-74% of the total fatty acids present (Holtz and Schisler, 1971). Maggioni et al.(1968) reported that the total lipid fraction of sporophores growing on nitrogen supplemented compost contained 71% linoleic acid and 15% palmitic acid. The percentage composition of fatty acids in the lipid extract was similar to that reported by Hughes (1962).

1.2.4 Minerals and Vitamins

Anderson and Fellers (1942) considered cultivated mushrooms to be deficient in calcium, although relatively high levels of potassium, phosphorous, iron and copper were present. These workers also noted that less than one third of the iron could be considered as utilisable by the human body.

Mushrooms are known to be good sources of the Vitamin B complex. In 1922 Orton, et al. found A.campestris to be a good source of these vitamins, a finding confirmed by Hara (1923) and by Quackenbush et al. (1935). Quantitative data on vitamin contents have been given by Cheldelin and Williams (1942) and Anderson and Fellers (1942) who concluded that the sporophores of A.campestris are an excellent source of nicotinic acid and riboflavin, and also contain significant quantities of pantothenic acid, and vitamins B, C and K.

Esselen and Fellers (1946) studied the effects of cooking, canning, drying and freezing on the content of the Vitamin B complex and showed that none of these vitamins were lost by processing.

1.3 Submerged culture of mushroom mycelium

Humfeld (1948) discovered that mushroom mycelium can grow in submerged culture. The purpose of growing mushroom mycelium in submerged culture has been either for its mushroom flavour, its possible nutritional value as human food, or feed stuff, or possibly as spawn for the cultivation of fruitbodies (Van Eybergen and Scheffers, 1972; and Dijkstra et al., 1972).

Humfeld (1948, 1950-1951), and Humfeld and Sugihara (1949, 1952) described processes for the production of mycelium of Agaricus campestris under submerged, aerated and agitated conditions in chemically defined media. They found that the presence of phosphorus, potassium, sulfur, magnesium, calcium and trace elements, of iron, manganese, zinc and copper were essential for optimum growth of the mycelium in a 5 per cent glucose medium with urea as the nitrogen source.

Styer (1928 , 1930) and Treschow (1944), using static liquid culture, Brock (1951), Humfeld and Sugihara (1952) and Willam et al. (1956), using submerged techniques, have demonstrated that mushroom mycelium can grow on a variety of carbohydrates supplemented with inorganic or simple organic nitrogen compounds and mineral salts. Litchfield and Overbeck (1963) and Litchfield et al. (1963a) showed that morel mushroom (Morchella crassipes, M.esculenta and M.hortensis) mycelium may be grown in submerged culture with glucose, maltose, lactose or wastes containing these sugars, as substrates. Fraser and Fujikawa (1958) studied the nutrient requirements of Agaricus bisporus (in standing liquid cultures) and reported the need for phenylalanine, methionine, proline and thiamine as growth factors, when glucose and asparagine were the major nutrients. Conversely Dijkstra et al. (1972) reported that, in defined media based on glucose, vitamins and minerals, asparagine was an unsatisfactory nitrogen source. Supplementation

with phenylalanine caused a three-fold increase in yield, while addition of other amino acids had no effect; they concluded that their organism strain differed from those used by Fraser and Fujikawa (1958).

The composition of the mycelium may also vary; Humfeld and Sugihara (1952) found that the mycelial protein content depended upon both the type and concentration of the nitrogen source. Guha and Banerjee (1970) studied the effect of various nitrogenous compounds on the submerged production of A.campestris mycelium; they obtained the highest yield using complex organic nitrogen sources. The protein content of the mycelium was also influenced by the nitrogen source, the highest protein being obtained with casein hydrolysate.

Dijkstra et al. (1972) reported that A.bisporus grew well in submerged culture in media containing malt-extract, phosphate and casein; they suggested that malt-extract or casein contain one or more growth factors lacking in corn step liquor and differing from amino acids or vitamins used in synthetic media.

The effect of pH, temperature, and other physical factors have also been studied (Litchfield, 1967).

The nature of the mycelium varies in submerged culture; mycelium may grow as small or large pellets, or as a dispersed or milky-type growth (Block, 1960). The type of agitation can influence the character of the growth and the size of the pellets, O'Neil (1956) related the pellet size not only to agitation and aeration but also to the culture medium sugar concentration. Sugihara and Humfeld (1954) reported that the yield was usually higher when the mycelium grew in the dispersed form than when it occurred in the pellet form, but the mushroom flavour content was absent.

The growth of A.bisporus mycelium has been stimulated by the addition of various lipids (olive oil, soyabean oil, corn oil etc.) to a

basal medium (Wardle and Schisler, 1969). These authors reported that the increased growth from the lipid addition was due to oleic and linoleic acids present in the lipids. Lehrian et al. (1976) reported that a 30 per cent stimulation of mycelial growth of A.bisporus was observed upon the addition of small amounts of sodium acetate or linoleic acid to a complex growth medium. They suggested that the supplemented linoleate is broken down to acetate units which are subsequently used in the synthesis of mycelial lipids.

Szuecs (1958) reported that submerged aerobic culture is a method of rapidly producing large quantities of mushroom mycelium at relatively low cost and from readily available substrates. Orange juice citrus press water (Block et al., 1953), yeast extract-corn syrup medium (Szuecs, 1958), waste materials discarded by the wood pulping industry (Cirillo et al., 1960), malt sprout extract (Moustafa, 1960), various soyabean whey media (Falanghe et al., 1964) and waste sulfite liquor (Kosaric et al., 1973) have been used as media for producing mushroom mycelium in submerged culture.

The composition of the medium influences mycelial flavour, Litchfield (1967); little or no flavour was noticeable in fried or toasted Agaricus blazei mycelium grown on a synthetic medium. A bitter flavour, which could be removed by water washing, was noted when orange juice medium was used (Block et al., 1953). Humfeld and Sugihara (1952) and Jennison (1956) reported that flavour appears to depend upon certain compounds in the culture medium and can be intensified by control of the kinds and amounts of these compounds. The age of the culture may also be a factor in determining the flavour content of the mycelium (Jennison, 1956).

Mycelium grown in submerged culture, like the mushroom fruitbody, was found to be nutritionally valuable as a source of amino acids, and

B complex vitamins (Humfeld and Sugihara, 1949; Block et al, 1953; Robinson and Davidson, 1959; and Block, 1960).

Humfeld (1948) determined the protein, fat and ash content of A.bisporus mycelium grown in submerged culture and reported that this material was of a similar composition to that of commercial mushrooms. Similarly Litchfield (1967) reported that the protein content of A.campestris mycelium was in the same range as that of the fresh mushroom fruitbody, Rehaček et al.(1962) found that the protein of submerged cultured mycelium of Boletus edulis changed both in quality and quantity depending upon the physiological state and age of the culture; methionine was absent during the early stages of incubation while valine decreased after 24 hours.

Litchfield et al.(1963b) analysed morel mushroom mycelium grown in submerged culture and reported values between 22.8-51.0% protein and 2.18-7.55% fat, which were dependent upon the species. Similarly to Humfeld and Sugihara (1952) he found that the protein content of the mycelium varied with the nitrogen content of the corn steep liquor medium. Similar results have been reported by Kosaric et al.(1973) for submerged cultures of morel mushroom mycelium grown in waste sulfite liquor.

Medium composition and growth conditions also influenced the protein composition of Tricholoma nudum (protein 15-57.8% ; Reusser et al., 1958), but, according to Worgan (1968) the quality of the protein, as measured by the amount and relative proportions of essential amino acids present, did not vary with the medium or conditions used.

A quantitative estimation of essential amino acids in the protein of Tricholoma nudum mycelium was carried out by Reusser et al.(1958) and Molitoris (1962). Tryptophan content was considerably higher than that reported for yeast by Prescott and Dunn (1959); all other essential

amino acids were present in lower concentrations than yeast protein.

Reusser et al. (1958) compared the protein and fat content of 10 strains of mushroom obtained by submerged culture, including four strains of Agaricus campestris, on a synthetic medium, molasses, and spent-sulfite liquor.

Amino acids may be important, not only from the nutritional point of view, but arginine, histidine and lysine may have a role in the determination of the characteristic flavour of Boletus edulis (Craske and Reuter, 1965).

A quantitative estimation of vitamins in the mycelium of A.campestris was made by Humfeld and Sugihara (1949); thiamine, riboflavin, niacin and trace amounts of vitamin C were found. In addition to these vitamins, in Agaricus blazei, pantothenic acid was found. A.blazei was considered to be a good source of B complex vitamins (Block et al.1953, 1956).

Similarly Litchfield (1964) reported that Morchella hortensis contains high amounts of vitamin B6, pantothenic acid and folic acid, but is low in riboflavin and biotin.

Section 2

MATERIALS AND METHODS

Section 2. MATERIALS AND METHODS

In view of the uncertainty of published data relating to the composition of mushroom fruitbodies, investigations were carried out to ascertain their composition under controlled conditions which incorporated modern methods of commercial culture.

2.1 Production of A.bisporus fruitbodies

Compost (10Kg) prepared from horse manure - straw mixtures according to the methods of Hayes and Randle (1969), and known as G.C.R.I. Formula 1, was filled into previously fumigated trays (45 cm x 45 cm x 13 cm). This was inoculated by thoroughly mixing in 100g of a commercial white strain of A.bisporus. Inoculated trays were then arranged on shelves in a room maintained at 80-90% RH (relative humidity) and 22-24°C. After 14 days, trays were cased with a layer (3.0 cm depth) of a peat-chalk casing soil (1:2, chalk:peat, w/w + water). This was followed by an incubation period of 10 days before the cultures were transferred to a room, where the temperature was maintained at 18°C and RH at 70-80%, for the remainder of the production period. Water was applied as required according to standard cultural procedure.

2.2 Harvesting, grading, trimming and sampling techniques

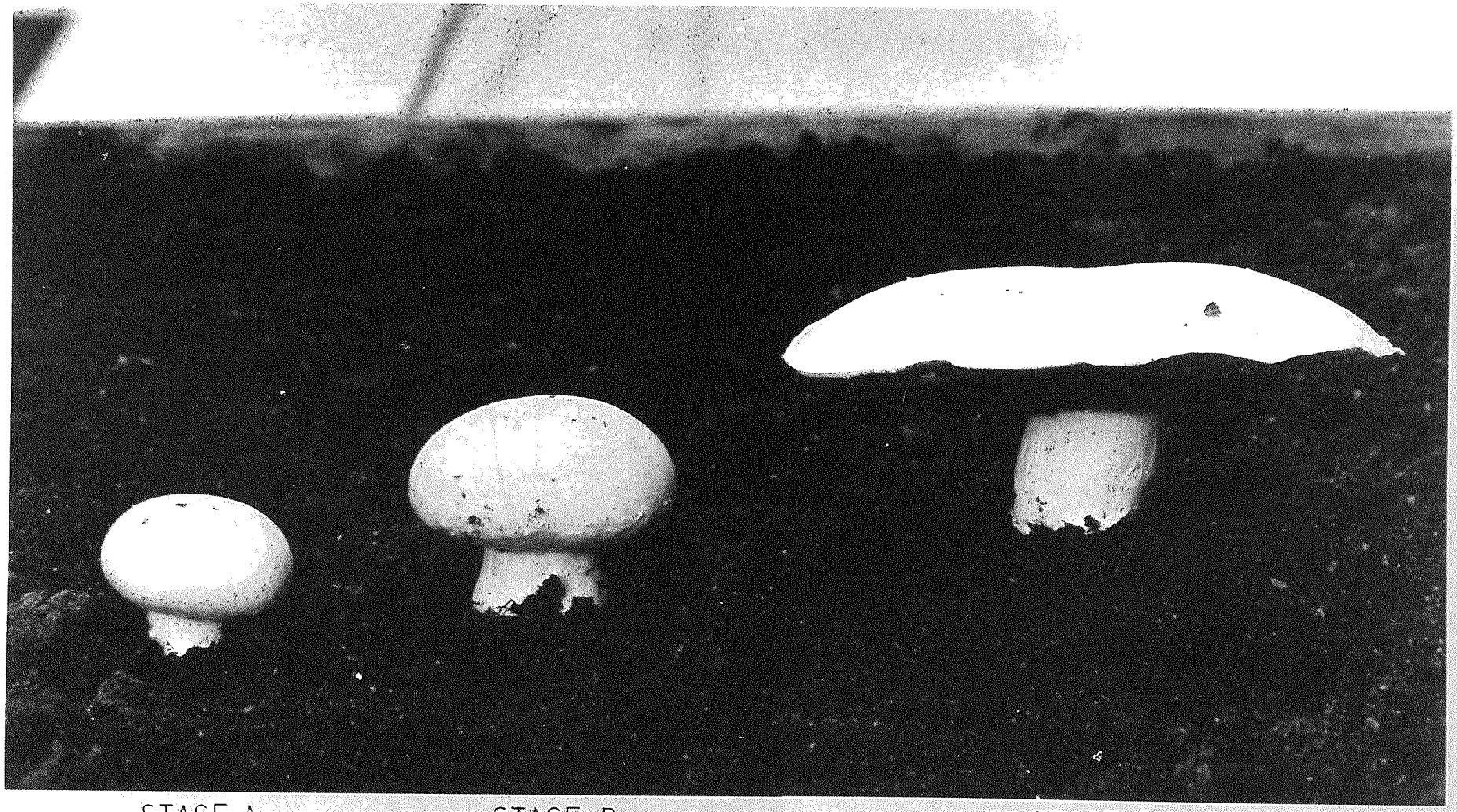
Mushrooms for harvest were classified into three groups which broadly reflected the usual stages of harvesting employed in commercial production and marketing in the U.K. These stages (Plate 2.1) were:-

Stage A. Cap diameter 2.5-3.5 cm with veil unbroken (commonly known as a button),

Stage B. Cap diameter 4.0-5.0 cm with veil partially broken (commonly known as a cup), and

Stage C. Cap diameter 6.5-7.5 cm; veil completely broken and the pileus flat. (Commonly known as a flat)

PLATE 2.1. DEVELOPMENTAL STAGES OF AGARICUS BISPORUS FRUITBODIES.



STAGE A STAGE B STAGE C

All fruitbodies were removed from the casing soil, by firstly twisting and then pulling. The bottom of the stalk, with the soil adhering was cut off, for each stage, 1 cm of stem length was left on the cap according to common commercial practice.

To obtain representative samples, standard techniques had to be developed (see Section 3.2.1, and Section 3.3.1).

2.3 Submerged culture of mushroom mycelium

2.3.1 Preparation of the inoculum

Pure stock cultures of mushroom mycelium were maintained on agar plates containing the basic synthetic medium used for the submerged growth cultures (Section 2.3.2). Several spawn grains were transferred aseptically onto the surface of agar plates with sterile forceps, and incubated at 24°C for 14 days.

The filamentous mushroom mycelium which developed was subsequently used to prepare subcultures. Cork borer plugs were transferred from the stock cultures onto agar plates using similar culture conditions as those described for the stock cultures. The mycelium scraped from these plates was employed as inocula for the liquid submerged culture media. Inocula were prepared fresh for each experiment.

2.3.2 The culture medium and culture methods

The basic synthetic medium was a modification of that developed by Hayes (1972) and contained (final concentration, $g.l^{-1}$); maltose, 23.4; glucose, 8.7; sucrose, 0.81; dextrin, 6.75; casein hydrolysate, 3.0; KCl, 0.2; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$,0.2; $FeSO_4.7H_2O$, 0.001; $Na_2HPO_4.2H_2O$, 0.06; biotin, 5 μgm; thiamine, 200μgm; ethyl acetate, 0.01 ml; deionized water to 1 litre.

50 ml of sugar solution was autoclaved (20 minutes at 1.05 $Kg.cm^{-2}$), cooled, and combined with 50 ml of autoclaved solution containing the

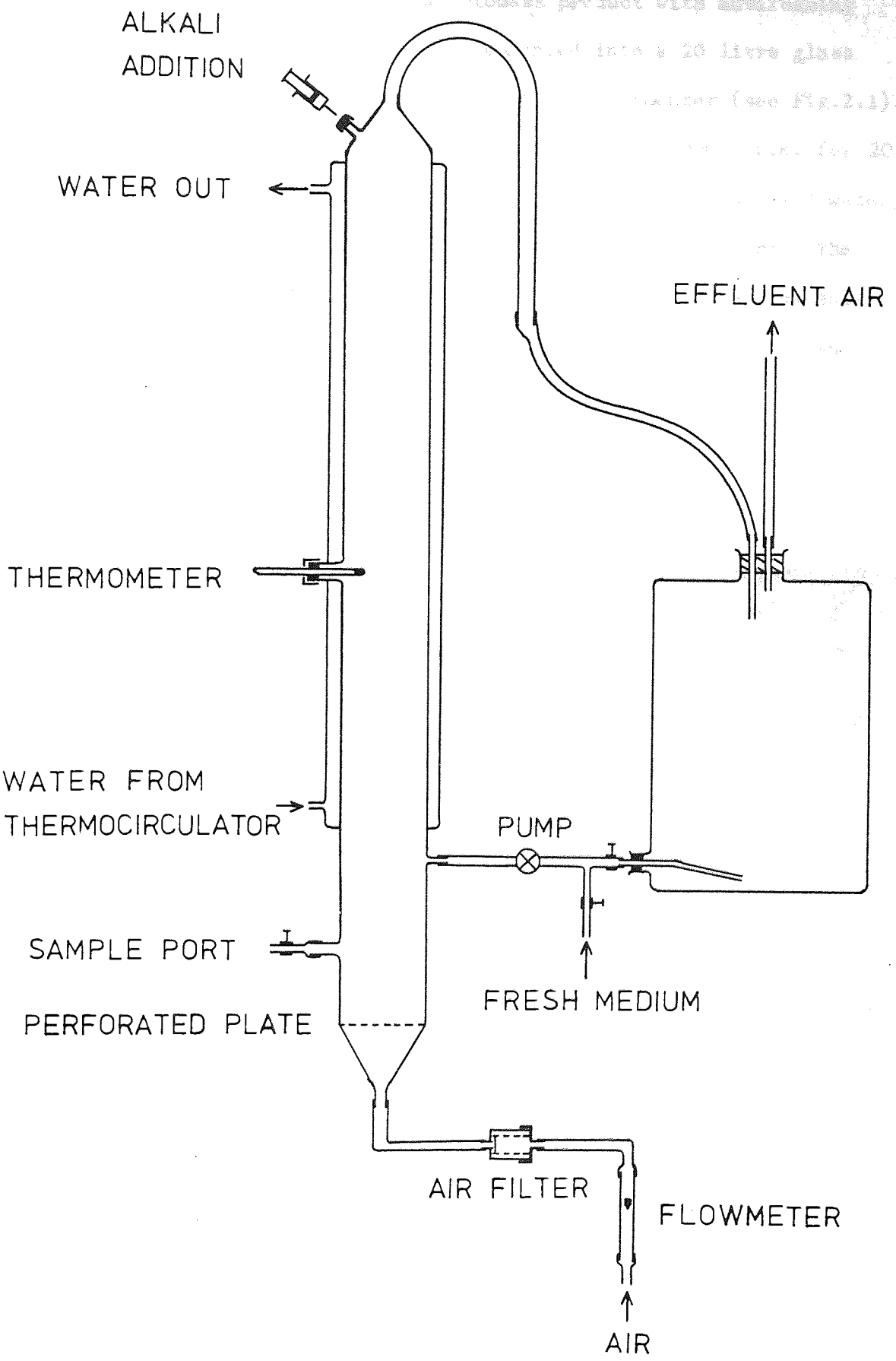
remaining nutrients in 250 ml Erlenmeyer flasks. The flasks were inoculated with mycelium (see Section 2.3.1) and incubated on a rotary shaker (at 180 r.p.m. and 24°C for 14 days).

2.3.3 Bacterial biomass production

Thermophilic bacteria were isolated from compost by washing with a sterile phosphate buffer saline solution (pH 7.3; NaCl, 8.0g; KH_2PO_4, 0.34g; K_2HPO_4, 1.21g; distilled water, to one litre). Serial dilutions of the liquid extract were made and inoculated onto nutrient agar plates. Incubation was at 50°C for 24 hours. Inocula for large scale cultures were prepared by inoculating four 250 ml Erlenmeyer flasks, containing 100 ml of sterile basic synthetic medium (see Section 2.3.2), with isolated bacteria and incubating on a rotary shaker at 50°C for 48 hours.

Large-scale bacterial biomass cultivation was in a 10 litre aerobic Tower fermenter (Fig. 2.1). The nutrient medium used was ($g.l^{-1}$); casein hydrolysate, 5.0; yeast extract, 5.0; NaH_2PO_4, 1.0; KCl, 0.5; $MgSO_4.7H_2O$, 0.25 and $CaCl_2$, 0.1, except the initial batch of medium which contained glucose in the place of casein hydrolysate and yeast extract, and included ammonium sulphate ($1.0g.l^{-1}$) as the nitrogen source. The initial batch of medium supported only minimal bacterial growth probably due to the formation of growth inhibitors in the presence of glucose and the high temperature of incubation. The fermenter was operated semicontinuously, i.e. after the bacterial biomass concentration had reached a suitable level a portion of broth (normally 5 litres) was drawn off and replaced with fresh medium. The culture broth temperature was maintained at 53°C, and the pH to a value of approximately 7.5 (by the manual addition of aliquots of $200g.l^{-1}$ NaOH). The aeration rate was approximately 5.0 litres per min.

FIG.2.1. DIAGRAM OF THE TOWER FERMENTER.



During the fermentation, continuous foaming of the culture occurred; because contamination of the final biomass product with antifoaming agents is undesirable, the foam was condensed into a 20 litre glass aspirator jar and continuously returned to the fermenter (see Fig.2.1).

The biomass was harvested by centrifugation (10,000 r.p.m. for 20 minutes), washed and recentrifuged several times with distilled water, and the bacterial paste dried in an oven at 60°C for 24 hours. The bacterial biomass samples were ground, combined, well mixed and subsequently used as a nutrient source for submerged cultures of mushroom mycelium (Section 3.6).

2.4 Analytical techniques

2.4.1 Dry weight of fruitbodies

A known weight of fresh mushrooms were dried to constant weight at 105°C. Dry matter and water content of different developmental stages of maturity were determined.

Samples for chemical analysis were dried at 60°C for 24 hours to avoid any changes in composition, ground to fine powder, placed in closed bottles and stored in a desiccator at room temperature to await analysis.

2.4.2 Dry weight of submerged mushroom mycelium

The culture broth was filtered through filter paper (Greens Hyduro 904), washed several times with distilled water, and dried in a freeze-dryer (Model 10P, Edwards High Vacuum Ltd., Manor Royal, Crawley, Sussex; Plate 2.2) the freeze-dried samples were weighed, (yield calculated as $g.l^{-1}$), ground and stored as in section 2.4.1.

2.4.3 Ash content

A known weight of dry mushroom powder (1-2 gm) in a porcelain crucible, was placed in a muffle furnace, which was preheated to 550°C, for 2 hours. Samples were transferred directly to a desiccator, cooled, and weighed, according to the methods of A.O.A.C. (1970).

2.4.4 Calorific value

Two methods are commonly used in calculating calorific values:-

(i) Calculation of the calorific value of a sample from its chemical composition

By this method the calorific value is calculated from the amounts of protein, fat and carbohydrate it contains, once these organic fractions have been determined, the values are then multiplied by factors representing the number of calories thought to be

PLATE 2.2. THE FREEZE DRYER.

produced in the body by one gram of each nutrient. The sum of these values gives the calorific value of the sample.

If the constituents forming the organic material in a sample are expressed as percentages, then the calorific value of the sample can be calculated according to McCance and Widdowson (1960) using the formula of Rubner, as follows:-

$$\frac{4.1\,P + 3.75\,C + 9.3\,F}{100} \quad K\ cal.g^{-1}$$

These conventional energy equivalents ("calorie conversion factors") are often called the Rubner coefficients, where P, C and F represent the percentage content of Protein, Carbohydrate and Fat, respectively. These "calorie conversion factors" do not represent the number of calories which one gram of protein, carbohydrate and fat would produce in a calorimeter, but are arrived at by applying to the values found by physical calorimetry various corrections allowing for losses occurring in digestion and absorption, and through incomplete oxidation. Since no two foods and no two people are ever exactly alike, and since these physiological corrections are based on averages, the calorie conversion factors do not have the same accuracy as the values for calories arrived at by physical calorimetry or the values for protein, fat and carbohydrate obtained by chemical determination (McCance and Widdowson, 1960). The results obtained are also influenced by the errors associated with determining the chemical composition.

Since, during their combustion, both in the bomb calorimeter and in the living organism, fats and carbohydrates are burned to the same products (CO_2 and H_2O), their physical calorific content is the same as their physiological one. However, the physical and physiological

calorific values of proteins and other nitrogenous compounds differ considerably because in living organisms nitrogenous food substances are not oxidised completely; consequently the physiological calorific equivalent of nitrogenous compounds is lower than their physical one (Winberg, 1971).

(ii) Direct calorimetry

The calorific value of food can be determined using a bomb calorimeter, which measures heat of combustion produced when the food is burned completely. This is also called the physical value.

The number of calories the body can derive from a food is less than the number of calories produced when the food is burned in a calorimeter because the calorie-producing nutrients, which are mainly protein, fat and carbohydrate, are not completely digested; the products of digestion, moreover, are not completely absorbed in the human gut, and the portion of the protein which is digested and absorbed is not completely oxidized to yield energy in the body.

It has been concluded by Winberg (1971), from a comparison of all the methods for determining calorific value, that the most accurate results are those obtained by direct calorimetry.

The principle on which this method is based consists of combusting a sample in the presence of excess oxygen under high pressure; the heat produced is absorbed by the bomb, raising its temperature. The heat energy is transmitted to a copper ring (attached to the heavy metal base of the instrument), and this, in turn, increases the temperature of the hot junctions of the thermocouples present. The difference in temperature between the two sets of thermocouple junctions produces a voltage which is indicated by a deflection of a potentiometer pen (Phillipson, 1964, and Grant, 1974).

In these studies a Phillipson bomb calorimeter was used. Complete combustion was ensured by compressing 8-10 mg of dry mushroom powder into a small pellet (pill) which was weighed on a clean platinum pan (cut from 0.001 inch thick platinum foil) and placed inside the bomb in contact with a piece of fuse wire 2 inches long (platinum fuse wire 0.006 inch diameter) joined between the two electrodes of the bomb. Distilled water (0.1 ml) was placed in the bottom of the bomb, before attaching to the head (containing the pellet). The bomb was filled with oxygen to a pressure of 425 p.s.i., and placed on the stand for firing and ignition of the pellet sample. Heat liberated during the combustion was measured by a potentiometric chart recorder. The calorific value of a known pellet weight was calculated from the predetermined calibration factor. The pertinent values are:-

1. The pre-fire change in lines per unit time — pre Δ
2. The post-fire change in lines per unit time — post Δ
3. Observed rise from firing to peak — peak height
4. Time necessary for 60% of rise — time from 0 to 60%
5. Time necessary for final 40% of rise — (60% point to peak).

Time was measured in chart paper units, 30 inch/hr.

Calculation of the time correction factor, used to calculate the total corrected rise (peak), was as follows:-

pre Δ		x	time from	0	to	60%	=	X
-post Δ		x	time from	60%	to	pk	=	(-)Y
(-)Y	+	(+) X	=	Z	Time correction factor			
Z	+	peak height	=		Total corrected peak			

$$\frac{\text{Total corrected peak} \times \text{calibration factor in calories/line}}{\text{Weight of sample in g.}} = \text{Cal/g}^{-1}$$

The calibration was carried out as follows:-

The total calories in a benzoic acid pill divided by the total corrected peak, gives the calibration factor in calories/line. (Lines on the graph paper).

A number of determinations were made with benzoic acid pills of 2, 4 and 10 mg and the results plotted. This was to establish a linear relationship over the potentiometer scale.

2.4.5 Nitrogen and protein estimations

(i) Kjeldahl nitrogen

Total nitrogen contents were determined by the micro-Kjeldahl method, according to Markham (1942). The catalyst used for digestion was a mixture of potassium sulphate, copper sulphate and selenium (ratio of 32:5:1 respectively).

Sample homogeneity was obtained by transferring a weighed portion of 150-200 mg of dry sample into a digestion flask with 2 ml concentrated sulphuric acid and catalyst. The mixture was digested for three hours. After cooling, the contents of the flask were washed with glass distilled water into a 100 ml volumetric flask and the volume made up to 100 ml with distilled water.

The distillation was carried out in a Markham Still using 10 ml of the diluted digest and 4 ml of 400 $g.l^{-1}$ NaOH.

The ammonia released was collected in 10 ml saturated boric acid solution, which was then titrated against 0.01N HCl, using Tashiro's indicator (methyl red, 2.0$g.l^{-1}$; methylene blue, 1.0 $g.l^{-1}$; in absolute alcohol.).

(ii) Protein estimation

There are several methods in use for protein estimation, all methods are either non-specific or measure a single component of the protein molecule, such as a particular element, grouping, or amino acid. In some methods other cell constituents interfere, e.g. nucleic acids. Consequently three methods of estimating the protein content were used during these investigations:-

(a) Crude protein from nitrogen determinations.

The total nitrogen obtained by the Micro-Kjeldahl technique was converted to crude protein by multiplying the nitrogen values by the constant factor 6.25.

(b) Colourimetric determination.

This method is based on the biuret reaction of protein with copper ions in alkali solution, and was employed according to the method of Lowry et al. (1951).

100 mg of dry sample was homogenised with 0.05 M phosphate buffer (di-sodium hydrogen orthophosphate dihydrate and potassium dihydrogen orthophosphate), pH 7.0 and made up to 100 ml solution with the same buffer. 0.3 ml of this was diluted with distilled water to 1 ml and used for analysis.

Bovine serum albumin ($200\,\mu g.ml^{-1}$) was used as a standard solution.

(c) Amino acid analysis

Amino acids were determined by using a Locarte automatic amino acid analyser, which operates on the principle described by Spackman et al. (1958). Samples were prepared for analysis as follows:-

Acid hydrolysis

Dry sample powder was weighed into a thick walled glass hydrolysis tube. To the sample an appropriate amount of nor-leucine was added as an internal standard. Concentrated hydrochloric acid was

then added, to give a solution of 6N HCl. The solution was then degassed by alternately freezing and thawing under high vacuum in order to avoid oxidative breakdown of some of the amino acids. After four freeze-thaw cycles, the tube was sealed under vacuum. The sample was then hydrolysed at 110°C for 24 hours (using a toluene bath). After hydrolysis, the hydrochloric acid was removed on a rotary evaporator at 40°C. The sample was then dissolved in a suitable volume of pH 2.2 citrate buffer.

Separation of the constituent amino acids was by ion exchange chromatography on a cation exchange resin of 8% cross linked, sulphonated, polystyrene beads (7 microns diameter) in the sodium form. The sample was applied to the resin at pH 2.2, thus all the amino acids were held by the resin as a thin band at the top of the resin column. Separation was achieved by eluting with a stepwise buffer gradient of increasing pH and ionic strength. The acidic amino acids were eluted first, followed by the neutral, then the basic amino acids. The amino acids are measured by reaction with ninhydrin , to give the characteristic purple colour (proline and hydroxyproline give a brown colour). Results were plotted directly with a three-channel, point-plot recorder. The amino acids are identified by their retention time. Calculation of the concentration of each amino acid was carried out by a Nova 1220 computer, on-line to the analyser. The amino acids (in dehydrated form) were expressed as $g.100g^{-1}$ dry sample.

Tryptophan

Since tryptophan is destroyed during the hydrochloric acid hydrolysis, this was extracted and determined separately. A Locarte automatic amino acid analyser was again used but hydrolysis was with p-toluenesulphonic acid (Liu and Chang, 1971).

Estimation of sulphur amino acids

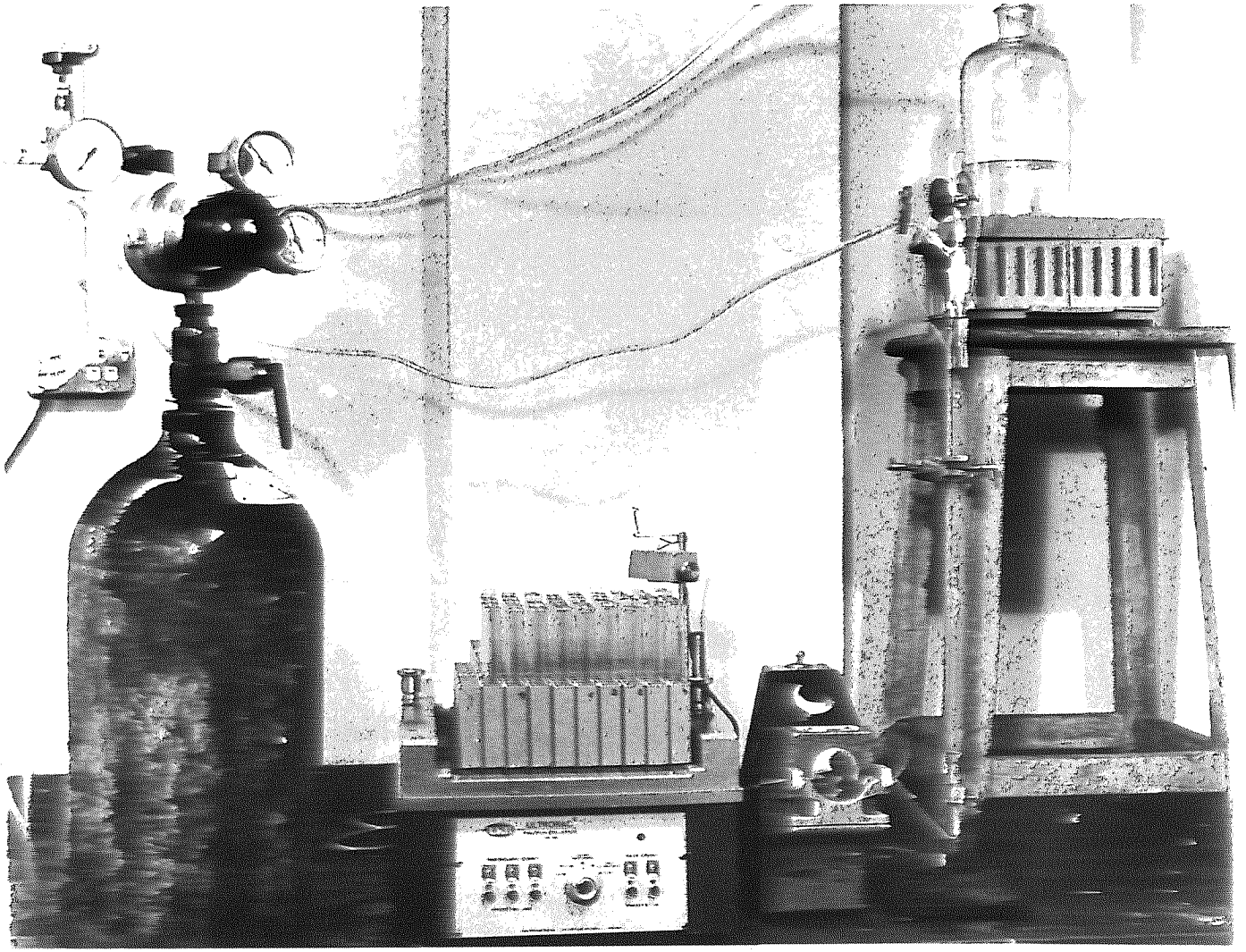
The estimation of sulphur amino acids is useful in the determination of the protein value, since they are often the limiting amino acids in human diets.

Methionine and cysteine were estimated as methionine sulphone and cysteic acid using performic acid oxidation and ion exchange chromatography; the method was based on that of Lewis (1966).

0.3 gm of powdered sample (containing approx. 15 mg nitrogen) was weighed into a 500 ml round bottomed flask and cooled to $0^{\circ}C$ in an ice bath. 10 ml of performic acid solution (5 ml of 30% hydrogen peroxide added to 45 ml of 90% formic acid and the mixture allowed to stand at room temperature for one hour to allow the maximum formation of performic acid) was cooled to $0^{\circ}C$, added to the material in the flask and oxidation allowed to continue for 16 hours at $0^{\circ}C$. 20 ml of ice-cold distilled water was then added and freeze-dried to remove the performic acid reagents. The freeze-dried residue from the oxidation reaction was hydrolysed with 6N HCl at $121^{\circ}C$ for 6 hours, cooled and diluted to 25 ml with distilled water. The hydrochloric acid was removed by freeze-drying. The residue was then dissolved in a few mls of 0.5 M, pH 2.8 citrate buffer (citric acid, 105g; NaOH, 40g; concentrated HCl, 65 ml; 0.5 per cent 'BRIJ' detergent solution, 4 ml; and distilled water to 2 Litres), filtered, and the volume made up to 10 ml with the same buffer solution.

The 40 cm column was packed with Zerolit 225, W.R. 1.2-1.6, 52-100 mesh, cation exchange resin (B.D.H. Ltd) (Plate 2.3) 1 ml of the hydrolysed sample was applied to the top of the resin column and washed in with 1 ml of citrate buffer (pH 2.8). A continuous flow of buffer was then started (using a Watson-Marlow Peristatic pump)

PLATE 23. THE CHROMATOGRAPHY COLUMN FOR SULPHUR AMINO ACIDS ANALYSIS.



from a reservoir at a rate of 20 $ml.h^{-1}$. Oxygen was excluded by bubbling a continuous stream of nitrogen through the reservoir. The separation was carried out at room temperature (18-25°C) and the elutriate from the column collected in 2 ml fractions in an L.K.B. fraction collector.

Cysteic acid was washed from the column at an elution volume of 40-60 ml and methionine sulphone at an elution volume of 140-180 ml. The concentration of the sulphur amino acids was determined colorimetrically by the ninhydrin method (Greenshields, 1966), using a Unicam Sp 500 at 570 mμ. Standard solutions, using L-methionine and cysteine hydrochloride were prepared as for the powdered samples.

2.4.6 Nucleic acid extraction

The nucleic acid extraction was carried out as follows: A sample of fresh mushroom fruitbodies, whose water content was determined (Section 2.4.1) was homogenized in a Waring blender for 10 minutes (1 minute intervals) with distilled water, the homogenate was then vacuum filtered through a Büchner funnel. The residue was suspended in distilled water and subjected to ultrasonic vibration for one hour (5 minute intervals); centrifuging of the sample was carried out for 15 minutes at 10,000 r.p.m.

The method of Ceriotti (1955), as modified by Daunter(1972) for the extraction of nucleic acids was followed. The residue was suspended in 50 ml of ice cold ethanol and allowed to extract for 1.0 hour at 0°C, the whole being gently agitated by a mechanical stirrer.

The ethanol extract was then discarded and the residue extracted three times with ethanol - ether (ratio 3:1) at 90°C for 3.0 minutes; the extract was discarded.

To this residue was added 25 ml of ice cold $20gl^{-1}$ perchloric acid and extracted for 20 minutes at 0°C; the extract was discarded

and the extraction repeated. The residue, now defatted and deproteinised, was then extracted twice with 70 ml of $100g.l^{-1}$ perchloric acid at $70^{o}C$ for 20 minutes to remove the nucleic acids. At the end of each extraction the supernatants were collected after centrifuging. The residue was washed with $100g.l^{-1}$ perchloric acid and added to the combined supernatants containing the nucleic acids.

Nitrogen determinations were carried out on 5 ml samples of the nucleic acid extract by the Kjeldahl method.

Calculation

The calculation of crude protein equivalent to nucleic acids nitrogen was determined from the following values:-

1) Fresh weight of mushroom sample taken for extraction,
2) Water content of the mushrooms, $g.100g^{-1}$,
3) Dry weight of mushroom sample in grams taken for extraction,
4) Final volume of nucleic acid extract, ml.,
5) Nitrogen content in 5 ml of this extract, g.,
6) Total nitrogen in the final volume of extract =

$$\text{nitrogen content in 5 ml} \times \text{final volume} \div 5$$

7) $g.100g^{-1}$ nitrogen due to nucleic acids =

$$\frac{\text{Total nitrogen in the final volume of the extract}}{\text{Dry weight of mushroom taken for extraction}} \times 100$$

8) $g.100g^{-1}$ crude protein equivalent to nucleic acid =

$g.100g^{-1}$ nitrogen equivalent to nucleic acids $\times$ 6.25

2.4.7 Protein digestibility

The digestibility of protein was determined by an *in vitro* technique described by Saunders *et al.*(1973). 1 g of dry material was suspended in 20 ml of 0.1N HCl and mixed with 50 mg of pepsin in 1 ml of 0.01 N HCl. The mixture was gently shaken at 37°C for 48 hours, then centrifuged. After removal of the supernatant, the solids were resuspended in 10 ml of distilled water and 10 ml of 0.1 M phosphate buffer (di-sodium hydrogen orthophosphate dihydrate and potassium di-hydrogen orthophosphate), pH 8.0, and treated with 5 mg of trypsin, the mixture was gently shaken at 23°C for 16 hours. The solids were separated by centrifuging and washed with water (5 x 30 ml), centrifuging, and removing supernatant after each washing. In this procedure, centrifuging was carried out for a period of 15 minutes at 15,000 r.p.m. The solids were finally filtered through Whatman No.1 filter paper, dried in the oven at 60°C, weighed and analysed for nitrogen using the micro-Kjeldahl method. The total nitrogen of the original mushroom sample was analysed by the same method.

Percentage digestibility of mushroom protein was calculated using the following formula:-

$$\% \text{ protein digestibility} = \frac{\text{g Nitrogen in mushroom sample} - \text{g Nitrogen in indigested fragment}}{\text{g Nitrogen in mushroom sample}} \times 100$$

2.4.8 Urea determination

Samples for analysis were prepared as in Section 2.4.9 . They were analysed by the spectrophotometric method of Ceriotti and Spandrio (1963) as modified by Kissmeyer *et al.*(1966). In this method a coloured product is formed, apparently from a double condensation of phenazone with diacetyl monoxime and urea.

2.4.9 Carbohydrate estimation

Most "total carbohydrate"methods are colorimetric and are derived from the well-known Molisch test for carbohydrate. They involve heating the material with strong (20N or more) sulphuric acid and a "colour developer" which is usually an aromatic amine or phenol.

Dry mushroom powder (100 mg) was homogenised with distilled water, using a hand Pestle homogeniser and diluted to 100 ml with distilled water (or 0.05 M phosphate buffer pH 7.0). 0.5 ml of this solution was added to 0.5 ml distilled water, and analysed using the phenol method of (Dubois et al., 1956, as described by Herbert et al., 1971). Glucose standards were used.

2.4.10 Fat extraction

Lipids were extracted by a modification of the method of Wilson and Crawford (1974).

1g of mushroom powder was transferred to a conical flask containing 20 ml of chloroform, 40 ml of methanol, and 16 ml of distilled water. The flask was then shaken with glass balls for 15 minutes and the contents vacuum filtered on a Büchner funnel through Whatman No.1 filter paper. 20 ml of chloroform was then allowed to filter through the tissue and the extract was then transferred to a measuring cylinder with 20 ml of distilled water. After standing for 15 minutes, the top layer was decanted, and the extract washed with two further 20 ml portions of chloroform to remove any remaining traces of lipid. The chloroform extracts were combined, any remaining traces of the alcoholic layer removed and the total volume of chloroform extracts recorded. 10 ml of this lipid extract was evaporated to dryness in a tared flask; evaporation was carried out in a vacuum desiccator over silica gel. The weight of the lipid residue was determined.

The lipid content of the sample was calculated using the following formula:-

$$\text{Lipid} = \frac{w.\ V}{W.\ v} \cdot 100 \quad (g.100g^{-1}) \text{ dry weight.}$$

where, w = weight of lipid in aliquot (grams)

V = Total volume of chloroform layer (lipid extract, ml)

v = volume of lipid extract (10 ml)

W = weight of dry sample (grams).

Section 3

EXPERIMENTAL

3.1 Statistical variation in the composition of Agaricus bisporus fruitbodies.

Previous work on the composition and food value of A.bisporus fruitbodies has not specified factors that contribute to any variation. In order to obtain reliable data on which realistic assessment can be made, the extent and nature of any variation in the composition of fruitbodies requires elucidation; this is especially important in the development of a standard sampling procedure.

A mushroom fruitbody consists of three recognisable structures, namely:- (a) stipe, or stalk which supports

(b) the pileus (cap), which, when mature, bears

(c) the hymenium (gills, the sporeforming tissues which bear the basidia, and the basidiospores).

Following the formation of primordia a proportion develop into first stage - Stage A - fruitbodies, which, if harvested, are commonly referred to as buttons. By delaying harvesting for a further 24 hours, fruitbodies develop to a second stage of development, referred to as Stage B or the cup stage. Stage C represents the final stage of fruitbody development, normally referred to as flats. This sequence is repeated at each successive break or flush; in normal commercial practice, there are five or six flushes.

In order to determine the extent of variation within a fruitbody, between fruitbodies within a given culture (in one tray), between different trays, between different developmental stages and between flushes, a series of six trays were set up in order to establish cultures according to standard commercial procedures (Section 2.1).

3.1.1 Variation within fruitbodies

Dry matter and total nitrogen determinations were performed on the stipe, pileus and hymenium tissues dissected from mature mushrooms (harvested at Stage C). While each region gave similar results for total nitrogen (i.e. no great variation between fruitbodies) there were significant differences between the regions (Table 3.1; Appendix 1.1). Nitrogen content and dry matter were both greatest in the hymenium, but least in the stipe and pileus respectively. This pattern of distribution may be of a reflection of the relative metabolic and transformation mechanisms associated with growth and differentiation.

Table 3.1 Mean values of dry matter and total nitrogen content of different tissues from single fruitbodies (Stage C).

Tissue	Dry matter $g.100g^{-1}$	Total Nitrogen $g.100g^{-1}$ dry weight
Stipe	7.11	2.63
Pileus	5.99	3.27
Hymenium	9.49	5.32

3.1.2 Variation between fruitbodies from one culture unit (tray).

Thirteen Stage A fruitbodies were selected at random from a single tray, at the first break, and analysed for dry matter. The dried sample provided material for nitrogen determinations.

Considerable variation in dry matter was found (Appendix 1.2), total dry matter ranging from 8.87-10.72 $g.100g^{-1}$ fresh weight. Less variation was obtained in the nitrogen contents, which ranged from 5.97-6.95 $g.100g^{-1}$ dry matter.

There was no obvious relationship between dry matter and nitrogen

content as found by regression analysis of the data (Appendix 1.2(a)).

This initial data, especially the extent of variation in dry matter, indicated the need for an adequate sampling procedure. In view of the limited size of samples obtained from single fruitbodies, for subsequent analysis ten fruitbodies from each tray culture were treated as a representative sample of that tray, this providing a minimum of 100g fresh weight or approximately 10 g dry weight.

3.1.3 Variation between culture units (trays)

Ten fruitbodies were harvested from each of six trays. Each fruitbody was quartered with a stainless steel knife. One quarter from each sporophore was taken for dry weight determination. The remaining quarters were combined, sliced to approximately 0.5 cm cubes, and dried at $60^{o}C$ for 24 hours. The dried product was ground by hand using a pestle and mortar so as to pass through a 40 mesh sieve, stored in McCartney bottles and placed in a desiccator.

Analyses obtained for dry matter and nitrogen content for first break fruitbodies harvested at Stages A, B and C are shown in Appendix 1.3.

Dry matter of samples varied from 7.0 - 9.2 $g.100g^{-1}$ for Stage A, 7.0 - 10.2 $g.100g^{-1}$ for Stage B, and 7.6 - 10.5 $g.100g^{-1}$ for Stage C fruitbodies.

There was little variation between the nitrogen contents of samples from Stage A fruitbodies. However, Stages B and C showed variations ranging from 4.72 - 5.64 $g.100g^{-1}$ and 3.80 - 4.88 $g.100g^{-1}$ respectively.

In general, there was an increase in dry matter, and a decrease in nitrogen content as the fruitbodies were allowed to mature.

These same trends were also detected in the analysis of second break fruitbodies harvested from the same trays (Appendix 1.4). The extent of the variation between samples obtained from different trays was similar with the exception of nitrogen content for Stage C, which was less pronounced than A and B.

3.1.4 Variation between fruitbody developmental stages

The mean values of dry matter and nitrogen content of Stages A, B and C fruitbodies obtained from six trays during the first and second breaks are presented in Fig. 3.1 and Appendix 1.5.

The differences in dry matter content between the three Stages A, B and C were not significant both in first and second breaks, but differences between nitrogen contents were highly significant, with the exception of Stages A and B second break samples (Appendix 1.5(a)).

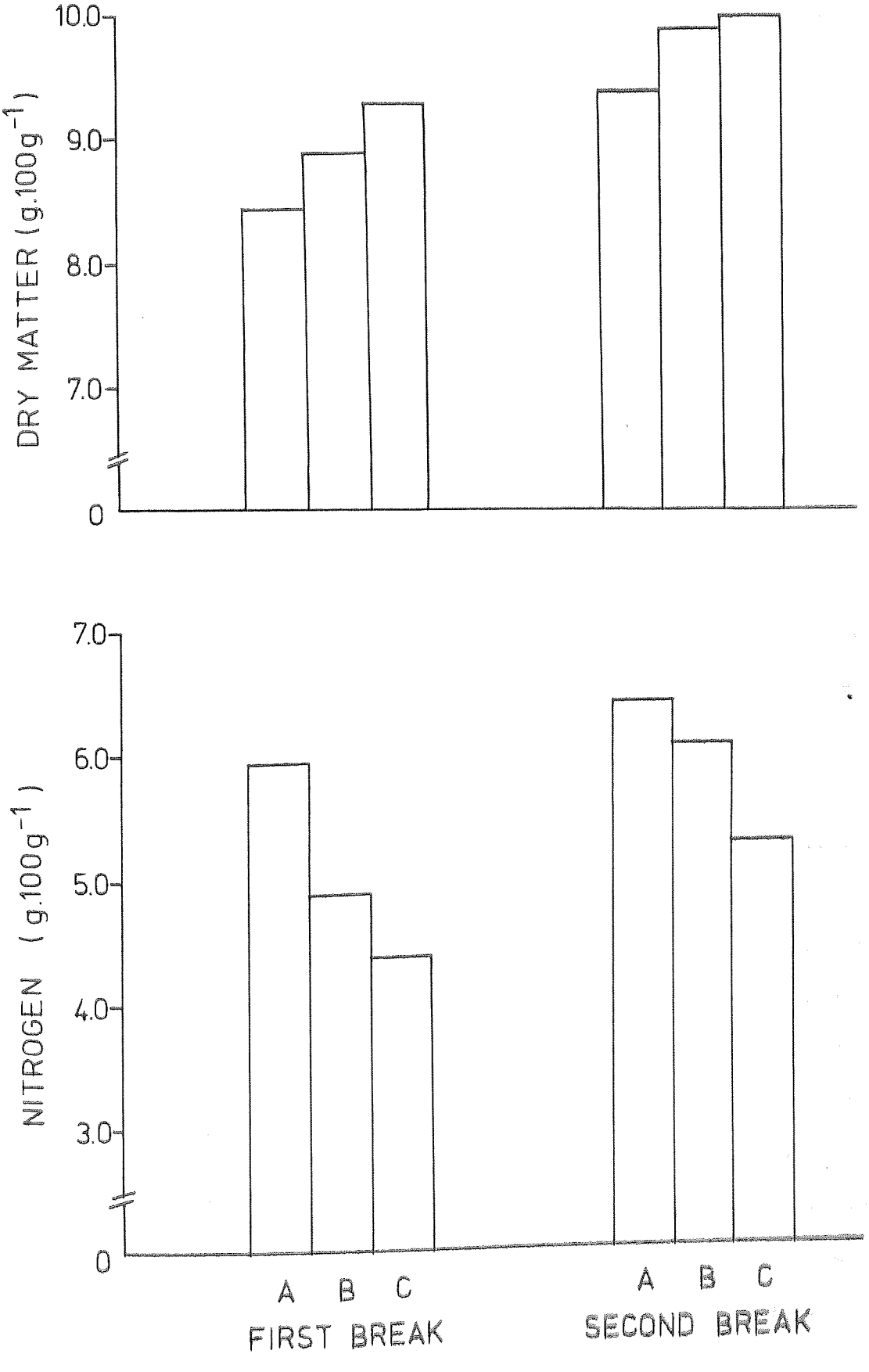
3.1.5 Variation between breaks

No significant differences were obtained in dry matter content of each stage from first and second breaks but differences in nitrogen content were significant. Nitrogen contents for all three stages were significantly greater at the second break (Appendix 1.5(b)).

3.1.6 Conclusions

During the study of mushroom fruitbody composition it is important to state the precise stage of development at which the fruitbodies are harvested (Stage A, B or C and break). Because of significant variation between individual fruitbodies, even when cultured, under identical conditions, it is essential to develop representative sampling techniques and also to ensure the correct statistical design of experiments.

FIG 3.1. DRY MATTER AND NITROGEN CONTENT OF STAGES A,B AND C FRUITBODIES AT FIRST AND SECOND BREAKS.



3.2 Nutritional value of A.bisporus fruitbodies.

An understanding of the nutritional value of a foodstuff requires a knowledge of its chemical composition. Proximate analyses, as elaborated by Atwater and Bryant (1906) do not determine particular elements or compounds (as in ultimate analysis) but rather estimate certain components, e.g. volatile matter, moisture, fat, carbohydrates, ash, nitrogenous matter (e.g. protein),vitamins etc.

Food analysis is considered to be a branch of analytical chemistry and values so obtained are applied in the development and enforcement of standards of identity, purity and value, in problems of decomposition under either normal or abnormal storage conditions, in studies designed to improve or control the quality of natural or processed foods, or in the determination of the nutritive value of foods for scientific, dietary or labelling purposes.

It was established in the investigations described in Section 3.1 that dry matter and nitrogen contents of A.bisporus fruitbodies varied according to the stage of development. In order to establish an overall food value of A.bisporus fruitbodies, it was necessary to confine analysis to a particular stage, considered to be representative of all stages of development. Consequently, the second stage of development (Stage B or cup fruitbodies) was considered to reflect the general food value. Since nitrogen contents varied according to the break, it was considered necessary to sample from each of the first, second and third breaks.

It is known that A.bisporus fruitbodies contain minerals and Vitamins (see Section 1.2.4), but emphasis is currently being given to the energy and protein value of foodstuffs. Since mushrooms, in particular A.bisporus, are now widely cultivated,and in view of the confusion that exists in the published literature, in these investigations emphasis was also given to the energy and protein values of A.bisporus fruitbodies.

3.2.1 Analysis of Stage B fruitbodies

Mushrooms were cultured according to the standard procedure (Section 2.1).

Samples consisted of ten Stage B mushrooms selected from three different trays at each of the first three breaks, giving a total sample of 30 fruitbodies per break. The sample was sliced, dried, combined with samples from the other breaks, and subsequently used in chemical determinations.

(i) Water and dry matter

The water content varied only slightly between the three breaks with a mean water content of 91.63g.100g^{-1} (Table 3.2). The total dry matter of the sample was therefore 8.37g.100g^{-1}. This is markedly different from data given by Anderson and Fellers (1942), MacConnell and Esselen (1946) and Chang (1972) (See Table 1.1).

(ii) Crude Protein

The mean crude protein value was 3.24g.100g^{-1} of the fresh weight (Table 3.2).

The values are lower than those reported by Anderson and Fellers (1942), MacConnell and Esselen (1946) and Chang (1972), which were 3.94, 3.95 and 4.88g.100g^{-1} fresh weight respectively.

(iii) Carbohydrates

In the chemical determination of food composition it is usual to estimate carbohydrate by subtraction, i.e. the dry matter remaining after subtraction of ash, fat and protein; this usually ascribed as "carbohydrate by difference". Because mushroom fruitbodies contain quantities of fibre, chitin and mannitol, the carbohydrate content was measured directly, and expressed as glucose, according to the recommendations of FAO/WHO Report (1973). A mean total carbohydrate content of 2.44g.100g^{-1} of the fresh weight was obtained (29.15g.100g^{-1} dry weight, Table 3.2).

Table 3.2 Composition of Stage B *A.bisporus* fruitbodies, $g.100g^{-1}$ fresh weight.

	1ST BREAK	2ND BREAK	3RD BREAK	MEAN
Water	91.15	91.89	91.87	91.63

Analysis of the dry matter was performed in triplicate by mean of three separate sub-samples.

	Sub-sample 1	Sub-sample 2	Sub-sample 3	Mean
Protein (Total Nitrogen×6.25)	3.24	3.27	3.21	3.24
Carbohydrate as monosacharide (glucose)	2.45	2.43	2.44	2.44
Lipid (chloroform-methanol extract)	0.39	0.40	0.40	0.40
Ash	0.92	0.91	0.89	0.90

(iv) Lipid

The sample contained 0.4g.100g^{-1} (fresh weight basis) of chloroform-methanol extractable lipid (Table 3.2); this value is approximately double that reported by Anderson and Fellers (1942), Hughes (1961) and Chang (1972) who used ethyl ether as the extracting solvent, and this may account for the lower values obtained by these workers. In view of the contribution made by lipids to the energy value of foods such differences are of significance.

(v) Ash

Ash represents the mineral content of the fruitbodies. The mean value obtained from the subsamples was 0.90g.100g^{-1} fresh weight (10.75g.100g^{-1} of the dry weight, Table 3.2). These values agree with those published previously by Watt and Merrill(1963).

(vi) Evaluation of the calorific value

Carbohydrate, fat and protein all act as sources of energy for the body. In the determination of the physiologically available energy, losses occurring during digestion, absorption and through incomplete oxidation are taken into account by applying the Rubner Coefficients (see Section 2.4.4(i)).

The alternative physical method, using a bomb calorimeter, measures the heat of combustion produced when the sample is burned and reflects the total energy value.

Using the Rubner Coefficients an energy value of 312.74 Cal.100g^{-1} dry weight was obtained (Table 3.3), showing that fruitbodies of A.bisporus may be classed as low calorie foods. Using physical analysis by bomb calorimeter, an energy value of 476.55 Cal.100g^{-1} dry weight was obtained; both these values are substantially greater than those quoted in standard food composition tables (Table 3.3).

Table 3.3 Calorific and protein values of Stage B fruitbodies.

	Values.100g^{-1} fresh weight	Values.100g^{-1} dry weight	Values given in McCance and Widdowson (1960), Manual of Nutrition (1970)/100g fresh weight
1 - using calorie conversion factors (refer to Table 3.2)	26.15 Cal	312.74 Cal	7 Cal
2 - Bomb calorimeter	39.84 Cal	476.55 Cal	-
PROTEIN			
(1) Crude protein	3.24	38.75	1.8
(2) Amino acid protein	2.99	35.75	-
(3) Soluble protein	2.83	33.85	-
(4) In vitro digestibility		82.05%	-

(vii) Evaluation of protein

Although it is recognised by the FAO/WHO Report (1973) that conclusions regarding protein needs are based on data from biological experiments in which the substance measured is nitrogen (protein and non-protein nitrogen), it is now recognised that the utilisation and value of dietary protein is determined by the amino acid composition and its digestibility. Consequently crude protein determinations do not accurately estimate nutritional values.

Biological values of protein are estimated directly be feeding animals diets in which protein is the single limiting nutritional factor, and measuring the percentage of ingested nitrogen retained for growth, repletion or maintenance. This estimate is referred to as net protein utilisation (NPU), which is a combined measure of digestibility and of the efficiency of utilisation of the absorbed amino acids. In some tests the digestibility and retention of absorbed nitrogen are evaluated separately, the latter being termed biological value (BV). It was suggested by the FAO/WHO (1973) that it would be necessary to consider the NPU of the dietary protein in comparison with that of milk or egg protein in estimating the amounts of dietary protein needed to meet human requirements. For valid comparison NPU values of egg or milk protein and that of the test protein should be established under identical conditions.

Other biological methods have been used to determine the quality of a protein. For example, the protein:efficiency ratio, PER, is the gain in body weight divided by weight of protein consumed.

During these investigations in vitro methods for the assessment of protein quality were used to determine digestibility (using the enzymes, pepsin and trypsin according to the technique of Saunders et al.(1973) as in Section 2.4.7. The amino acid composition was

determined using a Locarte automatic amino acid analyser (see Section 2.4.5(ii)(c)). For comparative purposes, the colourimetric method of Lowry *et al.* (1951) for the estimation of protein was also used.

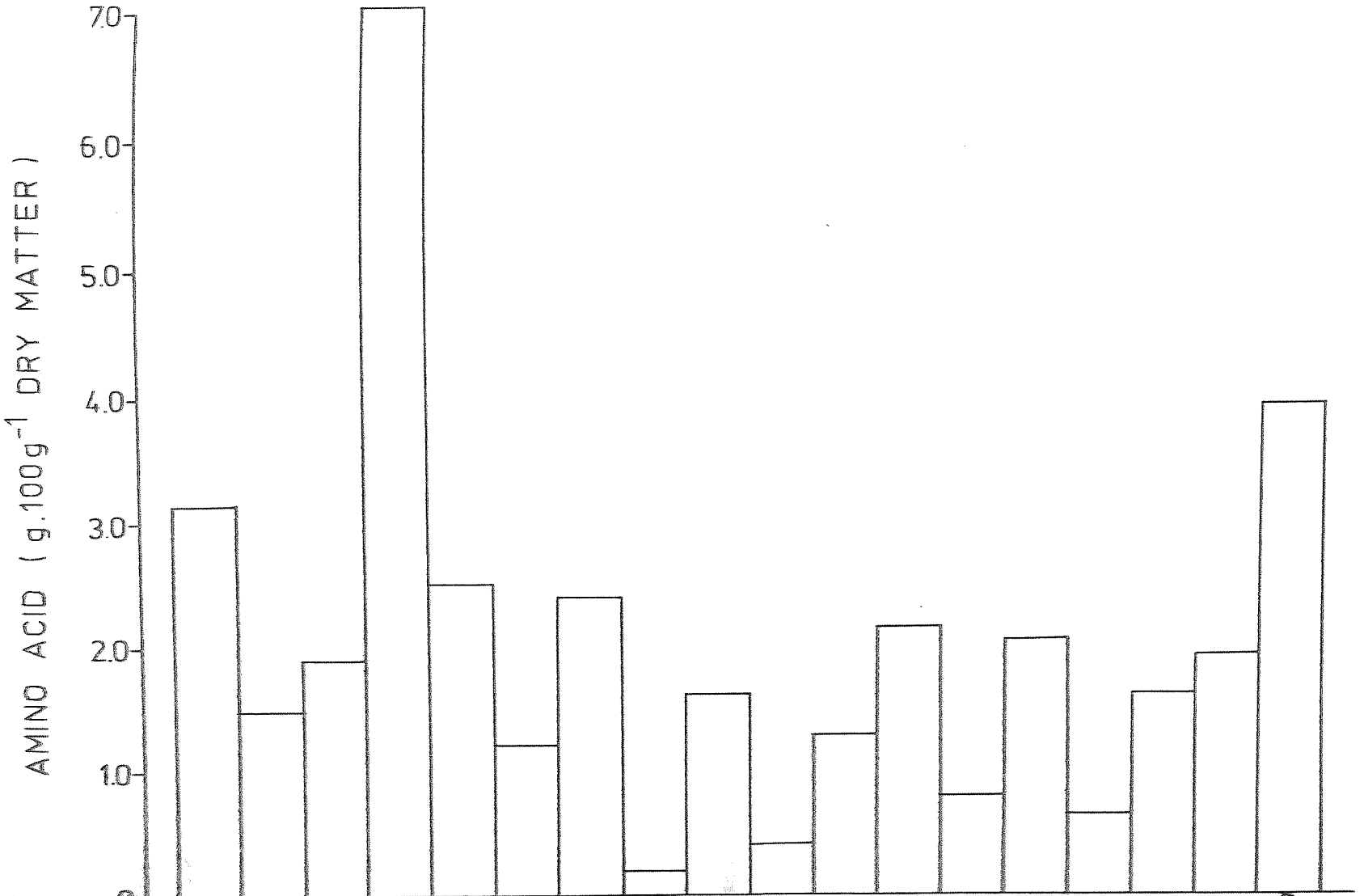
Different values were obtained for the protein content, according to the method of determination (Table 3.3). The true protein value obtained by amino acid analysis was 35.75g.100g^{-1} of the dry matter compared to a crude protein value of 38.75, the difference reflecting the amount of non-protein nitrogen, e.g. nucleic acid, glucosamine and urea. Nitrogen attributable to nucleic acid amounted to 0.19g.100g^{-1} dry weight of mushroom (equivalent to 3.04% of the crude protein). Soluble protein estimated according to the method of Lowry *et al.* was 33.85g.100g^{-1} dry matter.

Irrespective of the method used, the protein values obtained for Stage B fruitbodies were greater than those quoted in food composition tables (Manual of Nutrition, 1970). Additionally, the *in vitro* digestibility value of 82% indicates a comparatively high value protein.

The amino acids present, in order of decreasing concentrations, were glutamic acid, tryptophan , aspartic acid, proline, alanine, leucine, arginine, serine, valine, lysine, phenylalanine, threonine, isoleucine, glycine, tyrosine, histidine, methionine and cystine (Fig.3.2; Appendix 2.1).

The eight amino acids essential to Man were present, lysine and tryptophan were in high concentration, but, as in many micro-organisms, the sulphur containing amino acids, methionine and cystine, were present in only small amounts.

FIG. 3.2. AMINO ACID COMPOSITION OF STAGE B FRUITBODIES.



3.3 The influence of watering regime on the composition of A.bisporus fruitbodies.

The methods by which A.bisporus is cultured in industry varies not only from one production unit to another, but even within a given unit a number of variables in husbandry can be found. It is not known to what extent these variables affect the composition of fruitbodies.

The application of water to the culture is one of the most variable aspects of husbandry. Since it is understood that the purpose of watering is to compensate for losses due to evaporation, loss of water contained in the harvested crop, and losses due to respiration, no standard procedure can be applied during cultivation. Consequently watering is regarded as one of the "arts" of mushroom culture, and together with the preparation of the substrate (composting), represents the more difficult acquired skills of growing.

In order to establish the contribution of watering to the composition of fruitbodies, various watering regimes were used. It has already been established that the composition of any given sample of fruitbodies can vary according to (1) the stage of maturity, and (2) the time of harvest. It was therefore necessary to establish sampling techniques which allow for these variables. Since composition reflects directly the nutritional value of fruitbodies, analyses were concentrated on those components which relate to the dry matter, energy and protein values.

3.3.1 Experimental design

Fruitbodies were cultured according to standard procedures (Section 2.1). Samples were taken from the first three breaks. Low (LW), medium (MW) and high watering (HW) regimes were applied according to the following programme:

	Rates and time of water application to the casing soil
HW. High watering regime.	300 ml on alternate days 1-3-5-7 etc. Casing soil moisture maintained above 65%.
MW. Medium watering regime.	300 ml every third day 1-4-7 etc. Casing soil moisture maintained between 55-60%.
LW. Low watering regime.	300 ml every sixth day 1-6-12 etc. Casing soil moisture maintained between 45-50%.

In order to check that water contents were maintained within the range given, daily determinations were carried out so that minor adjustments could be made during the course of the experiment. (Two gram core samples, obtained with a large cork borer, were dried to constant weight at 105°C). Each watering treatment was done in triplicate.

First, second and third break fruitbodies were harvested at the three developmental stages (A, B and C). For analysis, fruitbodies from each replicate watering treatment and developmental stage were pooled and subdivided into three replicate groups. This procedure was adopted to minimise variations due to tray differences and was considered to be representative of harvesting and marketing practice in the U.K.. Samples were then prepared for analysis as in Section 3.1.3.

3.3.2 Results

(i) Dry matter and yield

Watering regime greatly affected the total dry matter content of the harvested fruitbodies (Fig. 3.3; Appendix 3.1).

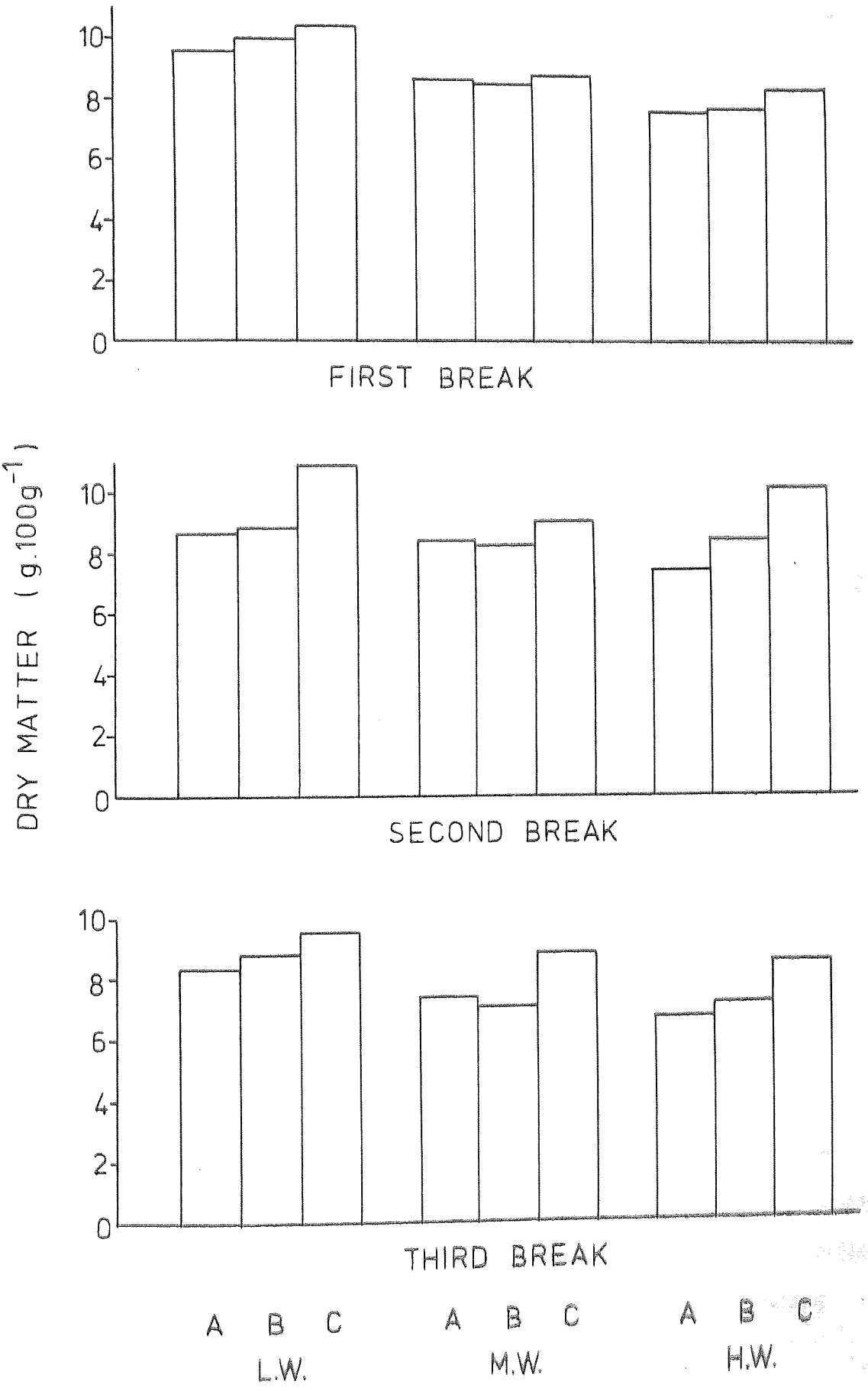
In general the dry matter content decreased progressively as the amount of water applied to the casing soil was increased (Appendix 3.1(a)).

In the low and high watering regimes irrespective of break, the dry matter content increased as the fruitbodies matured from Stages A to C. In the medium watering regime the increase was only significant between Stages A and C for the second and third breaks (Appendix 3.1(b)).

For the low watering regime, the dry matter content of Stages A and B fruitbodies declined progressively from first to third break, while for Stage C fruitbodies a significant increase in dry matter was observed in the second break (Appendix 3.1(c)).In the medium watering regime the decline in dry matter content was significant only for stages A and B between the first and third breaks. No significant difference was detected for Stage C. For the high watering regime for Stage A fruitbodies a similar pattern was found as in the low watering regime, while there was a significant increase in dry matter content for Stages B and C in the second break.

Yield, expressed as number of fruitbodies harvested, fresh, dry and mean weights per fruitbody, are given in Appendix 3.2. Greatly increased total fresh and dry weight yields were obtained by increasing the rate of water application to the casing soil; the medium rate of application increased yields by 30% but the high rate of water application increased yields by 89.58% on a fresh weight basis. However, increases in dry matter yield were not necessarily proportional to fresh weight increases. At the medium watering rate, dry weight

FIG.3.3. WATERING EXPERIMENT. FRUITBODY DRY MATTER CONTENT.



increase over the low rate of application was 34.52%, while the high rate of application resulted in an increase of only 54.47%. Thus it is seen that, while increasing the watering rate, yield and productivity of the culture was increased; at the medium watering rate this was reflected by an increase in dry weight. At the higher rate of application the dramatic increase in fresh weight yield was partly attributed to the increase in water content of the fruitbodies.

The increase in yield was due to the formation of larger fruitbodies, since the number of fruitbodies harvested was similar at all watering regimes. It was also noted that, at the low rate of application, successive breaks were more rapid than with medium and high rates of application.

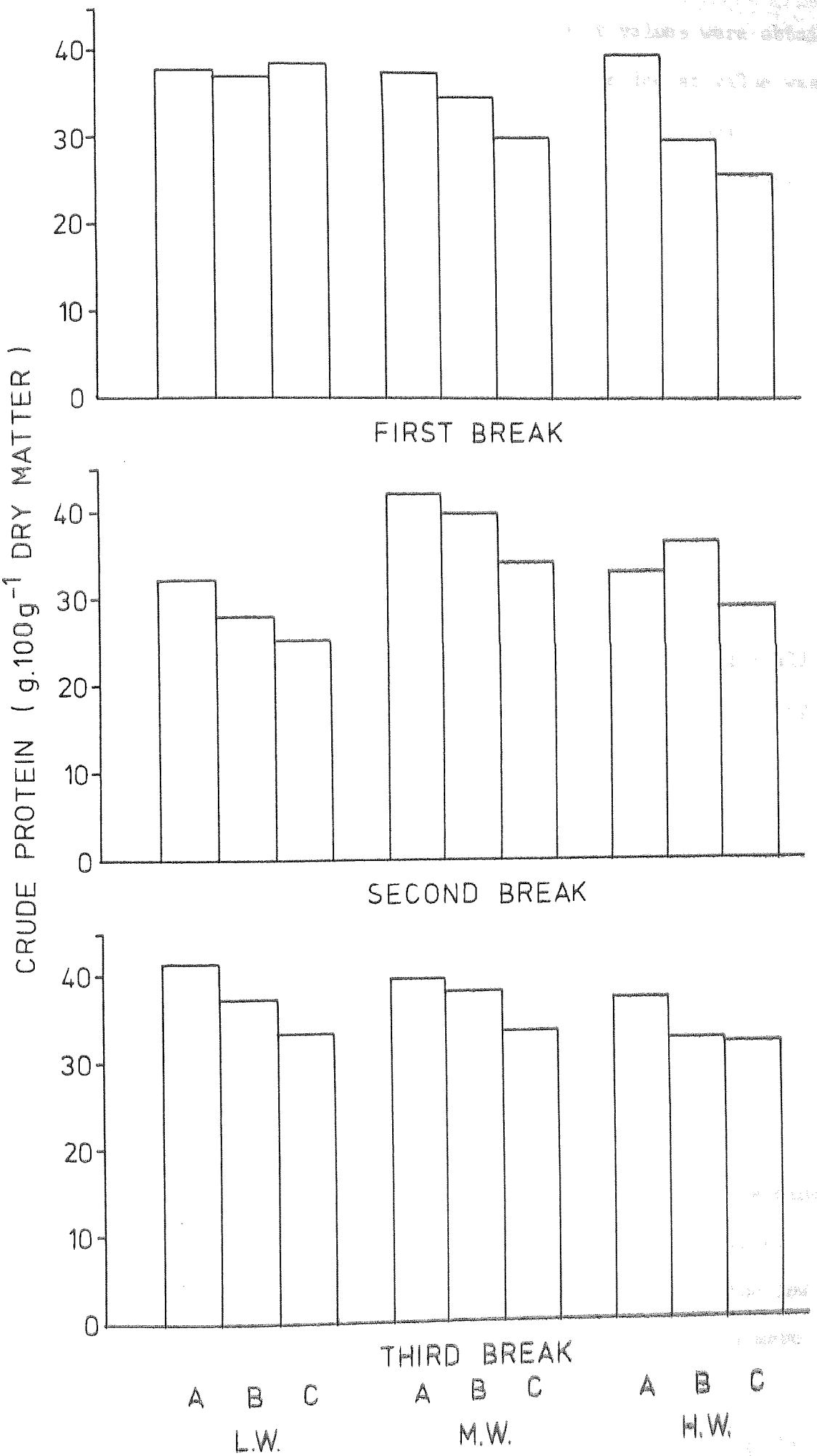
(ii) Crude protein

Watering regime was found to affect the crude protein content of fruitbodies at different stages of maturity and times of harvest (Fig. 3.4; Appendix 3.3).

Crude protein values generally declined as fruitbodies matured through the three developmental stages. No significant variation (Appendix 3.3(a)) was evident in first break fruitbodies cultured under the dry conditions (low watering regime), also, within the high watering regime second break, Stage B fruitbodies contained higher values than Stages A and C.

There were significant variations between breaks (Appendix 3.3(b)). In the second break of the low and high watering regimes, the crude protein of stage A fruitbodies was significantly less than of those harvested in the first and third breaks. In the medium regime (second break) Stage A fruitbodies were significantly higher in crude protein than those in the first and third break. For Stage B fruitbodies

FIG. 3.4. WATERING EXPERIMENT. CRUDE PROTEIN CONTENT.



from both medium and high watering regimes, highest values were obtained at the second break; in the low watering regime the lowest value was obtained at the second break. Similarly for Stage C fruitbodies (low watering regime) crude protein was lowest in the second break. For the medium and high watering regimes, crude protein content of Stage C increased from first to third break.

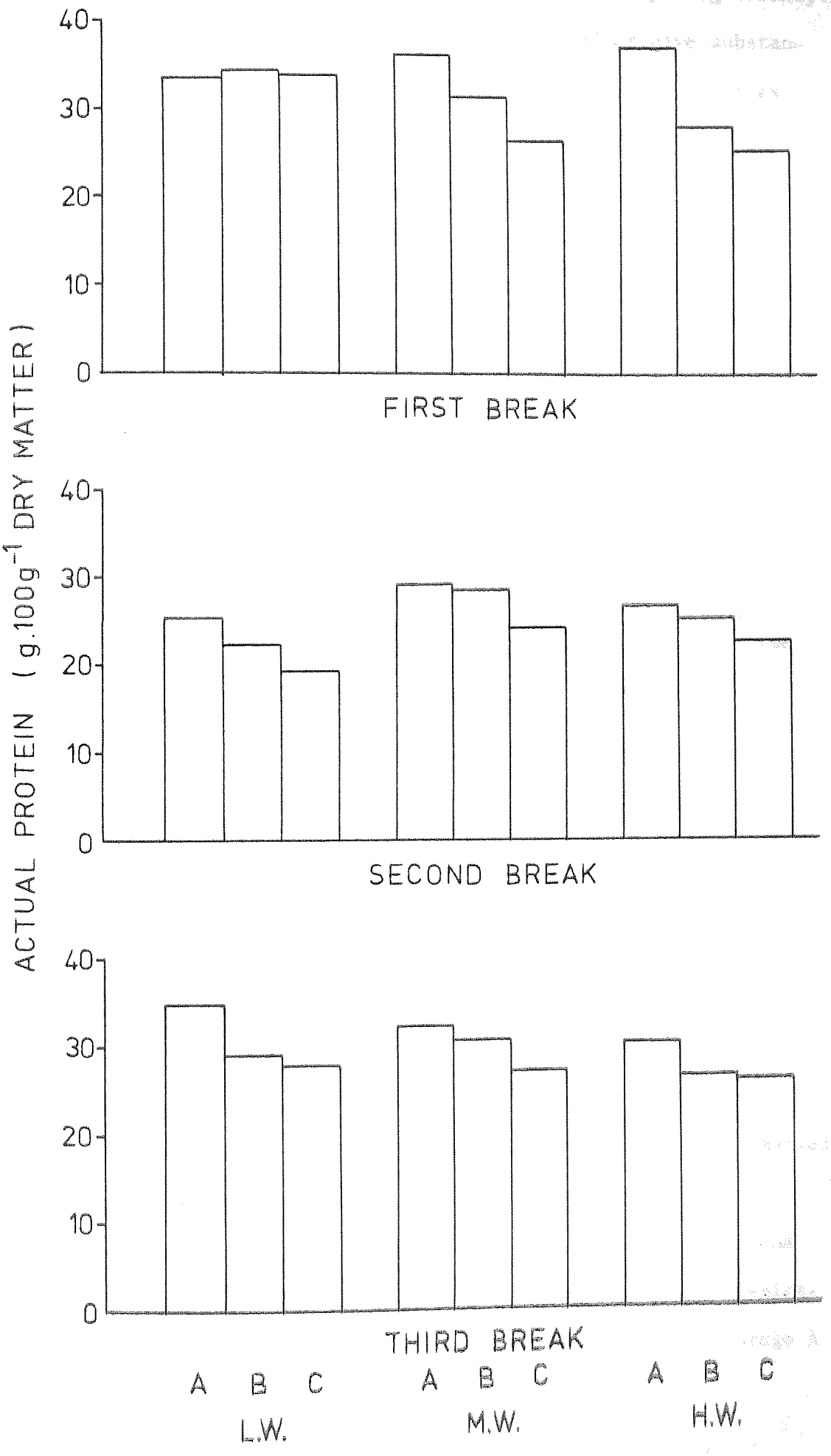
The crude protein content of the fruitbodies varied significantly with water application (Appendix 3.3(c)). In the first break, increasing water application to the higher level significantly increased the crude protein content of Stage A fruitbodies; this trend was reversed with stages B and C. In the second break there was a consistent pattern for all stages of maturity, the crude protein content at the medium watering regime being significantly greater than at the low and high watering regimes. In the second break there was a consistent pattern for all stages of maturity, the crude protein content at the medium watering regime being significantly greater than at the low and high regimes. In the third break, crude protein of Stage A fruitbodies gradually declined with increasing water application, whilst in Stages B and C similar values were obtained from the low and medium treatments, being higher than those obtained at the high watering regime.

These results show that considerable variation occurs in fruitbody crude protein (nitrogen) content during mushroom cultivation, when water is applied to the casing soil at various rates.

(iii) "Actual protein" - amino acid protein

In general, as for crude protein, "actual protein", as determined by summation of amino acids, declined as fruitbodies matured from Stages A to C, the exception was again in the first break of the low watering regime (Fig. 3.5; Appendix 3.4). Results followed a more

FIG. 3.5. WATERING EXPERIMENT. ACTUAL PROTEIN CONTENT.



consistent pattern than crude protein content when comparing breaks, thus, irrespective of stage, second break fruitbodies gave substantially lower values for actual protein than first and third break fruitbodies.

Watering itself had only a small effect on actual protein, this being generally highest in the low and medium watering regimes.

(iv) Amino acid composition

Amino acid contents obtained for each treatment are presented in Appendix 3.5 Eighteen amino acids were separated and quantitatively estimated. Glutamic acid and aspartic acid were present in great amounts, in all samples the essential amino acid methionine was present in low concentration. Of the other essential amino acids, tryptophan was found to be present in high concentration.

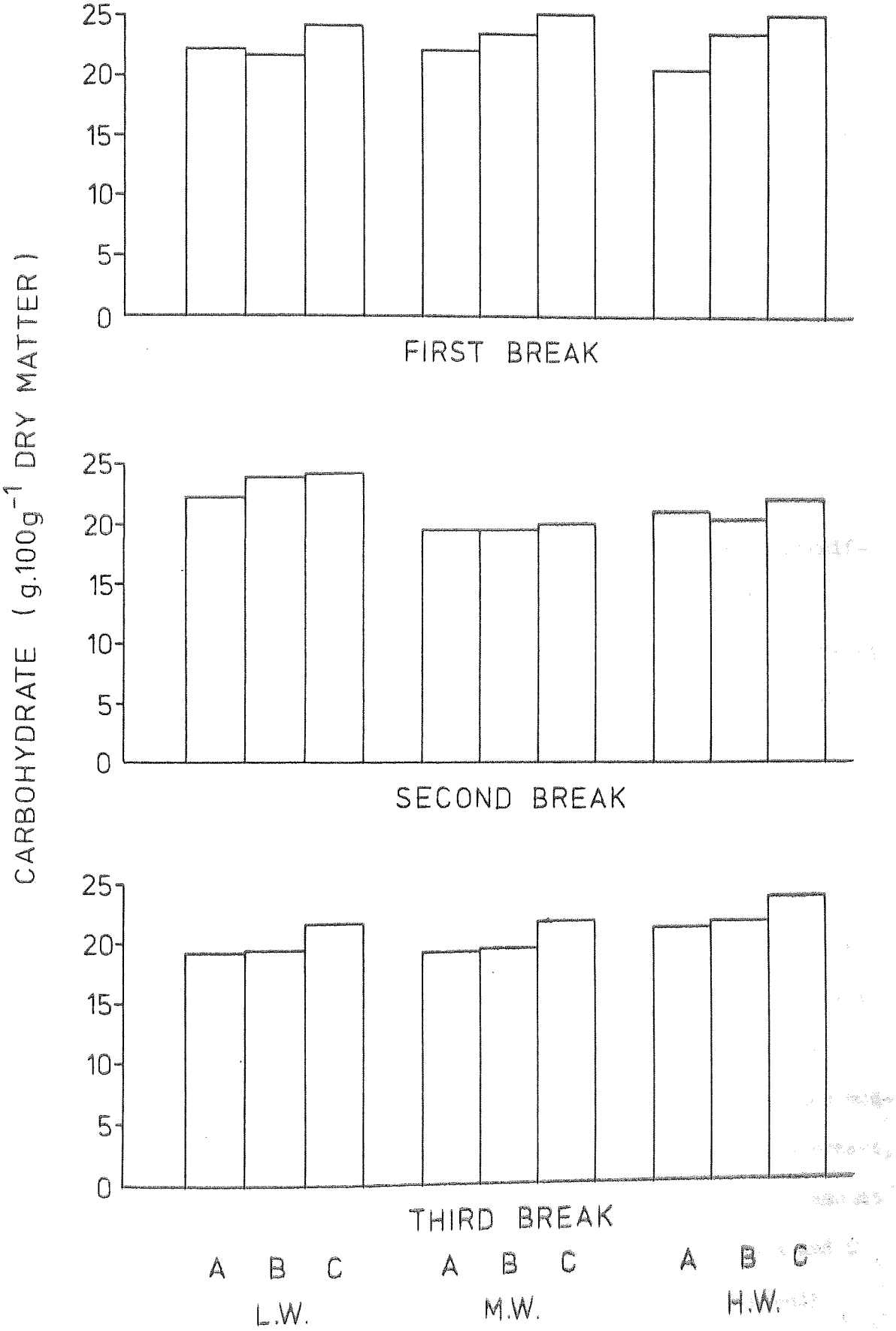
While Stage A fruitbodies contained greater quantities of the individual amino acids than Stages B and C, no general trend could be applied to each amino acid in relation to the time of harvest (breaks) or to watering regime.

(v) Carbohydrate

The carbohydrate content (expressed as glucose) increased significantly as fruitbodies matured from Stage A to C for all treatments, at first, second and third breaks, except in the second break of the medium watering regime when no significant differences were obtained (Fig. 3.6; Appendix 3.6 (a)).

In the low watering regime, carbohydrate content showed a marked decline from the first to the third breaks irrespective of stage, while in the medium watering regime the decline was found from the first to the second and third breaks. For the high watering regime, no significant differences were found between the breaks for Stage A

FIG. 3.6. WATERING EXPERIMENT. CARBOHYDRATE CONTENT.



fruitbodies, for Stages B and C, the carbohydrate content was significantly higher at the first break (Appendix 3.6(b)).

There was a consistent pattern for every stage within a break, e.g. in the second break the carbohydrate content was lowest at the medium watering regime for Stages A, B and C; the only exception was first break, Stage A fruitbodies (Appendix 3.6 (c)). The pattern was not, however, the same for all three breaks (Fig. 3.6; Appendix 3.6).

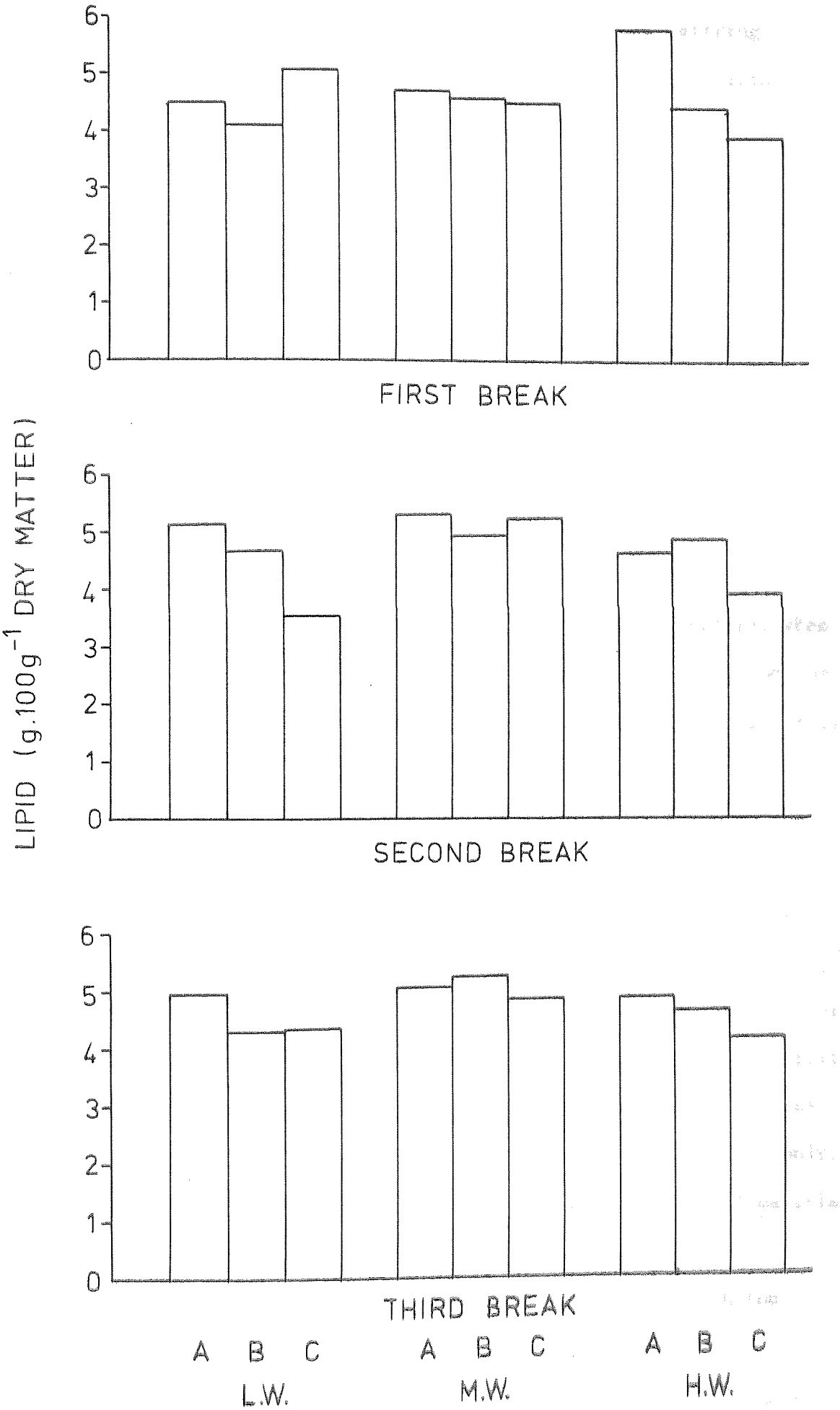
(vi) Lipid

Chloroform-methanol extractable lipid also showed significant variation with stage of maturity, time of harvest and watering regime. Values ranged from 3.54 to 5.87 $g.100g^{-1}$ dry matter (Fig. 3.7; Appendix 3.7).

In the low watering regime lipid content decreased significantly between Stages A and B at all three breaks (Appendix 3.7(a)). Significant differences were also seen between Stages A and C, but these variations did not follow a consistent pattern. For the high watering regime, there was a decline in lipid content between Stages A and C and B and C. There was no consistent pattern between Stages A and B. In general there were no differences in lipid content between the stages under the medium watering regime.

Significant variation in lipid content was seen between the three breaks for each stage of maturity, there was, however, no consistent pattern. In the low watering regime, lipid content was significantly greater at the second break for Stages A and B, but significantly lower in the second break for Stage C (Appendix 3.7(b)). Under the medium watering regime lipid content increased from first to third breaks, for Stage B fruitbodies, while Stage A and C contained greatest amount in the second break. For the high watering treatment Stages A and C fruitbodies contained least lipid in the second break; conversely

FIG. 3.7. WATERING EXPERIMENT. LIPID CONTENT.



Stage B fruitbodies contained higher amounts in the second break.

There was no correlation between lipid content and watering regime at the first break; for the second and third breaks lipid content was generally greater at the medium watering regime (Appendix 3.7(c)).

3.3.3 Conclusions and significance of water application on the composition of *Agaricus bisporus* fruitbodies.

The purpose of this experiment was to determine the effect of various watering regimes on the composition of *A.bisporus* fruitbodies. The watering regimes adopted were chosen to represent the entire range of water relations likely to be encountered during commercial culture. It should, however, be emphasised that the low watering treatment was an extreme condition and would only be found exceptionally in practice, for example, in areas of a growing house near to fans or heaters, when the culture would be exposed to high rates of evaporation. The medium and the high watering regimes were therefore more typical of commercial practice in the U.K.

There were many effects of the rate of water application on the cultivation of *A.bisporus* fruitbodies, the most significant being on the yield (measured as total fresh and dry weights). Thus, although the actual number of fruitbodies was not affected, yield increased significantly with increasing water application. The increase in total dry matter was not proportional to the fresh weight increase, because fruitbody dry matter content declined as the rate of water application was increased. This indicates that the increase in yield was due not only to the increased uptake of water, but also to the efficiency of material transfer from the substrate.

Composition of the fruitbodies varied inconsistently with the

different rates of water application, this demonstrating the fundamental role of water on the distribution and metabolism of substances within the fruitbody. It is not therefore possible to draw any specific conclusions regarding the composition of A.bisporus fruitbodies, as affected by watering regime.

3.4 Nitrogen supplementation of compost at casing

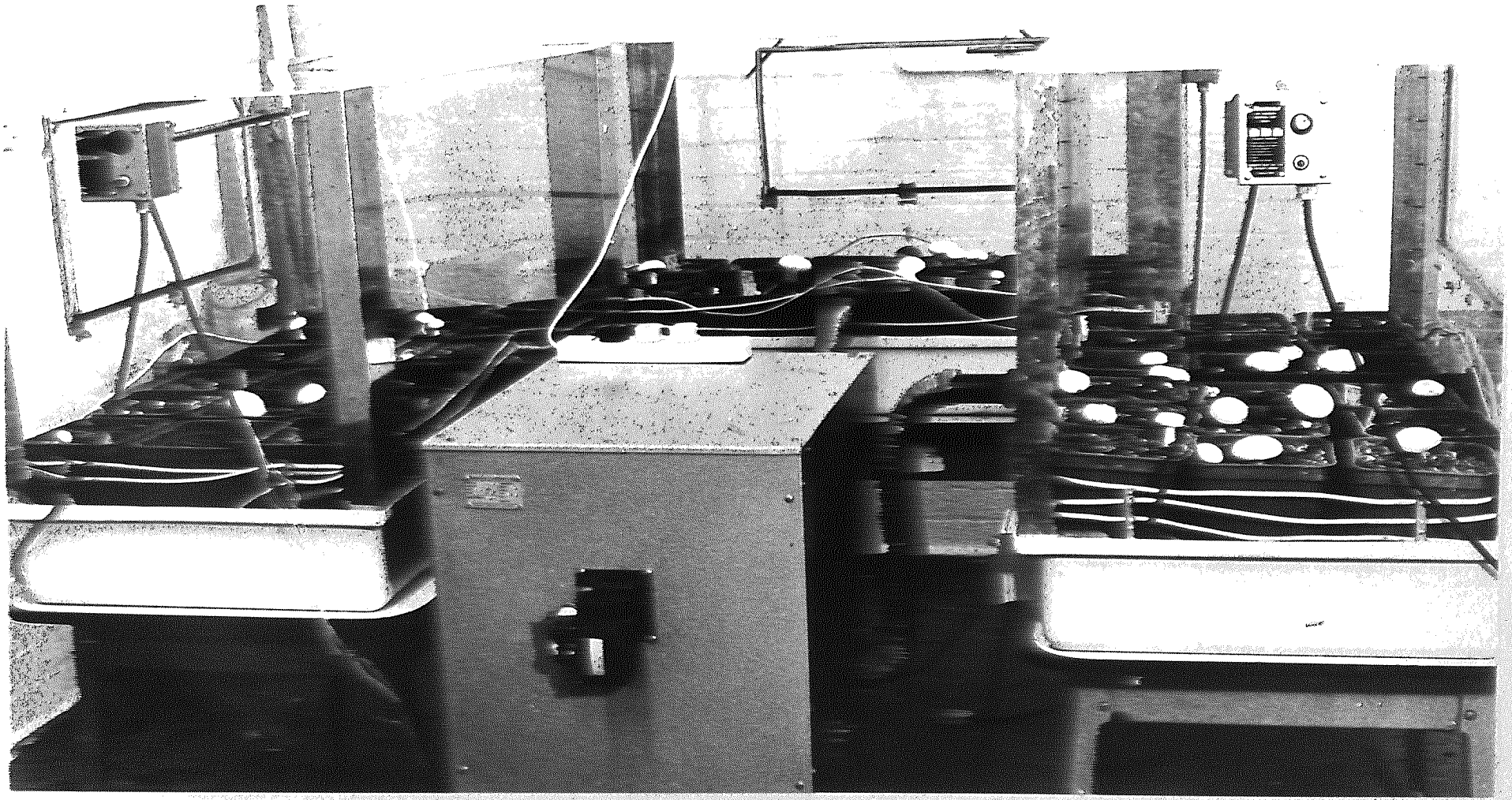
The effect of nitrogen supplementation of the compost at casing on the yield of A.bisporus fruitbodies has been investigated by Sinden and Schisler (1962) (see Section 1.1.2). In view of the significant changes that may occur in the composition of fruitbodies, it was considered valuable to extend this work by investigating the effects of various nitrogen supplements on the growth and composition (crude protein, amino acids, and urea contents) of the fruitbodies.

3.4.1 Experimental design

Four sources of organic nitrogen (dried blood, casein hydrolysate, soya bean meal and bacterial biomass (I.C.I. 'Pruteen') were added to mushroom compost at casing. Culture of the fruitbodies was by the general method described in Section 2.1. After fourteen days of spawn growth, the cultures were supplemented with the four different nitrogen sources at four rates of application (0.16, 0.32, 0.64 and 0.96 g nitrogen-equivalent per kilogram of freshly spawned compost. For amino acid analyses and nitrogen contents of the supplements see Appendix 4.1). There were three replicates of each. Each supplement was thoroughly mixed with freshly spawned compost, and one kilogram of freshly spawned supplemented compost filled in each box (18 cm x 18 cm x 13 cm). These boxes were cased with a 3 cm layer of casing soil, and placed in the production cabinets (Plate 3.1), which maintained constant conditions of temperature and humidity, for cropping.

Stage B fruitbodies were harvested at the first and second breaks. For analysis fruitbodies from each replicate treatment were pooled and subdivided into three groups (to minimise variation between the replicates). Samples were prepared for analysis as in Section 3.1.3.

PLATE 3.1. THE PRODUCTION CABINETS.



3.4.2 Results

(i) Yield and dry matter content

The fresh weight yield of fruitbodies increased following supplementation with casein hydrolysate. Increases were also observed on the addition of the higher concentrations of dried blood and soya bean meal. Results obtained from supplementation with bacterial biomass ('Pruteen') were inconclusive due to mould invasion of the casing layer (Fig. 3.8; Appendix 4.2).

For all treatments, the total number of fruitbodies increased over the control (Appendix 4.2).

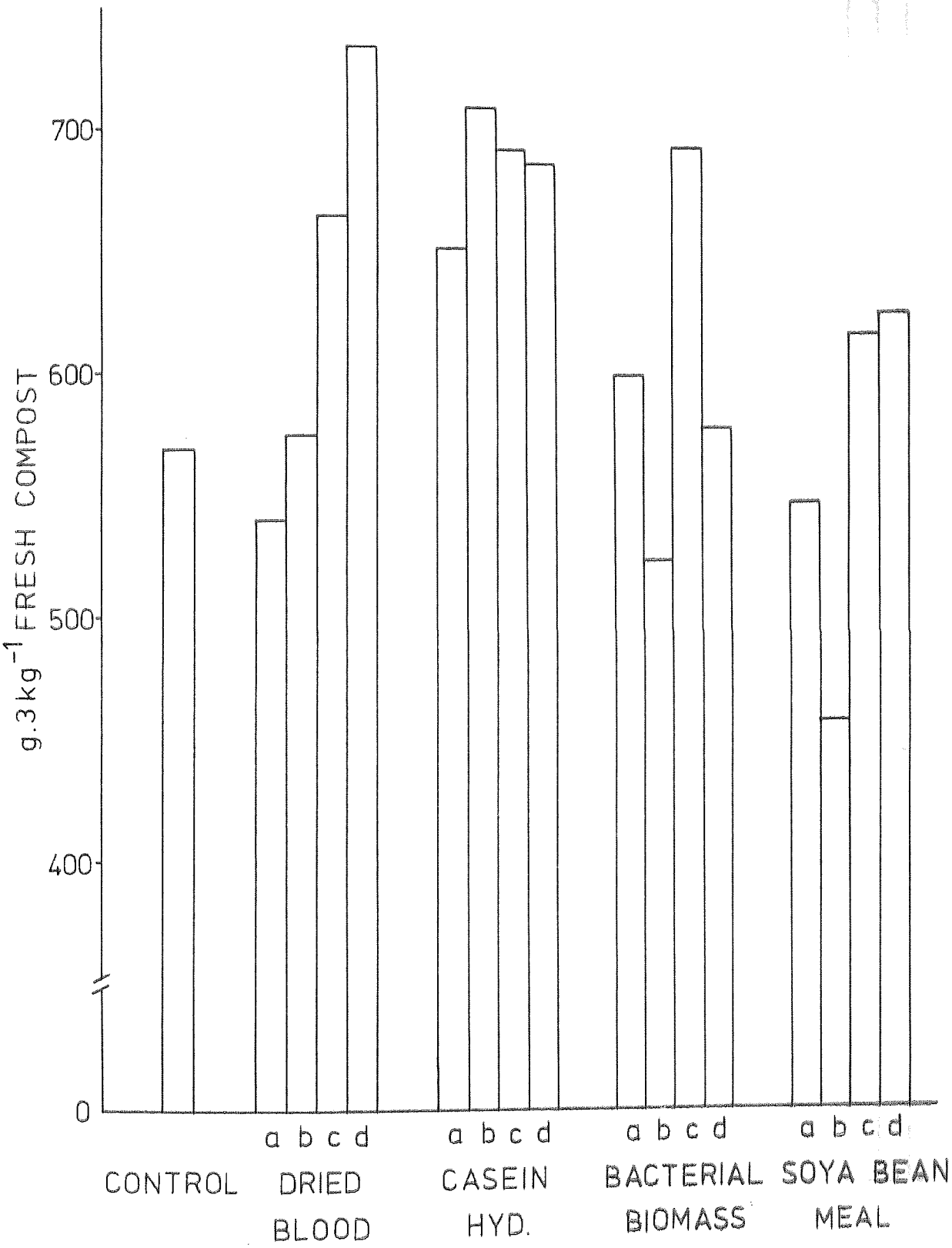
The fruitbody dry matter contents were generally unaffected by the individual nitrogen supplement and its concentration (Fig. 3.9; Appendix 4.3). Exceptions were found at the first break for the bacterial biomass ('Pruteen'), where dry matter was significantly lower than the control, and soya bean meal, where a highly significant increase in dry matter content was obtained at the higher level of application. In all cases dry matter content was higher at the second break; this was also noted in the initial experiments on fruitbody composition (Section 3.1.5; Appendix 1.5) and during the storage experiment (Appendix 5.1).

Dry weight yield is a function of the fresh weight yield and its dry matter content, consequently the dry weight yield for the soya bean meal supplement at the higher concentrations was approximately the same as dried blood and casein hydrolysate (Fig.3.10; Appendix 4.2). This was due to the high dry matter content of fruitbodies harvested from the two higher levels of supplementation at the first break, even though the fresh weight yield was comparatively low.

(ii) Crude Protein content

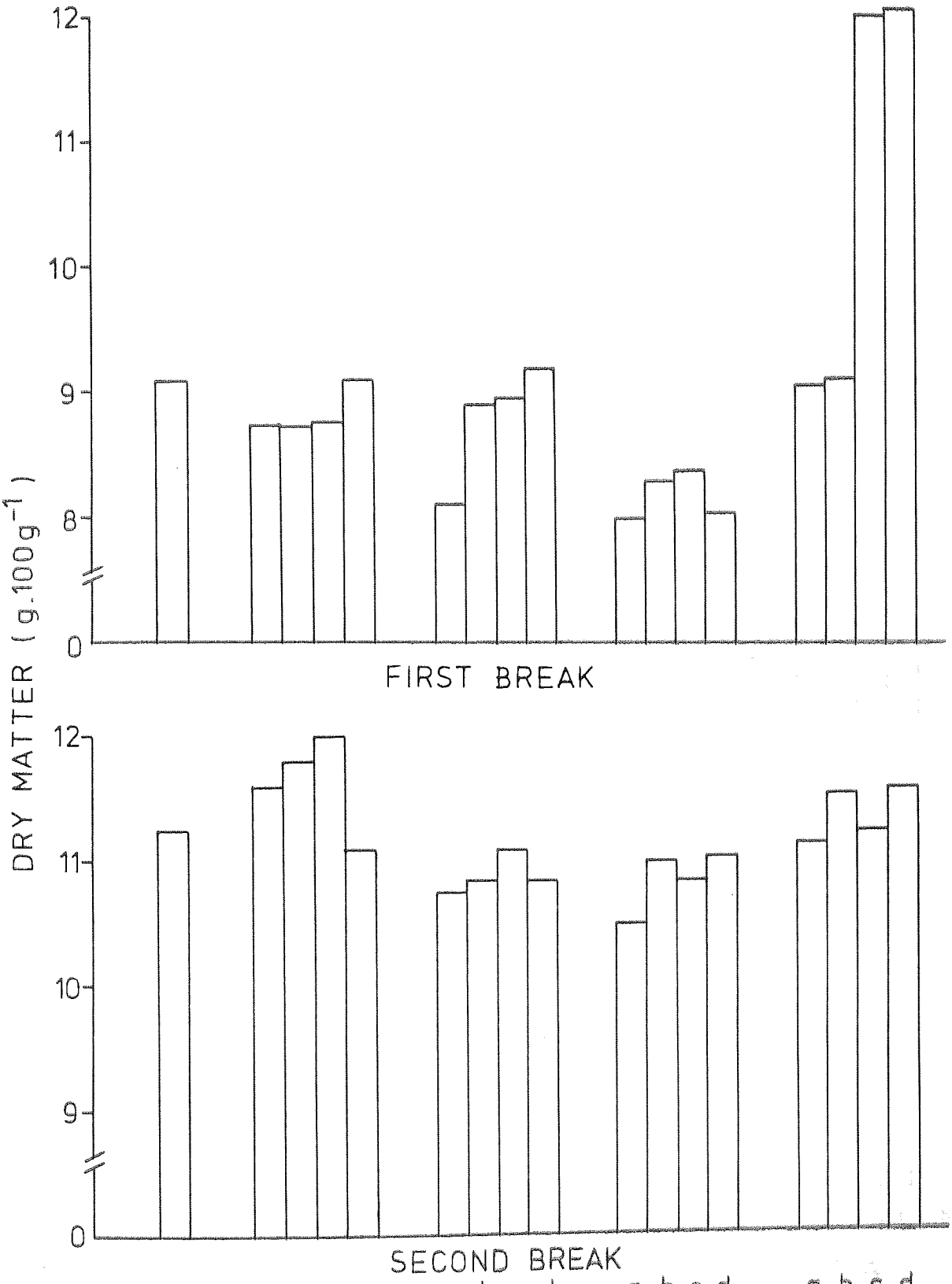
Crude protein content of the fruitbodies generally increased on addition of the nitrogenous supplements. During the first break

FIG. 3.8 NITROGEN SUPPLEMENTATION. TOTAL FRESH WEIGHT YIELD OF FRUITBODIES.



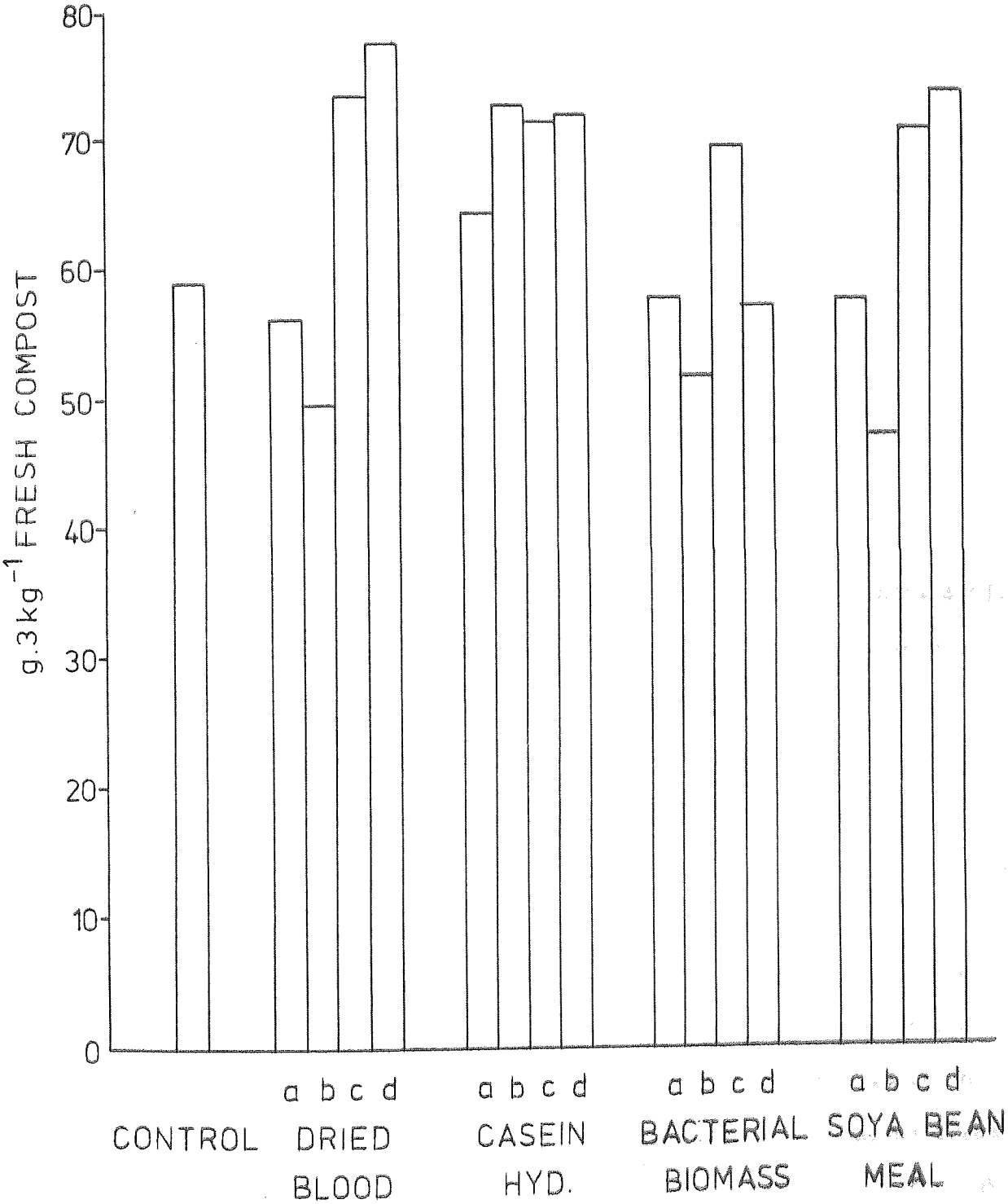
a, 0.16 ; b, 0.32 ; c, 0.64 ; d, 0.96 g NITROGEN-EQUIVALENT PER KILOGRAM FRESH COMPOST.

FIG.3.9. NITROGEN SUPPLEMENTATION. FRUITBODY DRY MATTER CONTENT.



a, 0.16 ; b, 0.32 ; c, 0.64 ; d, 0.96 g NITROGEN-EQUIVALENT PER KILOGRAM FRESH COMPOST

FIG. 3.10. NITROGEN SUPPLEMENTATION. TOTAL DRY WEIGHT YIELD OF FRUITBODIES.



a, 0.16 ; b, 0.32 ; c, 0.64 ; d, 0.96 g NITROGEN-EQUIVALENT PER KILOGRAM FRESH COMPOST.

differences were significant, except for the highest level of dried blood application (Fig.3.11; Appendix 4.4). At the second break differences were again highly significant, except for the two lowest concentrations of dried blood and the lowest concentration of casein hydrolysate, where there were no significant changes from the control, and the lowest concentration of soya bean meal, where crude protein was significantly reduced. As previously (Section 3.1.5), crude protein was generally higher at the second break.

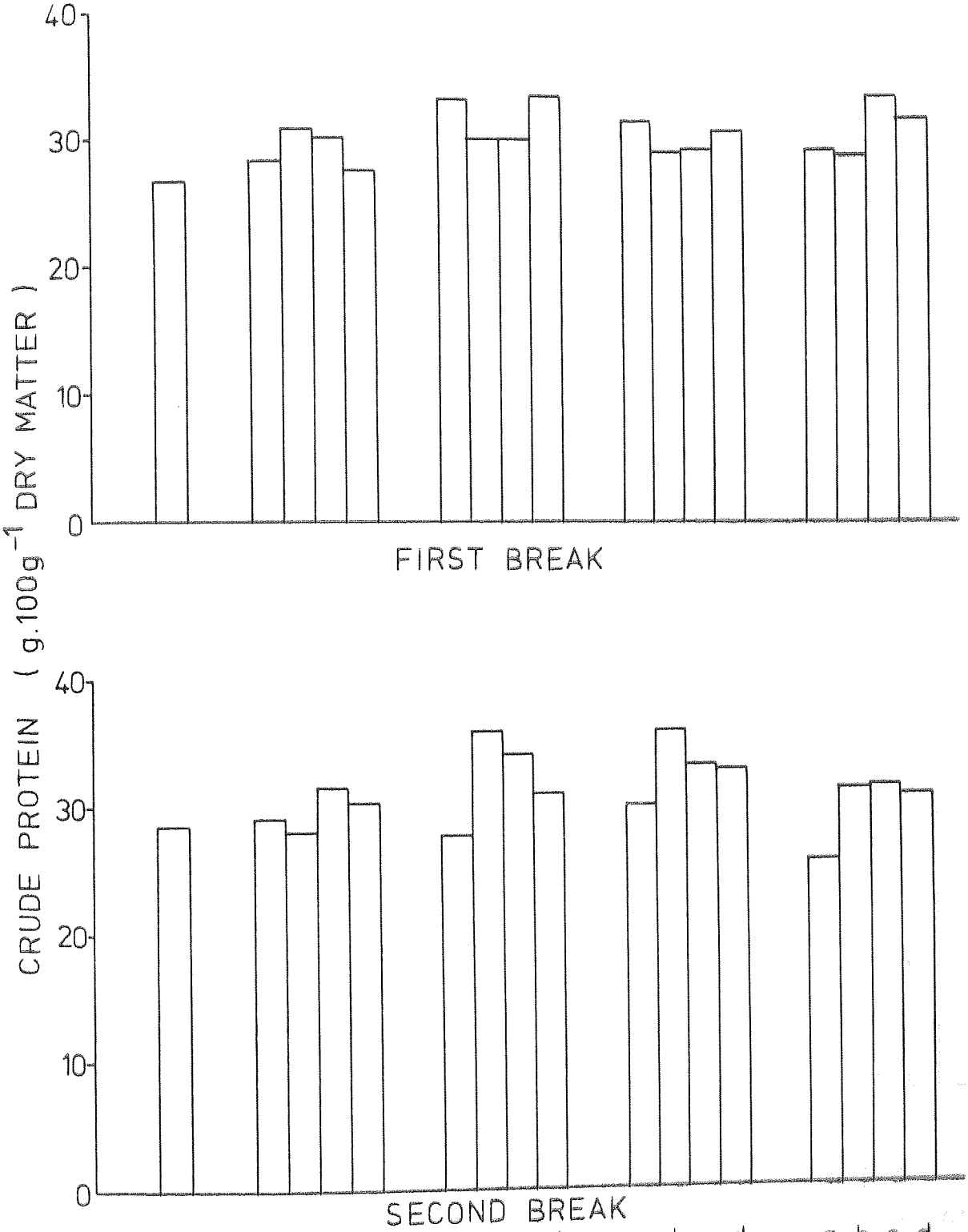
(iii) Amino acids and protein content

The protein content (by summation of amino acids) of the fruitbodies, and the amino acid composition was not affected by the addition of casein hydrolysate at all levels of application (Appendix 4.5). This was also confirmed by the soluble protein results, where there were no significant differences between the samples following the addition of the various concentrations of each supplement (Fig. 3.12; Appendix 4.6). Consequently, for amino acid analysis, the samples for each supplement were combined.

The actual protein contents of the fruitbodies harvested from the first and second breaks were also unaffected by the type of nitrogen supplement (Fig.3.13; Appendix 4.7); this was unlike the crude protein, where significant changes were found.

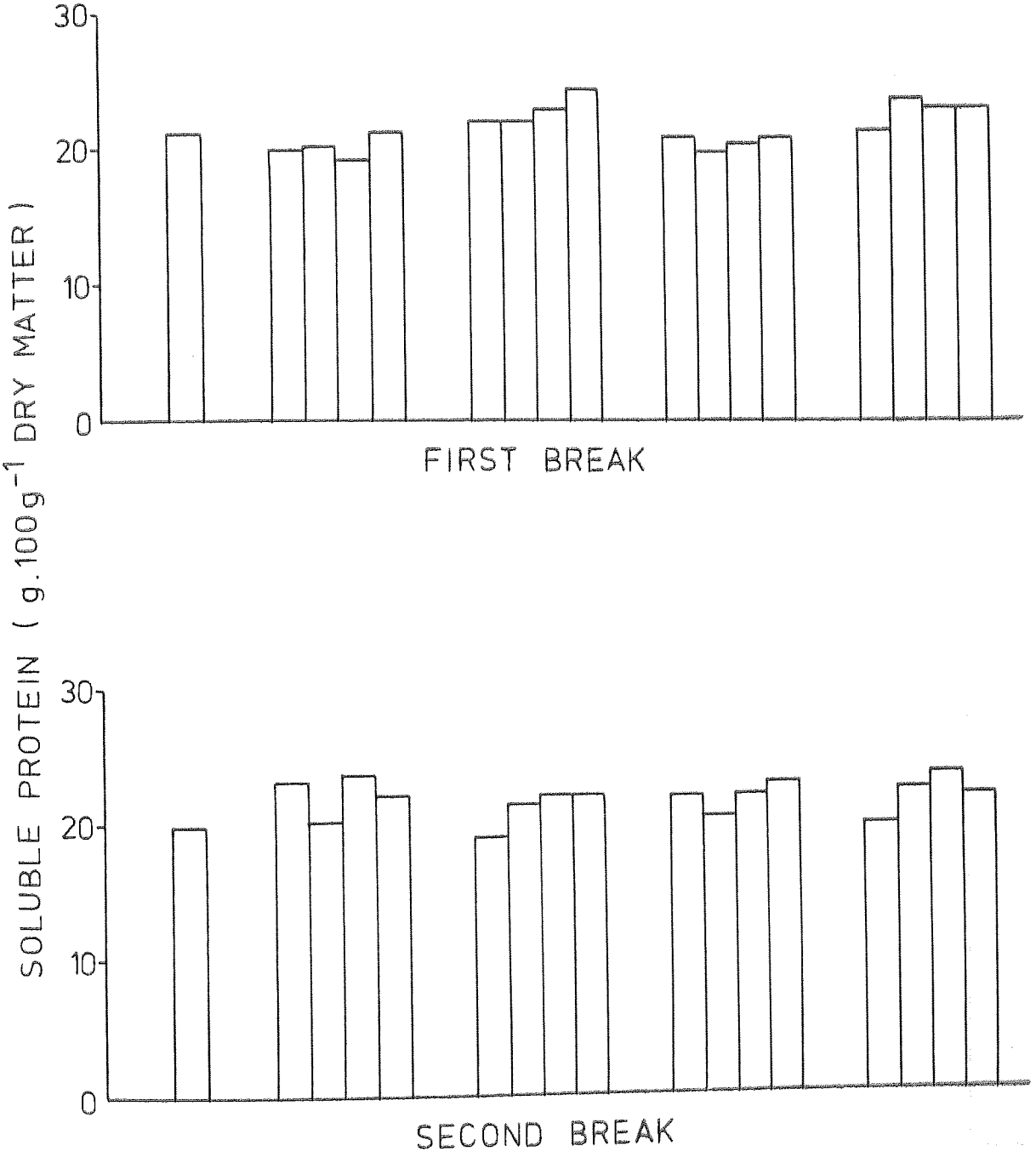
For each treatment there was variation in the amino acids profile from the control. Increases of approximately 30% were observed at the first break for proline, alanine, leucine and arginine on the addition of casein hydrolysate; no differences were observed at the second break (Appendix 4.8). Proline was also increased (by 22%), at the first break, on supplementation with bacterial biomass ('Pruteen'), however, a 28% decrease of methionine was obtained. In the second break, there was a 24% increase in valine. Dried blood and soya bean meal supplements

FIG.3.11. NITROGEN SUPPLEMENTATION. FRUITBODY CRUDE PROTEIN CONTENT.



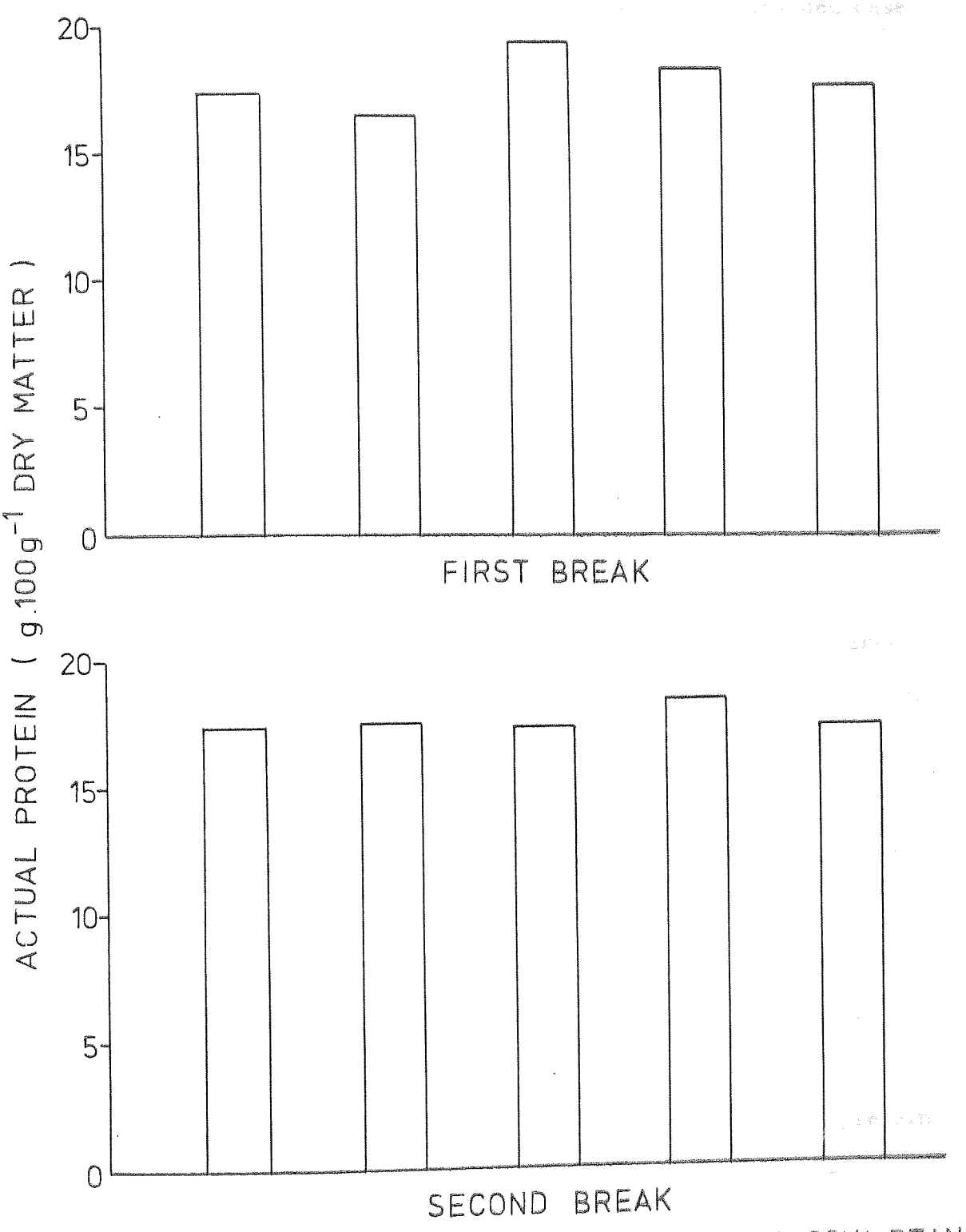
a, 0.16 ; b, 0.32 ; c, 0.64 ; d, 0.96 g NITROGEN-EQUIVALENT PER KILOGRAM FRESH COMPOST.

FIG. 3.12. NITROGEN SUPPLEMENTATION. FRUITBODY SOLUBLE PROTEIN CONTENT.



a, 0.16 ; b, 0.32 ; c, 0.64 ; d, 0.96 g NITROGEN-EQUIVALENT PER KILOGRAM FRESH COMPOST.

FIG.3.13. NITROGEN SUPPLEMENTATION. FRUITBODY ACTUAL PROTEIN CONTENT.(SUMMATION OF AMINO ACIDS).



-RESULTS DO NOT INCLUDE TRYPTOPHAN

-MEAN VALUE FOR EACH SUPPLEMENT.

appeared to have no great effect on the amino acids profiles at either the first or second breaks, except for a 20% increase in tyrosine at the second break on the addition of soya bean meal, and a 29% decrease of phenylalanine at the first break for dried blood.

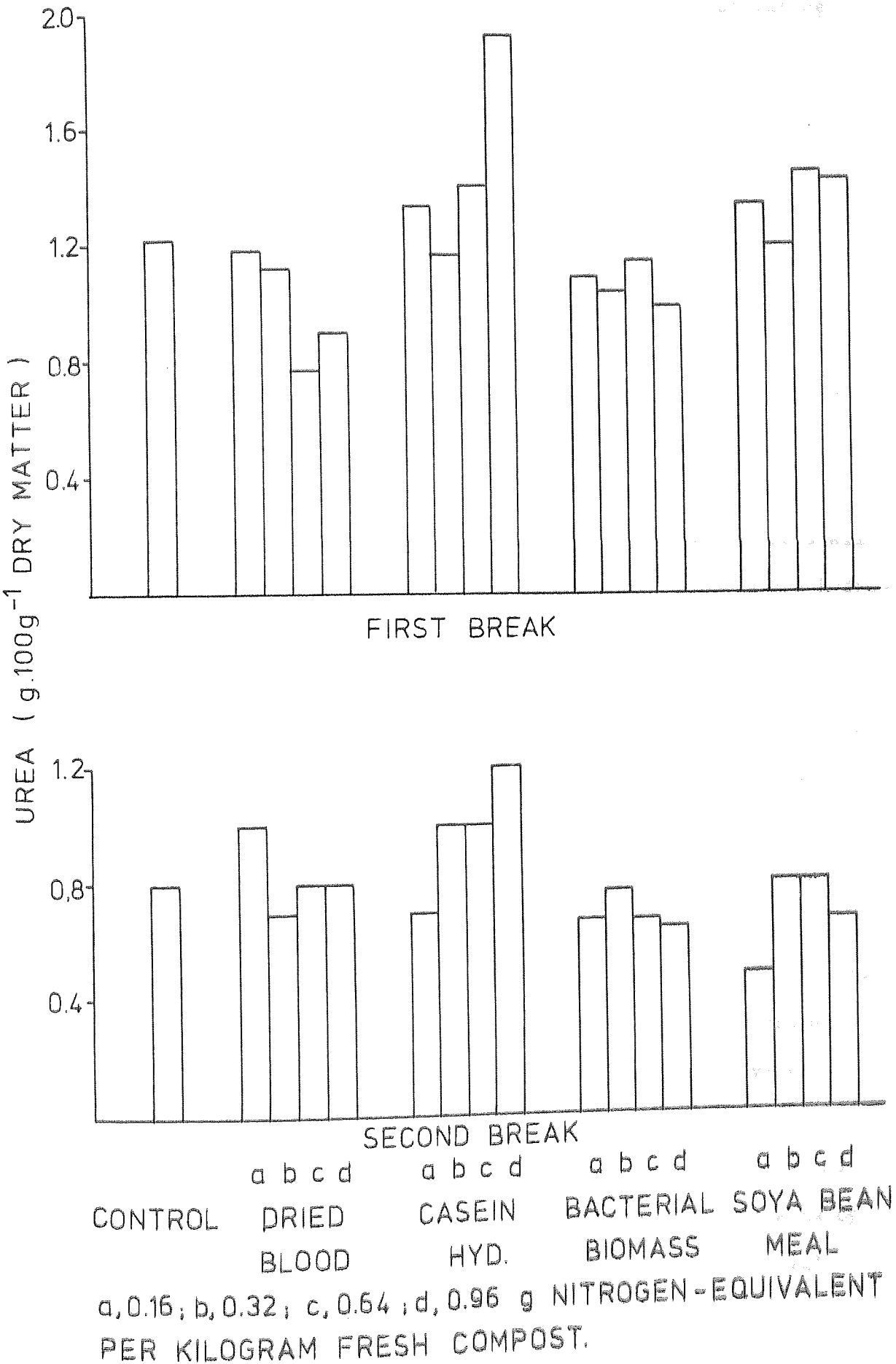
Variation in the amino acids profile between the breaks for each supplement was less pronounced. Proline and alanine increased from the first to the second break for the control (by 24% and 21% respectively). Proline was also affected by the addition of casein hydrolysate, but in this case, was decreased by 19%, decreases were also observed for leucine and valine (30% and 19% respectively). A dramatic increase (55%) of methionine was found using bacterial biomass ('Pruteen'). However, this does not represent an overall increase in methionine due to the supplementation, because methionine content at the first break was lower than the control. A similar effect was noted for phenylalanine following the addition of dried blood, there being a 22% increase from the first to second break. No major changes were found with soya bean meal supplementation.

(iv) Urea

The urea content was also subject to inconsistent variation, not only between the nitrogen supplements, but also between the various concentrations of each supplement. There was, however, a general decline in urea content from the first to the second break. (Fig.3.14; Appendix 4.9).

Urea content can not be correlated to the variation in non-protein nitrogen (crude protein minus soluble protein), consequently the concentration of the remaining non-protein nitrogen compounds (e.g. chitin) may also have been varying significantly.

FIG.3.14. NITROGEN SUPPLEMENTATION. FRUITBODY UREA CONTENT.



a, 0.16 ; b, 0.32 ; c, 0.64 ; d, 0.96 g NITROGEN-EQUIVALENT PER KILOGRAM FRESH COMPOST.

3.4.3 Conclusions

The yield and composition of fruitbodies was influenced by the addition of various nitrogenous supplements to the compost at casing. Soya bean meal gave substantially lower fresh weight yields when compared to the casein hydrolysate and dried blood supplements, however similar dry weight yields were obtained. This is attributed to the very high dry matter content of fruitbodies harvested at the first break following the addition of the higher concentrations of soya bean meal.

Sinden and Schisler (1962), concluded that protein supplements rich in phenylalanine, leucine, isoleucine and valine are most stimulatory to mushroom production. However, during the present work there was no relationship between yield increase and the amino acids profile of the supplements (Appendix 4.1). The increased yield may instead be related not only to the rich protein supplements, but also to the availability of the amino acids present in the supplements, e.g. casein hydrolysate, which gave the highest yields, contained lower concentrations of the individual amino acids when compared to dried blood protein.

There were no significant changes in protein content, although the amino acids profiles were highly variable. Since there was no common pattern between the treatments and the control, this suggests that there were significant differences in the amino acid metabolism; this was also observed in the watering regime experiment (Section 3.3.2 (iv)). There was no correlation between actual protein, crude protein and urea content of the fruitbodies, consequently other nitrogenous components (e.g. cell wall chitin, and nucleic acids) must have been varying significantly.

The changes in nitrogenous components observed in fruitbodies may not be the result of direct utilization of the nitrogen supplements by the mushroom mycelium, but may be indirectly attributed to microbial

activity (microbiological metabolism) occurring in the compost with a consequent transformation of the nitrogen supplied. This effect, which would be different for each supplement, would alter the apparent availability of the supplements, and hence fruitbody composition.

3.5 Storage conditions and the composition of Agaricus bisporus fruitbodies.

In the U.K. it is general practice to harvest fruitbodies and to convey them to the wholesale markets or retail outlets within 18 hours; it is usual for mushrooms to be purchased for consumption within 48 hours of harvest.

They are transported by road or rail in a variety of packages. In recent years prepacking and overwrapping with cellophane film has become popular, especially in direct sales to retail outlets.

It is known that mushrooms rapidly deteriorate after harvesting and soon become unfit for human consumption. After harvest, tissue respiration continues, changes in the total composition of fruitbodies can be expected. Similar factors operate in the processing of mushrooms by canning or "pickling", and are also relevant to the drying of mushroom fruitbodies commonly practised in the Orient.

3.5.1 Experimental design

An experiment was designed to determine the extent of changes in the gross composition of fruitbodies, stored at two different temperatures, over a six day time interval, which represents the time between harvesting and consumption.

Stage B fruitbodies were harvested from nine trays and selected to make 100g fresh weight before packing in standard commercial punnets and overwrapping with cellophane according to accepted commercial practice.

Punnets were stored at 3 ± 1°C and at 22 ± 1°C and analysed at days 0, 2, 4 and 6. Each punnet represented an individual sample and for each treatment three replicate punnet samples were analysed. Stage B fruitbodies from the first and second breaks were analysed separately. Thus the experiment may be summarised:-

2 temperature treatments (3°C and 22°C) x 2 breaks (first and second) x 4 sampling times (0, 2, 4 and 6 days) x 3 replicate punnet samples = 48 sample punnets.

3.5.2 Results

(i) Fresh and dry weight loss during storage.

Overwrapped Stage B fruitbodies from both first and second break harvests lost weight continuously over the 6 day storage period (Fig. 3.15; Appendix 5.1(a)). The extent of the loss was greater at 22°C, which represents ambient summer temperatures, than at 3°C, which corresponds to cold storage and refrigerator temperatures. At 3°C the percentage loss in fresh weight after six days was 7.36% and 3.96% in first and second break fruitbodies respectively, but the percentage loss for the parallel samples stored at 22°C amounted to 24.36% and 10.04%. After six days storage, first break fruitbodies were affected by decay and considered unfit for human consumption.

The data obtained for the loss of dry matter (which is a function of fresh weight lost and dry matter content of the fruitbodies) was more variable (Fig.3.16; Appendix 5.1 (b)); this reflected variations within the storage treatments together with inherent variations of individual fruitbodies. Losses in dry matter were also greater at 22°C. Losses occurring were due to fruitbody respiration and the growth of contaminants. Such losses are therefore of significance in the storage and marketing of mushrooms.

(ii) Protein content

(a) Crude protein. During storage, no significant changes occurred in the crude protein content of first or second break A.bisporus fruitbodies at 3°C (Fig.3.17; Appendix 5.2). A significant decline from day 0 to day 4 was found in the first break fruitbodies stored at 22°C,

FIG. 3.15. FRESH WEIGHT LOSS OF FRUITBODIES STORED AT TWO DIFFERENT TEMPERATURES.

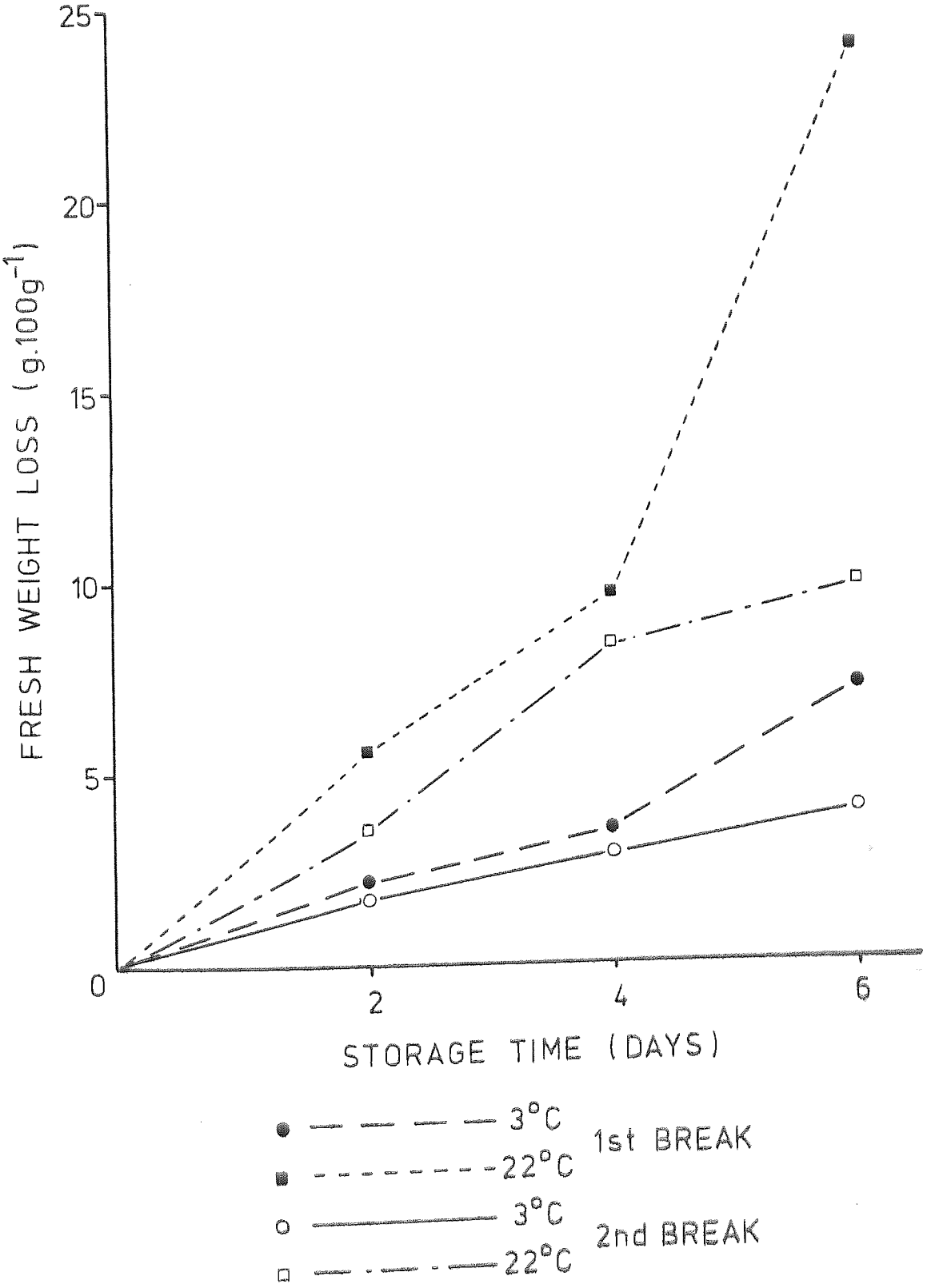


FIG. 3.16. DRY MATTER LOSS OF FRUITBODIES STORED AT TWO DIFFERENT TEMPERATURES.

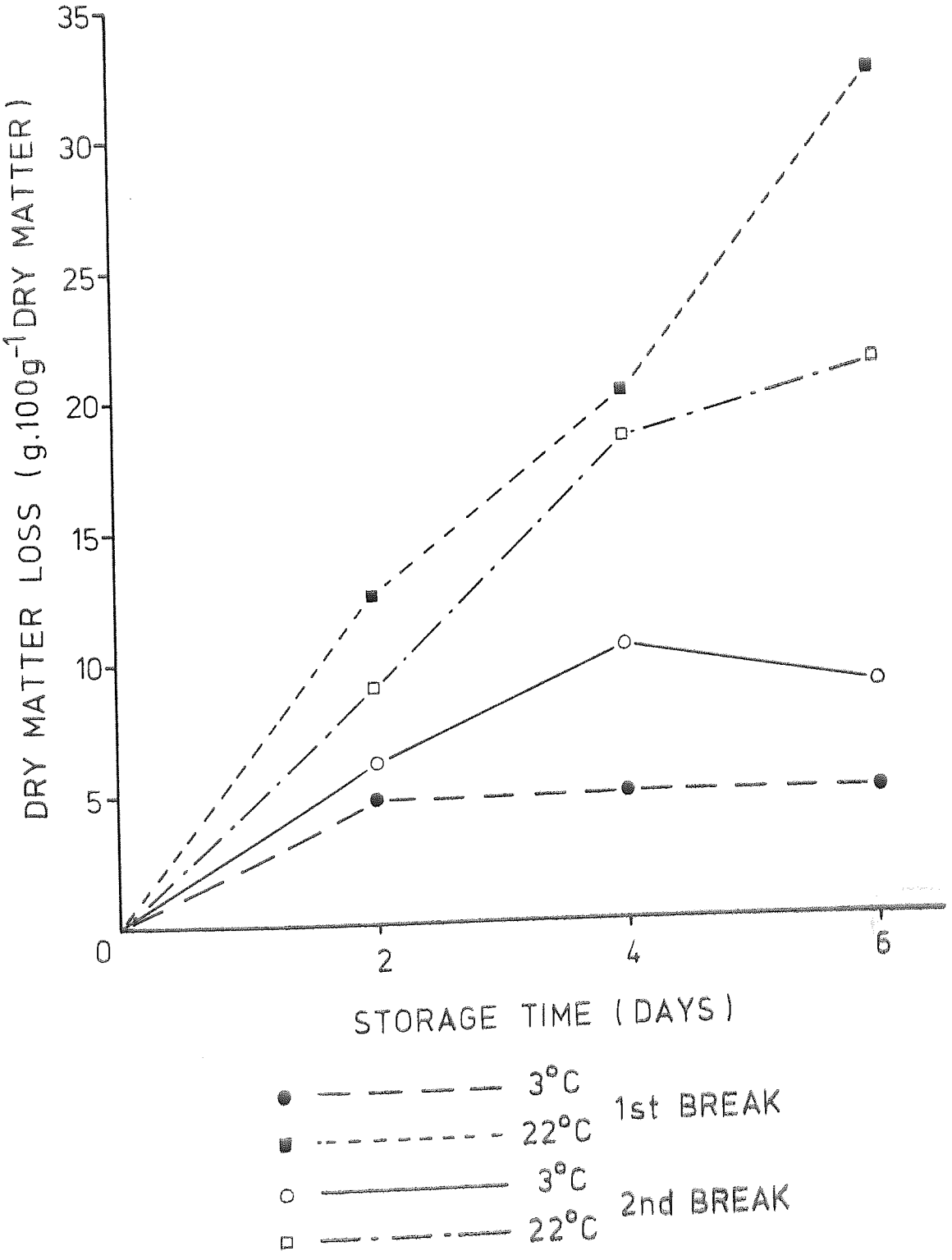
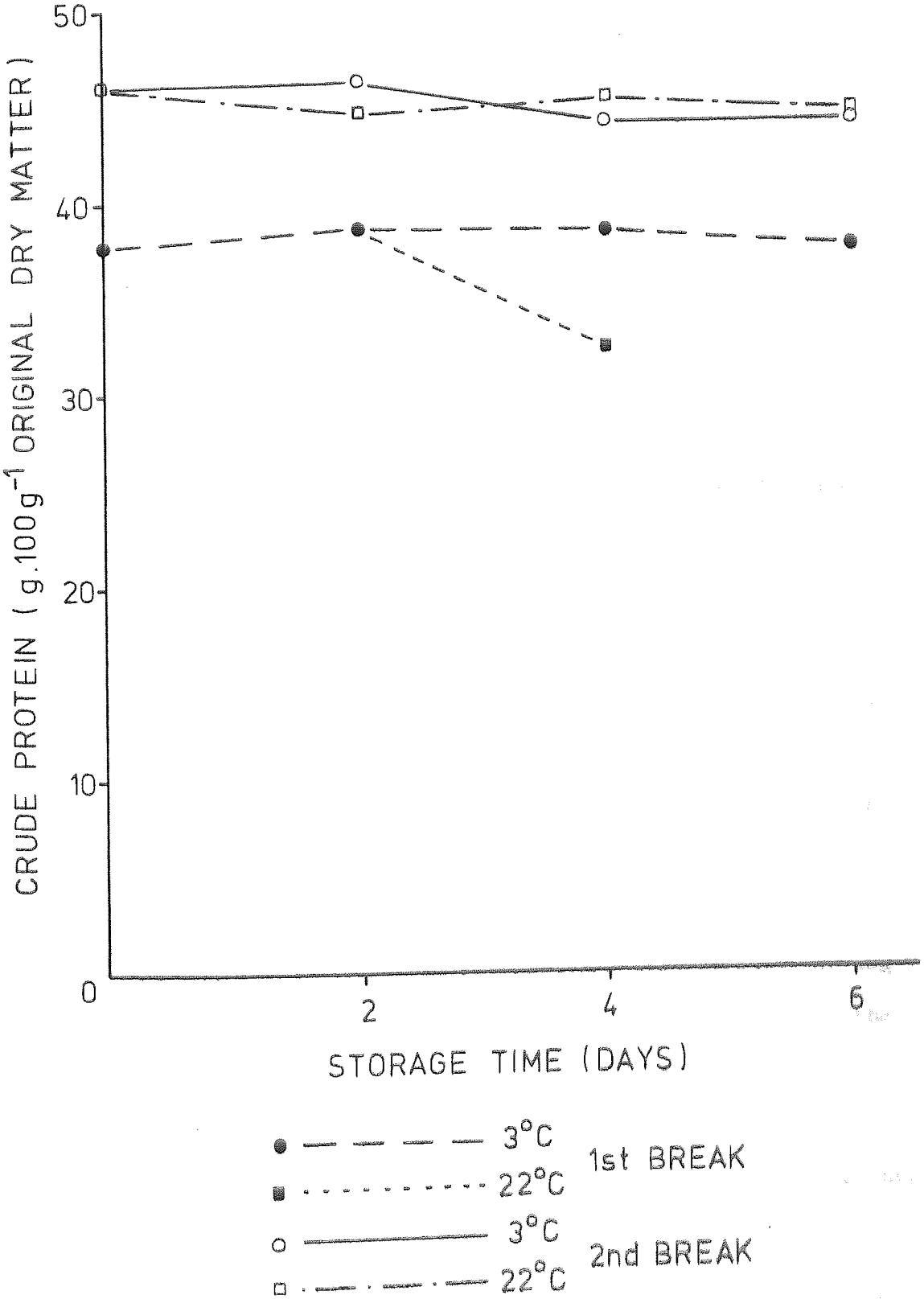


FIG. 3.17. CRUDE PROTEIN CONTENT OF FRUITBODIES STORED AT TWO DIFFERENT TEMPERATURES.



by the sixth day of storage, fruitbodies had decayed; this suggests the decline in crude protein was due to it's utilization by the contaminants. Second break fruitbodies showed no significant changes.

(b) Actual protein. While crude protein contents were generally unchanged during storage, actual protein, as determined by the summation of amino acids, showed a consistent decline (Fig. 3.18; Appendix 5.3). The decline was more marked at 22°C than at 3°C. The protein content, and the percentage of original protein loss were higher for the second break fruitbodies.

This marked decline in actual protein during storage, especially at high storage temperature, reflects a shift in the nitrogen components of fruitbodies to non-protein nitrogen due to amino acid degradation. This is of considerable importance to the methods employed in the post-harvest treatment when considering the food value of fruitbodies for human consumption.

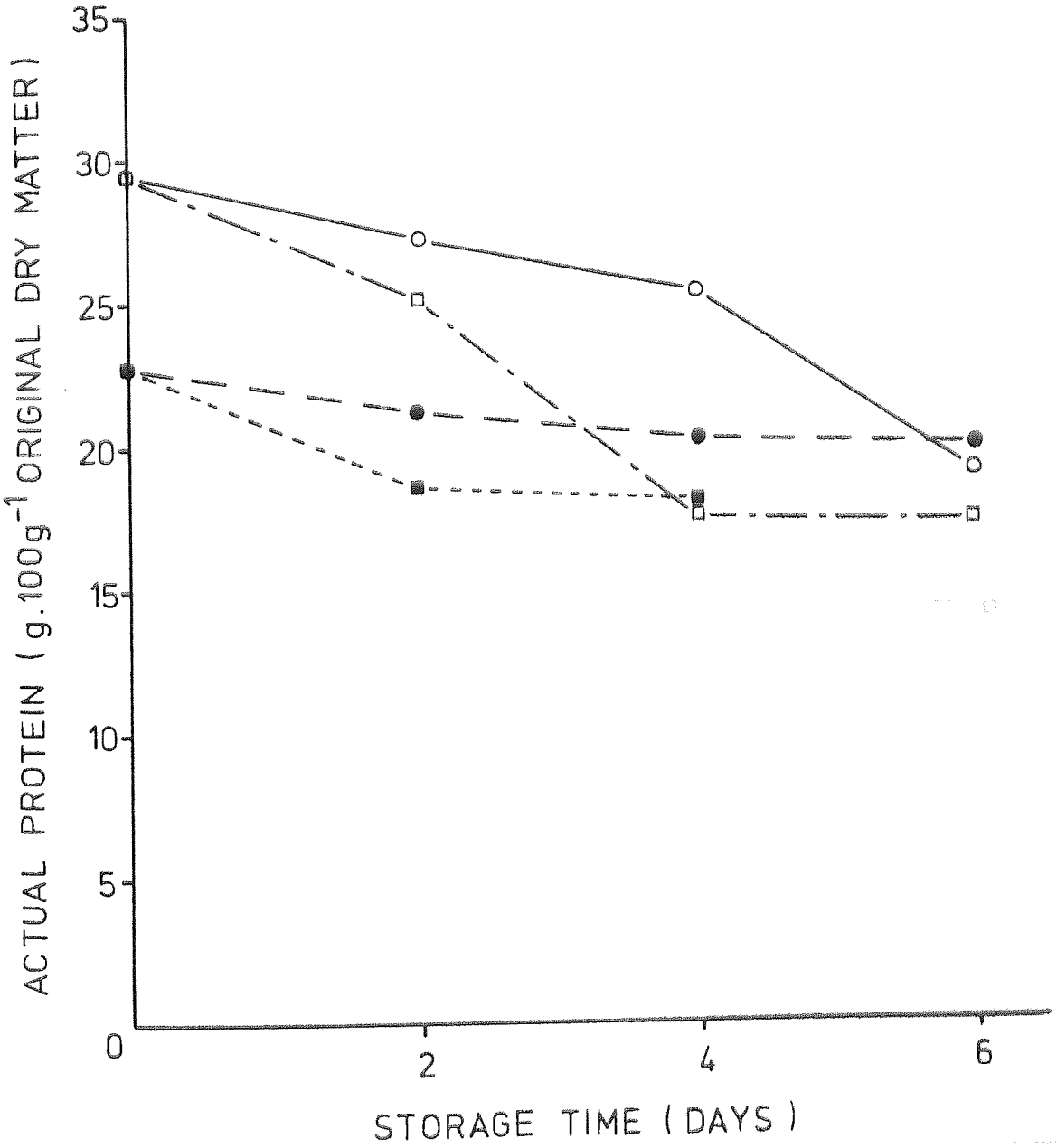
(iii) Carbohydrate content

The carbohydrate content of fruitbodies declined significantly during storage; again the decline was more marked at 22°C (Fig.3.19; Appendix 5.4). Losses of up to 40% of the original carbohydrate occurred over the 6 days of the experiment (second break, 22°C); losses which are again of significance to the food value.

(iv) Lipid content

As with carbohydrate, lipid content declined significantly during storage at 22°C, for both first and second breaks. In contrast to the carbohydrate content, no significant changes occurred in first or second break fruitbodies stored at 3°C (Fig. 3.20; Appendix 5.5). This suggests that fruitbody carbohydrates are metabolized in preference to the lipids.

FIG.3.18. ACTUAL PROTEIN CONTENT OF FRUITBODIES STORED AT TWO DIFFERENT TEMPERATURES.



- VALUES DO NOT INCLUDE TRYPTOPHAN

● — — — — 3°C 1st BREAK
■ - - - - - - - - 22°C
○ ——— 3°C 2nd BREAK
□ — · — · — · — 22°C

FIG.3.19. CARBOHYDRATE CONTENT OF FRUITBODIES STORED AT TWO DIFFERENT TEMPERATURES.

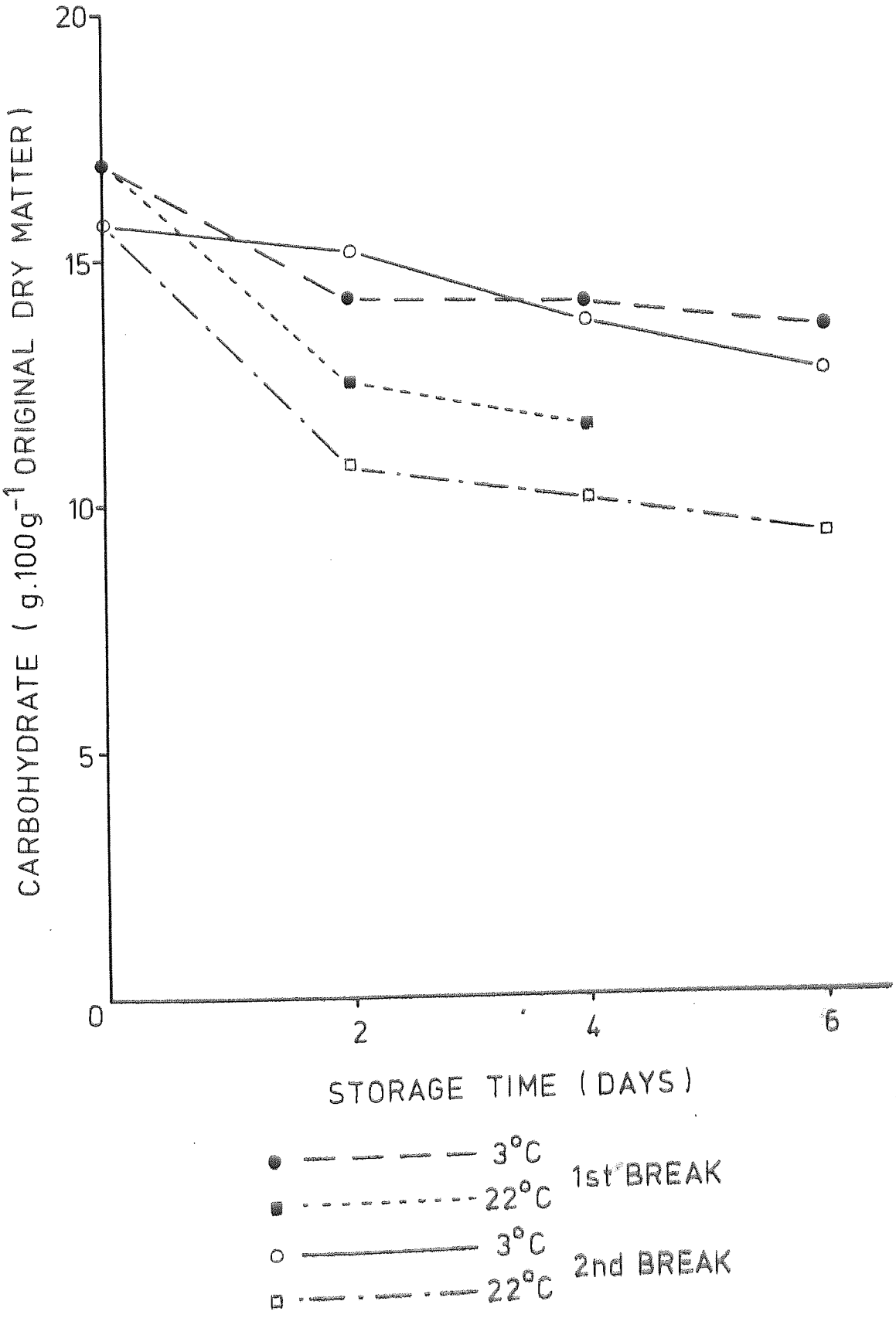
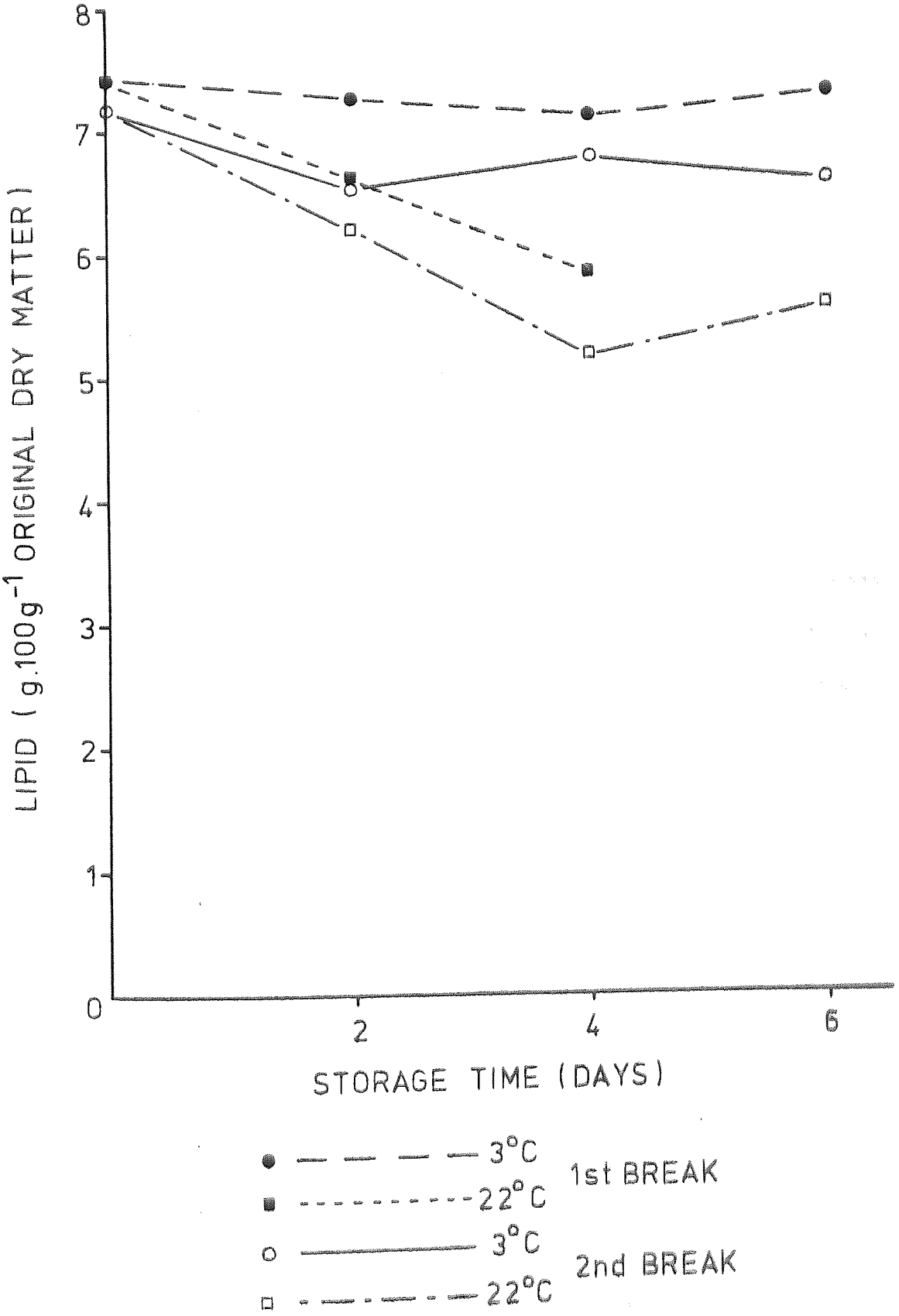


FIG. 3.20. LIPID CONTENT OF FRUITBODIES STORED AT TWO DIFFERENT TEMPERATURES.



(v) Ash content

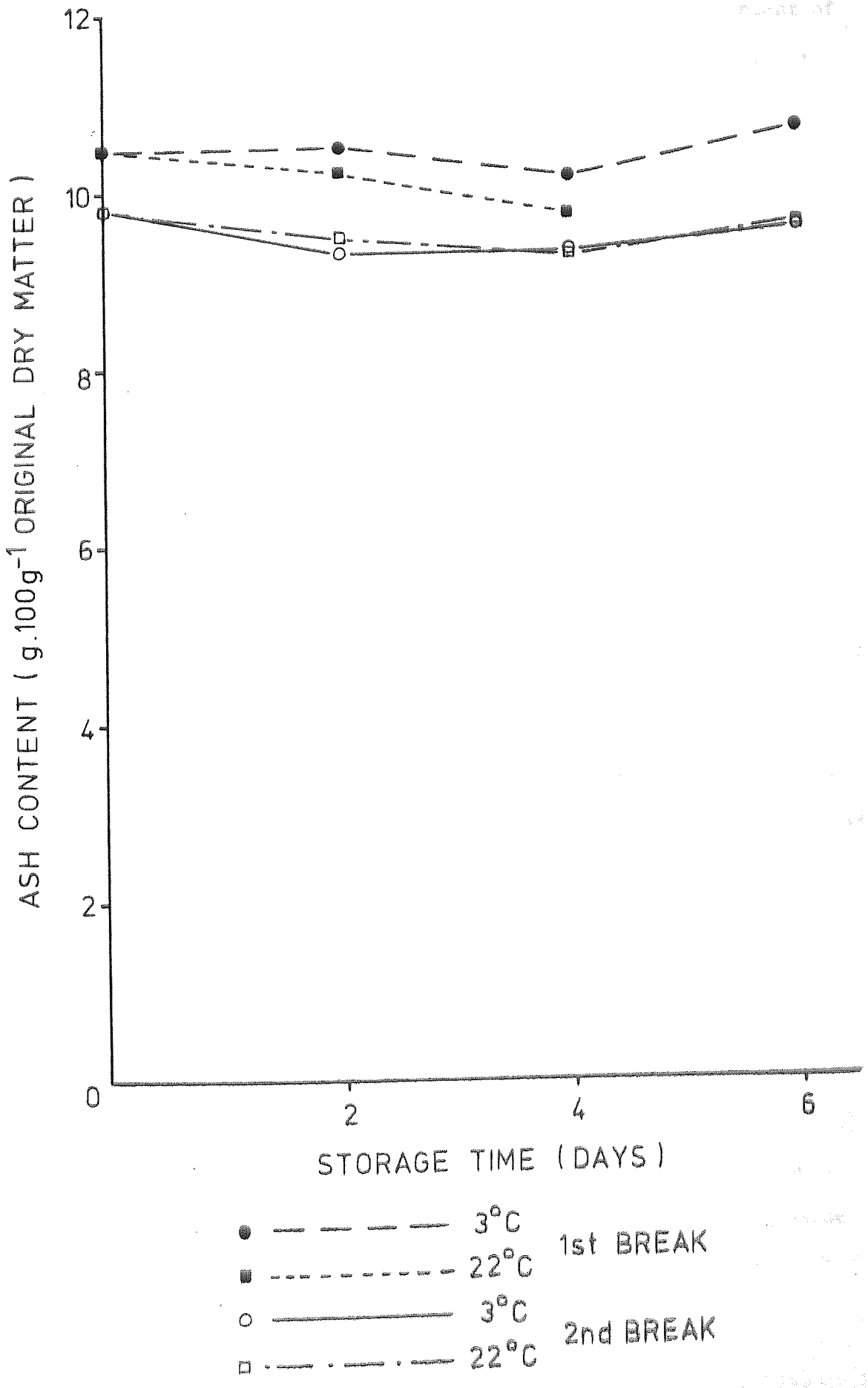
No significant changes in ash content were found at either storage temperature (Fig. 3.21; Appendix 5.6).

3.5.3 Conclusions

Storage conditions greatly affected the composition of A.bisporus fruitbodies. Losses of fresh weight, dry matter, actual protein, carbohydrate and lipid were greater at 22°C than at 3°C, which markedly affected the food value of the fruitbodies. No significant changes were found in total nitrogen unless the fruitbodies were decayed (when nitrogen was lost as ammonia). The apparent shift in the nitrogen components of the fruitbodies over a 6 day storage period, while not normally affecting the crude protein, affected greatly the quantity of actual protein present; consequently calorific values would have been greatly reduced.

The results of this investigation suggest that many of the methods generally accepted in the post-harvest handling and marketing of fresh mushroom fruitbodies are inadequate, and improvements to these methods would result in the preservation of food value.

FIG.3.21. ASH CONTENT OF FRUITBODIES STORED AT TWO DIFFERENT TEMPERATURES.



3.6 Submerged culture of mushroom mycelium

Sulphur-containing amino acids are known to be the first limiting amino acids in several foods. In view of the low methionine content of mushroom fruitbodies (see Section 3.2 to 3.5), a series of experiments were performed to investigate the effects of various nutrients on the composition of Agaricus bisporus mycelium, with particular reference to sulphur-containing amino acids.

Because of the unpredictable nature of compost as a medium, in this series of experiments, submerged culture techniques were used.

3.6.1 Effects of inorganic salts on yield and sulphur-containing amino acids biosynthesis.

It is known that most fungi can utilize inorganic sulphate as the sulphur source (Robson 1958; Cochrane, 1958; and Masselot and Robichon-Szulmajster, 1975), this is incorporated into the amino acids as follows:-

SO_4^{--} ⟶ SO_3^{--} ⟶ S^{--} or S_2O_3 ⟶ Cysteine ⟶ Methionine

Sulphate Sulphite Sulphide Thiosulphate

Chiao and Peterson (1953) have reported that the methionine content of Saccharomyces cerevisiae, grown on beet molasses medium, was increased by the addition of ammonium sulphate and ammonium dihydrogen orthophosphate. In view of this, an experiment was designed to investigate the influences of various inorganic salts on the yield, nitrogen, and the sulphur-containing amino acids content of the mycelium.

(i) Effect of salts mixtures

Mushroom mycelium was cultivated on a basic synthetic medium (Section 2.3.2) to which was added various concentrations of inorganic salts (ammonium sulphate, ammonium dihydrogen orthophosphate, magnesium sulphate and potassium dihydrogen orthophosphate; Appendix 6.1).

The flasks, in triplicate, were prepared and inoculated as in Section 2.3.2.

The cultures were harvested after 14 days of incubation. In all cases a mixture of spherical and ellipsoidal pellets of mycelium were formed, (Plate 3.2); these were similar to those obtained by Hou and Wu (1972).

Results

Dry weight yield

The dry weight yield of the mycelium was increased by 25% on addition of ammonium sulphate and ammonium dihydrogen orthophosphate, up to 0.22 $g.l^{-1}$ nitrogen added (Fig. 3.22; Appendix 6.1). Further addition of inorganic nitrogen caused a slight decrease in the yield. The addition of 1.0 $g.l^{-1}$ potassium dihydrogen orthophosphate increased the yield by a further 10%. Magnesium sulphate had a marked effect and reduced the yield of mycelium, so that, when magnesium sulphate and potassium dihydrogen orthophosphate were combined the yield was again reduced.

Nitrogen and sulphur-containing amino acids content of the mycelium

The nitrogen content of the mycelium was also increased by the addition of inorganic nitrogen, the highest nitrogen content being at the highest level of nitrogen addition (Fig 3.22; Appendix 6.1). This effect was subsequently observed during the Carbon:Nitrogen (C:N) ratio experiment (Section 3.6.3). Magnesium sulphate and potassium dihydrogen orthophosphate had no effect on the mycelial nitrogen content.

The mycelial methionine and cysteine content of the control was 0.33 and 0.55 $g.100g^{-1}$ dry matter respectively; this was increased to 0.41 and 0.65 $g.100g^{-1}$ on the addition of ammonium salts at the 0.44 $g.l^{-1}$ nitrogen level (Fig. 3.22; Appendix 6.1). This increase was associated with a high nitrogen content in the mycelium. Further addition of

PLATE 3.2. TYPICAL SUBMERGED CULTURE *AGARICUS BISPORUS* MYCELIAL PELLETS.

(mm scale)

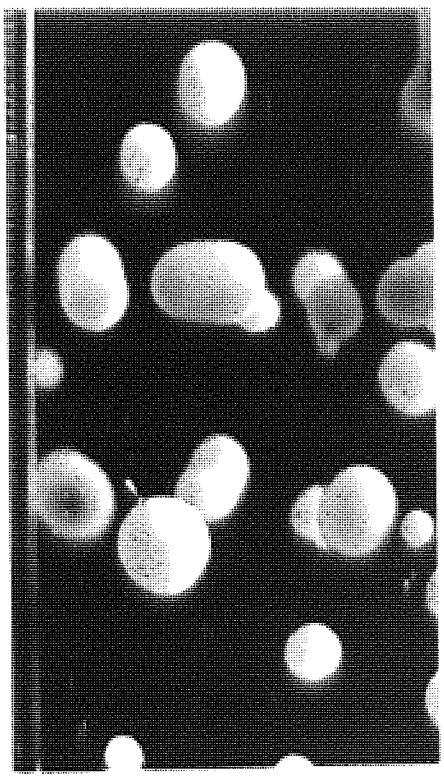
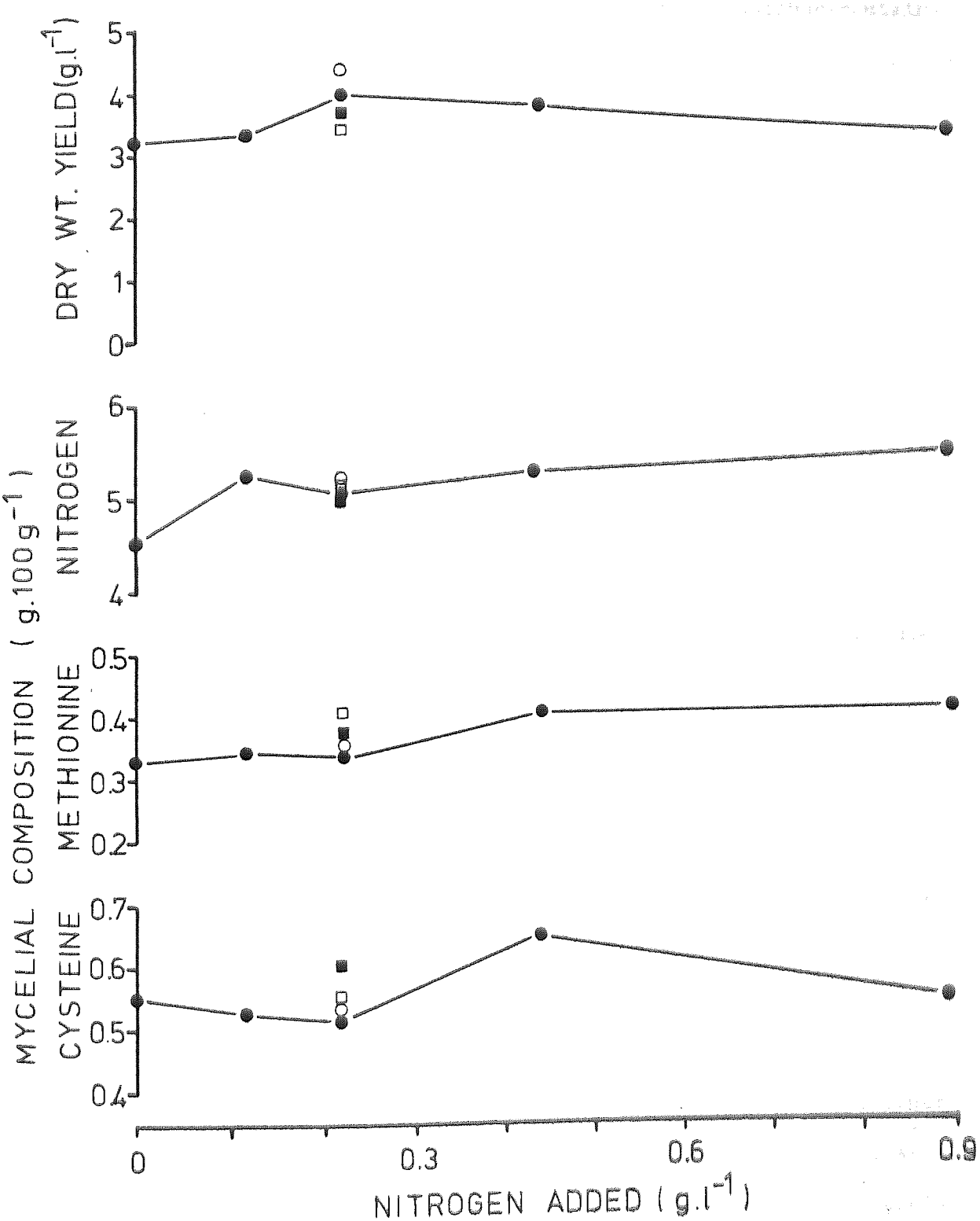


FIG. 3.22. EFFECTS OF INORGANIC SALTS MIXTURES ON THE SUBMERGED CULTURE OF A. BISPORUS.



ADDITION OF: ○ ; 1.0 g.l^{-1} KH_2PO_4

□ ; 2.5 g.l^{-1} $MgSO_4$

■ ; 1.0 g.l^{-1} KH_2PO_4 + 2.5 g.l^{-1} $MgSO_4$

-SEE APPENDIX 6.1.

ammonium salts maintained the high methionine content, although the cysteine content was reduced to that of the control. Potassium dihydrogen orthophosphate had no marked effect on the sulphur-containing amino acids content. While magnesium sulphate increased methionine by approximately 20%, it did not affect the cysteine content. When potassium dihydrogen orthophosphate and magnesium sulphate were combined an increase in methionine was again noted, but was less than when magnesium sulphate was used alone.

(ii) Effects of ammonium and magnesium sulphate

The above experiment showed that inorganic salts may significantly alter the yield and sulphur-containing amino acids content of submerged cultured A.bisporus mycelium. In view of this, a subsequent experiment was performed to further elucidate the effects of the sulphur source on the biosynthesis of the sulphur-containing amino acids.

Increasing concentrations of ammonium sulphate or magnesium sulphate were added to two series of cultures prepared in triplicate, as in Section 2.3.2 (Appendix 6.2 and 6.3 respectively).

Results

Dry weight yield

As the concentration of ammonium sulphate was increased, the dry weight yield of mycelium increased gradually, however, at the higher concentrations there was a dramatic decrease in yield (Fig. 3.23; Appendix 6.2). This was probably due to the effects of the different C:N ratios resulting from the addition of the ammonium sulphate, since this effect was also observed in the C:N ratio experiment at similar C:N ratios (Section 3.6.3; Fig.3.27). The dry weight yield of mycelium decreased as the concentration of magnesium sulphate was increased, except at the lowest rate of addition, when an increase from the control was noted (Fig. 3.24; Appendix 6.3).

FIG. 3.23. EFFECTS OF AMMONIUM SULPHATE ON THE SUBMERGED CULTURE OF A. BISPORUS.

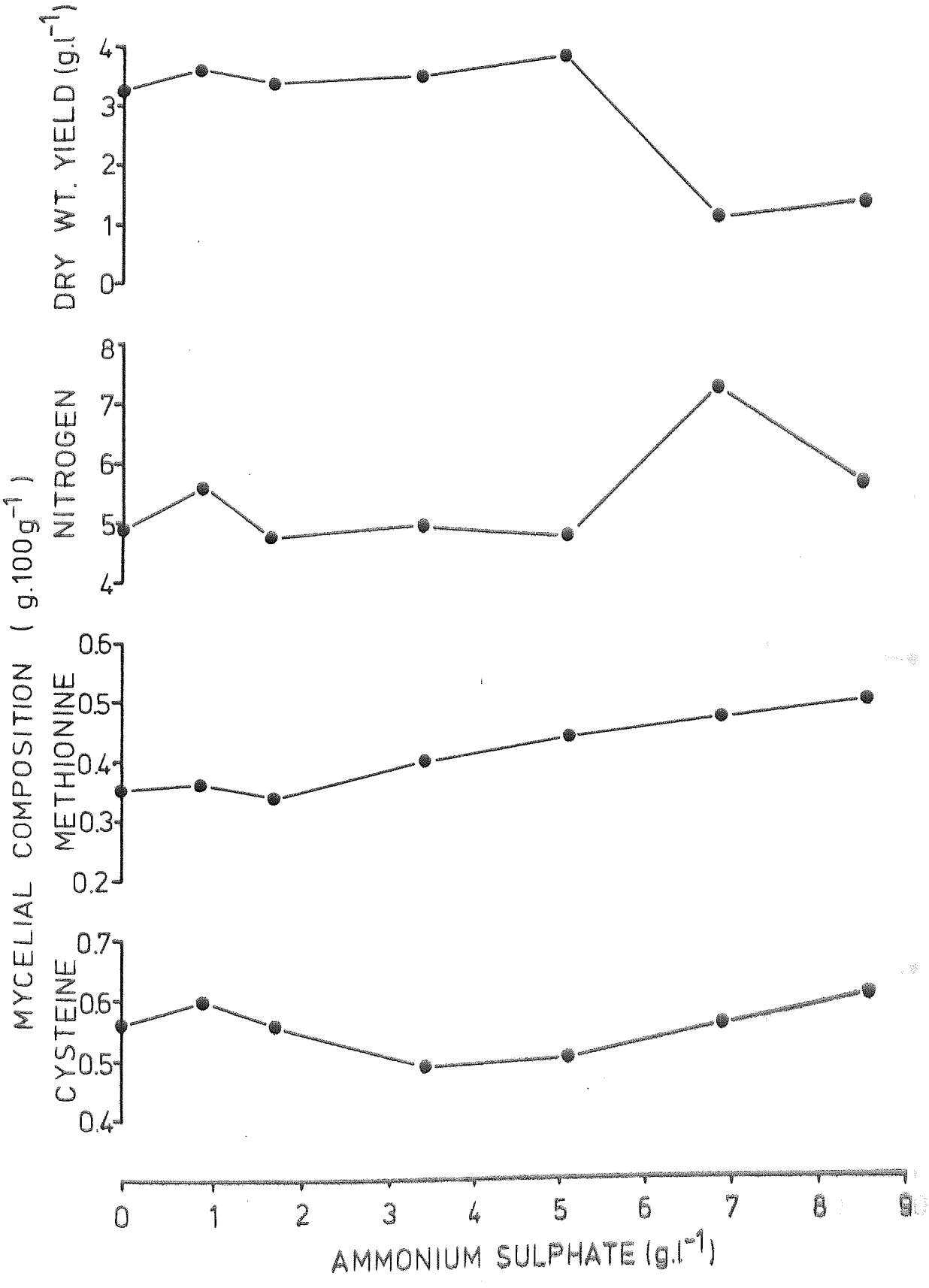
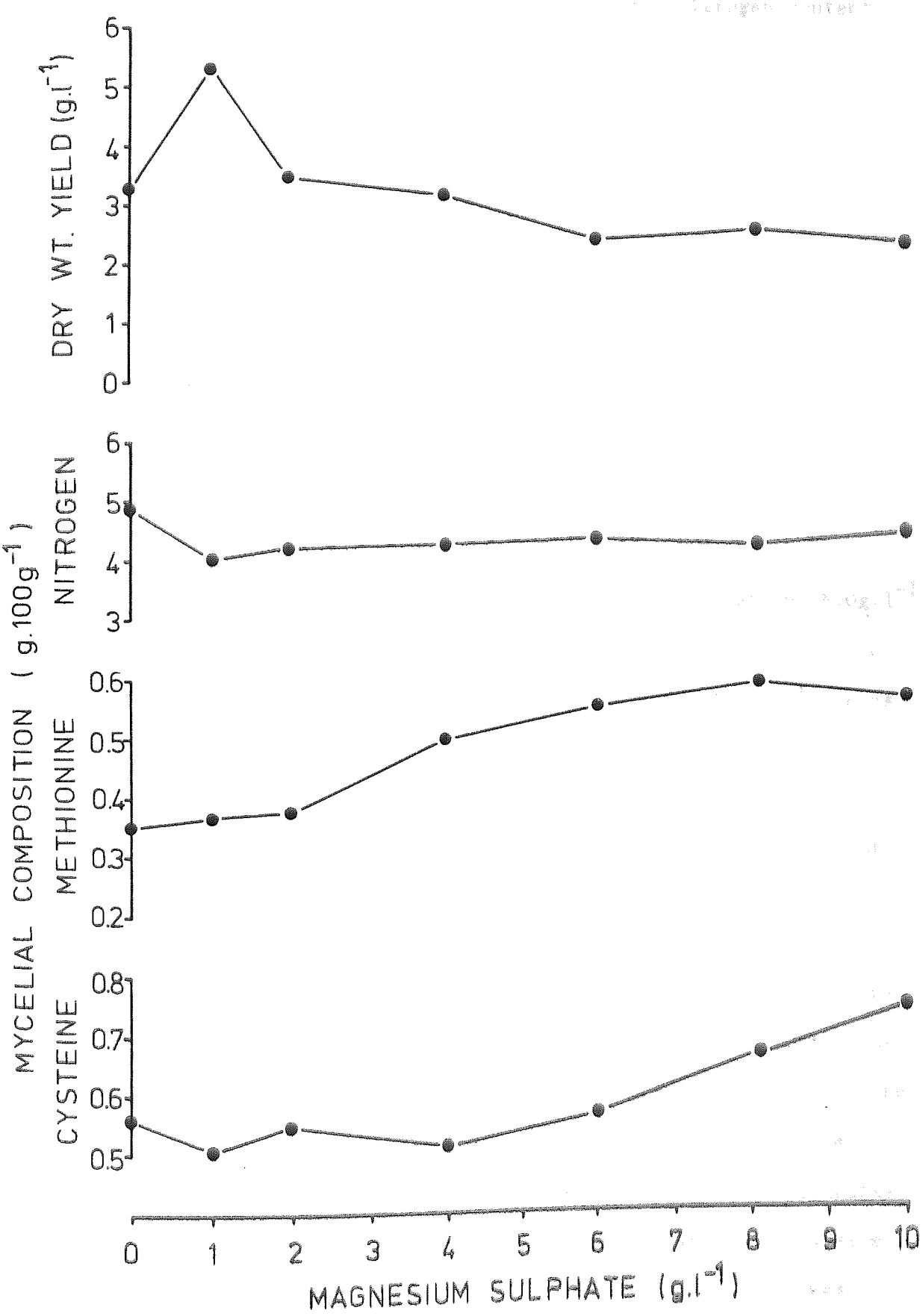


FIG.3.24. EFFECTS OF MAGNESIUM SULPHATE ON THE SUBMERGED CULTURE OF A.BISPORUS.



Nitrogen and sulphur-containing amino acids content of the mycelium

Unlike the previous experiment, where mycelial nitrogen content increased with increased nitrogen salts addition, on the addition of ammonium sulphate the results were more variable and showed no consistent trend (Fig.3.23; Appendix 6.2). This suggests that ammonium sulphate may not be the most beneficial nitrogen source. Mycelial cysteine was also more variable and again showed no consistent pattern. The methionine content was, however, influenced, and increased continually, even when ammonium sulphate was added at higher levels than in the previous experiment (Section 3.6.1.(i)).

The addition of magnesium sulphate again had no significant effect on the mycelial nitrogen content, although large increases were observed for the sulphur-containing amino acids (Fig. 3.24; Appendix 6.3). The methionine increased, by approximately 71%, to a maximum at the $8.0 g.l^{-1}$ magnesium sulphate level, and cysteine, after remaining relatively constant at the lower levels, increased continually above $4.0 g.l^{-1}$ magnesium sulphate.

Conclusions

The submerged culture experiments have shown that the growth and composition of A.bisporus mycelium may be altered significantly according to the type and concentrations of the inorganic nutrients.

The dry weight yield of mycelium was greatly affected by the concentrations of the inorganic nitrogen sources, probably due to the influences of the C:N ratio. The yield could be further increased by the addition of potassium dihydrogen orthophosphate. While magnesium sulphate decreased mycelium yield, the methionine content was significantly increased. When a mixture of magnesium sulphate and potassium dihydrogen orthophosphate was used, the increase in methionine was

minimised even though potassium dihydrogen orthophosphate had no effect; this suggests that the potassium dihydrogen orthophosphate was suppressing the advantageous effect of magnesium sulphate. The converse effect was observed on the dry weight yield, thus the anticipated increase in yield resulting from potassium dihydrogen orthophosphate addition (see above) was minimised by magnesium sulphate.

The cysteine content of the mycelium was less affected by inorganic salts addition, except at the higher concentrations of magnesium sulphate when it was markedly increased.

Mixed nitrogen sources promoted a higher mycelial nitrogen content. In contrast, ammonium sulphate alone appeared to be a less suitable nitrogen source, mycelial nitrogen not being consistently affected by its addition. At the higher concentrations of ammonium sulphate the methionine content continually increased, this efficient utilization of inorganic sulphate also being shown by the increase in culture pH after incubation (Appendix 6.2). This was caused by the rapid uptake of sulphate by the mycelium when compared to ammonium ion uptake (i.e. nett excess of ammonium ions increased culture pH). Mycelial methionine content could possibly be further increased by the addition of even higher concentrations of ammonium sulphate; this would not be advantageous, however, because the yield of mycelium would be too low.

3.6.2 Effects of free L-methionine, intermediates and its derivative N-acetyl-L-methionine on sulphur-containing amino acids biosynthesis

This experiment was designed to investigate the utilization of various methionine precursors by mushroom mycelium, and to observe their effects on mycelium composition, this having been already studied in animals, plants and micro-organisms (e.g. Aerobacter aerogenes, Schwartz and Shapiro, 1954; animals, plants and micro-organisms, Lehninger, 1971;

Saccharomyces cerevisiae, Masselot and Robichon-Szulmajster, 1975). Additionally L-methionine and its derivative N-acetyl-L-methionine (NAM), were used. Because these have been used to improve the biological quality of certain vegetable proteins deficient in sulphur-containing amino acids (e.g. soya bean protein, Boggs and Rotruck, 1975), they may also be useful as additives in the liquid culture medium.

(i) Experimental design

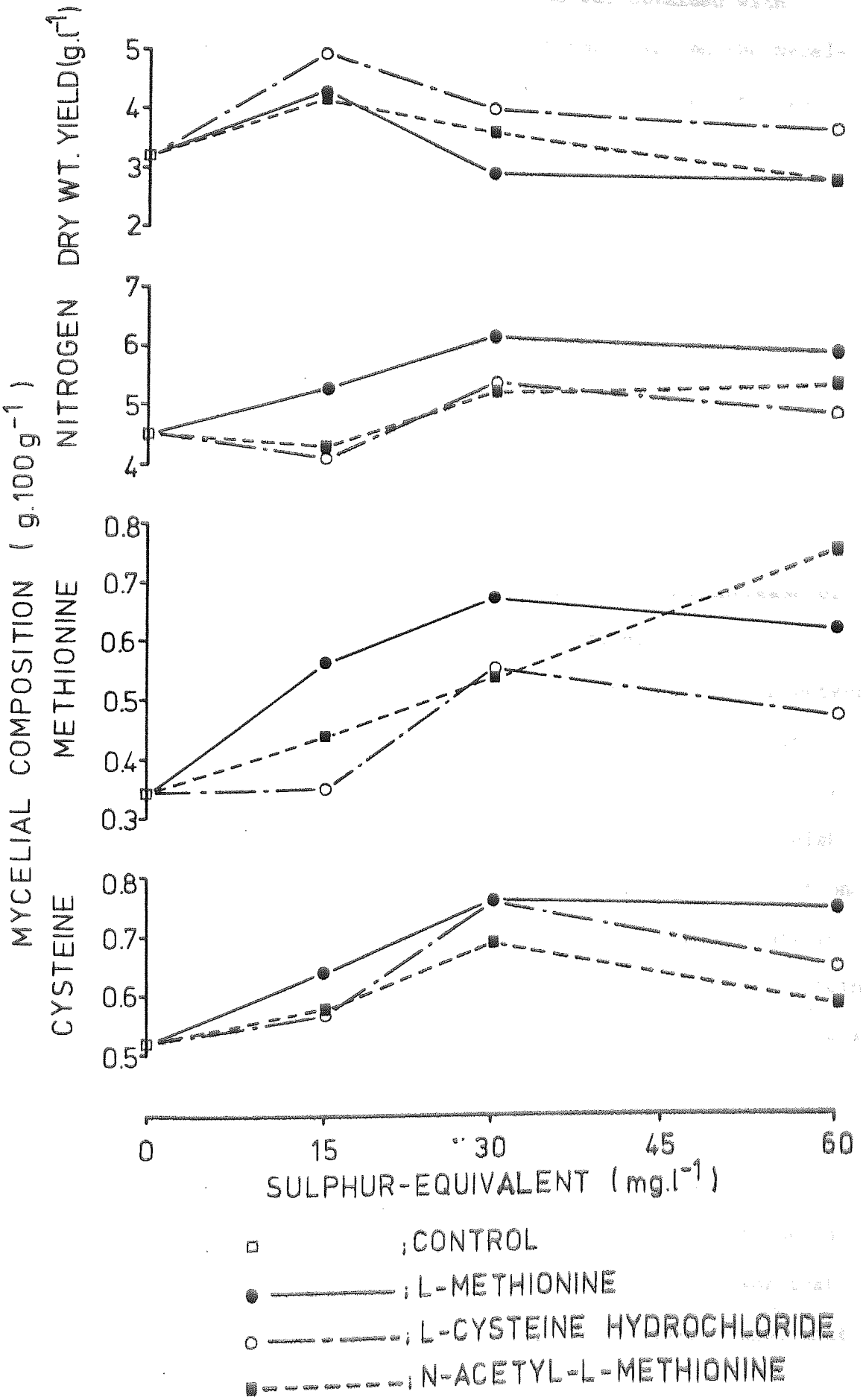
L-methionine, L-cysteine hydrochloride and N-acetyl-L-methionine were considered as sulphur sources. Three concentrations of each (15, 30 and 60 mg sulphur-equivalent.l^{-1}) were added to the basal synthetic medium (see Section 2.3.2). L-α-amino-n-butyric acid and L-homocystine were added to another series of flasks at concentrations of 40, 80 and 160 μmol.l^{-1}.

Three replicates of each concentration were prepared, each flask containing 90 ml of autoclaved basal synthetic medium (but containing nutrients required for 100 ml). Solutions of the supplements were filter sterilized, and 10, 5 or 2.5 ml of each solution dispensed singly into the flasks to give the required concentrations. After the addition of filter sterilized distilled water, to give final volumes of 100 ml, the flasks were inoculated and incubated as described in Section 2.3.2.

(ii) Results

The addition of free L-methionine, L-cysteine hydrochloride and NAM caused significant changes in the growth and composition of A.bisporus mycelium. The most dramatic effect was obtained with L-methionine supplementation, when mycelial nitrogen, methionine and cysteine contents all increased simultaneously to maxima as the free methionine concentration was increased (Fig. 3.25; Appendix 6.4). The methionine content was increased by approximately 100%, the cysteine content by 46%, and the nitrogen content by 41%. Mycelial yields were largely unchanged, except

FIG.3.25. EFFECTS OF L-METHIONINE, L-CYSTEINE-HCl AND N-ACETYL-L-METHIONINE ON THE SUBMERGED CULTURE OF A.BISPORUS.



at the lowest rate of methionine addition, when a 34% increase was observed. A similar pattern for mycelial yield was obtained with NAM. The most significant effect of this supplement was on the mycelial methionine content, which increased continually, the highest increase being 126% over the control. Mycelial nitrogen also increased (by approximately 25%), while no change was observed in cysteine content.

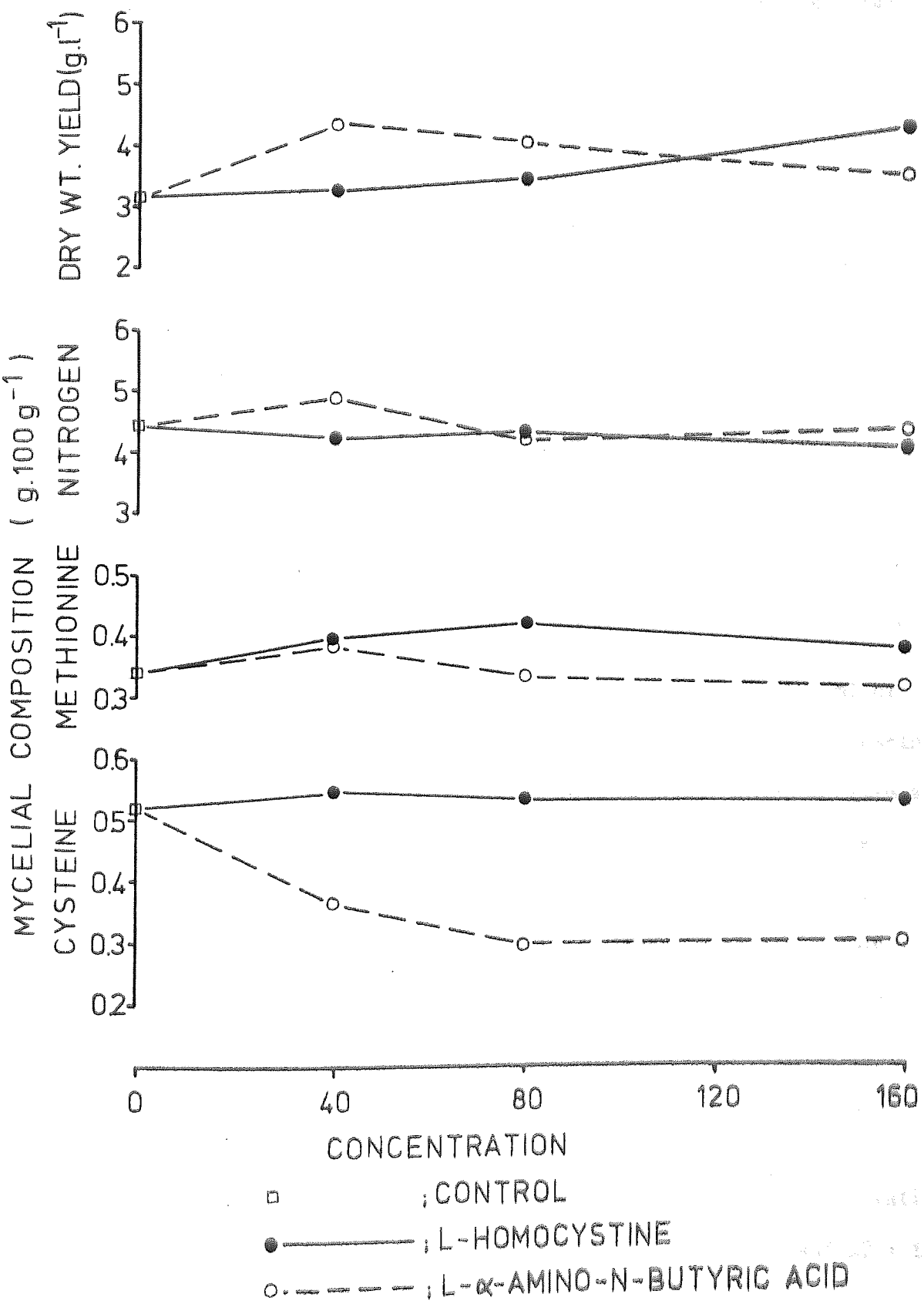
Cysteine hydrochloride increased yield at the higher concentrations (by approximately 25%), although the highest yield was again at the lowest rate of supplement addition. Cysteine content was significantly increased at the higher level of supplementation, the greatest increase (46%) over the control being similar to that observed upon methionine supplementation. Methionine and nitrogen contents were also significantly increased (by 64% and 21% respectively), the increase of the latter being similar to that seen on NAM addition.

The effects of the addition of L-homocystine and L-α-amino-n-butyric acid were less dramatic, thus, except for a significant decrease in cysteine content when L-α-amino-n-butyric acid was added, mycelial composition was largely unaffected (Fig. 3.26; Appendix 6.4). Dry weight yields were, however, altered, there was a 44% increase on the addition of the highest concentration of homocystine, while L-α-amino-n-butyric acid gave a similar pattern to that observed for L-methionine, L-cysteine hydrochloride and NAM, i.e. largest increase (of 38%) being at the lowest rate of application.

(iii) Conclusions

The most significant results were obtained on the addition of L-methionine and it's derivative N-acetyl-L-methionine; in both cases the methionine content of the mycelium was greatly increased over that of the control (by 100 and 126% respectively). These results show that

FIG.3.26. EFFECTS OF L-HOMOCYSTINE AND L-α-AMINO-N-BUTYRIC ACID ON THE SUBMERGED CULTURE OF A.BISPORUS.



there is some similarity between L-methionine and N-acetyl-L-methionine. This is probably due to cleavage of NAM to acetyl and L-methionine, the L-methionine released then being metabolically equivalent to the free L-methionine (which has already been shown in animal feeding trials by Rotruck and Boggs (1975).

L-methionine also caused the most significant increase in mycelial nitrogen content, therefore presumably increasing the overall protein content; this reinforces the views of Frazer and Fujikawa (1958) that methionine is one of the most active amino acids for the culture of A.bisporus mycelium.

L-homocystine and L-α-amino-n-butyric acid were found to be inadequate supplements, for, although increased mycelium yields were obtained, with respect to mycelial composition, the former had no significant effect, and the latter actually decreased the cysteine content.

3.6.3 Carbon:Nitrogen (C:N) ratio

(i) Experimental design

Twelve series of three flasks were used, each containing 100 ml of basic synthetic medium (Section 2.3.2). Various concentrations of casein hydrolysate (considered solely as a nitrogen source) were added to give a range of C:N ratios (C:N = 2 ——— 45 : 1; Appendix 6.5). The flasks were prepared, inoculated and incubated as described in Section 2.3.2, harvested (Section 2.4.2), and dry weight yield, total nitrogen (crude protein), soluble protein, total carbohydrate (as glucose), and sulphur-containing amino acids determined.

(ii) Results

Yield

The highest dry weight yield of mycelium was obtained at a C:N ratio of 18:1. The yield decreased gradually over the C:N ratios 21 and 27 : 1,

while at higher C:N ratios, no further significant decrease occurred. C:N ratios less than 18:1 gave a dramatic decrease in yield (Fig.3.27; Appendix 6.6).

Protein and sulphur-containing amino acids content of the mycelium.

As the C:N ratio was increased, the crude protein content of the dried mycelium decreased progressively, the decrease being especially pronounced at the lower C:N ratios (Fig.3.27; Appendix 6.6). There was a similar decrease of soluble protein, although at C:N ratios greater than 15:1 soluble protein was more variable. A minimum was observed in soluble protein between C:N ratios of 12 and 33:1, indicating possible significant changes in protein metabolism. The ratio of soluble protein : crude protein was also highly variable (Fig.3.28; Appendix 6.6), increasing gradually with C:N ratio up to C:N = 12:1 but again exhibiting a minimum between C:N ratios of 12 and 33:1. The methionine and cysteine contents also showed minima at the C:N ratios where the highest dry weight yields of mycelium were obtained (Fig. 3.29; Appendix 6.6).

Carbohydrate content

The maximum carbohydrate content (expressed as glucose) of the mycelium corresponded to the highest dry weight yield. (C:N = 18.1; Fig. 3.30; Appendix 6.6). At lower C:N ratios the carbohydrate content decreased progressively, while at the higher C:N ratios, after an initial decrease between C:N ratios of 18 and 21:1, carbohydrate remained relatively constant with increasing C:N ratio.

(iii) Conclusions

The C:N ratio of the synthetic medium significantly affected the growth of A.bisporus in submerged culture.

The highest mycelial dry weight yield was obtained at C:N = 18:1,

FIG.3.27. EFFECTS OF THE CARBON:NITROGEN RATIO ON THE SUBMERGED CULTURE OF A.BISPORUS.

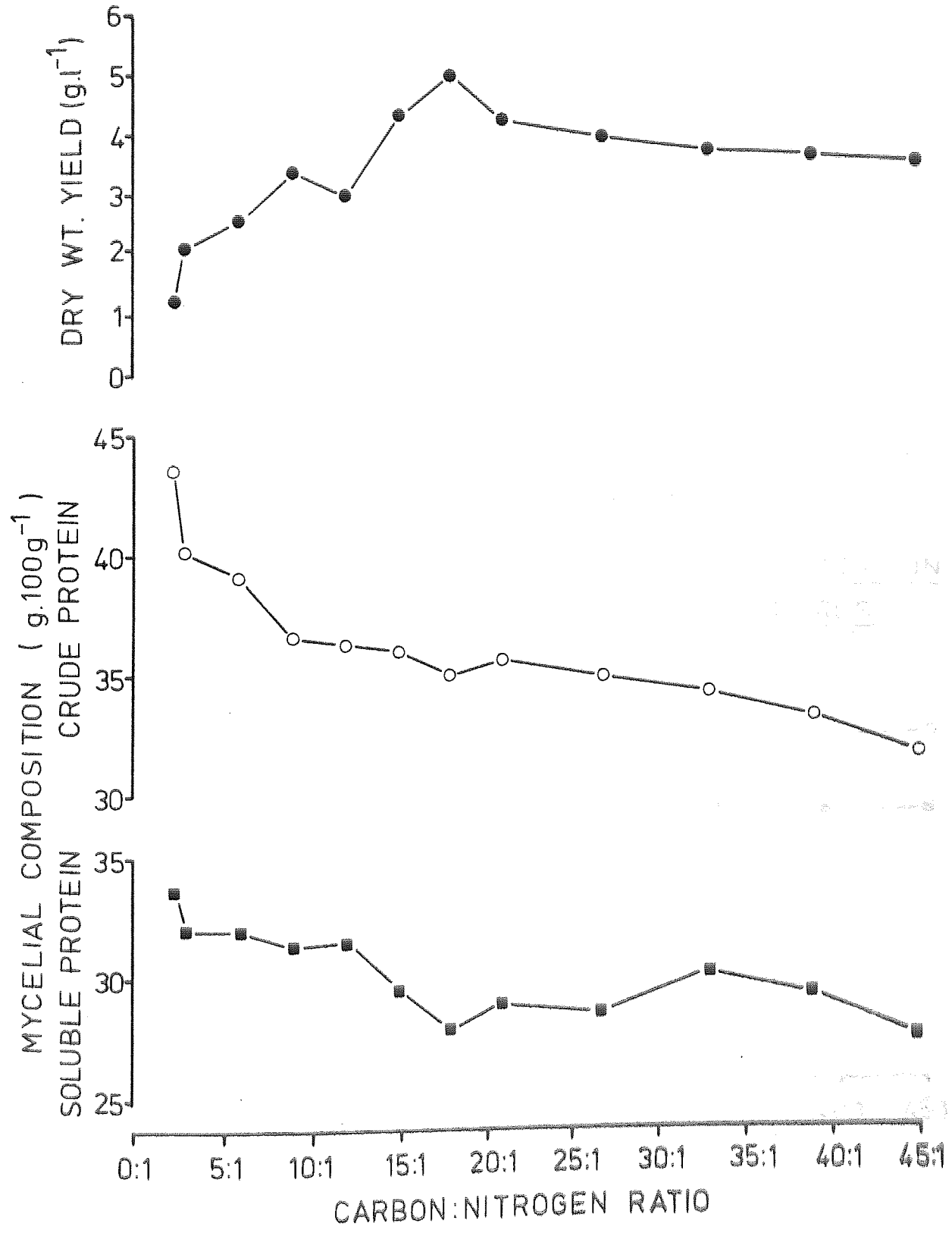


FIG.3.28. EFFECTS OF THE CARBON : NITROGEN RATIO ON THE SUBMERGED CULTURE OF A. BISPORUS.

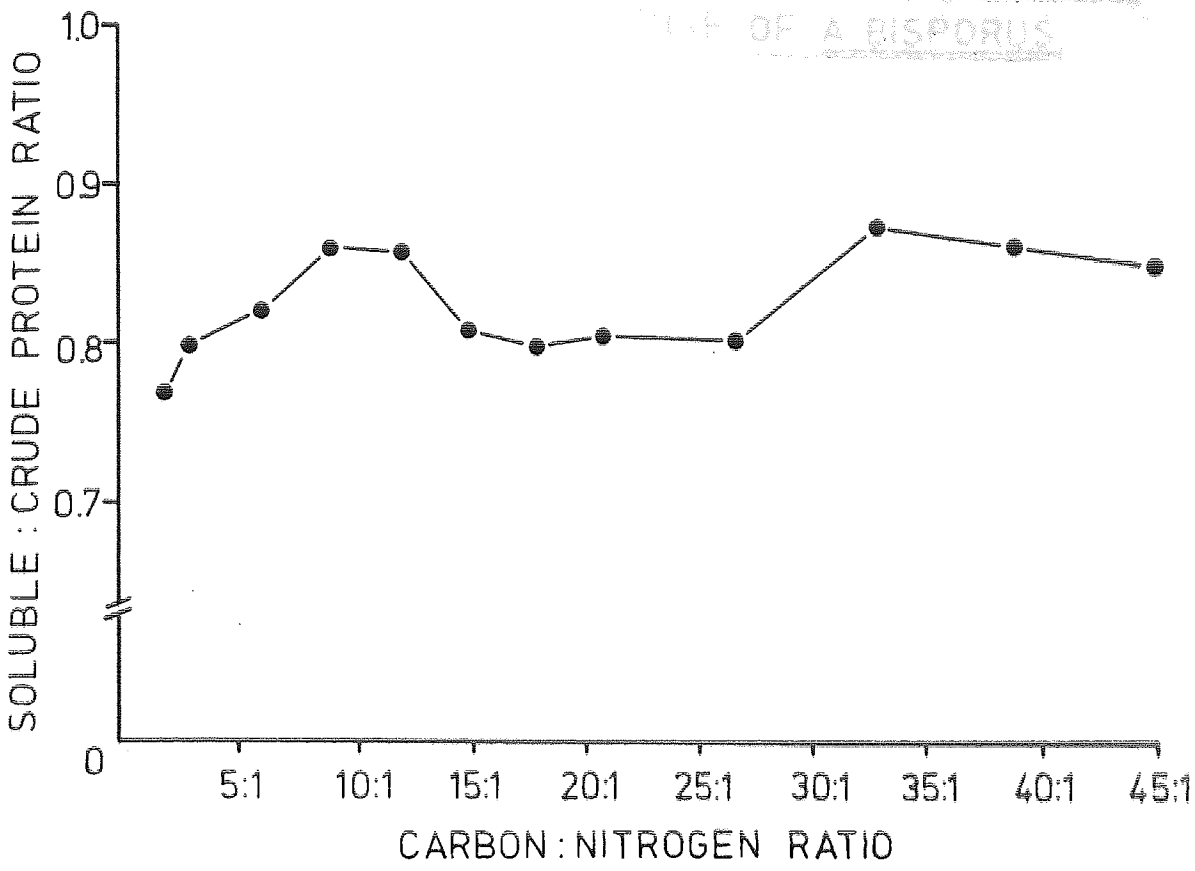


FIG.3.29. EFFECTS OF THE CARBON : NITROGEN RATIO ON THE SUBMERGED CULTURE OF A. BISPORUS.

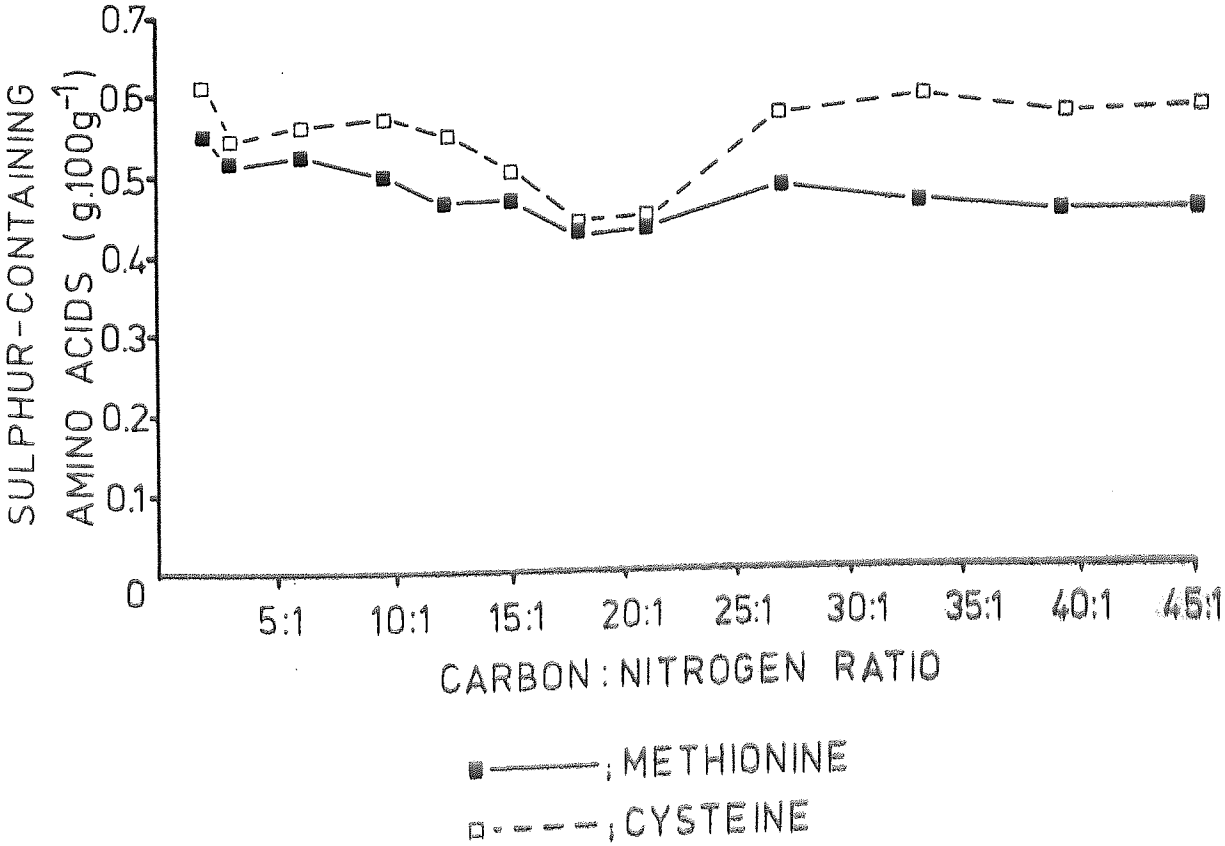
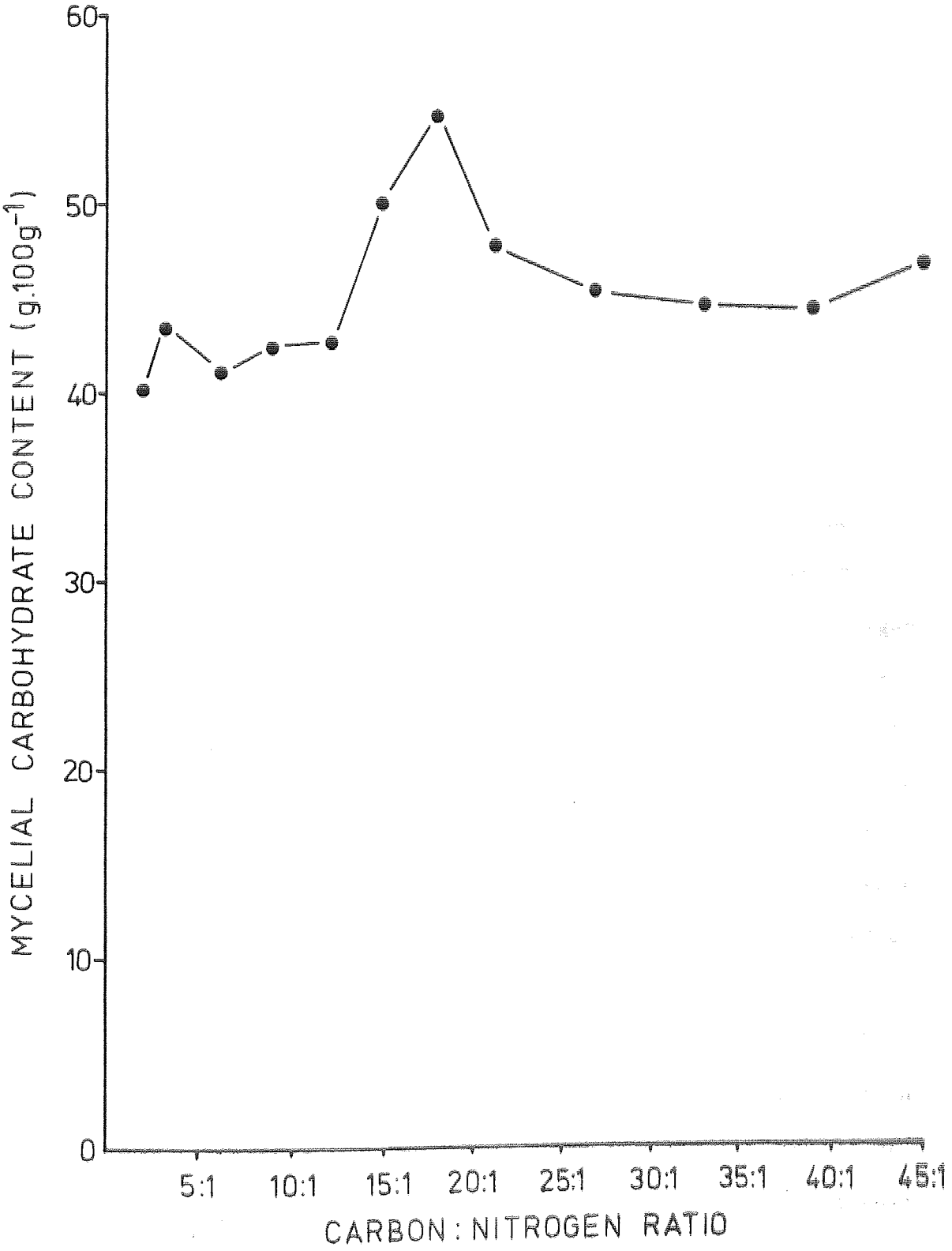


FIG.3.30. EFFECTS OF THE CARBON : NITROGEN RATIO ON THE SUBMERGED CULTURE OF A. BISPORUS.



ratios outside the optimum range caused a reduction in yield, which was especially pronounced at the lower C:N ratios.

The crude protein content of the dried mycelium was governed by the nitrogen concentration in the synthetic medium, i.e. the highest crude protein contents were observed at the lowest C:N ratios, which also corresponded to the lowest dry weight yields of mycelium. The overall reduction in protein content with increasing C:N ratio may have been due to the presence of higher lipid contents, Worgan (1968) having reported that the lipid (fat) content of Tricholoma nudum was proportional to the C:N ratio. The soluble protein (and consequently the soluble protein : crude protein ratio), methionine and cysteine contents were more variable, all exhibiting minima at the C:N ratios corresponding to the highest mycelial dry weight yields. In batch culture, the biomass yield after a fixed time is a function of the overall growth rate (i.e. assuming constant inocula, the dry weight yield of mycelium is proportional to the growth rate of the micro-organisms), these results may consequently be due to the influences of the mycelial growth rate, rather than directly to C:N ratio. The ratio of soluble protein : crude protein was determined by a complex interaction of several factors. At the low mycelial dry weight yields (when C:N $< 12:1$ and C:N > 33.1) the ratio probably decreased due to the presence of more cell wall material (primarily chitin). At the highest mycelial dry weight yields (C:N $\approx$ 18:1), a decrease of the ratio could be caused by the presence of higher concentrations of nucleic acids (this resulting in the minima observed in the soluble protein : crude protein ratio between C:N ratios of 12 and 33:1). The carbohydrate content of the mycelium, which increased with dry weight yield, may similarly have been influenced by the mycelial growth rate.

3.6.4 The use of thermophilic bacterial biomass as a nitrogen source

Investigations have shown that during the composting process, large populations of micro-organisms develop (Hayes, 1969; Laborde et al., 1969; Staněk, 1972; Imbernon and Leplae, 1972 and Lacey, 1973). Staněk (1969) and Hayes (1972) have drawn attention to the possible role of thermophilic micro-organisms present in compost in the growth of Agaricus bisporus.

The present experiment was designed to investigate the possible utilization of thermophilic bacterial biomass (culture originally isolated from pasteurized compost) by mushroom mycelium in submerged culture.

(i) Experimental design

Thermophilic bacterial biomass was produced as in Section 2.3.3 (for amino acid composition and total nitrogen content see Appendix 6.7).

Mycelium of A.bisporus was cultured (in triplicate) in 100 ml of basal synthetic medium (Section 2.3.2) to which was added various concentrations of the thermophilic bacterial biomass (as a nitrogen source, Appendix 6.8(a)) to give a range of known C:N ratios. In another series of flasks the thermophilic bacterial biomass was used as a sole nitrogen source, i.e. added to casein hydrolysate-free basal synthetic medium (Appendix 6.8 (b)).

After incubation the culture broth was filtered through cheese-cloth and washed several times with distilled water to remove any trace of bacterial biomass particles left in the medium. Mycelial dry weight yield, total nitrogen, amino acids and sulphur-containing amino acids were determined by the usual methods.

(ii) Results

The dry weight yield of mushroom mycelium increased dramatically on the addition of thermophilic bacterial biomass to the basal synthetic

medium (containing casein hydrolysate). The highest yield was obtained at C:N ratio of 9:1, where the highest bacterial biomass concentration was added, this being a 118% increase over the control (C:N = 44:1, when no bacterial biomass added. Fig.3.31; Appendix 6.8 (a)). Mycelial nitrogen content was not affected by the addition of bacterial biomass, except at the lowest rate of application, when an increase of 11% was observed (Fig.3.31). The mycelial methionine content increased gradually in the presence of increasing concentrations of the bacterial biomass, although at all concentrations this was significantly greater than the control. At the lowest rate of biomass addition the increase was 73% while, at the highest rate of addition, it increased to 100% over the control. The cysteine content of the mycelium showed a small overall decrease as the bacterial biomass concentration was increased. Amino acids analysis shows that, apart from the change in methionine, the mycelium cultivated at a C:N ratio of 9:1 (corresponding to the highest mycelial yield obtained) contained significantly higher concentrations of the essential amino acids isoleucine, leucine and lysine, than the control (increases of 27%, 25% and 34% respectively; Appendix 6.9) but less histidine (24% less than the control). The total amino acids content was similar in both cases.

Thermophilic bacterial biomass as sole nitrogen source gave greater yields of mycelium than when the mixture of casein hydrolysate and bacterial biomass was used (Fig.3.32; Appendix 6.8(b)). The optimum yield was at a C:N ratio of 18:1, and similar yields were maintained at lower C:N ratios (corresponding to greater bacterial biomass concentrations). The nitrogen and actual protein contents were unaffected by the rate of biomass addition (Appendix 6.9). The sulphur-containing amino acids contents were again high for mushroom mycelium, but both (cysteine and methionine) showed a slight overall decrease as the concentration of bacterial biomass was increased.

FIG.3.31. EFFECTS OF THERMOPHILIC BACTERIAL BIOMASS AND CASEIN HYDROLYSATE AS MIXED NITROGEN SOURCES IN THE SUBMERGED CULTURE OF A.BISPORUS.

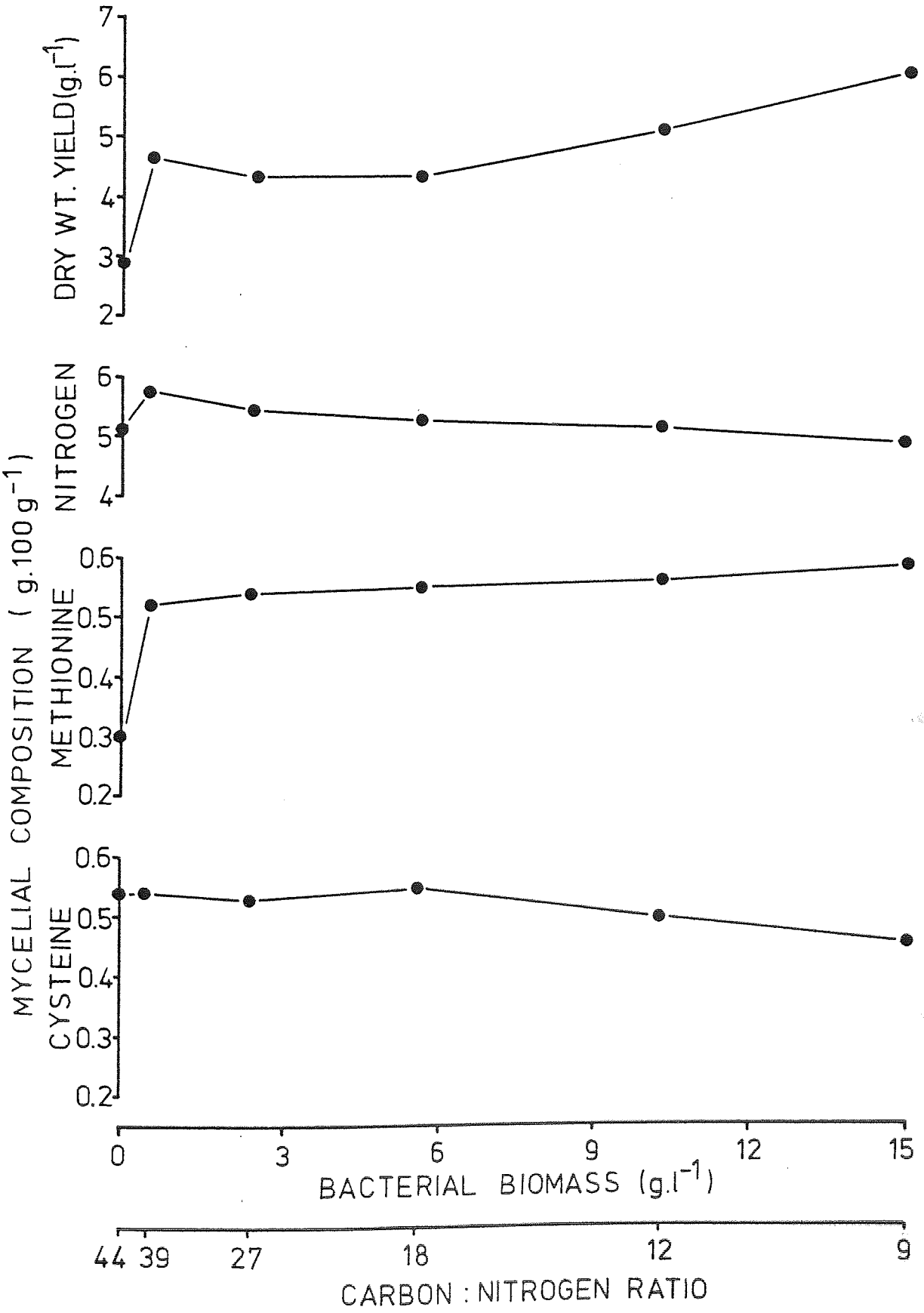
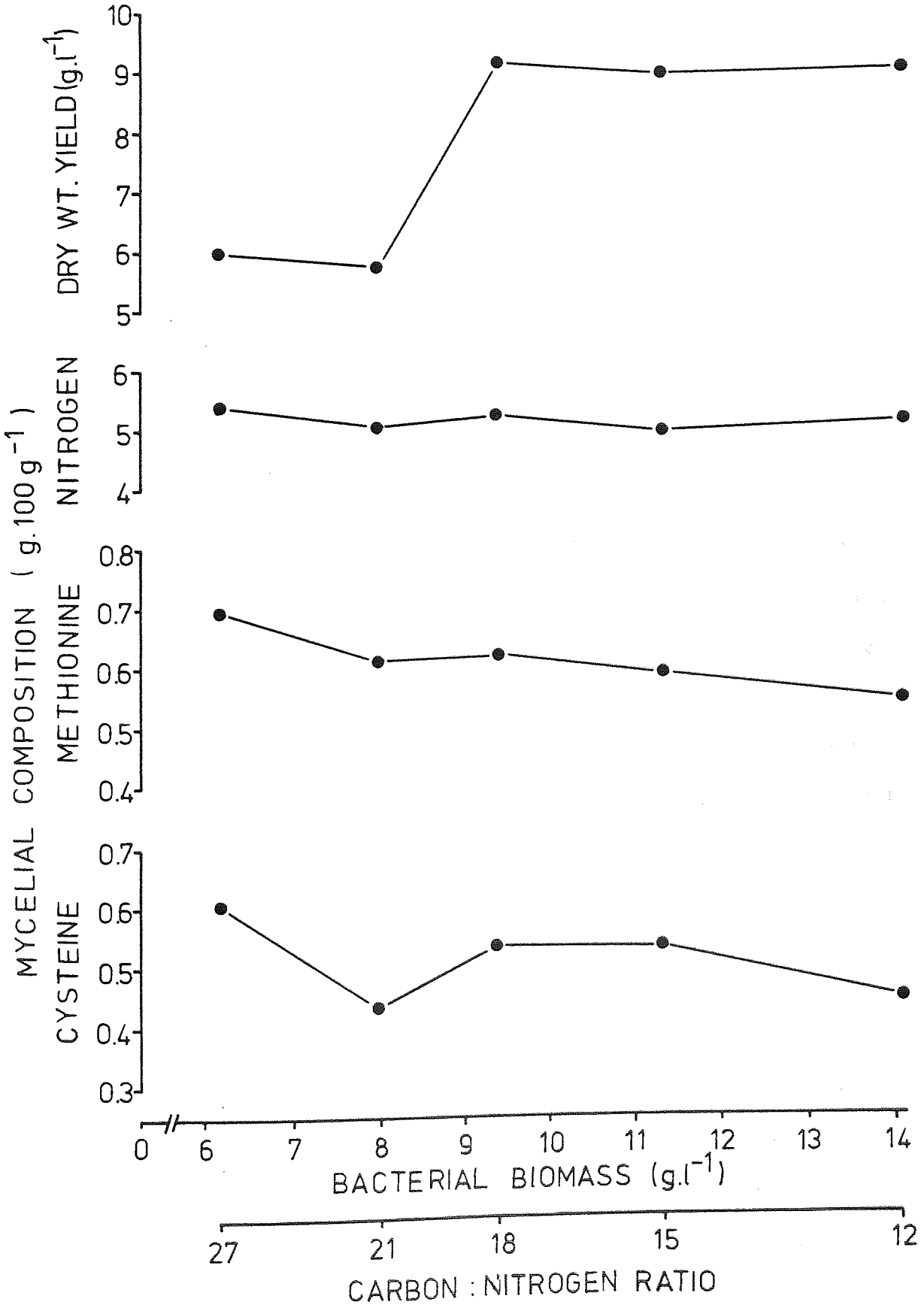


FIG.3.32. EFFECTS OF THERMOPHILIC BACTERIAL BIOMASS AS SOLE NITROGEN SOURCE IN THE SUBMERGED CULTURE OF A. BISPORUS.



(iii) Conclusions

The addition of thermophilic bacterial biomass significantly increased the yield of mushroom mycelium in submerged culture.

Mixed nitrogen sources (casein hydrolysate + thermophilic bacterial biomass) increased mycelial yield even at lower C:N ratios than the optimum, where a significant decrease in yield was found during the C:N ratio experiment (Section 3.6.3). When thermophilic bacterial biomass was the sole nitrogen source lower concentrations were needed to obtain the increased yields, i.e. casein hydrolysate reduced the efficiency of thermophilic bacterial biomass as a nitrogen source. This was presumably due to availability of the amino acids present in casein hydrolysate, the enzymes necessary for utilization of the bacterial biomass being produced by the mushroom mycelium in only small amounts (this minimising the yield). The enzymes necessary for bacterial biomass breakdown were presumably absent from the culture at the start of the experiment, for, especially in the cultures with bacterial biomass as sole nitrogen source, only minor growth occurred during the first week of culture. Subsequently rapid growth of mushroom mycelium occurred. This explains the sudden increase in yield observed between C:N ratios of 21:1 and 18:1 when bacterial biomass was the sole nitrogen source. With bacterial biomass as sole nitrogen source the optimum C:N ratio was 18:1, which is similar to that observed during the C:N ratio experiment; the yield did not decrease at lower C:N ratios, due to the over-riding effect of the bacterial biomass.

Addition of bacterial biomass (whether alone or in combination with casein hydrolysate) caused no major changes in the protein content or amino acids profiles, exceptions were significant increases in the concentrations of the essential amino acids isoleucine and lysine. Histidine and cysteine were decreased. The most significant effect,

however, was on the methionine content, which was dramatically increased even when small amounts of bacterial biomass were added to the culture medium.

In conclusion, the present experiment has shown that biomass of the thermophilic bacteria present in compost is actively and efficiently utilized by the mushroom mycelium, resulting, not only in greatly increased yields, but also in a higher grade mycelium containing especially high levels of methionine (up to a 3-fold increase on the values observed in mushroom mycelium cultured on other media, and from analyses of mushroom fruitbodies).

Section 4

DISCUSSION AND CONCLUSIONS

DISCUSSION AND CONCLUSIONS

Composition of fruitbodies

Variations in the composition of Agaricus bisporus fruitbodies could be attributed to two main factors, (a) those inherent to the fruitbody and to the system of artificial culture, and (b) those which were related to the conditions of artificial culture, e.g. the watering regime, and nutrition.

Within a given fruitbody it was found that the three distinct tissues (stipe, pileus and hymenium) showed marked differentiation with respect to the distribution of materials, more dry matter being deposited in the hymenium (the spore bearing tissue) than the remainder of the sporophore. Nitrogen content was also substantially greater in the hymenium than the stipe or the pileus, this difference being a reflection of the function of the tissue; since the hymenium tissue is concerned with spore production, the high level of metabolic activity resulted in the presence of large amounts of nitrogen. These differences were also observed from the results of soluble protein studies (Pegg 1973, Hammond and Nichols, 1975).

While variations between different regions of the fruitbody may be explained in terms of the biochemical processes involved in the development of tissues performing different functions, the cause of variations in the dry matter and nitrogen content of single whole fruitbodies harvested from a single culture unit (tray) reflects inherent variation in the growth process of the culture as a whole. The composition (nitrogen and dry matter contents) varied between fruitbodies within a culture unit, suggesting an uneven distribution of water and materials within the developing fruitbodies. In the culture of A.bisporus only a small proportion of the primordia develop into fruitbodies (Hein, 1930; Hayes, 1972). Hein suggested that some primordia are more favourably

positioned to initiate a directional flow of nutrients away from other primordia, this being due to a time difference in their initiation. This suggestion was supported by Madelen (1956a,b) in experiments with Coprinus lagopus. At any given time and over a given area of cropping, e.g. a tray, a limited amount of nutrients (including water) are available from the culture medium to permit the development of a certain number of primordia. If the time of initiation is variable (fruitbodies develop at any single break over a period of 3-5 days) it can be expected that individual fruitbodies from a tray culture can receive different amounts of nutrients from the substrate, this resulting in variation of composition.

Significant differences in the nitrogen content of fruitbodies were also found between individual tray cultures. This was probably caused by relatively minor differences in the composition of the substrate and/or small differences in the microclimate.

This study has identified consistent differences in composition between the different fruitbody developmental stages. Dry matter and carbohydrate contents increased as fruitbodies matured from Stages A (button) to C (flats), while nitrogen contents decreased during fruitbody development. In most cases the lipid contents also declined from Stages A to C. Such changes are clearly associated with the metabolic and cellular changes occurring during maturation of the fruitbody. No consistent trends were detected to suggest that composition was governed by the time (break) of harvest.

Nitrogen contents (crude protein) were shown in one experiment to be overall lower in the second break harvest, while second break fruitbodies grown at a different time were markedly higher in nitrogen than first break fruitbodies. This may be related to the release of nutrients from the substrate and the mobilisation of material reserves in the mycelium, phenomena which are not fully understood in mushroom culture.

During mushroom cultivation, the maintenance of the correct water regime, and the preparation of the main substrate (the compost) are procedures which are subjective, relying to a large extent on human judgement and skill. The observations of Flegg (1974), which related a high rate of water application to increased fresh weight yields, were also found in these investigations. This increase, however, could only be partly attributed to an increased water content of the fruitbodies. Dry weight yield also increased, but was not proportional to the fresh weight increase, which is in contrast to the results of Bonner et al. (1956). Additionally, the composition of fruitbodies varied according to the degree of watering, indicating the important role of water in the physiology of fruitbody development. Watering not only affected the distribution and metabolism of materials, but was also related to the inherent variation of the fruitbodies and the system of culture discussed above.

The composition of the fruitbodies was also greatly influenced by the nutrients present in the compost. This was demonstrated by supplementation of the compost after colonization with A.bisporus mycelium. Yield was increased following the addition of dried blood, casein hydrolysate and soya bean meal. With dried blood, yield response was directly related to the level of application, both fresh and dry weight yields increasing, and clearly emphasises the important role of proteins in the nutrition of A.bisporus. The yield increase was also associated with compositional differences which were directly related to the quality of fruitbody protein as indicated by amino acid analysis. While crude protein increased with the addition of supplements, 'actual protein' (by summation of amino acids) showed no marked changes. The amino acid profile was, however, affected, suggesting a relationship between the availability of nitrogen and the biosynthesis of amino acids.

No relationship could be found between the effects of supplements and their amino acid composition, which is in contrast to the conclusions of Sinden and Schisler (1962). Changes in amino acids metabolism were also found by Kissmeyer et al. (1966) following supplementation with gelatin and casein hydrolysate; this may have been related to the direct effect of the supplements on the microbiological activity in the compost.

A further demonstration of the changes in amino acids metabolism of A.bisporus mycelium, grown in a synthetic medium in submerged culture, was found when casein hydrolysate was substituted by the biomass of a thermophilic bacterium isolated from compost. In this case, the crude and 'actual' protein contents of the mycelium remained unchanged. The amino acids profile was, however, affected, histidine content being decreased, while increases in the essential amino acids leucine, isoleucine, lysine and the sulphur-containing amino acid methionine were detected. Mycelial yields were also greatly increased, suggesting a strong relationship between the thermophilic bacterial biomass and productivity of A.bisporus. Mycelial yield in submerged culture was influenced by the C:N ratio, irrespective of nitrogen source a C:N ratio of 18:1 being optimal. This ratio is approximate to that found by Smith and Hayes (1972) to be optimal for fruitbody formation (on compost).

In liquid culture organic sulphur was utilized in preference to inorganic sulphur sources. Mushroom mycelium was able to utilize free L-methionine, N-acetyl-L-methionine (probably by cleavage of this compound releasing L-methionine in a free form) and L-cysteine hydrochloride. The increase in yield could be related to the effect of these amino acids on the growth of mycelium, as demonstrated by Frazer and Fugikawa (1958). L-homocysteine was an inadequate supplement for sulphur-containing amino acids biosynthesis although yield was again increased.

This may have been due to the inefficiency of its utilization by the mushroom mycelium. It has been shown by Schwartz and Shapiro (1954) that L-α-n-amino butyric acid plays a vital role in methionine biosynthesis in Aerobacter aerogenes, in the present study, however, this compound had no significant effect on methionine biosynthesis, although the mycelial dry weight yield was affected at the lower concentration.

Nutritional value of fruitbodies

This study has already demonstrated that the composition of A.bisporus fruitbodies are subjected to variation, and this emphasises the difficulties of establishing food values of a fresh foodstuff. The crude and 'actual' protein values were substantially higher than those quoted by McCance and Widdowson (1960) and the Manual of Nutrition (1976), and are closer to those values of Anderson and Fellers (1942) and McConnell and Esselen (1946). Despite these difficulties a more accurate assessment of food value relating specifically to dry matter, protein and calorific values can now be made.

During post harvest storage, the environmental conditions affected fruitbody composition. Since endogenous respiration in the fruitbodies continues after harvest, losses in fresh and dry weights were detected, these being greater at a storage temperature of 22°C than at 3°C. The losses in dry matter were mostly accounted for by losses in carbohydrate and lipid; carbohydrates were used in preference to the lipid. Mannitol, which is normally used as a storage compound in fungi (Cochran 1958), has been reported by Hammond and Nichols (1975) to act as a major respiratory substrate after harvest. Mannitol is not normally regarded as being of nutritional significance for man and was not included in this study.

Significant changes in the nitrogenous components were also observed during storage. Crude protein was not affected, while 'actual' protein

declined gradually, reflecting a shift of amino acid nitrogen to non-protein nitrogen; this obviously affected the value of the fruitbodies as a protein source.

Alternative methods of marketing and preservation may be suggested as a result of this study. Modern methods of 'accelerated freezing', vacuum cooling and a traditional method of preservation in the Orient by air drying, are therefore relevant to the preservation of food values.

Although mushroom fruitbodies are considered a low calorific food-stuff, and contain large amounts of vitamins and minerals, it is the protein component which has given rise to conflicting opinions as to the nutritional value of edible mushrooms.

The nutritional value of proteins is primarily due to the amino acids profile. Biological availability of the amino acids may, however, be influenced by a number of factors and ideally should be determined in conjunction with animal feeding trials, which establish values for the protein efficiency ratio (PER). In lieu of animal feeding studies, three other factors have been considered as possible measures of nutritive value: in vitro digestibility, protein content, and its amino acid profile. In the absence of feeding studies, the most reliable measure presently available for estimating the nutritive value of mushrooms appears to be their amino acids content, especially the content of the essential amino acids.

Several methods may be used for determining or predicting the nutritional value of a food based on its amino acids content (FAO/WHO 1973). The essential amino acid index (EAA-index) has been used to assess dietary protein and is expressed in terms of the ratio of the essential amino acids content of a food relative to the essential amino acid content of a highly nutritive reference protein.

Another estimate of nutritive value used by some investigators, is the amino acid score or the chemical score. This is based on the amount of the most limiting amino acid present in the test protein, relative to the amount of that amino acid in the reference pattern. The amino acid score reflects the 'percentage of adequacy' of the protein and approximates the probable efficiency of utilization of the test protein. If the composition of an ideal protein (one containing all the essential amino acids in sufficient amounts to meet requirements without any excess) were known, then it should be possible to compute the nutritive quality of a protein by calculating the deficiency of each amino acid from the amount in the ideal protein. The most "limiting amino acid" would thus determine the nutritive value.

The amino acid score of a protein is calculated as follows:-

$$\text{Amino acid score} = \frac{\text{mg of amino acid in 1 g of test protein}}{\text{mg of amino acid in reference pattern}} \times 100$$

Lysine, total sulphur-containing amino acids or tryptophan are usually the most frequently limiting in foods and diets.

Digestibility of mushroom protein was 82% and the chemical score was 45 based on amino acids analysis and the provisional amino acids pattern (FAO/WHO, 1973; Appendix 7.1). This predicts that mushroom fruitbodies contain a sufficient amount of lysine when compared to whole grain wheat, cabbage leaves and rice, but are inferior to cow's milk, hen's eggs and meat, which are themselves considered to be excellent sources of amino acids. The sulphur-containing amino acids methionine and cysteine appeared to be limiting the nutritional value of mushrooms, which is similar to many other micro-organisms, e.g. yeasts (Van den Veen 1974).

In conclusion these studies have shown that both quantitative and qualitative aspects of growth are influenced by cultural practices. By maintaining the correct water relations and nutritional status of the substrate, not only yield but also composition may be influenced to the extent of significantly affecting nutritional values of this foodstuff. Additionally the significance of thermophilic bacterial biomass on the increased production of mycelium, containing especially high levels of the sulphur-containing amino acid methionine, in liquid (submerged) culture warrants further studies, which may have relevance to the application of fermentation techniques for the production of mushroom mycelium.

APPENDICES

Appendix 1.1 Dry matter and total nitrogen content of different tissues from single fruitbodies (stage C).

	Stipe		Pileus		Hymenium	
Fruit-body sample	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.
1	8.33	2.56	5.96	3.28	10.81	5.31
2	6.66	2.67	5.58	3.20	8.21	5.24
3	7.27	2.64	6.09	3.17	10.00	5.16
4	6.81	2.58	5.88	3.18	8.47	5.26
5	6.49	2.69	6.45	3.35	10.00	5.63
Mean	7.11	2.62	5.99	3.27	9.49	5.32
S.D.	0.74	0.066	0.31	0.077	1.11	0.181

Statistical analysis

	Dry matter	Total nitrogen
variations	t value	t value
stipe & pileus	**3.11	*14.25
stipe & hymenium	**3.99	*31.67
pileus & hymenium	*6.78	*23.63

* t value is significant at p = 0.01

** t value is significant at p = 0.05

*** t value is significant at p = 0.1

D.M. = Dry matter

Appendix 1.2 Dry matter and nitrogen content of single fruitbodies of A.bisporus (Stage A, 1st break).

Fruitbody sample	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ dry matter
1	9.07	6.09
2	8.87	6.57
3	9.44	6.00
4	9.66	6.59
5	10.69	6.65
6	9.61	5.97
7	9.01	6.77
8	9.20	6.79
9	10.72	6.95
10	9.66	6.25
11	10.01	6.43
12	9.07	6.06
13	9.21	6.12
Mean	9.55	6.40
SD	0.605	0.339

(a) Correlation between dry matter and nitrogen content

Let Nitrogen content = x

then Dry matter = y

$$Y = a + bx$$

$$Y = 5.26 + 0.670\ x$$

For significance

$$r = \frac{\xi dxdy}{(\xi dx^2 . \xi dy^2)}$$

$$r = 0.375$$

From table, when $p = 0.1$, $r = 0.458$

The calculated value of r does not exceed this, so there is no correlation between the variables.

Appendix 1.3 Dry matter and nitrogen content of first break fruitbodies harvested at Stages A, B and C.

	Stage A		Stage B		Stage C	
Tray number	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.
1	8.0	6.07	8.50	4.72	8.4	4.50
2	9.0	5.96	10.00	4.87	10.0	4.47
3	9.0	5.74	10.20	4.31	10.0	3.97
4	8.5	5.92	9.50	4.19	10.5	3.80
5	9.2	6.05	8.20	5.64	9.4	4.78
6	7.0	6.05	7.00	5.61	7.6	4.88
Mean	8.45	5.96	8.90	4.89	9.31	4.40
SD	0.83	0.12	1.22	0.62	1.10	0.43

D.M. = Dry matter

Appendix 1.4 Dry matter and nitrogen content of second break fruitbodies harvested at Stages A, B and C.

	Stage A		Stage B		Stage C	
Tray number	Dry m matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.	Dry matter $g.100g^{-1}$	T.N. $g.100g^{-1}$ D.M.
1	10.1	6.60	11.0	6.33	10.3	5.35
2	9.5	6.51	11.6	6.00	10.1	5.26
3	8.0	5.64	9.0	5.48	9.2	5.18
4	11.4	6.38	10.0	6.15	11.8	5.17
5	8.4	6.85	9.3	6.40	9.9	5.52
6	8.9	6.81	8.5	6.41	8.7	5.50
Mean	9.38	6.465	9.90	6.128	10.00	5.33
SD	1.24	0.441	1.20	0.355	1.06	0.153

D.M. = Dry matter

Appendix 1.5 Dry matter and nitrogen content of Stages A, B and C fruitbodies at first and second breaks.

	Dry matter $g.100g^{-1}$	Total nitrogen $g.100g^{-1}$ D.M.	Dry matter $g.100g^{-1}$	Total nitrogen $g.100g^{-1}$ D.M.
A	8.45	5.96	9.38	6.46
B	8.90	4.89	9.90	6.12
C	9.31	4.40	10.00	5.33

Mean of samples obtained from six replicate trays.

Statistical analysis

(a) t values for dry matter and nitrogen content of first and second breaks fruitbodies harvested at Stages A, B and C.

F I R S T B R E A K

Variations	Dry matter $g.100g^{-1}$	Total nitrogen $g.100g^{-1}$ dry matter
	t value	t value
Stage A & B	0.743	*4.150
Stage A & C	1.532	*8.523
	S E C O N D B R E A K	
Stage A & B	0.732	1.455
Stage A & C	0.923	*5.940

(b) t values for dry matter and nitrogen contents of fruitbodies harvested from first and second breaks.

Variation	Dry matter $g.100g^{-1}$	Total nitrogen $g.100g^{-1}$ dry matter
	t value	t value
Stage A, 1st & 2nd breaks	1.528	**2.676
Stage B, 1st & 2nd breaks	1.425	*4.234
Stage C, 1st & 2nd breaks	1.089	*4.96

*t value is significant at P = 0.01

**t value is significant at P = 0.05

***t value is significant at P = 0.10

D.M. = Dry matter

Appendix 2.1 Amino acid composition of Stage B fruitbodies of *Agaricus bisporus*.

Amino acids	$g.100g^{-1}$ dry matter
Aspartic acid	3.14
*Threonine	1.48
Serine	1.89
Glutamic acid	7.06
Proline	2.50
Glycine	1.20
Alanine	2.40
Cystine	0.18
*Valine	1.63
*Methionine	0.39
*Isoleucine	1.28
*Leucine	2.16
Tyrosine	0.78
*Phenylalanine	1.55
Histidine	0.64
*Lysine	1.62
Arginine	1.90
*Tryptophan	3.94
TOTAL	35.75

*Essential amino acids

Appendix 3.1 Dry matter in $g.100g^{-1}$ fresh weight of first, second and third break fruitbodies cultured under different watering regimes, harvested at Stages A, B and C.

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
	A	B	C	A	B	C	A	B	C
First break	9.45	9.90	10.34	8.60	8.54	8.78	7.65	7.77	8.41
Second break	8.63	8.87	10.88	8.42	8.23	9.03	7.43	8.43	10.17
Third break	8.33	8.77	9.53	7.36	7.05	8.75	6.63	7.14	8.50

Mean of three replicate samples

Statistical analysis

(a)

Variations	FIRST BREAK t-value	SECOND BREAK t-value	THIRD BREAK t-value
A low & A medium	*10.82	1.27	*10.15
A low & A high	*12.03	0.65	*19.12
A medium & A high	** 6.96	** 9.62	** 6.54
B low & B medium	*10.27	** 9.06	*16.94
B low & B high	*13.32	***3.89	*13.79
B medium & B high	**4.40	1.54	1.03
C low & C medium	*13.44	*26.21	**5.11
C low & C high	*12.77	** 8.06	**4.46
C medium & C high	2.20	*10.03	1.11

* t value is significant at p = 0.01
** t value is significant at p = 0.05
*** t value is significant at p = 0.1

(b)

Treatment	Variations	FIRST BREAK t-value	SECOND BREAK t-value	THIRD BREAK t-value
Low watering	A & B	***4.25	1.44	**4.33
	A & C	**9.60	*13.48	**9.57
Medium watering	A & B	0.56	2.21	***3.24
	A & C	1.76	**6.87	*10.68
High watering	A & B	0.62	**7.11	**4.78
	A & C	***3.99	*21.81	**8.73

(c)

Variations	LOW WATERING	MEDIUM WATERING	HIGH WATERING
A first & A second	**4.53	2.77	1.38
A first & A third	*13.16	*13.80	**6.68
B first & B second	*12.58	2.41	***3.75
B first & B third	**9.42	*12.79	***3.92
C first & C second	**8.69	2.04	*10.69
C first & C third	**6.19	0.26	0.35

* t value is significant at p = 0.01

** t value is significant at p = 0.05

*** t value is significant at p = 0.1

Appendix 3.2 The effect of water application on yield of fruitbodies per 30 kg fresh compost over 0.418 m^2.

		Low rate of application				Medium rate of application				High rate of application			
	Stage	Number	Fresh wt. in g.	Dry wt. in g.	Mn.Wt. per fruit-body	No.	Fresh wt. in g.	Dry wt. in g.	Mn.Wt. per fruit-body	No.	Fresh wt. in g.	Dry wt. in g.	Mn.Wt. per fruit-body
1ST BREAK	A	74	518	49.37	7.00	70	629	53.77	8.98	70	913	68.01	13.04
	B	15	293	29.14	19.53	54	973	85.25	18.01	50	1482	114.85	29.64
	C	6	130	13.57	21.66	31	838	75.00	27.03	18	867	75.34	48.16
	Total	95	941	92.08	14.39	155	1940	214.00	18.00	138	3262	258.2	30.28
2ND BREAK	A	55	307	26.49	5.58	38	193	16.2	5.07	40	291	21.50	7.27
	B	28	295	26.16	10.53	21	243	19.90	11.57	25	343	28.74	13.72
	C	43	587	63.92	13.65	39	533	48.28	13.66	17	453	39.90	26.64
	Total	126	1159	116.57	9.92	98	969	84.38	10.1	82	1169	90.14	15.87
3RD BREAK	A	25	117	9.78	4.68	15	117	86.81	7.8	25	122	8.10	488.0
	B	10	142	12.48	14.2	12	147	10.46	12.25	11	142	14.27	12.90
	C	18	185	17.74	10.27	13	193	16.96	14.84	18	185	13.37	10.20
	Total	53	444	40.00	9.71	40	457	36.1	11.63	54	449	35.76	9.32
GRAND TOTAL		274	2574	248.65		293	3366	334.48		274	4880	384.1	

Appendix 3.3 $g.100g^{-1}$ crude protein of first, second and third break fruitbodies cultured under different watering regimes, harvested at Stages A, B and C.

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
	A	B	C	A	B	C	A	B	C
First break	37.78	37.17	38.49	37.69	35.17	30.26	40.07	30.55	26.64
Second break	32.29	27.91	25.54	42.52	39.94	34.41	33.31	36.87	28.85
Third break	41.49	37.56	33.72	39.39	38.07	33.52	37.11	32.37	31.85

Mean of three replicate samples on dry matter basis

Statistical analysis

(a)

Treatment	Variations	FIRST BREAK t-value	SECOND BREAK t-value	THIRD BREAK t-value
Low watering	A & B	2.33	** 9.58	* 17.91
	A & C	2.10	*18.04	* 21.76
Medium watering	A & B	* 17.50	* 10.00	** 6.42
	A & C	* 48.72	* 35.12	* 34.28
High watering	A & B	* 56.49	* 20.56	* 20.06
	A & C	* 58.28	* 25.34	* 19.89

(b)

Variations	LOW WATERING t-value	MEDIUM WATERING t-value	HIGH WATERING t-value
A first & A second	*12.50	*19.46	*106.07
A first & A third	*11.80	**9.28	*12.70
B first & B second	*31.97	*29.88	*27.11
B first & B third	***3.00	*16.97	*10.47
C first & C second	*52.34	*34.01	**7.79
C first & C third	*12.63	*23.68	*19.88

(c)

	FIRST BREAK	SECOND BREAK	THIRD BREAK
A low & A medium	0.35	*23.67	**8.27
A low & A high	** 9.44	2.73	*14.27
A medium & A high	*20.78	*40.16	**8.34
B low & B medium	*13.94	*40.37	***3.17
B low & B high	*34.31	*28.19	*52.91
B medium & B high	*24.36	*14.67	*37.52
C low & C medium	*31.28	*107.77	0.67
C low & C high	*36.04	*18.15	**5.83
C medium & C high	*14.38	*17.63	*10.68

* t-value is significant at p = 0.01

** t-value is significant at p = 0.05

*** t-value is significant at p = 0.1

Appendix 3.4 *'Actual protein' (by summation of amino acids) of Agaricus bisporus fruitbodies of the first, second and third breaks, cultured under different watering regimes, harvested at Stages A, B and C.

	Low watering			Medium watering			High watering		
Harvesting time	A	B	C	A	B	C	A	B	C
FIRST BREAK	33.56	34.24	33.93	36.30	31.84	26.70	37.30	28.67	26.37
SECOND BREAK	25.25	22.17	19.21	29.14	28.79	24.42	26.90	25.43	22.79
THIRD BREAK	34.74	28.96	27.73	32.11	30.71	26.92	29.96	26.45	25.84

*Results expressed in g actual protein per 100g dry matter

-Data obtained from Appendix 3.5

Appendix 3.5 (a) Amino acid content of Agaricus bisporus fruitbodies of the first break, cultured under different watering regimes, harvested at Stages A, B and C.

Results expressed as g Amino acid.$100g^{-1}$ dry matter.

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
Amino acids	A	B	C	A	B	C	A	B	C
Aspartic acid	2.53	2.60	2.99	3.14	2.72	2.06	3.31	2.59	2.15
*Threonine	1.45	1.50	1.47	1.50	1.34	1.12	1.74	1.25	1.08
Serine	1.60	1.65	1.67	1.99	1.53	1.28	1.89	1.37	1.07
Glutamic acid	6.86	6.96	6.30	7.66	6.17	4.75	7.53	5.25	5.69
Proline	2.56	2.29	1.47	2.48	2.10	1.47	2.08	1.33	1.04
Glycine	1.20	1.23	1.31	1.26	1.12	0.96	1.42	1.04	1.90
Alanine	2.33	2.28	2.08	2.42	2.07	1.63	2.69	1.91	1.55
Cystine	0	0	0	0	0	0	0	0	0
*Valine	1.27	1.27	1.62	1.63	1.18	1.02	1.60	1.39	1.09
*Methionine	0.36	0.40	0.39	0.38	0.37	0.30	0.44	0.32	0.29
*Isoleucine	1.21	1.17	1.26	1.26	1.14	0.96	1.41	1.04	0.89
*Leucine	1.95	2.03	2.11	2.13	1.92	1.58	2.33	1.70	1.50
Tyrosine	1.16	1.21	1.26	0.79	0.72	0.91	0.69	0.71	0.76
*Phenylalanine	1.15	1.29	1.62	1.35	1.27	1.16	1.50	1.18	1.13
Histidine	0.62	0.62	0.64	0.65	0.57	0.47	0.73	0.54	0.47
*Lysine	1.41	1.49	1.50	1.51	1.37	1.10	1.72	1.25	1.06
Arginine	1.62	1.69	1.73	1.91	1.51	1.30	1.86	1.32	1.20
*Tryptophan	4.28	4.56	4.51	4.24	4.74	4.63	4.16	4.48	4.49

*Essential amino acids

(b) Amino acid content of Agaricus bisporus fruitbodies of the second break, cultured under different watering regimes, harvested at Stages A, B and C.

Results expressed as g Amino acid. $100g^{-1}$ dry matter.

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
Amino acids	A	B	C	A	B	C	A	B	C
Aspartic acid	1.99	1.67	1.43	2.33	3.39	2.04	2.09	2.08	1.66
*Threonine	1.07	0.89	0.72	1.19	1.16	0.99	1.09	1.11	0.92
Serine	1.16	0.97	0.76	1.25	1.08	0.95	1.18	1.18	0.98
Glutamic acid	4.54	3.94	3.15	5.84	5.21	4.74	6.20	4.57	5.1
Proline	1.14	1.00	0.84	1.84	1.82	1.36	1.09	1.25	1.01
Glycine	0.87	0.74	0.65	0.99	0.98	0.82	0.90	0.91	0.72
Alanine	1.49	1.27	1.16	1.95	1.93	1.57	1.63	1.68	1.33
Cystine	0	0	0	0	0	0	0	0	0
*Valine	0.98	0.84	0.64	1.12	1.01	0.85	0.91	1.08	0.76
*Methionine	0.24	0.23	0.19	0.31	0.31	0.26	0.27	0.26	0.25
*Isoleucine	0.83	0.73	0.59	0.97	0.93	0.78	0.88	0.87	0.72
*Leucine	1.44	1.19	0.97	1.55	1.52	1.26	1.43	1.46	1.20
Tyrosine	0.45	0.36	0.33	0.50	0.48	0.41	0.45	0.51	0.36
*Phenylalanine	0.89	0.77	0.64	0.98	0.97	0.82	0.85	0.67	0.75
Histidine	0.39	0.33	0.27	0.45	0.45	0.36	0.40	0.42	0.25
*Lysine	0.98	0.81	0.66	1.14	1.11	0.87	1.00	1.09	0.78
Arginine	1.16	0.94	0.80	1.43	1.34	1.09	1.17	1.19	0.91
*Tryptophan	5.63	5.47	5.41	5.30	5.10	5.25	5.36	5.10	5.10

(c) Amino acids content of Agaricus bisporus fruitbodies of the third break, cultured under different watering regimes, harvested at Stages A, B and C.

Results expressed as g Amino acid·100g^{-1} dry matter.

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
Amino acid	A	B	C	A	B	C	A	B	C
Aspartic acid	2.73	2.30	1.89	2.35	2.25	2.07	2.26	2.21	1.83
* Threonine	1.39	1.19	1.07	1.33	1.30	1.14	1.22	1.08	1.05
Serine	1.43	1.20	1.13	1.41	1.36	1.23	1.26	1.08	1.08
Glutamic acid	7.44	5.38	6.01	6.33	6.66	5.07	6.33	5.35	4.95
Proline	1.94	1.34	1.31	1.79	1.70	1.45	1.37	1.11	1.14
Glycine	1.11	0.89	0.83	1.02	0.98	0.88	0.92	0.84	0.80
Alanine	2.05	1.91	1.64	2.11	2.03	1.67	1.88	1.59	1.52
Cystine	0	0	0	0	0	0	0	0	0
* Valine	1.31	1.02	0.92	1.52	1.10	0.77	1.10	1.14	1.13
* Methionine	0.32	0.36	0.32	0.37	0.39	0.29	0.31	0.31	0.32
* Isoleucine	1.15	0.96	0.88	1.04	1.00	0.84	0.97	0.86	0.85
* Leucine	1.88	1.58	1.43	1.72	1.65	1.38	1.61	1.39	1.39
Tyrosine	1.63	1.25	1.10	0.81	0.80	0.79	0.81	0.75	0.75
* Phenylalanine	1.14	0.89	1.01	0.70	0.82	0.97	1.08	0.91	0.93
Histidine	0.56	0.49	0.48	1.26	0.49	0.47	0.55	0.43	0.65
* Lysine	1.30	1.28	1.21	1.27	1.21	1.02	1.22	1.00	0.97
Arginine	1.74	1.31	1.20	1.27	1.37	1.06	1.26	1.10	1.12
* Tryptophan	5.61	5.61	5.30	5.80	5.60	5.83	5.75	5.30	5.36

Appendix 3.6 $g.100g^{-1}$ total carbohydrate content of first, second and third break fruitbodies cultured under different watering regimes harvested at Stages A, B and C.

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
	A	B	C	A	B	C	A	B	C
FIRST BREAK	22.20	21.85	24.44	22.43	23.79	25.69	20.93	23.87	25.62
SECOND BREAK	22.12	24.04	24.09	19.67	19.76	20.08	21.22	20.47	22.31
THIRD BREAK	19.40	19.59	21.67	19.26	19.48	21.56	21.20	21.71	23.75

Mean of three replicate samples, on dry matter basis.

Statistical Analysis

(a)

Treatment	Variations	FIRST BREAK t-value	SECOND BREAK t-value	THIRD BREAK t-value
Low watering	A & B	1.36	** 7.01	1.38
	A & C	**9.14	*13.48	*12.32
Medium watering	A & B	**4.75	0.34	1.74
	A & C	*14.39	1.20	*27.08
High watering	A & B	*11.38	**4.34	***3.65
	A & C	*14.06	**4.31	*20.61

(b)

Variations	Low watering	Medium watering	High watering
A 1st & A 2nd	0.30	*11.97	1.25
A 1st & A 3rd	*11.73	*21.60	1.23
B 1st & B 2nd	** 8.47	*13.67	*15.95
B 1st & B 3rd	*13.78	*15.75	*10.68
C 1st & C 2nd	** 4.31	*16.92	** 9.37
C 1st & C 3rd	*14.52	*21.51	** 6.57

(c)

Variations	FIRST BREAK t-value	SECOND BREAK t-value	THIRD BREAK t-value
A low A medium	0.87	*10.13	1.39
A low A high	** 4.35	**4.51	*13.63
A medium A high	** 6.87	**6.50	*13.89
B low B medium	** 6.93	*15.56	0.68
B low B high	** 9.26	*14.03	*14.83
B medium B high	0.253	*** 3.93	*17.75
C low C medium	** 6.13	*14.66	0.60
C low C high	*** 4.03	** 8.32	* 11.66
C medium C high	0.216	** 6.45	* 39.11

* t value is significant at p = 0.01

** t value is significant at p = 0.05

*** t value is significant at p = 0.1

Appendix 3.7 $g.100g^{-1}$ total lipid content of first, second and third break fruitbodies cultured under different watering regimes, harvested at Stages A, B and C.

	LOW WATERING			MEDIUM WATERING			HIGH WATERING		
	A	B	C	A	B	C	A	B	C
FIRST BREAK	4.50	4.10	5.05	4.72	4.60	4.51	5.87	4.42	3.98
SECOND BREAK	5.11	4.64	3.54	5.30	4.94	5.26	4.65	4.90	3.90
THIRD BREAK	4.96	4.30	4.32	5.09	5.27	4.83	4.83	4.67	4.16

Mean of three replicate samples, on dry matter basis.

Statistical analysis

(a)

Treatment	Variations	FIRST BREAK t-value	SECOND BREAK t-value	THIRD BREAK t-value
Low watering	A & B	**9.28	* 11.40	** 8.58
	A & C	*17.56	* 35.12	* 10.22
Medium watering	A & B	1.37	2.10	2.71
	A & C	1.85	0.24	** 4.92
High watering	A & B	* 23.08	** 7.34	***3.01
	A & C	* 26.82	*19.26	*15.17

(b)

Variations	Low watering	Medium watering	High watering
A 1st & A 2nd	*12.54	*** 3.38	* 17.93
A 1st & A 3rd	*11.52	*** 3.86	* 14.71
B 1st & B 2nd	*15.60	*** 3.73	* 22.32
B 1st & B 3rd	2.59	*10.16	** 5.76
C 1st & C 2nd	* 65.36	**5.90	1.85
C 1st & C 3rd	* 12.65	*** 3.92	*** 3.97

(c)

	FIRST BREAK	SECOND BREAK	THIRD BREAK
	t-value	t-value	t-value
A low & A medium	2.58	1.22	2.45
A low & A high	* 21.08	** 8.69	2.56
A medium & A high	* 11.21	*** 4.26	*** 4.43
B low & B medium	** 8.62	*** 3.59	*11.20
B low & B high	** 8.02	*32.09	*** 4.60
B medium & B high	***3.40	0.513	** 9.36
C low & C medium	** 6.82	*16.93	** 8.40
C low & C high	* 25.78	*13.75	2.64
C medium & C high	** 6.29	*13.30	*18.13

* t value is significant at p = 0.01

** t value is significant at p = 0.05

*** t value is significant at p = 0.1

Appendix 4.1 Nitrogen supplementation. Amino acids composition and total nitrogen content of each supplement. ($g.100g^{-1}$ dry matter.)

Amino acids	Dried blood	Casein hydrolysate	Bacterial biomass (I.C.I. 'Pruteen')	Soya bean meal
Aspartic acid	6.88	3.29	5.12	3.83
Threonine	4.06	1.97	2.93	1.46
Serine	4.14	2.45	2.16	1.93
Glutamic acid	6.75	10.38	6.82	6.73
Proline	3.08	4.70	1.95	1.93
Glycine	2.56	0.86	2.69	1.34
Alanine	4.80	1.33	3.93	1.34
Cystine	0.29	0	0.22	0.38
Valine	5.78	2.80	3.39	1.83
Methionine	1.08	1.09	1.18	0.41
Isoleucine	0.74	2.05	3.10	1.71
Leucine	8.64	2.76	4.55	2.86
Tyrosine	2.28	1.40	1.75	1.29
Phenylalanine	5.36	1.59	2.44	2.03
Histidine	3.98	1.20	1.23	1.05
Lysine	7.17	4.04	4.21	2.51
Arginine	3.53	1.67	3.25	3.11
Total	71.13	43.58	50.95	35.77
Total nitrogen	13.02	11.98	11.76	7.25

Appendix 4.2 Nitrogen supplementation. Yield of fruitbodies per 3 Kg fresh compost over $0.032m^2$.

Control

	No. of fruitbodies	Fresh weight (g)	Dry weight (g)	Mean fresh weight per fruitbody (g)
First break	19	215.9	19.64	11.36
Second break	9	357.6	40.26	39.73
Total	28	573.5	59.90	20.48

Appendix 4.2 (Cont'd...)

g.Nitrogen-equivalent per Kilogram fresh compost		Dried blood				Casein hydrolysate			
		No.	Fresh wt. (g)	Dry wt. (g)	Mean fresh wt. per fruitbody (g)	No.	Fresh wt. (g)	Dry wt. (g)	Mean fresh wt. per fruitbody (g)
0.16	1st break	17	210.5	18.39	12.38	18	215.8	17.45	11.98
	2nd break	12	327.0	37.89	27.25	24	438.6	47.19	18.27
	Total	29	537.5	56.28	18.50	42	654.4	64.4	15.58
0.32	1st break	18	206.7	18.10	11.48	22	221.8	19.80	10.08
	2nd break	18	268.3	31.73	14.90	28	487.2	52.86	17.40
	Total	36	575.0	49.83	15.97	50	709.0	72.66	14.18
0.64	1st break	17	199.7	17.55	11.74	19	231	20.69	12.15
	2nd break	23	467.0	55.89	20.30	24	462.2	51.16	19.25
	Total	40	666.7	73.44	16.66	43	693.2	71.85	16.12
0.96	1st break	14	211.7	19.28	15.12	10	131.7	12.08	13.17
	2nd break	24	525.1	58.65	21.87	31	555.2	60.29	17.90
	Total	38	736.8	77.93	19.38	41	686.9	72.37	16.75

Appendix 4.2 (Cont'd...)

g.Nitrogen-equivalent per Kilogram fresh compost		Bacterial biomass (I.C.I. 'Pruteen')				Soya bean meal			
		No.	Fresh wt. (g)	Dry wt. (g)	Mean fresh wt.per fruitbody (g)	No.	Fresh wt. (g)	Dry wt. (g)	Mean fresh wt. per fruitbody (g)
0.16	1st break	16	189.4	15.11	11.83	15	175.50	15.19	11.70
	2nd break	20	411.0	43.15	20.55	17	375.92	42.02	22.11
	Total	36	600.4	58.26	16.67	32	551.42	57.93	17.23
0.32	1st break	22	216.8	17.99	9.85	19	210.5	19.19	11.07
	2nd break	12	310.2	34.12	25.85	15	24.70	28.47	16.46
	Total	34	527.0	52.11	15.50	34	457.5	47.66	13.45
0.64	1st break	21	227.2	19.10	10.81	21	239.0	28.94	11.38
	2nd break	27	469.49	50.98	17.38	14	379.6	42.74	27.11
	Total	48	696.69	70.08	14.51	35	618.6	71.68	17.67
0.96	1st break	21	219.0	17.58	10.42	18	206.2	25.01	11.45
	2nd break	18	360.3	39.84	20.01	14	425.55	49.36	30.39
	Total	39	579.3	57.42	14.85	32	631.75	74.37	19.74

Appendix 4.3 Nitrogen supplementation. Dry matter content of fruitbodies. ($g.100g^{-1}$ fresh weight.)

	FIRST BREAK			
g Nitrogen-equivalent per kilogram fresh compost	0.16	0.32	0.64	0.96
Nitrogen supplement				
Control	9.10			
Dried blood	8.74	8.76	8.79	9.11
Casein hydrolysate	**8.09	8.93	8.96	9.21
Bacterial biomass (I.C.I. 'Pruteen')	**7.98	**8.30	**8.41	**8.03
Soya bean meal	9.07	9.12	*12.11	*12.13
	SECOND BREAK			
Control	11.26			
Dried blood	11.59	11.83	11.97	11,17
Casein hydrolysate	10.76	10.85	11.07	10.86
Bacterial biomass (I.C.I. 'Pruteen')	10.50	11.00	10.86	11.06
Soya bean meal	11.18	11.53	11.28	11.60

* t value is significant when $p = 0.01$

** t value is significant when $p = 0.05$

Appendix 4.4 Nitrogen supplementation. Crude protein content of fruitbodies. ($g.100g^{-1}$ dry matter.)

FIRST BREAK

g Nitrogen-equivalent per kilogram fresh compost	0.16	0.32	0.64	0.96
Nitrogen supplement				
Control	26.80			
Dried blood	**28.48	*30.97	*30.32	27.63
Casein hydrolysate	*33.47	*30.22	*30.22	*33.80
Bacterial biomass (I.C.I. 'Pruteen')	*31.93	*29.61	*29.84	*31.23
Soya bean meal	*29.52	**29.38	*34.00	*32.41

SECOND BREAK

Control	28.67			
Dried blood	29.36	28.23	*31.72	*30.48
Casein hydrolysate	27.93	*36.00	*34.37	*31.26
Bacterial biomass (I.C.I. 'Pruteen')	*30.23	*36.02	*33.49	*33.14
Soya bean meal	*25.85	*31.58	*31.76	*31.12

* t value is significant when $p = 0.01$

** t value is significant when $p = 0.05$

Appendix 4.5 Nitrogen supplementation. Amino acid composition ($g.100g^{-1}$ dry matter) of first break fruitbodies following the addition of different concentrations of casein hydrolysate.

g.Nitrogen-equivalent per kilogram fresh compost	0.16	0.32	0.64	0.96
Amino acids				
Aspartic acid	1.96	1.78	2.06	1.98
*Threonine	0.93	0.85	0.97	0.92
Serine	0.99	0.97	1.17	1.01
Glutamic acid	4.89	4.23	4.49	4.45
Proline	1.02	0.94	1.14	1.42
Glycine	0.85	0.79	0.87	0.84
Alanine	1.40	1.37	1.51	1.61
Cystine	0.0	0.0	0.0	0.0
*Valine	0.99	0.92	0.99	0.95
*Methionine	0.32	0.24	0.26	0.23
*Isoleucine	0.84	0.77	0.86	0.83
*Leucine	2.11	2.01	2.13	2.01
Tyrosine	0.40	0.35	0.39	0.36
*Phenylalanine	0.85	0.74	0.79	0.77
Histidine	0.47	0.47	0.50	0.44
*Lysine	0.72	0.81	0.87	0.89
Arginine	1.06	1.02	1.11	1.44
TOTAL	19.79	18.27	20.11	20.15

* Essential amino acids.

Appendix 4.6 Nitrogen supplementation. (a)Soluble protein content of fruitbodies. ($g.100g^{-1}$ dry matter).

	FIRST BREAK			
g.Nitrogen-equivalent per kilogram fresh compost	0.16	0.32	0.64	0.96
Nitrogen supplement				
Control	21.30			
Dried blood	20.00	20.33	19.66	21.33
Casein hydrolysate	22.33	22.33	23.34	24.66
Bacterial biomass (I.C.I. 'Pruteen')	21.00	20.00	20.66	21.02
Soya bean meal	21.66	24.33	23.66	23.66
	SECOND BREAK			
Control	20.01			
Dried blood	23.33	20.33	23.66	22.30
Casein hydrolysate	19.33	21.66	22.30	22.30
Bacterial biomass (I.C.I. 'Pruteen')	22.00	20.66	22.33	23.36
Soya bean meal	20.00	22.66	23.66	22.00

(a) Estimated according to the method of Lowry *et al* (1951).

Appendix 4.7 Nitrogen supplementation. [a]Actual protein content of fruitbodies (summation of amino acids). ($g.100g^{-1}$ dry matter.)

Nitrogen supplement	First break	Second break
Control	17.39	17.45
Dried blood	16.61	17.70
Casein hydrolysate	19.59	17.45
Bacterial biomass (I.C.I. 'Pruteen')	18.62	18.68
Soya bean meal	18.18	17.48

Results do not include tryptophan.

(a) Data obtained from Appendix 4.8

Appendix 4.8 Nitrogen supplementation. Amino acid composition of fruitbodies. ($g.100g^{-1}$ dry matter.)

Nitrogen Supplement	FIRST BREAK					SECOND BREAK				
Amino acids	Control	Dried Blood	Casein Hydrolysate	Bacterial biomass ('Pruteen')	Soya Bean Meal	Control	Dried Blood	Casein Hydrolysate	Bacterial biomass ('Pruteen')	Soya Bean Meal
Aspartic acid	1.68	1.51	1.94	1.77	1.68	1.63	1.67	1.63	1.71	1.63
*Threonine	0.91	0.79	0.91	0.95	0.87	0.82	0.83	0.88	0.93	0.89
Serine	0.89	0.80	1.04	0.98	0.88	0.86	0.90	0.89	0.95	0.91
Glutamic acid	3.94	4.22	4.52	4.51	4.51	4.16	4.16	4.09	4.30	3.96
Proline	0.87	0.92	1.13	1.06	1.00	1.08	1.02	0.92	0.97	0.93
Glycine	0.83	0.81	0.84	0.86	0.85	0.82	0.82	0.82	0.86	0.81
Alanine	1.19	1.30	1.47	1.37	1.37	1.44	1.49	1.47	1.56	1.44
Cystine	0	0	0	0	0	0	0	0	0	0
*Valine	0.89	0.74	0.96	0.99	0.78	0.77	0.75	0.78	0.96	0.78
*Methionine	0.28	0.28	0.26	0.20	0.30	0.27	0.29	0.28	0.31	0.30
*Isoleucine	0.82	0.75	0.83	0.86	0.82	0.78	0.78	0.79	0.84	0.80
*Leucine	1.48	1.37	2.07	1.45	1.51	1.36	1.38	1.44	1.51	1.40
Tyrosine	0.43	0.37	0.38	0.41	0.41	0.37	0.42	0.41	0.42	0.45
*Phenyl alanine	0.84	0.59	0.79	0.83	0.77	0.72	0.72	0.74	0.82	0.73
Histidine	0.45	0.40	0.47	0.48	0.44	0.41	0.43	0.41	0.46	0.42
*Lysine	0.97	0.83	0.82	0.94	0.91	0.91	0.93	0.92	0.99	0.93
Arginine	0.91	0.92	1.16	0.95	1.07	1.04	1.11	0.98	1.08	1.08

* Estimated amino acids.

Appendix 4.9 Nitrogen supplementation. Urea content of fruitbodies ($g.100g^{-1}$ dry matter.)

	FIRST BREAK			
g Nitrogen-equivalent per kilogram fresh compost	0.16	0.32	0.64	0.96
Nitrogen supplement				
Control	1.22			
Dried blood	1.18	1.12	0.77	0.90
Casein hydrolysate	1.34	1.17	1.42	1.94
Bacterial biomass (I.C.I. 'Pruteen')	1.10	1.05	1.16	1.00
Soya bean meal	1.36	1.22	1.48	1.45
	SECOND BREAK			
Control	0.80			
Dried blood	1.00	0.70	0.80	0.80
Casein hydrolysate	0.70	1.00	1.00	1.20
Bacterial biomass (I.C.I. 'Pruteen')	0.67	0.77	0.67	0.64
Soya bean meal	0.48	0.80	0.80	0.67

Appendix 5.1 Storage experiment. Fresh weight and dry matter loss.

Percentage material losses were calculated according to the following equation:-

$$\frac{(\text{Fresh weight}_{t=0} \cdot \text{Dry Matter}_{t=0} \cdot \text{Nutrient content}_{t=0}) - (\text{Fresh weight}_{t} \cdot \text{Dry matter}_{t} \cdot \text{Nutrient content}_{t})}{(\text{Fresh weight}_{t=0} \cdot \text{Dry matter}_{t=0} \cdot \text{Nutrient content}_{t=0})} \times 100$$

Dry matter content of fruitbodies, time = 0, First break = 7.77g.100g^{-1} fresh weight.

Dry matter content of fruitbodies, time = 0, Second break = 8.74g.100g^{-1} fresh weight.

(a) Fresh weight loss of fruitbodies during storage at two different temperatures from first and second breaks.

	FIRST BREAK		SECOND BREAK	
Day of Storage	3°C	22°C	3°C	22°C
2	*2.16	*5.44	*1.60	*3.53
4	**3.53	*9.73	*2.83	*8.52
6	*7.36	*24.36	*3.96	*10.04

Mean value (g.100g^{-1}) of three replicate punnets.

(b) Dry matter loss of fruitbodies during storage at two different temperatures from first and second breaks.

Day of Storage	FIRST BREAK		SECOND BREAK	
	$3^{o}C$	$22^{o}C$	$3^{o}C$	$22^{o}C$
2	**4.76	**12.44	**6.13	**9.11
4	**4.97	*20.39	**10.60	*18.69
6	**4.96	$*32.81_{(d)}$	**8.66	*21.63

(d) denotes samples in decayed state.

Mean value ($g.100g^{-1}$) of three replicate punnets.

Significance between % loss from original sample within each temperature treatment indicated by:-

* t value is significant when p = 0.01

** t value is significant when p = 0.05

Appendix 5.2 Crude protein content (Total nitrogen x 6.25) of fruitbodies stored at two different temperatures from first and second breaks.

Day of Storage	FIRST BREAK		SECOND BREAK	
	3°C	22°C	3°C	22°C
0	37.84	37.84	45.99	45.99
2	38.74	38.86	46.74	44.53
4	38.80	*32.72	44.58	45.99
6	38.17	(d)	44.92	45.46

(d) denotes sample in decayed state.

Mean value of crude protein (g) in dry matter remaining after storage, when day 0 = 100g.

* t value is significant when p = 0.01

Appendix 5.3 Amino acid content of fruitbodies stored at two different temperatures from first and second breaks.

	FIRST BREAK					
			3°C		22°C	
Day of storage	0	2	4	6	2	4
(a) Amino acid						
Aspartic acid	2.53	2.26	2.25	2.03	1.49	1.83
*Threonine	1.10	1.17	1.00	1.14	0.69	0.93
Serine	1.18	1.05	1.01	1.05	0.82	0.89
Glutamic acid	5.96	5.74	5.54	5.32	5.04	4.77
Proline	1.48	1.61	1.61	1.39	1.38	1.42
Glycine	1.08	0.99	0.90	0.88	0.90	0.85
Alananine	1.75	2.00	1.92	2.09	1.74	1.79
Cystine	0	0	0	0	0	0
*Valine	1.01	1.01	0.82	0.91	0.89	0.84
*Methionine	0.21	0.18	0.19	0.18	0.40	0.17
*Isoleucine	0.91	1.00	0.92	1.01	0.80	0.86
*Leucine	1.63	1.50	1.40	1.38	1.50	1.22
Tyrosine	0.47	0.45	0.24	0.21	0.38	0.24
*Phenylalanine	0.86	0.85	0.54	0.55	0.55	0.41
Histidine	0.30	0.29	0.26	0.49	0.22	0.24
*Lysine	1.12	1.06	0.91	0.98	0.74	0.84
Arginine	1.33	1.15	1.02	0.76	1.05	0.92
Total	22.92	21.38	20.53	20.37	18.59	18.22
% loss from day = 0		6.7	10.42	11.1	18.89	20.50

(a)Amino acid (g) in dry matter remaining after storage, when day 0 = 100g

* Essential amino acids.

Appendix 5.3 (Cont'd...)

	SECOND BREAK						
			3°C			22°C	
Day of storage	0	2	4	6	2	4	6
(a)Amino acid							
Aspartic acid	3.06	2.52	2.30	1.80	2.49	1.65	1.45
*Threonine	1.44	1.44	1.40	1.10	1.39	0.95	0.99
Serine	1.38	1.27	1.22	0.92	1.19	0.91	0.84
Glutamic acid	6.89	7.01	6.55	4.85	6.11	4.69	4.27
Proline	2.77	2.14	1.85	1.38	2.09	1.29	1.29
Glycine	1.37	1.26	1.16	0.85	1.19	0.94	0.76
Alanine	2.34	2.50	2.24	1.91	2.15	1.35	1.72
Cystine	0	0	0	0	0	0	0.36
*Valine	1.54	1.43	1.39	1.07	1.41	0.96	1.02
*Methionine	0.19	0.29	0.30	0.17	0.23	0.14	0.16
*Isoleucine	1.26	1.13	1.11	0.85	1.06	0.71	0.76
*Leucine	1.85	1.69	1.68	1.28	1.62	1.11	1.22
Tyrosine	-	not resolved					
*Phenylalanine	1.33	1.32	1.27	0.89	1.22	0.84	0.84
Histidine	0.59	0.56	0.51	0.36	0.52	0 45	0.52
*Lysine	1.67	1.61	1.59	1.10	1.38	0.97	0.97
Arginine	1.81	1.21	1.13	0.75	1.07	0.72	0.69
Total	29.49	27.38	25.70	19.28	25.12	17.68	17.86
% loss from day = 0	-	7.15	12.85	34.62	14.81	40.04	39.43

(a)Amino acid (g) in dry matter remaining after storage, when day 0 = 100g.

* Essential amino acids.

Appendix 5.4 Carbohydrate content of fruitbodies stored at two different temperatures from first and second breaks.

	FIRST BREAK		SECOND BREAK	
Day of Storage	3°C	22°C	3°C	22°C
0	16.99	16.99	15.84	15.84
2	**14.22	**12.47	15.15	**10.87
4	**14.26	**11.58	*13.97	**10.12
6	*13.85	(d)	*12.80	*9.35

(d) denotes sample in decayed state.

Mean value of carbohydrate (g) in dry matter remaining after storage, when day 0 = 100g.

* t value is significant when p = 0.01

** t value is significant when p = 0.05

Appendix 5.5 Lipid content of fruitbodies stored at two different temperatures from first and second breaks.

Day of Storage	FIRST BREAK		SECOND BREAK	
	3°C	22°C	3°C	22°C
0	7.37	7.37	7.18	7.18
2	7.25	**6.60	*** 6.54	**6.21
4	7.19	**5.88	6.88	**5.23
6	7.42	(d)	6.72	**5.69

(d) denotes sample in decayed state

Mean value of lipid (g) in dry matter remaining after storage, when day 0 = 100g.

* t value is significant when p = 0.01

** t value is significant when p = 0.05

*** t value is significant when p = 0.10

Appendix 5.6 Ash content of fruitbodies stored at two different temperatures from first and second breaks.

	FIRST BREAK		SECOND BREAK	
Day of Storage	3°C	22°C	3°C	22°C
0	10.52	10.52	9.79	9.79
2	10.59	10.26	9.39	9.50
4	10.28	9.83	9.43	9.40
6	10.93	(d)	9.71	9.75

(d) denotes sample in decayed state.

Mean value of ash (g) in dry matter remaining after storage, when day 0 = 100g.

Appendix 6.1 Effects of inorganic salts mixtures on yield and sulphur-containing amino acids composition of *A.bisporus* mycelium.

No.	Addition to medium $g.l^{-1}$ (+)	Nitrogen in medium $g.l^{-1}$		Sulphur in medium $g.l^{-1}$		Dry weight yield of mycelium $g.l^{-1}$	Mycelial composition $g.100g^{-1}$ dry matter		
		Added	Total	Added	Total		Total nitrogen	*Methionine	Cysteine
1	None	0	0.36	0	0.025	3.2	4.57	0.33	0.55
2	0.5 $(NH_4)_2SO_4$ + 0.04 $NH_4H_2PO_4$	0.11	0.47	0.12	0.145	3.3	5.28	0.35	0.53
3	1.0 " + 0.08 "	0.22	0.58	0.24	0.265	4.0	5.10	0.34	0.52
4	2.0 " + 0.16 "	0.44	0.80	0.48	0.505	3.9	5.32	0.41	0.65
5	4.0 " + 0.32 "	0.89	1.25	0.96	0.985	3.6	5.61	0.42	0.55
6	No.3 + 1.0 KH_2PO_4	0.22	0.58	0.24	0.265	4.4	5.22	0.36	0.53
7	No.3 + 2.5 $MgSO_4.7H_2O$	0.22	0.58	0.56	0.585	3.4	5.17	0.41	0.55
8	No.3 + 1.0 KH_2PO_4 and 2.5 $MgSO_4.7H_2O$	0.22	0.58	0.56	0.585	3.7	5.14	0.38	0.61

Inorganic salts concentrations based on those of Chiao and Peterson (1953).

(+) Basal synthetic medium was that described in Section 2.3.2.

* This was measured using a locarte amino acid analyser, the column in Section 2.4.5 ii(c)Estimation of sulphur amino acids, appeared inadequate due to technical reasons associated with the resin mesh size, and temperature.

Appendix 6.2 Effects of ammonium sulphate on yield and sulphur-containing amino acids of A.bisporus mycelium.

No.	Addition to medium(+) $g.l^{-1}$ $(NH_4)_2SO_4$	Nitrogen in medium $g.l^{-1}$ Added	Total	Carbon: Nitrogen ratio	Sulphur in medium $g.l^{-1}$ Added	Total	pH before incubation	pH after incubation	Dry wt.yield of myeclium $g.l^{-1}$	Mycelial composition $g.100g^{-1}$ dry matter Total Nitrogen	*Methionine	Cysteine
1	None	0	0.36	44	0	0.025	6.5	5.4	3.3	4.92	0.35	0.56
2	0.84	0.17	0.53	30	0.2	0.225	6.2	5.3	3.6	5.60	0.36	0.60
3	1.69	0.35	0.71	22	0.4	0.425	6.1	6.3	3.4	4.80	0.34	0.56
4	3.39	0.71	1.07	15	0.8	0.825	5.9	6.0	3.6	5.00	0.40	0.49
5	5.09	1.07	1.43	11	1.2	1.225	5.8	6.4	4.0	4.85	0.44	0.50
6	6.83	1.44	1.80	9	1.6	1.625	5.7	6.3	1.3	7.40	0.48	0.56
7	8.53	1.80	2.16	7	2.0	2.025	5.7	6.3	1.6	5.80	0.51	0.61

(+) and * : see Appendix 6.1

Appendix 6.3 Effects of magnesium sulphate on yield and sulphur-containing amino acids composition of A.bisporus mycelium

No.	Addition to medium ($g.l^{-1}$) $MgSO_4.7H_2O$	Sulphur in medium (+) Added	Total	Dry weight yield of mycelium $g.l^{-1}$	Mycelial composition $g.100g^{-1}$ dry matter Total nitrogen	*Methionine	Cysteine
1	None	0	0.025	3.3	4.92	0.35	0.56
2	1.0	0.129	0.154	5.3	4.04	0.37	0.51
3	2.0	0.259	0.284	3.5	4.23	0.38	0.55
4	4.0	0.519	0.544	3.3	4.36	0.50	0.52
5	6.0	0.778	0.803	2.6	4.48	0.56	0.57
6	8.0	1.038	1.063	2.8	4.40	0.60	0.67
7	10.0	1.298	1.323	2.6	4.55	0.58	0.75

(+) and * : see Appendix 6.1

Appendix 6.4 Effects of intermediates, L-methionine and it's derivative N-acetyl-L-methionine on yield and sulphur-containing amino acids biosynthesis of A.bisporus mycelium.

Supplement	Sulphur in medium $mg.l^{-1}$ (+)		Supplement added $g.l^{-1}$	Dry weight yield of mycelium $g.l^{-1}$	Mycelial composition $g.100g^{-1}$ dry matter		
	Added	Total			Total nitrogen	*Methionine	Cysteine
Control	None	50	0	3.2	4.48	0.34	0.52
L-methionine	15	65	0.069	4.3	5.36	0.57	0.64
	30	80	0.139	3.0	6.32	0.68	0.76
	60	110	0.279	3.0	6.10	0.63	0.75
L-cysteine hydrochloride	15	65	0.073	5.0	4.11	0.35	0.57
	30	80	0.140	4.1	5.46	0.56	0.76
	60	110	0.290	3.9	5.00	0.48	0.65
N-acetyl-L-methionine	15	65	0.089	4.2	4.32	0.44	0.58
	30	80	0.179	3.7	5.34	0.54	0.69
	60	110	0.358	3.0	5.53	0.77	0.59

Appendix 6.4 (Cont'd...)

Supplement added	μmol.l^{-1}	Dry weight yield of mycelium g.l^{-1}	Mycelial composition g.100g^{-1} dry matter		
			Total nitrogen	*Methionine	Cysteine
L-homocystine	40	3.3	4.28	0.40	0.55
	80	3.6	4.41	0.43	0.54
	160	4.6	4.28	0.39	0.54
L-α-amino-n-butyric acid	40	4.4	4.92	0.39	0.37
	80	4.2	4.35	0.34	0.30
	160	3.8	4.54	0.33	0.31

(÷) and * : see Appendix 6.1

Appendix 6.5 Submerged culture. Medium composition for the Carbon:Nitrogen ratio experiment.

C : N ratio	Casein hydrolysate in medium $g.l^{-1}$	Total nitrogen in medium $g.l^{-1}$
2	66.30	7.94
3	44.20	5.29
6	22.10	2.64
9	14.73	1.76
12	11.05	1.32
15	8.84	1.05
18	7.36	0.88
21	6.31	0.75
27	4.91	0.58
33	4.01	0.48
39	3.40	0.40
45	2.94	0.35

All other medium constituents were as for the basal synthetic medium (see Section 2.3.2). Different C : N ratios were obtained by adjusting the concentration of casein hydrolysate, i.e:-

$$g.l^{-1} \text{ casein hydrolysate} = \frac{C}{C:N} \cdot \frac{1000}{11.98}$$

where C = carbon concentration = 15.9 $g.l^{-1}$

Nitrogen content of casein hydrolysate ($g.100g^{-1}$ dry matter) = 11.98

- Casein hydrolysate was considered solely as a nitrogen source.

Appendix 6.6 Effects of Carbon:Nitrogen ratio on the submerged culture of A.bisporus mycelium.

C:N ratio	Dry weight yield $g.l^{-1}$	Mycelial composition $g.100g^{-1}$ dry matter. Crude protein	Soluble protein	Total carbohydrate (as glucose)	*Methionine	Cysteine	Soluble protein / Crude protein
2	1.26	43.64	33.75	40.20	0.55	0.61	0.77
3	2.13	40.20	32.14	43.50	0.52	0.54	0.80
6	2.60	39.16	32.00	41.00	0.53	0.56	0.81
9	3.40	36.72	31.50	42.50	0.50	0.57	0.85
12	3.06	36.45	31.50	42.75	0.47	0.55	0.86
15	4.40	36.20	29.50	50.25	0.47	0.50	0.81
18	5.06	35.20	28.00	55.00	0.43	0.44	0.79
21	4.33	35.80	29.00	48.00	0.44	0.44	0.81
27	4.13	35.19	28.60	45.75	0.49	0.58	0.81
33	3.93	34.53	30.30	45.25	0.47	0.60	0.87
39	3.85	33.51	29.30	45.08	0.46	0.58	0.87
45	3.80	31.97	27.60	47.50	0.46	0.59	0.86

* See Appendix 6.1

Appendix 6.7 Amino acids composition and total nitrogen content of the* thermophilic bacterial biomass.

Amino acid	$g.100g^{-1}$ dry matter
Aspartic acid	3.44
Threonine	2.02
Serine	1.49
Glutamic acid	5.10
Proline	1.43
Glycine	1.66
Alanine	2.32
Cysteine	0.572
Valine	2.50
Methionine	1.04
Isoleucine	2.41
Leucine	3.23
Tyrosine	1.55
Phenylalanine	2.10
Histidine	0.90
Lysine	2.92
Arginine	2.01
Total	36.23
Total nitrogen	9.36

* The bacterial biomass was cultivated as described in Section 2.3.3.

Appendix 6.8 Utilization of thermophilic bacterial biomass as a nitrogen source.

(a) Bacterial biomass and casein hydrolysate as a mixed nitrogen source.

Carbon:Nitrogen ratio	Nitrogen in medium $g.l^{-1}$ (+)		Bacterial biomass added $g.l^{-1}$	Dry weight yield of mycelium $g.l^{-1}$	Mycelial composition $g.100g^{-1}$ dry matter		
	Added	Total			Total Nitrogen	*Methionine	Cysteine
44	None	0.36	None	2.9	5.20	0.30	0.54
39	0.05	0.40	0.53	4.73	5.78	0.52	0.54
27	0.23	0.58	2.45	4.33	5.49	0.54	0.53
18	0.53	0.88	5.66	4.46	5.33	0.55	0.55
12	0.97	1.32	10.36	5.33	5.29	0.57	0.50
9	1.41	1.76	15.06	6.33	5.07	0.60	0.46

(+) and * : see Appendix 6.1

Appendix 6.8 (Cont'd...)

(b) Bacterial biomass as sole nitrogen source.

Carbon:Nitrogen ratio	Nitrogen in medium(-) $g.l^{-1}$	Bacterial biomass added $g.l^{-1}$	Dry weight yield of mycelium $g.l^{-1}$	Mycelial composition $g.100g^{-1}$ dry matter		
				Total Nitrogen	*Methionine	Cysteine
27	0.58	6.19	6.00	5.41	0.70	0.61
21	0.75	8.01	5.76	5.18	0.62	0.44
18	0.88	9.40	9.33	5.36	0.63	0.54
15	1.06	11.32	9.16	5.13	0.60	0.54
12	1.32	14.10	9.33	5.37	0.56	0.45

(-) Casein hydrolysate-free basal synthetic medium was that described in Section 2.3.2

* See Appendix 6.1

Appendix 6.9 Amino acids composition ($g.100g^{-1}$ dry matter) of mushroom mycelium grown in basic synthetic medium (+) with casein hydrolysate and/or thermophilic bacterial biomass as a nitrogen source.

Thermophilic Bacterial biomass $g.l^{-1}$	0	15.06 + 3.0 casein hydrolysate	6.16	8.01	9.40	11.32	14.10
Carbon:Nitrogen ratio	44:1	9:1	27:1	21:1	18:1	15:1	12:1
Amino acids							
Aspartic acid	2.00	1.96	1.99	2.00	2.16	2.11	2.11
-Threonine	1.17	1.08	1.12	1.09	1.17	1.16	1.16
Serine	1.18	1.11	1.16	1.12	1.16	1.15	1.15
Glutamic acid	2.50	2.96	2.69	3.06	3.19	3.18	3.18
Proline	1.36	0.95	0.96	0.92	1.02	0.94	1.00
Glycine	0.90	0.89	0.97	0.90	0.92	0.95	0.95
Alanine	1.30	1.52	1.40	1.29	1.44	1.38	1.44
Cysteine	0.54	0.46	0.61	0.44	0.54	0.54	0.45
-Valine	1.10	1.28	1.25	1.24	1.39	1.28	1.34
*Methionine	0.30	0.60	0.70	0.62	0.63	0.60	0.56
-Isoleucine	1.03	1.31	1.36	1.28	1.41	1.32	1.37
-Leucine	1.63	1.90	2.08	1.90	2.12	2.01	2.04
Tyrosine	0.66	0.69	0.74	0.69	0.73	0.74	0.76
-Phenylalanine	1.08	1.18	1.26	1.16	1.31	1.19	1.26
Histidine	0.61	0.46	0.52	0.46	0.52	0.47	0.49
-Lysine	1.15	1.54	1.50	1.40	1.72	1.42	1.51
Arginine	1.33	1.35	1.80	1.37	1.44	1.43	1.43
Total	19.84	21.24	22.11	20.94	22.90	21.87	22.20

(+) and *: see Appendix 6.1

- Essential amino acids.

Appendix 7.1 Chemical scores of mushroom protein compared to various foodstuffs.

Essential amino acids	*A. bisporus fruitbodies	**Yeasts mixture	***Rice (brown or husked)	***Wheat whole grain	***Cabbage leaves	FAO/WHO 1973		***Beef & veal edible flesh	FAO/WHO 1973 Provisional amino acid scoring pattern mg.g. of protein
						Hen's Eggs	Cow's Milk		
Isoleucine	35.80	53.6	40.0	34.9	31.2	54	47	48.1	40
Leucine	60.4	75.1	86.4	71.3	53.7	86	95	81.0	70
Lysine	45.3	78.2	38.8	30.6	31.2	70	78	88.8	55
Methionine + cystine	15.9	30.8	35.6	43.2	21.8	57	33	39.7	35
Phenylalanine + tyrosine	65.1	83.4	90.8	80.3	49.3	93	102	79.9	60
Threonine	41.3	58.5	40.9	31.3	38.1	47	44	45.8	40
Tryptophan	110.2	13.4	-	-	-	17	14	-	10
Valine	45.5	59.9	57.7	47.2	42.5	66	64	50.0	50
Chemical score	45	88	70	55	56		94		
Limiting amino acid	Sulphur-containing amino acids	Sulphur-containing amino acids	Lysine	Lysine	Lysine		Sulphur-containing amino acids		

* Data obtained from Appendix 2.1

** Data obtained from Van den Veen 1974

*** Data obtained from FAO 1970, Amino acid content of foods and biological data on proteins, Rome, Italy. (FAO Nutritional studies, No.24).

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